International Journal of Neurology

ISSUE DEDICATED TO:

NEUROPEPTIDES IN THE BRAIN

9th International Symposium of the Fulton Society

VOLUME 14 NUMBERS 2-3-4

INTERNATIONAL JOURNAL OF NEUROLOGY

CONTENTS OF VOL. 14 — NUMBERS 2 - 3 - 4

Editorial

The impact of the investigations of Neuropeptides in Modern Neuropsychiatry

Man, without any bounds, to his scientific curiosity, having reached the moon, and searched the planets of the solar system by means of unmanned aircraft and having reached beyond his own galaxies to the limits of the Universe, by means of radiotelescopes must have on this occasion once more, the conviction that size is not linked to importance, and that the macrocosmos and the microcosmos hide to the intellectual curiosity many marvels beyond human understanding.

To find out its laws has been the task of generations of dedicated scholars who have benefited mankind with its most cherished treasure; knowledge.

It seems to be that the mission of man on earth is to witness, to the grandeur of creation, to macht it he ought to perfect his thinking methods, to unravel the intrincacies displayed to be mirrored in his understanding.

Nature has its own language, and the scientist who understands it, may entertain a life long enlightening and fruitful conversation.

From all the scientific avenues opened to intellectual curiosity, one of the most fascinating is the study of life itself, man scrutinizing nature, his own nature in all its multifarious áspects.

Nothing more complicated than to elucidate and interpret the multiplicity of a given function, in its mormal and impaired manifestations, and to intervene in the correct way to restore health.

The study of the "Neuropeptides in the Brain" is a topic of trascendental importance which will shed light on such crucial aspects of human life as, behavior, pain, memory, human growth, attention, learning schizophrenia and so on.

Electrochemistry is at the base of life and guide its future and evolution, above all it depends on his hormones, a better life can be achieved through the understanding of the ways and doings of peptide linked with important physical and psychological functions.

Mimicking nature, the man of science reproduces what he discovers in nature to his own bénefit and uses the same bricks to become a creator.

It is interesting to remark that while Woodward was in a train travelling to Saint Louis he began to think about the best way of making aminoacid molecules link themselves into chains in order to form a peptide.

Once in his laboratory, he began to search in the books and he found that the same ideas had ocurred to two chemists, Leuch and Geiger in 1908. *However, they missed the fact that the intervention of an enzime was necessary as a living catalist.*

Nowadays, synthetizing nature's own material, has become a wonderful trick of pharmacology in order to correct functional impairment, and to improve human life.

In the chapter on mental illnesses we are till facing the problem that our main difficulty is to know its etiology.

The studies on neurotransmitters and neuropeptides, have shed light on

many aspects of neuropsychiatry disorders and the words of L Pauling twice laureated with the Nobel Prize, have been prophetic, He said: "I believe that the majority of the mental illnesses are biochemical abnormalities."

The problem of the biochemical origin of certain psychotic ailments is posed due to the structural relationship that exist between psychopathogenic chemical substances and certain natural metabolic substances.

Thus, the investigator bq not facing anymore comparative studies of static structure, but by aiming to discover the biochemical perturbance hidden in the neurohormonal functions is able to explain the absence of anatomical lesions.

Not long ago some investigators proclamed that it will be ever impossible to relate the chemical transformation that occurs at the Level of the living cell and the phenomena of psychical dynamism.

Nowadays this concept has been modified with the advenement of new vistas about the role of neuropeptides.

As a living entity man is forced to use all his intelectual weapons so as to control his behavior, to face and overcome danger, or obstacles to tackle hisdecisions, to aspire at reaching a higher level.

This is a subject of utmost importance in the field of neurosciences Not only because it shed light on the normal and pathological functions of the nervous System, but it puts in evidence the close relationship between the Nervous and the Endocrinal System. This is determined by the direct action of the peptides originated in the hypothalamus, pituitary, and other hormones on the Central Nervous System This kind of studies have raised hopes as to the possibility that these investigations may provide new therapeutic tools for the treatment of the diseases of the nervous System.

The studies of the peptides on the Central Nervous System began in 1971 *with the studies if Plotnikoff in their basic aspects and in* 1972 *with Kasting and Barbeau in their clinical manifestations.*

Neuropeptides have called the attention of numerous investigators who are finding its implications in such passionate topics as memory, stress, attention, learning, Parkinson disease, depresion, psychophysiology, electrophysiology, sleep, body temperature regulation accunputure and so on.

It has been observed that the hypothalamic releasing factors act directly uppon the brain, besides the action determined by its endócrnal periphéral effects.

Investigations performed with the Everett Dopa pargyline potentiation test, puts in evidence that the hypothalamic releasing factors such as Melanocytic-stimulating hormone release inhibiting factor, Thyrotropin releasing hormone, Somatostasin and luteininzing hormone releasing hormone in their action give place to the potentialization of the influence upon the behavior of the Dopa in normal rat and also in those in which the hypophisis has been removed which means that the above mentioned peptides may be effective without the mediation of hormones coming from the hypophisis.

The peptides' action on the Central Nervous System may be expressed as neurotransmitters, or neuromodulators and in this aspect they participate in the regulation of neuronal excitability.

The neurohipophysary hormones and the substantia "P" acts particularly as neuromodulators.

The effects of the peptides of the neurohypophisis are greater when admi-

nistered intraventricularly than systematically which evidence that its action is central.

It is worthwhile to mention that a small quantity of peptides is to produce a desirable effect.

But we must also mention the difficulties to be overcome in order to get this substance. For example to obtain 1 *mgm of TRF it is necessary to get* 300.000 *sheeps' hypothalamus.*

A small amount of hypothalamic cells may control in its totality the dynamic biochemistry by which the thyroid regulated the metabolism of the cells all over the body.

In the Central Nervous System the peptides may act as neurotransmitters, many of them are sythetized in the C.N.S. and have an important biological specific action on the peripherical organs.

Concerning the neurons peptides relationships, the first that were evident were those pertaining to the Hypothalamus. It has been accepted that the interactions of neurons and peptides is very ample and cannot be restricted to the hypothalamus. Little quantities of peptides may elicit significant celular changes.

It is possible that the peptides acts in phychophysiological responses to environmental stress by means of the hypothalamus and the Limbic System.

The hypothalamus is involved in numerous vital functions. It receives myriads of messages from nervous and endocrinal sources and contributes to the regulation of the autonomic nervous system and to the endocrinal one.

Hormones that are principally peptides produced in the hypothalamus or the pituitary may participate in such a complex function as is the behavior.

The peptides may influence innate as well as learned patterns.

Concerning the acquisition and preservation of new patterns of behavior the peptides may be involved in the mechanisms of conditioning learning and memory. The process of learning a new kind of behavior is very frequently associated with a releasing of the pituitary hormone, ACTH, increasing the utilization of hormones.

This may provide biochemical mediatiors by means of which the proteic metabolism is activated and the plasticity of the nervous system facilitated, this is very important to maintain behavior.

IMPLICATIONS OF THE NEUROPEPTIDES IN STRESS

Stress may be inflicted upon the body by external forces such as changes in the temperature, information it is reveived and integrated by the nervous system and possibly delivered at the hypothalamus and the basal areas of the brain, The hypothalamus secretes corticotropine (CRP) and liberates another *factor that stimulates the pituitary wich in turn secretes the hormone ACTH. This stimulates the cortex of the suprarrenal gland to provoke the synthesis and excretions of hormones, besides CRF and ACTH which are both polipeptide and it seems to have a direct effect upon the brain. If the supra adrenal gland is removed the pituitary secretes abnormally great quantity of ACTH; it is believed that the absence of the suprarenal hormone allows a limitless secretion.*

Then the question is posed. Which is the function of the liberation of vasopressin to meet the adaptation to stress?

The liberation of vasopressin can be taken as a response to "fight" or "flight" like a factor that participates in the function of the preservation of

fluid that it may be useful in case that a hermorhage is originated. The vasopressin liberation facing symbols of danger may represent an anticipated response of adaptation and getting ready for fight.

VASOPRESSIN

The vasopressin besides acting as a peptide, is also acting as a neurotransmitter. Nervous cells of the hypothalamus rely on vasopressin in order to stimulate other nervous cells of the brain.

The vasopressin was considered first as an antidiuretic hormone factor, which acted in animals deprived of water ingestion diminishing the loss of water in the urine.

Afterwards it was observed that the antidiuretic hormone could increase the blood pressure acting as vaso constrictor, and then it was given its second name vasopressin.

Vasopressin and oxitocin. This last peptide promotes the contraction of the muscles of the uterus, In vertebrate animals, that are not mammals, it is represented by only one peptide called arginine-vasotocin In mammals both hormones are present, vasopressin and oxitocin.

Vasopressin and other peptides, corticotropin or endorphin affect learning and memory.

It has been accepted that vasopressin promotes changes of behavior.

Experiments have been made enploying long lasting analogues of vasopressin and those who have received it experienced an incresing awareness toward the environment and a better memory.

Research on such neuropeptides may suggest specific tools for the treatment of mental disorders related to disturbances in the functioning of the neurohypophyseal system.

Aside from this function of conservation of fluids, the vasopressin may have some other important functions. It has been taken as a bond between CNS and the liberation of ACTH.

Some authors have observed evidences that suggest bonds between the neurohypophisis and the adenohypophisis. In thie connection a basic peptide habilitated for the ACTH liberation has been described as a factor of liberation of corticotropinc.

It has been observed that besides its paticipation on blood pressure and water regulation the vasopressin has an important role in improving the memary at a long term range during the process of learning.

Studides on the mechanism of action of the peptides in stress situation have been directed to elucidate its possible interaction by trasmitting substances of the brain.

The norepinephrine levels have shown to be very sensitive to different kind of stresses. It has been suggested that the biogenic amines responses to stress may be hormonally mediated.

The hormones influence the cerebral function and specific kind of behavior may be related with sex behavior.

It has been indicated that gonadal hormones act upon the CNS in a different way in two different stages of evolution. Ist during the fetal life or neunatal and 2nd, during adult life.

On the other hand, it has been observed that hypothalamic peptides influences upon the animic states.

The Thyrotropin releasing hormones may convert the sedative effects of penthobarbital, chlorpromazine and ethanol.

Beside its influence in depression and schizophrenia has been observed, Significance has been assigned to the definition of subgroupe of depression which are based i nthe disminution of the response of Thyrotropin to the Thyrotropin releasing hormone.

Serotonin has also been studied and it has been observed that with it, are actives the Thyrotropin realising hormone and luteinizing hormone-releasing hormone. In its therapeutic action on Parkinson Diseases is has been studied the peptide melanocyte-stimulating hormone release inhibiting factor. It has been oberved that it originates a benefic effect upon rigidity, akinesia and tremor.

Melanocyte-stimulating hormone releases an inhibiting factor (MSH-R-IF) and potentiates the action of Dopa, it reverses tremor caused by oxotremorine and reduces the reaction provoked in monkeys by deserpidine. It also provokes stereotyped and compulsive behavior in cats.

Peptides are studdied with great interest because of their possibilities of being effective in reducing clinical anxiety and aggressive behavior, as antidepressants for the treatmont of learning and memory disorders, of Schizophremia and other mental diseases.

MEMORY

Vasopréssin ia a substances that may be liberated by collateral recurrents of the supraoptic cells at synapsis back on supraoptic cells, which explains the recurrent inhibitory pathways found in this system, which may determine a feedback regulation of vasopressin release.

Neurohypophyseal peptiddes have excitatory effects on invertebrate neurons Iontophoretic application of vasopressin to snail neurons determine changes in the properties of the neuronal membranes.

The neurohypophyseal hormones, particularly vasopressin, have perticipation in mechanism implicated in memory. Experimental studies demonstrate that the absence of vasopressin interferes, with the process of memory and learning the implication of catecholamines in the long term memory has been suggested.

The liberation of catecholamines in affective states may be a factor that facilitates the consolidation of learning with the stimulus of chemical processes at recently activated neurons.

The adrenergic systems are involved in the development of expression of long term memory.

The neurohypophyseal peptides have also implications in this process.

They may influence the noradrinergics systems by other means than by direct action upon the synthesis of norepinephrine, and even if the increasing of production of noradrenalin may influence memory consolidations this is not enough to explain the long term effects of neurohypophyseal in relationship with their central effects on memory.

Peptides that are liberated in the central nervous system by peptidergic neurons may interact with presinaptic receptors with the aim to increase the levels of cyclic adenosine 3,5 monophosphate (AMP) and in this way to stimulate tyrosine hydroxylase and to augment the norephinephrine synthesis.

The peptides, may also interact postsynaptically to modify the membrane properties and to determine a change in sensitivity of N.E. stimulating adem·lacetic cyclase (A.C.). Post synaptic effects of N.E. mat be mediated by cAMP

and this is probably to induce changes in membrane permeability, which will induce to the generation of postsynaptic potential.

Each one of the involved in the behavior seems to have a profile of different activities and each one may demonstrate to have a biochemical mechanism of specialized action, and also specific neuronal receptors.

Time is a significant tool to measure memory, and it has been stated that this is sensitive to hormonal influences.

Recent experiments suggest that maybe a central biogenic amine response to hormonal treatments, may mediate the hormonal influences of memory.

Trained rats to which an intermediate dosis of epinephrine 0.01 *to* 0.1 *mg/kg has been administrated had markly improved retentive performance. These results were time-dependant.*

Hormonal responses during the training may have a modulatory action on memory processing, helping to store the informative material derived from experience, which in normal conditions would be linked to hormonal secretion.

Almost all the pituitary hormones are released following different kinds of stress.

Retention in rats after the pituitary removal is greatly impaired.

If during two hours after training ACTH is injected, its retention abiiity improves. If the injection is administered 6 hours after training its performance is not any better.

In these experiments Dr. Paul E. Gold and James L. McGaugh found that posttrial administration of different kinds of hormones, counting among them ACTH may either improve or deteriorate retention in normal animals: just one injection of ACTH administrated to hypophysectomised animals may improve its poor performance. These findings favor the theory that hormonal responses to training which means post training events, may act as modulators in the storage of information of recent experiences.

It seems that the brain biogenic amine response to stress may be hormonally mediated since hormonal injections produce a diminution of brain norepinephrine concentration akin to that provoked by a stressful experience.

These results are considered as evidences of the brain state condition after the release of norepinephrine before its resynthesis by means of homeostatic mechanism.

The investigators suggest that the posttrial brain norepinephrine concentration is a good forecast for a good retention perfomance. Hormonal and neurochemical findings suggest that a training experience, even those of normal and un specific physiological consequences, may have importance in modulating in the storage, and retention of that experience.

The neurobiological basis that are implicated in retrograde amnesia and in intensifying memory storage are explained by the results of modulatory response to training.

Also, the time-dependent effects on posttrial memory put in evidence the quality of the synchronization between trainig experiences and modulatory actions. It is stressed thet hormones and neurotransmitters normally react to a training experience in a posttrial way.

It is concluded that these systems posses a modulatory influence in memory processes, and a mechanism of adaptation is set in motion, to elect from all the experiences those that are worthwhile fixing in the memory.

Some authors stated that the influences of peptides upon learning, are

oriented to performance, rather than learning since they are conspicuous for their participation in motivation, fatigue, stress, anxiety and arousal.

Thus some concluded that they influence instinctive behavior variable performance, sensitivity and attention, rather than learning.

SOMATOSTATIN

The results of the studides of R.L. Macdonald directly demonstrate that somatostatin (SRIF) has both pre-and post sypaptic actions on mammalian neurons in dissociatted cell culture. The postsynaptic action suggests the possibility that somatostatin could serve as an inhibitory neurotransmitter while the presynaptic suggests a presynaptic neuro-modulatory role.

MORPHIN LIKE NEUROPEPTIDES

Searching for pain killers Solomon Snyder and collaborators, have found the existence in all vertebrates of specific opiate receptors which clearly imply the presence of a substance born into the brain with a morphin like characteristic.

Experiments confirm the accuracy of this theory. John Hughes and Hans W. Kosterlitz of the University of Aberdeen were responsible for providing through their experiments a clear picture of this morphin like neuropeptides. The name of enkephalin meaning in Greek "from the Head" was adopted for this substance, formed by intimately related peptides both constituted by 5 amino-acid units.

One of the peptides was the so called methionine enkephalin; the other was known as leucine-enkephalin.

The slight differences between these two peptides may be reponsible to achieve a delicate balance in the function of the brain.

Specific neuronal systems are involved in the acting of enkephalins ruling the sensory information concerning emotional behavior, pain as well as some other still unknown specific functions.

Autoradiographic mapping of the distribution of enkephalin performed by some investigators revealed that the terminals are more dense in the substancia gelatinosa of the spinal cord, the amigdala and the limbic system and central part of the thalamus.

The presence of enkephalin in the intestinal tract, is attributed by some investigators to the embriologic fact that the gastrointestinal tract and the nervous system are developed from the same layer in foetal evolution. The same can be said of somatostatin, gastrin, vaso active intestinal peptide and substancia P.

The pituitary biological function concerning endorphins has still many enigmatic aspects. It is accepted that the enkephaline rol in regulating the pituitary function may be perturbed by opiates.

The analgesic property of enkephalin has raised hopes concerning the adquisition of a non addictive therapeutic tool.

Research performed by science investigators of the University of California, has shown that enkephalin and beta endorphin may exert an addiction syndrome when it is administered repeatedly in the ventricules of rat.

Withdrawal symptoms were evident in the rats such as diarrea and shaking, when the administration of enkephalin and beta enorphin were stopped. Still, new experiments are performed aiming to therapeutically profit these two substances by avoiding these undesirable effects.

The endorphines (called alpha, beta, gamma) are very large peptides more that the enkephalines and also contains in its constitution B-LHP. This suggests that the Beta-lipotropine is the precursor of different opiate peptides.

A great concentration of opiate peptides found in the central nervous system at the level of the pallidum and limbic systems suggest that it is possible that this substance has a role in controling motricity and affection. Thus its analgesic function would be only one of its many aspects.

It is interesting to notice that injections of beta-endorphin administrated intraventricularly in the rat is responsible for an analgesic condition, but at the same time it developed an adinamic state almost catatonic.

Terence and collaborators in 1976 *made studides on the spinalcord fluid of schizo phrenic patients which showed an increase of the endorphines activity in those suffering of evolutive schizophrenia. Other investigators are engaged in elucidating this fact and determining its importance.*

The enkephadin 5 *amino-acid sequence is contained in the* 31*-amino-acid sequence of beta-endorphin, which forms part of a bigger* 91*-amino-acid-polypeptide called beta-lipotropin. Betatropin reveals most no morphinlike activity. Dr. Guillemin from the Salk Institute and Sidney Udenfriend from Roche Laboratories informed separately that they found in the pituitary a polipeptide which has* 30.000 *molecular weight and submitted to hidrolization produces smaller peptides that posses morphinlike properties.*

Studies are being carryed on to determine if beta-lipotropin and some other with biological implications, may be found in this new big peptide.

Experiments conducted by Udenfriend in rat pituitaries which are frozen immediately after the animal dies, have demonstrated that there are 150 *times more molecules of beta-lipotropin than of beta-endorphin. If the pituitary were not immediately frozen ratio of lipotropin to endorphin decreases, demostrating that degradation has already taken place.*

Similar results may be obtained using other animals, such as sheep, cow, guinea pig, etc.

Maybe large polypeptide chains would be the way used by nature to store smaller molecules, At a given demand this peptide could be hydrolised to provide the organism with beta-endorphin and other molecules to fulfil their tasks.

A report appeared in the Proceedings of the National. Academy of Science saying that it seems to be that beta endorphin is related with body heat regulation.

It was already observed by a group of scientists that the beta endorphin helps the body to adapt to heat. Injections with small amounts of thispeptide. have been administrated which induced a litle elevation of temperature. Used in dogs it provokes at first wet-dog shakes, which are considered part of the pattern of heat-generating behavior, followed by abundant salivation, that corresponds a to heat loss behavior.

Experiments have been carried out by inducing rats to suffer acute or chronic heat. Half of the animals were used as control and received saline injections; the other half received naloxone. The rats that received saline injections adapted to the temperature by dimishing their body temperatures, while the others that had nalaxone did not.

This proved that nalaxone was interfering with the mechanism of heat adjustment, revealing that endorphine may act as the body's thermostat.

To clarify this point the investigators removed the pituitary gland from

some rats which is the site of previlege of endorphine and possibly its source in the brain, leaving the pituitary undamaged in others rats. Then they were submitted to acute heat, which elicited a high temperature in both groups of rats. Afterwards nalaxone was injected in both groups, the results were that nalaxone augmented even more the control rats, and not those without the pituitaries which suggests that endorphine acts as an endogenous thermostat and that nalaxone blokades. It is evident that endorphine's function is linked to the adaptive mechanism to heat.

So, it seems to be that endorphine acts as a modulator of heat and pain sensation acting upon the same neuronatomical pathways.

ANGIOTENSIN

The subcutaneous injection of angiotensin or into the cerebral ventricles determines an identical effect to the spontaneus drinking in species encompassing from lizards to primates.

The conditions activated by angiotensin are related to the following: low blood pressure, a low concentration of sodium, direct stimulation by nerve fibers, determine that certain cells from the kidneys segregate the renin enzime.

In the blood flow the renin acts upon a protein elaborated in the liver and liberates the decapeptide, angiotensin 1. *There is an enzime that acts in the blood that subtracts from the angiotensin* 1, *two aminoacids and the octapeptide that remains is the angiotensin II. Its physiological action consists in the constriction of the vessels that reach the skin and the kidneys, the dilatation of the vessels that reach the muscles and the brain, and the rise of the blood pressure.*

Besides, the angiotensin II stimulates adrenal cortex to increase its secretion of aldosterone, and in this way promotes the absortion of the sodium from the urine by renal action.

Another activity of the angiotensine II consists in facilitating the secretion of vasopressin from the posterior lobe of the pituitary. Besides the vasopressin produces the reabsortion of water from the urine.

SUBSTANCE P. VASOACTIVE INTESTINAL PEPTIDE, CHOLECYSTOKININ, SOMATOSTATIN

Many peptides which need to be considered as exclusively related to the gut or the endocrine system, have now been observed in the brain. Among these substances are: Substance P, vasoactive intestinal peptide, and cholecystokinin, and they have been found in local circuit neurons, in the cerebral cortex and in the hippocampus. Besides it was possible to observe that nerve fibers that contain substance P or the vasoactive intestinal peptide, innervate visceral tissues, such as parts of the lung. They also innervate the thyroid gland.

Somatostatin it is a peptide integrated by fourteen aminoacids. It has been found in the nervous system diseminated by all its structures from the cerebral cortex to the autonomic ganglia. In the gut, somatostatin appears in the cells that line the intestine. It is also found in the delta cells of the islets of Langerhan. In the pancreas it suppresses the secretion of insulin and glucagon.

The Substance P peptide of 11 *amino acids that Euler discovered in* 1931. *Forty years later it was radiscovered by Chang and Leeman in the hypothalamus of the "bovin" it was considered to be the sought mediator of the pain fibers.*

This believe was confirmed by the fact that the concentration of that substance P is 30 *times greater in the posterior corn of the spinal cord than in the anterior corn scrutinized by the electronic microscope and the immunofluo-*

rescence it has been observed that axonic endings form synapsis with many postsynaptic elements thet posess round vesicles of great size contaning P substance.

It has been registered in neuronal unts of the spinal cord, of the V layer of Rexed during painful stimulations which act differently to when morphine or met-enkephaline are directly applied at the level of the V layer or at the level of the gelatinous substance of Rolando (layer II and III formed by interneurones) When the morphinic substances are applied directly on the V layer this doesn't modify in any way the neurons behavior when there is a painful stimulation. It is quite different when the substance is administrated to the interneuronal level of the Rolando gelatinous substance, the morphine and the met-enkephaline exert a depresive influence on the neurons' activity of the V layer, action that is abolished by a previous administration of naloxone. The painful message is thus blockade to the level where it is preformed, the synapsis are contacted between the endings of fibers C and the dendrites of neurones of the layer V.

The results of these experiences have led Jessel and Iverson to propose the following hupothesis; the neuromediators of nervous fibers that transmit the painful sensation is the Substance P whose rele is to activate the spino-thalamic neurons specially those which belong to the V layer that projects their axons toward the superior centers. The interneurones located in the gelatinous substance of Rolando have the enkephalines which posses an inhibiting property that is exerted at the presynaptic level. According to this theory the task of the enkepaline would be to filter that paiful message at the entrance of the central nervous system. It is interesting to stress that the existence of a filter in the spinal cord was found after many Years of tests and arguments over the neurophysiological fact that was posed like the "theory of gate control" by Melzack and Wall in 1965 *Autoradiografic studies aided to determine the sites of election of the opiate receptors. It has been found that the same receptors wellcome substances an tagonists to the morphin like the naloxone.*

BOMBESIN

Bombesin extracted from the frog's skin is a peptide of 14 *aminoacid that has powerful properties. A small quantity of this peptide injected into the brain. diminishes the body temperature of rats already exposed to cold. Jean Rivier and Wyli Vale found that bombesin does not cool animal that are kept at room temperature. They think that probably bombesin interfere with the rat ability to produce heat of facilitate heat loss.*

Bombesin has never been isolated from the mamalian brain, but it is believed that this peptide may be important to normal function. They are investigating the interaction of bombesin with hormones that are engaged in coordinating the control of body temperature and to determine whether bombesin may be used as a therapeutic tool to prevent fever, or to induce hypotermia in the body to facilitate surgery.

LUTEINIZING HORMONE RELEASING HORMONE (LHRH)

The decapeptide called the luteinizing hormone releasing hormone is a hypothalamic substance that promotes the release of the luteinizing hormone from the anterior lobe of the pituitary.

It may be established that luteinizing hormone produces the ovulation.

It has been recently found in nerve cells pertaining to the autonomic ganglion that innervates the organs of reproduction.

Experiments have been carried in which the injection of the LHRH in rats have determined in the animal the adequate attitude for copulation.

This means that the above-mentioned peptide is implicated in all the aspects involved in the reproductive function.

ENKEPHALINS AND FACIAL FLUSHING

Besides being associated with pain relief, pleasure, learning, improving the memory, epileptic seizures, etc. the enkephalins are being studied together with the facial flushing that occurs after taking alcohol.

It has been accepted that facial flushing after ingesting alcohol has a genetic origin and it is principally found in persons in whose families there is a record of members suffering from non-insulin dependent diabetes.

Dr. R.D.G. Leslie of King's College Hospital in London and his collaborstors have found that a similar flushing can be obtained by injecting an enkephalin analogue.

And this effect may be blocked by administrating naloxone, the enkephaline antagonist.

It is thought that persons easily flushing after drinking may be experiencing an inherited sensitivity to enkephalins.

It has not been clarified why facial flushing is connected with non-insulin dependent diabetis, but it is thought that an exceptional, inherited sensitivity to enkephalin may be also linked to the latter. This hypothesis may be based on the fact that the enkephalin analogue used during his studies has increased the blood glucose in diabetics and non diabetics as well.

NATURAL SLEEP CHEMICAL

After peptides related to memory were isolated, chemically scrutinized and synthetized other virtues were assigened to the limelighted peptides Marcel Monnier and G. Shoenenberger of the University of Basel Switzerland, has discovered a peptide that trigers sleep.

Monnier discovered several years ago that by taking venous blood from sleeping rabbits filtering it and then injecting it into rabbits that are awabe it induces them to fall sleep. Because electroencephalic records taken from these rabbits has delta waves that eloquently evidence that they were in the middle of a deep sleep, he names this substances as "sleep peptide delta".

Afterwards Monnier and Shoenenberger isolated this sleep producing substance and identified it as a polipeptide containing nime amino acids.

PROBING SCHIZOPHRENIA WITH NALOXONE

While some scientists are engaged in treating schizophrenic patients with hemodialysis, other investigators are turning their interest in studying and applying the effects of naloxone in patients suffering from hallucinations.

An excesive ammount of endorphine was detected in the cerebtospinal fluid of patients suffering from severe maniac states, and schizophrenia. This kind of investigations were followed by others which have also implicated endorphine in schizophrenia, such as the finding of leucine endorphine in the blood od schizophrenic patients who have been submitted to hemodialysis.

Nancy Pritzker from Stanford University Behavioral Neurochemistry, reports that naloxone seems to aleviate principally auditory hallucinations in an acutely impaired group of schizophrenics.

According to Dr. Stanley Watson, from Stanford University, this indicates that naloxone interfere with the action of endorphines by altering several nerve receptors in the brain. Thus it is thought that the opiate system is in some way involved in certain cases of psychosis.

There have been some studies which contradict there findings, where the researchers have found no change in using naloxone, but in those studies a conparatively low dosis of naloxone was used 10 *times lower than the above mentioned studies.*

The patients were selected from among 1000 *psychiatric patients and the Stanford group chose only* 9 *that were clearly hallucinating.*

Separated by 48 *hours two injections were administrated one of naloxone, the other placebo, neither the doctors or the patients knew which one was the real one. The results were that six of the nine patients improved notably in their hallucinations, one had a borderline improvement, and two were indifferent.*

One objection at using naloxone as a therapeutic agent was that even at high dosis its effects have not long duration: even when two of the patients showed and improvement lasting from one to two days, most of the patients had hallucinations three to six hours after the injection.

It has been our aim to offer the reader a panoramic view of this fascinating subject that has attracted the atention of the most renowmed research centers of the world and we hope that our Editorial will serve as an introduction to the enlightened words of the outstanding group of speakers and discussers who have honored the memory of John Fulton in the ninth International Syw-posium that bears his name on "Neuropeptides in the Brain" that has been carried out in the hopitable City of Saint Louis, Missouri U.S.A.

It is with upmost satisfaction that we welcome in our Editorial Board two outstanding personalities of the Scientific circles. We are refering to Dr. Janice Stevens, Prof. of Psychiatry and Neurology of the University of Oregon and Dr. Sid Gilman Prof. of Neurology at Ann Arbour, Michigan, To both of them our heartiest welcome and I feel that their incorporation will enrich our publication with their wonderful personality.

Prof. Emeritus Dr. VICTOR SORIANO

Neurohypophyseal Hormones and Memory Function

Tj. B. van WIMERSMA GREIDANUS and A. R. WINTZEN *

Rudolf Magnus Institute for Pharmacology
Vondellaan 6, Utrecht, The Netherlands

The neurohypophyseal hormones vasopressin and oxytocin are well known for their peripheral effects. These consist of the antidiuretic action by reabsorption of water in the kidney and the rise in bloodpressure by peripheral vasoconstriction as far as vasopressin is concerned and of the induction of milk secretion by contraction of myoepithelial elements in the alveolae and the stimulation of contractions of the myometrium in the case of oxytocin. Moreover, vasopressin plays a role in the release of ACTH from the anterior pituitary, whereas the tail tripeptide of oxytocin under certain conditions modulates MSH release from the neuro-intermediate lobe.

The first report about behavioral involvement of neurohypophyseal principles concerned a facilitation of extinction of a learned response in rats after removal of the posterior lobe of the pituitary (De Wied, 1965). The rapid extinction of a conditiotioned avoidance response (CAR) in these animals could be restored by treatment with pitressin.

Extirpation of the whole pituitary gland resulted in a markedly impaired acquisition of an active avoidance response and this behavioral disturbance could be corrected by administration of pitressin or of synthetic vasopressin (lysine-8-vasopressin: LVP) (De Wied, 1969; Bohus et al., 1973).

Pitressin and LVP also induce marked behavioral changes in intact rats. Although effects on acquisition of a CAR are not so easily detected in these animals, maintenance of a learned response was strongly improved by vasopressin treatment (De Wied, 1969). A single subcutaneous injection of vasopressin results in a long term, dose dependent inhibition of extinction of a CAR, which lasted far beyond the actual presence of the injected peptide in the organism (De Wied, 1971; van Wimersma Greidanus et al., 1973). This suggests that vasopressin triggers a long term effect on the maintenance of a learned response, probably by facilitation of consolidation processes (De Wied et al., 1976). This effect is independent from the classical endocrine actions of vasopressin, since removal of the C - terminal glycinamide yields a product (desglycinamide lysine-8-vasopressin: DG - LVP), which is devoid of the effects on kidney function, on blood pressure and on ACTH release, but possesses still strong behavioral activity (De Wied et al., 1972).

The long term behavioral effects of vasopressin are also found when passive avoidance behavior is studied. The peptide induces a facilitation of retention of a passive avoidance response in rats, when administered either immediately after the single learning trial of electric footshock or prior to retention, which is usually performed 24 h after the acquisition trial (Ader and De Wied, 1971; Bohus et al., 1972).

Using an avoidance of attack paradigm where mice are trained to avoid attack by a trained fighter mouse, generally the same effects of vasopressin are obtained as in the

* Dept. of Neurology, State University Academic Hospital Leiden, The Netherlands.

avoidance of shock situation (Leshner et al., 1977).

Further evidence for the effects of vasopressin and its congeners on memory processes was obtained when it appeared that vasopressin and its analogs antagonize retrograde amnesia and protect against pyromycin induced memory loss (Lande et al., 1972; Rigter et al., 1972).

Comparable with the effect of vasopressin on passive avoidance behavior the peptide affects amnesia not only when injected after acquisition but also when given prior to the retention test. These data suggest that vasopressin not only enhances storage processes involved in memory consolidation but also promotes retrieval processes concerning the use of stored information.

Also other behaviors, in some of which learning and/or memory processes are involved, appeared to be affected by vasopressin administration. In addition various processes in the brain which may be related to behavior are influenced by the neurohypophyseal hormones (van Wimersma Greidanus and Versteeg, in press).

Oxytocin possesses behavioral activity opposite to that of vasopressin upon icv administration (Bohus et al., 1978a, 1978b). This effect however is not always present after systemic injection and using this latter route of administration behavioral influences of oxytocin have been reported which are of the same nature as those obtained after vasopressin treatment, although the potency of oxytocin was less (De Wied and Gispen, 1977). A different pattern of biotransformation after central or after systemic administration may account for this discrepancy (De Wied, 1976).

Following icv injection oxytocin induces an attenuation of passive avoidance behavior and a facilitation of extinction of active avoidance behavior (Bohus et al., 1978, 1978b). Time gradient studies reveal that oxytocin not only acts on consolidation but also exerts inhibitory effects on retrieval processes (Bohus et al., 1978b). Generally it is suggested that vasopressin and oxytocin modulate memory function in an opposite way by a direct action on the brain and oxytocin may be regorded as a naturally ocurring amnesic peptide.

Recently performed structure activity relationship studies reveal that the ring structure of vasopressin and oxytocin predominantly affect consolidation, while the linear tail portions of the molecules influence retrieval processes. The intact hormones are necessary for attenuation of consolidation and retrieval. This suggests that the neurohypophyseal hormones act as precursor molecules for neuropeptides with specific effects on memory consolidation or retrieval (Van Ree et al., 1978; De Wied and Bohus, 1978; Bohus et al., 1978c). Optimal brain function with respect to memory may depend on a delicate equilibrium between bioavailable amounts of neurohypophyseal hormones and their fragments in the brain.

The behavioral disturbance of posterior lobectomized rats suggested that vasopressin is physiologically involved in memory processes. Support for this idea originates from the observation that the hormone is effective in delaying extinction of a CAR upon intracerebroventricular (icv) administration of picogram amounts (De Wied, 1976).

Further and more definite information on the physiological role of neurohypophyseal hormones on memory processes was obtained from experiments with Brattleboro rats with hereditary hypothalamic diabetes insipidus. A homozygous variant (HO-DI) of this strain lacks the ability to synthetize vasopressin and in the plasma of these animals vasopressin is virtually absent whereas oxytocin levels are elevated (Dogterom et al., 1978). Memory function of HO-DI rats is impaired in a one trial passive avoidance task (De Wied et al., 1975), and extinction of a multiple trial active avoidance task is facilitated (Bohus et al., 1975). Treatment with vasopressin easily restores the avoidance behavior of HO-DI rats to normal (De Wied et al., 1975). The main disturbance of HO-DI rats is in the maintenance of the behavior rather than in learning itself, again suggesting that consolidation of memory is impaired in the absence of vasopressin, although a disturbance in the equilibrium between bioavailable amounts of vasopressin and oxytocin in the brain may be an attractive postulate as well (Bailey and Weiss, 1979).

Icv administration of antiserum to vasopressin after the single learning trial induces a marked deficit in retention of a passive avoidance response when tested 6 or more h later. No interference was found when retention was tested at less than 2 h after injection of the vasopressin antiserum (Van Wimersma Greidanus et al., 1975a; Van Wimersma Greidanus and De Wied, 1976). Intravenous injection of a hundred times as much vasopressin antiserum, which temporarily antagonizes vasopressin activity in the circulation did not affect passive avoidance behavior (Van Wimersma Greidanus et al., 1975a). These results illustrate the importance of centrally available vasopressin in relation to memory consolidation.

If the icv injection of vasopressin antiserum is postponed for maximally 2 h after the learning trial, the treatment still results in a marked disturbance in passive avoidance behavior, indicating that storage processes involved in memory consolidation last for several hours. Moreover, administration of vasopressin antiserum 1 h prior to the retention session also results in passive avoidance deficits. This again supports the hypothesis that vasopressin is involved in retrieval as well (Van Wimersma Greidanus and De Wied, 1977).

Treatment with vasopressin antiserum during acquisition of active avoidance behavior resulted in a tendency towards a retarted acquisition, although the animals reached the learning criterion. However, extinction of the avoidance response was facilitated in the group of rats which had been injected icv with the antiserum (Van Wimersma Greidanus et al., 1975b; Bohus et al., 1978a). The results reinforce the notion that learning takes place in the absence of vasopressin, but that the maintenance of an acquired response is disturbed.

In a learned helplessness paradigm as testing situation for memory function, animals display deficits in acquiring an escape task after an initial exposure of inescapable electric footschock. Mice treated icv with antiserum to vasopressin after this shock period did not show the later escape deficits. These data again support the hypothesis that endogenous vasopressin may be involved in memory (Leshner et al., 1978).

Moreover, neutralization of centrally available vasopressin by icv administration of vasopressin antiserum interferes with the development of tolerance to morphine. Time gradient studies suggest that endogenous vasopressin may be physiologically involved in the tolerance development in a way which is comparable with its role in memory processes (Van Wimersma Greidanus et al., 1978).

Neutralization of bio-available oxytocin in the brain by icv administration of specific oxytocin antiserum, either immediately after the learning trial or prior to retention, induced longer passive avoidance latencies than found in controls. Rats which received this antiserum prior to each acquisition session in an active avoidance situation, showed a weak but significant increase in resistance to extinction of the CAR (Bohus et al., 1978d). These results again point to an opposite effect of oxytocin and vasopressin on processes involved in memory function.

Although no significant correlation could be determined between the behavioral performance of rats in active and passive avoidance situations and the levels of radioimmunoassayable vasopressin in peripheral blood and/or cerebrospinal fluid (CSF) (Van Wimersma Greidanus et al., 1979), such a relationship was found between retention of a passive avoidance response and bio-assayable vasopressin in eye-plexus blood (Thompson and De Wied, 1973). This latter observation is particularly of interest with respect to recent findings of Legros et al., (1979), who reported a relationship between memory function and plasma neurophysin levels in humans (see below).

HUMAN STUDIES

Only recently central nervous system effects of neurohypophyseal hormones in humans have been reported. It has been suggested that vasopressin is involved in human affective disorders (Gold et al., 1978) and it has been found that plasma vasopressin levels were elevated in acutely psychotic patients and a positive correlation existed between the levels of vasopressin and the degree of psychosis (Raskind et al.,

1978). These results provide evidence for a role of vasopressin in central nervous system functioning. Of particular interest with respect to memory is the observation by Unger (1971) that oxytocin concentrations in the CSF are augmented after amnesia induced by electroconvulsive shock treatment.

Several observations on the beneficial effect of vasopressin on memory function have been reported. A remarkable improvement of memory has been found after the use of vasopressin in patients with Korsakoff syndrome. After two weeks of vasopressin treatment (nasal spray) a marked increase in memory was observed, whereas other less circumscribed improvements of memory function were found as well (Leboeuf et al., 1978).

However, improvement of the alcohol-induced amnestic state as part of the Wernicke-Korsakoff syndrome was not observed after vasopressin treatment in two patients as reported by Blake et al., (1978).

Return or improvement of memory and/or restoration of long-term memory has been reported after vasopressin treatment in patients suffering from post-traumatic retrograde and/or anterograde amnesia stemming from car accidents (Oliveros et al., 1978). Additionally, administration of the vasopressin analog DDAVP (desamino-D-arginine-8-vasopressin), which has weak behavioral activity in animals, improves the ability to learn a passive avoidance response in Lesch-Nyhan children (Anderson et al., 1979). One of the features of this disease is self-mutilation. Generally, skin shock is very effective in treating self-injurious behavior, but it is not in Lesch-Nyhan disease. This ineffectiveness seems to be due to an inability of these patiens to learn a passive avoidance task and this learning defect seems to be corrected by nasal application of DDAVP.

Furthemore, elderly patients, given vasopressin (nasal spray) performed better in tests involving attention and concentration and in memory tests than individuals in the placebo group (Legros et al., 1978).

Finally, vasopressin sensitive diabetes insipidus patients, who seem to have memory and attention disturbances, performed much better in a variety of psychological tests after treatment with LVP or DDAVP.

Improvements were observed in attention, concentration stability and memory function especially after chronic nasal application of DDAVP (Wagner et al., 1979).

In a pilot study we recently measured vasopressin in plasma and lumbal CSF in patients with and without memory deficits. Since vasopressin is assumed to enter the CSF intracranially and CSF samples (16 ml/patient) were collected from the lumbal dural sac, two separate portions of each sample were used for vasopressin determination: one representing the first 3 ml and the other one the 13-16th ml. Twelve patients had unequivocal memory disturbances (Wernicke-Korsakoff syndrome 5; presenile dementia 2; Huntington disease 2; other 4), whereas ten controls suffered from other non-amnesic neurological diseases. Table 1 summarizes the vasopressin levels in plasma and CSF in the various catagories of these patients. No significant differences between vasopressin levels in plasma of amnesic patients and non-amnesic controls were found. Furthermore, an absolute lack of correlation ($r = -0.117$, $n = 21$) was found between the individual values of vasopressin in plasma and CSF. The CSF levels of vasopressin appeared to be significantly lower than the levels in the plasma. In addition the 2nd portion of CSF tended to contain higher levels of vasopressin than the 1rst portion. Interestingly, in the 1rst portion CSF a significantly higher level of vasopressin was found in amnesic patients as compared with non-amnesic individuals, while this difference was less pronounced in the 2nd CSF portion. These data suggest an origin of CSF vasopressin which is different from that of blood vasopressin.

This also supports the view that the secretion of vasopressin into CSF or peripheral circulation is mutually independent and that different functions for vasopressin present in these two compartments may be involved (Luersson et al., 1977). The reason for the elevated levels of vasopressin in CSF of amnesic patients is not well understood. It may be that the fact that in these patients brain areas are damaged which are

TABLE 1

VASOPRESSIN LEVELS (PG/ML; MEAN ± SEM) IN PLASMA AND IN CSF OF AMNESIC AND NON-AMNESIC NEUROLOGICAL PATIENTS

	PLASMA	CSF I	CSF II	CSF I + II
AMNESIC	1.84 ± 0.50	0.59 ± 0.08**	0.72 ± 0.12*	0.65 ± 0.07***
NON-AMNESIC	1.78 ± 0.51	0.35 ± 0.04**	0.47 ± 0.07*	0.41 ± 0.04***
ALL PATIENTS .	1.81 ± 0.22	0.48 ± 0.05	0.60 ± 0.07	0.52 ± 0.05

* $.05 < p < .10$
** $p < .02$
*** $p < .01$

involved in memory and which may serve as the anatomical substrate for the action of vasopressin, results in an increased release of this hormone into the CSF in an attempt of the organism to restore the disturbed memory function. However, changes in enzymatic degradation may also account for the differences.

Recently Legros et al., (1979) reported a significant positive relationship between immunoreactive neurophysin (IRN) and item 7 of Rey's PRM memory test in normal individuals (aged >50 years). No relationhsip was found between IRN and other items of PRM, nor between those items and other endocrine parameters as plasma levels of LH, FSH, prolactin, cortisol or 17β-estradiol. These data suggest that in normal individuals a relationship exists between the activity of the neurohypophyseal system and consolidation processes involved in memory functioning. Differences in the used methods for measuring neurohypopyseal principles (radioimmunoassay vs. bioassay; vasopressin vs. neurophysin) may account for the discrepancies between the results concerning plasma levels of these entities, obtained in the animal experiments (see page 6) and those from human studies.

Neuropeptides belonging to the neurohypophyseal system determine the hormonal climate in the brain which is needed for processes related to memory function. The finding that vasopressin and oxytocin and/or their fragments exert opposite effects on storage and/or retrieval processes makes it tempting to assume that disturbances in the equilibrium between these entities result in changes in brain function. Consequently it is likely that certain disorders of psychopathological nature, particularly those in which memory deficits are apparent, may be caused by disfunctioning of the neurohypophyseal system. Development of neuropeptides derived from the classical neurohypophyseal hormones vasopressin and oxytocin which influence consolidation or retrieval processes may yield tools for the treatment of such mental disorders.

SUMMARY

Neurohypophyseal hormones are involved in the formation and maintenance of adaptive behavior. Removal of the pituitary gland results in a markedly impaired acquisition and maintenance of conditioned avoidance behavior. These behavioral disturbances can be corrected by administration of vasopressin.

In intact animals vasopressin and its analogs which are practically devoid of pressor, antidiuretic and ACTH releasing activity, facilitate the maintenance of active and passive avoidance behavior. Additionally these peptides antagonize retrograde amnesia and protect against puromycin induced memory loss. The long term nature

of the effect of vasopressin, suggests that the peptide facilitates consolidation processes.

Rats with hereditary diabetes insipidus which lack the ability to synthetize vasopressin due to a genetic failure, display disturbed avoidance behavior. This deficit is rather a disturbance in the maintenance than in the acquisition of a learned response. Moreover, temporary neutralization of vasopressin in the brain by intracerebroventricular (icv) administration of antiserum to this hormone causes marked behavioral disturbances.

These results indicate that vasopressin is physiologically involved in memory processes. Time gradient studies suggest that this involvement is at the level of storage of information as well as on that of retrieval of stored information.

Several observations in man illustrate the beneficial effect of vasopressin on memory function. A remarkable improvement of memory has been found after intranasal application of vasopressin in patients with Korsakoff syndrome, in elderly people and in patients suffering from retrograde and/or anterograde amnesia after car accidents. Vasopressin levels in blood of amnesic patients are similar to those of non-amnesic controls, whereas vasopressin levels in CSF of amnesic patients are slightly elevated as compared with those of non-amnesic individuals. Recent reports suggest a relation between plasma levels of neurophysin and memory function in elderly people.

Oxytocin has an effect opposite to that of vasopressin; i.e. oxytocin facilitates extinction of active avoidance behavior and attenuates passive avoidance behavior in rats. Time gradient studies in which small amounts of this neurohypophyseal hormone or antiserum to this peptide are icv injected point to an amnesic activity of oxytocin at the level of both storage and retrieval.

The effects of fragments of vasopressin and oxytocin on memory processes suggest that the neurohypophyseal hormones act as precursor molecules for neuropeptides with more specific effects on storage or retrieval.

Research on such neuropeptides, either stimulating or attenuating consolidation or retrieval processes, may yield more specific tools for the treatment of mental disorders which are the result of disturbances in the hormonal climate in the brain and particularly in the functioning of the neurohypophyseal system.

RESUMEN

Las hormonas neurohipofisarias intervienen en la formación y en el mantenimiento de la conducta de adaptación. La extirpación de la hipófisis provoca una neta alteración en la adquisición y el sostén de la conducta de precaución condicional. Estos transtornos de la conducta pueden corregirse con la administración de vasopresina.

En los animales intactos la vasopresina y sus derivados que prácticamente no actúan sobre la tensión, ni como antidiuréticos y no tienen actividad de liberación de ACTH, facilitan el sostén del comportamiento de precaución activa y pasiva. Además estos péptidos ejercen una acción antagónica sobre la amnesia retrógrada y protegen contra las pérdidas de memoria provocadas por la puromicina. La duración prolongada del efecto de la vasopresina incita a pensar que este péptido facilita los procesos de consolidación.

Las ratas afectadas de diabetes insípida hereditaria, incapaces de sintetizar la vasopresina como consecuencia de una carencia genética, presentan transtorno de la conducta de precaución. Este defecto constituye más bien transtorno del mantenimiento de una respuesta aprendida que de su adquisición. Por otra parte la neutralización temporaria en el cerebro de la vasopresina por la administración intracerebro ventricular (I C V) de antisuero de esta hormona provoca transtornos marcados de conducta. Estos resultados indican que la vasopresina está implicada fisiológicamente en procesos de memorización.

Estudios efectuados sobre el gradiente de tiempo sugieren que esta implicancia

tiene lugar a la vez en el mecanismo de acumular información y en el de restitución de la información guardada.

Varias observaciones efectuadas en el hombre ilustran acerca del efecto benéfico de la vasopresina en la memorización. Una apreciable mejoría de la memoria pudo ser comprobada después de la aplicación intranasal de vasopresina en pacientes afectados del Síndrome de Korsakoff, en personas de edad avanzada y en pacientes afectados de amnesia retrógrada y/o anterrógrada como consecuencia de accidentes automovilísticos. El nivel de vasopresina hallado en la sangre de pacientes amnésicos es semejante al de las personas no amnésicas; en cambio el nivel de vasopresina hallado en el líquido cefalorraquídeo de pacientes amnésicos es algo superior al de las personas no amnésicas. Estas referencias recientes sugieren de que existe una relación entre los niveles de neurofisina del plasma y la función de memorización en las personas de edad avanzada.

La oxitocina ejerce un efecto opuesto al de la vasopresina, es decir que la oxitocina facilita en las ratas la extinción de la conducta activa de precaución y atenúa la conducta pasiva de precaución. Estudios efectuados sobre gradientes de tiempo en el curso de los cuales pequeñas cantidades de esta hormona neurohipofisaria o de antisuero de este péptido son inyectados en forma intracerebro ventricular, señalan una actividad amnésica de la oxitocina tanto en el aspecto de guardar como de restituir la información.

Los efectos de fragmentos de vasopresina y de oxitocina sobre el proceso de la memorización hacen suponer que las hormonas neurohipofisarias tienen el papel de "moléculas precursoras" de los neuropéptidos, presentando efectos más específicos sobre el depósito o sobre la restitución de la información.

Es posible que las investigaciones sobre estos neuropéptidos, que estimulan o atenúan los procesos de consolidación o de restitución, suministran instrumentos más específicos para el tratamiento de desórdenes mentales que resultan de transtornos del ambiente hormonal del cerebro y en particular del funcionamiento del sistema neurohipofisario.

RÉSUMÉ

Les hormones neurohypophysaires interviennent dans la formation et dans le maintien du comportement adaptatif. L'ablation de l'hypophyse entraîne une nette altération de l'acquisition et du maintien du comportement d'évitement conditionnel. Ces troubles comportementaux peuvent être corrigés par l'administration de vasopressine.

Chez les animaux intacts la vasopressine et ses dérivés, qui sont pratiquement sans activité sur la tension, sans activité antidiurétique et sans activité libératrice d'ACTH, facilitent le maintien du comportement d'évitement actif et passif. De plus ces peptides exercent une action antagonique sur l'amnésie rétrograde et protégent contre les pertes de mémoire provoquées par la puromycine. La durée prolongée de l'effet de la vasopressine incite á penser que ce peptide facilite les processus de consolidation.

Les rats atteints de diabéte insipide héréditaire, incapables de synthétiser la vasopressine par suite d'une carence génétique, présentent des troubles du comportement d'evitement. Ce défaut constitue plutôt un trouble du maintien d'une réponse apprise que de son acquisition. D'autre part la neutralisation temporaire dans le cerveau de la vasopressine par l'administration intracérébroventriculare (ICV) d'antisérum de cette hormone entraîne des troubles comportementaux marqués. Ces résultats indiquent que la vasopressine est physiologiquement impliquée dans les processus de mémoration. Des études effectuées sur le gradient de temps suggérent que cette implication a lieu á la fois au niveau du stockage de l'information et à celui de la restitution de l'information stockée.

Plusieurs observations effectuées chez l'homme illustrent l'effet bénéfique de la vasopressine sur la fonction de mémoration. Une amélioration remarquable de la mémoire a pu être constatée après application in-

tranasale de vasopressine chez des patients atteints du syndrome de Korsakoff, chez des personnes âgées et chez des patients souffrant d'amnésie rétrograde et/ou antérograde à la suite d'accidents automobiles. Les taux de vasopressine trouvés dans le sang de patients amnésiques sont semblable à ceux de témoins non amnésiques, alors que les taux de vasopressine trouvés dans le liquide céphalo-rachidien de patients amnésiques sont légérement supérieurs à ceux d'individus non amnésiques.

Des rapports récents suggèrent qu'il existe une relation entre les taux de neurophysine du plasma et la fonction de mémoration chez les personnes âgées.

L'oxytocine exerce un effet opposé à celui de la vasopressine, c'est-à-dire que l'oxytocine facilite l'extinction du comportement d'évitement actif et atténue le comportement d'évitement passif chez les rats. Des études effectuées sur le gradient de temps, au cours desquelles de petite quantités de cette hormone neurophypophysaire ou, d'antisérum de ce peptide sont injectées de façon intracérébroventriculaire, indiquent une activité amnésique de l'oxytocine à la fois au niveau du stockage et à celui de la restitution de l'information.

Les effets de fragments de vasopressine et d'oxytocine sur les processus de mémoration font supposer que les hormones neurohypophysaires tiennent le rôle de "molécules-précurseurs" des neuropeptides tout en présentant des effets plus spécifiques sur le stockage ou sur la restitution de l'information.

Il est possible que des recherches sur ces neuropeptides, qui stimulent ou atténuent les processus de consolidation ou de restitution, fournissent des instruments plus spécifiques pour le traitement de désordres mentaux résultant de troubles du climat hormonal du cerveau et en particulier du fonctionnement du système neurohypophysaire.

ZUSAMMENFASSUNG

Neurohypophysische Hormone sind an der Formation und Aufrechterhaltung von adoptivem Verhalten beteiligt. Entfernung der Hypophyse resultiert in deutlich verringerte Erwerbsfaehigkeit und Aufrechterhaltung von bedingtem Vermeidungsverhalten. Diese Verhaltensstoerungen koennen durch Vasopressinapplykation korrigiert werden.

In Normaltieren erleichtern Vasopressin und seine Analogen, die praktisch pressorfrei sind und keine antidiuretische oder ACTH-abgebende Wirkung haben, die Beibehaltung von aktivem und passivem Vermeidungsverhalten. Zusaetzlich wirken diese Peptide ruecklaeufiger Amnesie entgegen und schuetzen vor Gedaechtnisverlust verursacht durch Puromycin. Die langanhaltende Dauer der Vasopressinwirkung suggeriert, dass dat Peptid Konsolodierungsprozesse erleichtert.

Ratten mit erblicher diabetes insipidus die auf Grund eines genetischen Versagens nicht imstande sind Vasopressin zu synthetisieren, zeigen gestoertes Vermeidungsverhalten. Dieses Fehlen zeigt sich eher in der Beibehaltung als in der Erwerbung einer erlernten Erwiderung. Weiterhin kann eine voruebergehende Neutralisation von Vasopressin im Gehirn durch eine intrazerebroventrikulae Applikation (icv) des Antiserums dieses Hormons auffallende Verhaltensstoerungen erzeugen. Die Resultate deuten an, dass Vasopressin physiologisch am Erinnerungsprozess beteiligt ist. Zeitabhaengigkeitsstudien deuten an, dass diese Beteiligung sowohl auf dem Niveau der Informationsspeicherung als auch auf dem der Erinnerung stattfindet.

Verschiedene Beobachtungen an Menschen illustrieren den nuetzlichen Effekt von Vasopressin auf die Gedaechtnisfunktion. Eine erstaunliche Verbesserung des Gedaechtnisses nach intranasaler Applikation von Vasopressin konnte bei Patienten mit Korsakoff Syndrome, bei aelteren Menschen und bei Patienten die an ruecklaufiger und/oder vorlaeufiger Amnesie nach Autounfaellen litten, beobachtet werden. Der Vasopressingehalt im Blut von Amnesiepatienten ist dem von nicht-amnesischen Kontrollen aehnlich, waehrend der Vasopressingehalt in CSF von Amnesiepatienten leicht erhoeht ist wenn verglichen met dem von Individuen ohne Amnesie. Neuren Be-

richten zufolge ist ein Zusammenhang zwischen Plasmagehalt von Neurophysin und Gedaechtnisfunktion bei aelteren Leuten angedeuted.

Oxytozin hat eine dem Vasopressin entgegengesetzte Wirkung, d.h. Oxytozin erleichtert die Aufhebung von aktivem Vermeidungsverhalten und verringert passives Vermeidungsverhalten in Ratten. Zeitabhaengigkeitsstudien, bei denen kleine Gaben dieses neurohypophysischen Hormons oder das Antiserum dieses Peptide intrazerebroventrikulaer injiziert wurden, zeigen eine amnesische Wirkung des Oxytozins sowohl auf dem Niveau der Speicherung wie auch auf dem der Erinnerung an.

Die Wirkungen von Vasopressinfragmenten und von Oxytozin auf die Erinnerungsprozesse deuten an, dass die neurohypophysischen Hormone als Precursor - Molekuele fuer Neuropeptide mit spezifischeren Wirkungen auf Speicherung und Erinnerung auftreten. Erforschung solcher Neuropeptide, die entweder stimulierend oder vermindernd auf Konsolidierungsprozesse wirken koennten mehr spezifische Werkzeuge sein um solche Geistesstoerungen zu behandeln, die das Resultat von Stoerungen im hormonalen Haushalt des Gehirns und besonders beim Funktionieren des neurohypophysischen Systems sind.

REFERENCES

1. *Ader, R.*, and *Wied, D.* de. Effects of lysine vasopressin on passive avoidance learning. Psychon. Sci. 29: 46-48, 1972.
2. *Anderson, L.T., David, R., Bonnet, K.*, and *Dancis, J.* Passive avoidance learning in Lesch-Nyhan disease: effect of l-desamino-8-argininevasopressin. Life Sci. 24: 905-910, 1979.
3. *Bailey, W.H.*, and *Weiss, J.M.* Evaluation of a «memory deficit» in vasopressin-deficient rats. Brain Res. 162: 174-178, 1979.
4. *Blake, D.R., Dodd, M.J.*, and *Grimley Evans*, J. Vasopressin in amnesia. Lancet, 1: 608, 1978
5. *Bohus, B., Ader, R.*, and *Wied, D.* de. Effects of vasopressin on active and passive avoidance behavior. Horm. Behav. 3: 191-197, 1972.
6. *Bohus, B., Gispen, W.H.*, and *Wied, D.* de. Effect of lysine vasopressin and ACTH 4-10 on conditioned avoidance behavior of hypophysectomized rats. Neuroendocr. 11: 137-143, 1973.
7. *Bohus, B., Wimersma Greidanus, Tj.B.* van, and *Wied, D.* de. Behavioral and endocrine responses of rats with hereditary hypothalamic deiabetes insipidus (Brattleboro strain). Physiol. Behav. 14: 607-615, 1975.
8. *Bohus, B., Urban, I., Wimersma Greidanus, Tj.B. van*, and *Wied, D.* de. Opposite effects of oxytocin and vasopressin on avoidance behaviour and hippocampal theta rhythm inthe rat. Neuropharmacology 17: 239-247, 1978a.
9. *Bohus, B., Kovacs, G.L.*, and *Wied, D. de.* Oxytocin, vasopressin and memory: opposite effects on consolidation and retrieval processes. Brain Res. 157: 414-417, 1978b.
10. *Bohus, B., Kovacs, G.L., Greven, H.* and *Wied, D. de.* Memory effects of arginine-vasopressin (AVP) and oxytocin (OXT): structural requirements. Neuro Science Letters, Suppl. 1: S80, 1978c.
11. *Dogterom, J., Wimersma Greidanus, Tj.B.* van, and *Swaab, D.F.* Evidence for the release of vasopressin and oxytocin into cerebrospinal fluid: measurements in plasma and CSF of intact and hypophysectomized rats. Neuroendocrinology 24: 108-118, 1977.
12. *Gold, P.W., Goodwin, F.K.*, and *Reus, V.I.* Vasopressin in affective illness. Lancet 1: 1233-1235, 1978.
13. *Krivoy, W.A., Zimmerman, E.*, and *Lande*, S. Facilitation of development of resistance to morphine analgesia by desglycinamide^9-lysinevasopressin. Proc. Natl. Acad. Sci. USA 71: 1852-1856, 1974.
14. *Lande, S., Flexner, J.B.*, and *Flexner, L.B.*, Effect of corticotropin and desglycinamide^9-lysine-vasopressin on suppresion of memory by puromycin. Proc. Natl. Acad. Sci. USA 69: 558-560, 1972.
15. *LeBoeuf, A., Lodge, J.*, and *Eames, P.G.* Vasopressin and memory in Korsakoff syndrome. Lancet 2: 1370, 1978.
16. *Legros, J.J., Gilot, P., Bruwiex, M. Fichefet, J.P., Dandoy, S.*, and *Moeglen, M. There* is a relationship between immunoreactive neurophysins, serum levels and item 7 of Rey's memory profile in the human aged more than 50. Proc. 10th International Congress. Int. Soc. Psycho-Neuro Endocrinology, Park City, Utah, 8-11 Aug., 1979.
17. *Leshner, A.I., Hofstein, R., Samuel, D.* and *Wimersma Greidanus, Tj.B.* van. Intraventricular injection of antivasopressin serum blocks learned helplessness in rats. Pharmacol. Biochem. Behav. 9: 889-892, 1978.
18. *Luersson, T.G., Shelton, R.L., Robertson, G.L.* Evidence for separate origin of plasma and cerebrospinal fluid vasopressin. Clinical Res. 25: 14A, 1977.
19. *Oliveros, J.C. Jandali, M.K., Timsit-Berthier*, M., Remy, R., Benghezal, A., Audibert, A.

and *Moeglen, J.M.* Vasopressin in amnesia. Lancet 1: 42, 1978.

20. *Raskind, M.A., Weitzman, R.E., Orenstein, H., Fisher, D.A.* and *Courtney, N.* Is antidiuretic hormone elevated in psychosis? A pilot study. Biol. Psychiat. 13: 385-390, 1978.
21. *Ree, J.M.* van, *Bohus, B., Versteeg, D.H.G.* and *Wied, D. de.* Neurohypophyseal principles and memory processes. Biochem. Pharmacol. 27: 1793-1800, 1978.
22. *Rigter, H., Riezen, H. van,* and *Wied, D.* de. The effects of ACTH and vasopressin-analogues on CO_2-induced retrograde amnesia in rats. Physiol. Behav. 13: 381-388, 1974.
23. *Thompson, E.A.,* and *Wied, D. de.* The relationship between the antidiuretic activity of rat eye plexus blood and passive avoidance behaviour. Physiol. Behav. 11: 377-380, 1973.
24. *Unger, H., Pommrich, G.,* and *Beck, R.* Der Oxytocingehalt im menschlichen pathologischen Liquor. Experientia 27: 1486, 1971.
25. *Wagner, A., Jardankazy, T., Laczi, F., Szilard, J. Telegdy, G.* and *Laszlo, F.A.* Study on the psychological effects of lysine vasopressin and DDAVP in diabetes insipidus patients. Acta Medica Acad. Sci. Hung. 36(3): abstract 87, 1979.
26. *Wied, D. de.* The influence of the posterior and intermediate lobe of the pituitary and pituitary peptides on the maintenance of a conditioned avoidance response in rats. Int. J. Neuropharmacol. 4. 157-167, 1965.
27. *Wied, D. de.* Effects of peptide hormones on behaviour. In: Frontiers in Neuroendocrinology, 1969. *W.F. Ganong* and *L. Martini* (eds.). Oxford University Press, pp. 97-140.
28. *Wied, D. de Long* term effect of vasopressin on the maintenance of a conditioned avoidance response in rats. Nature 232: 58-60, 1971.
29. *Wied, D. de.* Behavioral effects of intraventricularly administered vasopressin and vasopressin fragments. Life Sci. 19: 685-690, 1976.
30. *Wied, D. de.,* and *Bohus, B.* Modulation of memory processes by neuropeptides of hypothalamic-neurohypophyseal origin. In: Brain Mechanisms in memory and learning: from the single neuron to man, 1979. M.A.B. Brazier (ed). Raven Press, New York, pp. 139-149.
31. *Wied, D. de.,* and *Gispen, W.H.* Behavioral effects of peptides. In: Peptides in Neurobiology. *H. Gainer* (ed). Plenum Press, New York, 1977 pp. 397-448.
32. *Wied, D. de., Greven, H.M., Lande, S.,* and *Witter, A.* Dissociation of the behavioural and endocrine effects of lysine vasopressin by tryptic digestion. Br. J. Pharmacol. 45: 118-122, 1972.
33. *Wied, D. de., Bohus, B.,* and *Wimersma Greidanus, Tj.B.* van. Memory deficit in rats with hereditary diabetes insipidus. Brain Res. 85. 152-156, 1975.
34. *Wied, D. de., Wimersma Greidanus, Tj.B. van Bohus, B., Urban, I.,* and *Gispen,* W.H. Vasopressin and memory consolidation. In: Perspectives in Brain Research 45. M.A. Corner and D.F. Swaab (eds). Elsevier, Amsterdam, 1976, pp. 181-194.
35. *Wimersma Greidanus, Tj.B. van,* and *Versteeg,* D.H.G. Neurohypophyseal hormones; their role in endocrine function and behavioral homeostasis. In: Behavioral neuroendocrinology. *A.J. Dunn* and *Ch. B. Nemeroff* (eds). Spectrum Publ., Jamaica, N. Y. (in press).
36. *Wimersma Greidanus, Tj.B. van,* and *Wied D. de.* Modulation of passive avoidance behavior of rats by intracerebroventricular administration of antivasopressin serum. Behav. Biol. 18: 325-333, 1976.
37. *Wimersma Greidanus, Tj.B. van, Bohus, B.,* and *Wied, D. de.* Effects of peptide hormones on behaviour. In: International Congress Series Nº 273. Excerpta Medica, Amsterdam, 1973, pp. 197-201.
38. *Wimersma Greidanus, Tj.B. van, Dogterom, J., and Wied, D. de.* Intraventricular administration of anti-vasopressin serum inhibits memory consolidation in rats. Life Sci. 16: 637-644, 1975a.
39. *Wimersma Greidanus, Tj.B. van, Bohus, B.,* and *Wied, D. de.* The role of vasopressin in memory processes. In: Hormones, Homeostasis and the Brain. Progress in Brain Research 42. W.H. Gispen, Tj.B. van Wimersma Greidanus, B. Bohus, D. de Wied (eds). Elsevier Publ. Comp., Amsterdam, 1975b, pp. 135-141.
40. *Wimersma Greidanus, Tj.B. van, Fat-Bronstein, H.T.K.,* and *Ree, J.M. van. Antisera* to pituitary hormones modulate development of tolerance to morphine. In: Characteristics and Function of Opioids. J.M. van Ree and L. Terenius (eds). Elsevier/ North Holland Biomed. Press, 1978, pp. 73-74.
41. *Wimersma Greidanus, Tj.B. van, Croiset, G., Goedemans, H.,* and *Dogterom, J.* Vasopressin levels in peripheral blood and cerebrospinal fluid during passive and active avoidance behavior in rats. Horm. Behav. 12. 103-111, 1979.

Central Effects of Vasopressin in Man

A. AUDIBERT, J.M. MOEGLEN AND IOANA LANCRANJAN
Clinical Research Department, Sandoz Ltd., Basle/Switzerland

Vasopressin is one of the brain peptides which exert both endocrine and behavioral effects. Like oxytocin, vasopressin is a nonapeptide secreted by the neurohypophysis and synthesized in magnocellular neurons of the supraoptic (SO) and paraventricular (PV) nuclei. Vasopressin is also elaborated in the parvocellular neurosecretory neurons of the suprachiasmatic nucleus. In all species investigated so far oxytocin, vasopressin and their associated neurophysins are located in different neurons (1): vasopressin neurons are mainly in the ventral part of the SO nucleus and the medial part of the PV nucleus whereas the oxytocin neurons are in the dorsal part of the SO and the lateral part of the PV nucleus (1). Gainer, Sarne and Brownstein (2) have shown that vasopressin and its respective neurophysin are manufactured from a common precursor, a glucopeptide with a molecular weght of 20,000. The hypothesis-one hormone, one neurophysin, one cell - gained support when Burford et al (3) and Sunde and Sokol (4) demonstrated biochemically the absence of the neurophysin, which is the carrier of vasopressin (the bovine neurophysin II) in the brain of Brattleboro rats homozygous for diabetes insipidus.

The neurosecretory material synthetized in the magnocellular neurons is transported, packaged in granules, via the axons of the hypothalamo-pituitary tract to:

1) the posterior pituitary, where it is stored and released into the general circulation,
2) the external zone of the median eminence (ME) in contact with the portal vessels which reach the anterior pituitary, and
3) the third ventricle.

From nerve terminals which are in contact with the third ventricle vasopressin can be released into the CSF and then vehiculated to different areas of the brain. Vasopressin vehiculated by the CSF can account for some of its behavioral effects. Vasopressin is also released in the portal system as reported by Zimmerman et al (5) in the monkey and by Oliver et al (6) in the rat. The high amounts of vasopressin found in the portal blood may account for its possible effect on ACTH secretion, Indeed, Stillman et al (7) showed that neurophysn and vasopressin are increased in the external zone of the ME in adrenalectomized rats, whereas they are absent in adrenalectomized rats treated with prednisone. This suggests that vasopressin may act as a corticotrophin releasing factor. Vasopressin and its neurophysin stored in the posterior pituitary are released into the general circulation under the physiological stimulation of low osmotic pressure, postural hypotension, pain, apprehension and stress. Angiotensine II, nicotine and acetylcholine also stimulate the release of vasopressin.

In man, besides the hypothalamo-pituitary tract and its theree areas of distribution, vasopressin and oxytocin fibres from magnocellular neurons are also projected via the stria terminalis to the central amygdala, while descending fibres are distributed to the nucleus solitarius in the medulla

oblongata and to the central grey and lateral parts of the spinal cord. In addition, vasopressin fibres originating in the parvocellular neurons of the suprachiasmatic nucleus are directed to the lateral septum, posterior hypothalamus, interpeduncular nucleus, mediodorsal thalamus, lateral habenulae and preventricular grey of the brain stem. In addition, fine fibres are present in the medial amygdala and the ventral hippocampus (8).

The wide distribution of vasopressin fibres in the brain and their connection with the limbic system strongly suggest that vasopressin could have important neuromodulatory and behavioral effects in mammals. Indeed, a great deal of evidence has been accumulated supporting the role of vasopressin in learning and memory processes in rats (9). De Wied was the first to report that the extinction of a conditioned avoidance response in rats is markedly accelerated after the removal of the posterior lobe of the pituitary. Subsequent studies have shown that a posterior pituitary extract or vasopressin restores the disturbed extinction process (10, 11).

In intact rats, the s. c. injection of a posterior pituitary extract preparation or lysine vasopressin (LVP) had long term effects on the extinction of the conditioned avoidance response (9). Studies with vasopressin analogs have shown that the behavioral effects of vasopressin are independent of its endocrine action.

Indeed, desglycinamide —lysine-8-vasopressin—, which has almost no pressor or antidiuretic activity, is still centrally active, its central potency being 50% of that of arginin vasopressin (AVP). Administered i.v., LVP increased also avoidance latencies and retention in passive avoidance behavior studies. Furthermore, the fact that the intracerebroventricular (IVC) administration of VP inhibited the extinction f the pole jumping avoidance responce at doses 200 fold less than were needed for its systemic administration, suggests that VP may be physiologically involved in memory processes (10). The intraventricular administration of VP antiserum after a learning trial leads to a deficit in passive avoidance retention and the peripheral administration of large amounts of VP antiserum has no effects on behavior, which also supports the physiological implication of VP in memory processes (9).

Further evidence for the role of VP in memory processes was given by studies showing that vasopressin protects against puromycin induced memory loss, CO and electroshock induced amnesia (11). Therefore, a large body of convincing evidence has been accumulated showing that vasopressin is involved in memory processes in experimental animals.

Based on these animal data, we initiated several studies in man in order to assess whether or not vasopressin is involved in the modulation of memory processes in humans. Two groups of studies were carried out in various hospitals:

1) Studies in healthy volunteers using electrophysiological and psychometric methods and

2) studies in patients with either memory disturbances or psychiatric diseases, using appropriate scales.

I. Electrophysiological Studies in Normal Volunteers

I. 1. Influence of LVP on the spontaneous EEG in man

Timsit-Berthier et al. (12) have already reported the effects of intramuscular (i.m.) and intra-nasal administration of LVP on the spontaneous EEG of young healthy volunteers. A dose of 10 I.U. i.m. decreased the alpha activity and increased the delta activity 1-2h after the LVP injection. This effect lasted for 4-6h and was accompanied by clinical sedation. On the other hand 5.4 I.U. LVP nasal spray did not influence the EEG patterns, but 3 out of 4 subjects reported a "feeling of elation and well-being". In a crosss over, randomised placebo controlled study in 8 normal volunteers, Matejcek (data to be published) confirmed the effects of LVP on the spontaneous EEG. Indeed after placebo and 1 I.U. LVP i.m., no effect on EEG has been noticed, whereas after 2 and 4 I.U. LVP i.m., a dose-response effect has been recorded. This effect — a reduction of the alpha — activity and an increase of the delta and theta activity — was significant after 4

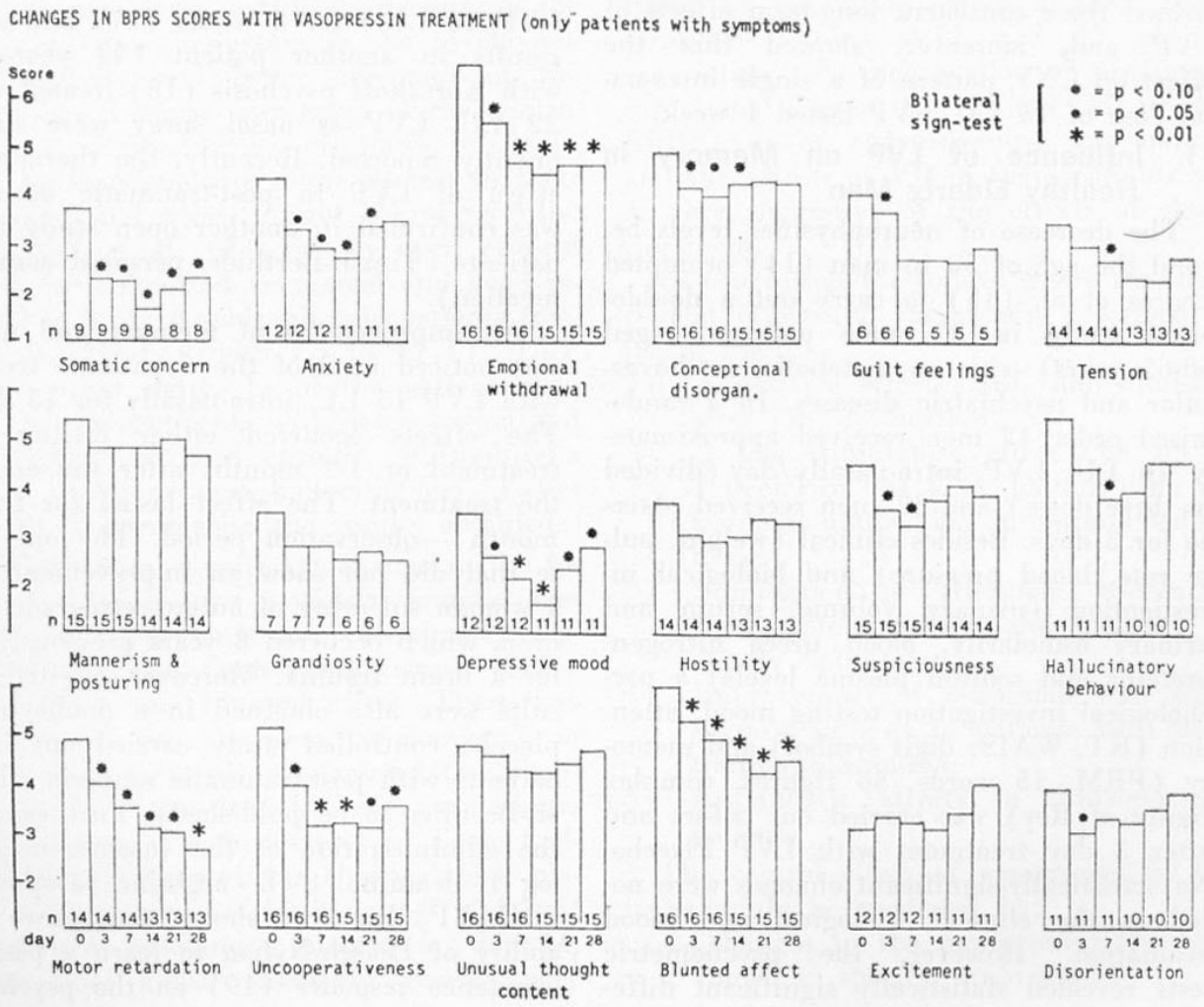
hours and lasted for up to 6 hours after the administration of LVP. On the other hand, 7.5, 15 and 30 I.U.LVP administered intranasally showed only a late effect, namely an increase in the alpha-activity which was statisticaly significant only 6 and 8 hours after the administration of 15 and 30 I.U. LVP.

I. 11. Influence of LVP on the CNV

The contingent negative variation (CNV) is considered as a sensitive and reliable indicator of the central effects of drugs with stimulant and depressant actions (13). It is a slow cortical potential recorded at the vertex, extracted from the spontaneous EEG by averaging several EEG fragments in the same phase in relation to the stimuli. The CNV is obtained by warning the subjects, by way of a first, conditioned stimulus (S1), of the arrival of a second, unconditioned stimulus (S2) to which he must give a response. Between S1 and S2 there is a negative slow potential, the CNV, which is developed in the frontal lobes of the brain and stopped by the occurrence of S2.

In the studies carried out by Timsit-Berthier et al. (13) the EEG was recorded for 30 min with the subject's eyes closed and 100 pairs of stimuli were delivered; S1 was a brief tone, and S2 a long tone that the subject had to stop by pressing a button. The interval between S_1 and S_2 was 1.5 sec and the interval between the pair of stimuli varied at random from

Table I.



10-20 sec. Each CNV was obtained by averaging 12 artefact-free vertex EEG fragments in phase-relation to 12 successive pairs of stimuli. So, for each subject a set of six to eight successive CNV was collected and analysed. It was thus possible to appreciate the evolution of CNV in time. 15 minutes after placebo administration a progressive decrease of CNV amplitude was noticed, whereas the administration of LVP induced a resistance to this habituation phenomenon and the amplitude of CNV remained constant.

Further evidence for the central effects of LVP has been given by Devos et al. (data to be published) also using the CNV recording. In a cross-over, double-blind study with placebo and a single 15 I.U. dose of LVP administered intra-nasally in 10 young healthy male volunteers, a significant increase of the wave P 300 2 hours and of the CNV 6 hours after the administration of LVP has been registered. Recent on-going studies carried out by Timsit-Berthier confirmed these consistent long-term effects of LVP and, moreover, showed that the effect on CNV pattern of a single intra-nasal dose of 15 I.U. LVP lasted 1 week.

II. Influence of LVP on Memory in Healthy Elderly Man

The decrease of neurophysines levels beyond the age of 50 in man (14) prompted Legros et al. (15) to carry out a double-blind study in 23 male patients (aged 50-65 years) with no metabolic, cardiovascular and psychiatric diseases. In a randomised order 12 men received approximately 16 I.U. LVP intra-nasally/day (divided in three doses) and 11 men received placebo for 3 days. Besides clinical (weight, pulse rate, blood pressure) and biological investigation (urinary volume, serum and urinary osmolarity, blood ureea nitrogen, proteins and sodium plasma levels) a psychological investigation testing mood, attention (KT, WAIS: digit symbol) and memory (PRM, 15 words, 30 figures, complex figure of Rey) was carried out before and after 3 day treatment with LVP/Placebo. No statistically-significant changes were noted in the clinical, biological and mood evaluation. However, the psychometric tests revealed statistically significant differences (Median-Test — 21 statistics) between the 2 groups of subjects. The subjects receiving LVP performed better in tests of attention, concentration and motor rapidity (KT Attention Test and Wais Digit Symbol Test) and in memory tests using visual graphic material for the measurement of visual retention (30 figures of Rey), recognition (sub-tests 2, 5, 3 and 4 of the PRM of Rey) and immediate and delayed free recall (sub-tests 6 and 7 of the PRM and complex figure of Rey). Using audioverbal material, it has been found that LVP improved attention and immediate memory (Wais Digit Span) as well as learning and recognition (15 words of Rey).

III. Influence of LVP in Amnesic Patients

Oliveros et al. were the first ti report the therapeutic effects of LVP administered intra-nasally in 4 patients with post-traumatic retrograde amnesia (16). Negative results n two patients (treated with LVP 16 I.U.) for 2 weeks (17) and good results in another patient (43 year-old) with Korsakoff psychosis (18) treated with 22 I.U. LVP as nasal spray were subsequently reported. Recently, the therapeutic effect of LVP in post-traumatic amnesia was confirmed in another open study in 6 patients (Timsit-Berthier, personal communication).

An improvement of memory and mood was noticed in 5 of the 6 patients treated with LVP 15 I.U. intra-nasally for 15 days. The effects occurred either during the treatment or 1-2 months after the end of the treatment. The effect lasted for the 6 month —observation period. The only case that did not show an improvement was a woman suffering of antero-retrograde amnesia which occurred 8 years previously after a brain trauma. Moreover, positive results were also obtained in a double-blind placebo controlled study carried out in 5 patients with post-traumatic amnesia (Timsit-Berthier to be published). Furthermore, the administration of the vasopressin analog 1 - deamino - 8 - D - arginine vasopressin (DDAVP) has been shown to improve the ability of Leasch-Nyhan to learn a passive avoidance responce (19) an the psycholo-

gical status of children under DDAVP therapy for central diabetes insipidus (20). Based on the analysis of a 61 - item questionnaire Waggoner et al. (20) concluded that DDAVP treatment "affords the child an enhanced energy level and out-look on life and permits the child freer use of imaginative and creative abilities". Moreover, Gilot et al. (21) and De Wied (personal communication) reported that patients with hereditary diabetes insipidus have a deficit of attention and memory which may be improved after the application of LVP.

IV. Influence of LVP Treatment in Psychiatric Patients

As early as 1952 Foriz published remarkable clinical results in schizophrenic patients treated with Pitressin 10 I.U. every 24 or 48 hours for 6 to 12 months. In two-thirds of the 62 patients treated most of them with chronic residual schizophrenia, there was a notable effect and in one third of the patients. the improvement was spectacular (22). The following beneficial effects have been described: increase of the sleeping time, reduction in the psycho-motor activity and better adequacy of the emotions, increase awareness of the environment, disappearance of ward problems, the patients showing more interest in their families and more insight. More recently, Vranckx et al. (23) in an open clinical trial have reported very good and good results in 8 of 16 schizophrenic patients treated for 28 days with 7.5 to 45 I.U. LVP nasal spray daily. 14 of the patients had chronic schizophenia with deterioration and the other two had chronic schizophrenia with delusions and hallucinations. Tables I and II summarise the results obtained.

A statistical significant change in the following items was noticed: somatic con cern, anxiety, emotional withdrawal, depressive mood, uncooperativeness, motor retardation and blunted affect. There was a tendency for the item "excitement" to deteriorate and this was related to discontinuation of the drug in one case, because of insomnia. The histograms in Table I also show that the effects observed took place very rapidly, i.e. from days 3 and 7 of treatment, reaching a plateau on day 14. The final global assesment reveals very good results in 3 cases, good in 5, inadequate in 6 and failures in 2. Compared to previous treatment, LVP was noted superior in 8 cases. 12 of the 16 patients had an unsatisfactory response to their previous treatment: in 5 of these 12 cases LVP had a good or very good therapeutic effect. In 4 patients the improvement observed was sufficient to allow discharge from hospital (Table II).

Apart from the quantitative data of the BPRS and from the final global evaluation, vasopressin seems to act at the level of the interactions with the therapist, other patients and the family, which fits with the remarcable changes in the items "emotional withdrawal", "uncooperativeness" and "blunted affects".

Comparison of efficacy of vasopressin with the previous drugs (neuroleptics in 15 cases and neuroleptic plus antidepressant in 1 case) has shown that in contrast with the sedative effect which neuroleptics usually have, VP has a contrary action, demonstrated by the improvement of the "motor symptoms (items 8,12 and 17 of the retardation". No aggravation of productive symptoms (items 8, 12 and 17 of the BPRS) or of aggressiveness was noticed and the effects of VP in these patients were very suggestive of the effects of shock treatment.

In conclusion the following are the main central effects of vasopressin described so far in man:

1) A sedative effect (EEG and clinical) after i.m. application;
2) A late stimulatorv effect (6h up to 1 week) on the CNV pattern after intra-nasal application in normal volunteers;
3) Stimulation of attention, concentration, motor rapidity and memory in elderly volunteers;
4) Improvement of retrograde and anterograde amnesia in patients with posttraumatic amnesia;
5) Antiautistic effects in schizophrenic patients.

The central effect of vasopressin in humans might be mediated by:

— changes in the neurotransmitter turnover in specific brain nuclei

— activation of prostaglandin synthesis or cAMP formation
— stimulation of critical enzymes in the brain
— stimulation of all or one of the hypothetical biochemical events involved in learning and memory processes, namely, RNA polymerase activity, RNA and protein synthesis and axonal transport,
— modulation of the phosphorylation of myelin
— stimulation of the endogenous ACTH secretion
— changes of the sleep pattern and nyctohemeral secretion of pituitary hormones
— possible relationships with the natural opiate peptides.

The central effects of vasopressin in man could be mediated by changes of neurotransmitters' turnover in specific brain nuclei

Increasing evidence shows that in animals the intracerebroventricular (ICV) administration of vasopressin causes significant changes in the turnover of catecholamines in specific brain nuclei. Tanaka et al. (24) reported that following the ICV administration of arginine-vasopressin (30 ng) to rats, noradrenaline turnover was increased in the dorsal septal nucleus, the anterior hypothalamic nucleus, the medial forebrain bundle, the parafascicular nucleus, the dorsal raphé nucleus, the locus coeruleus, the nucleus tractus solitarii and the Al-region. It has been previously shown that the following limbic structures are the most probably involved in the consolidation of memory and the retrieval of stored information: the rostral septal area, the region of the parafascicular nucleus of the thalamus and the dorsal hippocampus (9). In two of these regions, the dorsal septal nucleus and the parafascicular nucleus, NA turnover is enhanced by ICV administration suggesting that the effect of VP in memory processes is mediated by an increase of the NA turnover in these regions. Data recorded in Brattleboro homozygous rats showed that these rats have an altered brain catecholamine turnover, in a direction opposite to that of the changes induced by the ICV administration of vasopressin (25). Furthermore, the ICV administration of an antiserum antivasopressin induces a decrease of the NA turnover in the dorsal septal nucleus, the parafascicular nucleus and the rostral part of the nucleus tractus solitarii (9). A further support that the effect of VP on memory consolidation is mediated by the NA system was given by the work of Kovàcs et al. (26) who showed that the destruction of the ascending dorsal NA bundle by microinjection of 6-OH DA abolished the effect on memory consolidation in rats. Moreover, it has been found that bilateral injection of small amounts of vasopressin (25 pg) in regions containing terminals of the coeruleo-telencephalic noradrenergic system, viz. the dorsal septal nucleus, the dentate gyrus of the hippocampus and the dorsal raphé nucleus caused a facilitation of memory consolidation, whereas microinjections in the central amygdaloid nucleus and the locus coeruleus, which contain the cell bodies of this system, failed to do so (26). In contrast with this well documented evidence in animals supporting the modulatory effect of VP on the NA structures in the limbic system, no data on this subject are available in man. Ongoing studies will provide us material to appreciate whether or not in humans the effect of VP is mediated by changes of the neurotransmitter turnover. Particular attention will be payed to the cholinergic system, due to its critical involvement in senile dementia (27) and in memory processes in man (28). Indeed, the septal-hippocampal structures and pathways are included in the cholinergic system and are known to be involved in the storage of memory.

In man the amnesia produced by lesions of the hippocampal complex is characterized not only by the severity of memory storage impairment but also by the isolated occurrence of memory impairment. Furthemore, the pattern of memory cognitive changes in aging and dementias is similar to that seen with cholinergic blockade (28).

Vasopressin — Prostaglandins' Synte thesis and/or cAMP Formation

The effects of vasopressin on cAMP formation and prostaglandins' synthesis at the renal and bladder-level is well documented (29). Orloff and Handler (30) showed that cAMP mediated the antidiuretic action of VP and Bentley (31) showed that AVP stimulated the adenylcyclase whereas valinomycin blocked both the adenylcyclase and the action of VP on the bladder. Furthermore, Grantham and Burg (32) reported that AVP stimulated also the adenylcyclase in the renal medulla. A number of investigators showed that cAMP activates a proteinkinase which catalyse the phosphorylation of membrane proteins which in turn regulate membrane permeability (29). Finally, Barker (33) found that the iontophoretic application of VP into snail neurons produced changes in the permeability of the neuronal membranes. Thus, it is likely that VP via synthesis of cAMP can also change the permeability of neurons in the mammalian brain. Data have been also accumulated showing that VP stimulates PGE_2 synthesis in the toad urinary bladder and in vascular preparations (34, 35). On the other hand, there are no available data so far on whether vasopressin has similar effects on cAMP and PG synthesis in the brain.

Vasopressin — Stimulation of Critical Enzymes in the Brain

Knowing that hormones exert metabolic effects by stimulating key enzymes one may wonder whether VP has selective effects on one of the critical enzymes involved in the synthesis or metabolism of neurotransmitters and/or on enzymes involved in the RNA-protein synthesis. If one agrees with Rose and Haywood (36) that the biochemical events involved in learning and memory are increased RNA polymerase, increased RNA and protein synthesis and increased axonal flow to synapses, one should investigate the effects of vasopressin on all these processes.

Vasopressin — Modulation of the Phosphor lation of the Myelin

Another new biochemical finding is that in aged rats the in vivo phosphorilation of myelin in the cortex and hippocampus is increased. The administration in both young and old rats of 0.05 I.U. LVP/rat is followed by a reduction of the phosphorylation of the myelin in vivo in the cortex and hypothalamus (Hiestand, to be published). Additional experimental work is, however, requested for correlating the phosphorylation of the myelin with memory processes.

Vasopressin and ACTH secretion

The fact that VP releases ACTH after i.m. or i.v. injection and that ACTH has central effects in animals and man raises the question whether or not the central effects of VP are mediated by the release of ACTH. In a cross-over study carried out in 2 groups of 6 young male volunteers placebo and 3 doses of LVP were administered either i.m. or intranasally and ACTH plasma levels were measured in the subsequent 2 hours. A dose response ACTH releasing effect was noticed after the i.m. administration of 1, 5 and 10 I.U., whereas no effect on ACTH release was noticed after the intranasal admistration of LVP (7.5, 15 and 30 I.U.).

Moreover, a daily ACTH profile was made before and after 7 days treatment with LVP (15 I.U. intranasally daily) and showed that LVP had no effect on the ACTH pattern. Based on these data we ruled out the possibility that the central effect of LVP is mediated by the ACTH release. These conclusions are in agreement with De Wied's data showing that ACTH and VP have different central effects, ACTH being involved in attention, motivation and retrieval and not in memory storage (11).

Vasopressin and the Nyctohemeral Secretion of Pituitary Hormones

Vasopressin is a main constituant of the suprachiasmatic nucleus, and increased evidence support the idea that the suprachiasmatic nucleus is the site of the control of biological rhythms. Indeed, studies in adult and neonatal rats revealed the primary significance of the suprachiasmatic nucleus and the retinohypothalamic projection in the generation and maintenance of circadian rhythms (37).

It has been recently reported that the total ablation of the suprachiasmatic nucleus in 2 day-old rats permanently abo-

lishes circadian rhythms (38). On the other hand, it has been reported that deterioration in cognitive processes in schizophrenic patients is associated with a flattening of the diurnal rhythm of 17 ketosteroids or with a reversal of it (39). Moreover, Murri et al. and Vigneri et al. (40, 41) reported a disappearance of the nocturnal GH peak in schizophrenic patients when their slowave sleep is absent "as it seems to happen rather frequently in schizophrenia".

To test the mechanism of LVP action in schizophrenia, we first investigated the effect of LVP on the nyctohemeral rhythm of pituitary hormones.

The nyctohemeral rhythm of growth hormone (GH) and prolactin (PRL) secretion in two schizophrenic patients (Lancranjan et al., to be published) showed a normalisation after 3 weeks'treatment with LVP doses built-up to 22.5 I.U.

These results, in spite of the limited number of cases, support the hypothesis that one mechanism of LVP action in psychiatric diseases might be the normalization of the circadian rhythms. Moreover, we expect that LVP could also normalize the sleep pattern (stage III-IV of sleep) in schizophrenic patients due to the fact that the nocturnal GH peak is related with the slow wave sleep mainly during the first 2 hours. Animal data have also shown that the hippocampal theta activity in Brattleboro rats is significantly lower and could be temporarily normalized by the intraventricular injection of AVP (42).

Vasopressin — The Natural Opiate Peptides

Recent data strongly suggest that pituitary opiate peptides and neurohypophyseal hormones may participate in a regulatory loop in which each affects the other's release. It has been shown that plasma ACTH and beta-LPH rose in parallel in response to VP in normal subjects and in patients

Table II

FINAL OVERALL EVALUATION

Case no.	Previous treatment Type	Response	Vasopressin Response	Comparison
1	NL	(*)	*	LVP > NL
2	NL	(*)	(*)	LVP = NL
3	NL	(*)	(*)	LVP = NL
4	NL	0	(*)	LVP > NL
5	NL	*	** ①	LVP > NL
6	NL	(*)	(*)	LVP = NL
7	NL	(*)	0	LVP < NL
8	NL	(*)	(*)	LVP = NL
9	NL	*	0	LVP < NL
10	NL	(*)	(*)	LVP < NL
11	NL	(*)	** ①	LVP > NL
12	NL	*	*	LVP = NL
13	NL	*	** ①	LVP > NL
14	NL+AD	(*)	*	LVP >NL + AD
15	NL	(*)	* ①	LVP > NL
16	NL	(*)	*	LVP > NL

NL : neuroleptics
AD = antidepressants
① = discharged

0 = failure
(*) = poor
* = good
** = very good

with Cushing disease and Nelson syndrome (43). On the other hand, Weitzman et al. (44) reported that beta-endorphin stimulates secretion of arginine vasopressin in vivo, data in agreement with the antidiuresis produced in dogs and monkeys by opiates (45). Moreover, Simantov and Snyder (46) reported the presence of high concentrations of opiate receptors in membrane preparations of the posterior lobe of the pituitary, which might suggest a physiologic role of endogenous opiate beta LPH and beta-endorphin in the medulation of neurohormone secretion. Furthermore, Mata et al. (47) reported that dehydrated rats, with increased oxytocin and vasopressin release, have significantly decreased levels of pituitary opiate peptides, probably owing to depletion of their endogenous stores. Finally Rossier et al. (48) found a close anatomical relationship between enkephalin fibers from the PV and SO nuclei and the neuro intermediate pituitary lobe.

They found high amounts of enkephalin-like material in extracts of the neuropituitary, enkephalin cell bodies in the PV and SO nuclei and enkephalin fibres in the pars nervosa of the pituitary. Section of the pituitary stalk produced the disappearance of enkephalin-fibres in the posterior pituitary whereas lesions of the PV nucleus produced a decrease by only 40 per cent of the enkephalin content in the pituitary. Furthemore, the fact that dehydration decreased also enkephalin concentrations supported the idea that enkephalin fibres modulate the release of VP at the pituitary levels. Finally, the fact that there are opiate receptors in the pars nervosa suggests that enkephalin fibres control the secretion of VP in rats.

In order to assess whether or not there are functional relationships between endogenous opiates and VP in humans a pilot study using the CNV technique was carried out in 10 normal male volunteers (49). Eeach of them received 0.4 mg i.m. naloxone and 30 min. later either placebo or 15 I.U. LVP intranasally. The CNV was recorded three times: before injection. 5 min. and 30 min. after the single application of either placebo or LVP. Naloxone alone produced a marked and significant decrease of the wave P_{300} 5 min. after its injection. The effect was consistent but lasted only one hour.

Furthemore, after naloxone, LVP not only stopped the CNV habituation process but reversed it. The preliminary conclusion of this study was that LVP and natural opiates may have antagonist actions, at least on the CNV pattern.

In conclusion, preliminary data show that VP has central effects in man and this fact opened a new and extremely interesting field of research. However, much effort is still requested in order to clearly delineate these effects and to explain the central effects of VP in man.

SUMMARY

A great deal of evidence has been accumulated favouring the role of vasopressin, in learning and memory —processes in rats. Memory disturbances observed in Brattleboro rats and Wistar rats treated with vasopressin antiserum intraventriculary could be reversed by vasopressin and intra— and extrahypothalamic vasopressin pathways were described in rats giving anatomical support to behavioural effects of vasopressin. Finally, changes in neurotransmitters' turnover namely, NA and DA, in specific areas of the brain were observed after intra-ventricular administration of vasopressin in rats.

Based on these animal studies, we initiated several studies to test the psychotropic effects of L-vasopressin (LVP) in man. Two groups of studies were carried out in various university hospitals using LVP, administrated intra-nasally: A) studies in healthy volunteers using psychometric, electrophysiological and hormonal parameters and B) studies in patients with either memory disturbances or psychiatric diseases using appropriate scales.

Preliminary results in healthy volunteers have shown that LVP nasal spray a) improves attention, concentration, motor rapidity and memory. b) influences the

contingent negative variation pattern without having c) on the EEG profile and d) on the ACTH secretion the effects noticed after its i.m. administration.

In conclusion, in normal man, LVP has specific central effects which are not mediated by ACTH secretion.

Preliminary results in patients showed that LVP administrered intra-nasally has important therapeutic effects in postraumatic amnesic patients and in some chronic schizophrenics, strongly suggesting that LVP could be used as a therapeutic tool.

RESUMEN

Existe una clara evidencia en favor del papel de la vasopresina en el proceso del aprendizaje y de la memoria en ratas. Disturbios de la memoria observados en ratas Battleboro y ratas Wistar tratadas con suero antivasopresina por vía intraventricular, desaparecen después de la administración de vasopresina. Han sido descritas vías intra y extrahipotalámicas de vasopresina en la rata y ellas dan el sostén anatómico a los efectos comportamentales de esta sustancia. Además modificaciones del "turnover" de los neurotrańsmisores NA y DA han sido observados después de la administración intraventricular de vasopresina, en áreas específicas del cerebro.

Basados en estos resultados en animales, hemos iniciado varios estudios para probar los efectos psicotrópicos de la L-vasopresina (L V P) en el hombre. Dos tipos de estudios fueron realizados en varios hospitales universitarios utilizando LVP administrada por vía intranasal:

a) Estudios sobre voluntarios sanos, utilizando parámetros psicométricos, electrofisiológicos y hormonales.
b) Estudios sobre pacientes afectados por disturbios de la memoria o enfermedad psiquiátrica.

Los resultados preliminares en voluntarios sanos demostraron que la LVP administrada en pulverización nasal:

a) Mejora la atención, la concentración, la rapidez motriz y la memoria;
b) Actúa sobre la variación contingente negativa (V.C.N.) sin tener
c) Sobre el perfil EEG y
d) La secreción de A C T H

los efectos observados después de la administración i.m.

En conclusión, la LVP ejerce en el hombre normal efectos centrales específicos que no dependen de una mediación por la secreción de A C T H.

Los resultados preliminares observados en los pacientes muestran que la administración intranasal de LVP produce importantes efectos terapéuticos en la amnesia postraumática y en la esquizofrenia crónica lo que sugiere que puede ser empleado como recurso terapéutico.

RÉSUMÉ

L'influence de la vasopressine sur l'apprentissage et la mémoire chez le rat est désormais soutenue par un grand nombre de preuves. Les troubles de la mémoire observés chez les rats Brattleboro et Wistar après traitement intraventriculaire au sérum antivasopressine disparaissent après l'administration de vasopressine. Les voies intra— et extrahypothalamiques de la vasopressine chez le rat ont été décrites, et elles donnent, sur le plan anatomique, un support aux effects comportementaux de cette substance. Enfin, des modifications du turnover des neurotransmetteurs, NA et DA, ont été observées, après l'administration intraventriculaire de vasopressine, dans certaines régions cérébrales.

Compte tenu de ces études chez l'animal, nous avons entrepris plusieurs essais portant sur les effets psychotropes de la L—vasopressine (LVP) chez l'homme. Deux groupes d'études utilisant la LVP par voie intranasale ont été pratiquées dans divers hôpitaux universitaires:

a) études chez des volontaires sains, à l'aide de paramètres psychométriques,

électrophysiologiques et hormonaux;
b) études chez des patients présentant des troubles de la mémoire ou des maladies psychiatriques.

Les résultats préliminaires obtenus chez les volontaires sains, montrent que la LVP sous forme de pulvérisation nasale:
a) améliore l'attention, la concentration, la rapidité motrice et la mémoire;
b) agit sur la variation contingente négative (V.C.N.) sans avoir
c) sur le profil E.E.G. et
d) la sécrétion d'A.C.T.H.

les effets observés après administration i.m.

En conclusion, la LVP exerce chez l'homme normal des effects centraux spécifiques qui ne dépendent pas d'une médiation par la sécrétion d'A.C.T.H.

Les résultats préliminaires enregistrés chez des malades révèlent que l'administration intranasale de LVP produit d'importants effets thérapeutiques dans l'amnésie post-traumatique et dans la schizophrénie chronique, ce qui tendrait à suggérer son emploi en thérapeutique.

ZUSAMMENFASSUNG

Es wurden zahlreiche Beweise dafür gesammelt, dass Vasopressin Lernprozesse und Gedächtnisleistungen bei Ratten beeinflusst. Gedächtnisstörungen bei Brattleboro— und Wistar —Ratten, die nach intraventrikulärer Verabreichung von Vasopressin-Antiserum beobachtet wurden, konnten durch Vasopressin behoben werden. Ausserdem wurden intra— und extrahypothalamische Vasopressin— Bahnen an der Ratte beschrieben, welche die verhaltensmodifizierenden Wirkungen von Vasopressin anatomisch bekräwtigen. Schliesslich wurden nach intraventrikulärer Verabreichung von Vasopressin an Ratten Aenderungen des Neurotransmitter — Umsatzes (NA und DA) in bestimmten Hirnregionen beobachtet.

Auf diese Tierversuche gestützt, führten wir mehrere Studien durch, um die psychotropen Effekte von L — Vasopressin (LVP) am Mensch zu prüfen. Zwei Gruppen von Studien wurden in verschiedenen Universitätskliniken durchgefürht, wobei LVP intranasal verabreicht wurde: A) Studien an normalen Probanden, unter Verwendung psychometrischer, elektro-physiologischer und hormonaler Parameter: B) Studien an Patienten mit Gedächtnisstörungen oder psychiatrischen Krankheiten, unter Verwendung geeigneter Rating-Skalen.

Vorläufige Ergebnisse bei normalen Probanden zegt, dass LVP-Nasenspray
a) die Aufmerksamkeit, Konzentration, motorische Schnelligkeit und Gedächtnisleistung verbessert,
b) das Muster der kontingenten negativen Variation (CNV) beeinflusst, ohnedie nach i.m. Applikation zu beobachtenden Wirkungen,
c) auf das EEG-Profil und
d) auf die ACTH-Sekretion zu zeitigen.

Wir ziehen daraus die Schlussfolgerung, dass LVP am normalen Menschen spezifische zentrale Effekte hervorruft, die nicht durch die ACTH-Sekretion vermittelt werden.

Vorläufige Ergebnisse an Patienten zeigten, dass LVP bei intranasaler Verabreichung an Patienten mit posttraumatischer Amnesie und einige Patienten mit chronischer Schizophrenie beträchtliche therapeutische Wirkungen zeitigt. Diese Resultate legen dringend die Vermutung nahe, dass LVP als therapeutisches Mittel eingesetzt werden könnte.

REFERENCES

1. *Scharrer, E.* and *Scharrer, B.*: Hormones produced by neurosecretory cells. Rec. Prog. Horm. Rer. 10: 183-240, (1954).
2. *Gainer, H.; Sarne, Y.; Brounstein, M.*: Neurophysin biosynthesis. Conversion of a putative precursor during axonal transport. Science 195: 1354-1356 (1977).
3. *Burford, G.D., Jones, C.W.* and *Pickering, B.T.*: Relative identification of a vasopressin-neurophysin and an oxytocin neurophysin in the rat. Biochem. J. 124: 809-813, (1971).
4. *Sunde, D.A.* and *Sokol, H.W.*: Quantification of rat neurophysin by polyacrilamide

gel electrophoresis (PAGE): Application to the rat with hereditary hypothalamic diabetes insipidus. Ann. NY Acad. Sci. 248: 345-364, (1975).

5. *Zimmerman, E.A., Carmel, P.W., Husain, M.K., Ferin, M., Tannenbaum, M., Frantz, A.G.* and *Robinson, A.G.*. Vasopressin and neurophysin: High concentrations in monkey hypophyseal portal blood. Science 182: 925-927, (1973).
6. *Oliver, C., Mical, R.S.*, and *Porter, J.C.*: Hypothalamic-pituitary vasculature: evidence for retrograde blood flow in the pituitary stalk. Endocrinology, 100: 598-604, (1977).
7. *Stillman, M.A., Recht, L.D., Rosario, S.L., Seif, S.M., Robinson, A.G.* and *Zimmerman, E.A.*: The effect of adrenalectomy and glucocorticoid replacement of vasopressin and vasopressin-neurophysin in the zona externa of the median eminence of the rat. Endocrinology, 101: 42-49, (1977).
8. *Weindl, A.* and *Sofroniew, M.V.*. Immunohistochemestry of neuropeptides. XIIth Acta Endocrinologica Congress, June 26-30, 414, (1979).
9. *van Wimersma Greidanus* and *Versteeg, D.H.G.*: Neurohypophysial hormones; their role in endocrine function and behavioral homeostasis. In "Behavioral Neuroendocrinology" *Ch. B. Nemeroff* and *A.J. Dunn* eds. spectrum Publ. Inc. Jamaica (NY), USA, (1980) in press.
10. *de Wied, D.*: The influence of the posterior and intermediate lobe of the pituitary and pituitary peptides on the maintenance of a conditioned avoidance response in rats. Int. J. Neuropharmacol. 4: 157-167, (1965).
11. *de Wied. D., Bohus, B., Gispen, W.H., Urban, I.* and *van Wimersma Greidanus, Tj.B.*: Hormonal influences on motivational learning, and memory processes. Hormones, Behavior, and Psychopathology (Raven Press) 1-14, (1976).
12. *Timsit-Berthier, M., Audibert, A.* et *Moeglen, J.M.*: Influence de la lysine-vasopressine sur l'EEG chez l'homme. Resultats préliminaires. Neuropsychobiology, 4: 129-139, (1978).
13. *Timsit-Berthier, M., Audibert, A.* et *Moeglen, J.M.*: Modification de l'EEG et de la VCN sous l'effet d'une hormone post-hypophysaire: la lysine vasopressine (LVP). IIème Congrès Mondial de psychiatrie Biologique, Barcelona (1978). Abstract nº 438.
14. *Legros, J.J., Gilot, P., Seron, X., Claessens J., Adam, A., Moeglen, J.M., Audibert, A.* and *Berchier, P.*: Influence of vasopressin on learning and memory. The Lancet I, 41-42, (1978).
15. *Legros, J.J., Gilot, P.*: Vasopressin and memory in the human. In: "Brain Peptides: A new Endocrinology" *A.M. Gotto, Jr., E.J. Peck, Jr.* and *A.E. Boyd*, III eds. Biomedical Press, North-Holland/Elsevier/Amsterdam, (1979).
16. *Oliveros, J.C., Janadali M.K., Timsit-Berthier, M., Remy, R., Benghezal, A., Audibert, A.* and *Meoglen, J.M.*: Vasopressin in amnesia.The Lancet I, 41-42, (1978).
17. *Blake, D.R., Dodd, M.J.* and *Grimley, I.E.*: Vasopressin in amnesia. The Lancet I, 608, (1978).
18. *Le Boeuf, A., Lodge, J.* and *Eames, P.G.*: Vasopressin and memory in Korsakoff syndrome. The Lancet, II, 1370, (1978).
19. *Anderson, L.T., David, R., Bonnet K.* and *Dancis, J.*: Passive avoidance learning in Lesch Nyhan disease: Effect of 1-desamino-8-arginine-vasopressin. Life Sci. 24, 905-910, (1979).
20. *Waggoner, R.W., Slonim, A.E.* and *Armstrong, S.H.*: Improved psychological status of children under DDAVP therapy for central diabetes insipidus. Am. J. Psychiatry 135: 361-363, (1978).
21. *Gilot, P., Crabbe, J.* and *Legros, J.J.*: *Bilan* mnesique chez 5 sujets presentant un diabète insipide. Communication at the Symposium on "Clinical Psychoneuroendocrinology, Liège, (Sept. 1979).
22. *Forizs, L.* and *Butner*: The use of pitressin in the treatment of schizophrenia with deterioration. North Carolina Medical Journal: 76-80, (1952).
23. *Vranckx, 'C., Minne, Ph., Benghezal, A., Moeglen, J.M.* and *Audibert, A.*: Vasopressin and schizophrenia. In "Biological Psychiatry Today" J. Obiols, C. Ballus, E. Gonzales Monclus and J. Pujol, eds. Biomedical Press, North Holland/Elsevier/Amsterdam, (1979).
24. *Tanaka, M., de Kloet, E.R. de Wied, D.* and Versteeg, D.H.G.: Arginine^8-vasopressin affects cathecholamine metabolism in specific brain nuclei. Life Sci. 20: 1799-1808, (1977).
25. *Telegdy, G.* and *Kovacs, G.L.*: Role of monoamines in mediating the action of ACTH, Vasopressin, and Oxytocin. In "Central Nervous System Effects of Hypothalamic Hormones and Other Peptides", edited by Collu et al. Raven Press, New-York, (1979).
26. *Kovacs, G.L., Bohus, B., Versteeg, D.H.G., de Kloet, R.* and *de Wied, D.*: Effect of oxytocin and vasopressin on memory consolidation: sites of action and catecholaminergic correlates after local microinjection into limbic-midbrain structures. Brain Research 175: 303-314, (1979), (Biomedical Press, North Holland/Elsevier/Amsterdam).
27. *Perry, E.K.*: Biochemestry of the cholinergic system in Alzheimer's disease. Communication at the "Workshop on Biochemistry of the Dementias", March 29-30, 1979, Southampton.
28. *Sitaram, N., Weingartner, H., Caine, E.D.* and *Gillin, J.C.*: "Choline: Selective enhancement of serial learning and encoding of low imagery words in man. Life Sci. 22: 1555-1560, (1978).
29. *Edwards, C.R.W.*: Vasopressin "Clinical Neuroendocrinology" ed. *L. Martini* and

G.M. Besser, Academic Press, London pp527-567, (1977).
30. *Orloff, J.* and *Handler, J. S.*: J. Clin. Invest. 41: 702-709, (1962).
31. *Bentley, P.J.*: The effect of valinomycin on the toad bladder: antagonism to vasopressin and aldosterone. J. Endocr. 45: 287-295, (1969).
32. *Grantham, J.J.* and *Burg, M.B.*s Effect of vasopressin and cyclic AMP on permeability of isolated collecting tubules. Am. J. Physiol. 211: 255-259, (1966).
33. *Barker, J.L.*: "Peptides: Roles in neuronal excitability. Physiol. Rev. 56: 435-452, (1976).
34. *Zusman, R.M., Keiser, H.R.* and *Handler, J.S.*: Vasopressinstimulated prostaglandin E biosynthesis in the toad urinary bladder. J. of Clin. Invest. 60: 1339-1347, (1977).
35. *Karmazyn, M., Manku, M.S.* and *Horrobin, D.F.*: Changes of vascular reactivity induced by low vasopressin concentrations: Inteactions with cortisol and lithium and possible involvement of prostaglandins. Endocrinology 102: 1230-1236, (1978).
36. *Rose, S.P.R.* and *Haywood, J.*: Experience, learning and brain metabolism.in "Biochemical Correlates of Brain Structure and Function" ed. by A.N. Davison pp249-292, Academic Press, London (1977).
37. *Moore, R.Y.*: Central neural control of circadian rhytms. In W.F. Ganong and L. Martini eds. Frontiers in Neuroendocrinology. Raven Press, New York, 185-206, (1978).
38. *Mosko, S.S.* and *Moore, R.Y.*: Neonatal suprachiasmatic nucleus lesions: Effects on the development of circadian rhythms in the rat. Brain Research: 164: 17-38, (1979).
39. *Brambilla, F.* and *Penati, G.*: Schizophrenia: Endocrinological review. In: Perspectives in endocrine psychobiology ed. by F. *Brambilla, P.K. Bridges, E. Endroczi, G. Heuser*, pp309-421, *Jahn Wiley Sons, London* (1978).
40. *Murri* (1973) quoted by ref 39.
41. *Vigneri* (1974) quoted by ref. 39.
42. *Urban, I.* and *de Wied, D.*: Neuropeptides: Effects on paradoxical sleep and theta rhythm in rats. Pharmacol. Biochem. & Behavior 8: 51-59, (1977).
43. *Krieger, D.T.* and *Luria, M.*: Plasma ACTH and cortisol responses to TRF, vasopressin of hypoglycemia in Cushing's disease and Nelson's syndrome. J. Clin. Endocrinol. Metab. 44: 361-368, (1977).
44. *Weitzman, R.E., Fisher, D.A., Minick, S., Ling, N.* and *Guillemin, R.*s β—endorphin stimulates secretion of arginine vasopressin in vivo. Endocrinology 101: 1643-1646, (1977).
45. *de Bodo, R.C.*: Antidiuretic action of morphine and its mechanism. J. Pharmacol. Exp. Ther. 82: 74-76, (1944).
46. *Simantov, R.* and *Snyder, S.H.*: Opiate receptor binding in the pituitary gland. Brain Res. 124: 178-184, (1977).
47. *Mata, M.M., Gainer, H.* and *Klee, W.A.*: Effect of dehydration on the endogenous opiate content of the rat neuro-intermediate lobe. Life Sciences 21: 1159-1162, (1977).
48. *Rossier, J., Battenberg, E., Pittman, Q., Bayon, A., Koda, L., Miller, R., Guillemin, R.* and *Bloom, F.*: Hypothalamic enkephalin neurones may regulate the neurohypophysis. Nature 277: 653-655, (1979).
49. *Timsit-Berthier, M., Mantanus, H., Lancranjan, I.* and *Audibert, A.*: Possible relationship between vasopressin and natural opiate agonists: an investigation in humans. Communication at the IIIrd European Neuroscience Meeting, Rome, Sept. 11-4, (1979).

The Role of Neuropeptides in Modulating PRL and GH Secretion

IOANA LANCRANJAN, A. AUDIBERT AND * P. MARBACH

Clinical and * Preclinical Research Departments
SANDOZ BASLE, SWITZERLAND

Vasopressin and oxytocin, nonapeptides with a disulfide bridge, were the first neuropeptides characterized and synthesized in 1952 by du Vigneaud et al (1). These two hormones, considered for many years as hormones of the posterior pituitary are in fact synthetized by the neurons of the hypothalamic supraoptic and paraventricular nuclei and carried by the axoplasmic flow down to nerve endings in contact with capillary vessels in the posterior lobe of the pituitary. From there, vasopressin and oxytocin are released into the general circulation in response to a variety of physiological stimuli.

In recent years, in addition to vasopressin and oxytocin, several other peptides have been identified in central and peripheral neurons using histochemical (histofluorescence, immunocytochemistry) and biochemical techniques (enzimes isotopic methods, radioimmunoasays, mass spectrometry, liquid chromatography).

Table I shows the peptides identified so far in the central nervous system including the hypothalamus. Their concentrations are especially high in the median eminence, a fact which suggests that neuropeptides may be involved in the control or modulation of the anteropituitary secretion. Indeed, for many years, it has become evident from experimental studies using localized hypothalamic stereotaxic lesions and stalk section with transplantation of the pituitary under the renal capsule that the secretion of the adenohypophysis requires intact anatomical connections between the pituitary and the hypothalamus. That the hypothalamic control of the anterior pituitary is not mediated through neural pathways was suggested by the absence of an important nerve supply to the anterior pituitary gland.

In 1947 Green and Harris (3) postulated that hypothalamic substances transported via the hypophysial portal system described by Popa and Fielding in 1930 (4) control pituitary function. The neurohumoral concept of Harris and Green was thereafter confirmed by the isolation, characterization and synthesis of the first hypothalamic regulatory hormones: TRH, LH-RH (5) and somatostatin, GIH (6) by the groups of Guillemin in la Jolla and Schally in New Orleans. The discovery of these neurohormones formed the basis of the concept that anteropituitary hormones are physiologically regulated by releasing and release-inhibiting hypothalamic factors or hormones which are triggered by neurotransmitters e.g. noradrenaline (NA), adrenaline (A), dopamine (DA) and serotonin (5HT). Until recently it was thought that PRL and GH secretion are also modulated by releasing and release-inhibiting hypothalamic peptides: prolactin inhibiting factor (PIF), prolactin releasing factor (PRF), growth hormone releasing factor (GH-RF) and growth hormone release-inhibiting hormone (GIH) (8, 9). In spite of the fact that some data support the presence of PRF, PIF and GH-RH material in hypothalamic extracts (10, 11), attempts to identify these substances have thus far been unsuccessful and only GIH has now been isolated and structurally identified. On the other hand, a growing body of evidence suggests that DA is a PIF and, moreover, that other neuropeptideds found

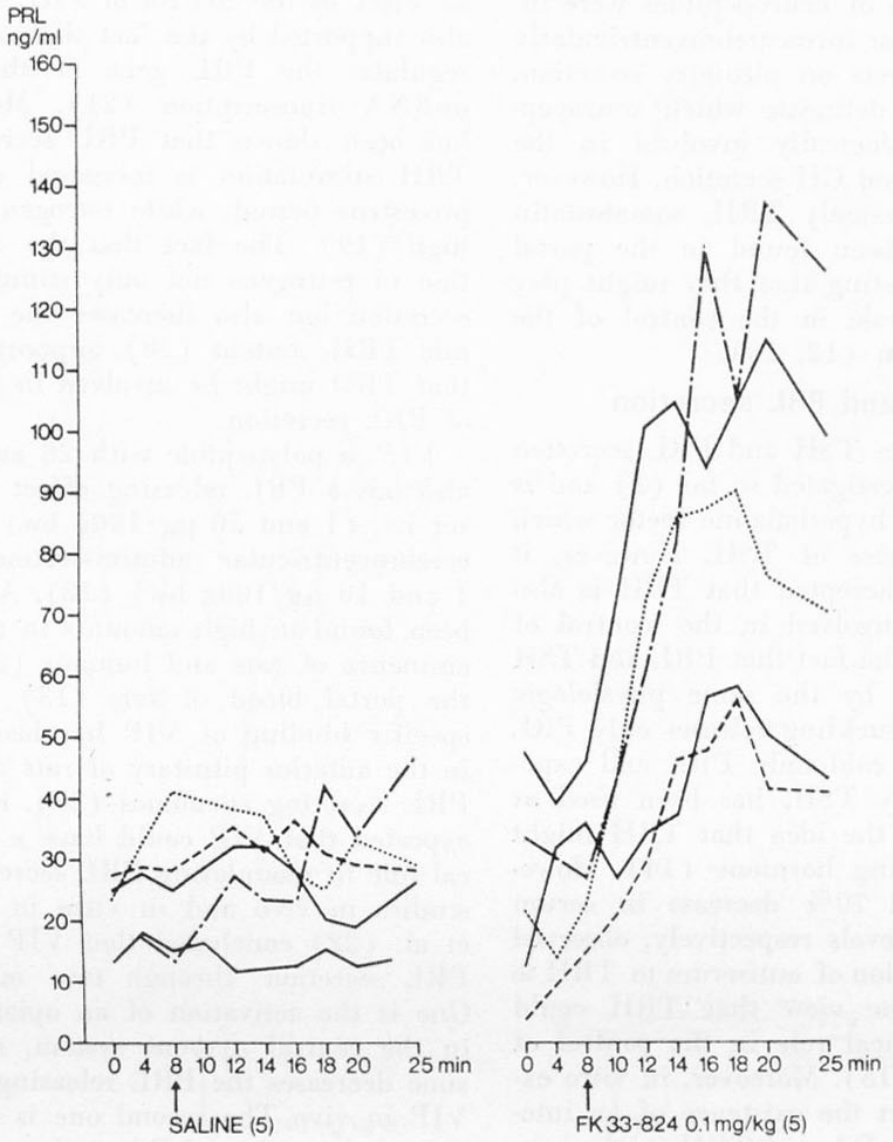


Fig. 1. — The effect of saline and FK 33-824 (0.1 mg/kg i.v.) on PRL secretion in conscious rats. Each line represents one animal.

in the hypothalamus namely opioid peptides, beta-endorphin and enkephalins, VIP, bombesin, neurotensin, substance P and TRH regulate GH and/or PRL secretion. The question then arises whether or not some of these neuropeptides might account for the releasing factors not yet isolated and identified.

At least two of the following criteria have to be fulfilled by a neuropeptide for it to be considered a hypothalamic releasing hormone:

1. it must stimulate the in vivo and in vitro secretion of one or several pituitary hormones;
2. it must be released by the hypothalamus into the portal blood;
3. it must change its turnover into the hypothalamu and its concentration into the portal blood in relation with physiological stimuli;
4. it must have specific receptors at the pituitary cell level.

Accordingly, we will review some of the available data on the effects of neuropeptides on GH and PRL secretion attempting to delineate whether or not some of them account for the hypothetical PRF and GH-RF.

Table II shows the effect on GH and PRL secretion of the neuropeptides tested so far in the rat. As in most studies phar-

macological doses of neuropeptides were injected either i.v. or intracerebroventricularly to test their effects on pituitary secretion, it is difficult to delineate which neuropeptides are physiologically involved in the control of PRL and GH secretion. However, three peptides, namely TRH, somatostatin and VIP have been found in the portal blood thus suggesting that they might play a physiological role in the control of the pituitary secretion (12, 13).

Neuropeptides and PRL secretion

TRH stimulates TSH and PRL secretion in all species investigated so far (1) and is the physiological hypothalamic factor which controls the release of TSH. However, it is not generally accepted that TRH is also physiologically involved in the control of PRL secretion. The fact that PRL and TSH are not released by the same physiologic stimuli, namely suckling releases only PRL and exposure to cold only PRL and exposure to cold only TSH, has been used as evidence against the idea that TRH might be a PRL-releasing hormone (14). However, the 50 and 70% decrease in serum PRL and TSH levels respectively, observed after administration of antiserum to TRH to rats, supports the view that TRH could play a physiological role in the control of both hormones (15). Moreover, in vitro experiments support the existence of an interrelation between DA and TRH in the control of PRL secretion. Hill-Samli and Mac Leod (16) found that addition of TRH to rat anterior hemipituitaries incubated in vitro had little effect on PRL secretion, but partially blocked the DA inhibition of PRL. Moreover, 17 β-estradiol which increases PRL secretion stimulates basal as well as TRH-induced PRL release, and simultaneously, causes an almost complete reversal of DA inhibition of PRL release (17).

In vivo, DA-TRH relationhips have been reported by Burrow et al. (18), who have shown that the PRL release response elicited by TRH in rats is blunted by a DA infusion starting 5 min. after the TRH bolus. In humans L-DOPA or bromocriptine pretreatment also blocks the PRL-releasing effect of TRH.

The possible physiological involvement of TRH in the control of PRL secretion is also supported by the fact that in rat TRH regulates the PRL gene at the level of m-RNA transcription (21). Moreover, it has been shown that PRL secretion after TRH stimulation is increased during the pro-estrus period, while estrogen levels are high (19). The fact that the administration of estrogens not only stimulates PRL secretion but also increases the hypothalamic TRH content (20), supports the idea that TRH might be involved in the control of PRL secretion.

VIP, a polypeptide with 28 amino acids, also has a PRL releasing effect in rats after i.v. (1 and 10 μg/100g bw) and intracerebroventricular administration (200ng, 1 and 10 μg/100g bw) (22). As VIP has been found in high amounts in the median eminence of rats and humans (23) and in the portal blood of rats (13) and, since specific binding of VIP has been reported in the anterior pituitary of rats and human PRL secreting adenomas (23), it might be expected that VIP could have a physiological role in modulating PRL secretion. From studies in vivo and in vitro in rats, Kato et al. (22) concluded that VIP stimulates PRL secretion through two mechanisms. One is the activation of an opiate receptor in the central nervous system, since naloxone decreases the PRL releasing effects of VIP in vivo The second one is the inhibition of the action of DA at the pituitary level since the addition of VIP (10^{-7}M) to the incubation medium significantly blocks the inhibition of PRL release induced by DA (10^{-7}M). Consequently, it seems that TRH and VIP act at the pituitary level in the same way, partially blocking the DA induced inhibition of PRL release.

Bombesin, a tetradecapeptide originally isolated from the skin of various anurans (24) and recently found to occur in mammalian brain (25, 26), has been shown to stimulate PRL secretion after either i.v. (o. 1 μg) or intracisternal (1 - 3 μg) administration (27). On a molar basis, bombesin and the related peptide alytesin are the most active releasers of PRL and GH. The PRL releasing effect of bombesin in vivo is not blocked by naloxone thus indicating that opiate receptors are not invol-

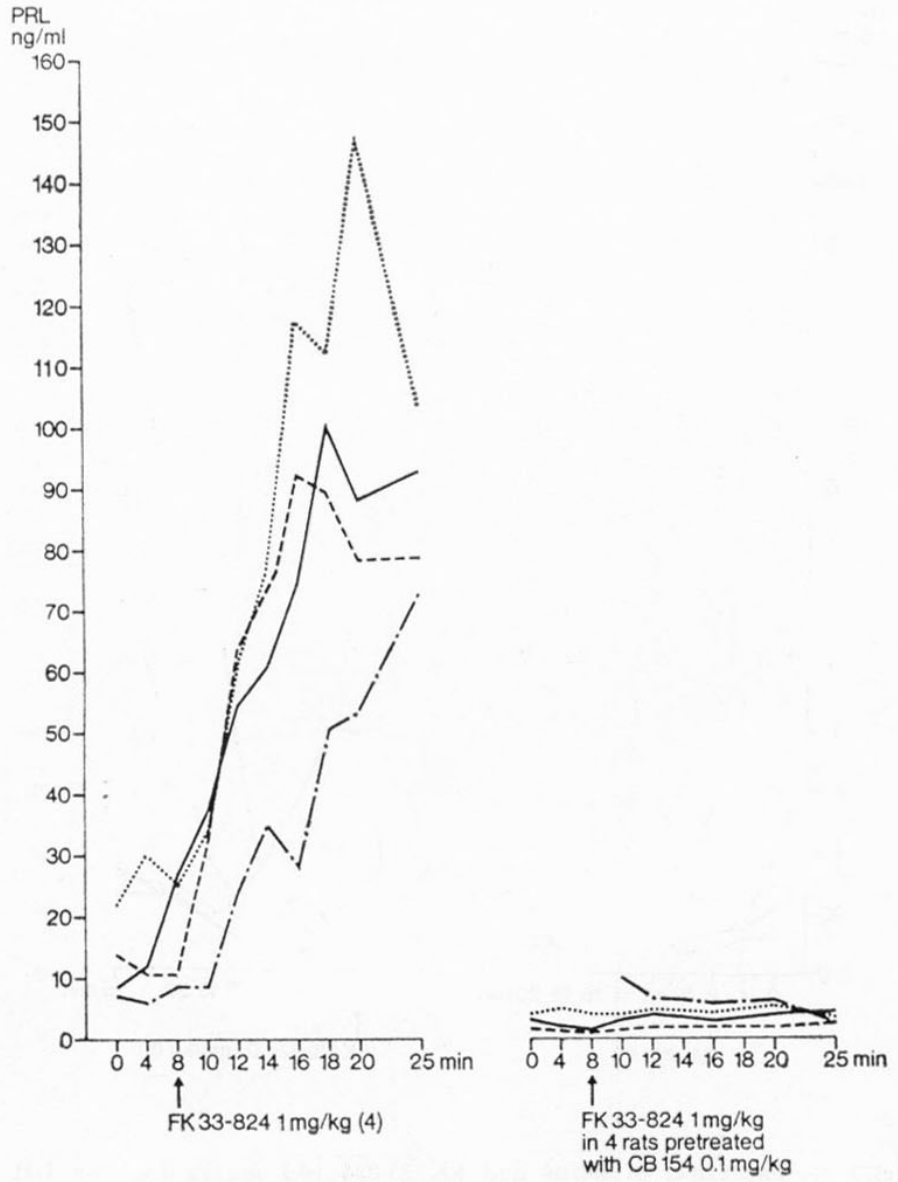


Fig. 2. — The effect on PRL secretion of FK 33-824 (1 mg/kg i.v) alone or after pretreatment with bromocriptine (0.1 mg/kg i.v.) in conscious rats. Each line represents one animal.

ved. The fact that in pituitary cultured cells bombesin has no effect on PRL secretion (27) suggests that bambesin probably acts via hypothalamic neurotransmitters to influence the release of PRL.

Substance P and Neurotensin

Rivier et al. (28) have reported that neurotensin, a tridecapeptide, and substance P, an undecapeptide isolated from bovine hypothalamus, injected i.v. (20 μg) stimulate the release of PRL in normal or estrogen-progesterone pretreated male rats. The PRL-releasing effect could not be blocked by naloxone but was inhibited by diphenhydramine, a histamine H_1 receptor antagonist. Further evidence that substance P (5 and 50 μg/100g bw) stimulates PRL release was obtained by Kato et al. (29). More recently, Chihara et al. (30) reported that substance P administered intraventricularly has no effect on PRL release. These results were partially confirmed by Vijayan and McCann (31) with a low dose (0.5 μg) of substance P. On the contrary, a higher dose (2 μg) injected intraventricularly caused a significant elevation

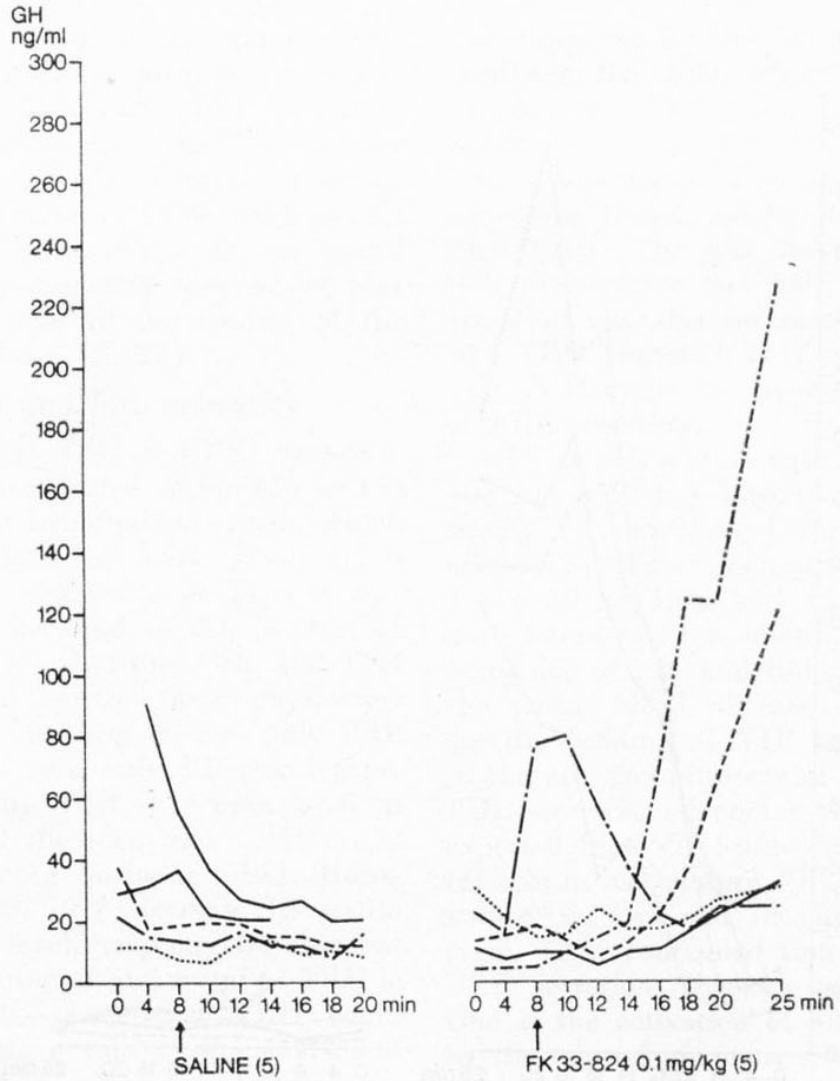


Fig. 3. — The effect of saline and FK 33-824 (0.1 mg/kg i.v.) on GH secretion in conscious rats. Each line represents one animal.

of PRL release. However, the physiological role of substance P, if any, in modulating PRL secretion is difficult to establish as long as there is no specific antagonist available. Neurotensin (0. 5 and 2 μg) administered intraventricularly decreases PRL secretion, whereas i.v. it enhances PRL release (32). Similiar discordant effects were noticed by Vijayan and MacCann (31) after *gastrin* administration.

Cholecystokinin (CCK) has been also reported to stimulate PRL secretion in rats (32) while *somatostatin* which is the GIH does not seem to be involved in the physiological control of PRL secretion. It has been reported that neither the basal levels of PRL nor the suckling induced rise of PRL in rats is influenced by somatostatin administration (34, 35). Moreover, in humans somatostatin administration reduces PRL levels only in acromegalic patients in whom it reduces also GH plasma levels (36).

Vasopressin and Oxytocin

Conflicting results have been reported as to whether or not neurohypophyseal hormones are able to stimulate PRL release. It has been suggested that oxytocin might initiate the release of PRL in rats, but Valverde et al. (37) showed in the rat that, whereas vasopressin had a marked stimulatory effect on PRL secretion, oxytocin had

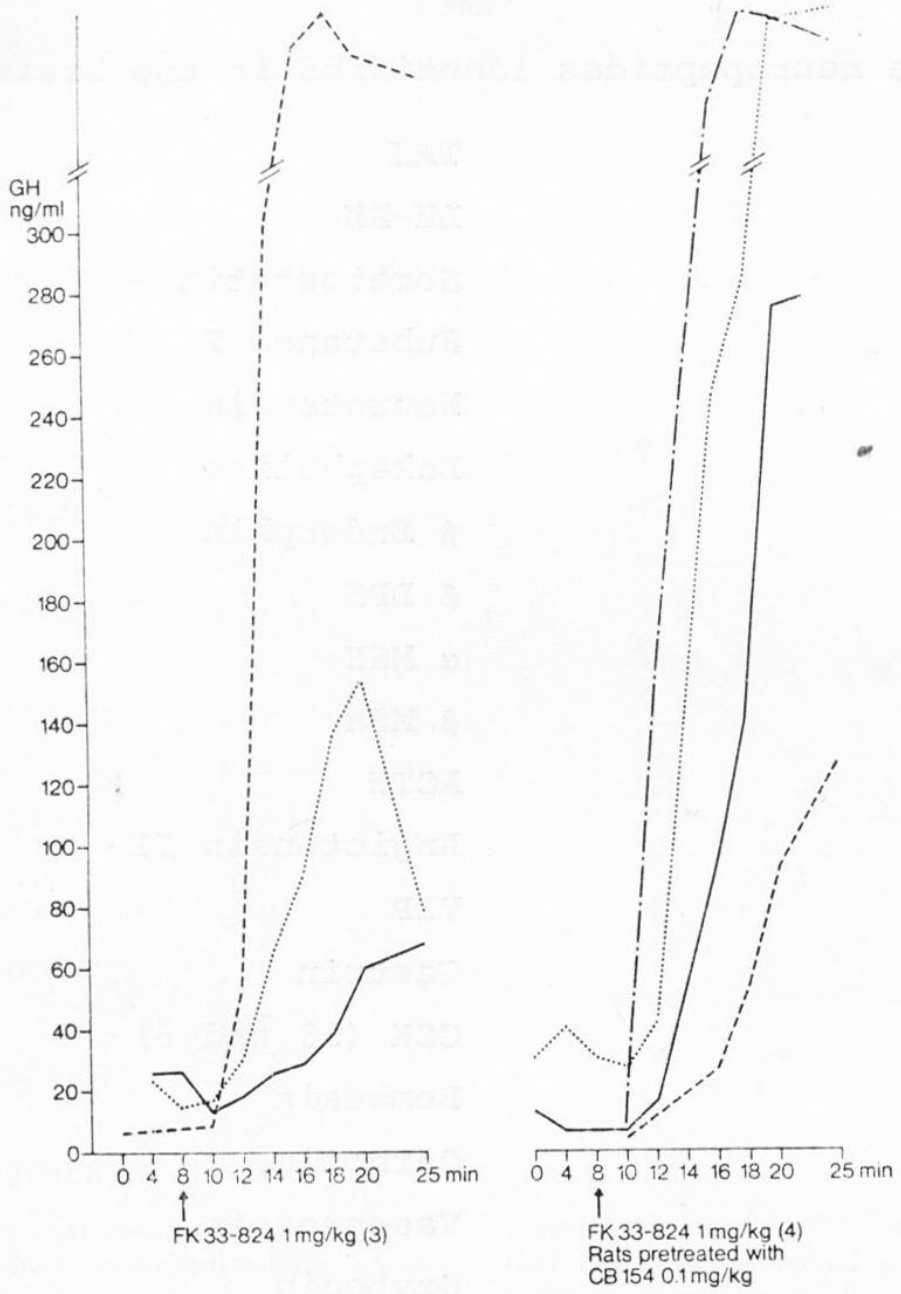


Fig. 4. — The effect on GH secretion of FK 33-824 (1 mg/kg i.v.) alone or after pretreatment with bromocriptine (0.1 mg/kg i.v.) in conscious rats. Each line represents one animal.

no effect. Further evidence that vasopressin releases PRL in the rat has been given by Vaughan et al. (38) who noticed a significant rise in plasma PRL after the administration of 1 ug of either arginine of lysine vasopressin. On the other hand, this effect has not been confirmed in man (39).

Opiate receptor agonists and PRL secretion

In 1976 Lien eet al. (40) reported that the pentapeptides methionine ond leucine-enkephalin stimulated PRL release in vivo at a dose of 5mg/kg/rat and in vitro in monolayer cultures of rat pituitary cells (Dose range 5 - 50 ng/ml). In 1977 Rivier et al. (41) reported that morphine sulfate and β-endorphin stimulated PRL release in steroid-primed and untreated male rats when injected intravenously or intracisternally. Morcover, it has been reported that on a molar basis β-endorphin is 20 times more potent than morphine in vivo

Table I

The neuropeptides identified in the brain

TRH
LH-RH
Somatostatin
Substance P
Neurotensin
Enkephalins
β Endorphin
β LPH
α MSH
β MSH
ACTH
Angiotensin II
VIP
Gastrin
CCK (33 and 8)
Bombesin
Carnosine
Vasopressin
Oxytocin
GH
PRL

(41). Since the effect of enkephalins and β-endorphin on PRL secretion has been reported, intensive investigations have been carried out to confirm this effect in animals and humans and to define the mechanism of action of opioid peptides. Of the opiate receptor agonists studied, FK 33-824, a stable, orally acting analogue of methionine-enkepthalin (Sandoz), exhibited potent PRL and GH releasing effects in rats and humans (42,43) in addition to its longlasting and potent analgesic action in animals.

The PRL-releasing effect of FK 33-824 could be blocked by pretreatment with naloxone and the DA agonist bromocriptine. Fig. 1 and 2 show the effect of placebo, FK 33-824 0.1 and 1mg/kg (20 - 200 μg/rat) administered i.v. in conscious rats before and after 0. 1mg/kg bromocriptine pretreatment. Complete blockade of the PRL-releasing effect of FK 33-824 was obtained in rats with bromocriptine, and si-

Table II

The effect of neuropeptides on GH and PRL secretion

Neuropeptide	Way of administration	Effect on the secretion of GH	PRL
TRH	i.v	-	↑
VIP	i.v	↑	↑
BOMBESIN	i.v	↑	↑
	i.c	↑	↑
SUBSTANCE P	i.v	↑ or -	↑
	i.c	↑ or ↓	- or ↑
NEUROTENSIN	i.v	↑	↑
	i.c	↓	↓
GASTRIN	i.v	↑	↑
	i.c	↓	↓
CCK	i.v	↑	↑
β ENDORPHIN	i.v	↑	↑
	i.c	↑	↑
ENKEPHALINS	i.v	↑	↑
	i.c	↑	↑
SOMATOSTATIN	i.v	↓	-

milar results were obtained in humans (del Pozo, personal communication). Consequently, in all species investigated (rat, dog, man) the opioid peptides enkephalins and β-endorphin have been shown to have a PRL releasing effect. Several data were also accumulated to explain the PRL-releasing effect of opioid peptides. A synergism in vitro between the PRL-releasing actions of TRH and leucineenkephalin has been reported by Mittler et al. (44) and a decreased DA turnover in the median eminence (ME) after methionine-enkephalin administration was reported by Labrie et al. (45). Moreover, Fuxe et al. (46) and Deyo et al. (47) reported that β-endorphin decreases also the DA turnover in the ME. Similar effects of morphine and D-Ala^2, D-leu^5-enkephalin (47) strongly suggest that the effect of opioid peptides on PRL secretion is mainly mediated via decreased DA turnover in the ME. Furthermore, Enjalbert et al. (48) reported that morphine, methionine-enkephalin and beta-endorphin also suppress the inhibitory effect of DA on PRL release in vitro. Accordingly, the opiate-DA interactions at both the hypothalamic and pituitary levels on the one hand and the synergistic effect of TRH and opioid peptides on the other could account for the PRL-releasing effect of opioids in all species investigated.

The fact that naloxone lowers PRL plasma levels in primates (49) and man (50) and blocks the suckling and stress induced PRL release (45) supports the idea that

opioid peptides are involved in the physiological release of PRL. In conclusion, TRH and natural opioid peptides stimulate PRL secretion in laboratory animals and man and evidence is accumulating suggesting that both neuropeptides are physiologically involved in the control of PRL release. The effect of substance P, neurotensin, VIP and bombesin on PRL release has only been reported in animals and some evidence suggest that VIP and bombesin (active in very low dose) might also be involved in the physiological contral of PRL release. The neuropeptides vasopressin and oxytocin have an action on PRL secretion in animals only in pharmacological doses and are without any effect in humans.

Neuropeptides and GH secretion

Krulich and collaborators were the first to notice the GH-inhibitory effects of the ovine hypothalamic extract (51) and thereafter Brazeau et al. (7) isolated, identified and characterized somatostatin, the GIH. This neuropeptide with 14 amino acids is equally active biologically in a linear and cyclic form. In rats somatostatin inhibits the rise of GH plasma levels observed after pentobarbital anesthesia, morphine, gentling, or the injection of NA into the ventromedial nucleus of the hypothalamus and the GH release induced by electrical stimulation of the basal medial hypothalamus (52). Moreover, somatostatin inhibits GH release from rat tumors and from human acromegalic pituitary adenomas (52).

The administration of somatostatin antiserum to rats resulted in increased basal levels of GH and TSH, prevention of stress-induced, in rat, GH decrease and enhanced TSH response to cold. (52), indicating that somatostatin plays a physiological role in the regulation of GH and TSH secretion in rats. The fact that higher somatostatin levels were measured in the hypophysial portal blood than in the systemic blood and that somatostatin levels in the hypophyseal portal blood are affected in the rat by anesthetics such as urethane, pentobarbital and althesin (53) that are known to influence GH plasma levels, gives new support to the hypothesis that somatostatin is the final inhibitory pathway of GH secretion. High concentrations of somatostatin were found in the arcuate nucleus and ME, in the ventro medial and suprachiasmatic nuclei, preoptic area of the hypothalamus and amygdala (54) and Patel et al. (55) found that 30% of the nerve terminals on the portal capillaries contain somatostatin. A large body of evidence has been accumulated showing that somatostatin has, in humans and animals, a strong inhibitory effect on GH released by all stimuli (36, 56). Furthermore, the data in animals and humans show that somatostatin inhibits not only the secretion of GH and TSH but also that of insulin, glucagon, gastrin, gastric acid, pepsin, secretin, pancreozymin and cholecystokinin secretion; it also inhibits gastric motility, the absorbtion of glucose, amino acids and triglycerides, food and water intake, ACh release from the myenteric plexus and firing rate of some neurons. It appears that somatostatin is not only the GIH but a "panhibin" as suggeste by Mc Cann (57).

Besides somatostatin, other neuropeptides have been reported to influence GH release. Enkephalins and β-endorphin, VIP, bombesin, gastrin and CCK, neurotensin and substance P administered i.v. in rats stimulate the release of GH. Vasopressin in pharmacological doses also releases GH in humans, monkeys and rats but has no physiological role in the modulation of GH secretion whereas TRH even in pharmacological amounts has no effect on GH release in rats and normal man (58). The GH releasing effect of substance P (5, 10, 20 μg i.v.) was first reported by Kato et al. (29). These workers also demonstrated that the simultaneous administration of either l-dopa or nicotin blunted GH release induced by substance P in male, urethane anasthetized rats with intact hypothalamus, thus suggesting that a DA and/or a ACh pathway is involved in this stimulation.

Neurotensin and substance P effects on GH release were also reported by Rivier et al. (28) in urethane anesthetized normal and estrogen-progesterone pretreated male rats. Neurotensin (10 μg) and substance P (20 μg) i.v. significantly increased GH secretion, an effect which was blocked by diphenhydramine Furthermore, Vijayan and

McCann (31) reported that 0.5 μg and 5 μg/rat substance P and neurotensin injected intraventricularly in conscious, ovariectomized rats elevated GH plasma levels, whereas their systemic administration was without any effect. In conscious, normal rats we were also unable to stimulate GH secretion with 5 μg substance P whereas a significant PRL release was recorded in the same animals. Finally, Maeda and Frohman (32) have shown that the intraventricular administration of 2 and 5 μg neurotensin inhibits and 30 μg/kg administered i.v. stimulates GH release.

In vitro incubation of hypothalamic fragments from male rats in the presence of neurotensin 10^{-5}M resulted in an increase in somatostatin release which could explain the GH release inhibiting effect of neurotensin administrated intraventricularly. These contradictory data suggest that indeed neurotensin administrated intraventricularly increases somatostatin release and consequently depresses GH secretion, whereas neurotensin administered i.v. has GH releasing effects, possibly via histamine pathways.

The central inhibiting effects of substance P have been reported by Chihara et tl., (30) who showed that antisubstance P in urethaneanesthetized male rats did not influence GH secretion, whereas the injection of substance P 10^{-9}M into the lateral ventricle suppressed serum GH levels in normal serum and antisubstance P-treated rats. This suppressant effect of substance P was not obtained in animals pretreated with serum anti-somatostatin. These results led Chihara et al. (30) to suggest that substance P may stimulate hypothalamic somatostatin release, thereby decreasing GH secretion. On the other hand, the same group reported that substance P potentiated the GH and PRL releasing effects of β-endorphin and morphine, data which support the complex modulatory action of neuropeptides.

However, the GH releasing effects of substance P and neurotensin administered i.v. cannot be attributed to a direct effect of these peptides at the pituitary level because a lack of effect in monolayer culture of isolated anterior pituitary cells has been reported for both (28).

The stimulating effects of *bombesin* on GH secretion have been reported by Rivier et al. (27). The i.v. administration of 0.1 μg and the intracisternal injection of 1 μg bombesin stimulates GH release, and naloxone and somatostatin reverse this GH-releasing activity. The lack of an effect in vitro on the pituitary, however, suggests that bombesin cannot account for a hypothalamic releasing hormone. It represents however the most activite peptide so far reported, capable of stimulating PRL and GH secretion via possibly central opioid receptors. One may also wonder whether or not bombesin acts at the hypothalamic levels like neurotensin and possibly substance P, by reducing the amount of somatostatin released in the portal blood and consequently increasing GH secretion.

GH releasing effects of opioid peptides in vivo have been also reported. Labrie et al. (17) were the first to show that like morphine 1000 μg methionine-enkephalin and 2 μg β-endorphin stimulated GH release in conscious rats, the PRL releasing effect of natural opioids being more sensitive.

When (D-Ala^2) methhionine-enkephalin was used the stimulation of GH release was obtained with 150 μg i.v. showing that the synthetic enkephalins are more potent that methionine-enkephalin itself. Indeed, using the long-acting enkephalin analog, FK 33-824, GH-releasing effects were obtained with doses of 0.1 and 1mg/kg (20-200 μg/rat) injected i.v. in conscious rats (Fig. 3 and 4). The effect of β-endorphin and enkephalins on GH secretion could be blocked by pretreatment with naloxone and by the blocker of cholinergic muscarinic receptors atropine (17, 59). These data suggest that central opiate receptors and cholinergic pathways are involved in the GH-releasing effects of opioid peptides. On the other hand, we found that pretreatment with bromocriptine potentiated the effect of FK 33-824 on GH secretion (Fig. 4), indicating that, unlike PRL, the GH-releasing effect of opioids cannot be explained by decreased DA turnover in the ME. Moreover, the fact that the pretreatment with antisomatostatin serum increa-

sed GH released by morphine in anesthetized rats (17) strongly suggests that opioids act not by inhibition of somatostatin secretion but by an increased release of hypothalamic GH-releasing material.

In humans, only somatostatin and opioid peptides have been investigated and their GH-releasing effects reported (42, 43).

In conclusion, to date no PRF and PIF hormones have been isolated and characterized in hypothalamic extracts. Instead compelling evidence has been accumulated supporting the hypothesis that DA is the PIF or a PIF. Indeed, the fact that DA lowers the PRL secretion in vivo and in vitro, that tuberoinfundibular nerve terminals end on portal capillaries, that variation of DA concentrations in the portal blood have been found to be correlated with physiological events and druginduced changes of PRL secretion and, also that DA receptors have been found at the lactotroph cell-levels, support the physiological role of DA as PIF (see rev. 60).

On the other hand, we suggest that some neuropeptides which have been found in the hypothalamus and been shown to have PRL-releasing effects may account for the PRF activity of hipothalamic extracts. Evidence is accumulating that TRH, VIP and opioid peptides have receptors at the pituitary levels and are released in the portal blood, suggesting that they are physiologically involved in the stimulation of PRL release. In conclusion, the PRF activity can be completed by different peptides released from the hypothalamus into the portal blood, neuropeptides which stimulate PRL secretion either by acting on their own receptors (e.g. VIP, TRH) or by changing the sensitivity or the number of DA receptors (e.g. the opioid peptides). The other neuropeptides which have no direct effect at the pituitary levels (neurotensin, substance P, gastrin, bombesin, CCK?), might act by changing DA turnover within the median eminence.

The GIF has been isolated, characterized and its physiological role acknowledged. On the other hand, the GH-RH has not been yet isolated and, furthermore, all neuropeptides with GH-releasing activity in rats have no direct effect at the pituitary level and, consequently, cannot account for the GH-RH.

From the neuropeptides with GH releasing effects investigated so far, neurotensin acts by reducing somatostatin release. Substance P and bombesin might have a similar mechanism of action whereas opioid peptides probably act by stimulating the release of a GH-RH. The screening studies reported so far, using mainly pharmacological doses of neuropeptides, have opened a new field of research. However, the studies to come should, using other approaches, better define the physiological role of neuropeptides. Changes in the turnover of hypothalamic neuropeptides and neurotransmitters, modulation of hypothalamic and pituitary receptor sensitivity as well as changes in the output flow of DA, somatostatin, VIP, TRH and possibly other neurotransmitters in relation with physiological stimuli (i.e., sleep, stress, suckling) would probably better define the role of neuropeptides in the control of pituitary secretion.

SUMMARY

Increasing evidence supports the role of neuropeptides in modulating PRL and GH secretion. In the recent years, it has been shown that endorphins and enkephalins, substance P and neurotensin bombesin, VIP, oxytocin and vasopressin could modulate GH and/or PRL secretion in rats.

As the effects of some neuropeptides are opposite after their intraventricular and i.v. application, a review of these aspects is given and their possible physiologic and/or pharmacologic effects is discussed.

Finally results obteined in humans are presented confirming the stimulatory effect of enkephalins on the PRL and GH secretion and showing the lack of effect of vasopressin and oxytocin on PRL and GH secretion in man.

RESUMEN

Pruebas siempre más numerosas, subrayan el papel que desempeñan los neuropéptidos en la regulación de la secreción de PRL y GH.

En el curso de los últimos años, ha sido demostrado que la secreción de GH y/o de PRL en la rata es modificada por las endorfinas y las encefalinas, la substancia P, la neurotensina, la bombesina, la ocitocina y la vasopresina.

Teniendo en cuenta que ciertos neuropéptidos tienen efectos diferentes después de su aplicación intraventricular e intravenosa estos aspectos son considerados y una delimitación de sus eventuales efectos fisiológicos y/o farmacológicos ha sido realizada.

En el hombre los estudios han conformado la acción estimulante de las encefalinas sobre la secreción de PRL y de GH, mientras que la vasopresina, la ocitocina y la calcitomina se han mostrado sin efectos sobre la secreción de PRL y de GH en el hombre.

RÉSUMÉ

Des preuves toujours plus nombreuses, soulignent le rôle que jouent les neuropeptides dans la régulation de la sécrétion de PRL et de GH. Au cours des dernières années, il a été démontré que la sécrètion de GH et/ou de PRL chez le rat est modifiée par les endorphines et les enképhalines, la substance P, la neurotensine, la bombésine, l'ocytocine et la vasopressine.

Etant donné que certains neuropeptides ont des effets différents après leur application intraventriculaire et intraveineuse, ces aspects sont revus et une délimitation de leurs éventuels effets physiologiques et/ou pharmacologique est faite.

Enfin, chez l'homme, les études ont confirmés l'action stimulante des enképhalines sur la sécrètion de PRL et de GH. Tandis que la vasopressine, l'ocytocine et la calcitonine sont montrés sans effets sur la sécrètion de PRL et de GH chez l'homme.

ZUSAMMENFASSUNG

Immer mehr Anhaltspunkte deuten darauf hin, dass Neuropeptide eine Modulation der PRL— und GH— Sekretion bewirken können. In den letzten Jahren wurde nachgewiesen, dass Endorphine und Enkephaline, die Substanz P und Neurotensin, Bombesin, VIP, Oxytocin und Vasopressin die GH— und/oder PRL-Sekretion an Ratten modulieren können.

Da einige Neuropeptide nach intraventrikulärer und i.v. Applikation eine gegensätzliche Wirkung zeitigen, werden wir zunächst diese Aspekte kurz beleuchten und versuchen, deren mögliche physiologische und/oder pharmakologische Auswirkungen zu skizzieren.

Abschliessend werden am Menschen gewonnene Daten vorgelegt, die den stimulierenden Effekt von Enkephalinen auf die PRL— und GH—Sekretion bestätigen und das Fehlen einer Wirkung von Vasopressin, Oxytocin und Calcitonin auf die PRL— und GH—Sekretion am Menschen beweisen.

REFERENCES

1. *Guillemin R.*: The Hormones of the Hipothalamus. Integrative Hipothalamic Activity — Progress in Brain Research, Ed. D.F. Schwaab, J.P. Schade, Elsevier Scientific Publishing Company, Amst./Oxf./NY. 41: 117-132, (1974).
2. *Harris G.M.*: Neural Control of the Pituitary Gl nd. Arnold, London, (1955).
3. *Green J.D. and G.M. Harris*: J. Endocrinol. 5, 136 (1947).
4. *Popa G.T.* and *V. Fielding*: *J. Anat.* 65, 88 (1930).

5. *Guillemin R., Burgus R.* and *Vale W.*: The hypothalamic hypophysiotropic thyrotropin releasing factor (TRF), Vitam. and Horm. 29, 1-39, (1971).
6. *Schally A.V., A. Arimura, Y. Baba, R.M. Debeljuk, W.F. White*: Biochem. Biophys. Res—Commun 43, 393 (1971).
7. *Brazeau P., W. Vale, R. Burgus, N. Ling, M. Butcher, J. Rivier* and *R. Guillemin.* Hypothalamic polypeptide that inhibits the secretion of immunoreactive pituitary growth hormone, Science 179, 77-89, (1973).
8. *Meites, J.*: Control of prolactin secretion in animals. In: Human Prolactin, edited by J.L. Pastells and C. Robyn, 105-118 (Excerpta Medica, Amsterdam, 1973).
9. *Schally V.A., Arimura A., Abba J. Kastin*: Hypothalamic Regulatory Hormones, Science 179, 341-350, (1973).
10. *Enjalbert, A., Moos, F., Carbonelli. L., Priam, M.* and *Kordon, C.*: Prolactin inhibiting activity of deopaminefree subcellular fractions from rat mediobasal hypothalamus. Neuroendocrinology 24: 147-161, (1977).
11. *McCann, S.M., Krulich, L., Ojeda, S.R.* and *Vijayan, E.*: Control of prolactin release by putative synaptic transmitters. In: Progress in prolactin physiology and pathology. C. Robyn and M. Harter editors. 137-148, (Elsevier, North-Holland Biochemical Press (1978).
12. *Gillioz P., Giraud, P., Conte-Devolx B., Jaquet P., Codaccioni J.L.* and *Oliver C.*: Immunoreactive Somatostatin in Rat Hypophysial Portal Blood, Endocrinology 104, 1407-1410, (1979).
13. *Said S.I.*, and *Porter J.C.*: Vasoactive intestinal polypeptide: Release into hypophyseal portal blood, Life Sciences 24, 227-230 (1978).
14. *Meites, J.*: Biogenic amines in the control of prolactin secretion: in James Endocrinology Proc. 5th Int. Congress of Endocrinology, Hamburg 1976, 144-147, (Excerpta Medica, Amsterdam, 1977).
15. *Koch, Y., Goldhaber, G., Fireman, L., Zor, E., Shani, J.* and *Tal, E.*: Supression of Prolactin and thyrotropin secretion in the rat by anti-serum to thyrotropin-releasing hormone. Endocrinology 100: 1476-1478, (1977).
16. *Hill-Samli, M.* and *McLeod, R.M.*: Interaction of thyrotropin-releasing hormone and dopamine on the release of prolactin from the rat anterior pituitary in vitro. Endocrinology 95: 1189-1192, (1974).
17. *Labrie, F., Beaulieu, M., Caron, M.G.* and *Raymond, V.*: The adenohypophyseal dopamine receptor: specificity and modulation of its activity by estradiol. In: Progress in Prolactin Physiology and Pathology, edited by C. Robyn and M. Harter, 121-136, (Elsevier, North-Holland Biochemical Press, Amsterdam 1978).
18. *Burrow, G.N., May, P.B.* and *Spaulding S.W.*: TRH and dopamine interactions affecting pituitary hormones secretion. J. Clin. Endocrinol. Metab. 45, 65-72, (1977).
19. *Ojeda, S.R., Castro-Vázquez, A.* and *Jameson, H.E.*: Prolactin release in response to blockade of dopaminergic receptors and to TRH injection in developing and adult rats: Role of estrogen in determining sex differences. Endocrinology 100, 427-439, (1977).
20. *Boyd III, A.E., Peck, E.J., Jr., Tytell, M.*, and *Johnson, S.I.*: Differential effects of estrogen on hypothalamic thyrotropin-releasing hormone (TRH) and somatostatin. The endocrine society, 60th annual meeting, Miami, June 1978, Abstract Nº 22.
21. *Perrone, M.H.* and *Hinkle, P.M.*: Relationship between TRH receptor number and response to TRH. In: The endocrine society: 60th annual meeting, Miami, June (1978).
22. *Kato, Y., Iwasaki, Y., Iwasaki, J., Abe, H., Yanaihara, N.* and *Imura, H.*: Prolactin release by vasoactive intestinal polypeptide in rats. Endocrinology 103, 554-558 (1978).
23. *Said, S. VIP*: possible functions as a neural peptide lecture at the symposium Regulation and Function of Neural Peptides, Gardone Riviera, Brescia Aug. 29 - Sept. 1, (1979).
24. *Brown, M., Allen, R., Villarreal, J., Rivier, J.* and *Vale, W.*: Bombesin-like activity: Radioimmunologic assessment in biological tissues. Life Sciences, 23, 2721-2728 (1978).
25. *Villarreal, J.A.*, and *Marvin R. Brown*: Bombesin-like peptide in hypothalamus: chemical and immunological characterization, Life Sciences 23, 2729-2734, (1978).
26. *Walsh, J.H., Wong, H.C.*, and *Graham J. Dockray*: Bombesin-like peptides in mammals, Federation Proc. 38, 2315-2319, (1979).
27. *Rivier, C., Rivier, J.* and *Vale, W.*: The effect of bombesin and related peptides on prolactin and growth hormone secretion in the rat. Endocrinology 102, 519-522, (1978).
28. *Rivier, C., Brown, M.* and *Vale, W.*: Effects of neurotensin, substance P and morphine sulfate on the secretion of prolactin and growth hormone in the rat. Endocrinology 100 751-754, (1977).
29. *Kato, Y. Chihara, K., Ohgo, S., Iwasaki Y., Abe, H.* and *Imura, H.*: Growth hormone and prolactin release by substance P in rats. Life Sciences 19, 441-446, (1976).
30. *Chihara, K., Arimura, A., Coy, D.H.* and *Schally, A.V.*: Studies on the interaction of endorphins, substance P, and endogenous somatostatin in growth hormone and prolactin release in rats. Endocrinology 102, 281-290, (1978).
31. *Vijayan, E.* and *McCann, S.M.*: Effects of intraventricular injection of substance P (SP), neurotensin (NT), and gastrin (G) on pituitary hormone release in conscious ovariectomized (OVX) rats. Neuroendocrinol. 25, 1500-2221, (1978).
32. *Maeda K.*, and *L.A. Frohmann*: Discordant effects of systemic VS central administration of neurotensin on the secretion of GH 60th

Annual Meeting of the endocrine society, Miami, 1978, Abs. 389.

33. *McCann S.M.*: The hypothalamo-pituitary unit. its physiology and chemistry lecture at the First International Symposium on Neuroactive drugs in Endocrinology Milan, Sept. 10-12, (1979).
34. *Vale, W., Rivier, C., Brazeau, P.* and *Guillemen, R.*: Effects of somatostatin on the secretion of thyrotropin and prolactin. Endocrinology 95, 968-977, (1974).
35. *Takahara, J., Arimura, A.*, and *Schally, A.V.*s Suppression of prolactin release by a purified porcine PIF preparation and catecholamines infused into a rat hypophysial portal vessel. Endocrinology 95, 462-465, (1974).
36. *Siler, T.M., Vandenberg, G.* and *Yen, S.S.C.*: Inhibition of growth hormone release in humans by somatostatin. J. Clin. Endocr. Metab. 37, 632-634, (1973).
37. *Valverde, R.C., Chieffo, V., Reichlin, S.*: Prolactin-releasing factor in porcine and rat hypothalamic tissue. Endocrinology 91, 982-993, (1972).
38. *Vaughan, M.K., Blask, DE., Johnson L.Y.* and *Reiter, R.J.*: Prolactin-releasing activity of arginine vasotocin in vitro. Hormone Res 6, 342-350, (1975).
39. *Dubey, L., Lancranjan, I., Rouselle, J.* and *Felber, J.P.*: Lack of effect of L-Vasopressin on PRL secretion in man. XIIth Acta Endocr. Congress Munich June 1979. Abstract Nº 158.
40. *Lien, E.L., Fenichel, R.L., Garsky, V., Sarantakis, D.* and *Grant, N.H.*: Enkephalin-stimulated prolactin release. Life Science 19, 837-840, (1976).
41. *Rivier, C., Vale, W., Ling, N., Brown, M., Guillemin, R.*: Stimulation in vivo of the secretion of prolactin and growth hormone by β-endorphin. Endocrinology 100, 238-241, (1977).
42. *Graffenried, B. von, Pozo, E. del, Roubicek, J., Krebs, E., Pöldinger, W., Burmeister, P., Kerp, L.*: Effects of the synthetic enkephalin analogue FK 33-824 in man. Nature 272, 729-730, (1978).
43. *Stubbs, W.A., Jones, A., Edwards, C.R.W., Delitala, G., Jeffcoate, W.J.*, and *S.J. Ratter*: Hormonal and metabolic responses to an enkephalin analogue in normal man, the Lancet, II, 1225-1227, (1978).
44. *Mittler, J.C., May, P.B., Manougian, A.* and *Ertel, N.H.*: Leucine-enkephalin modulates relative TSH or prolactin responsiveness to TRH. The endocrine society: 60th annual meeting, Miami, June 1978. Abstract Nº 551.
45. *Labrie, F., Cusan, L., Dupont, A., Ferland L.* and *Lemay, A.*: Opioids and anterior pituitary hormone secretion in characteristics and function of opioids, Van Ree and Terenius, 333-344 (Elsevier, North-Holland Biochemical Press, 1978).
46. *Fuxe, K., Andersson, K., Hökfelt, T., V. Mutt, Ferland, L., Agnati, L.F., Ganten, D., Said, S., Eneroth, P.*, and *Gustafsson, J.A.*: Localization and possible function of peptidergic neurons and their interactions with central catecholamine neurons, and the central actions of gut hormones, Federation Proc. 38, 2333-2340, (1979).
47. *Deyo, S.N., Swift, R.M.*, and *Miller R.J.*. Morphine and endorphins modulate dopamine turnover in rat median eminence Proc. Natl. Acad. Sci., 76, 3006-3009, (1979).
48. *Enjalbert, A., Ruberg, M., Arancibia, S., Priam, M., Kordon, C.*: Endogenous opiates block dopamine inhibition of prolactin secretion in vitro, Nature 280, 595-597 (1979).
49. *Gold, M.S., Redmond, E.E. Jr.* and *Donabedian, R.K.*: Prolactin secretion, a measurable central effect of opiate-receptor antagonists. Lancet I, 323-324, (1978).
50. *Rubin, P., Swezey, S.*, and *Blaschke, T.*: Naloxone lowers plasma-prolactin in man, the Lancet I, 1293 (1979).
51. *Krulich, L., A.P., S.D. Harival, S.M. McCann*: Stimulatory and inhibitory effects of purified hypothalamic extracts on growth hormone release from rat pituitary in vitro, Endocrinology, 83, 783-790, (1968).
52. *Martin, J.B.*: Brain Regulation of Growth Hormone Secretion, Frontiers in Neuroendocrinology, Vol. 4, Ed: Martini and Ganong, Raven Press, New York, (1976).
53. *Chihara, K. Arimura, A.* and *Schally, A.V.*: Immunoreactive Somatostatin in Rat Hypophyseal Portal Blood. Effects of Anesthetics, Endocrinology 104, 1434-1441, (1979).
54. *Brownstein, M., Arimura, A., Sato. H. Schally A.V.* and *Kizer, J.S.*: The regional distribution of somatostatin in the rat brain, Endocrinology 96, 1456-1461, (1975).
55. *Patel, Y.C., Weir, G.C.*, and *Reichlin, S.*: Anatomic distribution of somatostatin (SRIF) in brain and pacreatic islets as studied by radioimmunoassay (RIA), Endocrinology 96, A 127 (1975).
56. *Hall, R., Snow, M., Scanlon, M., Mora, B.* and *Gomez-Pan, A.*: Pituitary Effects of Somatostatin, Metabolism, 27, Suppl. 1, 1257-1262, (1978).
57. *McCann,*: Regulation and function of somatostatin lecture at the Symposium Regulation and Function of neural peptides, Gordone, Riviera, Brescia, Aug. 29-Sept. 1º (1979).
58. *Reichlin, S.*: Regulation of somatotrophic hormone secretion, Handbook of Physiology, Endocrinology IV, Part 2., Ed. by E. Knobil and W.H. Sawyer, 405-447, Williams & Wilkins, Baltimore, 1977.
59. *Felipe Casanueva, Roberto Betti, Clara Frigerio, Daniela Cocchi, Paolo Mantegazza*, and *Eugenio E. Müller*: Growth hormone-releasing effect of an enkephalin analog in the dog. Evidence for cholinergic mediation. (in press).
60. *Lancranjan, Ioana* and *H. Friesen*: The neural control of PRL secretion. (in press).

The Role of Substance P in the Nigrostriatal System

[1,2] **Richard L. Borison, M.D., Ph.D.**
[3] **Harold L. Klawans, Jr., M.D.**
[2] **Bruce I. Diamond, Ph.D.**

[1] Illinois State Psychiatric Institute
1601 West Taylor Street
Chicago, Illinois 60612 USA
[2] Anesthesia Department
Mount Sinai Hospital Medical Center
California at 15th Place
Chicago, Illinois 60608 USA

[3] Department of Neurological Sciences
Rush-Presbyterian-St. Luke's Medical Center
1725 West Congress Parkway
Chicago, Illinois 60612 USA

Send correspondence to:
Richard L. Borison, M.D., Ph.D.
Illinois State Psychiatric Institute
1601 West Taylor Street
Chicago, Illinois 60612
312-996-1361

At present there is ample anatomical, biochemical and physiological evidence to implicate the degeneration of a γ—aminobutyric acid (GABA) striatonigral pathway as being central to the pathophysiology of Huntington's disease.[9]

This GABA ergic pathway exerts an inhibitory influence on the firing of the nigrostriatal' pathway, and its loss accounts for a hyperactivity in this system, which then leads to the clinically observed 'hyperdopaminergic' behavior as characterized by choreiform movements. Although GABA is the inhibitory transmitter found in the striatonigral loop, it is believed that substance P may mediate a facilitatory effect in this system. Substance P is highly concentrated in the striatum and substantia nigra [3], and hemi-transection rostral to the nigra results in substance P depletion, thus supporting that it is stored in striatonigral neurons[4]. Moreover, Microiontophoretic application of substance P to the substantia nigra results in increased nerve firing[12].

It may well be that the pathophysiology associated with Huntington's disease may not necessarily be associated with the loss of GABA's inhibitory effect on the substantia nigra, but may rather reflect a relative substance P over-activity. To date, only few investigations have looked at the potential role of substante P in Huntington's disease and, moreover, few pharmacological studies have looked at the role of substance P in the extrapyramidal system. We thus propose to study what pharmacological role, if any, does substance P play in the striatum, and whether substance P can be responsible for the development of the choreiform movements seen in Huntington's disease.

METHODS

Albino male Sprague-Dawley rats (200-250 g) were used in all studies and housed in environmentally controlled quarters.

Animals were cannulated with stainless steel cannulae, 0.8 mm in diameter, and fitted with an indwelling obturator extending 1 mm beyond the inner end of the cannula. Animals were anesthetized with pentobarbital (40 mg/kg, i.p.) and placed into a David Kopf stereotaxic apparatus. Bilateral cannulae were stereotaxically positioned to a point corresponding to the zona reticulata of the substantia nigra according to the coordinates of Pelligrino and Cushman (—3.0 mm anterior, 1.9 mm lateral, and 8.9 mm ventral)[8].

The cannulae were fixed into place with dental acrylic cement after jeweler's screws were placed into the skull immediately an-

terior and posterior to the cannulae. To check cannulae placement, animals were sacrificed by cervical dislocation, and the brains fixed in 10% formalin and then cut into 50 μm slices on a cryostat and imbedded in paraffin. Data was used only from those animals where there was verification that the cannulae were in the zona reticulata.

All solutions for intracerebral injection were at pH 7.4 and were dissolved in artificial cerebrospinal fluid and infused at a rate of 0.5 μl/min. The total volume of intracerebral injection was 1 μl. Animals were placed into wire mesh cages after the intracerebral injections and observed by

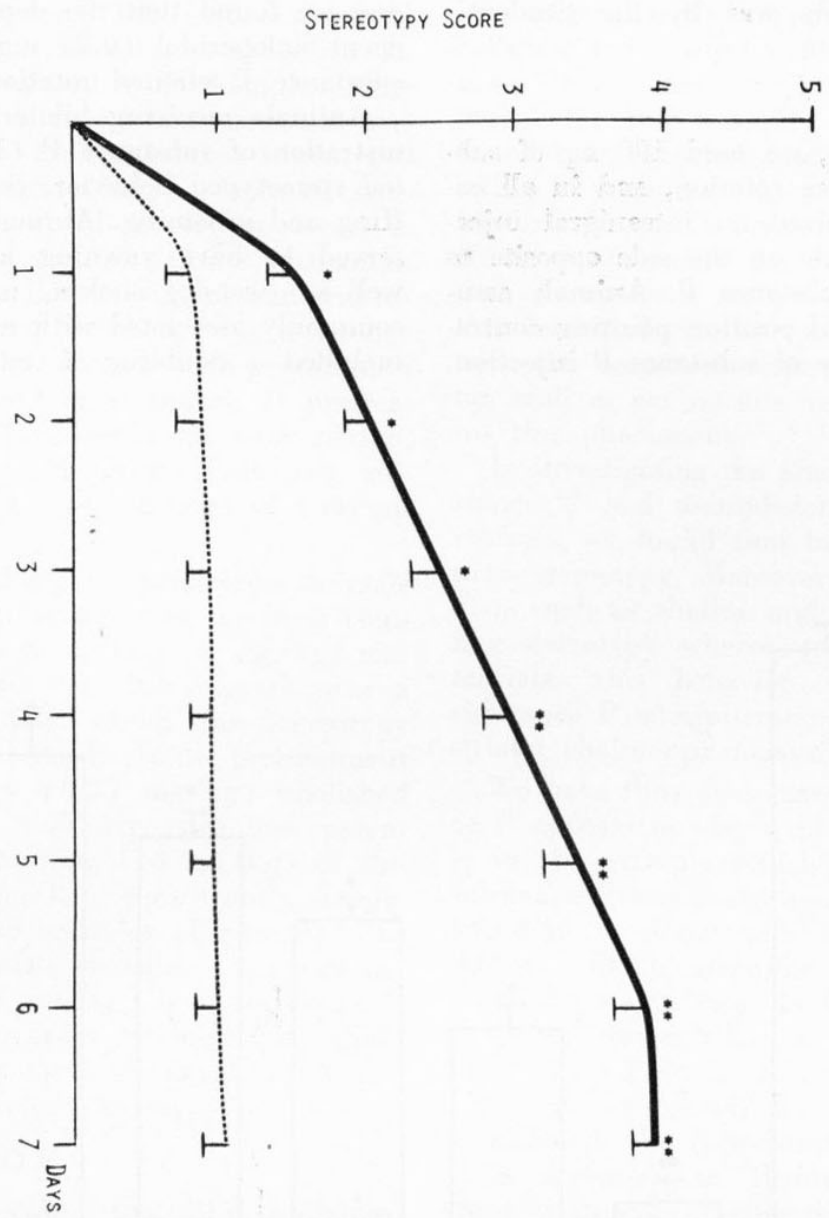


Figure 1. — Effect of seven day bilateral intranigral substance P on stereotyped behavior. ———Substance P (100 ng) bilateral intranigral administration. ***Bilateral intranigral administration of vehicle. On each day, animals received d-amphetamine (1 mg/kg, i.p.) five minutes after intracerebral injections and were then rated for stereotypy during the subsequent one hour. Stereotypy scoring sysem: O — no stereotyped behavior; 1 — increased exploratory behavior, grooming 2 — discontinuous stereotyped head-bobbing and sniffing; 3 — continuous stereotyped sniffing, discontinuous stereotyped head-bibbing; 4 — continuous sereoyped sniffing and head-bobbing, remains in one location for less than five minutes; 5 — continuous stereotyped sniffing and head-bobbing, remains in one location for over five minutes. *$p \leq 0.05$.
**≤ 0.01.

two blind independent observers using a rating scale for stereotypy and a checklist for general motor activity[2]. Observations were made for a minimum of one hour following every pharmacologic treatment. Agents used in pharmacologic tests were dissolved in saline and administered intraperitoneally in a volume of 0.1 ml/100 g. Statistical analysis was by the Student's t-test.

RESULTS

In our studies, we used 100 ng of substance P to induce rotation, and in all cases animals received an intranigral injection of the vehicle on the side opposite to that receiving substance P. Animals assumed a nose to tail position pointing contralateral to the side of substance P injection. Animals then began to rotate in tight circles, at a rate of three to four turns/min in a direction contralateral to the side of substance P administration. This rotational behavior ceased after approximately fifteen minutes, at which time the animal assumed a contralateral body assymetry for the subsequent 30 min. In pharmacological studies we found that the dopamine blocking agent haloperidol (0.25 mg/kg) abolished substance P elicited rotation.

Animals receiving bilateral nigral administration of substance P (100 ng) exhibited stereotyped behavior, consisting of sniffing and grooming. Animals were also observed to have yawning and bruxism as well as 'wet-dog shakes,' a behavior most commonly associated with narcotic withdra- included a doubling of total food intake.

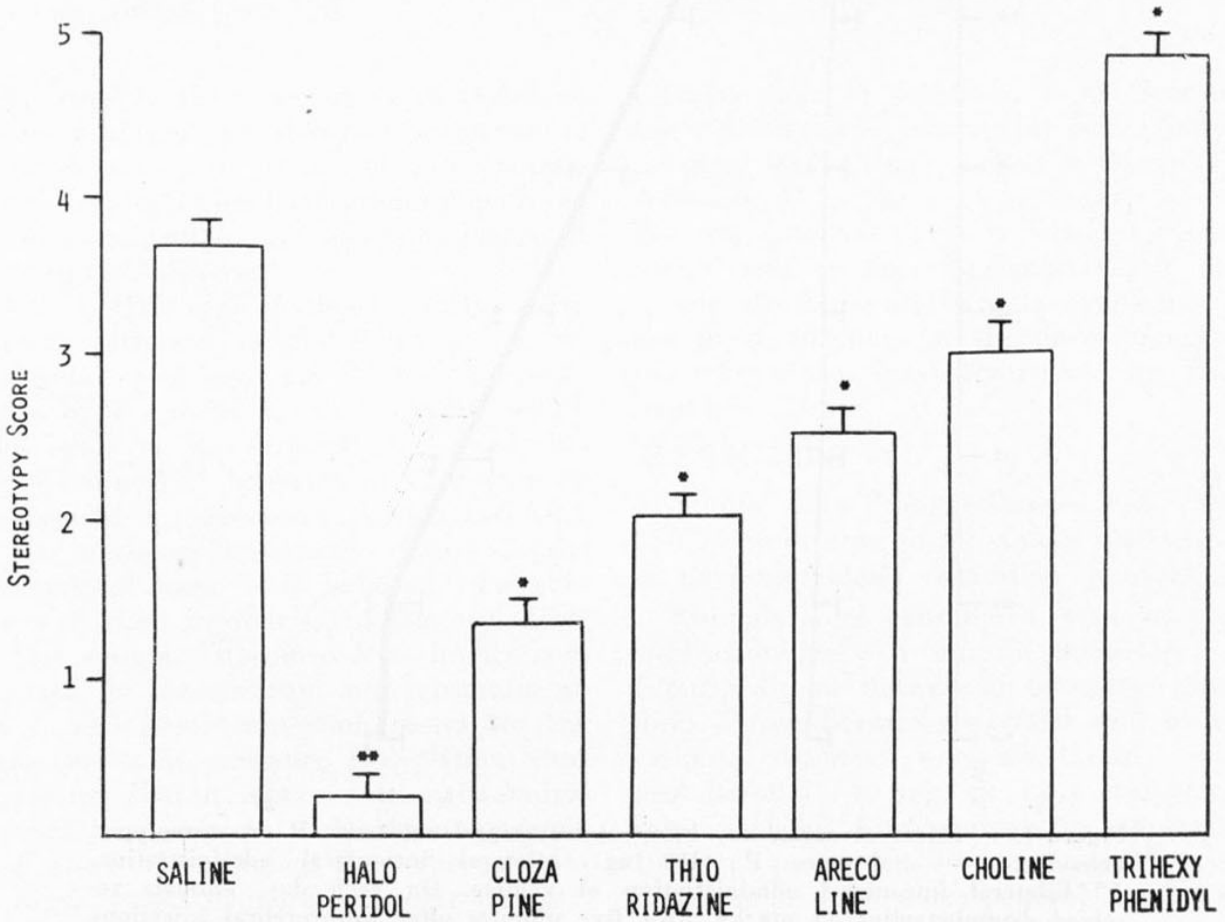


Figure 2. — Effects of various pretreatments on stereotypy induced by d-amphetamine (1 mg/kg, i.p.) in animals pretreated seven days with bilateral intranigral substance P (100 ng). Dosages of acute pharmacologic pretreatments are found in the text. Animals were observed for stereotyped behavior over a one hour period. The rating scale for stereotypy is found in the legend for Figure 1.

$^{*}p \leq 0.05$.

$^{**}p \leq 0.01$.

wal in animals. Other unusual behaviors included a doubling of total food intake, and involuntary beating movements of the forepaws which appeared to be 'choreic-like'. Also noted were head and neck tics.

When animals were pretreated with apomorphine (0.5 mg/kg),there was an attenuation of substance P - induced behavior, animals showing only increased exploratory behavior, with all stereotypies abolished. In contrast, pretreatment with d-amphetamine (2 mg/kg) in a dose which does not produce stereotypy, evoked intense stereotyped sniffing and head-bobbing in animals receiving substance P. Moreover, as shown in Figure 1, when d-amphetamine was administered to animals receiving intranigral saline or substance P, there was a reverse tolerance phenomenon to d-amphetamine's behavioral effects in substance P pretreated animals. Thus, over a one week period, substance P administration sensitized animals to the behavioral actions of substance P.

In pharmacological experiments, animals had all been pretreated with the drug combination of d-amphetamine (1 mg/kg) and substance P (100 ng, intranigral) over a one week period, at which time stereotypy scores were stabilized. Acute pretreatment with haloperidol (0.25 mg/kg) abolished all stereotypy. Similarly, two less potent neuroleptics, clozapine (10 mg/kg) or thioridazine (20 mg/kg), significantly antagonized stereotyped behavior (Figure 2). The cholinergic agonists arecoline (0.5 mg/kg) and choline (75 mg/kg) both significantly antagonized stereotypy, whereas the cholinergic blocking agent trihexyphenidyl (50 mg/kg) potentiated stereotypy.

DISCUSSION

Our results show that the unilateral application of substance P results in contralateral rotation, consistent with the data of others (7). This indicates that substance P activated nigrostriatal dopaminergic transmission. In support of this view, it has been demonstrated that intranigral substance P significantly increases levels of striatal homovanillic acid, a dopamine metabolite, only on the side ipsilateral to the injection[10]. We also found that the neuroleptic haloperidol abolished all rotatory behavior, even when administered in a noncataleptic dose.

Our results with the bilateral actions of substance P were most intriguing. We found that substance P itself was capable of producing stereotypy, and that this behavior was potentiated by d-amphetamine and antagonized by apomorphine, thus paralleling the clinical evidence on Huntington's disease and the kainic acid animal model for this disease[6]. Furthermore, we found that repeated administration of substance P produces a sensitization to the behavioral effects of d-amphetamine. It is difficult to reconcile an apparent sensitization of dopamine receptors as witnessed by the increased response to d-amphetamine, with the decreased response to apomorphine, and, as yet no one answer fully explains this phenomenon[1, 6, 11].

In investigating the pharmacology of substance P and d-amphetamine elicited stereotypy, we found that haloperidol antagonizes stereotypy. Moreover, cholinergic agonists such as choline and arecoline antagonize stereotypy, whereas trihexyphenidyl potentiates this behavior, thus implicating substance P administration as affecting the striatal cholinergic-dopamine axis.

We have thus demonstrated that substance P appears to play a pharmacological role in the extrapyramidal system, and that substance P administration results in a sensitization to dopamine's actions on motor activity, which resemble those found in Huntington's disease. Is there any reason to belive, however, that substance P overactivity is involved in the pathophysiology of Huntington's disease? We believe so, because although the concentration of substance P is decreased in Huntington's disease[5], there is an even greater decrease in GABA levels, thus resulting in a relative preponderance of substance P, which as we have shown affects the activity of brain dopamine in a manner consistent with the clinically observed motor disturbances in huntingtonian patients.

Furthermore, drugs with variable therapeutic efficacy in man, such as haloperidol, apomorphine, and choline antagonize the actions of substance P. It is therefore rea-

sonable to expect that agents with more specific actions on substance P systems may provide more powerful drugs in treating Huntington's disease.

ACKNOWLEDGEMENTS

This investigation was supported by the State of Illinois Department of Mental Health and Developmental Disabilities grant 8009-11. We thank H.S. Havdala, G.S. Sudakoff, J.E. Comaty, and O. Lobstein for their contributions.

SUMMARY

The bilateral intranigral administration of substance P to rats produces stereotyped behavior. This daily pretreatment leads to a dopaminergic behavioral sensitization response which is potentiated by d-amphetamine and antagonized by apomorphine. Furthermore, neuroleptic dopamine blockers and cholinomimetics decrease stereotypy, whereas anticholinergics increase stereotyped behavior.

These responses mimic the pharmacology of Huntington's disease and suggests that substance P may contribute to the clinically observed hyperdopaminergic behavior in this illness.

RESUMEN

La administración bilateral de la sustancia P a la sustancia nigra de las ratas produce una conducción estereotipada. Este tratamiento conduce a una exaltación de la conducta dopaminérgica, mientras que la anfetamina produce un aumento marcado, y la apomorfina produce una reducción de la respuesta a la sustancia P. También los neurolépticos que bloquean los receptores de la dopamina y los colinomiméticos producen una inhibición de la conducta estereotipada, mientras que los anticolinérgicos producen un aumento de la conducta estereotipada. Estas respuestas son como la farmacología de la enfermedad de Huntington y estos estudios indican que la substancia P desempeña un papel importante en la patogenia de los desórdenes motores desquinéticos e hiperdopaminérgicos.

RÉSUMÉ

L'administration bilatèrale de la substance P aux substance nigra des rats produit une conduite stéréotypique. Ce traitement produit un exaltation de la conduite dopaminergique, tandis l'anfetamine produit une augmentation marqueé, et l'apomorphine produit une reduction de la résponse à la substance P. Aussi, les neuroleptiques qui blocantes des récepteurs de la dopamine, et les cholinomimetiques produit une inhibition de la conduite stéréotypique, tandis les anticholinergiques produit une augmentation de la conduite stéréotypique. Ces résponses sont comme la pharmacologie de la maladie de Huntington, et ces études indiquent que la substance P jouet un rôle important dans la pathogénie de les disordres moteurs diskinétiques et hyperdopaminergique.

ZUSAMMENFASSUNG

Die beiseitige Verordnung der Substance P in Ratten erzeugt stereotypische Benehmungen. Die taegliche Behandlung fuehrt zu einer dopaminartigen sensitiziehrenden Ausartung. Diese Ausartung ist vom d-Amphetamine verstaerkt und vom Apomorphine entgegnet. Weiter kann man sagen dass neuroleptic dopamine Blocker und colinergische Mimiker die stereotypung verringern, ober gegen-cholinergische Medika-

mente erhohen das stereotypische Betragen. Diese Resultate nachahmen die Pharmacologie der Erkrankung des Huntington und geben zu verstehen dass die Substance P zu der clinischen sehbaren hyperdopamine Benehmung in dieser Krankheit beifraegt.

REFERENCES

1. *Borison, R.L.* and *Diamond, B.I.*: A new animal model for Huntington's disease. Adv. Neurol. 23: 669-677, (1979).
2. *Borison, R.L., Havdala, H.S.* and *Diamond, B.I.*: Chronic phenylethylamine induced stereotypy: A new animal model for schizophrenia? Life Sci. 21: 117-121, (1977).
3. *Brownstein, M.J., Mroz, E.A., Kizer, J.S., Palkowitz, M.* and *Leeman, S.E.*: Regional distribution of substance P in the brain of the rat. Brain Res. 116: 299-315, (1976).
4. *Hong, J.S., Yang, H.Y.-T., Racagni, G.* and *Costa, E.*: Projections of substance P containing neurons from neostriatum to substantia nigra.
5. *Kanazawa, I. Bird, E., O'Connell, R.* and *Powell, D.*: Evidence for a decrease in substance P content of substantia nigra in Huntington's chorea. Brain Res. 120: 1332-1334, (1977).
6. *Mason, S.T.* and *Fibiger, H.C.*: Kainic acid lesions of the striatum: Behavioral sequelae similar to Huntington's chorea. Brain Res. 155: 313-329, (1978).
7. *Olpe, H.-R.* and *Koella, W.P.*: Rotatory behavior in rats by intranigral application of substance P and eledoisin fragment. Brain Res. 126: 576-579, (1977).
8. *Pelligrino, L.J.*, and *Cushman, A.J.*: A Stereotaxic Atlas of the Rat Brain. New York, Appleton-Century-Crofts, 1967.
9. *Perry, T.J., Hansen, S.* and *Kloster, M.*: Huntington's chorea deficiency of γ-aminobutyric acid in brain. N. Eng. J. Med. 288: 337-343, (1973).
10. *Starr, M.S.*: Investigations of possible interactions between substance P and transmitter mechanisms in the substantia nigra and corpus striatum of rat. J. Pharm. Pharmacol. 30: 359-363, (1978).
11. *Tolosa, E.S.* and *Sparber, S.B.*: Apomorphine in Huntington's chorea: Clinical observations and theoretical considerations. Life Sci. 15: 1371-1380, (1974).
12. *Walker, R.J., Kemp, J.A., Yajima, H., Kitagawa, K.* and *Woodruff, G.N.*: The action of substance P on mesencephalic reticular and substancia nigra neurons of the rat. Experientia 32: 214-215, (1976).

"Enkephalinase", a Newly Characterised Dipeptidyl Carboxypeptidase : Properties and possible role in Enkephalinergic Transmission

J. C. SCHWARTZ, S. DE LA BAUME, B. MALFROY
G. PATEY, R. PERDRISOT and J. P. SWERTS
Unité de Neurobiologie
Centre Paul Broca de l'I.N.S.E.R.M.
2 ter rue d'Alésia 17014 Paris, France
and
M. C. FOURNIE - ZALUSKI, G. GACEL and B. P. ROQUES
Departement de Chimie Organique, ERA 613 (C.N.R.S.)
Université René Descartes
4 avenue de l'Observatoire 75006 Paris, France.

It is extremely likely that enkephalins (ENKs) play a neurotransmitter role in the CNS and therefore, that efficient and possibly specific inactivation system turn off the signal these peptides represent. Indeed, following their intraventricular administration ^{3}H-ENKs were shown to have a half-life of less than 1 min. in rat (28) as well as in mouse (J.P. Swerts and R. Perdrisot, unpublished observation).

Initial studies on the mode of breakdown of ENKs by brain tissues were conducted in various laboratories either with whole homogenates or soluble fractions, and have led to converging results: the first step consists in the cleavage of the Tyr-Gly bond, as demonstrated by the identification of Tyr as the firstly released amino-acid (13, 18, 19, 23, 24, 27, 28, 42) together with the tetrapeptide Gly-Gly-Phe-Met (19). That aminopeptidases are playipg an important role in the inactivation of exogenous ENKs is consistent with the observation that replacement of Gly_2 by D. Ala_2 leads to analogs possessing an enhanced duration of action (31). However, a variety of enzymes are involved in the ENKhydrolysing activity of rat brain extracts, asalready suggested by the heterogeneous pattern of inhibition by several agents when assessed in various subcellular fractions (24).

CHARACTERISATION AND PROPERTIES OF ENKEPHALINASE ACTIVITY

Enkephalinase activity was initially characterised as a highaffinity component of the total ENK-hydrolysing activity of striatal membranes, responsible for the cleavage of the Gly-Phe bond of these pentapeptides (25).

However, because this component represented less than 20% of the total ENK-hydrolysing activity in striatal membranes, even at low substrate concentration its properties could not be well investigated due to a high "background" of aminopeptidase activity i.e. Tyr-releasing enzyme activity. The observation that puromycin is a potent inhibitor of aminopeptidase activity (4, 23, 43) but does not affect the Tyr-Gly-Gly-releasing enzyme activity (26) pro-

vided a useful tool for such investigations. In a complementary way, the tetrapeptide Gly-Gly-Phe-Met inhibits almost completely at 0.1 mM the "enkephalinase" activity (Table 1). Thus two assays for "enkephalinase" activity could be developed. In both cases incubations are performed in the presence of puromycin and with low ^{3}H-Leu-ENK concentrations, and followed by either the selective estimation of ^{3}H-Tyr-Gly-Gly by thin-layer chromatography (26) or the evaluation of total ^{3}H-metabolites separated from unchanged ^{3}H-Leu-ENK on Porapak columns (43). In the latter case the hydrolysing activity not due to enkephalinase is estimated by a separate incubation in the presence of 0.1mM Gly-Gly-Phe-Met and this "blank" representing approximately 20% is subtracted. Results obtained with the two methods show excellent agreement.

Enkephalin dipeptidyl carboxypeptidase or "enkephalinase" activity is defined as the peptidase activity from brain tissues able to release the C-terminal dipeptide from ENKs in low concentrations. However a well-defined membrane-bound enzyme, peptidyl dipeptide hydrolase (EC 3.4. 15.1), i.e. angiotensin-converting enzyme (A.C.E.) has a broad substrate specificity and releases the C-terminal dipeptide not only from angiotensin I but also from a variety of other peptides (14). The possibility that "enkephalinase" is identical with A.C.E. has to be raised because this enzyme purified to homogeneity released the characteristic tripeptide Tyr-Gly-Gly from Met-ENK or Leu-ENK and this hydrolysis is completely blocked by 10^{-6} M of SQ 14,225 (Captopril, 2-D-Methyl-3-mercaptopropanoyl-L-proline) a potent inhibitor of this enzyme (15).

TABLE I

DIFFERENTIAL INHIBITION OF ENKEPHALIN-HYDROLYSING ENZYME ACTIVITIES FROM STRIATAL MEMBRANE BY VARIOUS COMPOUNDS

	Inhibition by		
	Puromycin (0.1 mM)	SQ 14,225 (1 μM)	Gly-Gly-Phe-Met (0.1 mM)
Aminopeptidase	90%	0	30%
Enkephalinase	0	0	90%
A.C.E.	0	100%	0

Aminopeptidase and "enkephalinase" activities evaluated by estimation of the initial rate of ^{3}H-Tyr and ^{3}H-Tyr-Gly-Gly formation from 10nM ^{3}H-Leu-ENK (thin-layer chromatography). A.C.E. activity represents the initial rate of His-Leu formation from 1mM Hip-His-Leu.

Nevertheless the use of compound SQ 14,225 allows to clearly distinguish between the release of ^{3}H-Tyr-Gly-Gly elicited by "enkephalinase" and A.C.E., respectively: in the presence of 10^{-6}M of this potent A.C.E. inhibitor (Ki around 10^{-8}M), ^{3}H-Tyr-Gly-Gly formation from concentrations of ^{3}H-Leu-ENK below 10^{-6}M is not affected whereas at higher substrate concentrations, ^{3}H-Tyr-Gly-Gly formation is diminished in a progressively higher proportion. These data indicate that the Km of ^{3}H-Leu-ENK for "enkephalinase" is 27 ± 3 μM whereas for A.C.E. it is around 1mM i.e. a 100-fold difference. The latter value is in agreement with the Ki value of ENKs for A.C.E., also in the mM range, when evaluated from the inhibition of Hip-His-Leu hydrolysis by the ame striatal membrane preparation (40). In addition, Benuck and Marks (6) have recently reported that the Km of Met-ENK for A.C.E. purified from rabbit brain is about 0.1mM. Similarly, large differences in the affinities of the

two potents A.C.E. inhibitors SQ 14,225 and SQ 20,881 (by 2-3 order of magnitude) can be noted regarding A.C.E. and "enkephalinase", respectively (40). Hence, these data show unambiguously that A.C.E. activity does not participate in ^{3}H-Tyr-Gly-Gly formation under the conditions of our assays i.e. when concentrations of the substrate, ^{3}H-Leu-ENK, are kept below 100nM.

A.C.E. is a chloride-activated enzyme, strongly inhibited by chelating agents which probably act by forming a complex with the Zn atom present in the active site of this glycoprotein (14). The exact catalytic mechanism of A.C.E. is not known but by analogy with that of carboxypeptidase A which has been elucidated following the complete determination of its structure (33), this Zn atom probably plays a key-role in the cleavage of the peptide bond of substrates (12). The strong inhibition of "enkephalinase" activity by EDTA and o-phenantroline as well as its restoration by addition of Zn^{++} (39) suggest that it is a metalloenzyme and that its catalytic mechanism might be similar to that of carboxypeptidase A or A.C.E. On the other hand no activation of the enzyme by Cl- occurs as reported for A.C.E. but it hould be noted that for the latter, the extent of the effect varies with the substrate (14).

INHIBITORY POTENCY OF VARIOUS PEPTIDES ON "ENKEPHALINASE" ACTIVITY

The specificity of peptidases can be indirectly evaluated, as long as these enzymes are not purified, by assessing the inhibitory potency of various peptides. Using this approach it was originally concluded that "enkephalinase" has a very restricted substrate specificity (25). But this conclusion was reached by analysis of biphasic saturation curves, an assay of low accuracy which, in addition, did not allow to determine the inhibitory potency of several peptides due to interference of the high aminopeptidase activity in the membrane preparation.

We have reinvestigated this problem using incubations in the presence of puromycin. It can first be noted that under such conditions a much lower Km value is found for Leu-ENK than previously reported (40) whereas the IC_{50} for Met-ENK is in the same range (Table 2).

In addition, it appears that "enkephalinase" does not exhibit an extremely stringent specificity towards a variety of peptides. This is evidenced by the IC_{50} in the micromolar range, like that of Met-ENK, of various peptides structurally unrelated to the ENK molecule (16).

This is the case for relatively large peptides like insulin, as well as for oligopeptides (angiotensin I, Gly-Gly-Phe-Met) or even dipeptides like Tyr-Gly. On the other hand a variety of other peptides, including β-endorphin, do not appear to be recognized by the enzyme.

This relatively broad specificity of "enkephalinase" may suggest that the entire molecule is not necessarily recognized by the enzyme. However the determination of inhibitory potencies of ENK analogues throws more light on this matter. The inversion of the configuration of the aminoacids either in position 3 (D.Ala$_3$-Met-ENK) or position 4 (D.Phe$_4$-Met-ENK) strongly reduces the inhibitory potency. Interestingly, although the effects is less marked when the inversion affects the aminoacid in position 2, it is still noticeable as shown by the significantly reduced potency of D.Ala$_2$-Met-ENK as compared to Met-ENK (Table 2).

The importance of a free carboxyle group is nicely shown by the reduced potency when this group is esterified and even more when amidified, suggesting its interaction with the active site of enkephalinase. Assuming for this dipeptidyl carboxypeptidase a catalytic mechanism analogous to that of carboxypeptidase A (33), this feature could be expected. A similar assumption could explain the low potency of Tyr-Arg and Tyr-Lys as compared to Tyr-Gly (16): in carboxypeptidase A the free carboxyle group of the substrate interacts with the Arg 145 residue, an effect which is conceivably inhibited when the C-terminal aminoacid of the substrate is of basic nature. The low potency of Tyr-L-Arg and Tyr-D-Arg (IC_{50} > 100μM) excludes that inhibition of "enkephalinase" accounts for

their analgesic activity (41). On the other hand the good inhibitory potency of dipeptides like Tyr-Gly ($IC_{50} = 1.6 \pm 0.9\mu M$) might explain that a mixture of dipeptides could protect endogenous ENKs released from depolarised brain slices by inhibiting "enkephalinae" (21).

TABLE II

INHIBITORY POTENCY OF VARIOUS ENKEPHALIN ANALOGS ON "ENKEPHALINASE" ACTIVITY FROM MOUSE STRIATUM

Compounds	IC_{50} (μM)
Tyr- Gly - Gly - Phe -Leu OH (Leu-ENK)	11.0 ± 1.0
Tyr- Gly - Gly - Phe -Met OH (Met-ENK)	1.4 ± 0.5
Tyr- Gly - Gly - Phe -Met OCH_3	8.3 ± 1.6
Tyr- Gly - Gly - Phe -Met NH_2	40.0 ± 15
Tyr-D.Ala- Gly - Phe -Met OH	3.8 ± 0.7
Tyr-D.Ala- Gly - Phe -Met NH_2	65.0 ± 8.0
Tyr-L.Ala- Gly - Phe -Met OH	2.3 ± 0.5
Tyr- Gly -L.Ala- Phe -Met OH	1.4 ± 0.4
Tyr- Gly -D.Ala- Phe -Met OH	32.0 ± 9.0
Tyr- Gly - Gly -D.Phe-Met OH	11.0 ± 2.0
Tyr- Gly - Gly -MePhe-Met OH	44.0 ± 8.0
Tyr-D.Met- Gly - Phe -Pro NH_2	310.0 ± 90
Tyr-D.Ala- Gly - MePhe-Met(0)-ol FK.33-824	420.0 ± 90

Enzyme activity determined as described in Table 2 in the presence of increasing concentrations of the various peptides. IC_{50} determined by iterative compute analysis, based on the least squares estimates.

The loss of potency in Tyr-Gly-Gly-Me Phe-Met could be due either to a steric hindrance by the methyl group in the vicinity of the amide bond to be cleaved or to the loss of an eventual hydrogen-bonded interaction with the enzyme at this level.

Taken together, these features may account for the increased biological activity (not explained by a higher affinity for receptors or resistance to aminopeptidase activity) in ENK analogues combining various modifications resulting in a lesser recognition by "enkephalinase". This is the case of Tyr-D-Met-Gly-Phe-Pro-NH_2 (3) a compound 20-times more active as an analgesic than Met-ENK and even more for FK 33-824 which is 250 times more potent (34). In connection with this, it is interesting to observe that the inhibition of aminopeptidase activity by replacement of Gly^2 by $D.Ala^2$ in ENKs leads to a three-time increase of the analgesic activity whereas the protection from enkephalinase activity by methylation of the Gly-Phe peptide bond produces a twelve time-enhancement of potency (35).

REGIONAL DISTRIBUTION AND LESION STUDIES

An important although indirect approach to evaluate the possible physiological significance of the various ENK-hydrolysing enzymes consists in determining whether their localisation, asssessed by regional distribution and lesion studies, are consistent with a presence within post-synaptic structures of putative enkephalinergic synapses.

The distribution of "enkephalinase"

shows a large variation between regions of mouse brain (26) the extreme values being found in cerebellum and striatum which differ by a factor of 3. This distribution parallels rather closely that of opiate receptors, as indicated by the correlation coefficient between the two sets of values ($r = 0.96$). Recently Sullivan et al. (38) have also determined the regional distribution of "enkephalinase" in rat brain and their data confirm that it parallels that of opiate receptors, although the enzyme activity they report is markedly lower than that we find under similar conditions.

In addition, opiate receptors appear to be located on membranes of a restricted number of neurons, the identity of which is progressively unraveled, especially by lesion studie (32). Following kainic acid injection into rat striatum a significant decrease in opiate receptor binding as well as "enkephalinase" activity is observed suggesting that both membrane components are present on post-synaptic neurons (26). Furthermore, lesioning specifically the nigrostriatal dopaminergic neurons results in a similar post-degenerative decrease in opiate receptors and "enkephalinase" activity in rat striatum (26). This indicates that the same class of neuronal cells i.e. dopaminergic neurons possess these two membrane components, a finding which is obviously consistent with their possible location in vicinity.

In contrats, the distribution of the other enkephalin-hydrolysing enzymes does not show any parallelism with that of opiate receptors. Thus, the aminopeptidase activity is rather homogeneously distributed between regions of mouse (26) and rat (6, 38) brain. On the other hand A.C.E. activity is distributed in a markedly heterogeneous manner between regions of rat brain (44) and kainic acid lesion data suggest its neuronal localisation (1, 37) like for "enkephalinase". However the distribution of A.C.E. differs markedly from that of "enkephalinase" and therefore also of opiate receptors. Recently Benuck and Marks (6) have reported that the regional distribution of Tyr-Gly-Gly forming enzyme was strictly parallel to that of A.C.E. (evaluated by measurement of Hip-His-Leu hydrolysis) and concluded that the two enzymes were identical. The discrepancy with our data can, in part, be explained by the high concentration of Leu-ENK used by these authors which is likely to make significant the contribution of A.C.E. in ENK-hydrolysing activity. However the reason for the discrepancy in the A.C.E. distribution they report and that found by Yang and Neff (44), the latter confirmed by our data (40) is unclear.

ONTOGENETIC DEVELOPMENT

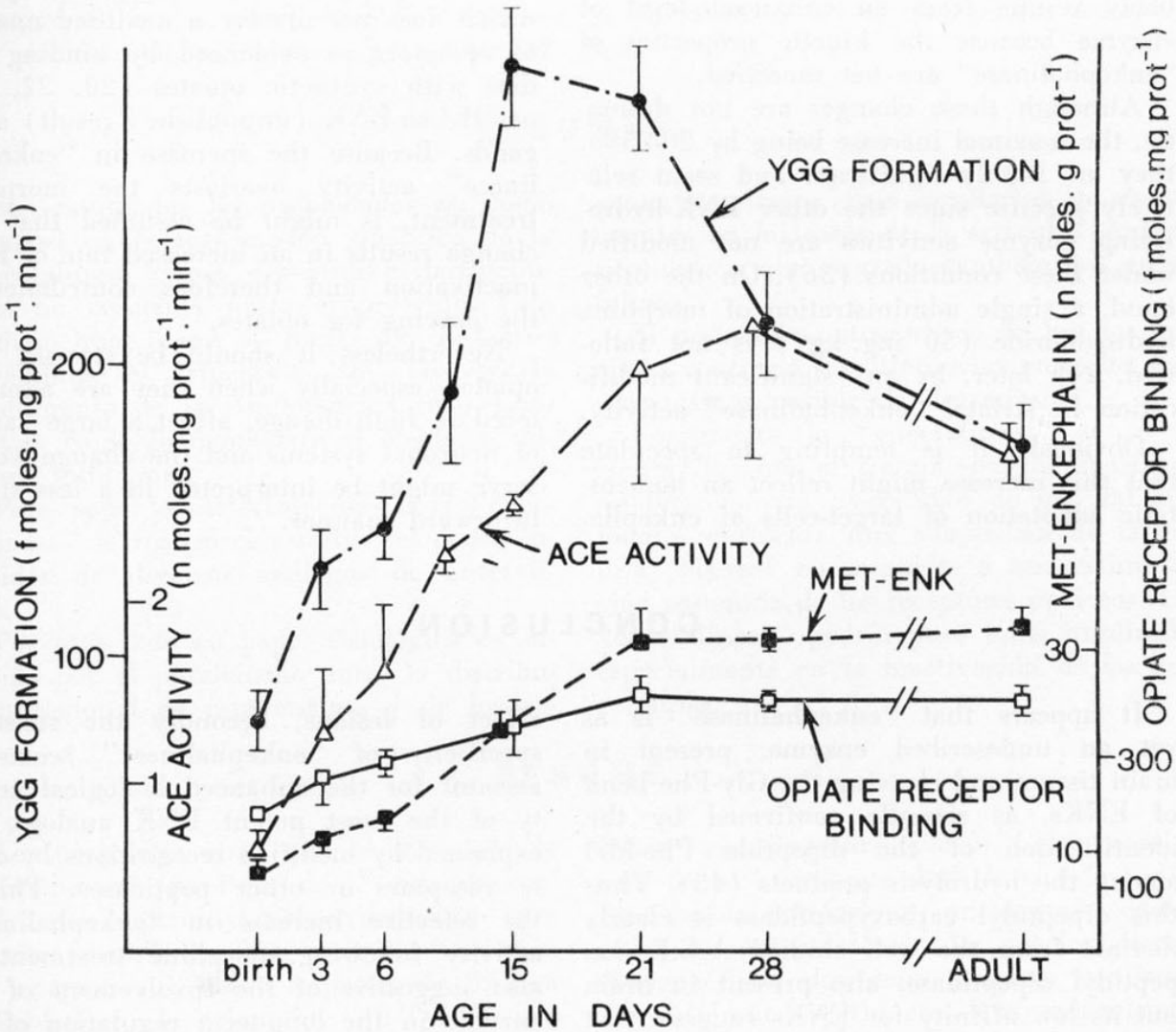
The knowledge of neurochemical changes occuring during the maturation of the CNS often helps to elucidate the functional role eof cerebral constituents. Hence we have recently compared (5) the post-natal changes in the levels of several parameters linked to the putative enkephalinergic transmission.

As shown in Fig. 1, striatal levels (expressed per mg of protein) of Met-ENK exhibit a 4-5 fold increase between birth and adulthood; this increase is comparable both in amplitude and time-course to the changes reported for several neurotransmitters in this region, a finding which is therefore compatible with the idea that ENKs are, indeed, playing a similar role.

In agreement with previous studies (2, 8, 10) the density of opiate receptors increases only about 2-fold during this period. It is interesting to note that the density of other classes of striatal receptors show a slightly more pronounced increase during development (11, 29). Approximately 1/3 of opiate receptors in the adult rat striatum are localised on dopaminergic neurons (32), the density of which increases by about 6-fold during ontogenesis (9). Taken together these data suggest that dopaminergic neurons might contribute predominantly in the post-natal increase in opiate receptor density in striatum. Like opiate receptors striatal "enkephalinase" activity also increases approximately 2-fold between birth and adulthood (Fig. 2) but the pattern of development is clearly different: by 15-21 days "enkephalinase" activity expressed per mg of protein is significantly higher than in adults whereas this is not the case for opiate receptors binding.

In fact it can be estimated that when "enkephalinase" activity is expressed per total striatum, it has already reached the adult level by 15 days. This feature probably reflects an early development of this enzyme activity during brain maturation and not a change in its properties because its Km for Leu-ENK at 21 days is not significantly different from that of adults. The developmental pattern of the other ENK-hydrolysing activities is distinct from that of "enkephalinase". The Tyr-releasing enzyme activity has the same value (per mg of protein) in the newborn as in the adult striatum (not shown) a feature which reflects a progressive increase paralleling that of the weight of the structure.

In contrast A.C.E. activity increases approximately 6 - fold between birth adulthood but its developmental pattern is markedly different from that of "enkephalinase": its delayed development is particularly demonstrated by comparison of levels of the two enzyme activities in the 15-day old animal (Fig. 2). This difference confirms that the two activities reflect two distinct enzyme species.



EFFECT OF MORPHINE TREATMENTS

The physiological implication of "enkephalinase" in enkephalinergic transmission can be indirectly assessed by evaluating the changes in this enzyme activity following a sustained stimulation of opiate receptors.

In mice implanted with morphine pellets, "enkephalinase" activity in striatum, cortex or hypothalamus increases progressively. This increase is maximal on the first day following pellet removal and disappears by the 2nd day. The increase probably results from an enhanced level of enzyme because the kinetic properties of "enkephalinase" are not modified.

Although these changes are not dramatic, the maximal increase being by 20-25%, they are highly significant and seem relatively specific since the other ENK-hydrolysing enzyme activities are not modified under these conditions (36). On the other hand, a single administration of morphine hydrochloride (50 mg. kg^{-1}) is not followed, 24h later, by any significant modification in striatal "enkephalinase" activity.

Obviously it is tempting to speculate that this increase might reflect an homeostatic adaptation of target-cells of enkephalinergic neurons to the chronic overstimulation of opiate receptors. This would imply that the same cells bear the opiate receptors and the "enkephalinase", as lesion studies suggest it. In the cholinergic system in brain, homeostatic changes in responsiveness to cholinergic agents appear to result from changes in acetylcholinesterase activity (7) whereas the density of muscarinic receptors does not appear to be modified under these circumstances. A parallel could be made with the development of tolerance to and dependence on opioids which does not involve a modified number of receptors as evidenced by binding studies with synthetic opiates (20, 22, 30) or ^{3}H-Leu-ENK (unpublished result) as ligands. Because the increase in "enkephalinase" activity overlasts the morphine treatment, is might be assumed that this change results in an increased rate of ENK inactivation and therefore contributes to the craving for opiates.

Nevertheless it should be stressed that opiates, especially when they are administered in high dosage, affect a large variety of neuronal systems and the change we observe might be interpreted in a less straightforward manner.

CONCLUSION

It appears that "enkephalinase" is as yet an undescribed enzyme, present in brain tissues and cleaving the Gly-Phe bond of ENKs, as recently confirmed by the identification of the dipeptide Phe-Met among the hydrolysis products (45). Thus this dipeptidyl carboxypeptidase is clearly distinct from the well studied A.C.E. i.e. peptidyl dipeptidase, also present in brain but its low affinity for ENKs suggests that it does not participate in the physiological inactivation of the pentapeptides.

Several features suggest that such might be the case. Firstly enkephalinase might be strategically located to have this function as is uggested by its regional distribution paralleling that of opiate receptors and ENK levels in rodent brain and by the effect of lesions. Secondly the substrate specificity of "enkephalinase" seems to account for the enhanced biological activity of the most potent ENK analogs, not explained by modified recognitions by opiate receptors or other peptidases. Thirdly the selective increase in "enkephalinase" activity following morphine treatments is also suggestive of the involvement of the enzyme in the long-term regulation of enkephalinergic transmission.

Other approaches are obviously needed to assess definitively whether "enkephalinase" is the "acetylcholinesterase of ENKs" but the important implications that inhibitions of the enzyme would have in Neurology and Psychiatry, if this is the case, make this aim particularly attractive.

SUMMARY

Properties as well as possible synaptic functions of "enkephalinase" a newly characterized dipeptidyl-carboxypeptidase is reported. This enzyme releasing ^{3}H-Tyr-Gly-Gly from ^{3}H-Leu-Enkephalin (Leu-ENK) differs from Angiotensin-Converting-Enzyme (A.C.E.), as seen by inhibition studies. To further characterize this "enkephalinase" the inhibitory potency of various Enkephalin analogs was tested.

On another hand its physiological role is suggested by the parallelism of its regional distribution as compared to that of opiate receptors. Such correlation was not found concerning aminopeptidase(s) which also degrade Leu-ENK. The difference between both Leu-ENK degrading activities is further assessed through ontogenetic development. Finally "enkephalinase" is increased during morphine treatment raising the possibility that such an increase represents an adaptation of the target-cell to an overtimulation of opiate receptors. Enkephalinase could thus be involved in the turning off of endogenous enkephalins.

RESUMEN

Son estudiadas las propiedades así como el papel en la transmisión sináptica de la "encefalinasa", una dipeptidil-carboxipeptidaza no descripta hasta el presente. Esta enzima que libera á partir de 3h-Leu—encefalina, el tripéptido 3h—Tir-Gly-Gly es diferente de la Angiotensina in — Convertasa como lo demuestra el efecto de diversos inhibidores.

Para poder caracterizar más a la "encefalinasa" se realizó el estudio del poder inhibidor de diversos análogos de encefalinas.

Por otro lado su papel fisiológico es sugerido por el paralelismo entre la distribución regional de esta enzima y de los receptores opiáceos. Esta correlación no se encuentra en los casos de la actividad aminopeptidásica que degrada también las encefalinas.

El desarrollo ontogenético de las dos enzimas confirma las diferencias entre los dos mecanismos posible de degradación.

Finalmente la "encefalinasa" se acrecienta durante el tratamiento de morfina, estableciendo la posibilidad de que tal aumento representa una adaptación de la célula "blanco" en respuesta a una estimulación sostenida de los receptores opiáceos. La "encefalinasa" podría pues estar implicada especialmente en la inactivación de las encefalinas.

RÉSUMÉ

Les propriétés ainsi que le rôle dans la transmission synaptique de l' "enképhalinase", une dipeptidyl-carboxypeptidase non décrite jusqu'à present, sont étudiées. Cette enzyme qui libère à partir de 3h-Leu enkephaline, le tripeptide 3h-Tyr-Gly-Gly est différente de l'Angiotensin-Convertase comme le prouvent l'effet de divers inhibiteurs.

Une caractérisation plus poussée de l' "enképhalinase" a été réalisée par l'étude du pouvoir inhibiteur de divers analogues des enképhalines. Par ailleurs son rôle physiologique est suggéré par le parallélisme entre la distribution régionale de cette enzyme et des récepteurs opiacés. Cette correlation ne se trouve pas dans le cas de l'activité aminopeptidasique qui dégrade également les enképhalines. Le développement ontogénétique des deux enzymes confirme des différences entre ces deux mécanismes possibles de dégradation. Enfin l'activité de l'enképhalinase est augmentée par un traitement chronique à la morphine ce qui pourrait représenter un mécanisme d'adaptation de la cellule cible en réponse à une stimulation soutenue des récepteurs opiacés. L'enképhalinase pourrait donc être impliquée spécifiquement dans l' inactivation des enképhalines.

ZUSAMMENFASSUNG

Die Eigenschaften, ebenso welche Rolle in der synoptischen Transmission der enzephalinase eine dipeptydyl-carboxypeptidase spielt, welche bisher noch nicht beschrieben ist, wird studiert.

Dieses Enzym, welches besonders 3h-Leuenzephalin, das tripeptid 3h-Tri-Gli-Gli frei macht, ist anders, als das angiotensin in konvertase, wie es der Effekt verschiedener inhibidose zeigt.

Zur besseren Charakterisierung dir "Enzephalinasen" ist das Studium der inhibierenden Kraft der verschiedenen Analogen der enzephalinasen gemacht worden.

Andrerseits ist seine physiologische Rolle durch den Parallelismus zwischen der regionalen Verteilung dieses Enzyms und der opiumalen Rezeptoren suggeriert worden. Diese Korrelation besteht nicht in den Fällen der aminopeptidasischen Aktivität, die auch die enzephalinasen degadiert.

Die ontogenetische Entwicklung der zwei Enzyme bestätigt die Differenz zwischen den zwei möglichen Meschanismen der Degradation.

Schliesslich nimmt die "enzephalinase" während der Behandlung von Morphium zu, wobei die Möglichkeit besteht, dass die Vermehrung eine Adaption der "weissen" Zelle darstellt als Antwort auf eine Stimulierung, hergestellt durch die opiumierten Rezeptoren. Die "enzephalinase" kann daher spezifisch in der Inaktivierung der enzephalinasen impliziert sein.

REFERENCES

1. *Arregui, A., Emson, P.C.* and *Spokes, E.G.* (1978): *Eur. J. Pharmacol.*, 52: 121-124.
2. *Auguy-Valette, A., Cros, J., Gouarderes, Ch., Gout, R.* and *Pontonnier, G.* (1978): *Br. J. Pharmac.*, 63: 303-308.
3. *Bajusz, S., Ronai, A.Z., Szekely, S.I., Dunai-Kavacs, Z., Berzelei I.* and *Graf, L.* (1976): *Acta Biochem. Biopys.*, 11: 305-309.
4. *Barclay R.K.* and *Phillips M.A.* (1978). *Biochem. Biophys. Res. Commun.*, 81: 1119-1123.
5. *De La Baume, S., Patey, G.* and *Schwartz, J.C.* (1979): Endogenous and exogenous opiate agonists and antagonists. Ed. E.L. Way, Pergamon Press, Oxford (in press).
6. *Benuck, M.* and *Marks, N.* (1979); *Biochem. Biophys. Res. Commun.*, 88: 215-221.
7. *Bird, S.J.* and *Aghajanian, G.K.* (1975): *Brain Res.*, 100: 355-370.
8. *Clendeninn, N.J., Petraitis, M.* and *Simon E.J.* (1976): *Brain Res.*, 118: 157-160.
9. *Coyle, J.T.* and *Campochario, P.* (1976). *J. Neurochem.*, 27: 673-678.
10. *Coyle, J.T.* and *Pert, C.B.* (1976): *Neuropharmacol.*, 15: 555-560.
11. *Coyle, J.T.* and *Yamamura, H.I.* (1976): *Brain Res.*, 118: 429-440.
12. *Cushman, D.W., Cheung, G.S. Sabo, E.F.,* and *Ondetti, M.A.* (1977) *Biochem.*, 16; 5484-5491.
13. *Dupont, A., Cusan, L., Garon, M., Alvarado-Urvina, G.* and *Labrie, F.* (1977): *Life Sci.*, 21. 907-914.
14. *Erdös, E.G.* (1977): *Fed. Proc.*, 36: 1760-1765.
15. *Erdös, E.G., Johnson, A.R.* and *Boyden, N.T.* (1978): *Biochem. Pharmacol.*, 27: 843-848.
16. *Fournié-Zaluski, M.C., Perdrisot, R., Gacel, G., Swerts, J.P., Roques, B.P.* and *Schwartz, J.C.* (1979): *Biochem. Biophys Res. Commun.*, 91: 130-135.
17. *Gros, C., Pradelles, P., Rouget C., Bepoldin, D., Dray, F., Fournié-Zaluski, M.C., Roques B.P., Pollard H., Llorens-Cortes, C.* and *Schwartz, J.C.* (1978): *J. Neurochem.*, 31. 29-39.
18. *Guyon, A., Barbet, J., Roques, B.P., Swerts J.C., Malfroy, B.* and *Schwartz, J.C.* (1979): *Biochem. Biophys. Res. Commun.*, 88: 919-926.
19. *Hambrook, J.M., Morgan, B.A., Rance, M.J.* and *Smith, C.F.* (1976): *Nature*, 262: 782-783.
20. *Harris, J.* and *Kamierowski, D.T.* (1975): *Life Sci.*, 16: 1831-1836.
21. *Henderson, G., Hughes, J.* and *Kosterlitz, H.W.* (1978): *Nature*, 271. 677-679.
22. *Klee, W.A.* and *Streaty, R.H.* (1974): *Nature*, 248: 61-63.
23. *Knight, M.* and *Klee, W.A.* (1978): *J. Biol. Chem.*, 253: 3843-3847.
24. *Lane, A.C., Range, M.J.* and *Walter, D.S.*, (1977): *Nature*, 269: 75-76.
25. *Malfroy, B., Swerts, J.P., Guyon, A., Roques B.P.* and *Schwartz, J.C.* (1978). *Nature*, 276: 523-526.

26. *Malfroy, B. Swerts, J.P., Llorens, C.* and *Schwartz, J.C.* (1979): *Neurosci. Lett.*, 11: 329-334.

27. *Marks, N., Grynbaum, A.* and *Neidle, A.* (1977): *Biochem. Biophys. Res. Commun.*, 74: 1552-1559.

28. *Meek, K.L., Yang, H.Y.T.* and *Costa, E.* (1977): *Neuropharmacol.*, 16: 151-154.

29. *Pardo, J.V., Creese, I., Burt, D.R.* and *SNYDER, S.H.* (1977): *Brain Res.*, 125. 376-382.

30. *Pert, C.B., Pasternak, G.W.* and *SNYDER S.H.* (1973): *Science*, 182: 1359-1361.

31. *Pert, C.B., Pert, A., Chang, J.K.* and *Fong, B.T.W.* (1976): *Science*, 194: 331-332.

32. *Pollard, H., Llorens, C., Schwartz, J.C., Gros, C.* and *Dray, F.* (1978): *Brain Res.*, 151: 392-399.

33. *Quicho, F.A.* and *Lipscomb, W.N.* (1971). *Adv. Prot Chem.*, 15: 1-48.

34. *Roemer, D., Blescher, H.M., Hill, R.C., Pless, J., Bauer, W., Cardinaux, F., Closses, A., Hauser, D.* and *Huguenin, R.* (1977): *Nature*, 268: 547-549.

35. *Roemer, D.* and *Pless, J.* (1979): *Life Sci.*, 24: 621-624.

36. *Schwartz, J.C., De La Baume, S., Malfroy, B., Patey, G., Perdrisot, R., Swerts, J.P. Fournié-Zaluski, M.C., Gacel, G.* and *Roques, B.P.* Advances in Biochemical Psychopharmacology. Ed. M.T. Trabucchi, Raven Press, N. Y. (in press).

37. *Singh, E.A.* and *Mc Geer, E.G.* (1978): *Ann Neurol.*, 4: 85-86.

38. *Sullivan, S., Akil, H.* and *Barchas, J.C.* (1979). *Commun. in Psychopharmacol.*, 2: 525-531.

39. *Swertz, J.P., Perdrisot, R., Malfroy, B.* and *Schwartz, J.C.* (1979): *Eur. J. Pharmacol.*, 53: 209-210.

40. *Swerts, J.P., Perdrisot, R., Patey, G. De La Baume S.* and *Schwartz, J.C.* (1979) *Eur. J. Pharmacol.*, 57: 279-281.

41. *Takagi, H., Shioni, H., Ueda, H.* and *Amawo, H.* (1979): *Eur. J. Pharmacol.*, 55: 109-114.

42. *Vogel, Z.* and *Altstein, H.* (1977): *Febs Lett.*, 80: 332-336.

43. *Vogel, Z.* and *Altstein, H.* (1979). *Febs Lett.*, 98: 44-48.

44. *Yang, H.Y.T.* and *Neff, N.H.* (1973): *J. Neurochem.*, 19: 2443-2450.

45. *Guyon, A., Roques, B.P., Guyon, F., Foucault, A., Perdrisot, R., Swerts, J.P.* and *Schwartz, J.C.* (1979): *Life Sci.*, 25: 1605-1612.

Neurological Effects of MIF-1, MSH, and Opiate Peptides in Clinical Studies

ABBA J. KASTIN, RUDOLPH H. EHRENSING, RICHARD D. OLSON, and DAVID H. COY

VA Medical Center, Tulane University School of Medicine, Ochsner Medical Institutions, and University of New Orleans, New Orleans, Lousiana.

INTRODUCTION

It could be said that the field of neurology has played an essential role in developing the concept of CNS effects of peptides found in the hypothalamus, i.e. brain peptides. Antiparkinsonian agents are active in the dopa-potentiation system used in animals to demonstrate that the pituitary gland is not required for potentiation of the effects of the tripeptide MIF-1 (Pro-Leu-Gly-NH_2) on motor activity (23). This led to the early evaluation of MIF-1 in patients with Parkinson's disease (14). Subsequently, other hypothalamic peptides have been found to have "extrapituitary" or "extra-endocrine" effects. The more recent discovery of the opiate peptides enkephalin and endorphin in the brain has focused even more attention upon the brain peptides. This brief review will discuss the clinical studies involving neurological findings that have been performed with MIF-1, MSH, and the brain opiates.

MIF — I

The series of animal studies that demonstrated the CNS effects of MIF-1 have been reviewed several places, including *Life Sciences* (17). In the initial clinical study of this peptide in Parkinson's disease, doses of 20-50 mg MIF-1 were administered iv for an hour orally for up to 3 months. Improvement in rigidity, akinesia and tremor averaged between 20-40 % (14). In 1974, two additional studies were reported. In one, 20 mg was infused over an hour in 6 patients and a "mild amelioratory effect in 3 of 6 untreated patients" was described (6). The second study of that year found that "the neurological assessment after 14 days of 30 mg MIF showed a mild but significant improvement in the entire status" (10).

More dramatic results were reported in 1975. One study more closely approximated the animal model of the dopa-potentiation test by using patients already receiving dopa (2). Barbeau found "the improvement obtained in almost all (5/6) cases of such magnitude that it far surpassed the clinical effect of any of the numerous antiparkinson drugs which we have tested in our laboratory over the past 15 years, including levodopa alone". That same year, Gerstenbrand *et al.* (11) used much larger doses of MIF-1 (400 mg) infused iv continuously for 10 days. He found 25-75 % improvement in 9 of 10 patients, concluding that "MIF-I is a very promising therapeutic agent in the management of Parkinson's disease" (11).

Two other investigations published the following year used MIF-1 alone. In one, the group receiving MIF-I orally for a month in the dose of 250 mg daily showed 24 % motor improvement compared with 6 % for the group receiving placebo, but this improvement disappeared as the study continued with higher doses (4). In the other, a single injection of 200 mg MIF-1 was given to 8 patients with Parkin-

son's disease by the iv route over a 5 minute period. Improvement began within 2 hours and persisted about 6 hours (3).

In 1978 Schneider *et al.* (26) pursued the interactions of MIF-1 and dopa by studyng 6 men and 6 women who were taking dopa. In this double-blind, cross-over design, they found statistically significant improvement, especially in fine motor performance, after the iv administration of 800 mg MIF-1 (200 mg every hour x 4). Gerstenbrand *et al.* (12) performed this second set of trials of MIF-1 also using patients receiving dopa. In patients injected iv with 400 mg MIF-1 daily for 10-15 days, a 25-75 % global improvement was observed (12).

The latest clinical study of MIF-1 in parkinsonism failed to find statistically significant improvement in the total disability score of 8 patients receiving dopa (5). Improvement in a rating of self-sufficiency, however, was statistically different from that found after placebo. Moreover, a definite trend toward improvement could be seen in total score, rigidity, and akinesia, but not in tremor, after the single iv administration of 200 mg MIF-1 (5).

The improvement in mood first observed by Ehrensing *et al.* (7,8) has been confirmed in several of the studies reported above (2, 10-12). The dose-related effects of MIF-1 in mental depression emphasize results obtained from animal studies, that "more" is not necessarily better. Dose-related effects of parenterally administered MIF-1 in parkinsonian patients on dopa have not been carefully explored. Moreover, problems involving the route of administration as well as stability of the peptide require more thorough evaluation.

It is not clear whether MIF-1 will ever have widespread clinical applicability in the treatment of Parkinson's disease. What is of greater importance at this time is the fact that a naturally occurring brain peptide can have any action whatsoever in a neurological disorder. The treatment of neurological diseases with peptides is an intriguing new approach. Whether alterations in brain levels of these peptides are involved in the etiology of neurological disorders also deserves consideration.

MSH

More than 10 years ago, in the first clinical study of the CNS effects of MSH, we found some changes in the EEG (15). In 1971, a second study reported a significant increase in the averaged somatosensory cortical evoked response after iv infusion of 10 mg α-MSH (16). These changes were found to be greatest when the subject was attending to the stimulus. A large number of studies in laboratory animals have now shown that MSH affects the processing of information, as in attention, rather than memory (18).

The next clinical study of MSH used the peptide's active core which consists of the fourth through the tenth amino acids of a sequence it shares with ACTH (22). The occipital EEG was passed through 4 bandpass filters and analyzed in terms of the power output of the filters. Those normal subjects receiving MSH/ACTH 4 - 10 showed an increase in the power output of the 12+ Hz and the 7 — 12 Hz bands together with a slight decrease in the output of the 3 — 7 Hz band (22). Moreover, subjects receiving the peptide showed more alpha (7 — 12 Hz) blocking. Changes in the magnitude of the contingent negative variation (CNV) were not significant in this (22) or another study (1).

Electrophysiological measurements were also made in another study of the effects of a single sc injection of MSH/ACTH 4—10 in students (21). Averaged evoked potentials were obtained from right and left occipital areas and analyzed visually and by power spectral analysis. The visual evoked potential data showed statistically significant increase in the latency and decrease in the magnitude of the negative component ocurring about 200 msec. In addition, an additional negative element which was absent during the control runs was observed at about 350 msec in subjects receiving the MSH peptide (21). EEGs obtained during the rest periods of a continuous performance task indicated a tendency toward lower amplitude and faster frequency alpha activity after MSH/ACTH 4—10.

These are the only clinical studies with MSH which have used electrophysiological measurements except for one investigation

which found no effect with MSH/ACTH 4—10 (9). The others, including a promising one in mentally retarded individuals (25), have focused on psychological and behavioral measurements. An additional potential application of MSH and its active core to the treatment of neurological disorders involves a stimulatory effect on muscles. This effect and some of the early clinical results, have been reviewed by Strand's group which has pioneered this approach (27).

The pharmaceutical company (Organon) which has provided the 4—10 sequence of MSH and ACTH for these clinical studies has more recently encouraged studies with a modified 4—9 analogue. Our group, however, believes that fragments and analogues of MSH which in one paradigm appear to differ only in potency may have independent effects which could be overlooked unless different paradigms are examined. There is evidence to support this with the MSH/ACTH peptides (24) as with the opiate peptides (28).

OPIATE PEPTIDES

Two clinical studies performed with the brain opiates have involved electrophysiological measurements. Other studies were more concerned with the possible beneficial effects of opiate peptides on schizophrenia and will not be discussed here. Sandoz, Ltd. has synthesized an analogue of Met-enkephalin with extremely potent analgesic properties. In one study, they found that administration of 0.5 mg of the analogue FK 33—824 to 8 normal men caused the following EEG effects: an increase in the abundance and amplitude of the EEG with a tendency to hypersynchronization and spindling in the alpha band, an increase in beta activity, and a simultaneous decrease of slow waves in the theta and delta band (20). These changes differed from those seen after placebo and from those known to occur after the opioid alkaloids. More importantly, the EEG changes were not reversed by nalorphine. The findings confirmed the somewhat similar EEG changes observed in 12 subjects in a previous study (13). The effects in this other clinical investigation also were not reversed by nalorphine.

The failure of nalorphine to reverse the changes induced by FK 33—824 in these 2 studies support the concept that the CNS effects of brain peptides can be dissociated from their narcotic effects. Although this concept was very unpopular when it was first proposed and experimentally supported in 1976 (19), it is now well on its way to being universally accepted, even by some of its former critics. The evidence for this concept as well as that for the CNS effects of peptides in general has recently been reviewed elsewhere (17).

SUMMARY

Clinical studies of CNS effects have been performed with several of the more promising brain peptides. The tripeptide MIF-1 (Pro-Leu-Gly-NH_2) has been shown effective, to varyin extents, in several studies of patients with Parkinson's disease. Electrophysiological changes have accompanied the improvement in attention seen after administration of MSH. Recent support for our concept of a dissociation between the CNS/behavioral effects of the brain opiates and their narcotic effects has been shown by the failure of nalorphine to reverse EEG changes found after injection of an enkephalin analogue in normal subjects. Peptides, therefore, may offer a new approach to the study and treatment of neurological disorders.

RESUMEN

Estudios clínicos de los efectos en el sistema nervioso central han sido realizados con varios de los más conocidos péptidos del cerebro. El Tripéptido MIF-1 (Pro-Len-Gly-NH_2) se ha mostrado efectivo en diversos aspectos en varios estudios pacien-

tes con enfermedad de Parkinson. Cambios electrofisiológicos han acompañado la mejoría en la atención observada después de la administración de MSH.

Apoyo reciente para nuestro concepto, de una disociación entre los efectos en CNS en relación con la conducta de los opiáceos del cerebro y sus efectos narcóticos, han sido mostrados por la falla de la nalorfina para invertir cambios electroencefalográficos hallados después de la inyección de un análogo de la encefalina en individuos normales. Los péptidos de este modo pueden ofrecer un nuevo enfoque al estudio y tratamiento de desórdenes neurológicos.

RÉSUMÉ

Etudes clinics des effets dans le système nerveux central furent faites avec plusieurs du plus connus peptides du cerveau.

Le Tripeptide M-I-F-1 (Pro-Len-gly-NH_2), il a aparut effectif dans divers aspects dans plusieurs études de patients avec la maladie de Parkinson. Changes electrophisiologiques ont eté observé avec l'amélioration de l'attention produit aprés l'administration de MSH.

En appui de nôtre opinion d'une dissociation entre les effets dans le systeme nerveux central en relation avec la conduite des opiaces du cerveau, et leurs effets narcotiques furent montrés por le défaut de la nalorfine pour transposer changements electroencephalographiques trouvés aprés l'injection d'un analogue de l'encéphaline en individus normales.

De cette façon les peptides peuvent offrir un nouveau point de vue dans l'étude et traitement des desordres neurologiques.

ZUSAMMENFASSUNG

K-inische Studien der Effekte im Zentralnervensystem sind mit verschiedenen der meisten bekannten Peptiden des Gehirns gemacht worden. Das Tripeptid MIF-I (pro-len-gly-nh_2) hat sich effektiv gezeigt in verschiedener Hinsicht beim Studium von Patienten mit der Parkinsonschen Krankheit. Elektrophysiologische Wechsel haben die Mehrheit bei der beobachteten Aufmerksamkeit nach der Anwendung von MSH.

Eine Stütze unserer Anschauung, die einer Disoziation zwischen den Effekten von CNS in Bezug auf die Beziehung des Konduktes der Opiate aufs Gehirn und seine narkotischen Effekte, sind gezeigt worden durch das Fehlen der Nallorphine, um elektroenzephalographische Wechsel zu invertieren, die nach der Injektion eines Analoges des enzephalins bei normalen Individuums. Die Peptide können auf diese Art einen neuen Lichtblick zum Studium und Behandlung neurologischer Unordnungen geben.

ACKNOWLEDGEMENTS

The studies reviewed here were supported in part by the Veterans Administration. We appreciate the encouragement of Dr. K.L. Bick.

REFERENCES

1. *Ashton, H.; J.E. Millman; R. Telford; J.W. Thompson; T.F. Davies; R. Hall; S. Shuster; A.J. Thody; D.H. Coy* and *A.J. Kastin*: Psychopharmacological and Endocrinological Effects of Melanocyte Stimulating Hormones in Normal Man. Psychopharmacology 55: 165-172 (1977).
2. *Barbeau, A.*: Potentiation of Levodopa effect by Intravenous L-prolyl-L-Leucyl-Glycine Amide in Man. Lancet 2: 683-684 (1975).
3. *Barbeau, A.; M. Gonce* and *A.J. Kastin*: Neurologically Active Peptides. Pharmac. Biochem. Behav. (Suppl. 1) 5: 159-163 (1976).
4. *Barbeau, A.; M. Roy and A.J. Kastin*: Double-Blind Evaluation of Oral L-Prolyl-L-

Leucyl Glycine Amide in Parkinson's Disease. Canad. Med. Assoc. J. 114: 120-122 (1976).

5. *Caraceni, T.; E.A. Parati; F. Girotti; I. Celano C. Frigeiro; D. Cocchi* and *E.E. Muller*: Failure of MIF-I to Affect Behavioral Responses in Patients with Parkinson's Disease Under L-Dopa Therapy. Psychopharmacology 63: 217-222 (1979).
6. *Chase, T.N.; A.C. Woods; M.A. Lipton* and *C.E. Morris*: Hypothalamic Releasing Factors and Parkinson Disease. Arch. Neurol. 31: 55-56 (1974).
7. *Ehrensing, R.H.* and *A.J. Kastin*: Melanocyte-Stimulating Hormone-Release Inhibiting Hormone as an Anti-Depressant: A Pilot Study. Arch. Gen. Psychiat. 30: 63-65 (1974).
8. *Ehrensing, R.H.* and *A.J. Kastin*: Dose-Related Biphasic Effect of Prolyl-Leucyl-Glycinamide (MIF-I) in Depression. Am. J. Psychiat. 135. 562-566 (1978).
9. *Ferris, S.H.; G. Sathananthan; S. Gershon; C. Clark* and *J. Moshinsky*: Cognitive Effects of ACTH 4-10 in the Elderly. Pharmac. Biochem. Behav. (Suppl. 1) 5: 73-78 (1976).
10. *Fischer, P.A.; E. Schneider P. Jacobi* and *H. Maxion*: Effect of Melanocyte-stimulating hormone release inhibiting hormone (MIF) in Parkinson's Syndrome. Europ. Neurol. 12: 360-368 (1975).
11. *Gerstenbrand, F.; H. Binder; C. Kozma; S. Pusch* and *T. Reisner*: Infusiontherapie Mit MIF (Melanocyte inhibiting factor) beim Parkinson-Syndrom. Wiener Klinische Wochenschift 87: 822-823 (1975).
12. *Gerstenbrand, F.; W. Poewe; F. Aichner* and *C. Kozma*: Clinical Utilization of MIF-I; in Central Nervous System Effects of Hypothalamic Hormones and Other Peptides, pp. 415-426. (Raven Press, New York, 1979).
13. *Graffenried, B.; E. del Pozo; J. Roubicek; E. Krebs; W. Poldinger; P. Burmeister* and *L. Kerp.* Effects of the Synthetic Enkephalin Analogue FK 33 824 in Man. Nature 272: 729-730 (1978).
14 *Kastin, A.J.;* and *A. Barbeau*: Preliminary Clinical Studies with L-Prolyl-L-Leucyl-Glycine Amide in Parkinson's Disease. Canad. Med. Assoc. J. 107: 1079-1081 (1972).
15. *Kastin, A.J.; S. Kullander; N.E. Borglin; L. Dyster Aas; B. Dahlberg; D. Ingvar; C.E.T. Krakau; M.C. Miller; C.Y. Bowers* and *A.V. Schally*: Extra-pigmentary Effects of MSH in Amenhorrheic Women. Lancet 1: 1007-1010 (1968).
16. *Kastin, A.J.; L.H. Miller; D. Gonzales-Barcena; W.D. Hawley; K. Dyster-Aas; A.V. Schally; M.L. Velasco-Parra* and *M. Velasco*. Psycho-Physiologic Correlates of MSH Activity in Man. Physiol. Behav. 7: 893-896 (1972).
17. *Kastin, A.J.; R.D. Olson; A.V. Schally* and *D.H Coy*: CNS Effects of Peripherally Administered Brain Peptides. Life Sci. 25: 401-411 (1979).
18. *Kastin, A.J.; C.A. Sandman; L.O. Stratton; A.V. Schally* and *L.H. Miller*: Behavioral and Electrographic Changes in Rat and Man after MSH, in Hormones, Homeostasis and the Brain, pp. 143-150. (Elsevier Acientific Publishing Co., Amsterdam, 1975).
19. *Kastin, A.J.; E.L. Scollan; M.G. King; A.V. Schally* and *D.H. Coy*: Enkephalin and a Potent Analog Facilitate Maze Performance after Intraperitoneal Administration in Rats. Pharmac. Biochem. Behav. 5: 691-695 (1976).
20. *Krebs, E.* and *J. Roubicek*: EEG and Clinical Profile of a Synthetic Analogue of Methionine-Enkephalin-FK 33-824. Pharmakopsychiat. 12: 86-93 (1979).
21 *Miller, L.H.; L. Harris; A.J. Kastin* and *H. Van Riezen*: A Neuroheptapeptide Influence in Attention in Man. Pharmac. Biochem. Behav. (Suppl. 1) 5. 17-21 (1976).
22. *Miller, L.H.; A.J. Kastin, C.A. Sandman; M. Fink* and *W.J. Van Veen.* Polypeptide Influence on Attention, Memory, and An-Anxiety in Man. Pharmac. Biochem. Behav. 2: 663-668 (1974).
23. *Plotnikoff, N.P.; A.J. Kastin; M.S. Anderson* and *A.V. Schally.* DOPA Potentiation by a Hypothalamic Factor, MSH Release-Inhibiting Hormone (MIF) Life Sci. 10: 1279-1283 (1971).
24. *Sandman, C.A.; B.E. Beckwith* and *A.J. Kastin.* Are Learning and Attention Related to the Sequence of Amino Acids in ACTH/MSH Peptides? Peptiles 1: 277-280 (1980).
25. *Sandman, C.A.; J. George; B. Walker; J.D. Nolan* and *A.J. Kastin.* The Heptapeptide MSH/ACTH 4-10 Enhances Attention in the Mentally Retarded. Pharmac. Biochem. Behav. (Suppl. 1) 5: 23-28 (1976).
26. *Schneider, V.E.; P.A. Fischer; P. Jacobi* and *W. Reh*: Der Einflub von MIF (Melanozyteninhibierender Faktor) auf Psychomotorik und Stimmung sceerhalten von Parkinsonkranken. Arzneimittel-Forschung Drug Res. 28: 1296-1297 (1978).
27. *Strand, F.L.; A. Cayer,; E. Gonzalez* and *H. Stoboy.* Peptide Enhancement of Neuromuscular Function. Animal and Clinical Studies. Pharmac. Biochem. Behav. (Suppl. 1) 5: 179-187 (1976).
28. *Veith, J.L.; C.A. Sandman; J.M. Walker; D.H. Coy* and *A.J. Kastin;* Systemic Administration of Endorphins Selectively Alters Open Field Behavior of Rats. Physiol. Behav. 20: 539-542 (1978).

Neurophysiological Aspects of Centrally Acting Peptides

LEO P. RENAUD,
Division of Neurology,
The Montreal General Hospital,
1650 Cedar Avenue,
Montréal, Québec, H3G 1A4, Canada

INTRODUCTION

Although nearly five decades have elapsed since a peptide substance was initially discovered in brain by von Euler and Gaddum (1931) and almost three decades have passed since it was first suggested that peptides might mediate transmission between neurons (see Lembeck and Zetler, 1962), most of our knowledge about brain peptides has resulted from developments within the past ten years. The rapid growth of interest in neuropeptides stems mainly from success in the isolation, structural characterization and synthesis of several endogenous brain peptides. With the introduction of electrophysiological techniques into neuropharmacology and their adaptation to the study of brain peptides, there has emerged new and exciting information on mechanisms of interneuronal communication and neuromodulation (c.f. Renaud and Padjen, 1978; Barker, these proceedings).

In this paper, we present a brief review of two closely related neurophysiological approaches, both based on in-vivo extracellular recording techniques, that we have utilized to understand the roles and mechanisms of action of neuropeptides. On the one hand, we have utilized electrical stimulation techniques to examine the localization and central connections of two identifiable peptidergic systems of hypothalamic origin. Evidence for peptide receptors on central neurons has been subsequently examined by microiontophoresis in search of peptide-sensitive neurons and evidence of interactions between peptides and other putative neurotransmitters.

PEPTIDE PATHWAYS OF THE HYPOTHALAMUS

Radioimmunoassay and immunohistochemical data indicate that the hypothalamus contains a wealth of peptides and peptide pathways. During the course of our studies on the connections of neurons in the hypothalamus (c.f. Renaud, Blume, Kearney, Mac Kenzie and Pittman, 1978) it was deemed relevant to attempt a more detailed examination of the electrophysiology of some of these peptidergic pathways, and therefore complement the neuropharmacological studies described elsewhere in this section. Within the hypothalamus, two peptidergic neural systems can be identified with reasonable certainty: the *neurohypophyseal system*, composed of cells that project to the posterior pituitary, and the *tuberoinfundibular system*, composed of neurons that project to the median eminence. The distribution of these pathways is schematically outlined in Fig. 1. Since both of these systems send axons to a termination site that is uniquely separate from the rest of the brain, these axon terminals can be stimulated electrically in relative isolation from adjacent neural pathways, and neurons belonging to each system can be identified according to techniques of antidromic activation.

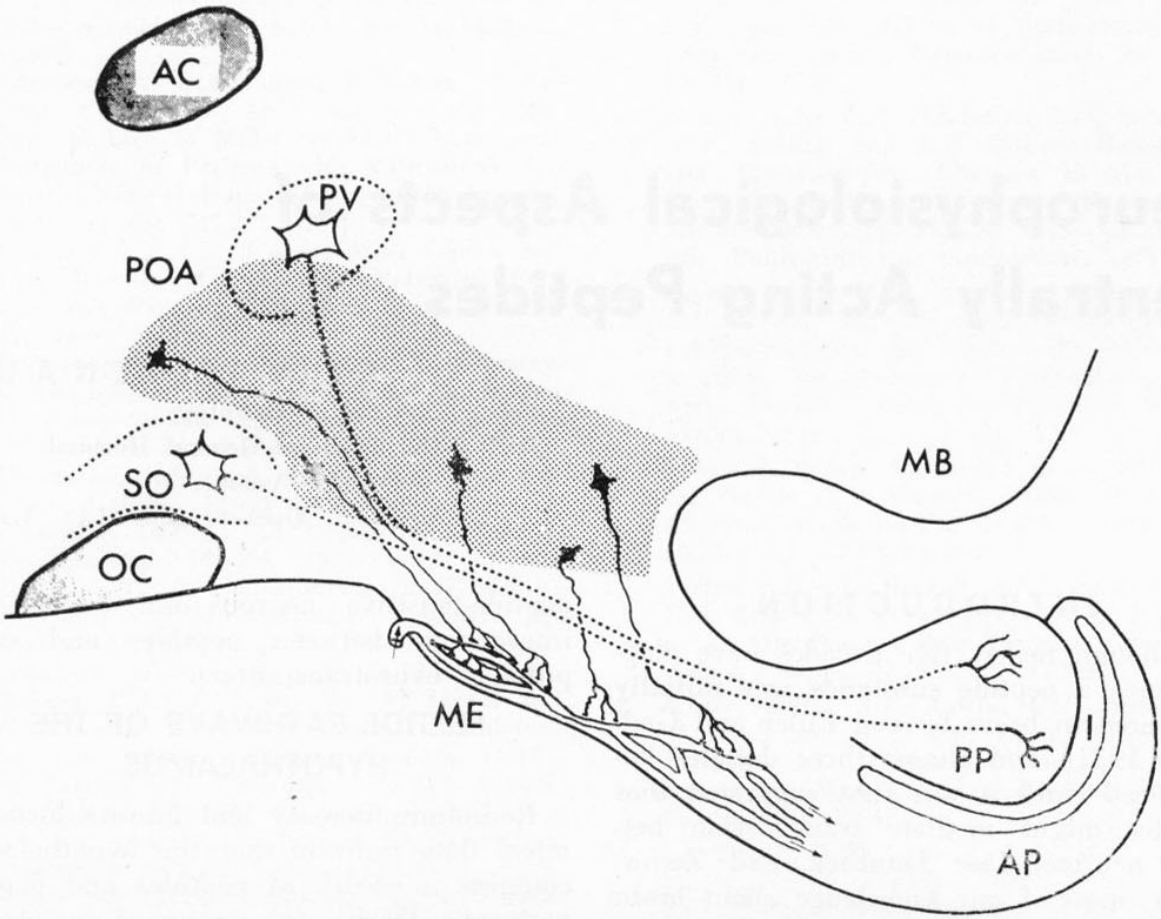


Fig. 1 — A simplified sketch of the rat hypothalamus in saggital section to illustrate the two classic neurosecretory pathways related to the pituitary: the *neurohypophyseal* tract is indicated as interrupted lines originating from magnocellular paraventricular (PV) and supraoptic (SO) neurons, terminating in the posterior pituitary (PP); the *tuberoinfundibular* pathway is seen to originate from parvicellular neurons in the medial hypothalamus, preoptic area (POA) and mamillary body region (MB), with axon terminations on portal capillaries in the median eminence (ME) for the release of factors that regulate anterior pituitary (AP) secretion.
Abbreviations: AC, anterior commissure; OC, optic chiasm; I, intermediate lobe. From Renaud, 1978.

THE TUBEROINFUNDIBULAR SYSTEM. The hypothalamus maintains a dominant influence on the secretion of anterior pituitary hormones. This is accomplished through a parvicellular neuronal system that projects to the portal capillary plexus in the median eminence. Axon terminals in this region secrete release or release-inhibiting factors into the pituitary portal circulation (Harris, 1955; Szentagothai, Flerkó, Mess and Halász, 1968; Blackwell and Guillemin, 1973; Schally, Arimura and Kastin, 1973). Although not yet concincingly demonstrated, release of catecholamines or peptides into the pituitary portal circulation is presumed to arise in response to appropriate neural stimuli and to be related to activity in the tuberoinfundibular system, analogous to events in the neurohypophyseal system that are associated with the release of vasopressin and oxytocin (c.f. Poulain, Wakerley and Dyball, 1977). Visualization of tuberoinfundibular neurons has been accomplished with current immunohistochemical techniques. However, relatively little is known of the neurophysiology of tuberoinfundibular neurons. Since an understanding of this neural system has an ultimate bearing on the release and action of TRH, LH-RH, somatos-

tatin and other releasing factors yet to be structurally characterized, considerable attention has been devoted to a study of the location and connections of tuberoinfundibular neurons using electrophysiologic techniques.

Discrete electrical stimulation applied to the surface of the median eminence through a transpharyngeal approach can evoke antidromic activation of neurons throughout the mediobasal hypothalamus and medial preoptic area (Fig. 2; Makara, Harris and Spyer, 1972; Sawaki and Yagi, 1973; Harris and Sanghera, 1974; Moss, Kelly and Riskind, 1975; Renaud, 1976; Renaud, Blume and Pittman, 1978). This distribution corresponds to the location of neurons that demonstrate immunoreactivity for a variety of peptides. The latencies for antidromic activation indicate that most tuberoinfundibular neurons conduct impulses at frequencies below 1.0 m/sec. Under conditions of general anaesthesia, the majority of tuberoinfundibular neurons show little or no spontaneous activity; some cells display random activity patterns; others display

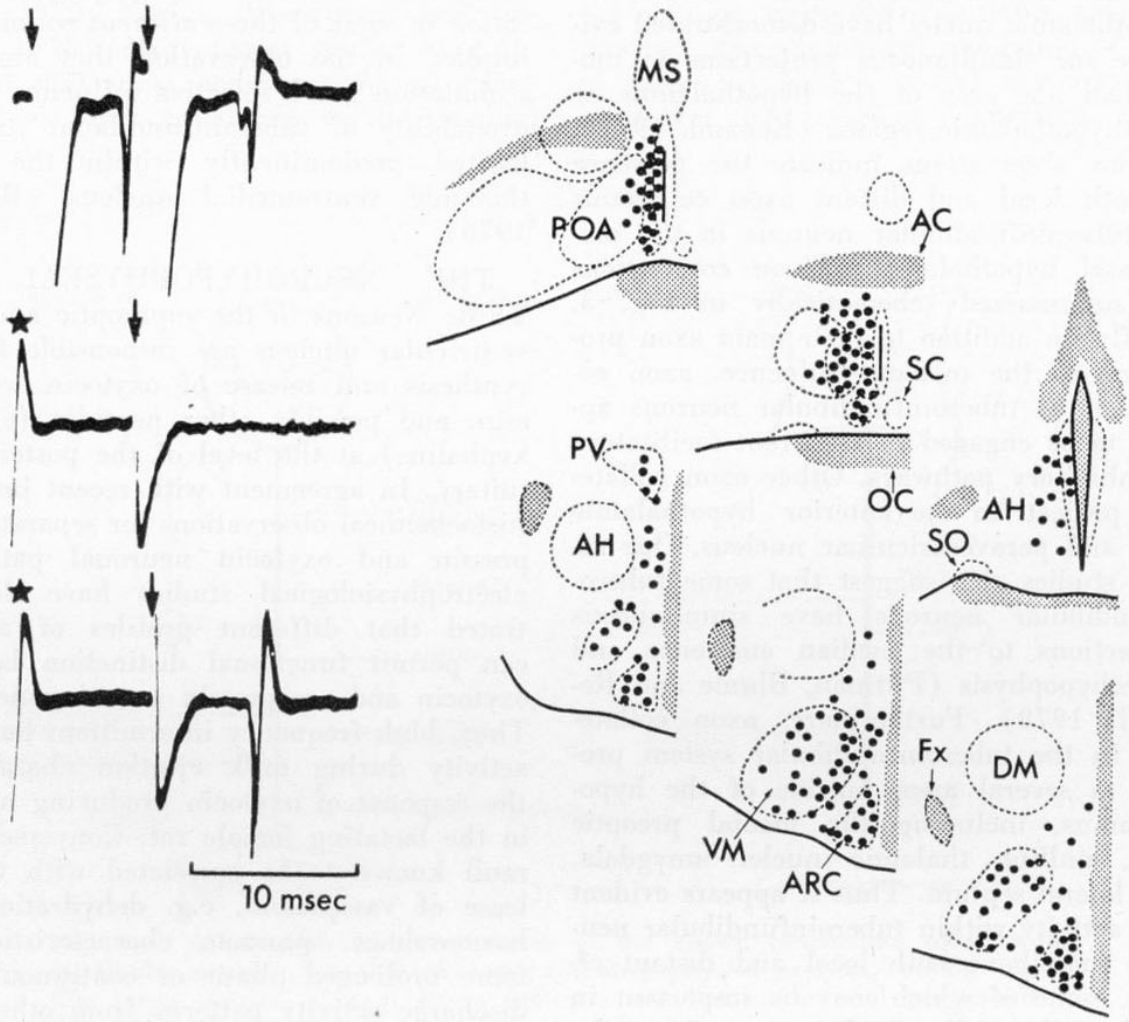


Fig. 2 — Identification and location of tuberoinfundibular neurons. The oscilloscope sweeps on the left illustrate antidromic invasion of a neuron in the ventromedial nucleus following stimulation of the surface of the median eminence (at arrows). This neuron demonstrates constant latency responses to paired median eminence stimulation at frequencies greater than 150 Hz (upper trace), and collisions at appropriate intervals between spontaneous (star) action potentials and antidromic evoked action potentials (lower traces). On the right, the black dots located throughout the medial hypothalamus and the medial preoptic area represent the distribution of tuberoinfundibular neurons identified using antidromic invasion criteria.
From Renaud, Blume and Pittman, 1978.

short bursts of action potentials, a pattern that is most pronounced among tuberoinfundibular neurons located within the area of the paraventricular nucleus (Blume, Pittman and Renaud, 1978). The latter resemble the phasic activity patterns considered characteristic of vasopressin secreting neurons (Poulain, Wakerley and Dyball, 1977).

Previous electrophysiological studies on neurons in the arcuate and ventromedial hypothalamic nuclei have demonstrated evidence for simultaneous projections to more than one area of the hypothalamus or extrahypothalamic regions (Renaud, 1977). Similar observations indicate the presence of both local and distant axon collaterals for tuberoinfundibular neurons in the mediobasal hypothalamus. These connections are summarized schematically in Fig. 3. Briefly, in addition to their main axon projection to the median eminence, axon collaterals of tuberoinfundibular neurons appear to be engaged in recurrent facilitatory or inhibitory pathways. Other axon collaterals project to the anterior hypothalamic area and paraventriculuar nucleus. Our recent studies also suggest that some tuberoinfundibular neurons have simultaneous projections to the median eminence and neurohypophysis (Pittman, Blume and Renaud, 1978). Furthermore, axon collaterals in the tuberoinfundibular system project to several areas outside of the hypothalamus, including the medial preoptic area, midline thalamic nuclei, amygdala, and lateral septum. Thus it appears evident that activity within tuberoinfundibular neurons may have both local and distant effects, some of which may be important in feedback loops involved in central regulation of the neuroendocrine system. It would also seem appropriate to consider that substances released from median eminence nerve terminals are simultaneously released from these central connections, implying that peptides may be released at central synaptic sites to affect the excitability of neurons in both hypothalamic and extrahypothalamic regions. Our results with microiontophoretic applications of peptides support this notion.

Numerous studies using lesion and stimulation techniques have indicated that several areas of the brain, notably within the limbic system, can alter or evoke secretion of anterior pituitary hormones. Recordings from single tuberoinfundibular neurons have confirmed that these cells receive powerful excitatory and inhibitory connections from extrahypothalamic areas, notably the amygdala, lateral septum, medial preoptic area and dorsal hippocampus. Furthermore, there may be a topographical organization in some of these afferent connections, implied in the observation that amygdala stimulation has a selective influence on the excitability of tuberoinfundibular neurons located predominently within the hypothalamic ventromedial nucleus (Renaud, 1976).

THE NEUROHYPOPHYSEAL SYSTEM: Neurons in the supraoptic and paraventricular nucleus are responsible for the synthesis and release of oxytocin, vasopressin, and possibly other peptides (e.g. enkephalins) at the level of the posterior pituitary. In agreement with recent immunohistochemical observations for separate vasopressin and oxytocin neuronal pathways, electrophysiological studies have demonstrated that different profiles of activity can permit functional distinction between oxytocin and vasopressin secreting neurons. Thus, high frequency intermittent bursts of activity during milk ejection characterize the response of oxytocin producing neurons in the lactating female rat. Conversely, stimuli known to be associated with the release of vasopressin, e.g. dehydration and haemorrhage, promote characteristic and more prolonged phasic or continuous high discharge activity patterns from other neurohypophyseal neurons, considered to secrete vasopressin (Arnauld, Dufy and Vincent, 1975; Poulain, Wakerley and Dyball, 1977). Therefore, the choice of an appropriate experimental model will permit functional differentiation between oxytocin and vasopressin secreting neurons, and permit electrophysiological studies to be conducted on each type of cell.

From a more general point of view, neurohypophyseal neurons have been identified through antidromic activation not only wi-

thin the supraoptic and paraventricular nuclei, but also in lesser numbers through the mediobasal hypothalamus (Pittman, Blume and Renaud, 1978). Evidence for axon collaterals in the neurohypophyseal system has also been described with electrophysiological methods indicating the existence of direct or indirect recurrent inhibitory pathways to these neurons (Barker, Crayton and Nicoll, 1971; Nicoll and Barker, 1971; Dreifuss and Kelly, 1972; Negoro and Holland, 1972; Negoro, Visessuwen and Holland, 1973). More recent studies have indicated that some neurohypophyseal axons appear to send collaterals to the median eminence (Pittman, Blume and Renaud, 1978). Furthermore, a small population of neurons within the periventricular region send axons to both the neurohypophysis and to the area of the midbrain periaqueductal gray (unpublished observations). Some afferent connections to the neurohypophyseal system have been defined arising in the amygdala, lateral septum, medial midbrain and doral hippocampus (Fig. 3). It is not yet known whether these are selective for oxytocin or vasopressin secreting neurons; however, the anatomical separation of each of these peptidergic neural networks suggests that their afferent connections would

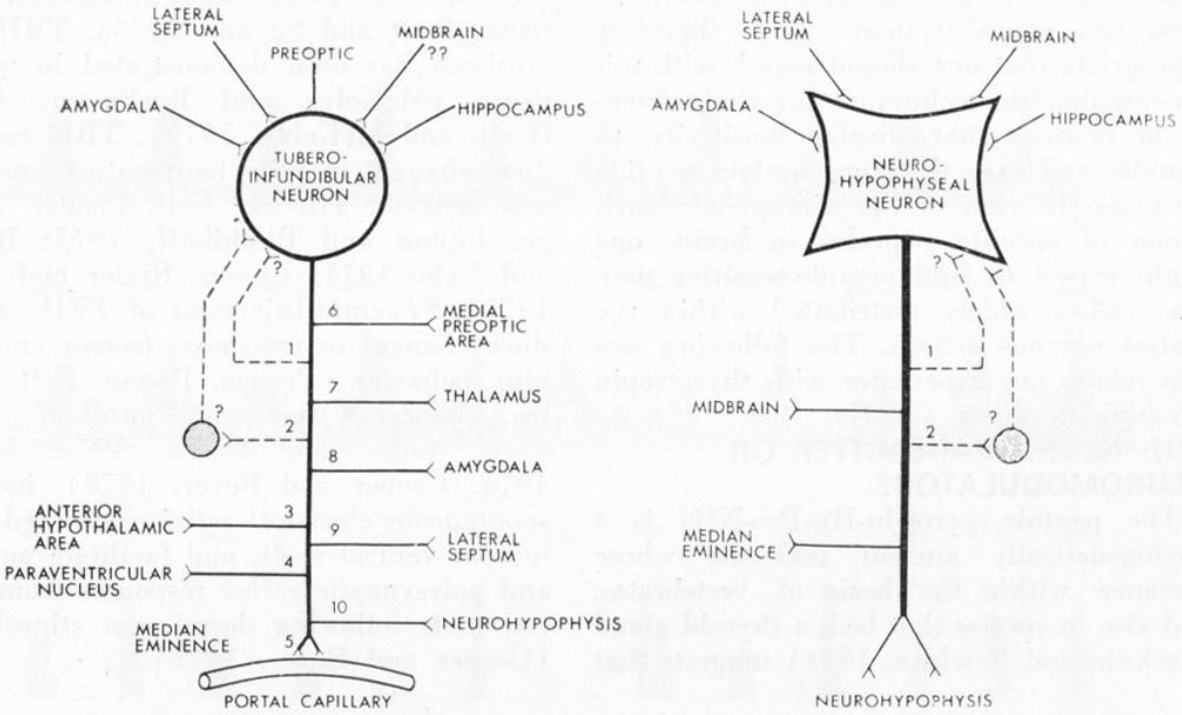


Fig. 3 — A schematic summary sketch of the known connections of tuberoinfundibular neurons (on the left) and neurohypophyseal neurons (on the right). The upper portion of each figure indicates sites known to send projections to the tuberoinfundibular or neurohypophyseal neurons respectively. The heavy vertical lines refer to the main peptidergic axons projecting to the median eminence portal capillary and neurohypophysis respectively, thus identifying each cell type. Additional efferent projections are indicated as axon collaterals of this main connection. Intrahypothalamic axon collaterals in recurrent pathways that either directly or indirectly (through local interneurons) engage the parent neuron, and whose transmitter agent is unknown (?) are depicted by numbers 1 and 2 in each system. Additional axon collaterals extend both within the hypothalamus and to extrahypothalamic areas, as indicated.
From Renaud, Pittman and Blume, 1979.

also be different.

The presence of neurophysin, vasopressin and oxytocin pathways in areas outside of the hypothalamus suggested the possibility that one and the same neuron might simultaneously project to both the posterior pituitary and to these extrahypothalamic regions. Our studies have indica-

ted that neurons in the paraventricular nucleus do indeed project to some of these areas, e.g., the amygdala and lateral septum, but none have demonstrated simultaneous connections with the neurohypophysis. Thus with the exception of the connections of periventricular cells to the neurohypophysis and midbrain region described above, these other projections of paraventricular neurons (presumably peptidergic in nature) appear separate from that to the neurohypophysis.

The presence of axon collaterals in the tuberoinfundibular and neurohypophyseal peptidergic systems suggest the presence of central synapses where peptides are likely to be released in association with activity in these two neural systems. It is therefore appropriate that one should search with microiontophoretic techniques for the existence of neurons that display sensitivity to peptides and may therefore contain peptide receptors. In view of the widespread distribution of specific peptides in brain, one might expect to find peptide-sensitive neurons rather widely distributed within the central nervous system. The following section relates our experience with thyrotropin releasing hormone (TRH).

TRH: NEUROTRANSMITTER OR NEUROMODULATOR?

The peptide pyroGlu-His-Pro-NH_2 is a phylogenetically ancient molecule whose presence within the brain of vertebrates, and also in species that lack a thyroid gland (Jackson and Reichlin, 1974) suggests that TRH may have a role in brain unrelated to its action on TSH secretion in the anterior pituitary. Several developments lend credence to a functional role for TRH at the synaptic level. TRH-like immunoreactivity is found within neurons and nerve fibres in specific areas of the central nervous system (Elde and Hökfelt, 1978). TRH is present within synaptosomes, and can be released from synaptosomes under appropriate circumstances (Edwardson and Bennett, 1977; Winokur, Davis and Utiger, 1977; Barnea, Parker, Neaves, Cho, Oliver and Porter, 1978; Jeffcoate, White, Bennett, Edwardson, Griffiths, Forkes and Kelly, 1979). High affinity TRH binding has been observed in rat brain subcellular fractions (Burt and Snyder, 1975). TRH biosynthesis has been demonstrated in neural tissue (McKelvy and Epelbaum, 1978; Hersh and McKelvy, 1979). TRH can induce changes in body temperature and animal behavior (Breese, Cott, Cooper, Prange, Lipton and Plotnikoff, 1975; Brown and Vale, 1975; Brown, Rivier and Vale, 1977). Systemic injections of TRH can induce changes in motricity, tremor and shaking behavior (Prange, Breese, Cott, Martin, Cooper, Wilson and Plotnikoff, 1975; Cohn, Cohn and Taylor, 1975; Kruse, 1976; Cooper and Boyer, 1978), increase spontaneous electrical activity recorded from lumbar ventral roots, and facilitate mono— and polysynaptic reflex responses from ventral roots following dorsal root stimulation (Cooper and Boyer, 1978).

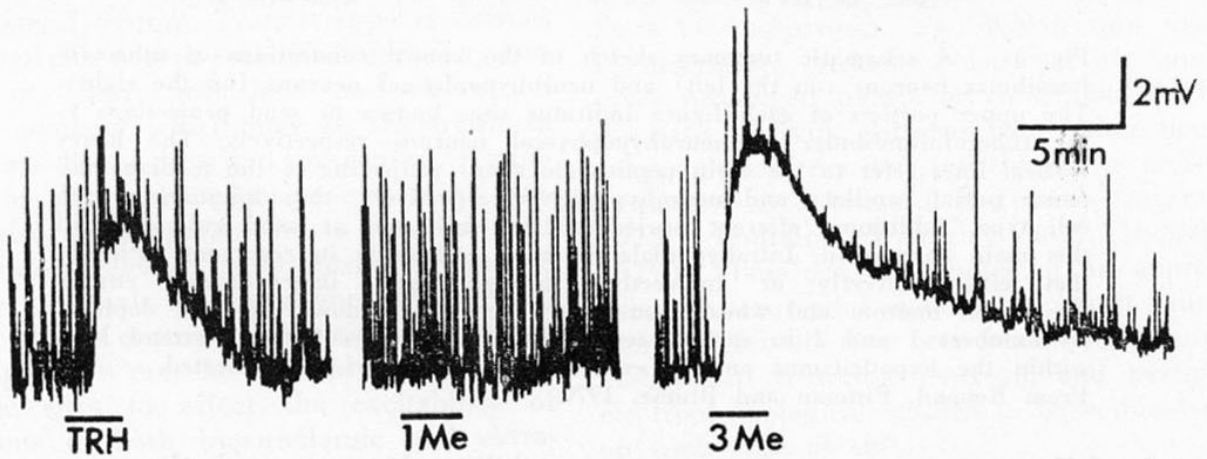


Fig. 4 — Effects of TRH and TRH analogs on isolated frog spinal cord motoneurons as indicated by ventral root recordings with sucrose gap. Depolarization is observed during perfusion with both TRH and the 3-methyl-histidine TRH analog, but not with the 1-methyl-histidine TRH analog. From Nicoll, 1978.

The hemisected frog spinal cord preparation in sucrose gap has been found suitable for direct and quantitative neuropharmacological studies of peptide actions. In this preparation, Nicoll (1977, 1978) has noted that TRH has a depolarizing action through both direct and indirect actions on motoneurons, and that the 3-methyl-histidine TRH analog is equal to or more potent than TRH, whereas the 1-methyl-histidine TRH analog is inactive (Fig. 4). Intracellular recordings have also suggested that a decrease in membrane resistance is associated with TRH actions, possibly due to an increase in sodium ion conductance. Nicoll has therefore suggested that in the spinal cord of this species, TRH may function in a background manner to facilitate transmission through pathways that subserve basic reflex activity (Nicoll, 1977).

In the vertebrate central nervous system, the early electrophysiological studies of Steiner (1972, 1975) yielded the initial evidence that microiontophoretic applications of TRH could provoke a decrease in spike discharge frequency of some spontaneously active neurons. Subsequent studies have illustrated that TRH does have a predominantly depressant effect on spontaneous or glutamate evoked activity of a cer-

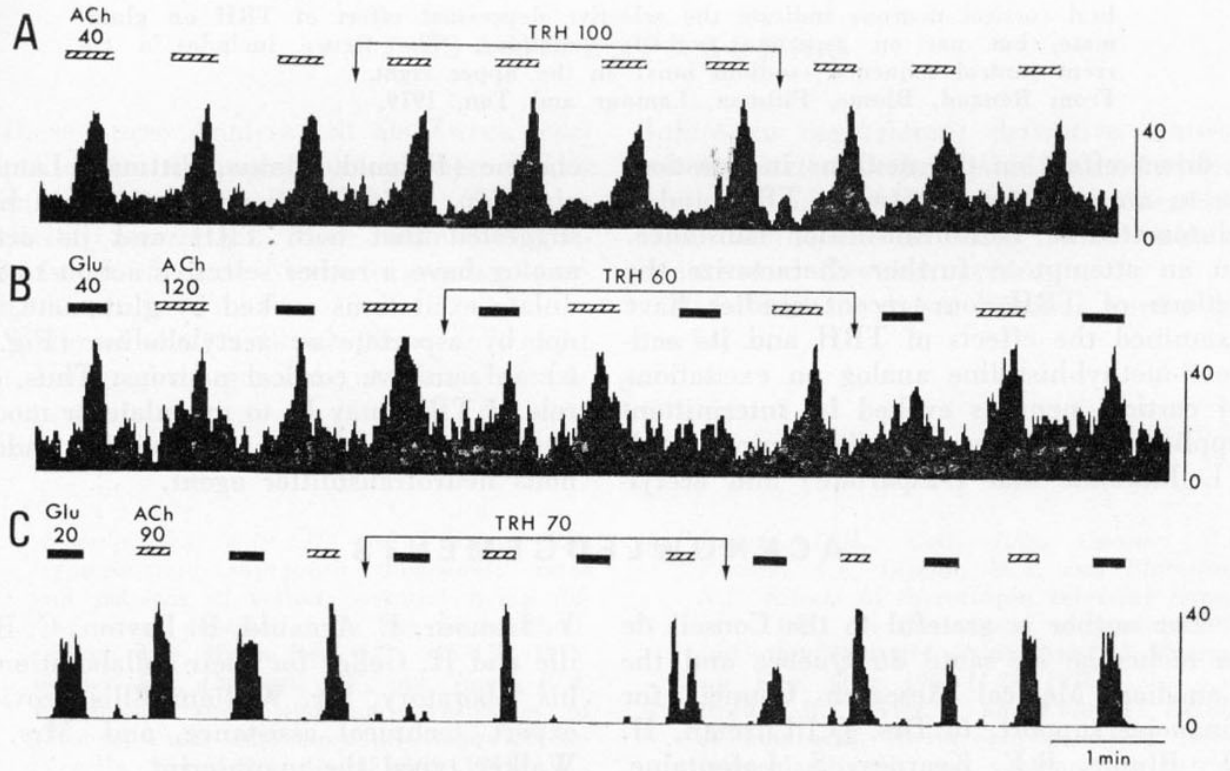


Fig. 5 — Upper and lower rate meter records obtained from two different neurons indicate the excitatory action of intermittent pulses of glutamate and acetylcholine on the excitability of neurons in the rat cerebral cortex. Numbers refer to microiontophoretic application currents. Note that during simultaneous application of TRH, the glutamate, but not the acetylcholine-evoked responses are diminished reversibly.
From Renaud, Blume, Pittman, Lamour and Tan, 1979.

tain percentage of neurons in the hypothalamus, cerebral cortex, cerebellum and brainstem (Dyer and Dyball, 1974; Renaud and Martin, 1975; Renaud, Martin and Brazeau, 1975, 1976; Winokur and Beckman, 1978). Some have reported an excitatory action with TRH (Moss, Dudley and Kelly, 1978; Mayer and McLeod, 1979). The depressant responses of TRH are generally brisk in onset and readily reversible, and are most evident on neurons whose activity is maintained by simultaneous applications of L-glutamate. It is uncertain whether these actions of TRH are due to

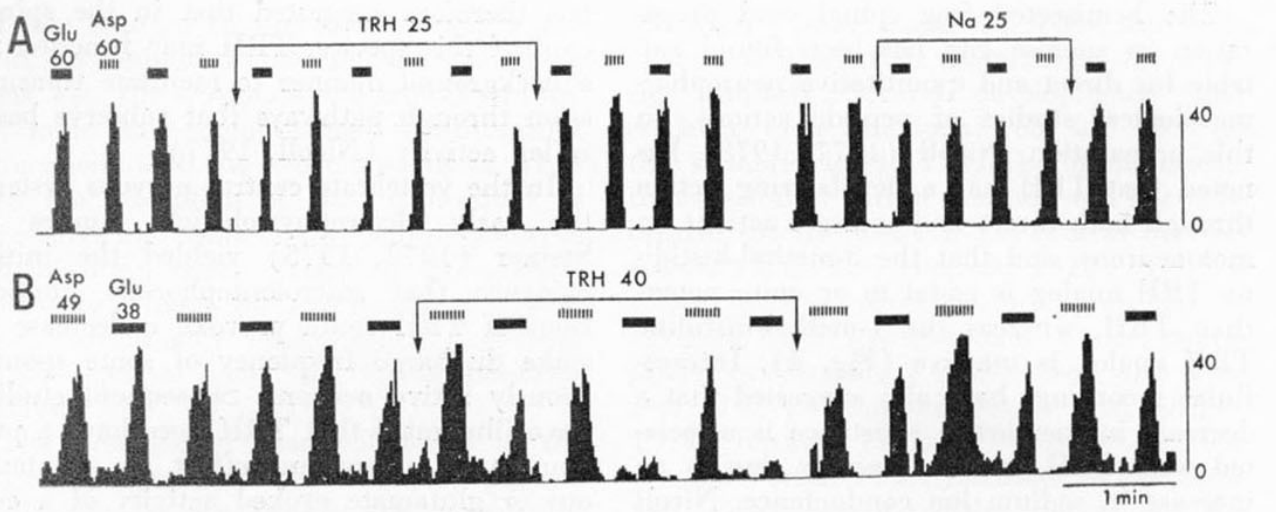


Fig. 6 — The upper and lower rate meter records from two different rat cerebral cortical neurons indicate the selective depressant effect of TRH on glutamate, but not on aspartate evoked excitations. The figure includes a current control sequence (sodium ions) in the upper right.
From Renaud, Blume, Pittman, Lamour and Tan, 1979.

a direct effect on the neurons in question, or to an interaction between TRH and a glutamate-like neurotransmitter substance. In an attempt to further characterize the actions of TRH, our recent studies have examined the effects of TRH and its active 3-methyl-histidine analog on excitations of cortical neurons evoked by intermittent applications of two acidic amino acids (L-glutamate and L-aspartate) and acetylcholine (Renaud, Blume, Pittman, Lamour and Tan, 1979). These investigations have suggested that both TRH and its active analog have a rather selective action to modulate excitations evoked by glutamate, but not by aspartate or acetylcholine (Fig. 5, 6) on sensitive cortical neurons. Thus, one role of TRH may be to modulate or modify postsynaptic actions of a specific endogenous neurotransmitter agent.

ACKNOWLEDGEMENTS

The author is grateful to the Conseil de la recherche en santé du Québec and the Canadian Medical Research Council for financial support, to Drs. Q.J.Pittman, H. W. Blume, R.E. Kearney, S. Lafontaine, Y Lamour, E. Arnauld, B. Layton, C. Bouillé and H. Geller for their collaboration in his laboratory. Mr. William Ellis provided expert technical assistance, and Mrs. M. Walker typed the manuscript.

SUMMARY

This brief review has attempted to indicate two aspects of the neurophysiological approach to the study of centrally acting peptides. New information has been provided by each method. While the in-vivo approach does not readily lend itself to intracellular recordings, definitive answers with the latter technique are likely to be achieved with the recent introduction of several in-vitro techniques i.e. brain slice and tissue culture. The results of such studies are reported elsewhere in this volume.

RESUMEN

Este breve análisis tiene como propósito indicar dos aspectos del enfoque neurofisiológico al estudio de los péptidos con actividad central. Nueva información ha sido

proporcionada por cada método. Mientras que el estudio in-vivo no se presta con facilidad a registros intracelulares, respuestas definitivas con la última técnica son probablemente logradas con la introducción reciente de varios métodos in-vitro, por ejemplo cortes de cerebro y cultivo de tejido.

Los resultados de tales estudios son referidos.

RÉSUMÉ

Ce bref analyse a comme propos faire avis aux aspects de l'étude neurophisiologue de l'activité central des peptides.

Nouvelle information nous fut donnée pour chaque methode. Tandis que l'étude "in vivo" me se prête pas avec facilité aux examen intracelulaires, réponses definitives avec la derniére tecnique sont probablement atteints avec l'introduction nouvellement fait de divers méthodes "in vitro" comme cuopes de cerveau et culture de tissus.

Les resultats de tous ces études sont rapportés.

ZUSAMMENFASSUNG

Diese kurze Analyse hat als Zweck zwei Aspekte des neurophysiolologischen Fokus beim Studium der Peptide mit Zentraler Aktivität anzuzeigen. Eine neue Information ist durch jede Methode gegeben worden.

Während das Studium "in vivo" nicht mit Leichtigkeit gegeben ist, um Intrazelluläre zu registrieren; definitive Antwort mit der letzten Technik sind wahrscheinlich erhältlich mit der Neueinführung verschiedener Methoden "in vitro", z.B. Gehienschnitte und Gewebskurturen.

Die Resultate dieser Studien werden gebracht.

REFERENCES

1. *Arnauld, E., Dufy, B.* and *Vincent, J.D.* Hypothalamic supraoptic neurones: rates and patterns of action potential firing during water deprivation in the unanaesthetized monkey. Brain Res. 100: 315-325, 1975.
2. *Barker, J.L., Crayton, J.W.* and *Nicoll, R.A.* Antidromic and orthodromic responses of paraventricular and supraoptic neurosecretory cells. Brain Res. 33: 353-366, 1971.
3. *Barnea, A., Parker, C.R., Neaves, W.B., Cho, G., Oliver, G.* and *Porter, J.C.* Subcellular distribution of neurohormones in the central nervous system. In: Biologie cellulaire des processus neurosécrétoires hypothalamiques — Cell biology of hypothalamic neurosecretion, J.D. Vincent and C. Kordon (eds.). Editions du Centre Nationale de la recherche Scientifique, Paris, 1978, pp 357-372.
4. *Blackwell, R.E.* and *Guillemin, R. Hypothalamic* control of adenohypophyseal secretions. Ann. Rev. Physiol. 35: 357-370, 1973.
5. *Blume, H.W., Pittman, Q.J.* and *Renaud,* L P. Electrophysiological indications of a "vasopressinergic" innervation of the median eminence. Brain Res. 155: 153-158, 1978.
6. *Breese, G.R., Cott, J.M., Cooper, B.R. Prange, A.J., Lipton, M.A.* and *Plotnikoff, N.P.* Effects of thyrotropin releasing hormone (TRH) on the actions of pentobarbital and other centrally acting drugs. J. Pharmacol. Exp. Ther. 193. 11-22, 1975.
7. *Brown, M., Rivier, J.* and *Vale, W.* Actions of bombesin, thyrotropin releasing factor, prostaglandin E_2 and naloxone on thermoregulation in the rat. Life Sci. 20: 1681-1688 1977.
8. *Brown, M. and Vale W.* Central nervous system effects of hypothalamic peptides. Endocrinology 96: 1333-1336, 1975.
9. *Burt, D.R.* and *Snyder, S.H.* Thyrotropin releasing hormone (TRH) apparent receptor binding in rat-brain membranes. Brain Res. 93: 309-328, 1975.
10. *Cohn, M.L., Cohn, M.* and *Taylor, F.H.* Thyrotropin releasing factor (TRH) regulation of rotation in the non-lesioned rat. Brain. Res. 96: 134-137, 1975.
11. *Cooper, B.R.* and *Boyer, C.E.* Stimulant action thyrotropin releasing hormone on cat spinal cord. Neuropharmacology 17: 153-156 1978.

12. *Dreifuss, J.J.* and *Kelly, J.S.* Recurrent inhibition of antidromically identified rat supraoptic neurones. J. Physiol. 220: 87-103, 1972.
13. *Dyer, R.G.* and *Dyball, R.E.J.* Evidence for a direct effect of LRF and TRF on single unit activity in the rostral hypothalamus. Nature 252: 486-488, 1974.
14. *Edwardson, J.A.* and *Bennett, G.W.* Hypothalamic hormones and mechanisms of neuroendocrine integration. In: Biologically Active Substances: Exploration and Exploitation, D.A. Hems (ed.), Wiley and Sons, New York (1977) pp 281-299.
15. *Elde, R.* and *Hökfelt, T.* Distribution of hypothalamic hormones and other peptides in the brain. In: Frontiers in Neuroendocrinology, Vol. 5, W.F. Ganong and L. Martini (eds.) Raven Press, New York (1978, pp 1-33.
16. *Euler, U.S.* von and *Gaddum, J.H.* An unidentified depressor substance in certain tissue extracts. J. Physiol. 72: 74-87, 1931.
17. *Harris, G.W.* Neural Control of Pituitary Gland. Edward Arnold, London, 1955.
18. *Harris, M.C.* and *Sanghera, M.* Projection of medial basal hypothalamic neurones to the preoptic anterior hypothalamic areas and the paraventricular nucleus in the rat. Brain Res. 81: 401-411, 1974.
19. *Hersh, L.B.* and *McKelvy, J.F.* Enzymes involved in the degradation of thyrotropin releasing hormone (TRH) and luteinizing hormone releasing hormone (LH - RH) in bovine brain. Brain Res. 168: 553-564, 1979.
20. *Jackson, I.M.D.* and *Reichlin, S.* Thyrotropin-releasing hormone (TRH) — distribution in hypothalamic and extrahypothalamic brain tissues of mammalian and submamma lian chordates. Endocrinology 95: 854-862, 1974.
21. *Jeffcoate, S.L., White, N., Bennett, G.W., Edwardson, J.A., Griffiths, E.C., Forbes, R.* and *Kelly, J.A.* Studies on the release and degradation of hypothalamic releasing hormones by the hypothalamus and other CNS areas in vitro. In: Central Regulation of the Endocrine System, K. Fuxe, T. Hökfelt and R. Luft (eds.), Plenum Press, New York, 1979, pp 61-70.
22. *KRUSE, H.* Thyrotropin releasing hormone (TRH): restoration of oxotremorine tremor in mice. Nauyn-Schmiedeberg's Arch. Pharmacol. 294: 39-45, 1976.
23. *Lembeck, F.* and *Zelter, G.* A polypeptide of possible physiological significance especially within the nervous system. Int. Rev. Neurobiol. 4: 159-215, 1962.
24. *Makara, G.B., Harris, M.C.* and *Spyer, K.M.* Identification and distribution of tuberoinfundibular neurones. Brain Res. 40: 283-290 1972.
25. *Mayer, M.L.* and *MacLeod, N.K.* The excitatory action of substance P and stimulation of the stria terminalis bed nucleus on preoptic neurones. Brain Res. 166: 206-210, 1979.
26. *Moss, R.L., Dudley, C.A.* and *Kelly, M.J.* Hypothalamic polypeptide releasing hormones: modifiers of neuronal activity. Neuropharmacology 17: 87-93, 1978.
27. *Moss, R.L., Kelly, M.* and *Riskind, P.* Tuberoinfundibular neurons: dopaminergic and norepinephrinergic sensitivity. Brain Res. 89. 265-277, 1975.
28. *McKelvy, J.* and *Epelbaum, J.* Biosynthesis, packaging, transport and release of brain peptides. In: The Hypothalamus, S. Reichlin, R.J. Baldessarini and J.B. Martin (eds.) Raven Press, New York, 1978, pp 195-211.
29. *Negoro, H.* and *Holland, R.C.* Inhibition of unit activity in the hypothalamic paraven tricular nucleus following antidromic activation. Brain Res. 42: 385-402, 1972.
30. *Negoro, N., Visessuwan, S.* and *Holland,* R.C. Inhibition and excitation of units in paraventriculuar nucleus after stimulation of the septum, amygdala and neurohypophysis. Brain Res. 57: 479-483, 1973.
31. *Nicoll, R.A.* Excitatory actions of TRH on spinal motoneurons. Nature 265: 242-243, 1977.
32. *Nicoll, R.* The action of Thyrotropin-Releasing hormone, Substance P and related peptides on frog spinal motoneurons. J. Pharmacol. Exp. Ther. 307: 817 824, 1978.
33. *Nicoll, R.A.* and *Barker, J.L.* The pharmacology of recurrent inhibition in the supraoptic neurosecretory system. Brain Res. 35: 501-511, 1971.
34. *Pittman, Q.J., Blume, H.W.* and *Renaud,* L.P. Electrophysiological indications that individual hypothalamic neurons innervate both median eminence and neurohypophysis. Brain Res. 157: 364-368, 1978.
35. *Poulain, D.A., Wakerley, J.B.* and *Dyball,* R.E.J. Electrophysiological differentiation of oxytocin — and vasopressin — secreting neurones. Proc. roy. Soc. B. 196. 367-384, 1977.
36. *Prange, A.J.Jr., Breese, G.R., Cott, J.M., Martin, B.R., Cooper, B.R. Wilson, I.C.* and *Plotnikoff, N.P.* Thyrotropin releasing hormone: antagonism of pentobarbital in rhodents. Life Sci. 14: 447-455, 1974.
37. *Renaud, L.P.* Tuberoinfundibular neurons in the basomedial hypothalamus of the rat: electrophysiological evidence for axon collaterals to hypothalamic and extrahypothalamic areas. Brain Res. 105: 59-72, 1976.
38. *Renaud, L.P.* Influence of amygdala stimulation on the activity of identified tuberin fundibular neurones in the rat hypothalamus. J. Physiol. 260: 237-252, 1976.
39. *Renaud, L.P.* Influence of medial preoptic-anterior hypothalamic area stimulation on the excitability of mediobasal hypothalamic neurones in the rat. J. Physiol. 264: 541-564 1977.

40. *Renaud, L.P.* Neurophysiological organization of the endocrine hypothalamus. In: The Hypothalamus, S. Reichlin, R.J. Baldessarini and J.B. Martin (eds.), Raven Press, New York, 1978, pp 269-301.

41. *Renaud, L.P., Blume, H.W., Kearney, R.E. MacKenzie, B.W.* and *Pittman, Q.J.* Physiological Anatomy of the Hypothalaamus. In. Current Studies of Hypothalamic Function, Vol. 2, W.L. Veale and K. Lederis (eds.), Karger, Basel, 1978, pp 1-16.

42. *Renaud, L.P., Blume, H.W.* and *Pittman,* Q.J. Neurophysiology and neuropharmacology of the hypothalamic tuberoinfundibular system. In: Frontiers in Neuroendocrinology, Vol. 5, W.F. Ganong and L. Martini, (eds.), Raven Press, New York, 1978, pp 135-162.

43. *Renaud, L.P., Blume, H.W., Pittman, Q.J., Lamour, Y. and Tan A.T.* Thyrotropin releasing hormone (TRH) selectively depresses glutamate excitation of cerebral corti cal neurons. Science 205: 1275-1277, 1979.

44. *Renaud, L.P.* and *Martin, J.B.* Thyrotropin releasing hormone (TRH) —depressant action on central neuronal activity. Brain Res. 86: 150-154, 1975.

45. *Renaud, L.P., Martin, J.B.* and *Brazeau, P.* Depressant action of TRH, LH-RH and somatostatin on activity of central neurones. Nature 255: 233-235, 1975.

46. *Renaud, L.P., Martin, J.B.* and *Brazeau, P.* Hypothalamic releasing factors: physiological evidence for a regulatory action on cen tral neurons and pathways for their distribution in brain. Pharmacol. Biochem. Behav. (Suppl. 1) 5: 171-178, 1976.

47. *Renaud, L.P.* and *Padjen, A.* Electrophysiological analysis of peptide actions in neural tissue. In: Centrally Acting Peptides, J. Hughes (ed.), The MacMillan Press Ltd., 1978, pp 59-84.

48. *Renaud, L.P., Pittman, Q.J.* and *Blume, H.W.* Neurophysiology of hypothalamic peptidergic neurons. In. Central Regulation of the Endocrine System, K. Fuxe, T. Hökfelt and R. Luft (eds.) Plenum Press, New York, 1979, pp 119-136.

49. *Sawaki, Y.* and *Yagi, K.* Electrophysiological identification of cell bodies of the tuberoinfundibular neurones in the rat. J. Physiol. 230: 75-85, 1973.

50. *Schally, A.V., Arimura, A.* and *Kastin, A.J.* Hypothalamic regulatory hormones. Science 179: 341-350, 1973.

51. *Steiner, F.A.* Effects of locally applied hormones and nuerotransmitters on hypothalamic neurons. Proc. 4th Int. Congr. Endocrin., Washington, 1972. Int. Congr. Ser. Nº 273, Excerpta Medica, Amsterdam, 1973, pp 202-204.

52. *Steiner, F.A.* Electrophysiological mapping of brain sites sensitive to corticosteroids, ACTH and hypothalamic releasing hormones. In: Anatomical Neuroendocrinology, W.E. Stumpf and L.D. Grant (eds.), Karger, Basel, 1975, pp 270-275.

53. *Szentágothai, J., Flerkó, B., Mess, B.* and *Halász, B.* Hypothalamic Control of the Anterior Pituitary, Akademiai Kiado, Budapest.

54. *Winokur, A.* and *Beckman, A.L.* Effects of thyrotropin releasing hormone, norepinephrine and acetylcholine on the activity of neurons in the hypothalamus, septum and cerebral cortex of the rat. Brain Res. 150: 205-209, 1978.

55. *Winokur, A., Davis, R.* and *Utiger, R.D.* Subcellular distribution of thyrotropin-releasing hormone (TRH) in rat brain and hypothalamus. Brain Res. 120: 423-434, 1977.

Somatostatin actions in primary dissociated cell cultures of mammalian spinal cord neurons

ROBERT L. MACDONALD
Department of Neurology
University of Michigan Medical Center
Ann Arbor, MI 48109
and
LINDA M. NOWAK
Neurosciences Program
University of Michigan
Ann Arbor, MI 48109

INTRODUCTION

Somatostatin (somatotropin-release inhibiting factor: SRIF) is a tetradecapeptide initially isolated from hypothalamus and shown to inhibit release of growth hormone from anterior pituitary (8,10) as well as other endocrine and exocrine secretory processes (30,46,48) in anterior pituitary (43,47), pancreas (12,13,17,29) and gastrointestinal tract (7). In addition to its action as a hormone, it has been suggested that SRIF may be a neurotransmitter since SRIF-like immunoreactivity has been demonstrated in axons and cell bodies of dorsal root ganglion (24) and sympathetic neurons (23), intrinsic neurons in the gastrointestinal system (1,21,31) and neurons in the central nervous system (3,9,22,28, 36, 37,39). In addition, SRIF has been localized to synaptic terminals and synaptic vesicles (4,16,39) and calcium-dependent release of SRIF from various regions of the CNS (2,5,26,38) and from dorsal root ganglia (27,33) has been demonstrated.

Attempts to characterize the physiological actions of SRIF have, however, been inconclusive. SRIF has been demonstrated to inhibit neuronal firing of cortical (42), hypothalamic (42), cerebellar (42), spinal cord dorsal horn (40) and myenteric neurons (49) and to excite hippocampal pyramidal (15) and sensorimotor cortical neurons (25) (Table I). Both pre-and postsynaptic sites of SRIF action have been proposed (Table II). A presynaptic action of SRIF was suggested since SRIF inhibited release of acetylcholine (11,19,20) and norepinephrine (11,32) from peripheral smooth and skeletal neuromuscular junctions without altering postjunctional neurotransmitter sensitivity. SRIF also decreased the calciumdependent component of action potentials recorded in dorsal root ganglion neuron (33). If SRIF had a similar action at the synaptic terminals, calcium entry and thus transmitter release would be depressed. In rat cortical neurons in cell culture however, SRIF increased release of neurotransmitter (14). A postsynaptic action for SRIF was implied by the finding that SRIF inhibited firing of myenteric neurons when synaptic transmission had been elimited by bathing the preparation in calcium-free medium (49). In addition, SRIF depolarized hippocampal pyramidal cells *in vitro*, a presumed postsynaptic action (15). Finally, in the isolated perfu-

TABLE I

Somatostatin Actions on Neuronal Activity

Preparation	Investigators
Inhibition of neuronal firing	
cat cerebellar, cortical and hypothalamic neurons	Renaud, Martin and Brazeau (1975)
cat dorsal horn neurons	Randic and Miletic (1978)
guinea pig myenteric plexus neurons	Williams and North (1978)
Excitation of neuronal firing	
cat hippocampal pyramidal neurons	Dodd and Kelly (1978)
rabbit sensorimotor cortical neurons	Ioffe et al (1978)

sed frog spinal cord, SRIF increased ventral root potentials evoked by dorsal root stimulation and produced direct but small hyperpolarizations recorded from both ventral and dorsal roots suggesting that SRIF might have both pre-and postsynaptic actions (35). We have studied the actions of SRIF on murine spinal cord neurons grown in primary dissociated cell culture using intracellular recording and report that SRIF had presynaptic actions to modify release of neurotransmitter.

Methods

Neuronal cell cultures were prepared from spinal cords and attached dorsal root ganglia removed from 12.5 to 14 day old fetal mice as described previously (41). Intracellular recordings were made from spinal cord neurons using high resistance (25-50 MΩ) glass micropipettes under visual control on the modified stage of an inverted phase contrast microscope which was heated to maintain a culture temperature of 35°-37°. SRIF was applied to the surface of neurons (which had been impaled by a recording micropipette) using blunt (5-20μ) micropipettes (miniperfusion (MP) pipettes) filled with SRIF. The open end of the MP pipette was connected to a pressure regulator via a voltage activated three way valve. By using an electronic timer, SRIF could be applied for known durations. SRIF (Cal Biochem, Sigma) was dissolved in phosphate buffered saline, 0.002N HCl, 0.002 N HAc or distilled water to make a stock solution which was then diluted at least 10-fold with phosphate buffered saline to achieve the final SRIF concentration (0.2 to 25 μM). In all experiments pH was confirmed to be between 7.2 and 7.4.

Results

In some but not all neurons, SRIF applied by MP evoked a volley of action potentials (Fig. $1A_1$) produced by excitatory postsynaptic potentials (Fig. $1A_2$). The hyperpolarization and reduction of spontaneuos activity shown in the specimen records were artifacts of pressure application (Fig. $1B_1$, $1B_2$). Application of SRIF and control solutions did not alter membrane resistance. Constant current pulses (50 msec) were applied through the recording micropipette and the voltage produced was dependent upon neuronal membrane resistance. SRIF application did not alter the amplitude of these pulses (Fig. $1A_2$) and therefore did not produce a change in mem-

TABLE II

Synaptic Site of Somatostatin Actions

Action	Preparation	Investigators
	Presynaptic	
increased DR-VRP	frog spinal cord	Padjen (1977)
Increased transmitter release	spinal cord neurons in cell culture	Macdonald and Nowak (this study)
increased transmitter release	rat cortical neurons in cell culture	Delfs et al (1979)
decreased calcium action potential	chick DRG neurons in cell culture	Mudge, Leeman and Fischbach (1979)
decreased —adrenergic transmission	rat vas deferens	Cohen et al (1978); Magnan et al (1979)
decreased cholinergic transmission	guinea pig ileum	Guillemin (1976); Cohen et al (1978); Furness and Costa (1979)
	Postsynaptic	
hyperpolarized VR	frog spinal cord	Padjen (1977)
depolarized membrane potential	cat hippocampal pyramidal neurons	Dodd and Kelly (1978)
inhibited activity	guinea pig myenteric neurons	Williams and North (1978)

brane resistance. In some cells, hyperpolarization of membrane potential associated with an increase in conductance occurred. However, this was infrequently observed and thus its significance was unclear. The synaptic potentials that were evoked were of varying amplitudes and rise and fall times (Fig. 2) suggesting that release of neurotransmitters was evoked from several different synaptic terminals. When release was evoked, it was dose-dependent (Fig. 3). Application of 25 μM SRIF (Fig. 3B) produced both higher frequency of release and larger amplitudes of individual synaptic potentials than did 10 μM SRIF (Fig. 3A). Also, both excitatory and inhibitory synaptic potentials were evoked. In a single neuron at a resting membrane potential of —40 mV, SRIF evoked hyperpolarizing inhibitory synaptic potentials (Fig. 4A, filled circle). Hyperpolarization of membrane potential to —80 mV converted all synaptic potentials to depolarizing synaptic potentials as would be expected if the cell was hyperpolarized past the reversal potential for the inhibitory synaptic potentials. In addition, the surface of individual neurons was not uniformly sensitive to SRIF. As shown in one representative cell (Fig. 5), application of SRIF to three different sites evoked different synaptic potentials. At site 2, several excitatory synaptic potentials were evoked with one being 17 mV while at site 1, a few small excitatory synaptic potentials less than 3 mV were evoked. SRIF application at site 3 produced an intermediate response with two 12 mV excitatory postsynaptic potentials being evoked.

Release of neurotransmitter was abolished by tetrodotoxin (TTX). TTX blocks the regenerative inward sodium current of

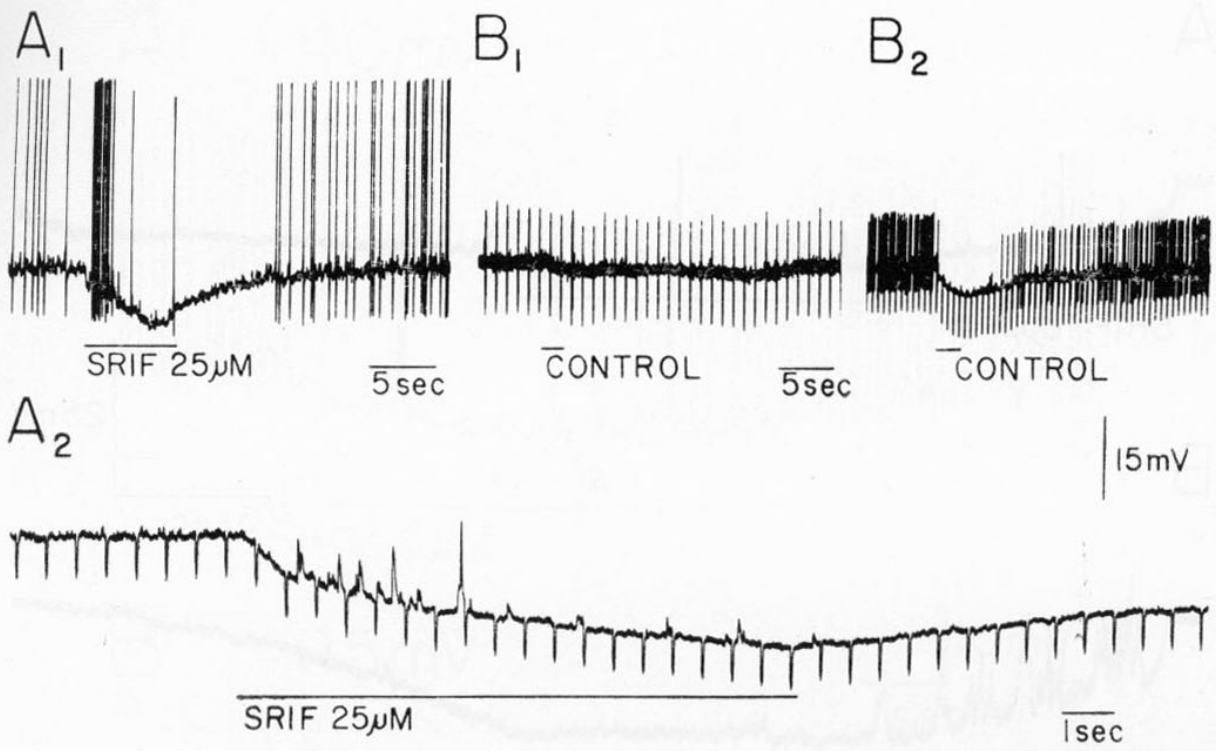


Figure 1. *Somatostatin (SRIF) evoked transmitter release.* A.) SRIF evoked release of neurotransmitter from synaptic terminals demonstrated by (A_1) an increase of action potential frequency (RMP = —36 mV) and (A_2) an increase in the number and amplitude of postsynaptic potentials that ocurred without any change of membrane resistance as indicated by a constant amplitude of the responces to brief (30 msec) periodic (600 msec interval) hyperpolarizing constant ocurrent (1 nA) pulses. Membrane potential was hyperpolarized to —40 mV by DC current. B.) Control solutions sometimes caused an apparent hyperpolarization without changing membrane resistance (B_1 and B_2 — brief periodic hyperpolarizations due to constant current pulses) and B_2) occasionally blocked action potentials if miniperfusion pipette tips were large.

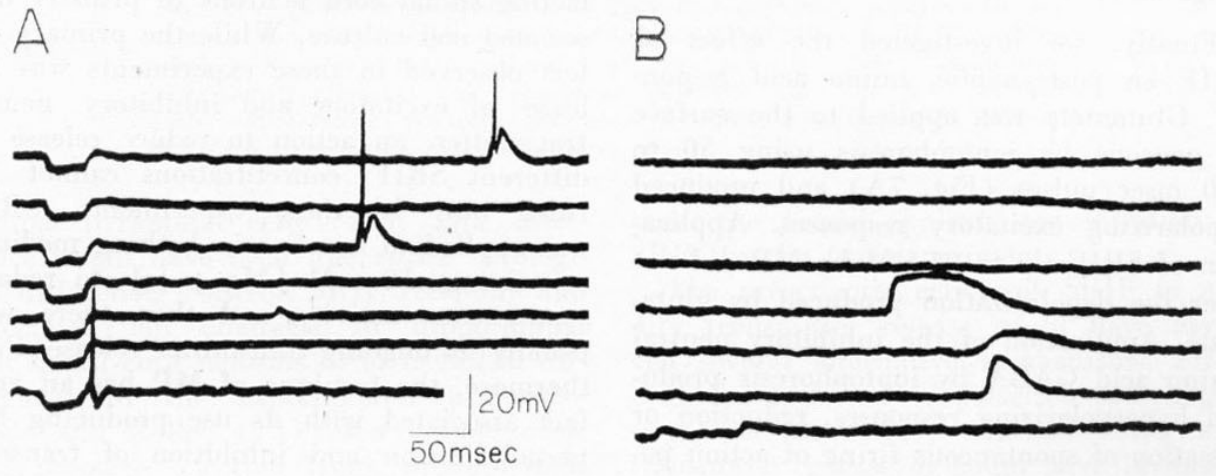


Figure 2. *SRIF evoked postsynaptic potentials (PSPs).* Action potentials (A) and PSPs (A, B) were recorded during miniperfusion of 100 μM SRIF (Cal Biochem.). Rastered records were photographed from the oscilloscope screen. The initial hyperpolarizations observed in A. were evoked by constant-current pulses.

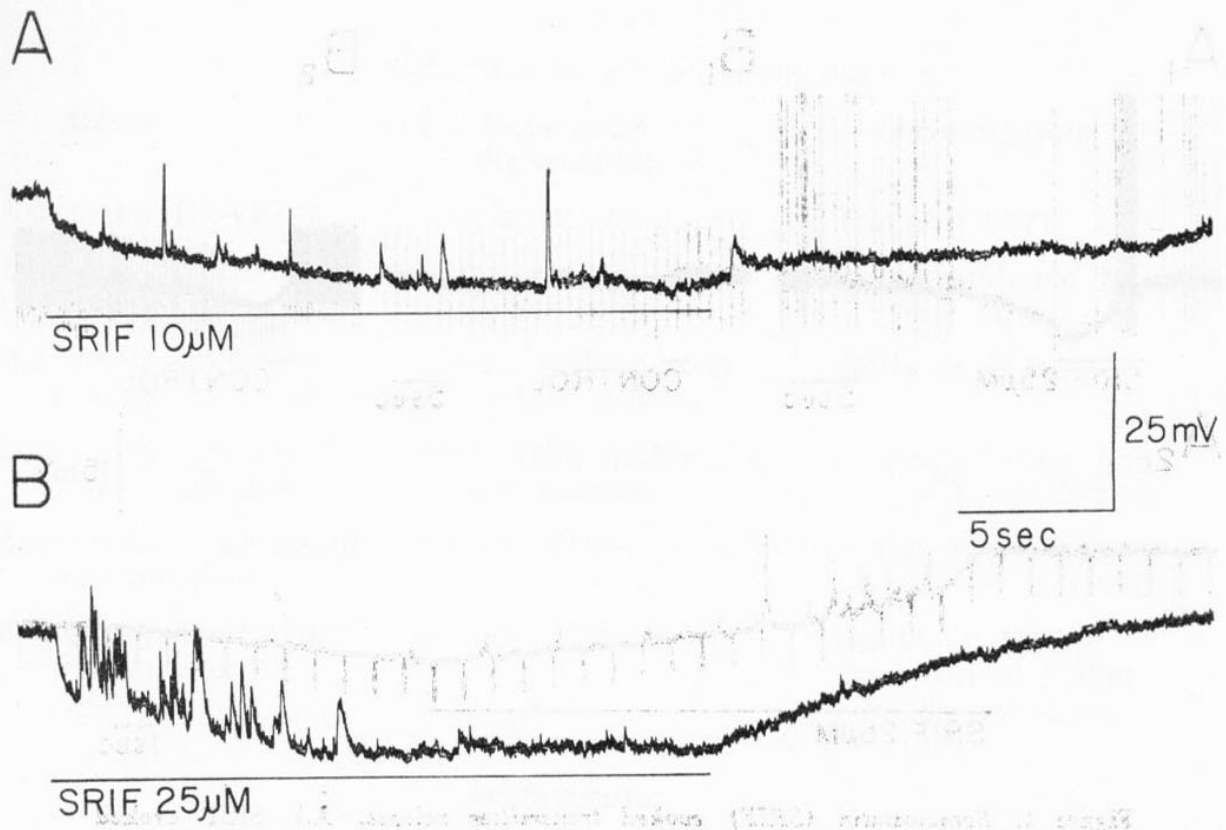


Figure 3. *SRIF evoked dose-dependent release of neurotransmitter.* A.) 10 μM SRIF and B.) 25 μM SRIF evoked PSPs during successive applications of peptide on the same neuron from different miniperfusion pipettes. PSP amplitude and frequency were dose-dependent.

action potentials. While good release was obtained in medium containing 10 mM [Mg++] (Fig. 6A), no release was evoked in medium containing 1 μM TTX (Fig. 6B).

Finally, we investigated the effect of SRIF on postsynaptic amino acid responses. Glutamate was applied to the surface of neurons by iontophoresis using 50 to 100 msec pulses (Fig. 7A) and produced depolarizing excitatory responses. Application of SRIF up to 25 μM by MP did not alter the depolarization produced by glutamate. Application of the inhibitory neutral amino acid GABA by iontophoresis produced hyperpolarizing responses, reduction or cessation of spontaneous firing of action potentials (Fig. 7B) and an increased membrane conductance. Application of SRIF up to 25 μM by MP (5 μM SRIF is shown in Fig. 7B) did not alter the GABA-responses.

Discussion

In the present study we have demonstrated that SRIF modified release of neurotransmitter from synaptic terminals contacting spinal cord neurons in primary dissociated cell culture. While the primary effect observed in these experiments was release of excitatory and inhibitory neurotransmitter, an action to reduce release at different SRIF concentrations cannot be ruled out. In these experiments, SRIF was applied to neurons in bathing medium containing 10 mM [Mg++] to reduce spontaneous activity and thus there was usually no ongoing transmitter release. Furthermore, the tecnique of MP has an artifact associated with its use producing hyperpolarization and inhibition of transmitter release. Thus, we can only conclude that SRIF has presynaptic actions to modify release of neurotransmitter.

Our findings are in contrast with the report that SRIF directly depolarized hippo-

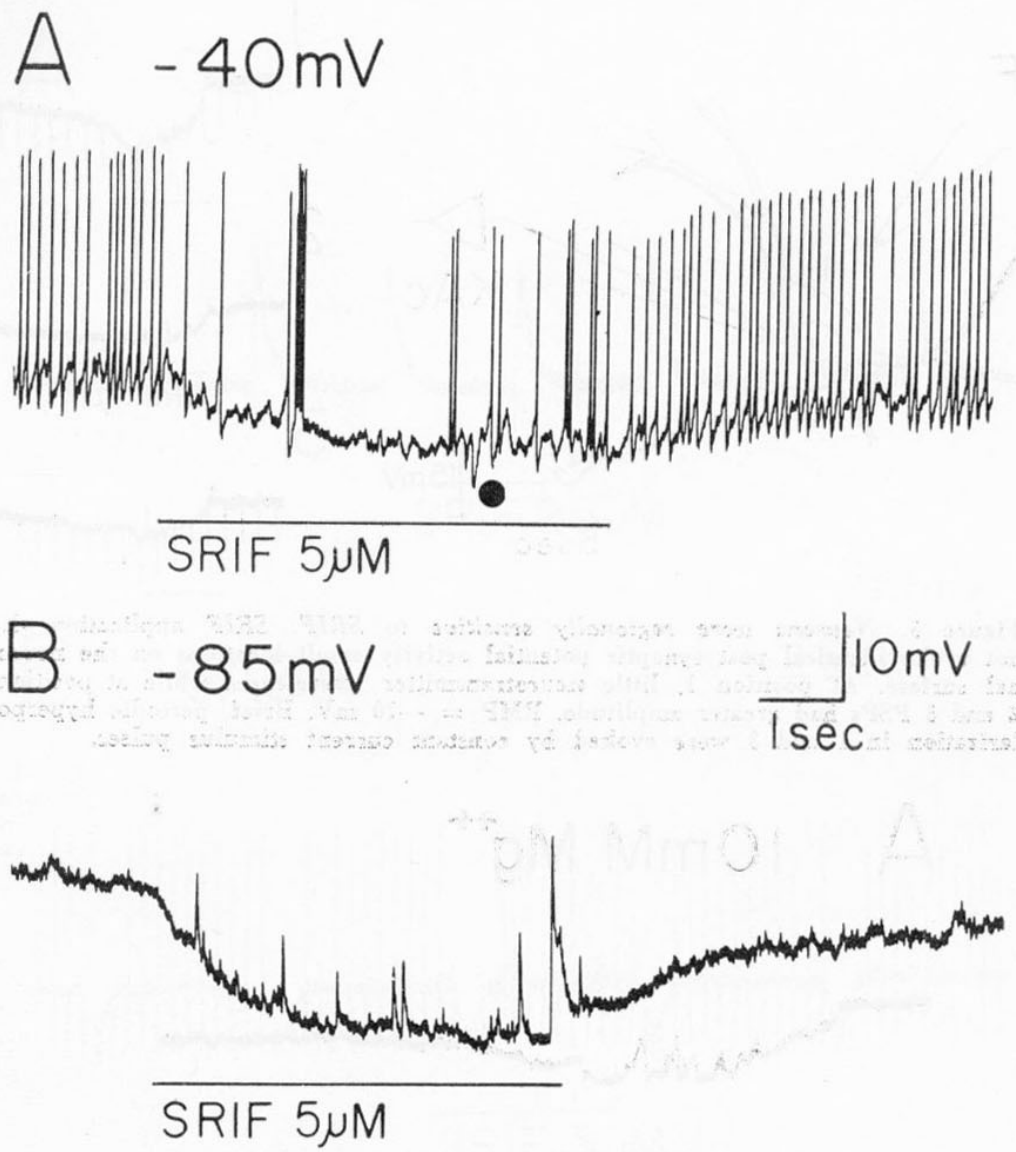


Figure 4. *SRIF evoked both inhibitory and excitatory postsynaptic potentials.* A.) SRIF application produced both EPSPs and IPSPs (filled circles) at a membrane potential of —40 mV. B.) SRIF application after hyperpolarizing cell membrane potential (to —88 mV) evoked only depolarizing synaptic potentials (EPSPs and inverted IPSPs). KAc-filled recording electrodes were used.

campal pyramidal cells (15). This difference might have been due either to biogical differences between SRIF receptors and associated ion channels in hippocampal and spinal cord neurons or to technical differences in the experimental protocols. Dodd and Kelley (15) used iontophoresis and pressure ejection from micropipettes containing 3 mM SRIF and 5 mM sodium-acetate while in our experiments we used only pressure application of peptide from pipettes containing 0.2 to 25 μM concentrations of SRIF. Thus, it is difficult to compare the actual applied concentration of SRIF in the two experiments.

The presynaptic action of SRIF to modify transmitter release could have involved several alternative mechanisms. SRIF could have directly depolarized presynaptic terminals to produce terminal firing and transmitter release or could have increased the quantal content of synaptic potentials which were not detectable due to the noise level of the recordings. In addition, SRIF could have modulated calcium entry into presynaptic terminals to alter quantal con-

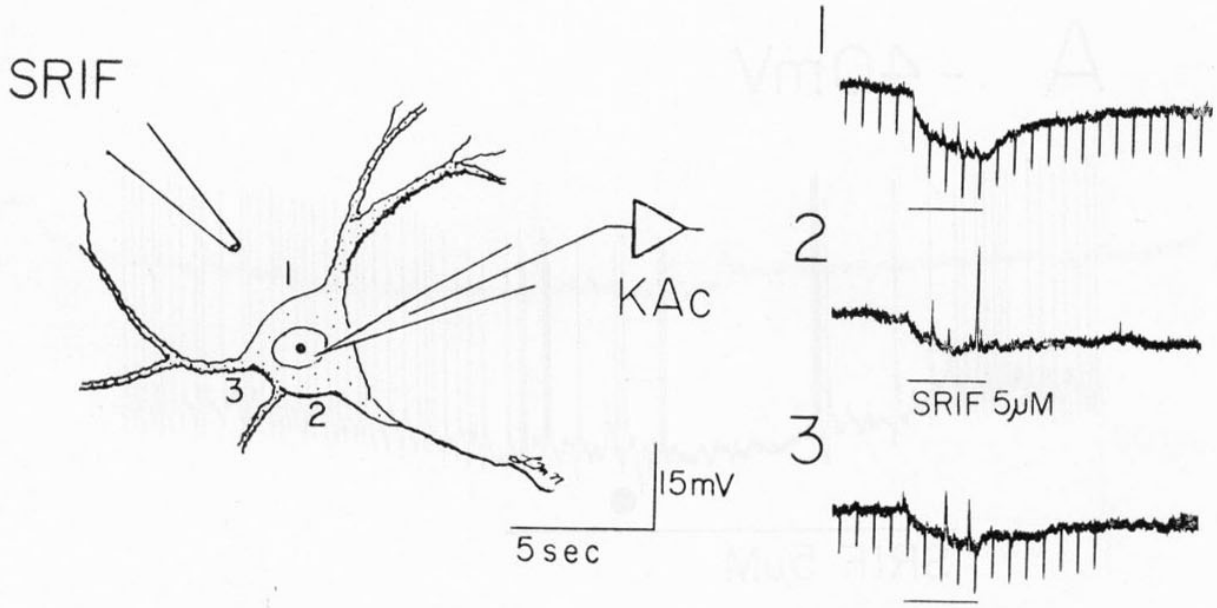


Figure 5. *Neurons were regionally sensitive to SRIF. SRIF* application did not evoke identical post synaptic potential activity at all locations on the neuronal surfase. At position 1, little neurotransmitter wasreleased while at positions 2 and 3 PSPs had greater amplitude. RMP = —70 mV. Brief, periodic hyperpolarization in 1 and 3 were evoked by constant current stimulus pulses.

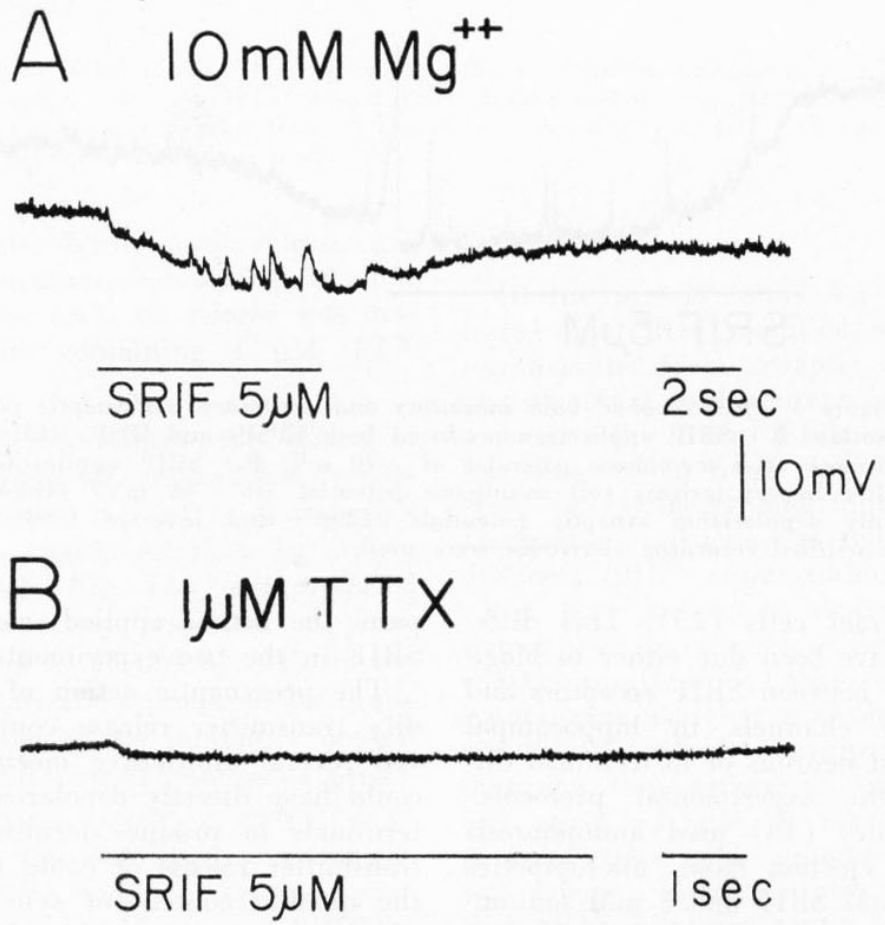


Figure 6. *Neurotransmitter release stimulated by SRIF was blocked by tetrodotoxin (TTX).* PSPs evoked by SRIF (A.) were not observed after TTX (1 μM) was added to the recording medium (B.). The same miniperfusion pipette was used to apply peptide to successive neurons in A and B.

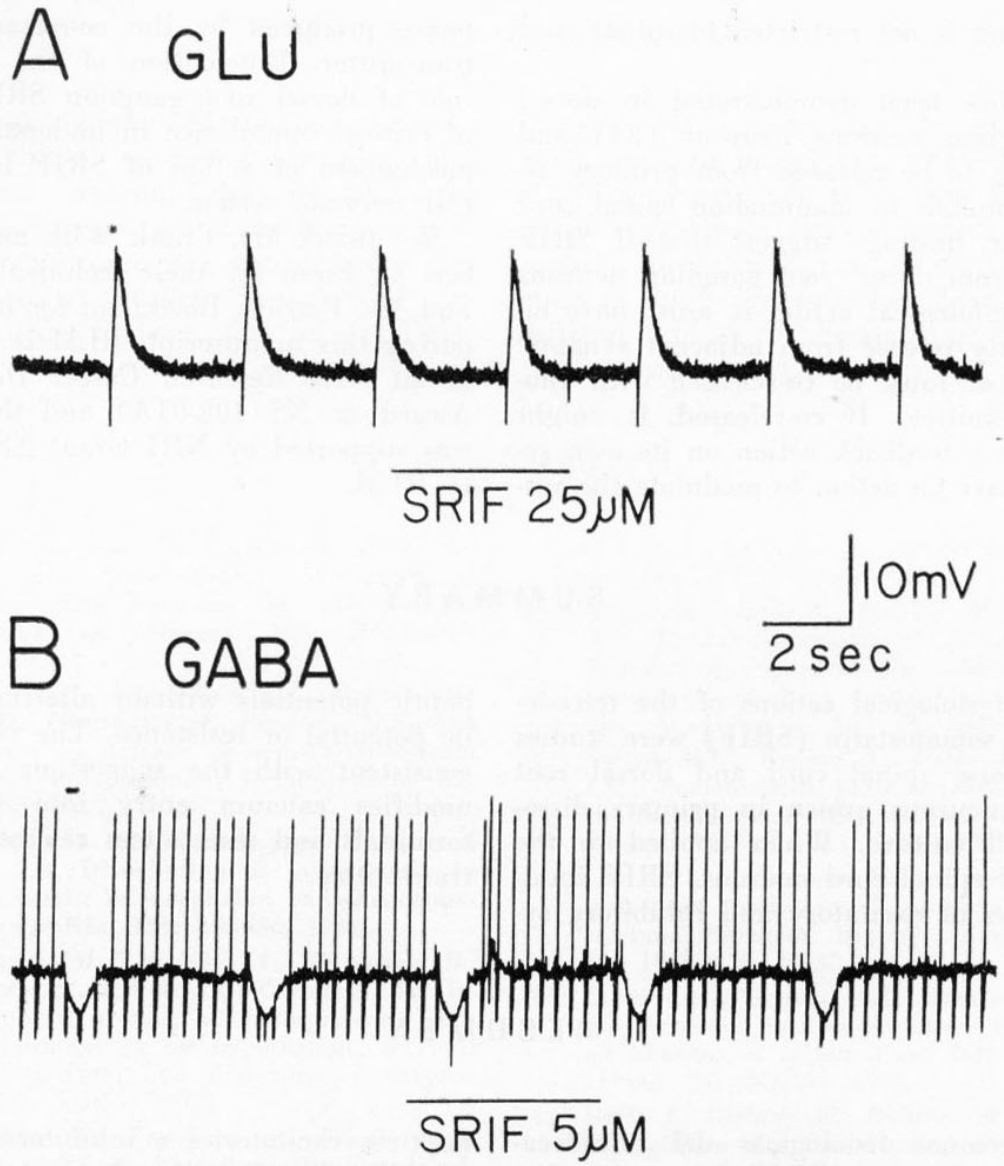


Figure 7. *SRIF did not appear to modulate actions of amino acid neurotransmitters.* A.) Miniperfusion of neuronal membranes with 25 μM SRIF during glutamate iontophoresis did not alter amplitude or duration of glumate-evoked (100 msec, 32 nA) depolarizations. RMP was —54 mV. B.) Miniperfusion of neuronal membranes with 5 μM SRIF did not change the duration or amplitude of GABA-evoked (200 msec, 8 nA) hyperpolarizations. GABA mediated changes of membrane resistance were also unaffected although SRIF may have increased PSP activity. RMP was —51 mV.

tent and thus increase or decrease resultant evoked synaptic potentials. SRIF has been shown to impair the uptake of 45Ca2+ by pancreatic islet cells (6,34,44) and SRIF inhibition of insulin secretion was reversed by elevation of extracellular calcium (12,13) and by the calcium ionophore A23187 (18). In contrast, SRIF increased glutamate-induced 45Ca2+ accumulation in cortical synaptosomes (45) and blocked release of a portion of the stored calcium in cortical synaptosomes (45). Thus the most likely mechanism of action for SRIF in our experiments was that SRIF modulated calcium entry into presynaptic terminals and thus modified neurotransmitter release. A similar presynaptic action of SRIF has also recently been described in cortical neurons in primary dissociated cell culture (14) suggesting that

this finding is not restricted to spinal cord neurons.

SRIF has been demonstrated in dorsal root ganglion neurons neurons (24) and was shown to be released from primary afferent terminals in mammalian spinal cord (27). Our findings suggest that if SRIF released from dorsal root ganglion neurons has a physiological action it must have either modify release from adjacent synaptic terminals or must be co-released with another transmitter. If co-released, it might then have a feedback action on its own release or have an action to modulate the response produced by the co-released neurotransmitter. Elucidation of the functional role of dorsal root ganglion SRIF will be of critical importance in understanding the mechanism of action of SRIF in the central nervous system.

We thank Mr. Frank Wilk and Mr. Robert C. Frere for their technical assistance and Ms. Patricia Blackman for help in preparing this manuscript. RLM is a recipient of an NIH Research Career Development Award # NS 408-01A1 and the research was supported by NIH Grant NS 14704-01 to RLM.

SUMMARY

The physiological actions of the tetradecapeptide somatostatin (SRIF) were studies using mouse spinal cord and dorsal root ganglion neurons grown in primary dissociated cell culture. When applied to the surface of spinal cord neurons, SRIF induced volleys of excitatory and inhibitory synaptic potentials without altering membrane potential or resistance. The findings are consistent with the suggestion that SRIF modifies calcium entry into presynaptic terminals and thus alters release of neurotransmitter.

RESUMEN

Las acciones fisiológicas del tetradecapéptido somatostatin SRIF fueron estudiadas empleando neuronas de la médula espinal y del ganglio de la raíz dorsal de ratones en cultivo de célula primaria disociada. Cuando fue aplicada SRIF a la superficie de neuronas de la médula espinal se produjeron descargas de potenciales sinápticos excitatorios e inhibitorios sin alterar el potencial de membrana o resistencia. Estos hallazgos son consistentes con la hipótesis de que SRIF modifica la entrada de calcio en las terminales presinápticas y de este modo altera la liberación del neurotransmisor.

RÉSUMÉ

Les actions physiologiques du tetradecapeptide somatostatin SRIF ont été étudiées sur des neurones de la moelle épiniere et du ganglion de la racine dorsale de rats, en culture de cellule primaire dissociee. Apres application de SRIF a la surface de neurones de la noelle épiniere il se produisit des décharges de potentiels synaptiques excitantes on inhibitrices sans alérer le potentiel de membrane on résistance. Ces déconvertes vont de pais avec l'hypothèse que SRIF modifie l'entrée du Calcium dans les terminaisons présynaptiques et altère ainsi la liberation du neurotransmetteur.

ZUSAMMENFASSUNG

Die physiologische Aktion der Tetradecapeptide, SRIF ausgesetzt, wurden studiert indem Neurone des Rückenmarks und des Ganglions der Dorsalwurzel von Mäusen in Kulturen der getrockneten Primarzelle benutzt wurden. Wenn SRIF angewandt wurde, bildeten sich an der Oberfläche der Neuronen des Rückenmarks potentielle synaptische Entladungen exitatorisch und inhibitatorisch, ohne das Potential der Membranen oder den Widerstand zu ändern. Dieser Befund bestätigt die Hypothese, dass SRIF den Eintritt von Kalcium in die präsynaptischen Terminale modifiziert und so die Freisetzung des Neurotransmissors ändert.

REFERENCES

1. *Arimura A., Sato H., Dupont A., Nishi N., Schally AV.* Somatostatin: Abundance of immunoreactive hormone in rat stomach and pancreas. Science 189: 1007-1009, 1975.
2. *Bennett G.W., Edwardson J.A., Marcano de Cotte D., Berelowitz M., Pimstone B.L.,* and *Kronheim S.* Release of somatostatin from rat brain synaptosomes. J. Neurochem. 32: 1127-1130, 1979.
3. *Bennet-Clarke C., Romagnano M.A.,* and *Joseph S.A.* Distribution of somatostatin in the rat brain: telencephalon and diencephalon. Brain Res. 188: 473-486, 1980.
4. *Berelowitz M., Hudson A., Pimstone B., Kronheim S., Bennett, G.W.* Subcellular localization of growth hormone release inhibiting hormone in rat hypothamus, cerebral cortex, striatum and thalamus. J. Neurochem. 31. 751-753, 1978.
5. *Berelokitz M., Kronheim S., Pimstone B.* and *Sheppard M.* Potassium stimulated calcium dependent release of immunoreactive somatostatin from incubated rat hypothalamus. J. Neurochem. 31: 1537-1539, 1978.
6. *Bhathena S., Peirino P., Voyles N., Smith S., Wilkens S., Coy D., Schally A., Recant L.* Reversal of somatostatin inhibition of insulin and glucagon secretion. Diabetes 25: 1031-1040, 1976.
7. *Bloom S.R., Mortimer CH., Thorner M.O., Besser G.M., Hall R., Gomez-Pan A., Roy V.M., Russell R.C.G., Coy D.H., Kastin A.J., Schally A.V.* Inhibition of gastrin and gastric-acid secretion by growth-hormone release-inhibiting hormone. Lancet 2: 1106-1109, 1974.
8. *Brazeau P., Vale W., Burgus R., Ling N., Butcher M., Rivier J., Guillemin R.,* Hypothalamic polypeptide that inhibits the secretion of immunoreactive pituitary growth hormone. Science 179: 77-79, 1973.
9. *Brownstein M., Arimura A., Sato H., Schally A.V., Kizer J.S.* The regional distribution of somatostain in the rat brain. Endocrinology 96: 1456-1461, 1975.
10. *Burgus R., Ling N., Butcher M., Guillemin R.,* Primary structure of somatostatin, a hypothalamic peptide tha inhibits the secretion of pituitary growth hormone. Proc. Nat. Acad. Sci. (USA) 70: 684-688, 1973.
11. *Cohen M.L., Rosina E., Wiley K.S., Slater I.H.,* Somatostatin inhibits adrenergic and cholinergic neurotransmission in smooth muscle. Life Sci. 23. 1659-1664, 1978.
12. *Curry D.L., Bennett L.L.,* Reversal of somatostatin inhibition of insulin secretion by calcium. Biochem. Biophys. Res. Common. 60: 1015-1019, 1974.
13. *Curry D.L., Bennett L.L.* Does somatostatin inhibition of insulin secretion involve two mechanisms of action. Proc. Nat. Acad. Sci. (USA) 73: 248-251, 1976.
14. *Delfs J., Dichter M., Robbins R., Connolly J., Reichlin S.,* Somatostatin in rat cortical neurons in cell culture: synthesis and physiological effects. Neurosci. Abst. 5: 526, 1979.
15. *Dodd J., Kelly J.S.* Is somatostatin an excitatory transmitter in the hippocampus? Nature 273: 674-675, 1978.
16. *Epelbaum J., Brazeau P., Tsang D., Brawer J., Martin J.B.,* Subcellular distribution of radioimmunoassayable somatostatin in rat brain Brain Res. 126: 309-323, 1977.
17. *Fujimoto W.,* Somatostatin inhibition of glucose-, tolbutamide-, theophyline, cytochalasin B— and calcium-stimulated insulin release in monolayer cultures of rat endocrine pancreas. Endocrinology 97: 1497-1509, 1975.
18. *Fujimoto W.Y., Ensinck J.W.,* Somatostatin inhibition of insulin and glucagon secretion in rat islet culture: reversal by ionophore A23187. Endocrinology 98: 259-262, 1976.
19. *Furness J.B., Costa M.,* Actions of somatostatin on excitatory and inhibitory nerves in he intestine. Eur. J. Pharm. 56: 69-74, 1979.

20. *Guillemin R.*, Somatostatin inhibitis the release of acetylcholine induced electrically in the myenteric plexus. Endocrinology 99: 1653-1654, 1976.

21. *Hokfelt T., Efendic S., Hellerstrom C., Johansson O., Luft R., Arimura A.* Cellular localization of somatostatin in endocrine like cells and neurons of the rat with special reference to the A_1 cells of the pancreatic islets and to the hypothalamus. Acta Endocrinol, Cophn 80 Suppl 200, 1-41, 1975.

22. *Hokfelt T., Efendic S., Johansson O., Luft R., Arimura A.* Immunohistochemical localization of somatostatin (growth hormone release-inhibiting factor) in the guinea pig brain. Brain Res. 80: 165-169, 1974.

23. *Hokfelt T., Elfvin L.G., Elde R., Schultzberg M., Goldstein M., Luft R.* Occurrence of somatostatin immoreactivity in some peripheral sympathetic nonadrenergic neurons Proc. Nal. Acad. Sci. (USA) 74: 3587-3591, 1977.

24. *Hokfelt T., Johansson O., Luft R., Arimura A.* Immunohistochemical evidence for the presence of somatostatin, a powerful inhibitory peptide, in some primary sensory neurons. Neurosci. Lett. 1:231 235, 1975.

25. *Ioffe E., Havlicek V., Friesen H., Chernick V.* Effect of somatostatin (SRIF) and L-glutamate on neurons of the sensorimotor cortex in awake habituated rabbits. Brain Res. 153: 414-418, 1978.

26. *Iversen L.L., Iversen S.D., Bloom F., Douglas C., Brown M., Vale W.* Calciumdependent release of somatostatin and neurotensin from rat brain in vitro. Nature 273: 161-163, 1978.

27. *Jessell T., Mudge A., Leeman S.E., Yaksh T.L.* Release of substance P and somatostatin. in vivo, from primary afferent terminals in mammalian spinal cord. Neurosci Abst. 5: 611, 1979.

28. *Kobayashi R.M., Brown M., Vale W.* Regional distribution of neurotensin and somatostatin in rat brain. Brain Res. 126: 584-588, 1977.

29. *Koerker D.J., Ruch W., Chideckel E., Palmer J., Goodner C.J., Ensinck J., Gale C.C.* Somatostatin: hypothalamic inhibitor of the endocrine pancreas. Science 184: 482-484, 1974.

30. *Luft R., Efendic S., Hokfelt T.* Somatostatin-Both hormone and neurotransmitter? Diabetologia 14: 1-13, 1978.

31. *Luft R., Efendic S., Hokfelt T., Johansson O., Arimura A.* Immunohistochemical evidence for the localization of somatostatin-like immunoreactivity in a cell population of the pancreatic islets. Med. Biol. 52: 428-430, 1974.

32. *Magnan J., Regoli D., Quirion R., Lemaire S., St. Pierre S., Rioux F.* Studies on the inhibitory action of somatostatin in the electrically stimulated rat vas deferens. Eur. J. Pharm. 55: 347-354, 1979.

33. *Mudge A.W., Leeman S.E.* and *Fischbach G.D.* Enkephalin inhibits release of substance-P from sensory neurons in culture and decreases action potential duration. Proc. Nat'l Acad. Sci. (USA) 76: 526-530, 1979.

34. *Oliver J.* Inhibition of calcium uptake by somatostatin in isolated rat islets of Langerhans. Endocrinology 99: 910-913, 1976.

35. *Padjen A.L.* Effects of somatostatin on frog spinal cord. Neurosci. Abstr. 3: 411, 1977.

36. *Parsons J.A., Erlandsen S.L., Hegre O.D., McEvoy R.C., Elde R.P.* Central and peripheral localization of somatostatin immunoenzyme immunocytochemical studies. J. Histochem. Cytochem. 24: 872-882, 1976.

37. *Patel Y.C., Reichlin S.* Somatostatin in hypothalamus, extrahypothalamic brain and peripheral tissues of the rat. Endocrinology 102: 523-530, 1978.

38. *Patel Y.C., Zingg H.H., Dreifuss J.J.* Calcium-dependent somatostatin secretion from neurosecretary axons in rat neurohypophysis. Nature 267: 852-853, 1977.

39. *Pelletier G., Leclerc R., Dube D., Labrie F., Puviani R., Arimura A., Schally A.V.* Localization of growth hormone-release-inhibiting hormone (somatostatin) in the rat brain. Amer. J. Anat. 142: 397-401, 1975.

40. *Randic M., Miletic V.* Depressant actions of methionine-enkephalin and somatostatin in cat dorsal horn neurones activated by noxious stimuli. Brain Res. 152: 196-202, 1978.

41. *Ransom B.R., Neale E., Henkart M., Bullock P.N. and Nelson P.G.* Mouse spinal cord incellculture I morphology and intrinsic neuronal electrophysiologic properties. J. Neurophysiol. 40: 1132-1150, 1977.

42. *Renaud L.P., Martin J.B., Brazeau P.* Depressant action of TRH, LH-RH and somatostatin on activity of central neurones. Nature 255: 233-235, 1975.

43. *Siler T., Yen S.L., Vale W., Guillemin R.* Inhibition by somatostatin on the release of TSH induced in man by thyrotrophin-releasing factor. J. Clin. Endocrin. 38: 742-745, 1974.

44. *Taminoto T., Seino Y., Goto Y., Imura H.* Interaction of somatostatin and calcium in regulating insulin release from isolated pan-

creatic islets. Biochem. Biophys. Res. Commun. 66: 928-934, 1975.

45 *Tan A.T., Tsang D., Renaud L.P., Martin J.B.* Effect of somatostatin on calcium transport in guinea pig cortex synaptosomes. Brain Res. 123: 193-196, 1977.

46. *Vale W., Brazeau P., Rivier C., Brown M., Boss B., Rivier J., Burgus R., Ling N., Guillemin R.* Somatostatin. Recent. Prog. Horm. Res. 31: 365-397, 1975.

47. *Vale W., Rivier C., Brazeau P., Guillemin R.* Effects of somatostatin on the secretion of thyrotropin and prolactin. Endocrinology 95: 968-977, 1974.

48. *Vale W., Rivier C., Brown M.* Regulatory peptides of the hypothalamus. Ann Rev. Physiol. 39: 473-527, 1977.

49. *Williams J.T., North R.A.* Inhibition of firing of myenteric neurones by somatostatin. Brain Res. 155: 165-168, 1978.

Multiple Modes of Communication by Substance P

JANE A. HARKINS, JOHN M. STEWART
and WILLIAM A. KRIVOY

Department of Biochemistry
University of Colorado Health Sciences Center
Denver, Colorado 80262

National Institute on Drug Abuse
Division on Research, Addiction Research Center
Lexington, Kentucky 40583

INTRODUCTION

The list of neuroactive compounds has grown in the last decade to 40 or more and most of the latest additions are peptides. The amine and amino acid transmitters are characterized by certain criteria: they are synthesized in neurons and released from neuronal terminals; their excitatory or inhibitory action at the post-synaptic membrane has a short latency and is of short duration; and they have an enzymatic or reuptake system for termination or recycling. The peptides deviate somewhat from these rules, particularly with respect to biosynthesis and electrophysiology. This review explores the latter process dealing with a variety of actions of Substance P on excitable membranes, as well as the apparent neurosecretory properties of this peptide.

NEUROMODULATION

Substance P was suspected to mediate synaptic transmission as long ago as 1953 when Lembeck[24] noted its presence in the dorsal horn of the spinal cord where primary sensory neurons were thought to release transmitter substances. Improvements in elecrophysiological techniques to identify the effect of Substance P on neurons and the characterization, sequencing and subsequent synthesis of pure Substance P in 1970 and 1971[2, 35] have allowed more precise investigations of its role(s) in transmission. Since then, cells in several brain areas and in the spinal cord of frogs, guinea pigs, mice, cats and rats, *in vitro* and *in vivo*, have been shown to respond in an excitatory fashion to the application of Substance P[1, 5, 11, 21, 23, 28, 30 37]. However, compared with the biogenic amine and amino acid transmitters, relatively slow excitatory responses of long duration were often noted as was an occasional inhibitory effect.

Another effect was observed by Krivoy *et. al.*[20]: Substance P influenced synaptic excitability, altering the efficiency of the transmission process. This effect was called "synaptic modulation" and was observed in the decerebrate cat spinal α-montoneuron preparation[20, 21]. When these cells are stimulated orthodromically at threshold intensity and at a repetition rate of 0.5 Hz, recordings of single α-montoneurons show action potentials (detonation) in response to a certain percentage of the pulses. Iontophoresis of Substance P causes an increase (facilitation) or decrease (inhibition) in the percent of the stimuli resulting in action potentials. Sustained ejection of Substance P at a current of 40 nanoamperes (nA) resulted in an increase in the number of spikes produced by electrical stimulation. As the iontophoresis current was decreased to 10 nA synaptic activity dropped below control levels. The α-motoneurons were, in effect, ignoring the stimula-

TABLE I

COEXISTENCE OF AMINES AND PEPTIDES WITHIN INDIVIDUAL NEURONS

AMINE	PEPTIDE
serotonin	Substance P
serotonin	Thyrotropin releasing hormone (TRH)
serotonin	Substance P + TRH
norepinephrine	somatostatin
norepinephrine	enkephalin
norepinephrine	neurotensin
acetyl choline	enkephalin
acetyl choline	vasoactive intestinal peptide
dopamine	enkephalin
dopamine	cholecystokinin

tory signals from the dorsal root when low amounts of Substance P were applied and responding with increased synaptic excitability with higher peptide concentrations.

Another example of neuromodulation involves a change in the magnitude and kinetics of the conductance and current responses to the neurotransmitters acetylcholine and glutamate by Substance P. The sensitivity of Renshaw cells to acetylcholine is reduced by Substance P[7, 22, 31] and the glutamate excitatory responses of cultured mouse neurons are attenuated by the peptide[22].

TACHYPHYLAXIS TO SUBSTANCE P

Cultured mouse spinal neurons respond to pressure or iontophoretic application of Substance P[36]. The response is rapid and excitatory and applications of 50 milliseconds duration depolarize the cell membrane above the threshold for generation of action potentials. However, with sustained Substance P release, the membrane first becomes less sensitive to Substance P, and then within one second is completely desensitized.

Desensitization, or tachyphylaxis, to the excitatory effect of Substance P is also observed *in vivo* in cells of the substantia nigra[5]. The distinction between desensitization and neuromodulation is subtle. The examples of modulation above showed Substance P altering synaptic events mediated by other neurotransmitters. One could easily argue that *in vivo* desensitization in the substantia nigra is a result of modulation of synaptic responses to Substance P by serotonin, GABA, norepinephrine, dopamine or peptides known to exist in that brain area. However, the *in vitro* demonstration of tachyphylaxis in dispersed single cells in culture suggests that the desensitizing effect of Substance P is a membrane (receptor) phenomenon not requiring the intervention of other neurotransmitters or neuronal circuitry. Such cellular desensitization may explain the tachyphylaxis to Substance P contractions in the isolated guinea pig ileum and even behavioral desensitization seen in rats where rearing behavior to Substance P injections in the substantia nigra disappears after repeated injections[17].

COEXISTENCE OF SUBSTANCE P AND SEROTONIN IN NEURONS

Hökfelt, who has mapped a number of Substance P pathways, has discovered that the peptide "coexists" with serotonin (5-HT) in the pars α of the nucleus reticularis gigantocellularis, the nucleus intrafascicularis hypoglossi and in several raphé nuclei in the medulla oblongata of rats[13]. Other amine-peptide pairs have been reported recently[12, 14] and are presented in table I.

It is too early to know the significance of these amine- peptide pairs. Kosterlitz and Hughes[18] proposed a functional relationship for neurons containing a classical amine transmitter and the peptide enkephalin where the amine acts postsynaptically while enkephalin controls further release by acting on presynaptic receptors of the 'amine-enkephalin' neuron terminals. Further study of the Substance P-5-HT neurons should reveal if Substance P's role in these pathways is to regulate serotonin release.

NEUROSECRETION OF SUBSTANCE P

Another study of Substance P by immunocytological methods shows that it is secreted into the bloodstream from neurons and therefore may act as a neurohormone. Chan-Palay and Palay[3] identified Substance P-reactive cells in rat sensory ganglia. In the axons of these cells, Substance P is found in granules 100-300 nm in diameter while in the axon terminals it is contained in smaller, round vesicles sized in groups 45-60, 90-110 and 110-150 nm across. The larger vesicles (110-150) were clumped together in peripheral portions of the axon terminal.

The Substance P-reactive cell bodies in the ganglia send fine varicose processes into the surrounding extracellular spaces between ganglion cells and around blood vessels. Electron micrographs show large diameter (100-300 nm) granules moving intact through endothelial cells of the capillary and into the bloodstream.

The target of the secreted Substance P is unknown. Intravenous Substance P injections in rats produce an increase in growth hormone and prolactin[16] whereas intraventricular Substance P stimulates somatostatin release causing decreased growth hormone secretion and no effect on prolactin[4]. The discrepancies in these results may be due to the route of Substance P administration. Besides the histological evidence, Nilsson[26] has measured Substance P in the plasma of man and dogs. Since Substance P is one of the most potent vasodilators known, its release concomitant with nerve stimulation may lead to functional hyperemia of the organ involved. Since Substance P is stable on passage through the pulmonary circulation, it is well situated to have some physiological role as a circulating hormone. It is also found in high concentration in the hypothalamus, along with a number of peptides which at as releasing hormones in the hypothalamo-hypophysial axis.

CONCLUSIONS

We have reviewed a number of possible modes of communication by Substance P. Depending on the cells studies and the experimental conditions, Substance P can excite nerve cells to detonation, inhibit neuronal firing, modulate synaptic transmission and desensitize single cells isolated in culture or brain cells *in situ*. Substance P can be secreted into the bloodstream and coexists with the amine neurotransmitter serotonin in specialized neural pathways. Are these processes physiologically operational? Although it is too early to answer this question comprehensively, the processes provide useful mechanisms to explain some observed effects. Administration of Substance P produces a wide variety of effects in animals. A partial list includes: hypotension[9], gut contraction[9], salivation[2], sedation[32, 33], analgesia[10, 25, 32, 34] hyperalgesia[10, 27], behavioral posturing such as rearing and rotation, [15, 17, 29] release or inhibition of hypothalamic-hypophysial hormones[4, 16] and inhibition of drinking[8]. Considerable attention has been given to the role of Substance P in the afferent transmission of pain. Noxious input from the periphery is relayed to cells in the spinal cord by sensory neurons. It is generally accepted that the intensity of a stimulus transmitted by a single neuron is coded by the rate of its spiking, and that an increase in spiking is positively related to the amount of transmitter that is released from that neuron's terminals. If Substance P is being released, it could have a desensitizing effect on the spinal cells such as that found *in vitro* in cultured mouse spinal neurons or a biphasic, modulating effect also observed in cord α-motoneurons. In the first instance, where the cells are desensitized by prolonged application of the peptide, the spinal cells appear to receive information from Substance P neurons about brief events only. Sustained release of Substance P would turn the cells off. On the other hand, the

α-motoneurons which control muscle contraction and avoidance of further stimuli are more readily depolarized in response to increasing Substance P concentration. Substance P also reaches the cord via decending neurons from the brain stem. The medullary raphé projections to the dorsal horn of the spinal cord are involved in mediating centrally-induced analgesia, presumably by inhibiting primary afferents in the dorsal horn[13]. Serotonin was thought to mediate such analgesic effects. However, since Substance P and serotonin are found together in these decending pathways, the regulation of information about pain by the decending neurons may involve the coordinate release of both Substance P and serotonin.

Several of the synaptic properties of Substance P are also exhibited by other peptides. First, single cells in culture become desensitized to leucine - enkephalin as well as to Substance P[1]. Second, synaptic transmission is modulated by peptides other than Substance P. For example, β-MSH facilitates submaximally evoked reflexes in the cat[19] and enkephalin reduces acetylcholine-induced excitation of Renshaw cells[6]. Third, several peptides are found in neurons containing amine neurotransmitters[14].

Thus, Substance P appears to possess characteristics common to a growing group of neuroactive peptides which are capable of a variety of neuroregulatory functions in many parts of the body.

SUMMARY

We have reiewed a number of possible modes of communication by Substance P. Depending on the cells studied and the experimental conditions. Substance P can excite nerve cells to detonation, inhibit neuronal firing, modulate synaptic transmission and desensitize single cells isolated in culture or brain cells *in situ*. Substance P can be secreted into the bloodstream and coexists with the amine neurotransmitter serotonin in specialized neural pathways.

Considerable attention has been given to the role of Substance P in the afferent transmission of pain. Noxious input from the periphery is relayed to cells in tho spinal cord by sensory neurons.

Substance P also reaches the via decending neurons from the brain stem.

Thus, Substance P appears to possess characteristics common to a growing group of neuroactive peptides which are capable of a variety of neuroregulatory functions in many parts of the bbody.

RESUMEN

Hemos pasado revista a un número de posibles modos de comunicación por la Substancia P. Dependiendo de las células estudiadas y las condiciones experimentales, la Substancia P puede excitar células nerviosas hasta llegar a la detonación, inhibir descargas neuronales, modular la transmisión sinóptica y desensibilizar células aisladas en cultivo o células del cerebro *in situ*. La Substancia P puede ser segregada en el torrente sanguíneo y coexiste con el neurotransmisor serotonina en vías neurales especializadas. Se le ha prestado mucha atención al papel de la Substancia P en la transmisión aferente del dolor. Influjos nocivos desde la periferia son transmitidos a células en la médula espinal por neuronas sensitivas.

La Substancia P también alcanza la médula por las neuronas descendentes desde el tronco cerebral.

La Substancia P. posee características comunes a un grupo creciente de péptidos neuroactivos que poseen una variedad de funciones neurorreguladoras en muchas partes del cuerpo.

RÉSUMÉ

Nons avons passé en revue nn certain nombre de possibles modes de communication grace a la substance P.

Selon les cellules étudiées et les conditions expérimentales la substance P peut exciter les cellules nerveuses jusqu' á la détonation, inhiber des décharges neuronales, moduler la transmission synaptique et désensibiliser des cellules isolées en culture ou des cellules du cerveau in situ. La substance P peut être sécrétée dans le torrent sanguin et coexiste avec le neurotransmetteur sérotonine dans les voies neurales spécialisées.

On a considéré très attentivement le rôle de la substance P dans la transmission, afférente de la donleur.

Des niflux nocifs venus de l'extérieur sont transmis á des neurones de la moelle épiniére par des neurones sensitifs. La substance P atteint aussi la moelle par les neurones descendants renant du tronc cérebral.

La substance P posséde des caractéristiques communes a un groupe croissant de peptides neuroactifs qui possédent une diversité de fonctions neurorégulatrices in diverses parties du corp.

ZUSAMMENFASSUNG

Wir haben wegen der möglichen Arten der Mitteilungen der Substanz P Umschau gehalten. Abhängig von den studierten Zellen und den experimentalen Bedingungen, kann die Substanz P nervöse Zellen reizen bis zur Explosion, neuronale Entladungen hindern, die synaptische Transmission modulieren und einzelne isolierte Zellen in Kulturen oder Gehirnzellen desensibilisieron oder *in situ* lassen. Die Substanz P kann in den Blutkreislauf segrediert werden und mit dem serotonischen Neurotransmissor in spezielle Neuralwege zusammengebracht werden. Grosse Aufmerksamkeit hat man der Rolle gewiedment von der Substanz P in der Übertragung des Schmerzweges. Schädliche Beeinflusigungen von der Peripherie aus werden nach Zellen der Medula spinal durch sensitive Neuronen transportiert.

Die Substanz P besitzt gewöhnliche Charakteristik zu einer wachsenden Gruppe von neuroaktiven Peptiden, die eine verschiedene neuroregulierende Funktion in vielen Stellen des Körpers haben.

REFERENCES

1. *Barker, J.L.* and *T.G. Smith Jr.* 1979. Three modes of communication in the nervous system. In: Modulators, mediators and Specifiers in Brain Function. Eds. Y.H. Ehrlich, J. Volavka, L.G. Davis and E.G. Brunngraber. Plenum, New York.
2. *Chang, M.M.* and *S.E. Leeman.* 1970. Isolation of a sialogogic peptide from bovine hypothalamic tissue and its characterization as Substance P. J. Biol. Chem. 245: 4784-4790.
3. *Chan-Palay, V.* and *S.L. Palay.* 1977. Ultrastructural identification of Substance P cells and their processes in rat sensory ganglia and their terminals in the spinal cord by immunocytochemistry. Proc. Natl. Acad. Sci. 74: 4050-4054.
4. *Chiahara, K., A. Arimura, D.H. Coy* and *A.V. Schally.* 1978. Studies on the Interaction of Endorphins, Substance P, and Endogenous Somatostatin in Growth Hormone and Prolactin Release in Rats. Endocrinology 102: 281-290.
5. *Davies, J.* and *A. Dray.* 1976. Substance P in the substantia nigra. Brain Research 107: 623-627.
6. *Davies, J.* and *A. Dray.* 1976. Changes in amino acid sensitivity during polypeptide "desensitization". Nature 262: 603-609.
7. *Davies, J.* and *A. Dray.* 1977. Substance P and opiate receptors. Nature 268. 351-352.
8. *de Caro, G., M. Massi* and *L.G. Micossi.* 1978. Antidipsogenic effects of I C injections of Substance P in rats. J. Physiol. Lond. 279: 133-140.
9. *Euler, U.S. von,* and *J.H. Gaddum.* 1931. An unidentified depressor substance in certain tissue extracts. J. Physiol. London 72: 74-87.

10. *Frederickson, R.C.A., V. Burgis, C.E. Ha* rrell and J.P. Edwards. 1978. Dual actions of Substance P on Nociception: Possible Role of Endogenous Opioids. Science 199: 1359-1352.

11. *Henry, J.L., K. Krnjevic* and *M.E. Morris* 1975. Substance P and spinal neurons. Can J. Physiol. Pharmacol. 53: 423-432.

12. *Hökfelt, T., L.G. Elfvin, R. Elde, M. Schultzberg, M. Goldstein* and *R. Luft.* 1977. Occurence of somatostatin-like immunoreactivity in some peripheral sympathetic noradrenergic neurons. Proc. Natl. Acad. Sci. 74: 3587-3591.

13. *Hökfelt, T.A. Ljungdahl, H. Steinbusch, A. Verhofstad, G. Nilsson, E. Brodin, B. Pernow* and *M. Goldstein.* 1978. Immunohistochemical evidence of Substance-P-like immuno-reactivity in some 5 hydroxtryptamine-containing neurons in the rat central nervous system. Neuroscience 3: 517-538.

14. *Hökfelt, T., O. Johansson, A. Ljungdahl, J.M. Lundberg* and *M. Schultzberg.* 1980. Peptidergic Neurones. Nature 284. 515-521.

15. *James, T.A.* and *M.S. Starr.* 1977. Behavioral and biochemical effects of Substance P injected into the substantia nigra of the rat. J. Pharm. Pharmac. 29: 181-182.

16. *Kato, Y., K. Chihara, S. Ohgo, Y. Iwasaki, H. Abe and E. Imura.* Growth hormone and prolactin release by Substance P in rats. Life Sci. 19: 441-446.

17. *Kelley, A.E.* and *S.D. Iversen.* 1979. Substance P infusion into the substantia nigra of the rat: behavioral analysis and involvement of striatal dopamine. Eur. J. Pharmacol. 60: 171-179.

18. *Kosterlitz, H.W.* and *J. Hughes.* 1975. Some thoughts on the significance of enkephalin, the endogenous ligand. Life Sci. 17: 91-96.

19. *Krivoy, W.* and *R. Guillemin.* 1961. On a possible role of melanocyte stimulating hormone (β-MSH) in the central nervous system of the Mammalia: an effect of β-MSH in the spinal cord of the cat. Endocrinology 69: 170-175.

20. *Krivoy, W., M. Lane* and *D. Kroeger.* 1963. The actions of certain polypeptides on synaptic transmission. Ann. N.Y. Acad. Sci. 104. 312-318.

21. *Krivoy, W.A., J.R. Crouch, J.L. Henry* and *J.M. Stewart.* 1979. Syn tic modulation by Substance P. Federation Proc. 38: 2344-2347.

22. *Krnjevic, K.* and *D. Lekic.* 1977. Substance P selectively blocks excitation of Renshaw cells by Ach. Can. J. Physiol. Pharm. 55: 958-961.

23. *Le Gal La Salle, G.* and *Y Ben-Ari.* 1977. Microiontophoretic effects of Substance P on neurons of the medial amygdala and putamen of the rat. Brain Res. 135: 174-179.

24. *Lembeck, F.* 1953. Zur Frage der controlen Ubertragung affterenter impulse. III. Das Vorkommen und die Bedeutung der Substanz P in den dorsalen Wurzeln des Rückermarks.

25. *Malick, J.B.* and *J.M. Goldstein.* 1978. Analgesic activity of Substance P following intracerebral administration in rats. Life Sciences 23: 835-844.

26. *Nilsson, G., B. Pernow, G.H. Fischer* and K. Folkers. 1975. Presence of Substance P-like Immunoreactivity in Plasma from Man and Dog. Acta. Physiol. Scand 94: 542-544.

27. *Oehme, P., J. Bergmann, M. Bienert, H. Hil* se, L. Piesche, P. Minh Thu and E. Scheer. 1977. In: Substance P. U.S. von Euler and B. Pernow, Eds. Raven Press, New York pp. 327-335.

28. *Ogata, N.* 1979. Substance P causes direct depolarization of neurones of guinea pig interpeduncular nucleus *in vitro.* Nature 277: 480-481.

29. *Olpe, H-R.* and *W.P. Koella.* 1977. Rotatory behavior in rats by intranigral application of Substance P and an eledoisin fragment. Brain Res. 126. 576-579.

30. *Otsuka, M.* and *S. Konishi.* 1976. Substance P and excitatory transmitter of primary sensory neurons. Cold Spring Harbor Symp. Quant. Biol. 40: 135-143.

31. *Ryall, R.W.* and *G. Belcher.* 1977. Substance P selectively blocks nicotinic receptors on Renshaw cells: a possible synaptic inhibitory mechanism.

32. *Starr, M.S., T.A. James* and *D. Gaytten.* 1978. Behavioral depressant and antinocep tive properties of Substance P in the mouse: possible implication of brain monoamines. Eur. J. Pharmacol. 48: 203-212.

33. *Stern, P.S. Catovic* and *M. Stern.* 1974. Mechanism of action of Substance P. Naunyn-Schmiedeberg's Arch. Pharmacol. 281: 233-239.

34. *Stewart, J.M., C.J. Getto, K. Nelder, E.B. Reeve, W.A. Krivoy* and *E. Zimmermann.* 1976. Substance P and analgesia. Nature 262: 784-785.

35. *Tregear, G.W., H.D. Niall, J.T. Potts, S.E. Leeman* and *M.M. Chang.* 1971. Synthesis of Substance P. Nature 232: 87-89.

36. *Vincent, J.D.* and *J.L. Barker.* 1979. Substance P. Evidence for diverse roles in Neuronal function from Cultured Mouse Spinal neurons. Science 205. 1409-1412.

37. *Walker, R.J., J.A. Kemp. H. Yajima, K. Kitagawa* and *G.N. Woodruff.* 1976 The action of Substance P on Mesencephalic Reticular and Substantia Nigral Neurons of the rat. Experimentia 32: 214 215.

Behavioral Effects of Substance P in Rats

A. BARBEAU, M.D., F.R.C.P. (C)
D.B. RONDEAU, PH.D. *
F.B. JOLICOEUR, PH.D.

Department of Neurobiology
Clinical Research Institute of Montreal
* Present address: Department of Psychology
University of Moncton

Address for Correspondence & Reprints:
Prof. A. Barbeau
Clinical Research Institute of Montreal
110 Pine Avenue West
Montreal, Quebec, Canada
H2W 1R7

During the last decade, several peptides extracted from the pituitary gland and from various regions of the brain have been characterized and synthetized. New therapeutic approaches involving peptides or analogs in the management of a variety of disorders can be foreseen. Some peptides, like luteinizing hormone - releasing hormone (LHRH), thyrotropin - releasing hormone (TRH) and β-endorphin, may have some clinical usefulness in the treatment of endocrine, neurological and mental disorders. Already, significant improvement of symptoms of Parkinson's disease has been observed in patients who received intravenous infusions of L-prolyl-L-leucylglycine amide (PLG or MIF-I) in combination with L-Dopa or anticholinergic drugs (for review see 1, 2). The first clinical trials of PLG, a tripeptide with unconfirmed melanocyte-stimulating hormone (MSH) inhibitory properties, by Kastin and Barbeau (17) were based on experimental data which had indicated that this substance potentiated the actions of L-Dopa and oxotremorine in animals (26), a finding later confirmed by numerous pharmacological studies. Recently, PLG was found also to potentiate apomorphine actions in rats treated with 6-hydroxydopamine in order to produce experimentally symptoms of parkinsonism (2, 20). The study of the role of a given peptide in the central nervous system and of its possible implication in the pathophysiology of human disorders includes the examinations of the behavioral effects of this peptide in animals. The present paper will review some data obtained in our laboratory concerning the behavioral effects of substance P (SP) in rats and its interaction with various treatments modifying catecholaminergic systems.

Functional roles for substance P in the central nervous system are suggested by a large number of pharmacological actions of the extracted or synthetic peptide. For instance, electrophysiological and neurochemical evidence is accumulating that SP acts as a transmitter or modulator of some but not all primary sensory neurons (for review see 23, 32). Intraventricular injections of SP in very low doses produced naloxone-reversible analgesia in mice while injections of high doses of SP in combination with the well known morphine antagonist produced hyperalgesia; these findings suggested a possible relationship between SP and the brain opiate peptides, endorphins and enkephalins (10). Besides its most probable involvement in nociception, SP might play a physiological role in the control of motor functions, especially via interactions with the putative neurotransmitters of the basal ganglia.

High levels of SP have been detected in the substantia nigra of rats and human

brain (3, 15) and the existence of a striatonigral pathway of SP containing cells originating in the anterior striatum has been demonstrated (4, 12, 16). There is electrophysiological evidence that SP is released from the substantia nigra (14) and that it exerts excitatory actions on neurons in this area (9). An increasing number of neurochemical studies supports the hypothesis of an excitatory input of SP on dopaminergic cells in the substantia nigra (6, 7, 8, 33); there is also one report suggesting that SP neurons may be under the tonic inhibitory influence of dopaminergic neurons (11). Indirect evidence that SP may exert an excitatory action on nigrostriatal dopaminergic neurons was provided by the observation that intranigral application of SP in rats caused contralateral rotations, a behavior primarily but not solely attributed to an asymmetric activation of the dopaminergic nigrostriatal pathway (13, 24). Administration of doses of SP higher than 10 μg/rat into the right ventricle of naive rats induced within a few minutes horizontal rotations to the left; and then, rotations along the length axis of the body; the rotations were followed by a period of general excitation (6).

We undertook an experiment to establish the dose related effects of intraventricular (IVT) administration of synthetic SP (Peninsula Labs, San Carlos, Calif.) on motor activity in rats. Doses ranging from 0.07 to 80.00 μg/rat were studied. Activity scores were determined by photocell counts recorded for a 15 min test period which followed a 5 min pre-injection adaptation period. A rigid posture with extension of the limbs, interrupted by several barrel rolling rotations was a characteristic behavioral response to IVT infusion of SP

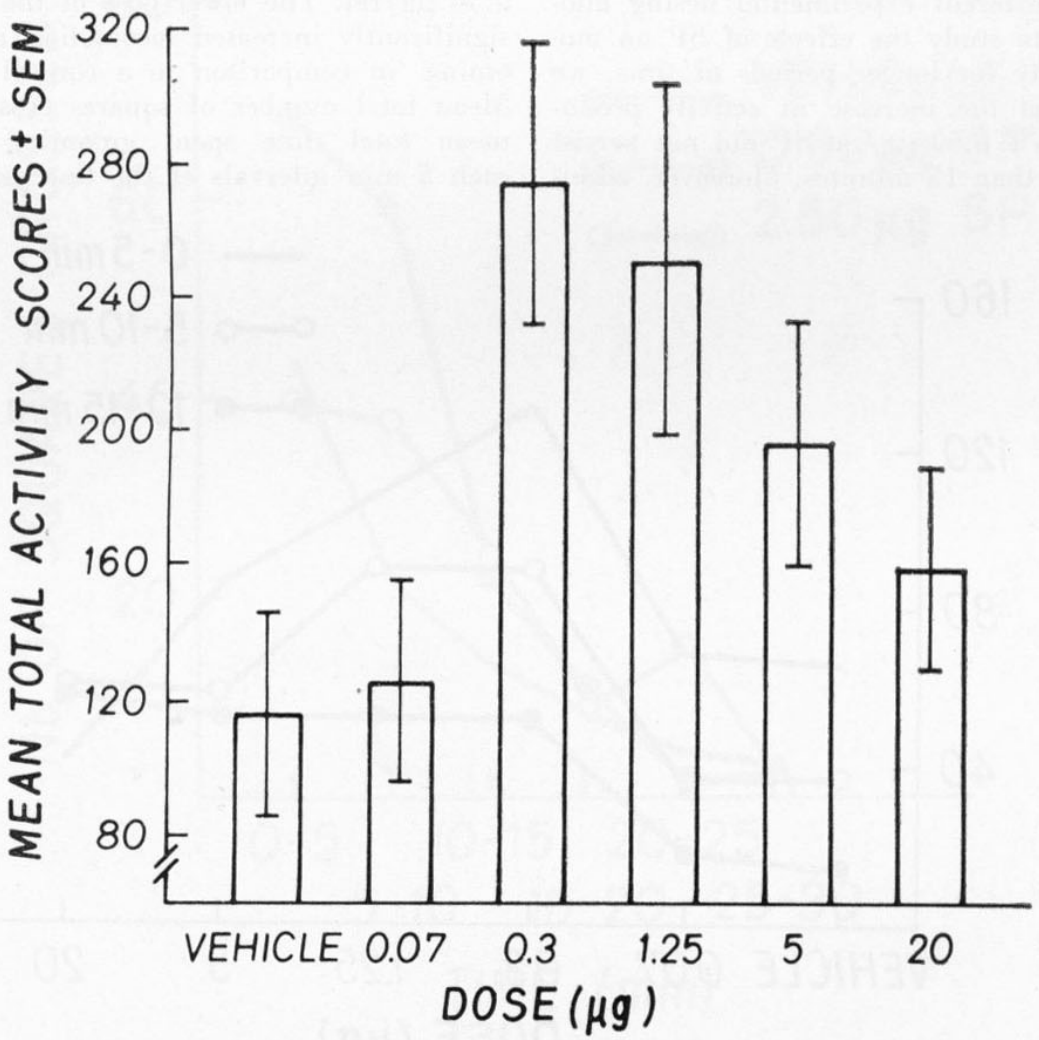


Fig. 1. — Mean total activity scores during the 15 min test session following IVT administration of substance P. Eight rats per group.

in the 40-80 μg dose range. Similar abnormal postures were observed to a lesser extent with 20 μg/rat. An analysis of variance revealed significantly greater activity scores for the group of animals which received 0.30 and 1.25 μg doses of SP. This increase in activity induced by SP is illustrated in Figure 1 in which mean total activity scores during the 15 min test session are plotted for each dose of SP. Further examination of the data indicated that, at these doses, SP increased motor activity during all three 5 min intervals of the session. Mean activity scores for those intervals are presented in Figure 2. A significant increase in the total number of reasings was found also in the animals injected with 0.30 μg SP. Grooming was observed, often accompanied by hypersalivation, but animals did not spend more time than control rats in such activity.

In a different experimental desing allowing us to study the effects of SP on motor activity for longer periods of time, we found that the increase in activity produced by IVT 0.60 μg/rat SP did not persist for more than 15 minutes. Moreover, administration of the peptide, at this dose, did not potentiate or reduce the stereotypy and increased activity induced by 2 mg/kg d-amphetamine and 1 mg/kg apomorphine, injected simultaneously or 30 min after SP. Results of experiments in mice confirmed that SP shows little activity in the L-Dopa and 5-HT potentiation tests (25) and indicated that the peptide does not antagonize or accentuate tremors induced by oxotremorine.

Photocell counts obtained when animals are placed in an activity meter are an index of gross motor activity. It is not easy from this measurement to distinguish between locomotor activity and the fine movements of grooming behavior. Therefore, we studied the behavioral effects of SP in rats placed in a 16 squares open field (88 X 88 X 60 cm) for 30 minutes. SP was administered IVT in doses of 0.60 and 2.50 μg/rat. The lower dose of the peptide significantly increased locomotion and grooming in comparison to a control group. Mean total number of squares crossed and mean total time spent grooming during each 5 min intervals of the test period are

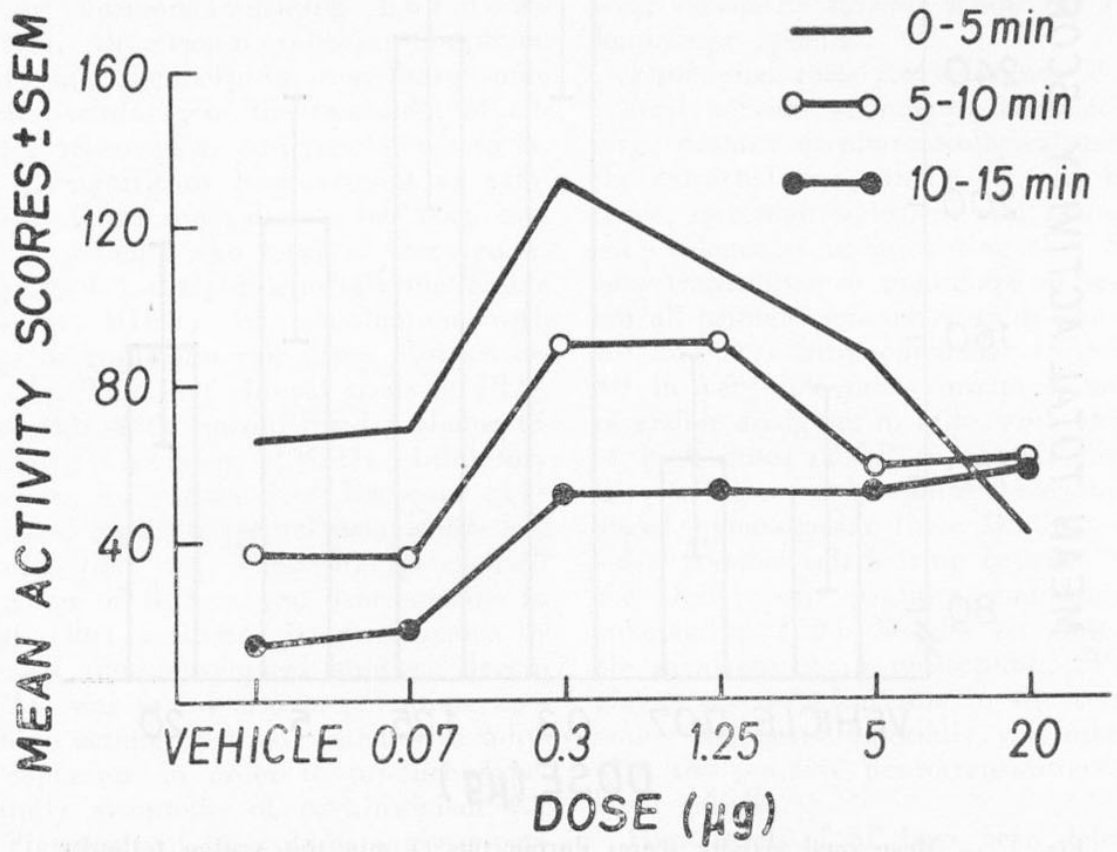


Fig. 2. — Mean activity scores for each 5 min intervals of the test sessions.

presented in Figures 3 and 4 respectively. Analyses of variance and appropriate post hoc tests revealed that locomotor activity in the 0.60 μg SP group was increased significantly for the first 10 min of the test session while grooming activity remained significantly greater almost throughout the 30 min. Those results suggest that a greater frequency of grooming movements was probably an essential component of the SP induced activity initially observed when rats were tested in an activity meter.

In order to study at the behavioral level the possible interaction of SP with the catecholaminergic systems, various groups of rats were administered the following treatments at appropriate times before IVT injections of the peptide: 250 mg/kg α-methyl-para-tyrosine, a tyrosine hydroxylase inhibitor, 20 mg/kg phenoxybenzamine, a predominantly post-synaptic α-adrenergic receptor antagonist, 25 mg/kg FLA-63, a relatively specific dopamine-β-oxidase inhibitor and 0.1 mg/kg haloperidol, a dopamine receptor antagonist. All four treatments produced hypokinesia in SP-vehicle treated rats, as evidenced by decreases of similar magnitude in locomotor activity measured in the open field. SP in doses of 0.60 and 2.50 μg/rat did not affect the behavioral depression produced by α-methyl-para-tyrosine, phenoxybenzamine and FLA-63. However, the peptide systematically reversed the hypokinesia of the haloperidol pretreated rats. An analysis of variance and the appropriate post-hoc tests revealed that 0.60 μg SP increased locomotor activity during the whole test session while the higher dose of the peptide, 2.50 μg, produced such effect only during the first

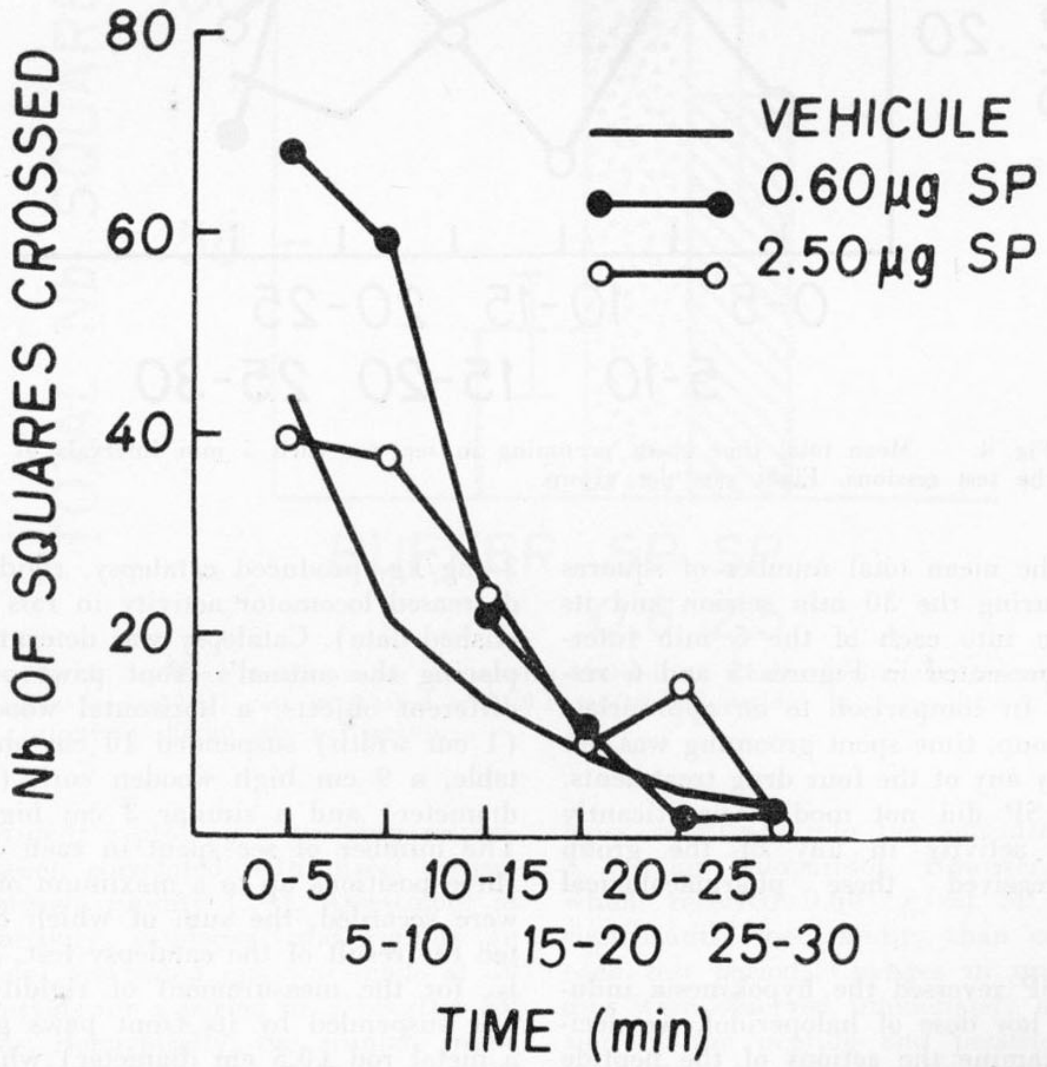


Fig. 3. — Mean total number of squares crossed for each 5 min intervals of the test sessions. Eight rats per group.

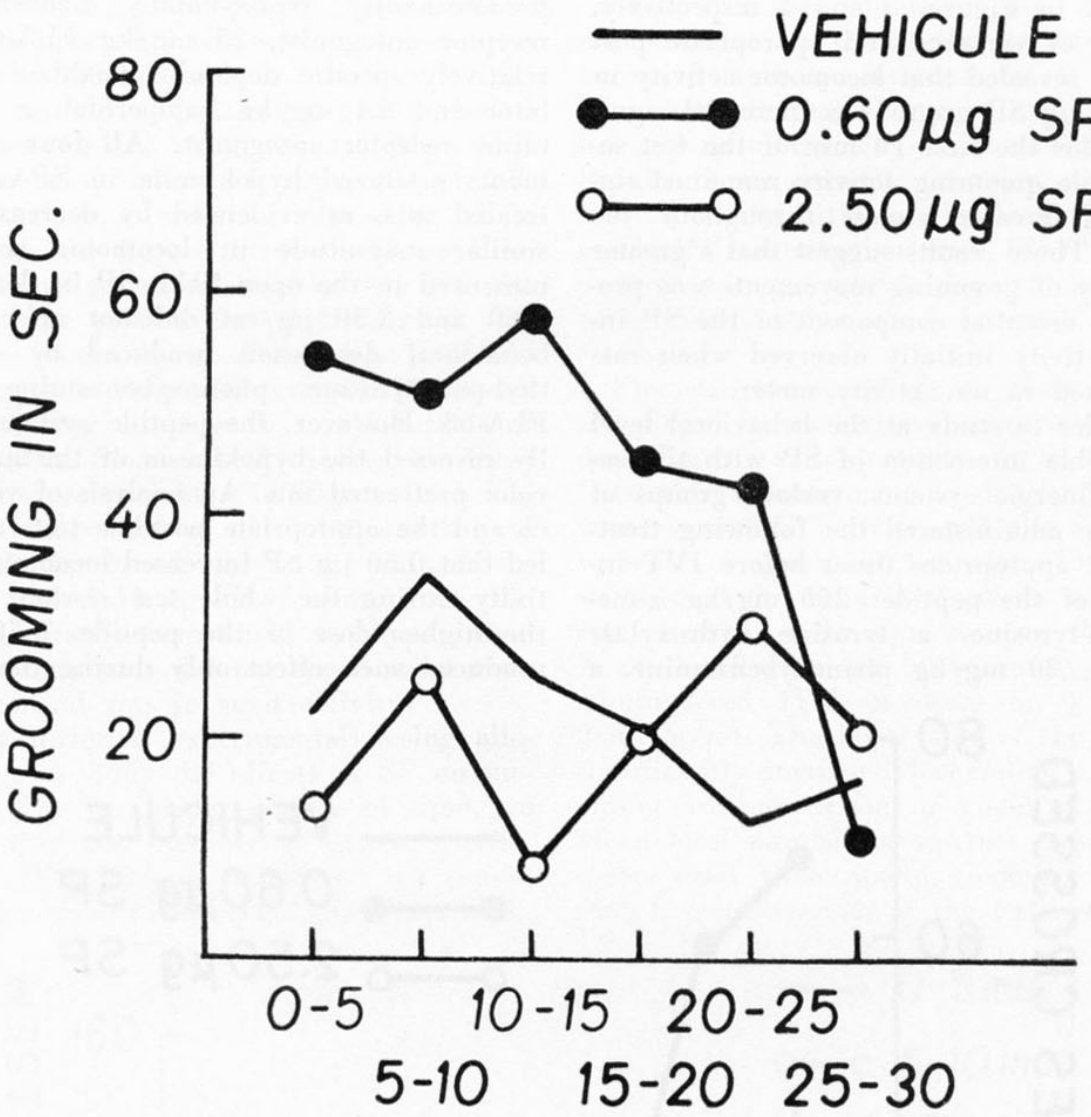


Fig. 4. — Mean total time spent grooming in sec. for each 5 min intervals of the test sessions. Eight rats per group.

5 min. The mean total number of squares crossed during the 30 min session and its breakdown into each of the 5 min intervals are presented in Figures 5 and 6 respectively. In comparison to an appropriate control group, time spent grooming was not reduced by any of the four drug treatments. However SP did not modify significantly grooming activity in any of the group which received these pharmacological agents.

Since SP reversed the hypokinesia induced by a low dose of haloperidol, we decided to examine the actions of the peptide on some other behavioral effects of the neuroleptic. A higher dose of haloperidol, 3 mg/kg, produced catalepsy, rigidity and decreased locomotor activity in rats (unpublished data). Catalepsy was determined by placing the animal's front paws on three different objects: a horizontal wooden bar (1 cm width) suspended 10 cm above the table, a 9 cm high wooden cork (2.5 cm diameter) and a similar 3 cm high cork. The number of sec spent in each of these three positions up to a maximum of 60 sec were recorded, the sum of which constituted the result of the catalepsy test. Similarly, for the measurement of rigidity a rat was suspended by its front paws grasping a metal rod (0.5 cm diameter) which was held about 50 cm above the table and the time the animal remained in such position

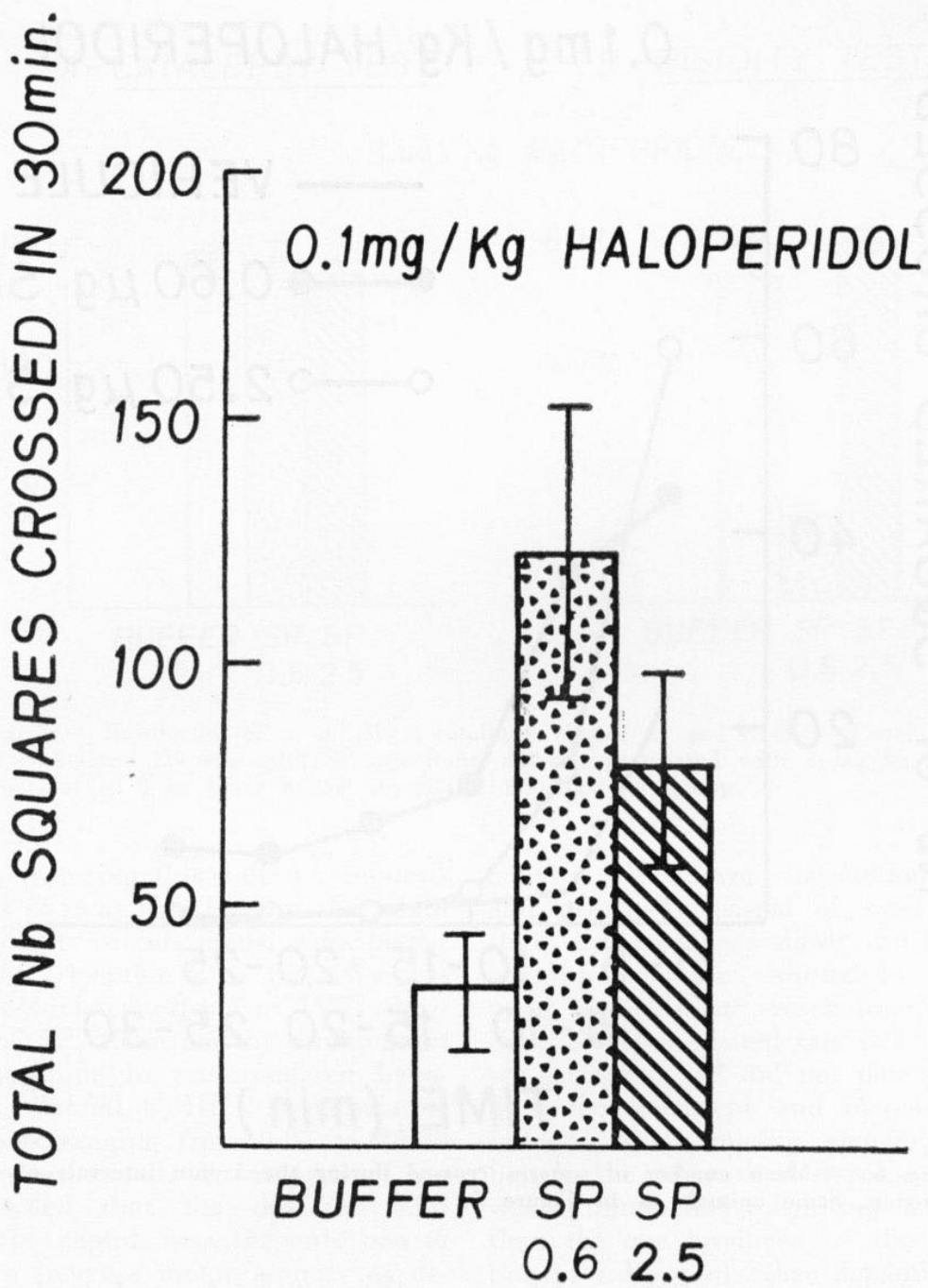


Fig. 5. — Mean total number of squares crossed ± S.E.M. during the 30 min test session. All rats pretreated with 0.1 mg/kg haloperidol ip 2 hr prior SP injections. Eight rats per group.

was recorded; a prolonged grasping response has been correlated with direct measures of muscle rigidity (29). Assessment of motor activity, catalepsy and rigidity in haloperidol pretreated rats was made at 30 min intervals for a period of 3 hr after SP injections. Behaviorally, the animals injected with IVT 0.60 and 2.50 μg/rat SP did not differ from controls; the peptide did not counteract the hypokinesia and catalepsy resulting from the administration of 3 mg/kg haloperidol. However, animals which received 0.60 μg/rat SP displayed significantly less rigidity than controls at each test period. Changes in muscle rigidity were observed immediately after infusion of the peptide and persisted for at least 150 min. Results at the catalepsy and rigidity tests obtained 120 min after SP injections are presented in Figure 7. It ap-

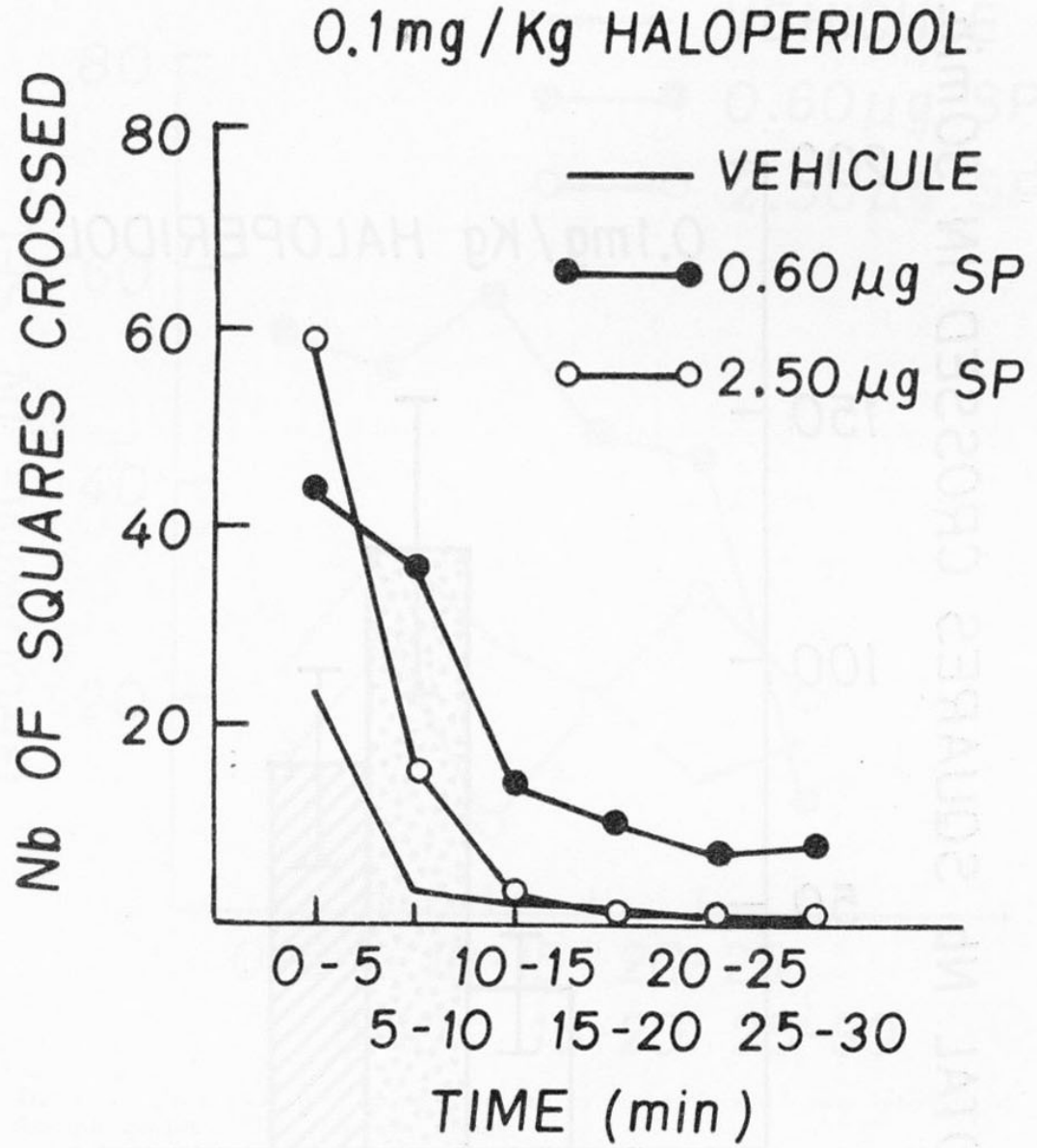


Fig. 6. — Mean number of squares crossed during the 5 min intervals of the session. Same animals as in Figure 5.

pears that the occurrence of the symptoms induced by a high dose of haloperidol, catalepsy, rigidity and a considerable reduction in motor activity, cannot be attributed to a decrease of SP content in the substantia nigra. In fact, it has been reported recently that chronic administration of haloperidol significantly reduced SP content of the substantia nigra, but also, that an acute treatment with a dose of 2 mg/kg of the neuroleptic failed to produce such neurochemical change (11). Nevertheless, our results indicated that SP exerted differential effects on the symptoms induced by haloperidol.

Destruction of catecholamine containing neurons by microinjections of 6-hydroxydopamine (6-0HDA) into the substantia nigra and/or along the dopaminergic nigrostriatal pathway have been employed to create animal models of parkinsonism (28, 31). Bilateral injections of 6-0HDA in the anterolateral hypothalamus produced a hypokinesia in rats which is accompanied by a generalized reduction in brain noradrenaline levels and a reduction of dopamine in the striatum and cerebral cortex (5).

The putative dopamine receptor agonist apomorphine and several drugs used in the treatment of Parkinson's Disease were

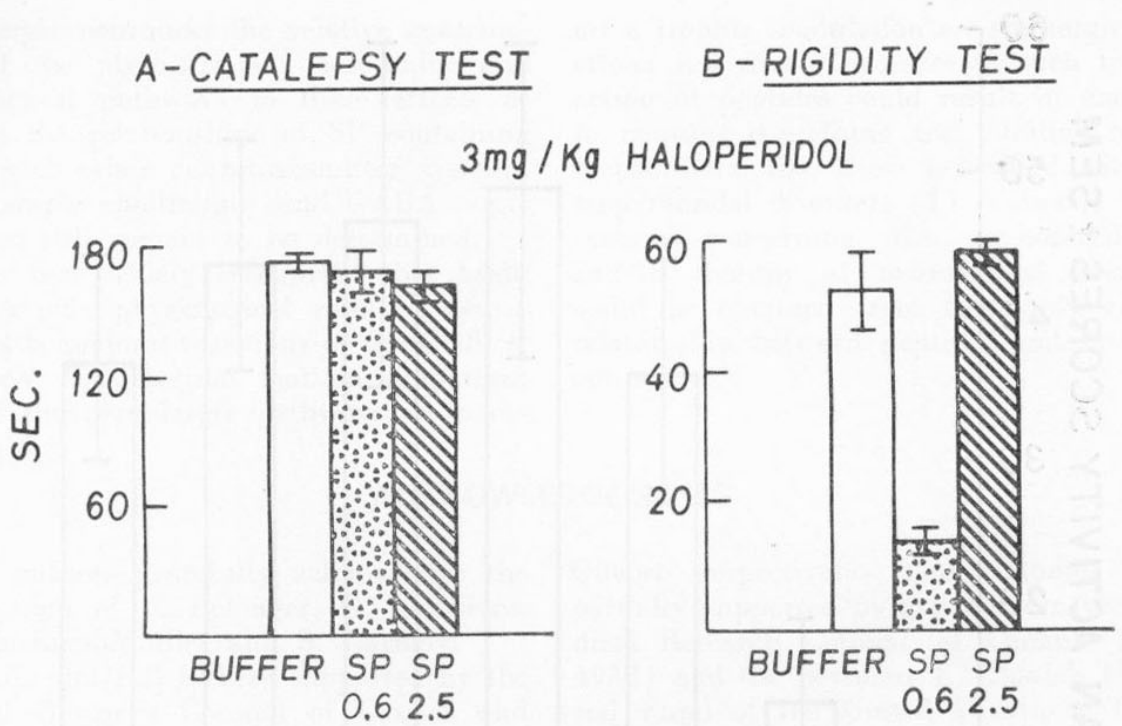


Fig. 7. — Results in sec ± S.E.M. at catalepsy (Panel A) and rigidity (Panel B) tests obtained 120 min after SP injections. All rats pretreated with 3 mg/kg haloperidol ip 2 hr prior to SP injections. Six rats per group.

effective in reversing this 6-0HDA induced hypokinesia (5); as mentioned earlier, apomorphine effects in this model were potentiated by the tripeptide PLG (2). We studied the dose-related effects of IVT administration of SP, alone and in combination with apomorphine in rats rendered hypokinetic by bilateral 6-0HDA hypothalamic lesions. Doses ranging from 0.07 to 20.00 μg/rat were examined. An analysis of variance revealed that the dose of 0.30 μg/rat of the peptide was the only one to significantly increase motor activity as determined by photocell counts in a 5 min test session immediately after SP administration. This is illustrated in Figure 8 in which mean activity scores during the test session are plotted for each dose of SP.

Behavioral observations indicated that the significantly greater activity in the 0.30 μg/rat SP group as well as the higher mean activity counts in other groups which received SP were not necessarily related to the induction of locomotor activity in the hypokinetic rats. Grooming was observed in all animals injected with 0.30 μg/rat SP while only 3 out of 8 showed locomotor activity. Whenever locomotion occurred, gait of rats consisted of extremely short steps, executed very slowly. Such abnormal walking has been reported to occur following injections of anticholinergics in akinetic 6-0HDA treated rats (27). At all doses examined, SP did not potentiate or reduce the stereotypy and increased activity induced by 1 mg/kg apomorphine. Although the reversal of hypokinesia by SP was of much less magnitude and duration than the one produced by the administration of L-Dopa or other dopaminergic agonists (5), our finding demonstrated that IVT injection of SP elicits a behavioral activation in rats with 6-0HDA hypothalamic lesions.

In summary, we observed that IVT injections of low doses of SP, between 0.30 and 1.25 μg/rat, induced behavioral activation in rats characterized by increased locomotor activity and grooming.

Similar behavioral changes have been reported following administration of SP by this route in rabbits (22) and rats (21) and following intranigral injections (18). It has been demonstrated recently that ap-

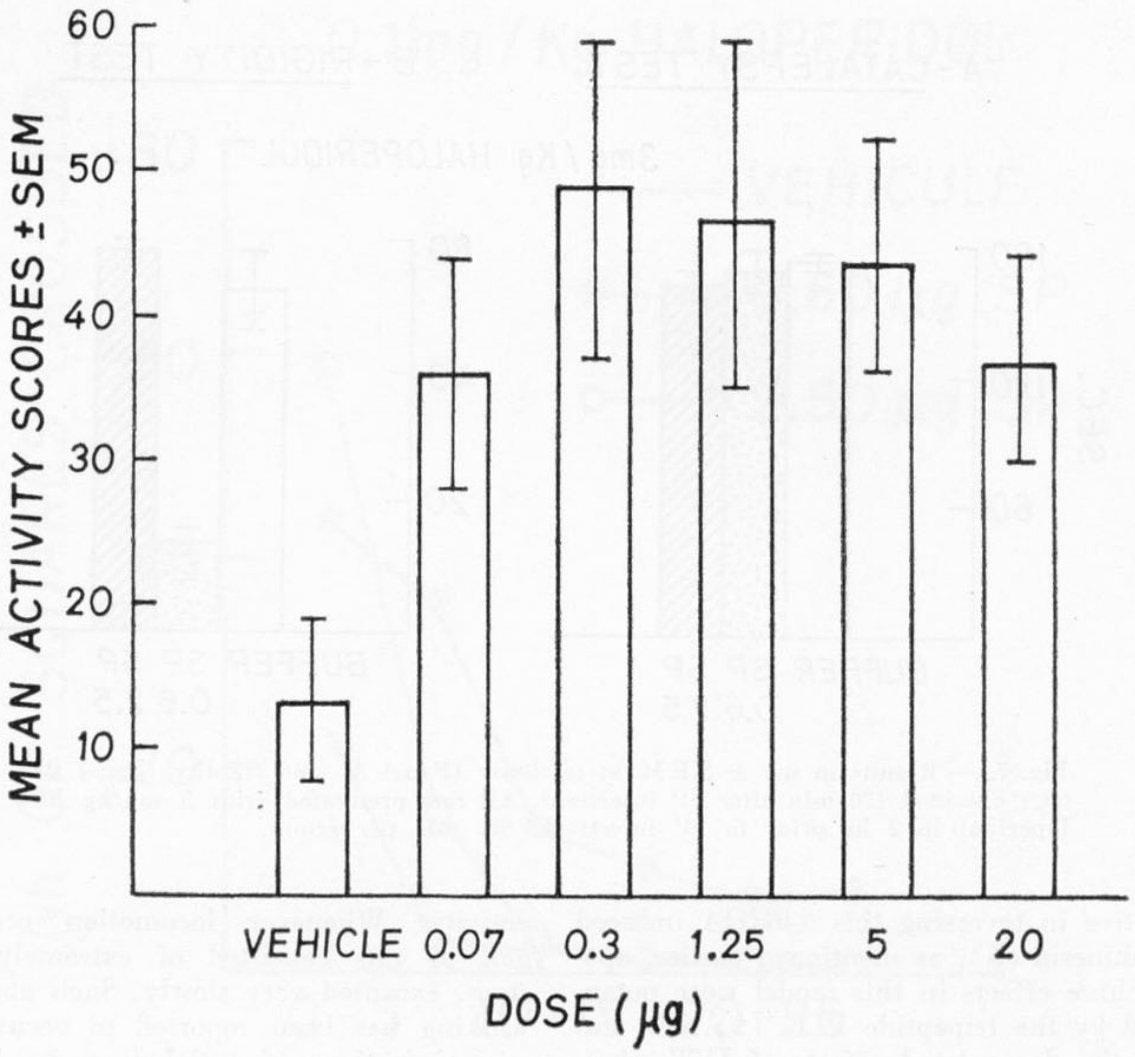


Fig. 8. — Mean activity scores during the 5 min test session following IVT administration of SP in 6-OHDA treated rats. Eight rats per group.

plication of SP into the ventral tegmental area (VTA) elicits an increase in locomotion and exploration without affecting grooming (30); a potentiation of the effects of d-amphetamine on activity by previous infusion of SP into the VTA was also reported (30). The latter result is in contrast with our finding that IVT injection of SP did not potentiate the stereotypy and behavioral arousal produced by amphetamine and apomorphine.

Since we administered the peptide into the ventricles, it cannot be established precisely upon which brain structures SP might have exerted its action. The use of two doses of haloperidol which produced distinct behavioral symptoms in rats allowed us to study separately the effects of SP on hypokinesia and rigidity. The peptide reversed the rigidity induced by a high dose of the dopamine receptor antagonist but did not affect catalepsy and hypokinesia. SP did reverse the hypokinesia induced by a dose of 0.1 mg/kg haloperidol but did not affect the behavioral response to α-methyl-para-tyrosine, phenoxybenzamine and FLA-63.

Locomotion and grooming were briefly observed after SP injection in rats rendered hypokinetic by 6-OHDA hypothalamic lesions. It has been demonstrated that the increase in locomotor activity resulting from SP infusion into the VTA could be blocked by infusion of haloperidol into the nucleus accumbens or by 6-OHDA lesions of the ascending A10 neurones (19). These findings suggest that SP induces its behavioral effects through activation of do-

paminergic neurones; the relative contribution of the nigro-striatal, mesolimbic and mesocortical pathways to these effects as well as the relationships of SP containing fibers with other neurotransmitter systems, for example cholinergic and GABA ergic neurons, still remain to be determined.

It is now widely recognized that brain peptides play physiological roles in neuronal and behavioral functions of the CNS. It has been hypothesized that an important role of the peptidergic pathways is to exert a trophic modulation on aminergic functions and that a decrease in such trophic action of peptides could result in damages to neurons containing the putative neurotransmitters, like those associated with extrapyramidal disorders (1). Valuable information concerning the pathophysiology and/or therapy of neurological disorders could be obtained from the study of the relationship between peptides and biogenic amines.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge the contribution of F. Bélanger, G. Fouriezos, C. Robichaud-Maillet and S. Gariépy.

D.B.R. and F.B.J. were supported by the Medical Research Council of Canada and the Conseil de la Recherche en Santé du Québec respectively. These studies were partially supported by grants from the Medical Research Council of Canada (MA-4938) and the Seymour E. Clonick Memorial Fund of the United Parkinson Foundation.

SUMMARY

In recent years, many peptides have found a progressively more important role in the biochemistry and physiology of the brain in addition to their definite involvement in hormonal regulation. There is growing evidence that, among these peptides having CNS effects, the undecapeptide substance P (SP) may functionally interact with the neurochemical mechanisms of the basal ganglia. A series of experiments were undertaken to determine the behavioral effects of SP in rats. Intraventricular injections of SP in doses ranging from. 30 to 1.25 μg/rat induced motor activity in naive rats placed for 15 min in an activity meter, locomotion and grooming in a 16 squares open field were also increased temporarily. Doses over 40 μg/rat produced immobility, rigidity and barrel rolling rotations.

The interaction of SP with various treatments modifying catecholaminergic systems was investigated. Administration of SP, .60 μg/rat in combination with 30 min after injections of 2 mg/kg d-amphetamine and 1 mg/kg apomorphine did not potentiate or reduce the hyperactivity and the stereotyped behavior induced by these two drugs. SP did not affect the hypokinesia produced by the tyrosine hydroxylase inhibitor, α-methyl-p-tyrosine (250 mg/kg), the α-adrenergic receptor antagonist, phenoxybenzamine (20 mg/kg) and the dopamine-β-hydroxylase inhibitor, FLA-63 (25 mg/kg). However, SP, in a dose of 0.60 μg/rat, sistematically reversed the behavioral depression and the decrease in locomotor activity induced by a relatively small dose of haloperidol (1 mg/kg), a dopamine receptor antagonist. On the other hand, SP did not counteract the catalepsy resulting from the administration of a higher dose of this neuroleptic (3 mg/kg).

Intraventricular injection of SP, 0.30 μg/rat, increased motor activity in rats rendered hypokinetic by bilateral microinjections of 6-hydroxydopamine into the anterolateral hypothalamus; behavioral observations indicated that grooming and not locomotion was mainly responsible for the greater activity scores. The reversal of the hypokinesia produced by administration of apomorphine to these 6-0HDA treated animals was not potentiated by SP.

Brain peptides may modulate catecholaminergic functions and may contribute to

the pathogenesis of extrapyramidal disorders such as Parkinson's disease and Huntington's chorea. The possible involvement of SP in the control of motor functions is discussed.

RESUMEN

En los últimos años, muchos péptidos han encontrado un papel progresivamente más importante en la bioquímica y fisiología del cerebro agregado a su definitiva implicancia en la regulación hormonal. Existe una evidencia creciente que entre estos péptidos que tienen influencia sobre el sistema nervioso central, el undecapéptido sustancia P (SP) puede actuar funcionalmente en los mecanismos neuroquímicos de los ganglios basales. Se realizaron una serie de experimentos para determinar los efectos en la conducta de la substancia P en ratas. Inyecciones intraventriculares de SP en dosis fluctuantes entre 30 a 1.25 Mg/rata inducían actividad motora en ratas sin práctica de conducta colocadas durante 15 minutos en un medidor de actividad, locomoción y acicalamiento en un espacio abierto de 16 cuadrados que fueron también acrecentados en el tiempo. Dosis por encima de 40 Mg/rata produce inmovilidad, rigidez y rotaciones.

Se investigó la interacción de SP con varios tratamientos que modifican los sistemas catecolaminérgicos.

La administración de SP. 60 Mg/rata en combinación con inyecciones 30 minutos después de 2 mg/kg de anfetamina y 1 mg/kg de apomorfina no acrecentó o redujo la hiperactividad y la conducta estereotipada inducida por estas dos drogas.

La substancia P. no afecta la hipokinesia producida por el inhibidor de la tirosina hidroxilasa α-metil-p-tirosina (250 mg/kg), el antagonista del receptor α-adrenérgico, fenoxibenzamina (20 mg/kg) y el inhibidor de la dopamina B-hidroxilasa, FLA-63 (25 mg/kg). No obstante SP en una dosis de 0.60 Mg/rata invirtió la depresión de conducta y la disminución en actividad locomotora inducida por una dosis relativamente pequeña de haloperidol (1 mg/kg), antagonista del receptor de dopamina. Por otro lado, SP no neutralizó catalepsia resultante de la administración de una dosis mayor de este neuroléptico (3 mg/kg).

La inyección intraventricular de SP, 0.30 Mg/rata, acrecentó la actividad motora en ratas vueltas hipokinéticas por microinyecciones bilaterales de hidroxidopamina en el hipotálamo anterolateral; observaciones de conducta indicaron que al acicalamiento y no a la locomoción correspondían los mayores resultados de actividad. La inversión de la hipokinesia por la administración de apomorfina a estos animales tratados por 6-0HDT no fue potencializada por SP.

Los péptidos del cerebro pueden modular las funciones catecolaminérgicas y pueden contribuir a la patogénesis de los desórdenes extrapiramidales tales como enfermedad de Parkinson y corea de Huntington. Se discute la posible implicancia de SP en el control de las funciones motoras.

RÉSUMÉ

On cours des dernieres années, de nombreux peptides se sont montrés de plus en plus importants dans la biochimie et la physiologie du cerveau, outre leur rôle dans la régulation hormonale. Il est de plus en plus évident que parmi les peptides qui ont une influence sur le systeme nervoux central, l'undecapeptide substance P (SP) peut agir sur le fonctionnenent des mécanismes neurochimiques des ganglions basaux. Des expériences ont été faites pour déterminer le rôle de la substance P chez le rat.

Des injections intraventriculaires de SP a doses variant de 30 a 1,25 μg/rat provoquaient une activité motrices chez des rats neufs, placés pendant 15 minutes dans un appareil mesurant l'activité, la locomotion

et la parure dans un espace ouvert de 16 carrés qui furent augmentés avec le temps. Des doses supérieures a μg40/rat produisent immobilité, raidissenent, rotation.

On a étudié l'interaction de SP et de divers traitements modifiant les systémes catécholaminergiques. L'administration de SP 60μg/rat combinée avec des injections 30 minutes plus tard de 2 mg/kg d'anphétamine et 1 m/kg d'apomorphine n'a ni augmenté ni réduit l'hyperactivité et la conduite stéréotypée induite par ces deux drogues.

La substance P n'affecte pas l'hypokinese produite par l'inhibiteur de la tyrosine hydroxylase, 1 méthyl-p-tyrosina (250 mg/kg), l'antagonique du récepteur adrénergique phénoxiybenzamine (20 mg/kg), et l'inhibiteur de la dopamine B-hydroxylase FLA-63 (25 mg/kg). Cependant, SP a la dose de 0,60 μg/rat a inverti la dépression de conduite et la diminution de l'activité locomotrice provoqué par une dose relativement faible de haloperidol (1 mg/kg) antagonique du récepteur de dopamine. D'autre part, SP n'a pas neutralisé la catatepsie provoqué par L'administration d'une dose plus porte de ce neuroleptique (3 mg/kg).

L'injection intraventriculaire de SP (0,30/rat) a augmenté l'activité motrice chez des rats rondus hypokinétiques par des microinjections bilatérales de 6 hydroxydopamine dans l'hypethalamus antéro lateral. L'observation de la conduite indique que á la parure et non a la locomotion correspondaient les résultats d'augmentation d'activité. L'inversion de l'hypokinésie résultant de l'administration d'apomorphine a ces animaux traités par 6-OHDA n'a pas été potentialosée par SP.

Les peptides du cerveau peuvent moduler les fonctions catécholaminergiques et peuvent contribuer a la pathogénese des désordres extrapyraminaux tels que la maladie de Parkinson et chorée de Huntington. On discute le possible rôle de SP dans le contrôle des fonctions motrices.

ZUSAMMENFASSUNG

In den letzten Jahren haben viele Peptiden ein fortschreitend wichtiges Papier in der Biochemie und Physiologie des Gehirns zugehörig zu seiner hormonalen Regulierung. Es besteht eine wachsende Beziehung zwischen diesen Peptiden, die Einfluss haben auf das Zentralsystem; die undecaptische Substanz P (Sr) kann funktionell handeln in dem neurochemischen Mechanismus der Basalganglien. Es wurde eine Serie Experimente gemacht, um die Effekte in der Leitung der Substanz P bei Ratten zubestimmen. Intraventrikulare Injektionen von Sp in Schwankungen der Dosis zwischen 30 bis 1,25 mg/Ratte induzierten motorische Aktivität bei Ratten ohne Führunsprakis während 15 Minuten in einem Messgerät der Aktivität, Lokomotion und Polierung in einem offenen Raum von 16 Quadraten, die auch in der Zeit vermehrt wurden. Eine Dosis von 40 mg/Ratte mehr verursacht eine starre Bewegungslosigkeit und Retationen.

Die Interaktion von Sp bei verschiedenen Behandlungen wird untersucht wegen der Modifikation der Katecolaminergikas. Die Anwendung von Sp.60 mg/Ratte in Kombinattion von 30 Minuten nach 2 mg/Kg von Amfetamin und 1 mg/kg von Apomorphin erhöht oder reduziert nicht die Hyperaktivität und die esteriopatische Führung, eingeführt durch diese zwei Drogen.

Die Substanz F. greieft nicht die Hypokinese, produziert durch den Inhibidor der Tirosinhydroxilase, α - Methyl - p - thyrosin (250 mg/kg), den Antagonisten des Rezeptors α-adreniko, Phenoxibenzamin (20 mg/kg und den Inhibidor der Dopamin-B -hydroxilase, FLA -63 (25 mg/kg). Trotzdem hat SPm in einer Dosis von 0,60 mg/Ratte die Depression des Konduktes invertiert und die Minderung in der lokomotorischen Aktivität durch eine relativ kleine Dosis von Haloperidel (1 mg/kg), des Antagonisten des Rezeptores von Dopamin. Andrerseite, Sp hat nicht die entstandene Katalepsie neutralisiert nach der Anwendung einer grösseren Dosis dieses aminoleptikos

(3 mg/kg).

Die intraventrikulare Injektion von Sp, 0,30 mg/Ratte, beschleunigte die motorische Aktivität bei Ratten, die hypokinetisch geworden waren durch Mikroinjektionen beiderseits von Hydroxidopamin in den anterolateralen Hypothalamus; Observationen des Konduktus ergaben, dass die Hervorhebung und nicht die Lokomotion die grössten Resultate der Aktivität entsprachen. Die Inversion der Hyperkinese durch die Anwendung von Apomorphin bei diesen mit 6-OHDA behandelten Tiere wurde nicht durch Sp verstärkt.

Die Peptiden des Gehirns könen die Katekolaminergischen Funktionen ändern und können zur Pathogenese der extrapyramidalen Störungen beitragen wie die Parkinson sche Krankheit und Correa von Huntington. Die mögliche Implikation von Sp in der Kontrolle der motorischen Funktion wird diskutiert.

REFERENCES

1. *Barbeau, A.* Role of peptides in the pathogenesis and treatment of Parkinson's Disease. In: Central Nervous System Effects of Hypothalamic Hormones and Other Peptides, edited by R. Collu, A. Barbeau, J. R. Ducharme and J.G. Rochefort. New York: Raven Press, 1978, pp. 403-414.
2. *Barbeau, A. and A.J. Kastin.* Polypeptide therapy in Parkinson's Disease. A new approach. In Advances in Parkinsonism, edited by W. Birkmayer and O. Hornykiewicz Basle: Editiones Roche, 1976, pp. 483-487.
3. *Brownstein, M.J., E.A. Mroz, J.S. Kiser, M. Palkovits* and *S.E. Leeman.* Regional distribution of substance P in the brain of the rat. Brain Res. 116: 299-305, 1976.
4. *Brownstein, M.J., E.A. Mroz, M.L. Tappaz* and *S.E. Leeman* On the origin of substance P and glutamic acid decarboxylase (GAD) in the substantia nigra. Brain Res. 135: 315-333, 1977.
5. *Butterworth, R.F., F. Belanger* and *A. Barbeau.* Hypokinesia produced by anterolateral hypothalamic 6-hydroxydopamine lesions and its reversal by some antiparkinson drugs. Pharmac. Biochem. Behav. 8: 41-45, 1978.
6. *Carlsson, A., T. Magnsson, G.H. Fisher, D. Chang* and *K. Folkers.* Effect of synthetic substance P on central monoaminergic mechanisms. In. Substance P, edited by U.S. Von Euler and B. Pernow. New York: Raven Press, 1977, pp. 201-205.
7. *Cheramy, A., R. Michelot, V. Leviel, A. Nieoullon, J. Glowinski* and *B. Kerdelhue.* Effect of immunoneutralization of substance P in the cat substantia nigra on he release of dopamine from dendrites and terminals of dopaminergic neurons. Brain Res. 155: 404-408, 1978.
8. *Cheramy, A., A. Nieoullon, R. Michelot* and *J. Glowinski.* Effects of intranigral application of dopamine and substance P on the in vivo release of newly synthesized (^{3}H)-dopamine in the ipsilateral caudate nucleus of the cat. Neurosci. Lett. 4: 105-109, 1977.
9. *Davies, J.* and *A. Dray.* Substance P in the substantia nigra. Brain Res. 107: 623-627, 1976.
10. *Frederickson, R.C.A., V. Burgis, C.E. Harrell* and *J.D. Edwards.* Dual actions of substance P on nociception: Possible role of endogenous opioids. Science 199: 1359-1362, 1978.
11. *Hong, J.S., H.-Y.T. Yank* and *E. Costa.* Substance P content of substantia nigra after chronic treatment with antischizophrenic drugs. Neuropharmac. 17: 83-85, 1978.
12. *Hong, J.S., H.-Y.T. Yang, G. Racagni* and *E. Costa.* Projections of substance P containing neurons from neostriatum to substantia nigra. Brain Res. 12: 541-544, 1977.
13. *James, T.A.* and *M.S. Starr.* Behavioural and biochemical effects of substance P injected into the substantia nigra of the rat. J. Pharm. Pharmac. 29. 181-182, 1977.
14. *Jessell, T.M.* Inhibition of substance P release from the isolated rat substantia nigra by GABA. Brit. J. Pharmacol. 59: 486P., 1977.
15. *Kanazawa, I., E. Bird, R. O'Connell* and *D. Powell.* Evidence for a decrease in substance P content of substantia nigra in Huntington's chorea. Brain Res. 120: 387-392, 1977.
16. *Kanazawa, I., P.C. Emson* and *A.C. Cuello.* Evidence for the existence of substance P containing fibers in striato-nigral and pallido-nigral pathways. Brain Res. 119: 447-453, 1977.
17. *Kastin, A.J.* and *A. Barbeau.* Preliminary clinical studies with L-prolyl-L-leucyl-glicine amide in Parkinson's disease. Can. Med. Ass. J. 107: 1079-1081, 1972.
18. *Kelley, A.E.* and *S.D. Iversen.* Behavioural response to bilateral injections of substance P into the substantia nigra of the rat. Brain Res. 158: 474-478, 1978.
19. *Kelley, A.E., L. Stinus* and *S.D. Iversen.* Behavioural activation induced in the rat by substance P infusion into ventral teg-

mental area: implication of dopaminergic A-10 neurons. Neurosc. Lett. 11: 335-339, 1979.

20. *Kostrzewa, R.M., A.J. Kastin* and *S.K. Sobrian.* Potentiation of apomorphine action in rats by L-prolyl-L-leucyl-glicine amide. Pharmac. Biochem. Behav. 9: 375-378, 1978.
21. *Kubicki, J.* Some behavioral effects of SP_{6-11} hexapeptide C-terminal of substance P injected into the cerebral ventricles in rats. Acta Phisiol. Pol. 28: 489-492, 1977.
22. *Melo, J.C.* and *F.G. Graeff.* Effect of intracerebroventricular bradykinin and related peptides on rabbit behavior. J. Pharmac. Exp. Ther. 193: 1-10, 1975.
23. *Mroz, E.A.* and *S.E. Leeman.* Substance P. Vitam. Horm. 35: 209-281, 1977.
24. *Olpe, H.R.* and *W.P. Koella.* Rotatory behavior in rats by intranigral application of substance P and an eledoisin fragment. Brain Res. 126: 576-579, 1977.
25. *Plotnikoff, N.P.* and *A.J. Kastin.* GH-RIF, LH-RH, TRH, MIF-I, Substance P and Angiotensin - Central nervous system studies. In: Peptides: Chemistry, Structure and Biology, edited by R. Walter and J. Meinhofer. Ann Arbor: Ann Arbor Science Publ., 1975, pp. 645-649.
26. *Plotnikoff, N.P., A.J. Kastin, H.S. Anderson* and *A.V. Schally.* Dopa potentiation by a hypothalamic factor, MSH release-inhibiting hormone (MIF). Life Sci. 10: 1279-1283, 1971.
27. *Schallert, T., I.Q. Whishaw, V.D. Ramirez* and *P. Teitelbaum.* Compulsive, abnormal walking caused by anticholinergics in akinetic, 6-hydroxydopamine-treated rats. Science 199: 1461-1463, 1978.
28. *Smith, G.P.* and *R.C. Young.* A new experimental model of hypokinesia. In: Advances in Neurology, vol. 5, edited by F.H. McDowell and A. Barbeau. New York: Raven Press, 1974, pp. 427-432.
29. *Steg, G.* Efferent muscle innervation and rigidity. Acta Physiol. Scand. 61, Suppl. 225: 5-53, 1964.
30. *Stinus, L., A.E. Kelley* and *S.D. Iversen.* Increased spontaneous activity following substance P infusion into A10 dopaminergic area. Nature 276: 616-618, 1978.
31. *Ungerstedt, U., A. Avemo, E. Avemo, T.L. Ljungberg* and *C. Ranje.* Animal models of parkinsonism. In: Advances in Neurology, Vol. 3, edited by D.B. Calne. New York: Raven Press, 1973. pp. 257-271.
32. *Von Euler, U.S.* and *B. Pernow* (editors). Substance P. New York: Raven Press, 1977.
33. *Waldmeier, P.C., R. Kam* and *K. Stocklin.* Increased dopamine metabolism in rat striatum after infusions of substance P into the substantia nigra. Brain Res. 159: 223-227, 1978.

Possible Involvement of Taurine and Gaba in Morphine-Like Peptide Actions

KANJI IZUMI, M.D. [1]
EISUKE MUNEKATA, Ph D. [2]
HIROAKI YAMAMOTO, PhD. [3]
TAKAO NAKANISHI, M.D. [1]
and ANDRE BARBEAU, M.D. [4]

1) Department of Neurology, Institute of Clinical Medicine, University of Tsukuba, JAPAN
2) Institute of Applied Biochemistry, University of Tsukuba
3) Institute of Social Medicine, University of Tsukuba
4) Department of Neurobiology, Clinical Research Institute of Montreal, CANADA

INTRODUCTION

Methionine enkephalin, amino acid sequence 61-65 of β-lipotropin (β-LPH) and its related peptide, leucine enkephalin are known to be endogenous peptides with morphine-like properties[1,2]. These peptides administered intraventricularly in rats[2] and mice[6] possess a short-lasting analgesic action. Studies on structure activity relationship of enkephalin analogues showed that D-Ala^2 - Met-enkephalinamide (Tyr-D-Ala-Gly-Phe-Met-$CONH_2$) has a similar pharmacological character to morphine or β-endorphin (β-LPH_{61-91}); the enkephalin analogue is more potent in the induction of analgesia than methionine enkephalin[23] and elicits catalepsy in rats[27] as β-endorphin.

On the other hand, taurine is an ubiquitous sulfar containing amino acid and is proposed to be a neurotransmitter or a modulator in the mammalian central nervous system (CNS)[1]. Recently, it has been shown that taurine can counteract with a certain action of morphine; the increment in plasma growth hormone values in rats induced by morphine administration was completely blocked by the intraventricular injection of taurine[8]. On the basis of this observation reported, we decided to investigate whether or not taurine interferes with another symptoms such as akinesia or catalepsy or analgesia induced by an acute administration of morphine-like peptide in rats. Studies on effect of γ-aminobutyric acid (GABA) on akinesia and analgesia were also included.

INTRAVENTRICULAR INJECTION OF D-ALA^2-MET-ENKEPHALINAMIDE AND AMINO ACIDS

In male Wistar rats (220-310g of body weight), cannulation of the left lateral ventricle was carried out under sodium pentobarbital anesthesia one day before behavioral observation. The high quality stainless needle (0.8 mm outer and 0.5 mm inner dia-

Correspondence: Dr. K. Izumi, Department of Neurology, Institute of Clinical Medicine, University of Tsukuba, IBARAKI 305, JAPAN

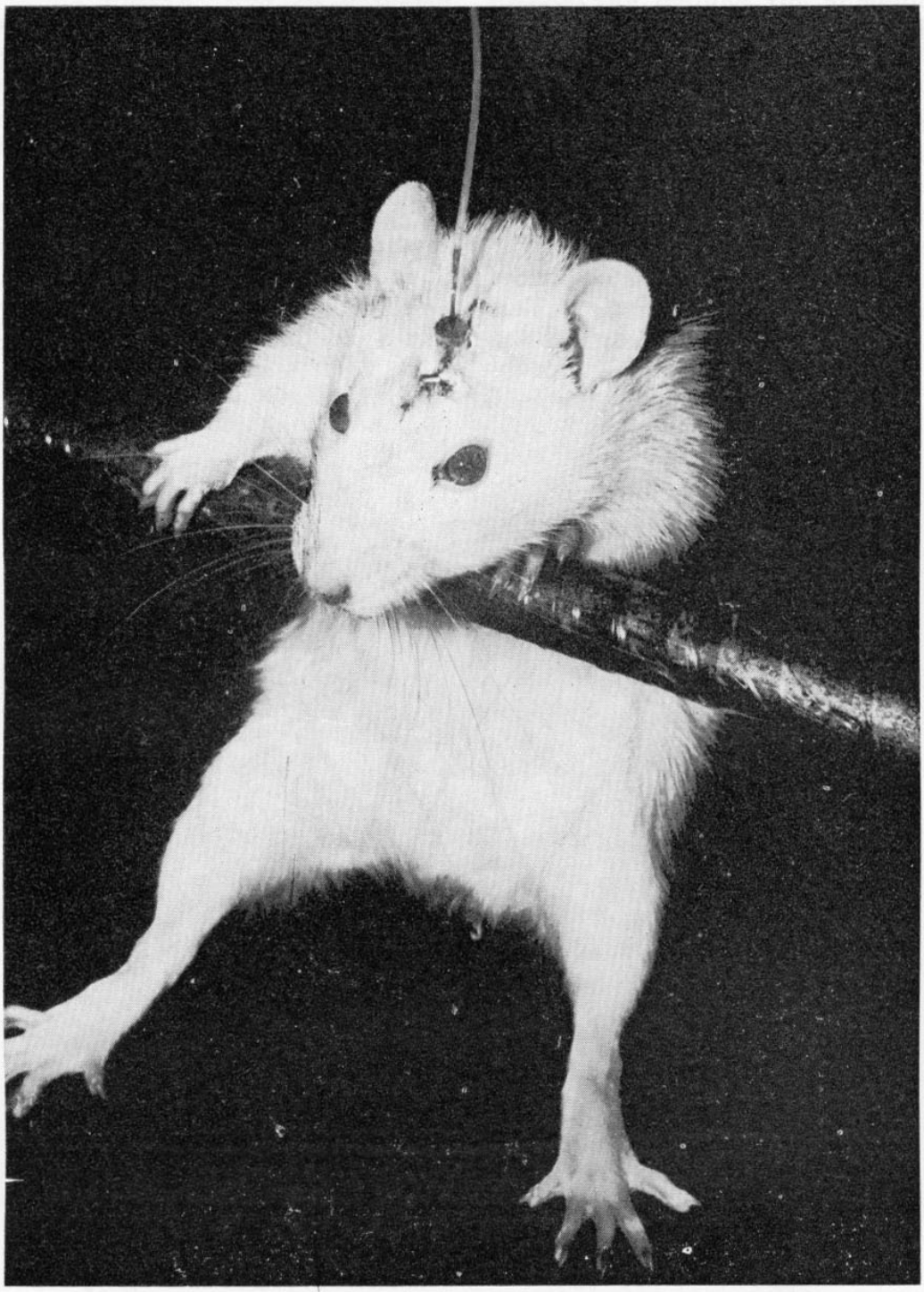


Fig. 1. The picture shows akinesia induced by the intraventricular administration of D-Ala^2-Met-enkephalinamide (50 μg/10 μl) in rats. The photograph was taken 60 min after the injection of enkephalinamide.

meter) of which the tip length is 4.4 mm from the stopper attached to the needle was stereotaxically implanted into the lateral ventricle. The needle point was determined at 1.0 mm lateral and 1.5 mm posterior to the bregma. After insertion, the needle was cemented. The correct location of the needle was confirmed by an injection of dye and tissue slices from coronal section of the brain in the preliminary experiments. The outer top of the needle was connected with a 8.0 cm polyethylene tube (Fig. 1). The

desired volume of any solution was injected using a Hamilton microsyringe through the tube.

D-Ala^2-Met-enkephalinamide was synthesized by the classical solution methods and the product was chromatographically and electrophoretically homogeneous[22]. In a preliminary experiment, the biological activity of the peptide product intraventricularly injected in rats was tested and was confirmed by naloxone to be mediated through opiate receptors. D-Ala^2-Met-enkephalinamide was dissolved in 0.85% saline solution. Each amino acid used was dissolved in 5 mM phosphate buffer in NaCl solution and made to iso-osmotic (280-290 mosM) and neutral (pH 7.2-7.4). The injection volume for all substances studied was 10 µl. Either taurine or GABA was given 10 min prior to the injection of 50 µg of D-Ala^2-Met-enkephalinamide. This dose of enkephalinamide is equivalent to the ED_{99} for both akinesia and analgesia. The doses of amino acids injected in the present study which varied

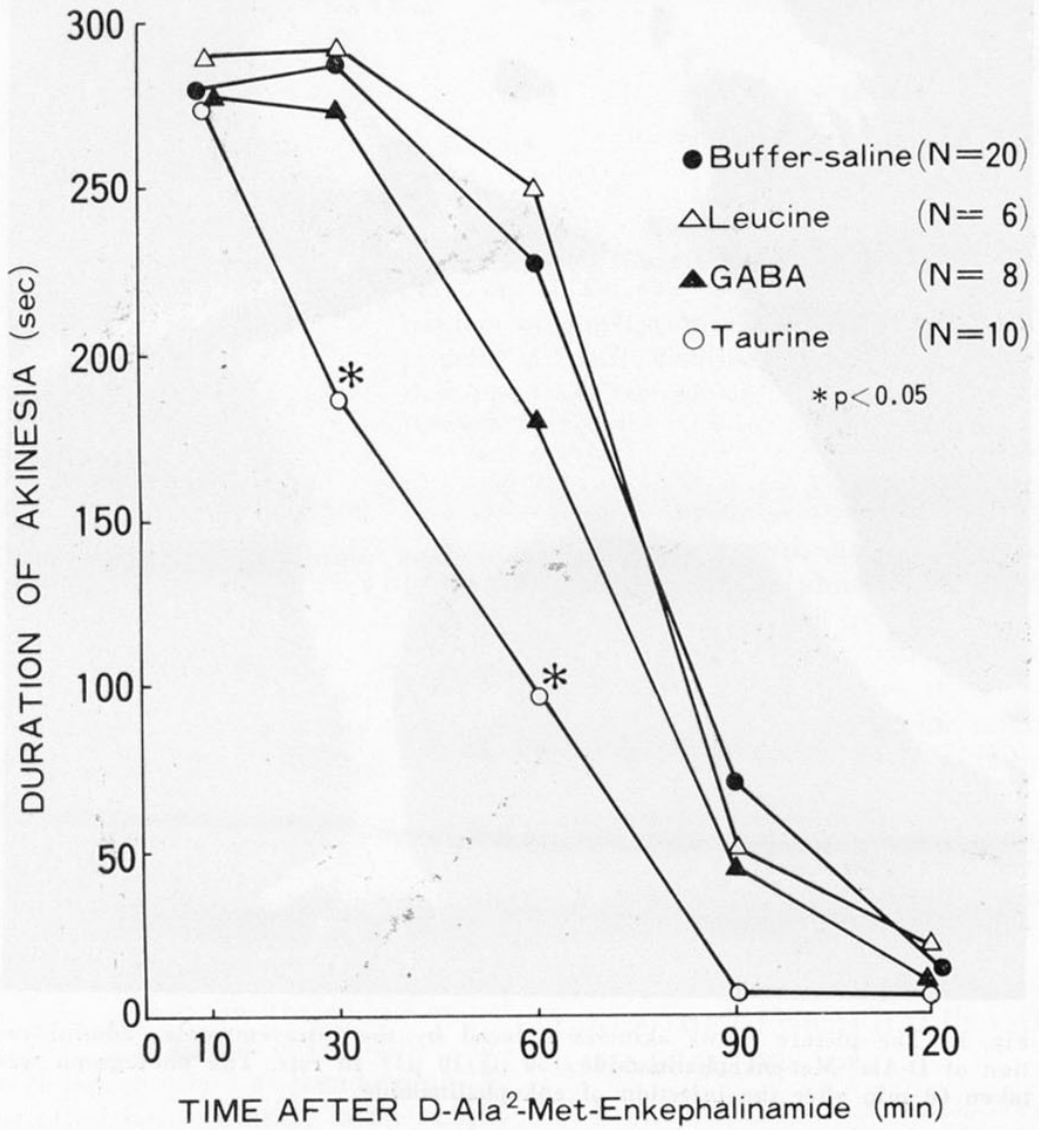


Fig. 2. Effects of amino acids on akinesia induced by D-Ala^2-Met-enkephalinamide. Test rats received each amino acid (9.5 x 10^{-2}M/10 µl) 10 min prior to the administration of D-Ala^2-Met-enkephalinamide (50 µg/10 µl). Control animals received 10 µl of 5 mM phosphate buffer in NaCl solution and 10 min later the same dose of enkephalinamide. All substances were administered intraventricularly. The maximum time of duration for the estimation of akinesia was determined to be 300 sec as the cut-off time. Significant difference between the duration of akinesia in test rats and that in control animals is shown by*: $p < 0.05$. N: number of animals.

from 2.375 x 10^{-2}M/10 μl to 9.5 x 10^{-2}M/10 μl were chosen from our previous data on suppressive action of taurine against experimental epilepsies [14,15,16]. L-Leucine, at the same molar concentration as that of the solution of taurine or GABA, was chosen as a control in each of the present experiments because it monitors the factors of osmolarity and injection volume better than a buffer saline solution alone.

EVALUATION OF AKINESIA AND ANALGESIA

Immobility[4,7] and muscle rigidity in animals induced by morphinelike peptide has been often described as catatonia[5,25,27] or catalepsy[9,19,21]. Catatonia is a well-recognized psychiatric condition characterized by negativistic reactions, phases of stupor or excitement and impulsive behavior which are observed in patients with schizophrenia. The similar state of immobility and muscle rigidity are also seen as the cardinal symptoms in patients with parkinsomism; immobile state is described as akinesia in this disorder, although akinesia is not well defined yet.

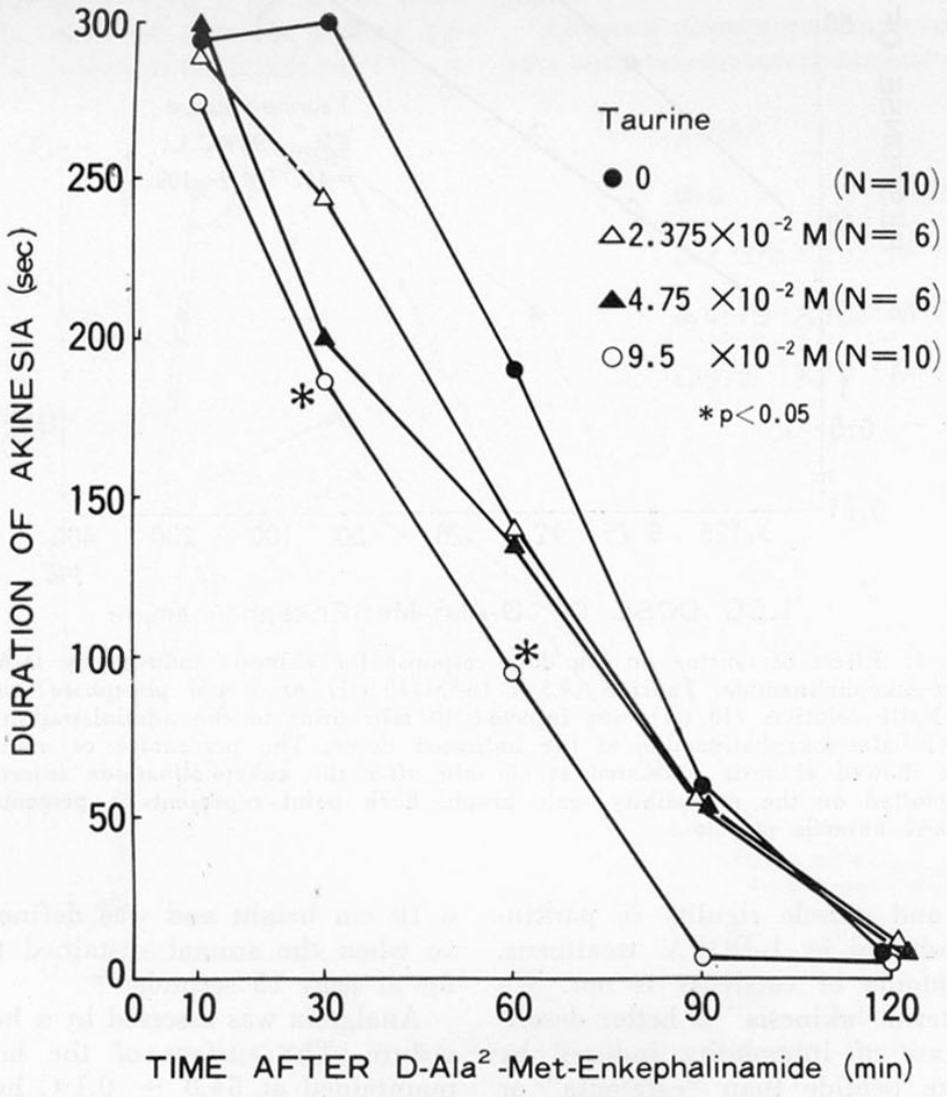


Fig. 3. Dose-dependent effects of taurine on akinesia induced by D-Ala²- Met-enkephalinamide. Different doses of taurine with a volume of 10 μl was injected 10 min prior to the administration of D-Ala²-Met-enkephalinamide (50 μg/10 μl) in rats. The animals with taurine 0 received the identical volume of 5 mM phosphate buffer in NaCl solution. The other experimental conditions were the same as those described in legend for Fig. 2. Significant difference between the duration of akinesia in rats with and without taurine is shown by*: $p < 0.05$. N: number of animals.

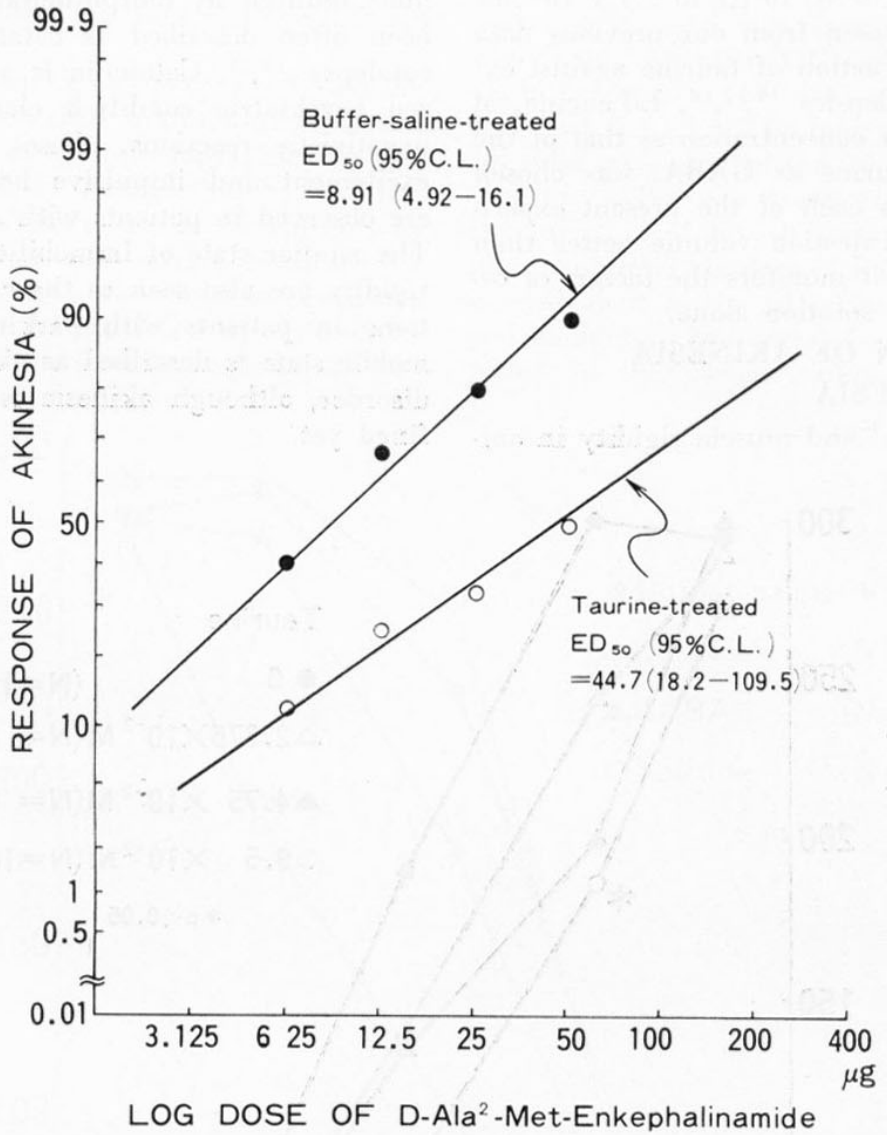


Fig. 4. Effect of taurine on log dose response for akinesia induced by D-Ala^2-Met-enkephalinamide. Taurine (9.5 x 10^{-2}M/10 μl) or 5 mM phosphate buffer in NaCl solution (10 μl) was injected 10 min priot to the administration of D-Ala^2-Met-enkephalinamide at the indicated doses. The percentage of animals that showed akinesia evaluated at 60 min after the enkephalinamide injection is plotted on the probalibity scale graph. Each point represents a percentage of 6-12 animals per dose.

Akinesia and muscle rigidity in parkinsonism is relieved by L-DOPA treatment, whereas catatonia or catalepsy is not. We feel that a term "akinesia" is better describing the state of immobility induced by morphine-like peptide than "catatonia" or "catalepsy" in rats, because this immobile state with muscle rigidity is completely cured by L-DOPA[21] or apomorphine[17], a dopamine receptor stimulant.

Akinesia was evaluated by placing the forepaws of the rat on a horizontal bar with a 10 cm height and was defined as positive when the animal sustained the position for at least 15 seconds.

Analgesia was assessed by a hot-plate procedure. The surface of the hot-plate was maintained at 54.0 ± 0.1°C by a thermoregulated water-circulating pump. The time between placing the rat on the hot-plate and the first appearance of any reaction such as licking paws, rearing or rapid movement was counted for estimation of duration of analgesia. Analgesia was defined

as positive when the animal placed on the hot-plate showed no response for at least 10 seconds.

EFFECT OF AMINO ACID ON AKINESIA INDUCED BY D-ALA^2-MET-ENKEPHALINAMIDE

Immediately after the intraventricular administration of taurine and GABA at a dose of 9.5 x 10^{-2}M/10μl, all eighteen rats showed slightly decreased muscle tonus. Hypotonus lasted for several minutes and was normalized by 10 min after the injection of each amino acid. At a smaller dose (2.375 x 10^{-2}M — 4.75 x 10^{-2}M) of these amino acids, none of animals showed hypotonus. The injection of L-leucine (9.5 x 10^{-2}M/10 μl) or 5 mM phosphate buffer in NaCl solution (10 μl) did not alter muscle tonus.

1. Effects of taurine (Fig. 2, 3, 4)

The duration of akinesia estimated at 10 min after the administration of D-Ala^2-Met-enkephalinamide in rats which had been pretreated with 9.5 x 10^{-2}M taurine was almost identical to that in control animals with 5 mM phosphate buffer in NaCl solution. Subsequent observation demonstrated that taurine-treated rats showed a significant reduction of the duration of akinesia compared with that in control animals.

Akinesia disappeared in seven out of 10 rats with taurine pretreatment at a 90 min

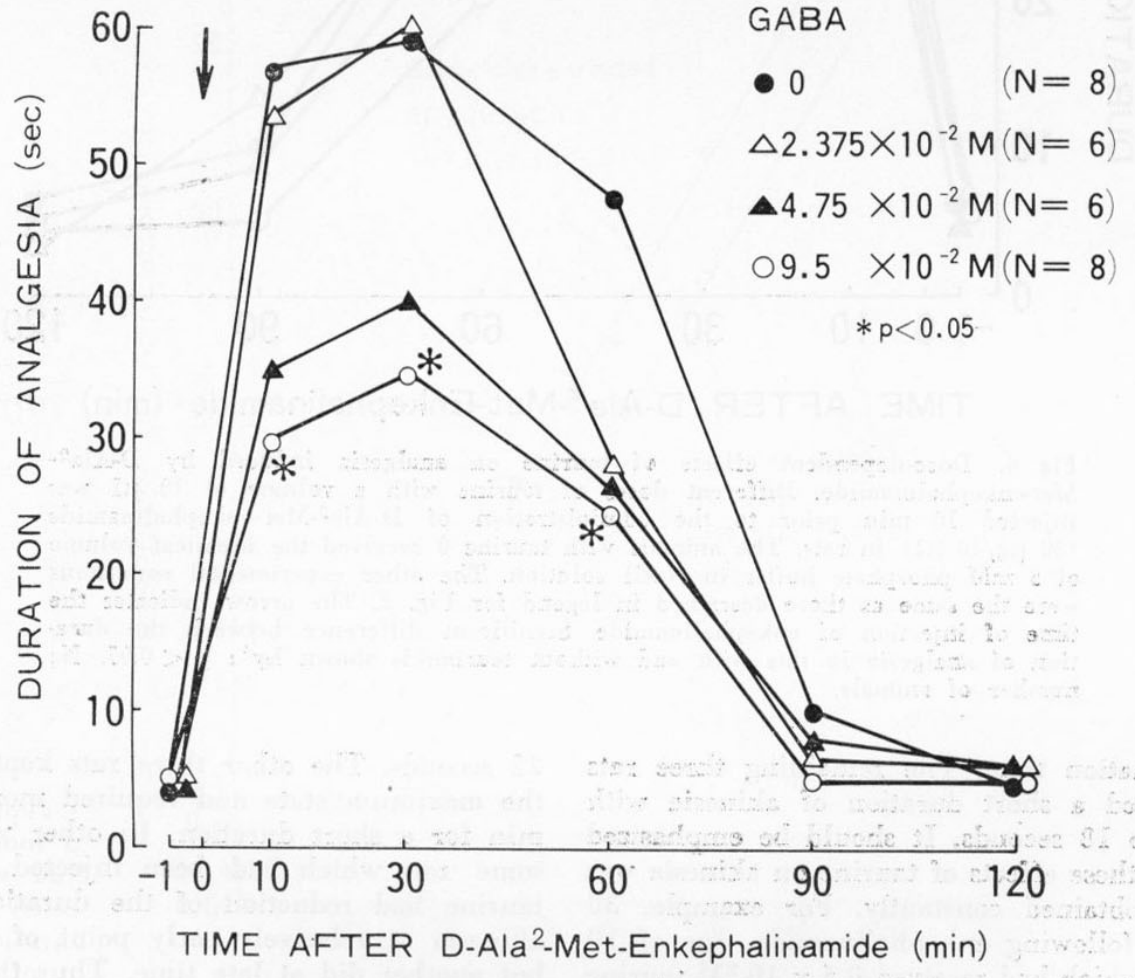


Fig. 5. Effects of amino acids on analgesia induced by D-Ala^2-Met-enkephalinamide. One min before the enkephalinamide injection, all rats were erved for the estimation of analgesia as basal value. The maximum time of duration for the estimation of analgesia was determined to be 60 sec as the cut-off time. The other experimental conditions were the same as those described in legend for Fig. 2. The arrow indicates the time of injection of enkephalinamide. Significant difference between the duration of analgesia in test rats with amino acid and that in control animals with 5 mM phosphate buffer in NaCl solution is shown by*: $p < 0.05$. N: number of animals.

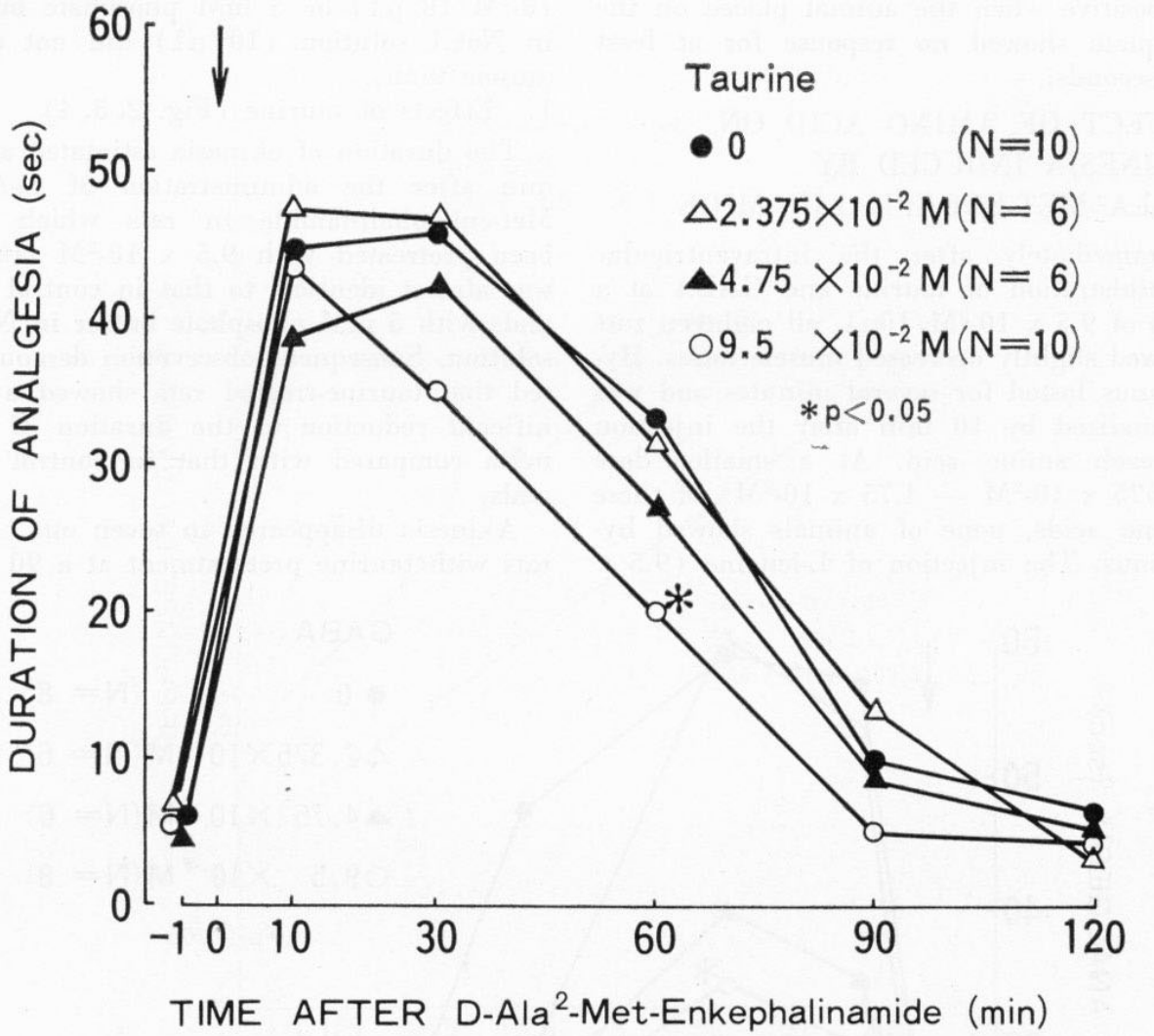


Fig. 6. Dose-dependent effects of taurine on analgesia induced by D-Ala^2-Met-enkephalinamide. Different doses of taurine with a volume of 10 µl was injected 10 min prior to the administration of D-Ala^2-Met-enkephalinamide (50 µg/10 µl) in rats. The animals with taurine 0 received the identical volume of 5 mM phosphate buffer in NaCl solution. The other experimental conditions were the same as those described in legend for Fig. 2. The arrow indicates the time of injection of enkephalinamide. Significant difference between the duration of analgesia in rats with and without taurine is shown by*: $p < 0.05$. N: number of animals.

estimation time. The remaining three rats showed a short duration of akinesia with 16 to 18 seconds. It should be emphasized that these effects of taurine on akinesia was not obtained constantly. For example, 30 min following enkephalinamide, one of 10 rats which had received 9.5 x 10-^{2}M taurine showed already no akinesia and three of them had a markedly short duration of akinesia with 16 to 36 seconds. The other six rats had still the maximum state of immobility. At 60 min, one of these six animals was completely released from akinesia and two of them had a short duration each with 22 seconds. The other three rats kept still the maximum state and required more 30 min for a short duration. In other words, some rats which had been injected with taurine had reduction of the duration of akinesia at relarively early point of time, but another did at late time. Thus the variation of the averaged duration of akinesia in rats pretreated with taurine, which is not represented on the figures, became rather great.

Nevertheless, effects of taurine on decreasing the duration of akinesia were observed in a dose-dependent manner, at least at

the estimation time between 30 and 60 min following the injection of D-Ala^2-Met-enke phalinamide (Fig. 3). To confirm these effects of taurine, a ED_{50} of D-Ala^2-Met-enkephalinamide for akinesia was determined at 60 min following the injection of enkephalinamide in two grous of rats. As shown in Fig. 4, the ED_{50} of enkephalinamide was five times greater in the taurine-treated rats than that in the buffer-saline-treated animals. A calculated potency ratio (P.R.) was 5.0 and the factor for P.R. was 2.94, indicating that the administration of taurine decreased significantly the occurrence of akinesia in rats evaluated at 60 min after the injection of D-Ala^2-Met-enkephalinamide.

2. Effects of GABA (Fig. 2)

The duration of akinesia in rats induced by D-Ala^2-Met-enkephalinamide was not modified by pretreatment with 9.5 x 10^{-2}M GABA during a 120 min observation period. A ED_{50} of enkephalinamide for akinesia estimated at 60 min after the injection of the peptide analogue was 11.3 μg with 4.72 to 27.1 μg of 95% confidence li-

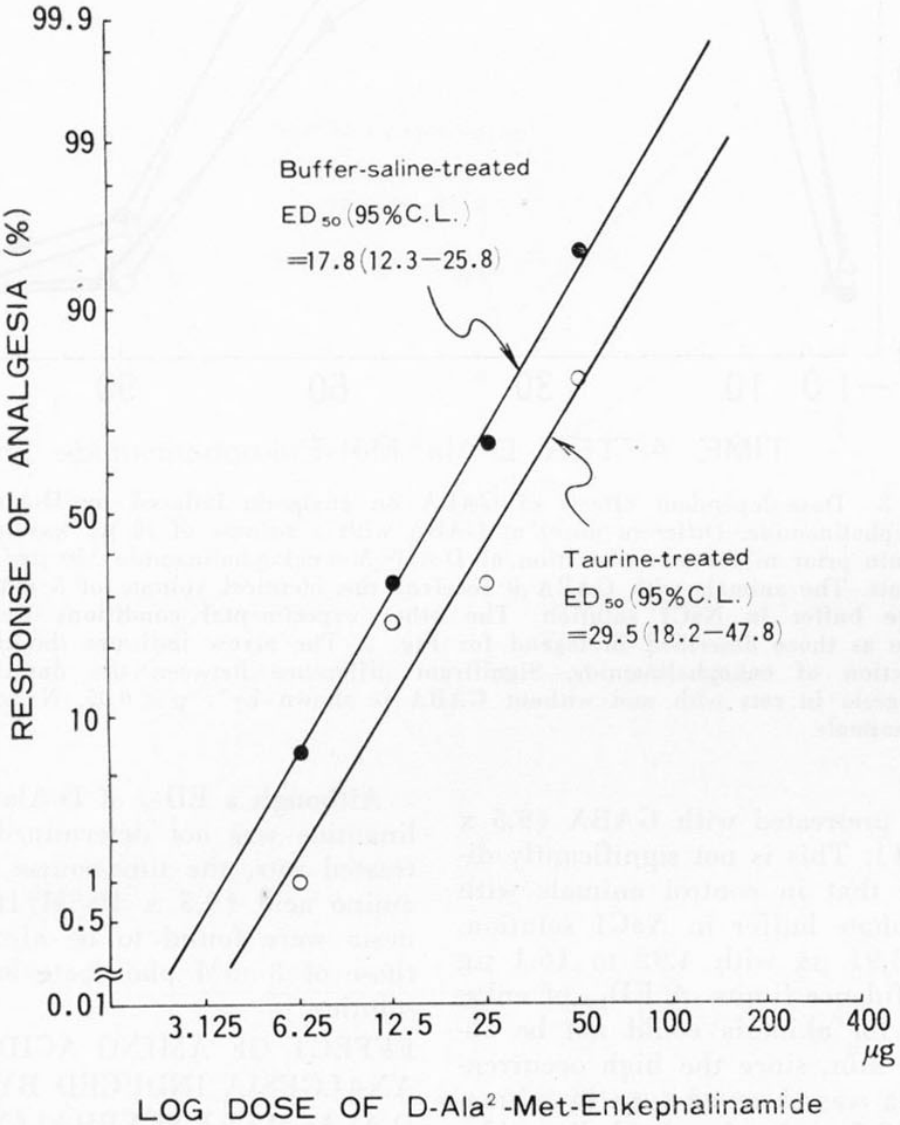


Fig. 7. Effect of taurine on log dose response for analgesia induced by D-Ala^2-Met-enkephalinamide. Taurine (9.5 x 10^{-2}M/10 μl) or 5 mM phosphate buffer in NaCl solution (10 μl) was injected 10 min prior to the administration of D-Ala^2-Met-enkephalinamide ot the indicated doses. The percentage of animals that showed analgesia evaluated at 60 min after the enkephalinomide injection is plotted on the probability scale graph. Each point represents a percentage of 6-12 animals per dose.

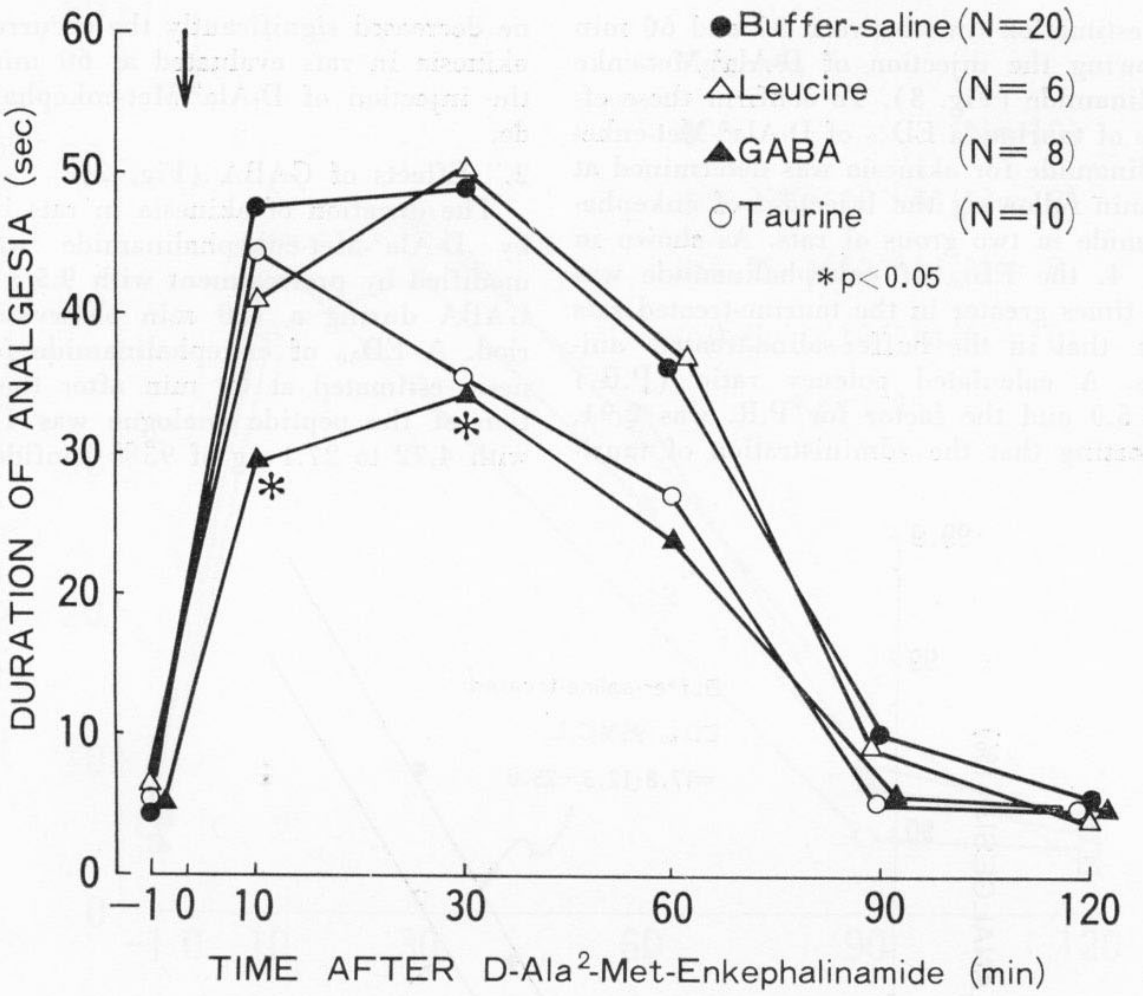


Fig. 8. Dose-dependent effects of GABA on analgesia induced by D-Ala²-Met-enkephalinamide. Different doses of GABA with a volume of 10 μl was injected 10 min prior to the administration of D-Ala²-Met-enkephalinamide (50 μg/10 μl) in rats. The animals with GABA 0 received the identical volume of 5 mM phos phate buffer in NaCl solution. The other experimental conditions were the same as those described in legend for Fig. 2. The arrow indicates the time of injection of enkephalinamide. Significant difference between the duration of analgesia in rats with and without GABA is shown by*: $p < 0.05$. N: number of animals.

mits in rats pretreated with GABA (9.5 x 10^{-2}M/10 μl). This is not significantly different from that in control animals with 5 mM phosphate buffer in NaCl solution, which was 8.91 μg with 4.92 to 16.1 μg of 95% confidence limits. A ED_{50} of enkephalinamide for akinesia could not be obtained at 30 min, since the high occurrence of akinesia was observed even by a smaller dose (12.5 μg) of enkephalinamide; 83% of rats pretreated with GABA and 67% of those with 5 mM phosphate buffer in NaCl solution showed akinesia by enkephalinamide.

3. Effects of L-leucine (Fig. 2)

Although a ED_{50} of D-Ala²-Met-enkephalinamide was not determined in L-leucine-treated rats, the time-course effects of this amino acid (9.5 x 10^{-2}M/10 μl) on akinesia were found to be almost similar to those of 5 mM phosphate buffer in NaCl solution.

EFFECT OF AMINO ACID ON ANALGESIA INDUCED BY D-ALA²-MET-ENKEPHALINAMIDE

Effect of each amino acid alone upon nociperceptive sensation was tested at one min prior to the injection of D-Ala²-Met-enkephalinamide. None of amino acids used which were administered intraventricularly

9 min before evaluation caused either analgesia or hyperalgesia in rats (Fig. 5).

1. Effects of taurine (Fig. 5, 6, 7)

The duration of analgesia estimated at 10 min after the administration of D-Ala^2-Met-enkephalinamide in rats which had been pretreated with 9.5 x 10-^{2}M taurine was almost identical to that in control animals with 5 mM phosphate buffer in NaCl solution. Subsequently, a tendency to decrease in the duration of analgesia was obtained at 30 and 60 min after the injection of enkephalinamide in taurine-treated rats. This tendency at these times was observed in a dose-dependent manner for this sulfar containing amino acid (Fig. 6). Individual case analysis revealed that there seemed to be a similar pattern for analgesic response by taurine pretreatment to that for akinesia. For example, 30 min following enkephalinamide, four out of 10 rats which had received 9.5 x 10-^{2}M taurine showed a markedly short duration of analgesia with 11 to 13 seconds. The other six rats had long duration with 25 to 60 seconds. At 60 min, two rats among them which

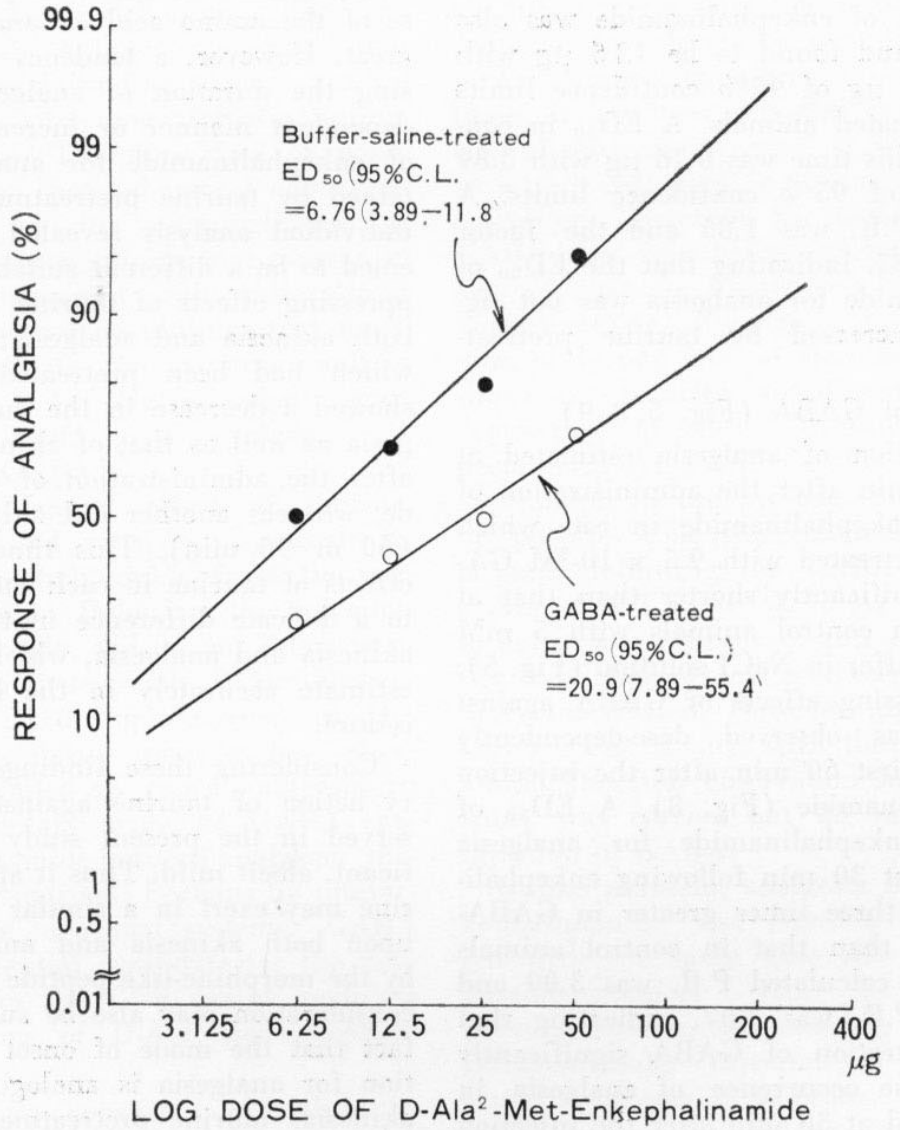


Fig. 9. Effect of GABA on log dose response for analgesia induced by D-Ala^2-Met-enkephalinamide. GABA (9.5 x 10-^{2}M/10 μl) or 5 mM phosphate buffer in NaCl solution (10 μl) was injected 10 min prior to the administration of D-Ala^2-Met-enkephalinamide at the indicated doses. The percentage of animals that showed analgesia evaluated at 30 min after the enkephalinamide injection is plotted on the probability scale graph. Each point represents a percentage of 6-8 animals per dose.

had a duration of 25 or 48 seconds at a 30 min estimation time showed normal response against pain stimulation. The prolonged duration (60 sec) of analgesia in the remaining four animals was not altered by taurine pretreatment during a 120 min observation period.

A ED_{50} of D-Ala^2-Met-enkephalinamide for analgesia determined at 60 min following enkephalinamide was slightly greater in taurine-treated rats than that in buffer-saline-treated animals (Fig. 7).

A calculated P.R. was 1.66 and the factor P.R. was 1.84, indicating that the two curves are not significantly different. At 30 min, a ED_{50} of enkephalinamide was also determined and found to be 12.5 μg with 5.69 to 27.5 μg of 95% confidence limits in taurine-treated animals. A ED_{50} in control rats at this time was 6.76 μg with 3.89 to 11.8 μg of 95% confidence limits. A calculated P.R. was 1.85 and the factor P.R. was 1.87, indicating that the ED_{50} of enkephalinamide for analgesia was not significantly increased by taurine pretreatment.

2. Effects of GABA (Fig. 5, 8, 9)

The duration of analgesia estimated at 10 and 30 min after the administration of D-Ala^2-Met-enkephalinamide in rats which had been pretreated with 9.5 x 10-^{2}M GABA was significantly shorter than that at each time in control animals with 5 mM phosphate buffer in NaCl solution (Fig. 5). This suppressing effects of GABA against analgesia was observed dose-dependently during the first 60 min after the injection of enkephalinamide (Fig. 8). A ED_{50} of D-Ala^2-Met-enkephalinamide for analgesia determined at 30 min following enkephalinamide was three times greater in GABA-treated rats than that in control animals (Fig. 9). A calculated P.R. was 3.09 and the factor P.R. was 3.07, indicating that the administration of GABA significantly decreased the occurrence of analgesia in rats evaluated at 30 min after the injection of D-Ala^2-Met-enkephalinamide.

3. Effects of L-leucine (Fig. 5)

Although a ED_{50} of D-Ala^2-Met-enkephalinamide was not determined in L-leucine-treated rats, the time-course effects of this amino acid (9.5 x 10-^{2}M/10 μl) on analgesia were found to be almost similar to those of 5 mM phosphate buffer in NaCl solution.

DISCUSSION

Among amino acids used at the limited dose, L-leucine was only the substance not to modify both akinesia and analgesia induced by D-Ala^2-Met-enkephalinamide in rats. These findings implicate that actions of taurine and GABA observed in the present study may be specific.

1. Effects of taurine

Although suppressive effects of taurine on akinesia in rats caused by D-Ala^2-Met-enkephalinamide was clearly observed, those of the amino acid on analgesia was not great. However, a tendency toward decreasing the duration of analgesia in a dose-dependent manner or increasing the ED_{50} of enkephalinamide for analgesia was obtained by taurine pretreatment. Additional individual analysis revealed that there seemed to be a different suitable time for suppressing effects of taurine in each rat on both akinesia and analgesia; some animals which had been pretreated with taurine showed a decrease in the duration of analgesia as well as that of akinesia at 30 min after the administration of enkephalinamide, whereas another did only at later time (60 or 90 min). This time difference in effects of taurine in each rat might be due to a delicate difference in the intensity of akinesia and analgesia, which we could not estimate accurately in the behavioral procedure.

Considering these findings, the inhibitory action of taurine against analgesia observed in the present study may be significant, albeit mild. Thus it appears that taurine may exert in a similar way its action upon both akinesia and analgesia elicited by the morphine-like peptide analogue. This consideration may also be supported by the fact that the mode of onset of taurine action for analgesia is analogous to that for akinesia; taurine pretreatment did not alter either duration of akinesia or that of analgesia at the most early point of time (10 min) after the enkephalinamide injection, but altered those at 30 min or more later. This implies that taurine promotes a recovery from akinesia and analgesia indu-

ced by D-Ala^2-Met-enkephalinamide in rats.

The relatively delayed effect of taurine suggests that its action may not be mediated by a competition at opiate receptor sites between the sulfar amino acid and the morphine-like peptide analogue. It has been demonstrated that β-endorphin has a selective depleting action on calcium content in synaptic membranes and synaptic vesicles, probably by inhibiting the binding of calcium to synaptic membrane[24]. Calcium ion is indispensable for the neurotransmitter release mechanisms. Taurine can also change intracellular Ca++ content in the CNS[13,18,20] and modify the release of norepinephrine and acetylcholine[20]. Recently, Yamamoto et al. (in preparation) found that the decreased binding capacity to synaptic membrane by morphine was normalized by taurine. Therefore, one of possibilities could be that behavioral counteraction observed by the administration of D-Ala^2-Met-enkephalinamide and taurine in rats may be associated with intracellular mechanism. Another possibilities that taurine might accelerate the excretion and/or metabolism of D-Ala^2-Met-enkephalinamide from the CNS could not be excluded.

2. Effects of GABA

Unlike the delayed effect of taurine, GABA suppressed analgesia caused by D-Ala^2-Met-enkephalinamide from the early time (10 min) following the injection of enkephalinamide. We do not know whether GABA competes with D-Ala^2-Met-enkephalinamide at the opiate receptors. However, this possibility may be less, since GABA alone failed to cause analgesia in rats. The inhibitory action of GABA against enkephalinamide-induced analgesia, therefore, may be indirect. Perhaps by interacting on other neuronal systems, GABA which is a potential candidate for the neurotransmitter in the CNS would modulate analgesic actions of D-Ala^2-Met-enkephalinamide.

Our results are consistent with those by Ho et al.[11] who demonstrated that elevating the GABA content of the brain by aminooxyacetic acid (AOAA, an inhibitor for GABA transaminase) or inhibiting GABA uptake in the neurons by 2,4-diaminobutyric acid antagonized the analgesic action of morphine in mice. However, other conflicting data have also been previously reported the administration of AOAA to mice[28] or that of muscimol (a stimulant for GABA receptor) to mice and rats[3] potentiated morphine analgesia; decreasing the GABA content by semicarbazide (an inhibitor for GABA synthesizing enzyme)[28] or inhibiting GABA receptors by bicuculline[28] attenuated analgesia in mice. It is difficult to delineate at the present time the difference between our results, together with those of Ho et al.[11], and those conflicting data mentioned above. Difference in strain of animals may be an important factor responsible for different analgesic response by morphine or morphine-like peptides[10].

With respect to effects of GABA on akinesia, it has been reported that the injection of muscimol to rats[3] or that of AOAA to mice[26] enhanced "catalepsy" induced by morphine. Therefore, the administration of GABA was expected to prolong the duration of akinesia caused by D-Ala^2-Met-enkephalinamide, although the subconvulsive dose of bicuculline or picrotoxin has been shown not to affect significantly morphine "catalepsy" in mice[26]. In spite of this expectation, GABA did not alter the duration of akinesia in the present study. It might be possible that the estimation time for akinesia following the GABA injection at a dose of 9.5 x 10^{-2}M/10 μl was too late to detect any efficacy of this neurotransmitter candidate which is thought to be repidly inactivated. However, this possibility may be less, if any, with the following reasons. The first observation in the present study was carried out 20 min after the injection of GABA.

Unlike akinesia, analgesia caused by enkephalinamide was significantly suppressed with the same time schedule for the administration of GABA. Moreover, in our previous studies, this time lag followins the intraventricular injection of GABA was found to be enough to protect rats from running seizures induced by ouabain, cardiac glycoside[14].

When drugs that activate the GABA neuronal system in the brain are used, behavioral evaluation for akinesia should be accounted carefully, since such drugs can

decrease muscle tonus; the intensity of akinesia evaluated would be possibly overlaid by hypotonus and might look as if akinesia was potentiated. We did not examine this possibility, because muscle tonus in rats pretreated with GABA was normal at least at the time of the injection of D-Ala^2-Met-enkephalinamide in the present study.

ACKNOWLEDGMENTS

We are greatful to Miss M. Hirose and Mrs. M. Shibanuma for their excellent technical assistance. We also thank Mrs. M. Izumi for typing. These studies were supported in part by grants from the Ministry of Health and Welfare and from Taisho Pharmaceutical Co.

SUMMARY

Effects of taurine or γ-aminobutyric acid (GABA) on akinesia and analgesia induced by D-Ala^2-Met-enkephalinamide were investigated in rats. Administration of taurine (2.375 x 10-^{2}M — 9.5 x 10-^{2}M/10 μl) into the left lateral ventricle 10 min prior to the injection of D-Ala^2-Met-enkephalinamide (50 μg/10 μl) showed a dose-dependent reduction of duration of akinesia and to some extent that of analgesia estimated at 30 and 60 min following the enkephalinamide injection; at the first point of estimation time (10 min), taurine did not alter either the duration of akinesia or that of analgesia. The median effective dose (ED_{50}) of D-Ala^2-Met-enkephalinamide for akinesia determined at 60 min after enkephalinamide was 5 times greater and that for analgesia assessed at the same time was 1.7 times greater in taurine-treated rats than each that in control animals.

Administration of GABA under similar experimental conditions showed a dose-dependent reduction of duration of analgesia from the initial estimation time (10 min) following the injection of D-Ala^2-Met-enkephalinamide. The ED_{50} of D-Ala^2-Met-enkephalinamide for analgesia determined at 30 min after enkephalinamide was 3 times greater in GABA-treated rats than that in control animals. Unlike the effects of taurine, GABA did not alter the duration of akinesia. Neither the duration of akinesia nor that of analgesia was modified by taurine or GABA alone in rats tested 9 min after the injection of each amino acid.

These finding indicating that taurine may promete a recovery from both akinesia and analgesia, while GABA suppress only analgesia induced by morphine-like peptide in rats.

RESUMEN

Se investigó en ratas los efectos de la taurina o el acido γ-aminobutírico sobre la akinesia y analgesia inducida por D-Ala^2-Met-encefalinamid. La administración de taurina (2.375 10 M-9.5 x 10 M/10μl) en el ventrículo lateral izquierdo 10 minutos antes de la inyección de D-Ala -Met- encefalinamida (50μg/10/μ^1) mostró una reducción de la duración de la akinesia dependiente de la dosis y también de la analgesia estimada a los 30 y 60 min. después de la inyección de encefalinamida. Cuando se observa a los 10 min. la taurina no altera la duración de la akinesia o de la analgesia. La dosis media efectiva (ED_{50}) de D-Ala-Met-encefalinamida para la akinesia determinada a los 60 min. fue 5 veces mayor que aquella empleada para la analgesia tomada al mismo tiempo, fue 1.7 mayor en ratas tratadas por taurina.

La administración de GABA bajo condiciones experimentales similares mostró una reducción de la duración de la analgesia dependiente de la dosis a los 10 min., después de la inyección de D-Ala^2-Met-encefalinamida. El ED_{50} de D-Ala^2-Met-encefalinamida para analgesia determinada a los 30 minutos después de la inyección de en-

cefalinamida fue 3 veces mayor en las ratas tratadas por GABA que en los animales de control. Distinto a los efectos de la Taurina, GABA, no alteró la duración de la akinesia. Ni la duración de la akinesia de la analgesia fueron modificadas por la taurina o GABA solas en ratas sometidas a prueba 9 min. después de la inyección de cada aminoácido.

Estos hallazgos indican que taurina puede promover una recuperación de ambas, akinesia y analgesia, mientras que GABA suprime sólo analgesia inducida por péptidos del tipo de morfina en ratas.

RÉSUMÉ

On a clerché chez le rat les effects de la taurine on l'acide γ-aminobutyrique sur l'akinésie et analgésic produite par D-Ala²-Met. encéphalinamide. L'administration de taurine (2.375x10-²M/10μ¹) dans le ventricule latéral gauche 10 minutes avant l'injection de D-Ala²-Met. encéphalinamide (50μg/10μ¹) a donné une réduction de la durée de l'akinesia dependant de la dose et également de l'analgesie 30 a 60 min. aprés l'injection de L'encéphalinamide.

Quand on observe aprés 10 min. la taurine n'altere pas la durée de l'akinésie ni de l'analgésie. La dose meyonne efficace (ED_{50}) de D-Ala²-Met. encéphalinamide, pour l'akinésie déterminée après 60 min. a été 5 fois plus grande que celle employée pour l'analgésie prise en mémé temps. Elle a été 1,7 fois plus grande pour les rats traités avec la taurine.

L'administration de GABA dans des conditions expérimentales similaires a montré une réduction de la durée de l'analgésie, dépendant de la dose, 10 minutes apres l'injection de D-Ala²-Met. encéphalinamide. Le ED_{50} de D-Ala²-Met. encéphalinamide, pour l'analgésie déterminée 30 min. aprés l'injection de l'encéphalinamide a été 3 fois plus grande dans les rats traités par GABA que dans les animaux de controle.

Contrairement aux effects de la taurine GABA n'a pas alteré la durée de l'akinesie.

Ni la durée de l'akinesie ni celle de l'analgésie ont été modifiées par la taurine ou le GABA chez des rats nus en expérience 9 min. aprés l'injection de chaque aminoacide.

Ces résultats indiquent que la taurine peut proroquer une récupération de l'akinésie et de l'analgésie, tandis que GABA supprime seulement l'analgésie induite par des peptides du geure morphine chez le rat.

ZUSAMMENFASSUNG

Bei Ratten wird der Effekt des Taurins oder der γ-aminobutyrisäure auf die Akinese und Analgie, verursacht durch D-Ala²-Met-enzephalinamid, untersucht. Die Anwendung von Taurin (2.375x10 M-9,5x10 M/10μ¹) in den lateralen linken Ventrikel 10 minuten vor der injektion von D-Ala² Met-Enzephalinase (50mg/10μl) zeigte eine Reduktion der Dauer der Akinese, abhängig von der Dosis und auch der Analgesie, geschätzt auf 30 und 60 Minuten, nach der Injektion des Enzephalinamids. Nach der Beobachtung nach 10 Minuten ändert Taurin nicht die Dauer der Akinese in der Analgese. Die mittlere wirkhame Dosis (Ed_{50}) des D-Ala²-Met-enzephalinamids für die Akinase bestimmt nach 60 minuten, war 5 mal grösser als die angewandte für die Analgese, die zur selben Zeit genommen wurde, und 1,7 mal grösser bei Ratten, mit Taurin behandelt.

Die Anwendung von GABA unter experimentalen Bedingungen ähnlicher Art zeigte eine reduktion in der Dauer der Analgesie, abhängig von der Dosis nach 10 Minuten nach der Injektion von D-Ala²-Met-enzephalinamid. Das Ed_{50} von D-Ala²-Met-enzephalinamid für die Analgesie, bestimmt nach 30 minuten nach der Injektion von enzephalinamid war 3 mal grösser bei Ratten mit GABA behandelt als bei den Kontrolltieren. Verschieden von den Effekten des taurins, GABA ändert nicht die dauer der akinese. Weder die dauer der

akinese noch die analgie wurden durch das taurin oder GABA allein geändert bei ratten, nach der Probe 9 minuten nach der Injektion jeder aminosaeure.

Diese befunde zeigen an, dass taurin eine rekuperation bei der akinese und analgesie hervorrufen kann, während GABA nur analgesie unterdrückt hervorgerufen durch peptide vom typ morphium bei ratten.

REFERENCES

1. *Barbeau, A.* and *Huxtable, R.J.* (Eds.): Taurine and Neurological Disorders, Raven Press, New York, 1978.
2. *Belluzzi, J.D., Grant, N., Garsky, V., Sarantakis D., Wise, C.D.* and *Stein, L.*: Analgesia induced in vivo by central administration of enkephalin in rat, Nature, 260. 625-626, 1976.
3. *Biggio, G., Della Bella, D., Frigeni, V.* and *Guidotti, A.*: Potentiation of morphine analgesia by muscimol Neuropharmacol., 16: 149-150, 1977.
4. *Bloom, F., Segal, D., Ling, N.* and *Guillemin, R.*: Endorphins: profound behavioral effects in rats suggest new etiological factors in mental illness, Science, 194: 639-632, 1976.
5. *Bradbury, A.F., Smyth, D.G., Snell, C.R., Deakin, J.F.W.* and *Wendlandt, S.*: Comparison of the analgesic properties of lipotropin C-fragment and stabilized enkephalins in the rat, Biochem. Biophys. Res. Comm., 74: 748-754, 1977.
6. *Buscher, H.H., Hill, R.C., Romer, D., Cardinaux, C., Closse, A., Hauser, D.* and *Pless, J.*: Evidence for analgesic activity of enkephalin in the mouse, Nature, 261: 423-435, 1976.
7. *Chang, J.K., Fong, B.T.W., Pert, A.* and *Pert, C.B.*: Opiate receptor affinities and behavioral effects of enkephalin: structure -activity relationship of ten synthetic peptide analogues, Life Sci., 18: 1473-1482, 1976.
8. *Collu, R., Charpenet, G.* and *Clermont, M.J.*: Antagonism by taurine of morphine induced growth hormone secretion, Can. J. Neurol. Sci., 5: 139-142, 1978.
9. *Feldberg, W.* and *Smyth, D.G.*: C-fragment of lipotropin — an endogenous potent analgesic peptide, Br. J. Pharmacol., 60: 445-453, 1977.
10. *Frigeni, V., Bruno, F., Carenzi, A., Racagni, G.* and *Santini, V.*: Analgesia and motor activity elicited by morphine and enkephalins in two inbred strains of mice, J. Pharm. Pharmacol., 30: 310-311, 1978.
11. *Ho, I.K., Loh, H.H.* and *Way, E.L.*: Pharmacological manipulation of gamma-aminobutyric acid (GABA) in morphine analgesia, tolerance and physical dependence, Life Sci., 18: 1111-1124, 1976.
12. *Hughes, J., Smith, T.W., Kosterlitz, H.W., Fothergill, L.A., Morgan, B.A.* and *Morris, H.R.*: Identification of two related pentapeptides from the brain with potent opiate agonist activity, Nature, 258: 577-579, 1975.
13. *Izumi, K., Butterworth, R.F.* and *Barbeau, A.*: Effect of taurine on calcium binding to microsomes isolated from rat cerebral cortex, Life Sci., 20: 943-950, 1977.
14. *Izumi, K., Donaldson, J., Minnich, J.L.* and *Barbeau, A.*: Ouabain-induced seizures in in rats: suppressive effects of taurine and γ-aminobutyric acid, Can. J. Physiol. Pharmacol., 51. 885-889, 1973.
15. *Izumi, K., Igisu, H.* and *Fukuda, T.*: Suppression of seizures by taurine-specific or non-specific?, Brain Res., 76: 171-173, 1974.
16. *Izumi, K., Igisu, H.* and *Fukuda, T.*: Effects of edetate on seizure suppressing actions of taurine and GABA, Brain Res., 88: 576-579, 1975.
17. *Izumi, K., Motomatsu, T., Chretien, M., Butterworth, R.F., Lis, M., Seidan, N.* and *Barbeau, A.*: β-Endorphin induced akinesia in rats: effect of apomorphine and α-methyl-p-tyrosine and related modifications of dopamine turnover in the basal ganglia, Life Sci., 20: 1149-1156, 1977.
18. *Izumi, K., Ngo, T.T.* and *Barbeau, A.*: Metabolic modulation in the central nervous system by taurine, In Taurine and Neurological Disorders, edited by A. Barbeau and R.J. Huxtable, Raven Press, New York, 1978, pp. 137-149.
19. *Jacquet, Y.F.* and *Marks, N.*: The C-fragment of β-lipotropin: an endogenous neuroleptic or antipsychotogen?, Science, 194: 632-635, 1976.
20. *Kuriyama, K., Muramatsu, M., Nakagawa, K.*, and *Kakita, K.*: Modulatin role of taurine on release of neurotransmitters and calcium transport in excitable tissues, In Taurine and Neurological Disorders, edited by A. Barbeau and R.J. Huxtable, Raven Press, New York, 1978, pp. 201-216.
21. *Motomatsu, M., Lis, M., Seidah, N.* and *Chretien, M.*: Cataleptic effect of 61-91 beta-lipotropic hormone in rat, Can. J. Neurol. Sci., 4: 49-52, 1977.
22. *Munekata, E., Ishiyama, N., Higa, F., Ohtaki T. and Izumi, K.*: Synthesis and pharmacological properties of enkephalino igomers, Peptide Chemistry (in press).
23. *Pert, C.B., Pert, A., Chang, J.-K.* and *Fong, B.T.W.*: [D-Ala^2]-Met-enkephalinamide: a

potent, long-lasting synthetic pentapeptide analgesic, Science, 194: 330-332, 1976.

24. *Ross, D.H.* and *Cardenas, H.L.*: Nerve cell calcium as a messenger for opiate and endorphin actions, In Neurochemical Mechanisms of Opiates and Endorphins, edited by H.H. Loh and D.H. Ross, Raven Press, New York, 1979, pp. 301-336.

25. *Tseng, L.-F., Loh, H.H.* and *Li, C.H.*: Human β-endorphin: development of tolerance and behavioral activity in rats, Biochem. Biophys. Res. Comm., 74: 390-396, 1977.

26. *Vanderwende, C.* and *Spoerlein, M.T.*: Morphine-induced catalepsy in mice. Modification by drugs acting on neurotransmitter system, Neuropharmacol., 18: 633-637, 1979.

27. *Wei, E.T., Tseng, L.F., Loh, H.H.* and *Li., C.H.*: Comparison of he behavioral effects of β-endorphin and enkephalin analogs, Life Sci., 21: 321-328, 1977.

28. *Yoneda, Y., Takashima, S.* and *Kuriyama, K.*: Possible involvement of GABA in morphine analgesia, Biochem. Pharmacol., 25: 2669-2670, 1976.

Hypothalamic Releasing Factor in Primary and Situational Depression

YVES F.L. DEKEYSER, MD
Psychiatric Health Center
Welterhof
J.F. Kennedylaan 301
Heerlen
The Netherlands.

Ever since 1972, when Prange and his associates (1) first noted a definite antidepressive effect following administration of thyrotropin-releasing hormone (TRH), increasing attention has focused on the possible therapeutic role of other hypothalamic releasing hormones, particularly the decapeptide luteinizing hormone- releasing hormone (LHRH), in depressed patients. The evidence from these studies has been unclear and sometimes conflicting; some investigators (2) have substantiated the antidepressive effect of LHRH, whereas others (3) have failed to replicate these findings. Controversy has centered principally on whether the patients treated were suffering from primary depression or from that due to situational stress. For example in German and Stampfer's work (4) in 19 endogenous depressives and 8 with stress-associated depression, the eight situational depressives responded dramatically to a single 0.5 mg injection of LHRH (HRF Ayerst), whereas in those with primary affective illness the effect was minimal.

In order to help clarify this rather ambiguous situation, and to arrive at appropriate criteria for patient selection for the therapeutic use of LHRH (HRF Ayerst) in depressed patients, we have initiated a systematic study, of double-blind, placebo-controlled, crossover design, comprising 18 patients who have met the research diagnostic criteria for primary major depressive disorder, in addition to six normal controls. This study will be reported in detail elsewhere. We have completed a preliminary study in three patients with major depressive disorders, the results of which suggest some possible variations in therapeutic approach.

Two of our patients, both women, fully met the research diagnostic criteria for probable or definite endogenous major depressive disorder, while the third, a male, was diagnosed as having a major depressive disorder associated with stress. A pretreatment psychiatric profile of each patient was established by Zung and Hamilton ratings, and repeated psychiatric evaluations were made at intervals during treatment and at its conclusion.

All three patients showed a clear, positive response to treatment with HRF, with eventual clearing of symptoms and return to normal life. The rapidity of such response, however, varied greatly among the three, one — the situational depressive — responding within hours, the others not for several days or even weeks.

Although the injections given varied from 0.1 mg to 0.5 mg of HRF, no connection could be established between degree of response and size of dose. Rather, the determining factor seems to be frequency of dosing.

In the patient whose depression was stress-related, his disorder dated from six years earlier, when his wife left him for another man, taking their children with

her; he never saw them again. In this patient, dramatic subjective improvement occurred within 5 to 8 hours after the first injection and persisted for a wew days. Depressive symptomatology then returned, however so the reported subjective improvement could not be established by Hamilton rating during the first psychiatric evaluation. By the fourth injection (sixteen days after the start of treatment), improvement in mood and behaviour was established objectively by Hamilton rating. Moreover, the patient reported that he felt better at that moment than he had done in the previous five years. This time, too, the complete resolution of the depression lasted only a few days. During the whole treatment almost each injection caused the therapeutic effects just mentioned, but four to seven days after the injection depressive symptomatology returned. The therapeutic effect of the injections seemed to be best maintained by grouping the injections in pairs, one day apart.

The subjective as well as the objective response was more delayed in the two patients with primary major depression. One patient reported less anxiety and weeping after the third injection, but her Hamilton scores remained at pretreatment levels until the eighth injection. From the tenth injection on, she showed more definite and more rapid improvement, and this positive clinical evaluation was confirmed by the patient's husband.

The third dpatient, who was hospitalized following a vaginal hysterectomy — the ovaries were intact — was treated for two weeks in a doubleblind, placebo-controlled manner, and showed no improvement on placebo. By the third HRF injection, however, her Hamilton scores began to show definite improvement; at this point we switched to an open treatment mode, for a total of 9 HRF injections separated by intervals ranging from 1 tot 25 days. Subjective marked improvement was noted after seven injections; the Hamilton ratings continued to decrease to a more marked degree. After 11 HRF injections given over approximately five weeks, her depression was completely resolved.

None of our patients reported side effects; it should be noted that improvement in sleep patterns was not reported, in contrast to the findings of some investigators. (4) In all three patients, despite the difficulty of studying the widely varying patternsof response to the repeated injections, we are convinced that the observed response, as objectively established by Hamilton rating, was too well differentiated and characteristic to be a plavebo effect.

The results are in contrast to those reported by Benkert et al (3) and by Amsterdam et al (5), who could not establish a therapeutic effect of HRF in primary depressive disorders when the agent was administered as a single injection. Even German and Stampfer (4), giving monthly injections, obtained no very impressive response to that regimen in their endogenous depressives. We feel that it is important to give an adequate number of injections, with relatively close intervals between the early injections. The amount of the dose does not seem to correlate with the degree of response.

The effects observed in these three patients suggest that HRF can exert a beneficial effect in more than one type of depression, but that further research is needed to determine appropriate patient selection and to develop an effective dosage schedule. We thus feel amply justified in undertaking a larger study to help establish the practical significance of HRF in the treatment of various depressive disorders.

SUMMARY

The author presents his first successfully treated depressed patients with LH-RH (HRF Ayerst) out of a series of doubleblind placebo controlled cross-overed study in progress.

Evaluation has been based on repeated psichiatric examinations including the objective Zung and Hamilton ratings. Emphasis is given in discussing his findings with those already reported in the literature and

the differences in the rapidity of responses to HRF in primary and stressed associated depression. In the latter, response is observed earlier with symptomatic improvements within hours, in the former however the subjective as well as the objective responses is delayed and requires several injections. No correlation could be established between the degree of response and the size of dosage. The determining factor seems to be the frequency of dosing in both types of depression.

RESUMEN

El autor presenta sus primeros pacientes tratados con éxito con LH-RH (HRF Ayerst) en un estudio que se realiza en series de placebo doble ciego controlados.

La evaluación se ha basado en exámenes psiquiátricos repetidos incluyendo las valoraciones objetivas de Lung y Hamilton. Se da énfasis en la discusión de sus hallazgos con aquellos ya referidos en la literatura y las diferencias en la rapidez de respuesta al HRF en la depresión primaria y la asociada al stress. En la última, la respuesta es observada antes con mejorías sintomáticas en el transcurso de horas, en la primera la respuesta subjetiva lo mismo que la objetiva es demorada y requiere varias inyecciones. No se pudo establecer una correlación entre el grado de respuesta y la magnitud de la dosis. El factor determinante parece ser la frecuencia de la dosificación en ambos tipos de depresión.

RESUMÉ

L'auteur présent ses premiers patients traités avec succés par LH-RH (HRF Ayerst) dans une étude réalisée en une série de placebo double aveugle, contrôlés.

L'évaluation a été basée sur des examens psychiatriques répétes, y compris les valorations objetives de Lung et Hamilton. On insiste dans la discussion des résultats sur ceux déjà contenus dans la littérature et sur les différences concernant la rapidité de la réponde au HRF dans la dépression primaire et celle qui est associée au stress. Dans cette desniére la réponse est observée d'abord avec des améliorations systématique au cours de quelques heures, dans la première la réponse subjective, de même que la réponse objective est retardeé et demande plusieurs injections. On n'a pas pu établis de corrélation entre le degré de la réponse et l'importance de la dose. La facteur déterminant semble être la fréquence de la dosification dans les deux sortes de dépression.

ZUSAMMENFASSUNG

Der Autor berichtet von seinen ersten erfolgreich mit LH-Rh (HRF Ayerst) behandelten Patienten in einer Studie, die in Serien durchgefuehrt wird unter doppelter Blindkontrolle mit Placebo.

Die Beurteilung ist erfolgt durch wiederholte psychiatrische Untersuchungen einschliesslich objektiver Beurteilung nach Lung und Hamilton. Man legt Wert auf die Diskussion seiner Befunde mit den anderen in der Literatur schon berichteten und auf die Unterschiede in der Raschheit der Reaktion auf das HRF in der primaeren Depression und der mit Stress Verbundenen. Bei der Letzteren wird die Wirkung frueher beobachtet mit symptomatischer Besserung im Lauf von Stunden. In der Ersten braucht die subjektive Wirkung ebenso wie die objektive laenger und benoetigt mehrere Injektionen Man konnte kein Verhaeltnis zwischen dem Grad der Wirkung und der Hoehe der Dosis feststellen. Der bestimmende Faktor scheint die Haeufigkeit der Dosierung bei beiden Arten zu sein.

REFERENCES

1. *Prange A.J. Jr., Lara P.P., Wilson I.C., Alltrop L.B., Breese G.R.* effects of thyrotropin-releasing hormone in depression. *Lancet* 1972; ii: 999 - 1002.
2. *Garcia J.A., Santos M., Camacho G.C.* Treatment of depressions with hypothalamic decapeptide. *Folha Medica* 1977; 74.
3. *Benkert O,. Gordon A., Martschke D.* The comparison of thyrotropin-releasing hormone, luteinizing hormone-releasing hormone, and placebo in depressive patients using a double-blind cross-over technique. *Psychopharmacologia* 1974; 40: 191 - 98.
4. *German G.A., Stampfer H.G.* Hypothalamic releasing factor for reactive depression. *Lancet* 1979; ii: 789.
5. *Amsterdam J.D., Winokur A., Mendels J., Caroff S.* Effect of gonadotropin-releasing hormone on depressed mood. *Lancet* 1979; ii: 1138.

Fragments of Cholecystokinin and Gastrin as neurotransmitters in mammalian peripheral and central nervous system (Abstract)

J. S. KELLY and **JANE DODD**

MRC Neurochemical Pharmacology Unit, Department of Pharmacology, Medical School, Hills Road, Cambridge CB2 2QD.

The recent introduction of radioimmunoassay has led to the discovery, in many regions of the brain and peripheral nervous system, of peptides which were previously regarded simply as hormones of the gastrointestinal system. The presence of several of these peptides in the hippocampus has allowed us to examine their postynaptic actions on cortical neurones thought to be innervated by specific peptidergic pathways. Experiments, in which fmolar per sec quantities were applied by pressure ejection from a multibarrelled micropipette placed in the vicinity of the recording microelectrode, showed somatostatin, vasoactive intestinal polypeptide, bombesin and cholecystokinin to be potent excitants of pyramidal cells.

Perhaps more significantly, brief applications of the biologically active, tetra —and octa— peptide, C - terminal fragments of cholecystokinin evoked abrupt and rapidly reversible depolarizations which were accompanied by marked increases in excitability. Comparison of the actions of these peptides with those of glutamate and acetylcholine, released from the same multibarrelled micropipette, showed that the rate of onset of the cholecystokinin-evoked response was similar to that of the response evoked by glutamate and very much faster than that evoked by acetylcholine. However, the desulphated form of the cholecystokinin octapeptide was inactive, as was substance P, a peptide not normally found in the hippocampus. Since a number of authors have shown pyramidal cells to be excited by morphine and enkephalin, despite the fact that opiate binding in the hippocampus is poor our experiments might suggest that these excitations may be mediated by one or more populations of receptors which are not normally activated by enkephalin but by a variety of other endogenous hippocampal peptides.

The Role of Substance P in Primary Sensory Transmission an Nociception (Abstract)

T. M. JESSELL

Dept. of Pharmacology, Harvard Medical School
Boston, Mass. U.S.A.

Substance P is contained within one population of small diameter sensory neurons that have their terminal fields within laminae I and II of the dorsal horn. The iontophoretic application of substance P excites dorsal horn neurons that respond to noxious peripheral stimuli, providing some evidence that substance P may play a role in pain transmission. To examine in more detail the role of substance P as a sensory transmitter, release of the peptide was measured from mammalian spinal cord *in vivo* in response to the specific activation of primary senory neurons. Rats and cats were anaesthetized and a polyethylene cannula inserted into the subarachnoid space to the caudal margin of the lumbar spinal cord. Outflow was collected from a cannula in the cisterna magna or by a concentric cannula opening at the level of T-12 in cats. The spinal cord was then superfused with CSF and samples collected and assayed for substance P by radioimmunoassay. In rats, the release of substance P detectable in control periods was 1-2 fmol/min. Superfusion of the spinal cord with CSF-containing 40mM potassium for 10 min evoked a 4-fold increase in substance P release. In cats, bilateral stimulation of the sciatic nerve at intensities sufficient to activate only rapidly conducting, low threshold afferents did not increase the release of substance P. However, increasing the stimulus intensity to recruit A delta and C fibers produced a 2-4 fold increase in substance P release.

Opiate receptors and enkephalin-containing neurons are alo highly concentrated within laminae I-III of the dorsal horn. The release of substance P from the central terminals of primary sensory neurons was inhibited by the administration of opiate alkaloids and opioid peptides in a naloxone-reversible manner. The direct spinal analgesic action of opiates may therefore be mediated by the activation of presynaptic opiate receptors, causing an inhibition of substance P release.

Release of substance P can be evoked *in vivo* and *in vitro* by the homovanylic acid derivative capsaicin, which appears to exert a specific activation of C-fibres. Furthermore, a single intrathecal injection of 30 µg capsaicin into rats depleted substance P from primary sensory neurons and produced a prolonged elevation in the thermal and chemical pain threshold with no discernable change in response to noxious mechanical stimuli.

These results provide further evidence that substance P is directly involved in nociception and may be related specifically to C-fibre mediated pain.

The Distribution of Peptide Pathways in the Brain A Review of Immunocytochemical Studies (Abstract)

EARL A. ZIMMERMAN, M.D.
Neurological Institute of New York
College of Physicians and Surgeons
Columbia University

In recent years some important information concerning the localization of more than 17 peptides in the central nervous system has been obtained by regional assay and immunocytochemical procedures. By application of immunofluorescence and immunoperoxidase methods it has become apparent that different peptides are formed in different neuronal pathways. As a result of the identification and synthesis of each peptide and production of specific antiserum to it, a new chemical neuroanatomical map for each peptide is now being constructed. Many of these are widespread and complex. A remarkable explosion of new data, generally incomplete, on the subject of peptide pathways is available, and promises to become much more complex as additional peptides are discovered or studied.

At present it appears that there are peptide systems with cell bodies of origin which are relatively localized in one area of the brain and their destruction leads to a loss of their peptide in branches to other regions. Examples of these include several hypothalamic sites: the vasopressin, oxytocin, neurophysin system of the magnocellular paraventricular region which projects to many forebrain sites including amygdala and the brainstem and spinal cord; the ACTH, β-LPH, α-MSH, β-endorphin cells in the periarcuate region projecting to the forebrain and midbrain central grey; and the LHRH system in preoptic area ending in brainstem and other regions. Other peptides are generally found in perikarya and are presumably formed in more widespread brain regions: somatostatin, TRH, enkephalin, substance P, neurotensin, and vasoactive intestinal polypeptide (VIP), for examples. Some of these are probably contained in both long and short pathways. Although the distribution of several peptides shows remarkable similarities in some areas such as innervation of the central nucleus of the amgydala and bed nucleus of the stria terminalis by enkephalin, neurotensin and VIP, there is not yet convincing evidence for two different peptide systems in the same cell. ACTH and β-LPH and probably their products (α-MSH, β-endorphin) are found in the same arcuate neurons, but they are derived from the same common precursor. Despite overlaps of a number of peptide maps in certain regions, the overall distribution of each appears to be a unique and separate system.

Training Neurologists For The Future

JAMES F. TOOLE, M.D.
Chairman, Department of Neurology
Bowman Gray School of Medicine
Winston-Salem, North Carolina 27103

"A neurologist must learn to know his diseases not as a zoologist knows species, genera and orders — by descriptions of comparative characters — but as a hunter knows his lions and tigers." Critchley (1).

How should neurology be taught now that we have computerized tomography, arteriography, ultrasound, electrodiagnostic, studies, and soon nuclear magnetic resonance for making precise anatomic, and perhaps chemical localization of central nervous system disease? Because they are quicker and more precise for diagnosis and treatment why then should we train neurologists? Will there be too many (2)? Similar to Lubbites are we waging a battle against technology or should we embrace it as our own? Will neurologists become extinct just as phthisiologist and syphilologists? These and questions related to the core of information that house officers must know so that they can recognize and treat disease of the nervous system are very appropriate questions for current leaders in the field to consider before they recruit and train its future generations.

For me the answer is apparent — the field of neurology will grow but the role of the neurologist must change if this is is to occur. The medical technology revolution has created a machine and image oriented health care system but there is an accelerating need for more humanistic, patient oriented physicians who will advise, select and supervise appropriate studies and tretment of the patient (3).

Many have written that the health care system is now so large and complex that it has become impersonal and I agree. Therefore the neurologist, as head of a health care team, must lend his personality and be the link between patient and the system. How can this be taught to future neurologists?

Let us divide the elements necessary for training of a neurologist into:

Faculty
Curriculum
Trainees
Academic environment

Faculty

Members of a training program have many functions. (1) recruiter, (2) supervisor, (3) dean of curriculum, (4) role model and (5) disciplinarian.

The requirements for faculty include far more than knowledge of the field. Cutting edge knowledge in one area is, of course, the basic ingredient but in addition he has many other functions. I will be able to consider only a few.

Most of todays teachers are products of an era when the bedside ritual was the key to correct diagnosis. Consummate use of eye, hand and brain aided by hammer, ophthalmoscope, pin, and key were the measure of one's prowess. Stethoscope was eschewed and laboratory tests used only as an adjuvant to one's clinical impression. Those of us who trained with Critchley, Carmichael, Symonds, Brain and Walshe of England or Merritt, Wolff, Denny-Brown, Gammon or Adams in the USA remember their ability to elicit subtle neurologic abnormalities by detailed testing and to predict location and pathology which would subsequently be confirmed by autopsy. Alas! Their meticulous and laborious techniques have been supplanted by the accuracy of arteriography, computerized cranial

tomography, and ultrasonography. No longer is the neurologist dependent upon exhaustive bedside examination for localization because it is now possible to locate anatomically and to identify etiology more accurately with special diagnostic tests. As a consequence the prolonged investment of time for training in bedside technique seems superfluous. Yet the orientation of neurologic training programs have not yet changed radically to adjust to the new era. Curriculum:

It is very enlightening to review the perspective on the development of neurology which was written by one of the luminaries in American Neurology, H. Houston Merritt in 1975 on the occasion of the 100th anniversary of the founding of the American Neurological Association (4). He wrote, "Fifty years ago all practioners of the specialty were neuropsychiatrists. It was a common expression of the day that the so-called neuropsychiatrist got his pleasure from the study and treatment of patients with organic disease of the nervous system but he made his living by treatting psychiatric patients. There were only a few residencies in neurology available to young graduates at that time. Even as late as 1936 there were only 16 hospitals listed in the United States as being approved for training for residency in neurology. In addition, most of the physicians who took a residency in neurology went on to practice neuropsychiatry. A few chose internal medicine or neurosurgery. This was perhaps due to two factors: first the training they received in neurology was very scant, usually only a year, and as a result they did not know much more about the diseases of the nervous system than did the internist who received several years of training in internal medicine. In addition there was a great pressure for the care of patients with mental illnesses and most of those who had any training in psychiatry gradually became almost totally concerned with the treatment of psychoneurotic and psychiatric patients." Dr. Merritt goes on to recall that "Before the birth of the National Institute of Neurologic Diseases and Blindness in 1947 there was 32 residency positions in neurology in less than a dozen hospitals. In less than 25 years this has increased over 30 fold to include more than 1,000 residency positions and 108 university-affiliated hospitals." (4) This dramatic change in emphasis and in manpower which occurred during the span of one neurologists career will, I predict, occur once again during the succeeding twenty-five years.

Because of rapid evolution of technology, how to train the aspirant neurologist is now an unsettled matter. Only seven years ago Critchley (1) published a program for training his ideal neurologist.

1 Year — Internship in medicine, surgery, gynecology and ophthalmology.

18 mos — Internal medicine

18 mos — Basic sciences including 6 months of genetics and statistics, 12 months of anatomy, physiology, biochemistry, or epidemiology

4 years — Clinical neurology of which 1 year shauld be spent in a laboratory in German speaking country to make friendships to last a lifetime and to develop fluency in this tongue

1 year — Completion of neurological training in the mother institution.

Note that Dr. Critchley's program encompasses nine years only five of which include bedside neurology and none of which incorporates training in diagnostic procedures. Along the way he advocated acquisition of Latin, Greek, French, and Queens English in order to provide finesse and polish to the product. He did not favor training in psychiatry but considered psychology to be an option. How quaint this sounds just seven years later!

In the United States today the requirements for residency training are half that recommended by Dr. Critchley but far more complicated (5). The training period must expand once more to at least 6 years because of the burgeoning of information and skills which the neurologist of the future must have.

The American Board of Psychiatry and Neurology requires only four years at present. It is of interest that they reaffirm the traditional linkage between psychiatry and neurology despite the stresses and strains of divisive forces within each discipline. Nevertheless the continued existence of one

Board for the two specialties is tangible expression that there are common roots from which each discipline grows.

The current training requirements are: one year of post-graduate experience emphasizing internal medicine, pediatrics, or family practice followed by three years of specialized training in neurology. The requirements for admission to the Board Examination include clinical neurology, neuropathology, neurochemistry, neuropharmacology, neuroanatomy, neurophysiology, neuroradiology, computerized axial tomography, EEG and evoked responses, EMG, visual fields, ocular fundi, neuro-otology, cerebral spinal fluid, radioisotopic scans, sonography, neuro-immunology, neuro-virology, and neuroendocrinology.

The psychiatric portion of the examination includes questions on growth and development, psychopathology, biological psychiatry, psychosocial psychiatry, and diagnostic procedures. The candidate who successfully passes the written examination which includes the areas described above is then eligible for the part II oral examination question which includes the examination of patients under the supervision of an examiner. The manner of examining patients, and the reasoning and deductions therefrom constitute an important part of the examination. Knowledge of basic science principles, special diagnostic procedures, and management recommendations and risks are essential parts of this examination which is focused on the evaluation of clipatient and his findings with their examinical skills.

Candidates are expected to discuss the patient and his findings with their examiner responding to questions which cover differential diagnosis, treatment and prognosis.

The patient examination includes psychiatry, and child and adult neurology. In the near future this examination will be conducted largely with audio-visual systems particularly with videotapes.

The Liaison Committee on Graduate Medical Education of the American Medical Association (5) describes approved training programs in neurology as having the objective to train physicians skilled in the care of patients with neurological diseases. This training should be based on supervised clinical work with increasing responsibility for out-patients and in-patients. It should include not only specific diseases of the nervous system of different age groups but also the neurological complications of medical and surgical conditions.

There shoul be organized instruction in the basic and clinical sciences relating to neurology. This is often best obtained by participating in and performing laboratory procedures and attending instructional exercises in which clinical correlation with laboratory data are emphasized.

Residents should have instruction and practical experience in the critical, orderly, and detailed patient interview and recording of the clinical history. There must also be instruction and experience in the methods of clinical examination of patients. A trainee should undestand the performance and interpretation of various diagnostic procedures including radioisotopic studies and computerized axial tomography; electroencephalography; electromyography and nerve conduction studies; psychological testing methods; and ophthalmologic and otolaryngologic investigations pertinent to clinical neurology. They should learn to correlate the information derived from these techniques and for other laboratory tests with the clinical histories and with those data from bedside observation informulating a differential diagnosis and a plan of treatment for patients with disorders of the nervous system. Instruction in neurodiagnostic techniques must be directed toward this goal.

In addition to the supervised experience with in-patients and out-patients on the neurology service, the residents should participate in consultations and other appropriate liaison operations with the medical surgical, pediatric, and psychiatric services and their sub-specialties. The neurological out-patient clinics and consultation service should be supervised by an experienced neurologist and where feasible a close relationship should be maintained with other clinics. There should be an especially close relationship with neurosurgery so that the residents can follow their patients

throughout the neurosurgical operation.

During their training residents should have experience with problems in child neurology including neurological examination of newborns and infants. Particular emphasis should be given to the changes in growth and development of the nervous system of a child. This training is best obtained on a child neurology service under the supervision of a qualified child neurologist.

It is important that the residents have sufficient opportunity to acquaint themselves with the techniques, procedures, and services of physical medicine and rehabilitation. Acquisition of the knowledge of community resources for patients with neurological diseases is essential for the proper care, rehabilitation, and placement of patients.

Residents in neurology should have organized instruction in the examination of the mental status of patients and should be acquainted with the symptomatology and differential diagnosis of the more frequently encountered psychiatric syndromes, particularly these associated with lesions of the nervous system. Trainees should be made aware of the psychological aspects of the patient-physician relationship and the importance of personal, social, and cultural factors in disease processes and their clinical expression.

Using the past as recalled by Merritt and the present requirements for training can we project requirements for training the neurologist of the future? I believe that he will continue to be a skilled historian, will a new role as coordinator of a management team, supervisor of procedures and will provide critical judgment as to which among many remedial actions to choose.

Because of time constraints and increased knowledge training programs will become ever more rigidly constructed to ensure a core of knowledge. For example, we must teach the techniques of team management and instill the difficult art of interviewing patients (6, 7). These will be added to the continued necessity to teach a detailed knowledge of the most complex part of the human body knowledge of which is forever expanding. For these reasons the length of training must increase.

We will have to contend with the concept of brain death and the problems of access to scarce or rationed resources so we must provide time for training in ethics, teach scientific objectivity for evaluation of new therapies and, crass at it sounds, business methods, so that he will possess a skill which will be sought in the marketplace. If he also learns empathy and a desire to communicate with the global community of his colleagues, one will have trained him well.

Trainees

Our specialty encompasses all symptoms and disorders referable to the nervous system with the realization that disease of almost any organ can affect the brain. Therefore, a neurologist must first be skilled in general medicine before specializing in neurology.

Because most patients are frightened, he must have patience, understanding and empathy. His function is not only to solve diagnostic conundrums but to give hope and solace to patients. Therefore potential neurologists should be selected for these aptitudes in measure equal to their knowledge and then their different talents and aptitudes must be developed. Some are action oriented, others contemplative, some self assured, others hesitant. Some would order tests rather than use their brains. Recognizing this, the program director must select trainees, not because patient care responsibilities are to be covered, but because the candidate has the prerequisite character traits, the most important of which is the desire to listen, observe carefully, to learn to lead and to be adaptible.

The beginner must become a skilled observer, using his eyes and ears to gather information which his brain can synthesize and use for the patients' benefit (8). Because listening is not synonymous with hearing; he must develop intuitive skills at evaluating, interpreting, and responding. To my knowledge this essential feature of a neurologist's training is taught only in psychiatry programs and it is for this reason that I require a period of time to be spent in rotation through our companion specialty. Melvin Yahr said it best when

describing Dr. H.H. Merritt's skills as a clinician (9). "Houston Merritt's ability as a diagnostician is legendary. This unusual capability was founded on a remarkable understanding of the complexities of the nervous system, a broad range of medical knowledge, and keen clinical skills. He was masterful in elicting and assessing the essentials of a diagnostic problem, separating the wheat from the chaff, in both history and examination. Coupling this with an astonishing ability to recall instantaneously comparable cases he had encountered, he rendered the most complex diagnostic dilemma approachable. At first sight, Dr. Merritt's abilities seemed to be almost intuitive, for his conclusions were decisive and reached with lightning speed. However, it did not take long to appreciate that his skills were deeply based and indicative of a combination of talents rarely encountered in our profession."

Part of the process involves disciplining one's self to judge content and not method of delivery or deportment of the patient, to concentrate on what the patient is saying and to resist distraction, to control one's emotions, and to focus one's attention entirely on the patient. I make it a point to teach this skill by emphasizing that skilled listeners recognize four levels of communication.

1. Informal: conversation that establishes rapport.
2. Informative: one gathers information.
3. Cathartic: the patient ventilates feelings, problems and frustrations.
4. Persuasive: attitudes are reinforced, motivation is provided, and leadership is exert.

Haw best does one teach a harried resident distracted by the responsibility for 20 or more patients, and a variety of procedures, yet determined to learn the skills necessary for becoming a neurologist (10,11). There is no one formula, but my method evolved during 30 years is first to be a role model and second to use Socratic methods for developing logic and knowledge. The focus is on the patient and his problem and is done with patient present in Oslerian tradition. I elicit the history from students and house officers who have evaluated the patient and confirm its accuracy and completeness by quizzing each, corroborating it with the patient, and if possible a family member who has observed the patient closely. I have found it best to begin by inviting the most inexperienced member of the group to recite the history then asking in sequence the more senior house officers, concluding by asking patient and family members to verify the accuracy of information. It amazes me to discover haw frequently three interviewers have been given (or have interpreted) information quite differently.

After examining the patient, my role then becomes that of group leader and coordinator. I use the data base for Socratic dialogue utilizing: 1) leading questions, 2) questions which require reasoning and judgement not facts, 3) yes/no responses which I program into algorhythms, particularly the interpretation of symptoms and signs which I elicit during my examination until finally the woof and warp of facts and findings are woven into a clinical diagnosis which becomes a hypothesis to be proved by the selection of appropriate tests. We discuss these tests and their utility for reinforcement of the hypothesis or to eliminate other possibilities, and I emphasize that the culmination of the diagnosticians art lies in the selection only of tests which are sensitive, specific and cost-efficient and which do not expose the patient to unnecessary risk. We consider risk/benefit ratios with the understanding that the best physician is the one who is able to make the correct diagnosis most safely in least time at lowest cost, and who institutes appropriate therapy, which results the quickest cure. Young physicians must be shown this technique in action, so that they become cognizant of the ramifications of their decisions. There must be no ordering of superfluous, expensive or hazardous tests. Sensibilities of the patient and family must be paramount and most important of all trainees must be made to realize that they will someday be the leader of a team of people engaged in the most difficult and demanding profession, which coordinates the activities of large numbers of skilled

workers in what I consider to be the most hazardous environment on earth — the hospital. These institutions, which are the meeting ground for complex technology and human misery must be balanced by the wisdom and humanity of the physician who is continuously torn between sympathy for patients and the need for scientific detachment in order to achieve correct diagnosis and cure.

It seems to me that some trainees are educated in theory beyond their understanding. In the search for scientific detail (12) and in the effort to outstrip their fellows they may forget that the great human values of medicine lie in simplicity. The inexperienced physician will all too often choose the difficult approach while the experienced neurologist simplifies problems as much a possible.

The Curriculum

There is a raging debate about the length of time which is necessary for training a competent neurologist. Because the majority of student neurologists are destined to be practitioners and only the minority clinical researchers and academicians, I believe that the content of our programs should be so tailored Faculty must understand the environment in which neurologists practice and what colleagues will expect of him in order to train neurologists to fit properly into a community. Foremost a neurologist must be able to recognize early phases of neurologic illness and to differentiate it from general medical or psychiatric disorders; second we must recognize that the majority of his care will be delivered to ambulatory patients. Third we must recognize him as a leader of a team who will be the most proficient physician in the community for performance and interpretation of diagnostic procedures such as EEG, EMG, CCT, arteriography, and ultrasonography. The training program should be constructed with the above in mind.

Because undergraduate medical curriculum varies so greatly, there is no standard core of neurologic knowledge for graduates of our medical schools. Therefore, the first post-graduate year must be adjustible to suit individual needs but, in general, a solid base of pediatrics or internal medicine preferably two but a minimum of one year is necessary. Responsibility for hospitalized patients with vascular, infectious, renal, neoplastic, and hematologic disorders provides a solid base upon which to build one's special knowledge of the nervous system and its diseases.

The next two years of neurologic specialization should be spent on rotations such as: 14 months of ambulatory and hospitalized patient care of children and adults, 3 months each of electrodiagnostic procedures, neuroradiology including CCT, myelography, arteriography, and ultrasound, 3 months of psychiatry and of neuropathology.

During the fourth and final year some will be competent to be fully responsible for patient care with minimal supervision, others will need extra time before achieving this. There should be available during this time about 6 months for elective work which for some would be clinical research, for others travel, and still others the honing of special diagnostic skills. If the trainees wants knowledge in depth about a special aspect of neurology a fifth year of fellowship is necessary.

I attempt to teach my house officers to spend their lime with patients using eyes, ears, nose, and hands, acquiring bedside skills, for therein lies the secret of diagnosis, not in a battery of tests or in the contents of books. After all, it is diagnostic ocumen and judgement which they must acquire. This is founded on the observation partly motor and sensory in the use of hands, eyes, ears, or nose and is partly interpretive. The interpretation is only partly logical depending to an important extent upon memory, and even more, perhaps, upon intuition, or hunch, which springs from unconscious memory.

Lord Russell Brain said it well:

"Most young physicians, when they begin practice, have a brain full of knowledge that may be quite correct, but most of which is complicated beyond any hope of applicability. This complexity is neither necessary nor desirable. Rather, let the young man know the basic principles of disease with which he must struggle, and know them well; let him know the value (or

lack of it) of the simple, basic procedure of diagnosis and therapy. And, most important, let him know the true value of the physician himself, which, whether we of the profession approve it or not, understanding the distinction between the biologic events of disease and the human events of illnes and the importance of care that gives attention to both. Third, ambulatory care experience permits the trainee who has had inpatient experience with discrete, major illness events sharpen his or her competence by focusing on minor manifestations of disease in relation to major diagnostic possibilities. A fourth gain is experience in efforts to forestall or avoid hospitalization."

REFERENCES

1. *Critchley M*: The training of a neurologist. International Journal of Neurology 9:308, 1975.
2. *Menken M*: The coming oversupply of neurologists in the 1980s: implications for neurology and primary care. JAMA 245: 2401 2403, 1981.
3. *Barondes JA*: The training of the internist with some messages from practice. Ann of Internal Med 90: 412-417, 1979.
4. *Merritt HH*: The development of neurology in the past fifty years. Centennial Anniversary Volume, American Neurological Association, D Denny Brown (Ed), New York, 1975.
5. Essentials of approved residency programs. Liaison Committee on Graduate Medical Education, 1980. American Medical Association, Chicago, Illinois.
6. *Stevenson I*: Medical history taking. Hoeber Medical Division, Harper and Row, New York, 1960.
7. *Gardner M*: Science good, bad, and bogus. Prometheus Books, Buffalo, New York, 1981.
8. *Barrows HS, Hamilton,* and *Bennett K*: The diagnostic (problem solving) skill of the neurologist. Arch Neurol 26: 273-278, 1972.
9. Clinical ecompetence in internal medicine. American Board of Internal Medicine. Ann of Internal Med 90: 402-411, 1979.
10. *Levitt LP*: Neurological education of nonneurologists. Neurology 29: 1514-1516, 1979.
11. *Feinstein AR*: What kind of basic science for clinical medicine? Special article. Clinical Med 283: 847-852, 1970.

HISTORY OF MEDICINE

Medicine And Air Supremacy

John F. Fulton, M.D.†
NEW HAVEN, CONNECTICUT

* The Shattuck Lecture, delivered at the annual meeting of the Massachusetts Medical Society, Boston, May 26, 1942.

From the Laboratory of Physiology, Yale University School of Medicine.

GEORGE CHEYNE SHATTUCK, the younger (1813-93), whose father, George Cheyne Shattuck, the elder (1784-1854), left the bequest that led to the founding of this lectureship, died early in 1893, and Osler,[1] who gave the fourth lecture of the series in that year, chose for his subject "Tuberculous Pleurisy", a theme in which the younger Shattuck had been interested since his early days in Paris, when he studied under the great French clinician, Louis. Shattuck's son, Frederick Cheever Shattuck (1847-1929), was, like his father and grandfather, a great force in New England medicine. The Shattucks were men of humor, forthright candor and passionate loyalty to the traditions of this country. Their humor is well illustrated in a lively encounter between Frederick Cheever Shattuck and Harvey Cushing, who gave the Shattuck Lecture in 1913.[2] Dr. Shattuck had read Cushing's account of the Western Reserve and its traditions,[3] and was horrified to find the word tomahawk misspelled. Dr. Cushing, his secretariat and the Cleveland proofreaders had all passed "tommy-hawk" — spelled like "tommy-gun." This was too much for Frederick Cheever, who immediately commandeered conveyance to to the Peter Bent Brigham Hospital, and, wearing a pair of enormous plus fours, dashed in the side door of Dr. Cushing's office to tell him that the Moseley Professor of Surgery, who had been born in the Western Reserve out among the Indians, should know the spelling of tomahawk; not content with this, he wrote Cushing a letter referring him to the Century Dictionary.

* * *

I have said that the Shattucks were men of intense loyalty to this country's traditions, and when your committee requested aviation medicine as the subject of this discourse, it seemed obviusly a theme wholly appropriate for a lecture devoted to the memory of this remarkable line of American physicians; moreover, a topic with military implications is not without precedent, for just twenty-five years ago, — in June, 1917, — Dr. Walter B. Cannon gave a Shattuck Lecture on traumatic shock.[4] In accepting the honor, I have, however, taken on a heavy responsibility, and one that for various reasons is embarrassing.[5]

The National Research Council and the Office of Scientific Research and Development have followed the policy of classifying as "confidential" or "secret" all topics having to do with offensive instrumentalities of war. The airplane is clearly such an instrumentality, as are many of the devices within the plane designed to improve the performance of the pilot in his rapid, high-altitude maneuvers; so that medicine, perhaps for the first time in its history, has come to be divided, so far as war is concerned, into offensive and defensive spheres. Advances that have to do with increasing the effectiveness of human performance in combat become military secrets, and cannot

now be openly discussed. Defensive measures, on the other hand, designed for treating the wounded, either civilian or military, or for prophylaxis, as by inoculation, fall into the category of defensive measures and can be freely described. Aviation medicine falls squarely across the broad categories of offense and defense, and I am therefore obliged to devote attention primarily to the defensive phases of the subject.

It has become obvious, even to the most casual observer, that air supremacy will determine the outcome of the present war. Shipping still has vast importance and we look carefully to our tonnage, but all the ships of the United Nations would become virtually useless without command of the air. Supremacy in aviation is not wholly a question of more and faster planes with greater firing power than the enemy. This, to be sure, is important, but equally so is the problem of securing well-selected, well-trained and adequately protected flying personnel. The performance of modern aircraft has far outstripped the physiological limitations of the pilot. The newer combat planes can fly higher than is compatible with life, even when the fliers are breathing pure oxygen. They can perform maneuvers causing centrifugal force of such intensity that blood tends to be drawn away from the brain, a condition that results in transient blindness (blacking-out) and unconsciousness. And, finally, the range of the modern four-motored bombers — some of which can remain for twentyfour hours in the air — has raised problems of pilot fatigue, severe stresses and strains from cold, psychological tension and loss of sleep that impair the performance of flying personnel. It is the responsibility of medicine in its broadest sense, including psychology, psychiatry, physiology and the special branches of clinical medicine, to protect flying personnel from these and many other hazards that they face. The role of the physician in both the offensive and defensive phases of the war effort has therefore become increasingly vital for broad military strategy.

Air supremacy involves not only flying personnel but ground personnel. It is estimated that for every man in the air there are nine or ten men on the ground, both in civilian airlines and in military aviation; men on the ground are as essential as the men in the air, and if the Army should wish 100,000 pilots it must recruit 1,000,000 men. Air supremacy also extends to the men in the aircraft factories, who are exposed to special hazards peculiar to aircraft production. I cannot speak of industrial hazards in aircraft plants, but they are real and their successful handling rests with industrial physicians. In an aviation plant recently visited, 600 from a total of 30,000 employees were treated daily for accidents or illness occurring in the plant — that is, 2 per cent of the total personnel became ill or were injured each day. This is far higher than one would wish or anticipate, and yet with the vast expansion of the past twelve months, such injuries are to some extent inevitable. In a plant that is not expanding, injury rates diminish, but usually only as rapidly as the measures taken for their prevention. The need for industrail physicians in all phases of the war effort continues to be enormous.

To give a more general idea of the scope of aviation medicine, I shall describe a classified bibliography of the subject that is now in the process of publication.

LITERATURE OF AVIATION MEDICINE

In recent months, my associates, Dr. and Mrs. Ebbe C. Hoff, and I have had the responsibility of searching out, listing and classifying all available literature bearing on the medical aspects of aviation. The project was proposed nearly eighteen months ago, and the labor is now completed, for the bibliography will be published within a few weeks.[6] The subject matter covers a vast range, the principal topics being indicated by the main chapter headings of the bibliography:

1. History and General Aspects of Aviation Medicine.
2. The Special Physiology of Aviation. (This section is divided into nineteen subsections including all the organ systems and special senses.)
3. The Special Pharmacology of Aviation.
4. The Special Psychology of Aviation.
5. Aeromicrobiology. (Bacteriology and immunology in aviation and high altitudes.)

6. Diseases and Accidents in Aviation and Conditions Simulating Flight.
7. Selection and Assessment of Efficiency of Flight Personnel.
8. Training, Performance and Fatigue of Flight Personnel.
9. Protection of Flight Personnel: Preventive medicine and therapeutics of aviation.
10. Aviation and Public Health.
11. Organization of Aviation Medicine.
12. Special Problems.
13. General Studies in Aviatiton Medicine.
14. Bibliographies.

It may be of interest that, although approximately six thousand separate items were found, the author index contains some nineteen thousand names, from which one must infer that those who write on the subject generally write in trios. And this expresses what some of us had gradually come to realize: that research endeavor in this field is inevitably co-operative. The flight surgeon uses a pilot or some fellow flight surgeon as a subject of an experiment, sometimes in the air, sometimes in a decompression chamber and sometimes in a human centrifuge. When decompression experiments are involved, five or six people generally constitute a team, and their names may appear as co-authors of the report.

The bibliography itself cuts across the scientific periodical literature of all phases of science in all countries, articles from about eight hundred journals having been cited. Of these, less than half are medical journals.

In passing, one may mention that from the bibliographical standpoint it would be impossible to cite a vast literature of this sort if one restricted abbreviations to a system worked out purely for medical journals. On this point, we were fortunately forewarned and at the start adopted the conventions of *A World List of Scientific Periodicals*[7] as a basis for abbreviations; this made possible the ready citation in conveniently abbreciated form of any scientific journal in any language, without serious confusion.

In surveying this literature, we were impressed by the large number of Japanese articles on aviation medicine. Much more striking, however, was the fact that about thirty Russian journals were represented in the bibliography, embodying a vast and well co-ordinated literature on the subject— far ahead, incidentally, of that of Japan.

There is a widespread feeling that bibliography is a dull preoccupation reserved for spinsters and old maids of the male sex. Actually, it is far from that, for careful analysis of the literature of any subject reveals trends of research, and in the bibliography under consideration, it has exposed trends and emphasis of far-reaching international significance. The Germans, for example, began publishing papers on the effects of high acceleration in aircraft five years before the flight surgeons of the United Nations had given any general consideration to the problem, and everyone must realize what the dive bomber has meant to the Axis war effort. For better or for worse, the Allies have depended largely on horizontal bombing, but with our fast fighters we are quickly learning the significance of high acceleration, and are studying the modes of counteracting its effects on aircraft personnel.

PROBLEM OF ANOXIA

The responsibility of carryng on research in the more academic phases of aviation medicine falls largely to the civilian laboratories, although one looks forward to research institutes within the military services that will continue with active investigative endeavor in times of peace. But in the present war crisis, it is clearly up to the civilian scientists to undertake the long-range problems, and in aviation medicine the most basic of these is a study of the adjustments of the body to anoxia. There are many aspects of the problem as yet imperfectly understood, — individual variations, variations of the individual, — factors that aid the body in making the adaptations, all of which involve fundamental physiological, biochemical and endocrinological research; the aim in view is to increase knowledge of the processes involved and to search out ways of improving human performance in the higher altitude ranges. To use the language of aviation, the basic problem is to raise the aviator's "ceiling." But from the purely academic standpoint, we wish first to extend our knowledge of the processes involved.

In his excellent monographic review on the effects of anoxia in the body, Van Liere[8]

gives a broad picture of the manifold changes that occur when the body is exposed to low oxygen partial pressure. In adjusting, for example, to a fall of half an atmosphere, giving an equivalent altitude of 18,000 feet, there is a veritable ionic cataclysm between blood and tissues and renal tubules, accompanied by a shift of the blood pH to the alkaline side, with an extensive loss of sodium and chloride ions in the urine. Van Liere, however, makes little attempt to elucidate the important problem of how these ionic shifts are integrated. What organ responds in the first instance to the lowered oxygen partial pressure? From the work of Cannon[9] and of Gellhorn and his collaborators,[10,11] it is known that the sympathetic system is exquisitely sensitive to anoxia and that many of the adjustments arise from the direct stimulating action of low oxygen tension on the central neurons of the sympathetic system. From the sympathetic comes the reflex mobilization of idle red blood cells from spleen, bone marrow and other reservoirs, and a vast series of vasomotor readjustments designed to improve the circulation of vital organis is brought about, also reflexly, through interaction of the simpathetic and parasympathetic systems. No one, however, appears previously to have suggested that the ionic shifts essential for anoxic acclimatization are likewise mediated through reflex channels. The evidence to date is incomplete, but suggestive, and it turns largely on recent developments bearing on the part played by the adrenocortical hormone in anoxia.

Anoxia and Adrenal Cortex

Two papers published by French flight surgeous at the end of the last war suggested that the asthenia that certain aviators developed after repeated missions to high altitudes was due to adrenal insufficiency. Ferry[12] observed urinary retention of nitrogen and alkali, low blood pressure and pathological heart sounds in a group of overfatigued aviators, and he was led on the basis of these findings to the conclusion just mentioned. The paper of Josué[13] was based on a study of physiological and psychological alterations in fatigued pilots. But since at that time there was no clear distinction between the adrenal medulla and the cortex, the suggestion can remain only of historical interest. More recently, Armstrong and Heim[14] found on exposing rabbits for four hours a day to an atmosphere equivalent to 18,000 feet that, in the early stages, hypertrophy of the adrenal gland resulted and was followed later by degenerative changes in the adrenal cortex. In his well-known book on aviation medicine, Armstrong[15] later pointed out that overfatigued pilots, especially those subjected to many high-altitude missions, developed symptoms strikingly similar to those seen in early Addison's disease.

Sundstroem,[16,17] whose early studies on the adaptation of man to high altitudes are well known, was led some years ago to study the relation of the adrenal glands to acclimatization and, independently of Armstrong and Heim, confirmed the existence of adrenal hypertrophy resulting from anoxia; in his monograph about to appear from the University of California Press, he[18] shows that the degree of adrenal hypertrophy can be roughly correlated with the extent to which the oxygen partial pressure is diminished. All animals exposed to diminished atmospheric pressure during the period of acclimatization tend to lose weight. This loss of weight is shared according to Sundstroem, by all organs of the body except the adrenal cortex (and possibly the kidney); the adrenal hypertrophy is therefore regarded as something specific to the anoxic state. On the basis of the hypertrophy, Sundstroem asked himself whether this might not indicate increased secretion of the glands. He set out to obtain a direct answer to the question in two ways. In the first place, adrenal steroids were extracted from tissues, such as the heart and liver, from control animals at sea level and from groups exposed to the high-altitude ranges; the tissues of the latter animals invariably showed a larger proportion of adrenal steroid than the corresponding tissues in animals at sea level.

More impressive, however, was the study of Giragossintz and Sundstroem,[19] in which it was found that adrenalectomized animals could not survive in the high-altitude ranges and that it took twenty times more crude extract of the adrenal cortex to maintain rats at 20,000 feet than it did at sea level. This clearly suggested that to maintain the body at high altitude increased secretion of adrenocortical extract is essential.

The problem has recently been taken up anew in my laboratory by Langley and Clarke,[20,21] who have confirmed the fact that adrenal hypertrophy develops in rats exposed to 20,000 feet; and in adrenalectomized animals, they find that at sea level the maintenance dosage for an average adult rat is 05 cc. of total extract a day (Wilson), or 0.03 mg. of desoxycorticosterone acetate. At 20,000 feet, a rat on this maintenance dose rapidely loses weight and dies, and Langley and Clarke find that 2 or 3 cc. of total extract is essential at that altitude and that 1 mg. of desoxycorticosterone is required. When acclimatization has taken place, however, after one week at 20,000 feet, the maintenance dose can be reduced to the sea-level amount.

Langley found, as had Gerald Evans,[22, 23] that exposure of a fasting rat to an altitude of 20,000 feet for twenty-four hours causes an elevation of both the bloou-sugar and liver-glycogen levels. This suggests that Compound E, the carbohydrate fraction of the adrenocortical secretion, is mobilized in conditions of anoxia. But the desoxycorticosterone fraction appears also to be mobilized, since Langley has found in dogs exposed to an altitude of 20,000 feet that a marked increase occurs in sodium and chloride and also in potassium excretion. Following adrenalectomy, dogs subjected to anoxia failed to show the sodium and chloride excretion, although potassium loss continued. The failure of the sodium, chloride and carbohydrate adjustments in adrenalectomized animals exposed to anoxia indicates that the presence of adrenal extract is apparently essential to make the bodily adjustments to altitude, and one naturally wishes to know how the adrenal cortex is specifically activated — whether directly by the blood stream or in some way through the nervous system. Langley,[20] in discussing the question, remarks: "It is possible that the increase in sodium chloride and urine volume observed in the normal animal exposed to anoxia was brought about by increased excretion of these specific fractions [desoxycorticosterone] of the adrenal cortex. This observation suggests that the adrenal cortex is capable of secreting certain components of the whole extract independently of the others."

The recent important work of Dr. George Thorn,[24] the newly appointed Hersey Professor of Medicine at the Harvard Medical School, has also established in animals that a large increase in sodium, chloride and potassium excretion occurs on exposure to anoxia, and he has found conspicuous nitrogen retention in man under these conditions. Treatment of adrenalectomized animals with the so-called "carbohydrate-regulating" factor caused a striking increase in sodium, chloride and water excretion, but no increase in potassium. Thorn and his collaborators[25–27] have just given an account of the effect on rats, rabbits and dogs of intermittent exposure to altitudes equivalent to 18,000 and 27,000 feet. They have confirmed Armstrong and Heim's[14] observation that adrenal hypertrophy develops in consequence of such repeated exposure in rabbits (and also rats); they have found moreover, that the adrenalectomized animal fails to survive repeated "flights" to these altitudes, and that their capacity for adjustment can be restored by administration of adrenocortical hormone.

From the studies of Collip[28] and his students, it appears probable that the adrenal cortex is normally activated, not by the blood stream directly, but rather by the adrenotropic hormone of the anterior pituitary. The ingenious work of Uotila[29,30] indicated that the thyrotropic hormone is under the direct control of nerve centers in the hypothalamus whose axons passed down the pituitary stalk, and that the reaction to cold results from therman stimulation (via the blood) of the hypothalamic centers. Since the adrenal cortex also plays a large part in the reaction to cold and to anoxia it is likely that the primary activation of the adrenal cortex comes from the hypothalamus through the adrenotropic hormone. Favoring this is the fact, originally disclosed by Gerald Evans[22] and recently confirmed by Catchpole[31] that the chronically hypophysectomized rat has no greater altitude tolerance than the adrenalectomized animal.

All this brings one to a far clearer concept of the mode of integration of the bodily adjustments to altitude. The part played by the respiratory center in the medulla has long been recognized. Mobilization of red cells and the reflex adjustments of the heart and circulation arise in part from direct stimulation of the chemoreceptors of the ca-

rotid body, as well as from the direct effect of lowoxygen tension on the sympathetic system; there appears to be further reflex control, through the centers in the hypothalamus, of the ionic pattern and carbohydrate level of the blood. Undoubtedly, when the complete picture has been put together, the posterior pituitary gland will also be found to play a part in these adjustments through the influence of its antidiuretic hormone on the kidney tubules. This strongly suggests that the bodily adjustments to anoxia are in large measure integrated by the central nervous system.

SAFETY IN CRASHES

The military phases of aviation medicine are rigidly practical. General academic research is encouraged at some of the larger bases and institutions, but for the immediate purposes of the war effort a group of practical problems has arisen for which solution is required in a matter of months. Combat fliers, for example, are constantly exposed to rough landings under blackout conditions, or to crash landings when machines are disabled, and the question arises whether mechanical factors for safety, similar to those introduced within the past few years in automotive design, cannot be adapted to aircraft. This raises the question of the factors responsible for injuries, fatal and otherwise, in air crashes. Close study of the large literature on air crashes indicates that impact of the body, especially the head, with some solid part of the aircraft is generally the cause of death or of serious injury, even in minor accidents. When the body or the head strikes something that yields, as when the flier is thrown through a fabric roof or the windshield, the victim generally escapes serious injury. What, then, are the basic factors that govern the degree of injury in such circumstances?

The most significant clues have come from two sources: De Haven's[32] analysis of nonfatal suicidal leaps from high buildings, and a study, for which Denny-Brown is largely responsible, of the effects of sudden acceleration on the head.

Nonfatal suicidal leaps. In a series of recent papers, De Haven[32,33] has drawn attention to some remarkable cases of suicidal leaps from high buildings that proved not to be fatal. A number of such cases — in which all data were available concerning the exact distance of the fall, the position of the body during the fall and on landing, and the character of the surface that the body struck — permitted him to draw certain generalizations: in the nonfatal leap, the victim generally landed flat on the back or flat on the stomach, so that the long bones or the head was not driven into the trunk. But more interesting is the fact that a slight degree of cushioning of the head, as in landing in a garden plot instead of on a cement sidewalk, prevented concussion and serious injury of other parts. A typical case may be cited[33]:

A twenty-one-year-old woman, mentally depressed because of an amorous disappointment, took a room on the tenth floor of a hotel, consumed half a bottle of whiskey, and leap in her nightdress to the street below — a free fall of 93 feet. She landed squarely on her back in a small garden in which the etrth had been freshly turned, her head, back and legs sinking into the earth to a depth of 4 to 6 inches. A hand, which struck the cement border of the garden plot, sufered a fracture to a small bone in the wrist, but except for this and a fractured rib she was uninjured, suffered no concussion, and could walk without assistance. Her height was 5 feet, 7 inches, and her weight 115 pounds.

The important point about this and similar cases is that the head experienced a brief interval of deceleration, instead of an abrupt impact on a rigidly solid object. De Haven calculates that the girl's body was falling at a rate of 73 feet a second (50 miles an hour) at the time of the impact, and that the deceleration distance, which amounted to 4 to 6 inches of garden turf, must have taken place in a small fraction of a second; the rate of deceleration was 166 g (1 g = 32 feet per second per second). There is a vast difference between being decelerated from 50 miles an hour in 0.001 second and being decelerated in 0.1 or even in 0.01 second. Little attempt has been made so far to measure these brief but vital deceleratory time intervals in relation to injury.

A more complex case ocurred several months ago in New York and is mentioned because of the relatively long distance of the fall. A woman leaped from the seventeenth floor, falling 144 feet, and landed in a "steamer-chair" position on a metal

ventilator box 24 inches wide, 18 inches high and 10 feet long. The force of her fall, De Haven points out, crushed the structure to a depth of 12 to 18 inches. Both arms and one leg extended beyond the area of the ventilator, with resultant fractures of both bones of both forearms, the left humerus and the left os calcis. The woman remembered falling and landing, but had no marks on her head or subsequent loss of consciousness. She sat up and asked to be taken back to her room. No evidence of abdominal or intrathoracic injury was found, and x-ray films failed to reveal other fractures. The minimum gravity increase in this case was 80 g (average, 100 g).

Stunt drivers. A practical application of the principle of gradual deceleration has long been used by circus performers and stunt drivers, who deliberately drive a car at 60 miles an hour into a brick wall. Their trade secret is to jump into the back seat of the car and lie hard against the rear of the front seat, a hand or an elbow being placed between the side of the head and the back of the front seat. The car then crashes into a solid object and the superstructure crumples up against the wall, but in a finite time interval sufficient to give adequate deceleration of the head and the rest of the body. If the head or the body were thrown without having the benefit of the car's own crumpling deceleration, "Reckless Peter", one of the best known of these stunt drivers, could be reckless no longer.

Aircraft[34] and automobiles, at the instigation of the National Safety Council, have been studied from the point of view of diminishing hazards to the head in the event of crash, and flying personnel are being indoctrinated with the principles of how to "take" crashes. For some pilots, this is instinctive, but the fact that the head and body should be placed hard against some solid part of the structure of the machine when a crash is anticipated is not commonly appreciated. Any yielding substance placed between the head and the solid area of superstructure has cushioning value in making deceleration more gradual; but if the head is free and hurled against the solid object at the time of a crash, the injury sustained is inevitably severer. Those stationed in the rear of the automobile or plane when it strikes a solid object have more opportunity for deceleration than personnel situated farther forward.

Experimental concussion. Denny-Brown and Russell[35] have approached the problem in the reverse direction, — namely, by analyzing factors of acceleration in relation to injury rather than through deceleration, — but the principles in the two approaches are the same. Denny-Brown and his collaborator have found that when the head of an animal is struck by a moving pendulum, concussion does not occur unless the head is free to move free to be accelerated. If a head, hard against an anvil or a brick wall, is accidentally struck, a nasty fracture may result, but the subject is not rendered unconscious; for this an acceleration of the head in space is essential. In Denny-Brown's experiments, the rate of acceleration essential to cause concussion was relatively high: a critical value of 46,000 feet per second per second. If the head is cushioned, as by a helmet, the same blow may give the same ultimate velocity after the head has moved 5 mm., but if it does not start off with the same high acceleration, concussion is prevented. The implications of this in relation to crash helmets for absorbing blows from falling debris and flying bomb fragments are obviuos, and it is no longer a secret that both the British and the Germans have a mandatory regulation to wear crash helmets in all operations of mechanized units, especially motorcycles. Such helmets have enormously diminished the number of serious head injuries sustained on being thrown, especially during blackout conditions.[36]

Ruptured intervertebral disks. A syndrome common in the military services, especially in airforce personnel, that is not often diagnosed is that of the ruptured intervertebral disk.[37] When men are maneuvering in aircraft, accelerations as great as 8 or 9 g may occur, that is, eight or nine times the normal acceleration of gravity, which cause strains on the vertebral column of intense character, making the weight of the torso at the lumbosacral articulatión equal to about 800 pounds if the body weight of the pilot is 200 pounds. One means of lessening the physiological effects of high acceleration is the assumption of a crouched posture, which brings the lower extremities nearer the heart and thus diminishes the length of the hydrostatic column of

blood subjected to acceleratory force.[38] There is no doubt that the assumption of such a posture increases tolerance to high degrees of acceleration, but it also greatly increases strain on the lumbar vertebrae, the annulus fibrosus and the pulposus nuclei between the vertebrae. In these circumstances, one or more of the nuclei may rupture and herniate into the spinal canal, and thus may give rise to pain from compression of sensory-nerve trunks and rootlets. This accident, which is also common in civil life, especially in young adults, is one of the most frequent causes of acute and incapacitating sciatic pain.

The syndrome of the ruptured intervertebral disk is one with which every flight surgeon should be familiar, for the injury not only occurs as a result of high acceleration in aircraft but also is a common complication of injuries sustained as a result of a crash landing. Mild cases improve on simple immobilization, but there is a growing conviction among neurosurgeons, especially Spurlin,[37] of Louisville, and Love,[39] of the Mayo Clinic, that when pain is enduring even with immobilization, operative removal of the ruptured disk is the only satisfactory therapy. Group Captain Symonds,[40] of the Royal Air Force, reports on the British experience with ruptured disks and expresses his doubts of the wisdom of operation, for in his experience few, if any, cases can be returned to active service. Spurling,[41] on the other hand, reports that in his noncompensation group fully 75 per cent have returned to their former occupations within three months; the details of treatment must, however, be left to the neurologist and to the neurosurgeon.

From the point of view of aviation medicine, the importance of the intervertebral disk lies in the fact that one must be familiar with the condition so as to make positive diagnosis possible, and the flight surgeon should be interested in any procedure or device that will lessen the incidence of this accident in combat operations. Various forms of mechanical restraint, such as seat belts and shoulder harnesses, have been proposed to prevent flying personnel from being thrown or overstressed during landings and high-speed maneuvers, but there is as yet no unanimity of opinion on this point, and the matter is one that clearly deserves intensive study, not only for the purpose of diminishing lumbosacral injuries but also to reduce the large numbers of unnecessary injuries to the head sustained in combat maneuvers and crash landings.

AFTERMATH OF INJURY

The flight surgeon and other physicians who attend air-corps personnel not only must heed the problems of the air cadet in training or the pilot engaged in combat operation, but he must also consider the management of incapacitated flying personnel. Some may be wounded by machine- gun bullets, and others may be hurt less seriously by a crash landing or a violent air maneuver. Still others may deteriorate from fatigue, or from too many missions at a high altitude. A medical officer in charge of any command must be able quickly to distinguish the three types of incapacity and must know how best to manage each one — whether it is a physical injury from gunfire, an injury from crash or maneuver, or a psychological insult from anoxia.

Watson-Jones[42] in a recent stimulatin paper, one of the few released for public consumption from the Royal Air Force, has discussed the ultimate problem with which all flight surgeons will sooner or later be faced namely, the rehabilitation of personnel disabled by combat operation. He begins his article with a story of an injured air gunner.

An air gunner was admitted to a civilian orthopaedic hospital in November 1940, for the treatment of a torn and displaced semilunar cartilage. In August 1941, no less than ten months after admission, he was still in hospital and still totally incapacitated. Why was recovery so long delayed? What possible explanation could there be? The diagnosis had been correctly made and a skilful operation permormed. The wound had healed by first intention; there was no infection, arthritis, or surgical complication. Daily massage had been continued, but the muscles were still wasted and weak. Two manipulations had been performed under anaesthesia, but movement was only half of normal. The gait was slow and hesitant; he limped; he could not run — he had never tried to run. The medical officer blamed him because 'he would not co-operate,' because he was disinterested, depressed, and

resentful. He was certainly depressed, for after ten months the incapacity was more complete than on the day of admission. He was disinterested because, in his own words, 'nobody takes any notice, and it looks os if it is hopeless.' He was resentful because he could not believe that the fault was his. Had he not been told that 'the nerve to his knee was cut'?

He was transferred to one of the orthopaedic rehabilitation centres of the R. A. F. Medical Service. He saw the sky, the sea, the open spaces. For many months he had seen only the stone walls of hospital wards, the stone walls of massage rooms, the stone walls of many corridors. In his new surroundings there was a lounge and writing-room; there were tasteful decorations and flowers, a varied menu, and an atmosphere of well-being and contentment. After a few days he smiled. There was sometimes a sparkle in his eye. He sensed a spirit of optimism and was reassured. His difficulties were explained, and he was taught special exercises. He learned to walk and then to run. He became an enthusiast and worked in the gymnasium, played on the fields, swam in the pool, cycled on the track. In the evenings he attended lectures and concerts, or played billiardds and table-tennis. Time raced past, for he was busy. He became bronzed and fit. He laughed and was full of the joy of life. In seven weeks he returned to his unit and to full duty. The 'nerve in his knee' was forgotten.

Ten months — total incapacity; seven weeks — full recovery: that is the story of rehabilitation in one air gunner. But is this an isolated case from which no conclusion should be drawn?

We must face the fact that our air forces will bear the sting of heavy casualty; convalescent homes for study and rehabilitation of air-force personnel must be developed on a national scale, with a well-planned program for analysis of injuries peculiar to modern air combat, as well as facilities to meet the needs — physical and spiritual — of rehabilitation.

Watson-Jones's recommendations concerning the injuried man in the air service are essentially conventional, at least conventional to our nonmilitary eyes in this country. But many military hospitals cannot study their cases from a scientific standpoint, and there may be many that would do for ten months what was done for the air gunner of Watson-Jones's report. It is highly important that the injured men from Pearl Harbor, Bataan, Corregidor, Cebu, Panay, Australia, Singapore, Java, India, Africa, the Mediterranean and the North Atlantic sea lanes be given a sense of the importance of the contribution they have rendered, be given a sense of the part that they may still be able to contribute if put back into active service. I am not speaking as a psychiatrist, or even as a practical surgeon, but essentially as a layman. I have, however, had opportunity to survey the literature and have een hospitals filled with sicke men, seriously injured men, of the fighting forces of Britain; I cannot too vigorously emphasize the value of maintaining the morale of the injured man, of allowing him to take part in the care of others more seriously incapacitated than himself, and of giving him opportunity to discuss combat problems with those who have been placed before their injury in military situations similar to his own.

* * *

Expansion of the air corps of both the United States Navy and Army has created an unprecedented need for medical personnel. The Navy within the year expects to complete training of more than 1000 air medical officers including several hundred flight surgeon, and Colonel David Grant. Air Surgeon of the Army, authorizes me to say that the Army Air Forces have now in service some 2300 medical officers and that an expansion is expected within the year to bring a total of 19,000 flight surgeons and aviation medical officers. If this demand is filled, it would alone absorb all the graduates of Class A medical schools in the United States during the past three years.

This war is probably more challenging to the physician than any other conflict in the world's history. Those who serve, especially those who serve the air forces, must have special knowledge; they must be cognizant of this, cognizant also of the part that they can play in maintaining air supremacy, and of re-establihing the right of free men to live in peace.

REFERENCES

1. Osler, W. Tuberculous Pleurisy. Boston M. & S. J. 129:53-57, 81-85, 109-114, 134-138, 1893.

2. Cushing, H. Concerning diabetes insipidus and the polyurias of hypophysial origin. Boston M. & S. J. 168:901-910, 1913.

3. *Idem. The Western Reserve and Its Medical Traditions.* 33 pp. Cleveland: Privately Printed, 1924.

4. Cannon, W. B. The physiological factors concerned in surgical shock. Boston M. & S. J. 176:859-867, 1917.

5. Fulton, J. F. Physiology and high altitude flying: with particular reference to air embolism and the effects of acceleration. *Science* 45:207-212, 1942.

6. Hoff, E. C., and Fulton, J. F. *A. Bibliography of Aviation Medicine.* Prepared for the Committee on Aviation Medicine, National Research Council. Springfield, Illinois: Charles C. Thomas (in press).

7. Smith, W. A. *A World List of Scientific Periodicals Published in the Years* 1900-1933. Second edition. 779 pp. London: Oxford University Press, 1934.

8. Van Liere, E. J. *Anoxias Its effect on the body.* 269 pp. Chicago: University of Chicago Press, 1942.

9. Cannon, W. B. *The Wisdom of the body.* Second edition. 333 pp. New York: W. W. Norton & Co., 1939.

10. Gellhorn, E. Fundamental principles in the adjustment reactions of the organism to anoxia. Ann. Int. Med. 14:1518-1532, 1941.

11. Gellhorn, E., and Lambert, E. H. The vasomotor system in anoxia and asphyxia. *Illinois M. & Dent. Monogr.* 2(3):1-71, 1939.

12. Ferry, G. Les signes prémonitoires de l'asthénie des aviateurs. *Compt. rend. Soc. de biol.* 82:637, 1919.

13. Josué, O. L'asthénie des aviateurs. *Comp. rend. Soc. de biol.* 82:641-643, 1919.

14. Armstrong, H. G., and Heim, J. W. The effect of repeated daily exposures to anoxemia. *J. Aviation Med.* 9:92-96, 1938.

15. Armstrong, H. G. *Principles and Practice of Aviation Medicine.* 496 pp. Baltimore: Williams & Wilkins Co., 1939.

16. Sundstroem, E. S. Studies on adaptation of man to high altitudes. *Univ. California Publ., Physiol.* 5:121-132, 1919.

17. *Idem.* The physiological effects of tropical climate. *Physiol. Rev.* 7:320-362, 1927.

18. *Idem. Adrenal Cortex in Adaptation to Altitude, Climate and Cancer.* Berkeley: University of California Press (in press).

19. Giragossintz, G., and Sundstroem, E. S. Cortico-adrenal insufficiency in rats under reduced pressure. *Proc. Soc. Exper. Biol. & Med.* 36:432-434, 1937.

20. Langley, L. L. *The Role of the Adrenal Cortex in the Reaction to Low Atmospheric Pressure.* A dissertation presented to the Faculty of the Graduate School, Yale University, in candidacy for the degree of Doctor of Philosophy, 1942.

21. Langley, L. L., and Clarke, R. W. The role of the adrenal cortex in the reactions to low atmospheric pressure. *Yale J. Biol. & Med.* 14:529-546, 1942.

22. Evans, G. The effect of low atmospheric pressure on the glycogen content of the rat. *Am. J. Physiol.* 110:273-277, 1934.

23. *Idem.* The adrenal cortex and endogenous carbohydrate formation. *Am. J. Physiol.* 114: 297-308, 1936.

24. Lewis, R. A., Thorn, G. W., Koepf, G. F., and Dorrance, S. S. The role of the adrenal cortex in acute anoxia. *J. Clin. Investigation* 21:33-46, 1942.

25. Thorn, G. W., Jones, B. J., Lewis, R. A., Koepf, G. F., and Mitchell, E. R. The role of the adrenal cortex in anoxia. The effect of repeated daily exposures to reduced oxygen pressure. I. Experiments on rats. *Am. J. Physiol.* (in press).

26. Jones, B. F., Thorn, G. W., Lewis, R. A. and Kennedy, T. J., Jr. The role of the adrenal cortex in anoxia. The effect of repeated daily exposures to reduced oxygen pressure. II. Experiments on rabbits. *Am. J. Physiol.* (in press).

27. Thorn, G. W., Jones, B. F., Lewis, R. A., and Eisenberg, H. The role of the adrenal cortex in anoxia. The effect of repeated daily exposures to reduced oxygen pressure. III. Experiments on dogs. *Am. J. Physiol.* (in press).

28. Collip, J. B., Anderson, E. M., and Thomson, D. L. Adrenotropic hormone of anterior pituitary lobe. *Lancet* 2:347, 1933.

29. Uotila, U. U. The regulation of thyrotropic function by thyroxin after pituitary stalk section. *Endocrinology* 26:129-135, 1940.

30. Idem. Hypothalamic control of anterior pituitary. *A research Nerv. & Ment. Dis., Proc.* (1939) 20:580-588, 1940.

31. Catchpole, H. R. Personal communication.

32. De Haven, H. Miraculous safety. *Air Facts* 4:21-26, 1941.

33. *Idem.* Mechanical analysis of survival in falls from heights of fifty to one hundred and fifty feet. *War Medicine* (in press).

34. Committee on Aircraft Accidents. *Aircraft Accidents Method of Analysis.* United States National Advisory Committee for Aeronautics Report Nº 576. 10 pp. Washington: Government Printing Office, 1941.

35. Denny-Brown, D., and Russell, W. R. Experimental cerebral concussion. *Brain* 64:93-164 1941.

36. Cairns, H. Head injuries in motor-cyclists: the importance of the crash helmet. *Brit. M. J.* 2:465-471, 1941.

37. Bradford, F. K., and Spurling, R. G. *The Intervertebral Disc with Special Reference to Rupture of the Annulus Fibrosus with Herniation of the Nucleus Pulposus.* 158 pp. Springfield, Illinois: Charles C Thomas, 1941.

38. Grow, M. C., and Armstrong, H. G. *Fit to Fly: A medical handbook for fliers.* 387 pp. New York: D. Appleton-Century Co., 1941.

39. Love, J. G., and Walsh, M. N. Intraspinal protrusion of intervertebral disks. *Arch. Surg.* 40:454-484, 1940.

40. Symonds, C. P. Sciatic pain. *Lancet* 1:186, 1942.

41. Spurling, R. G. Personal communication.

42. Watson-Jones, R. Rehabilitation in the Royal Air Force. *Brit. M. J.* 1:403-407, 1942.

HISTORY OF MEDICINE

Aeromedical Research at Yale

Effects of High Altitudes Studied in Decompression Chamber

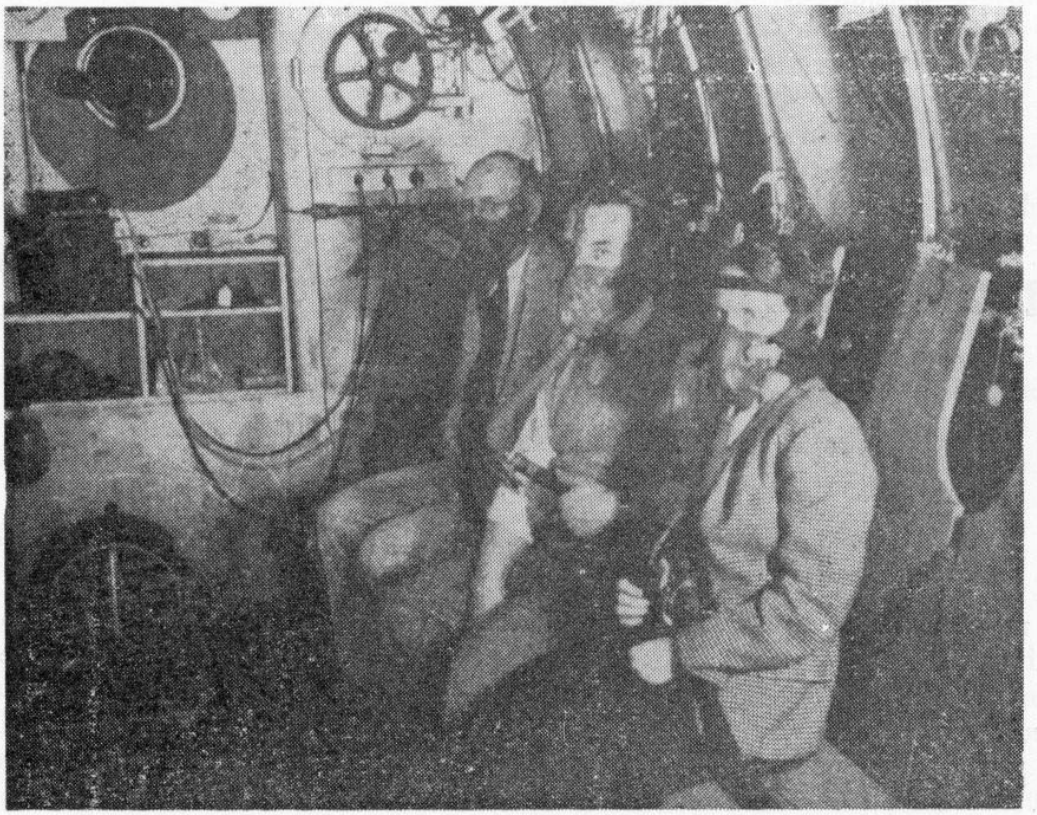
JOHN F. FULTON and **LESLIE F. NIMS**

Sterling Professor and Associate Professor of Physiology.

The rapid development of commercial and military aviation has focused attention upon the many physiological problems encountered by men who fly. Unaided man cannot function effectively at altitudes above 10,000 feet; indeed he mannot even survive unassisted at the altitudes at which some aircraft now routinely fly. The success of our air forces in World War II was in large part due to the successful development of accessory equipment; for example, oxygen supply systems and heated clothing, based upon sound physiological principles, have enabled men to withstand the rigors of the stratophere. Much of this development unfortunately took place under the stress of the war years. In 1939 our country was gravely unprepared for the war emergency and it was only by the intense effort of many men both in the services and out, that partial solutions to the biological problems of high altitude flight were reached. It must be recognized that despite the success already achieved, many of the problems are only incompletely solved and much more study, both fundamental and practical, is required before man will completely conquer the stratosphere and before he can reap in full the benefits of the age of flight.

In 1940, requests for information on medical problems began to come to the National Research Council from the military services. It was early realized that to answer some of these requests, research work by qualified civilian investigators would be essential. On June 30, 1941, the Office of Scientific Research and Development (OSRD) was created, which not only coordinated the activities of the civilian scientists in problems relating to the war effort, but also financed much of the civilian scientific work in these fields. Through its two divisions, the National Defense Research Council (NDRC) and the Committee on Medical Reearch (CMR), the OSRD began to function effectively by September 1941, and in early October, 1941, the Committee on Aviation Medicine (CAM) endorsed a proposal from the Department of Physiology of the Yale University School of Medicine for the construction of a refrigerated low pressure chamber for human beings. In late November the proposal was acted upon favorably by the CMR and the day before the attack on Pearl Harbor the University signed a contract with the York Ice Machinery Company for the construction of the decompression chamber.

The plans for the decompression chamber, the principal item of equipment for the Yale Aeromedical Research Unit, had been drawn in detail during the previous summer and were based in part upon a survey of the engineering features of existing installations conducted by Lt. Col. Pharo Gagge formerly of the John B. Pierce Laboratories of New Haven, at the request of the CAM. A special design for rapid cooling which had been proposed by the engineers of York Ice Machinery Company was incorporated. The Yale chamber

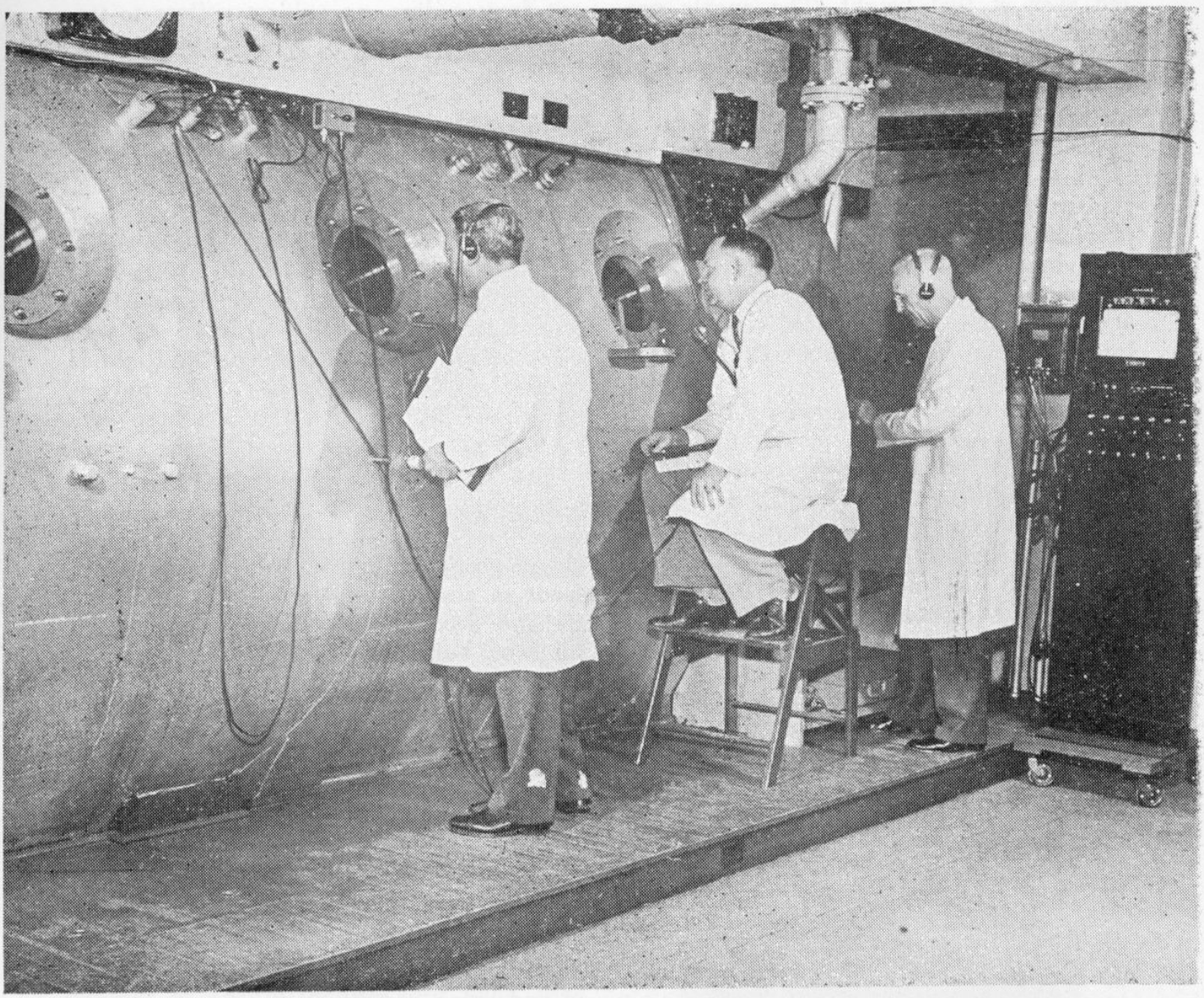


The interior of the decompression chamber. Eight subjects can be taken to altitude at a time.

was the first to include the rapid cooling feature and it served as a prototype for the many chambers subsequently constructed for the military services and the aircraft companies. In the Yale chamber, it is possible to reach a simulated altitude of 40,000 feet and simultaneously to drop the air temperature from plus 70°F. to minus 70°F. within four minutes, rates of change of pressure and temperature much more rapid than would be encountered in the present combat aircraft. Six months were required to fabricate the chamber at York, Pennsylvania, and after delivery, a further three months were required to install it. Formal operations began in November, 1942, and during the period up to the first of January, 1946, when the contract terminated, the Yale Aeromedical Research has been supported by the OSRD through repeated renewals of the original contract recommended by the CAM. Space to house the Aeromedical Research Unit was made available in the laboratories of the Physiology Department and the Unit consists of the decompression chamber and its accessory equipment, a small, well equipped machine shop, a biochemical laboratory, and a physiology laboratory. During the war, the investigations were of necessity carried on under the security regulations but many of the reports have been released for publication and will eventually appear in the various scientific journals.

Biological Problems

In aviation the body must make continouos adjustments to rapid changes in the environment and it is a study of mechanisms of adjustment for which the physiologist is well qualified. The duty of the avia tion physiologist is twofold. He must learn how the comepnsatory mechanisms of the body work to bring about the necessary adjustments in order to aid these mechanisms by suitable external apparatus. He must also set the limits of the stresses to which a human body, aided or unaided, can be safely exposed. The stresses encountered by the aviator in normal flight are esentially of four types. The first type of stress results from marked variations in barometric pressure. The pressure fluctua-



The exterior of the decompression chamber. A staff of five is required to operate the chamber with subjects inside.
Professors Fulton, Nims and Miles studying the effect of high altitude on aviators.

tions can affect the ears, the sinuses,—in fact, any of the gas containing cavities of the body. A reduction in external pressure allows the expansion of gases in these cavities. For example, the intestinal gases may expand more than sixfold in going from sea level to an altitude of 40,000 feet. At altitudes above 25,000 feet, a second consequence of the reduced pressures may develop. The tissues of the body are saturated with gases much like a bottle of charged water and when the pressure around the body is greatly reduced, these gases are released from solution and form bubbles in the tissues and blood vessels giving rise to a group of symptoms which have become well known as characteristic of decompression sickness. Fortunately, the aviator,unlike the deep sea diver or the caisson worker, has a simple maneuver to relieve him of these symptoms. By decreasing his altitude he increase the pressure and the painful manifestations rapidly disappear.

A second stress is the fall in the oxygen partial pressure of the air as altitude is gained. Without oxygen equipment, no one can fly successfully for long above 15,000 feet since performance is seriously impai-

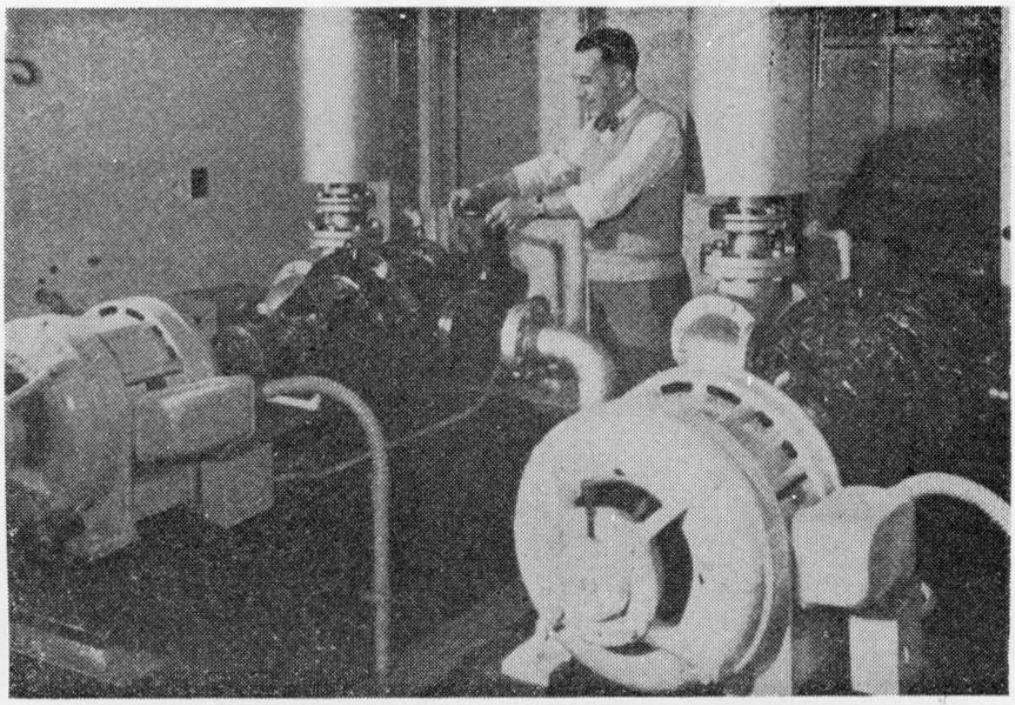
red even at that height. The anoxia produced in unprotected men at altitudes above 20,000 feet can rapidly cause unconsciousness and death. Many of the unexplained accidents in aircraft have in all probability been due to men trying to fly at altitudes where their performance is seriously inadequate because of anoxia. The problems of anoxia are peculiarly difficult involving as they do adjustments in all of the physiological mechanisms of the body, and much more investigation is needed before all of the effects of anoxia are thoroughly understood.

A third stress encountered by high altitude flyers is the extreme cold. At altitudes of 10,000 feet, the temperatures commonly range from 0° to minus 10°F. at 40,000 feet the temperatures may be as low as minus 70°F. Aviators must, therefore, be protected from the cold by special heating devices, and at extreme altitudes special clothing is required to protect flying personnel.

A fourth stress arises from the high speeds and maneuverability of the aircraft particularly fighter planes and jet propelled craft of all types. A sharp turn or pull out from a dive can subject a flyer to tremendous acceleratory forces, far greater than the compensatory mechanisms of the body can successfully combat. With acceleratory forces greater than four times that of gravity, the heart cannot pump sufficient blood to supply the tissues with oxygen. The central nervous system suffers first and loss of vision, followed by unconsciousness, develops rapidly. With the advent of jet propulsion and the greater speeds of aircraft, the acceleration problem as well as other physiological problems of flight is much intensified, and much more study is needed to insure the safety of those who man all types of high speed planes.

A subjeet equipped for altitudes of 35,000 feet or more and temperatures as low as minus 70°F. receiving a final check from Miss Eleanor M. Eddy, fomerly of the Navy Altitude Training Unit at Cherry Point, North Carolina.

The New Haven group was given the task of studying the effects of reduced pressures and anoxia on man and animals. The major portion of the time was therefore spent in studying the effects of mild anoxia and decompression sickness on human subjects. Volunteers from the medical classes, the Divinity School, and the high schools served as subjects for the majority of experiments. To carry out these experiments with the maximum of safety, the personnel who conducted the chamber runs were thoroughly trained in decompression chamber operation both in the Yale unit and at other centers, military and civilian. All prospective subjects were given a thorough physical examination, including electrocardiograms, electroencephalograms, blood and urine examinations before being accepted. While the actual procedures in the chamber were eminently safe with a well trained crew, all precautions were taken to insure that the subjects would not be injured in any way by their participation in the experiments. Three main conclusions resulted from the work on decompression sickness. All persons exposed to high altitudes for sufficient length of time develop signs of decompression sickness, but the young, physically well trained subject is much more resistant than an older person in poor physical condition. The variation in susceptibility is such that selection procedures are useful in picking a group of men who can be counted upon to perform creditably at high altitudes. However, the average susceptibility of all men is such



The vacuum pumps, which reduce the pressure in the decompression chamber, being started by Mr. Jack Marshall, Engineer for the Unit.

that in addition to selection, a protective procedure is necessary in order to insure the greatest freedom from the manifestations of decompression sickness at altitude.

The cause of the trouble arises from the tissue nitrogen, and since nitrogen comprises about four-fifths of the air we breathe, it is possible to rid the body of nitrogen by breathing pure oxygen for a variable period of time before ascent. This is not a perfect solution of the difficulties, for such methods are time-consuming since nitrogen is washed out of the body relatively slowly. However, by combining a selection and a protection procedure, it is possible to obtain a group of men who have relatively little difficulty in withstanding the effects of ascent to 38,000 feet for practically useful periods of time.

Many studies were made if the effects of anoxia. One of the more important conclusions of the work on this phase of aviation physiology was the objective demonstration of a thesis propounded with great emphasis by the late Professor Yandell Henderson. The effects of mild anoxic-anoxia are in large part due to a disturbed carbon dioxide equilibrium in the body. If this disturbance in the carbon dioxide content of the blood is prevented, as can easily be done by suitable means, many of the consequences of mild anoxia can be ameliorated. Studies have been made of blood sugar, lactic acid and phosphate, liver glycogen, adrenal cholesterol ester, urinary excretion, the electroencephalogram and the physiological performance of animals and men exposed to anoxia. In all instances, with mild anoxia, the addition of carbon dioxide to the breathing mixture would completely or partially prevent the effects thought to be due to a reduced pressure of oxygen. Certain aspects of this work were of such fundamental interest, that plans have been made to continue the anoxia studies in a more thorough and basic level. Particularly is it important to clarify more completely the interrelations between the two respiratory gases, oxygen and carbon dioxide, in the general economy of the body as a whole.

One of the difficulties in carrying on particular experiments contemplated by the research group was the obtaining of suitable subjects for these experiments. At the request of the United Aircraft Company, the group decided to indoctrinate the flying personnel of the Pratt and Whitney, the Chance Vought, and Hamilton Propeller

Companies. In return for this indoctrinationin the use of high altitude equipment and the practices and procedures of high altitude flight, the men were used as subjects in whatever series of experiments the Unit was conducting. The indoctrination procedure was extremely successful, for it not only furnished the Unit with a group of several hundred men who were well motivated and had an intelligent interest in the experiments, but it also provided a means of direct and valid comparison between the conditions in the chamber and those obtained in actual flight. Much of the success of the experiments was the result of the enthusiastic cooperation of this group of men.

The future of the Unit is at present somewhat uncertain. Efforts are being made to continue fundamental investigations on the effects of anoxia on man and animals. This field of investigation, while of immediate importance to the well being of those who fly, is of fundamental significance to many problems in the physiology of man and the practice of medicine. The country is entering upon an air-minded age and it is hoped that the Yale Unit can contribute in a substantial manner to the physiological knowledge of man so much needed to make aircraft flight the safe· comfortable means of transportation it is destined to become.

NOTE: The work described in this report was done under a contract recommended by the Committee of Medical Research between the Office of Scientific Research and Development and Yale University 1946.


Martinique
Fishermen

On Vacation...

Martinique

Time is conquered by speed that reduces it as the distance shrinks. So a few hours after we left New York we were arriving at the "Martinique". One of the lesser Antillas Islands which go from Puerto Rico to Trinidad. They form what is known as "The Caribean Arc" because they form half a circle and in the most prominent part of the arc is La Martinique, half way from Puerto Rico and Trinidad.

A taxi took us from the Airport to the Hotel. The road was winding and going up and down all the time amid beautiful landscapes with all the shades of greens splashed by bright flowers. There were lakes like pools of light reflecting the sky.

Emerging and fading mountains were seen at every turn of the road, some of them were going into the sea, others emerged from it surrounded by water. We had rented a bungalow right on the beach. Behind us the horizon was closed by mountains. In front of us the sea was voluptuously swaying to and fro its silky waters. Then standing in front of it I felt foreign to that environment. I still had all my being perfused with the wild rhythm of New York. My blood was still throbbing at the fast pulse of the big City. I was still a piece of that environment of very high mountain of steel glass and concrete created by man of the mad race of quivering subways, compelling everyone to run... run... run... Faster... Higher... Higher... Faster... Faster... Faster...

The placid atmosphere didn't permeate myself yet, I felt uncomfortable like a gear out of place.

It took some time to wash that sensation away. But it was serenely dissolved in that world where the rhythm of Nature prevails with a quieter but more intense life.

As soon as we arrived at our temporary shelter, we were trying to make of the bungalow a provisional home. Hanging clothes; exploring where the key to every bulb of light was, and placing on the desk some books to be read, gave us a precarious sense of stability so needed in a fast moving trip.

We were at this task when there reached us the sound of music and the voices of people speaking aloud. Soon we realized that the big covered space we passed on going to our bungalow was a movie house, and that raised our respect for the place. "So, they also have a movie house", I thought.

The dialogue sounded tense. A harsh voice was shouting, another tremolous reflected fear. A cornered life I guessed. After several minutes of moun-

ting tension it climaxed with three Bang!... Bang!... Bang!... And I could hear the steps of a person running down the stairs.

Afterwards a shrill cry informed us that the crime had been discovered. We heard distinctly the police cars siren with a cry of a wounded beast, and the sound of wildly running wheels, whizzing in every curve. A musical background was effectively chosen increasing a sense of danger, fright, dispair and anguish involved in the persecution of man chasing man.

Something happened that brought the wheels to a halt. Afterwards we heard shouting voices and the rattle of a machine gun, which sounded as if Death was macabrely dancing with castagnets.

A soft soothing music informed us that on the silver screen was played a love scene and that the story was brought to a happy end.

We didn't mind that time to hear an unseen movie. But soon we realized that this was the only movie they had, and it was played over and over again everytime a new customer arrived to impress him. So we knew when we had new neighbors at the bungalows, because we were going again through the dramatic argument the Bang...! Bang...! Bang...! the car races, etc.

In our bungalow there was no hot water or air conditioning but it was new and clean, I say with certain reluctance that it was clean, because everyone who does not like a place immediately says "But it is clean" and we liked our little bungalow. We had dinner and lunch at an arbour in front of the sea.

We were never told what we were going to eat but we always liked it.

There are in the Martinique beautiful big hotels, with air conditioning and swimming-pools. All the comfort you can find in United States. But, who cares to travel far away to feel always the same sensations? Some people carry their comfort like a protective shell and will feel uncomfortable naked without it. We came here, decided "to feel" the tropics and My God! We were not deceived. If we wanted to have a steam bath, we only had to shut the windows and doors and we were melting like a Christmas candle lit the whole night.

But if we opened the doors we were immediately relieved by the trade winds (vents alizés) which blow the whole year continuously East-North-East from the Azores toward the Equator. They are produced by the rotation of the Earth and the movement of the Air toward the equatorial regions in the course of its circulation between the warmer and the colder portions of the Earth. We enjoyed Nature's air-conditioning especially on this side of the tropics where it is so needed and it is working all the time.

One feels wonderfully caressed by its freshness.

But we were not so happy about the sudden storms that took us unaware when we were enjoying our getting acquainted with the Island. But this are the tropics. Sometimes even storms may look wonderful.

And that was the case when sitting in front of our bungalow, with the mountains at our back and the sea at our feet, we felt something like the drumming of a far distant assembling of players. At their calling, blackened clouds began to gather covering the bluishness of the sky.

The drumming grew louder, lightnings shed an intermittent sinister glow, zigzagging like a scintillating spade, a thunderbolt wounded the firmament which groaned with pain.

Sheets of rain fell over the mountains and the little houses on their slopes, over the trees and the sea.

Everything blurred under the aqueous curtains; it seems a wet water-color with its colors running and the palm trees had their tops madly streched by the wind.

Slowly the rain was getting rid of the shadows, the atmosphere got clearer and clearer, until the sun shone again, and everything looked nicer than before.

The trees covered with shining drops of water looked greener.

The flowers with more intense hues. And I felt part of that nature, happier, cleaner, alive.

Elation ran through my veins, the air carried mingled the smell of the salt sea and a concoction of perfumes from the humid vegetation. Newly washed, some clouds immaculately white were hanging from the sky drying to the sun.

From the green depth of the treès, like the organ of a chapel, the tiny pipes of exalted birds elevated a Hymn to Nature.

Courting a glittering flower, a brightly colored humming bird stood streamlined in the air nimble by the vibrating halo of its wings.

FORT DE FRANCE

The Capital city of La Martinique is on the other side of the bay just in front of Trois Illets. A small boat takes us there every day. A crossing of more or less twenty minutes which is in itself very rewarding. You get a wonderful view from the sea of the Island, in which the roofs palm trees and the colorful flowers rioting the mountains seem to dance from the goings up and down of the boat.

What a gift for a painter's eyes!

No wonder Gauguin, sick and tired of the artificial life of big cities, left Paris to come to La Martinique. Even though he needed to work as a digger in the Panama Canal to pay his way to that lovely Island. "We are going to dig the Canal —he said— and in a couple of months we will have enough to get to Martinique, and live on for a long time".

And La Martinique didn't deceive him. A paradise compared with Panama. Everything there appealed to his sensibility. The exultant freshness of the nature, the beautiful women dressed in flamboyant colors. The mountains covered with green hues and crowned with misty clouds, the palm trees and the banana plantations serve as a suitable background to the natives so naturally integrated with it.

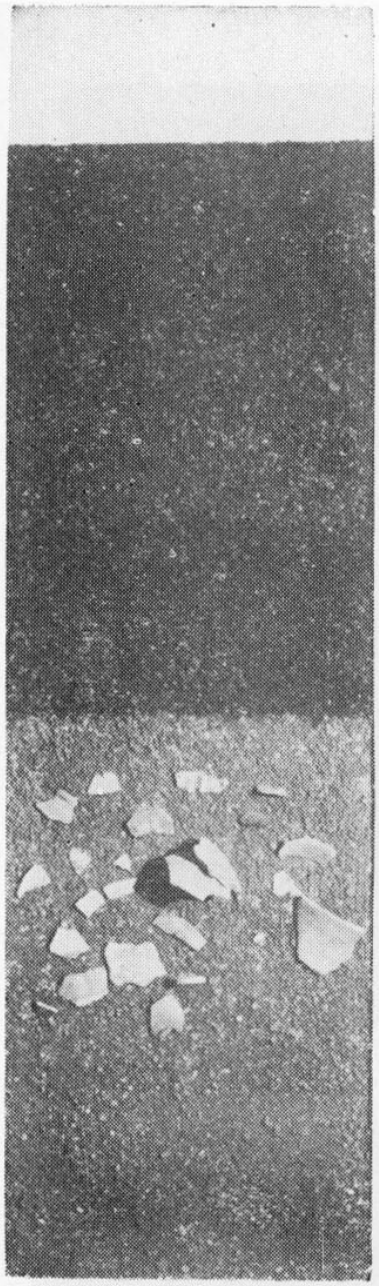
On his return to Paris, Degas bought some paintings by Gauguin among them a landscape of La Martinique.

Arriving at Fort de France we were immediately attracted by groups of people selling the most beautiful shells and handicrafts in the Island.

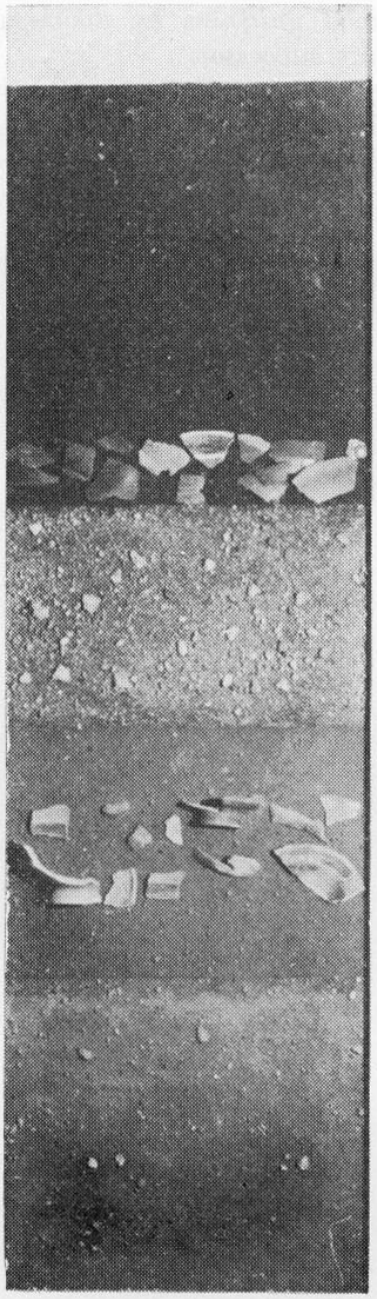
In the middle of the square that is just in front of the Port was an imposing monument erected to Josephine by her grandson Napoleon III. Nearby there is a very interesting Museum that records the life of the inhabitants of the Island from prehistoric times to the present day.

Going through it came to my mind the biblical words. "Generations come and generations go and the Earth always remains".

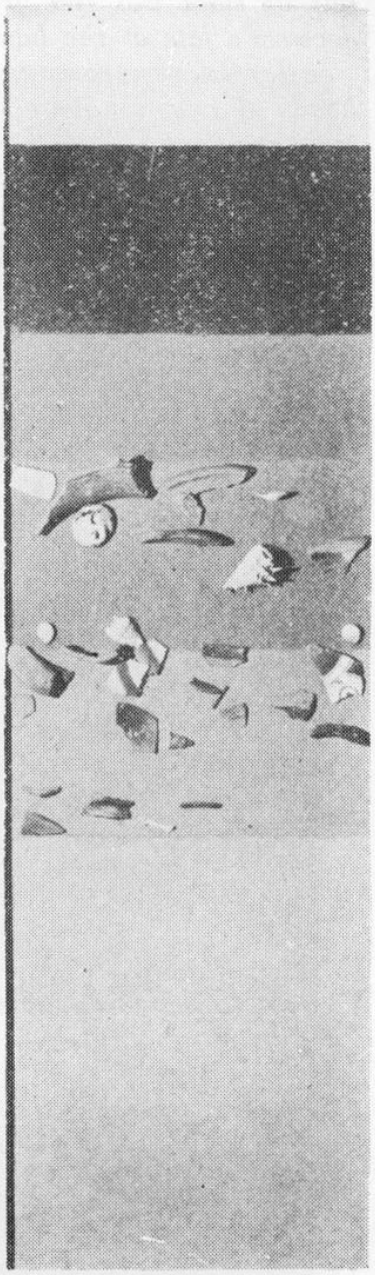
Fig. 1. — *POTTERY FROM THE 2x SECOND ARAWAK PERIOD (1660 to 1000 B.C.)*



a) *Cut from an excavation's quarry. First Arawak Period demonstrates that the inhabitants left the place after a volcanic eruption.*



b) *..Cut of a quarry sited in the North East of the Island, representing the two Arawak Periods separated by volcanic soil.*



c) *..Cut of an excavation quarry Arawak Implantation (Second Period) followed by a Caraibe implantation.*

There, in a few broken pieces of pottery was the Indian Arawak, speaking a voiceless language from the remote past, peaceful, industrious, humble, leading a quiet life. Then came the Indian Caraibes who from the coast of the Orinoco invaded the Caribean Island. Warriors, ferocious, cannibals, they easily defeated and were merciless with the defenseless Arawaks and La Martinique became their possession. But sooner or later they would pay for their crime, because in History no one is free from also becoming the sufferers of the same pain they have inflicted. Remembering Arnold Toynbee when he related the ritual of the Golden Bow at Nemi the priest who slew the slayer and shall him-

self be slain was free from blood guiltness by dooming himself ti suffer predecessor's fate at the hand of his successor.

In the same way the Caribeans were going to suffer for their sins at the hands of new conquerors. And that happened when Pierre d'Estambuc, a Norman gentleman, took La Martinique in 1637 *and declared in the name of his Majesty, La Martinique a French possession.*

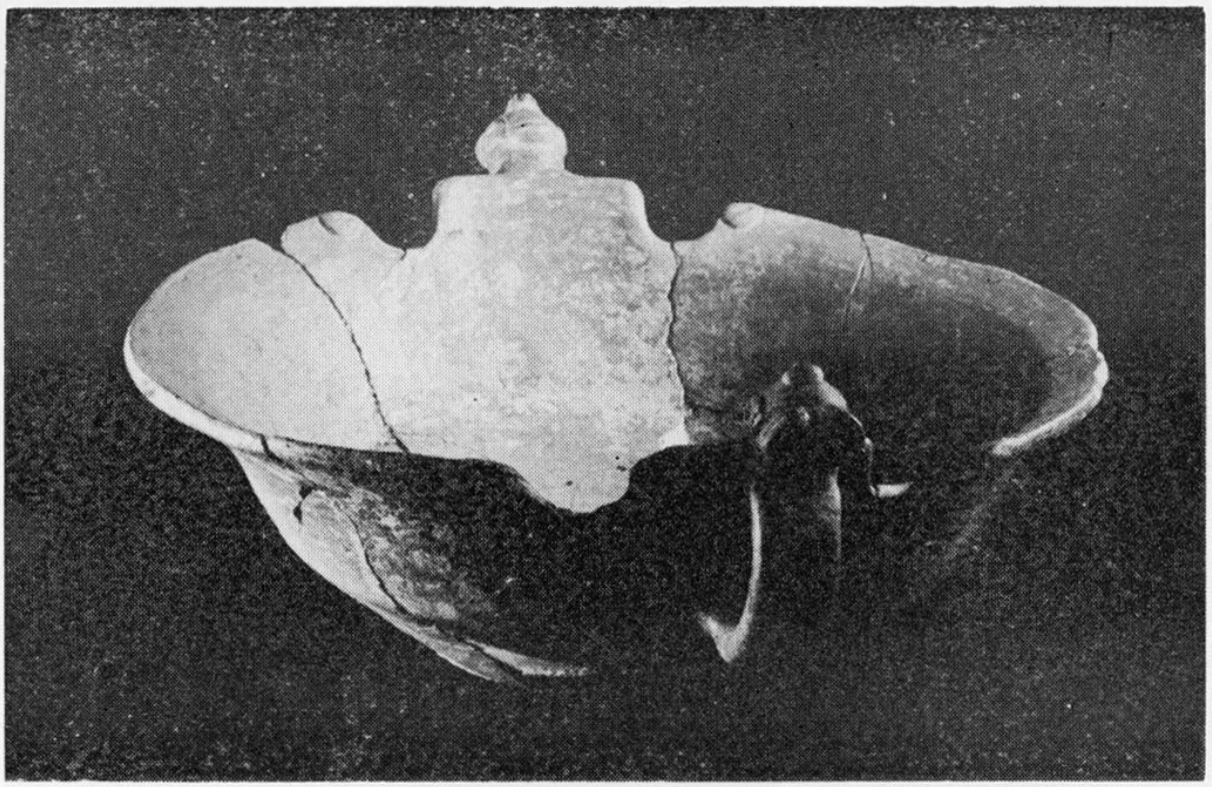


Fig. 3. — *Pottery from the first Arawak Period. 180-450 A.C.*

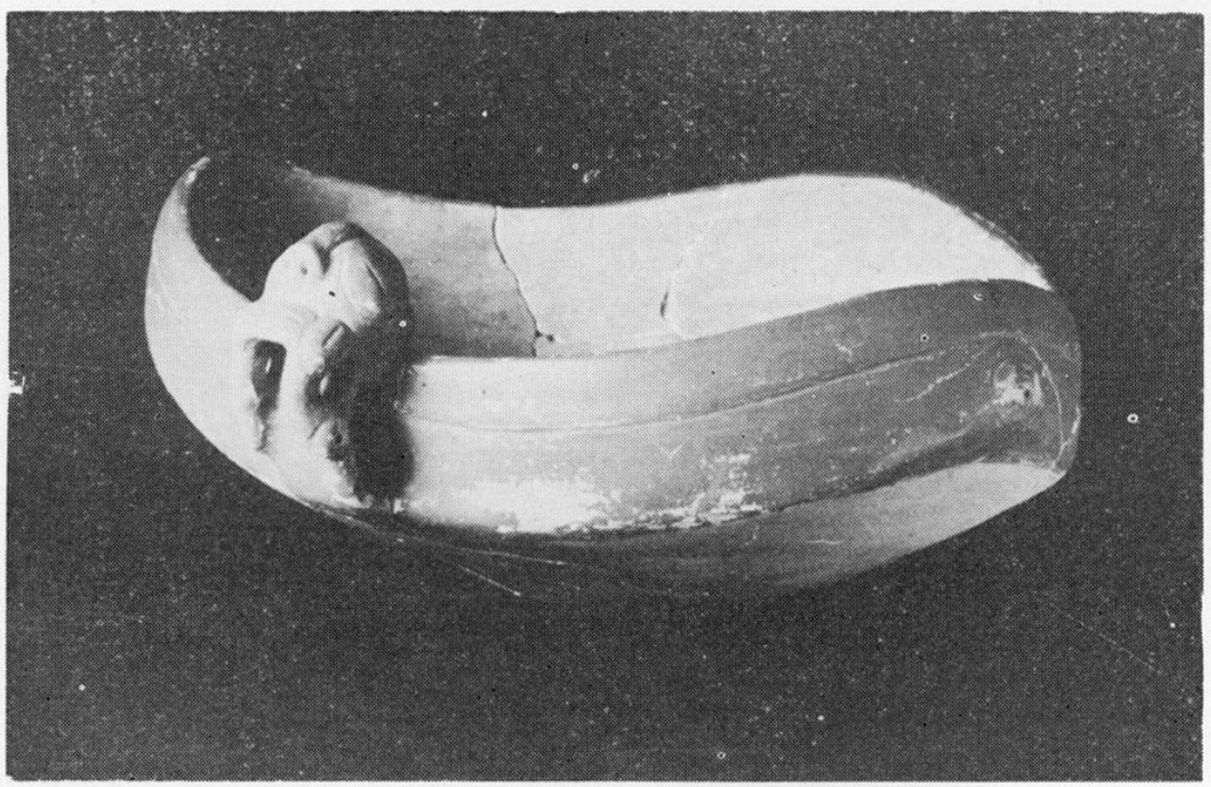


Fig. 4. — *Pottery form the first Arawak Period. 180 to 460 A.C.*

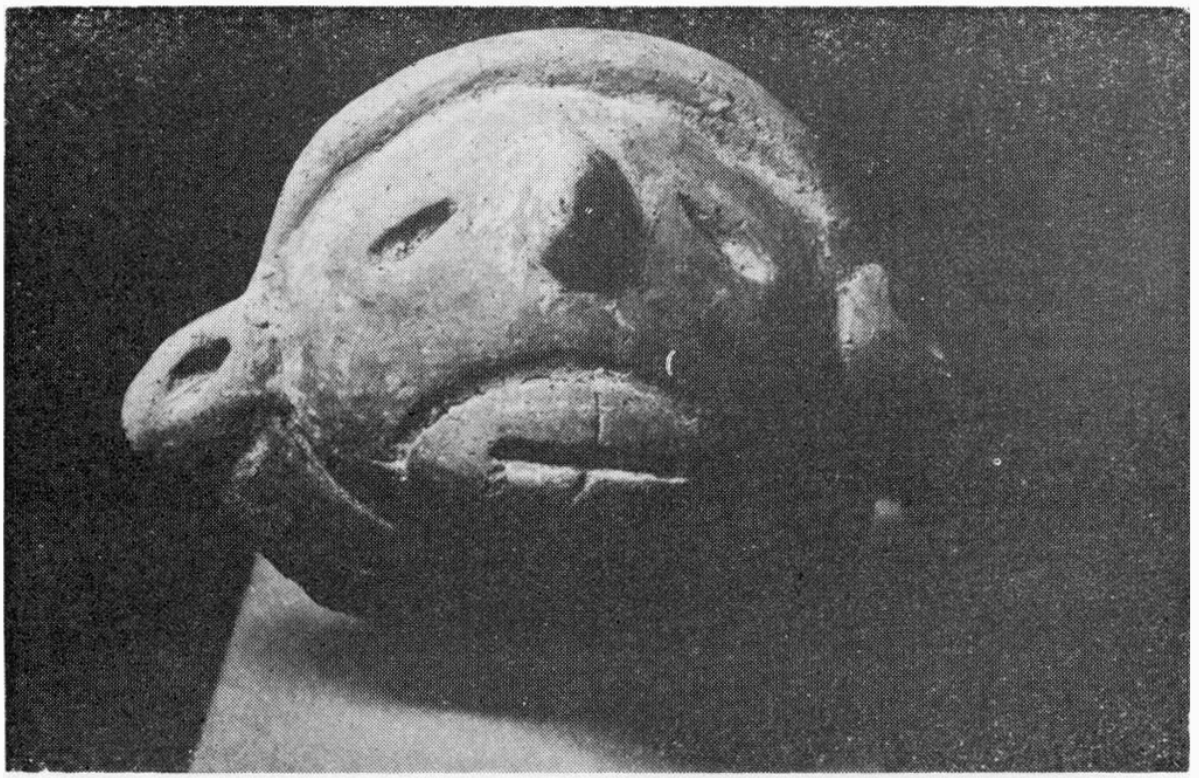


Fig. 5. — *Antropomorphic sculpture from the Second Arawak Period.*

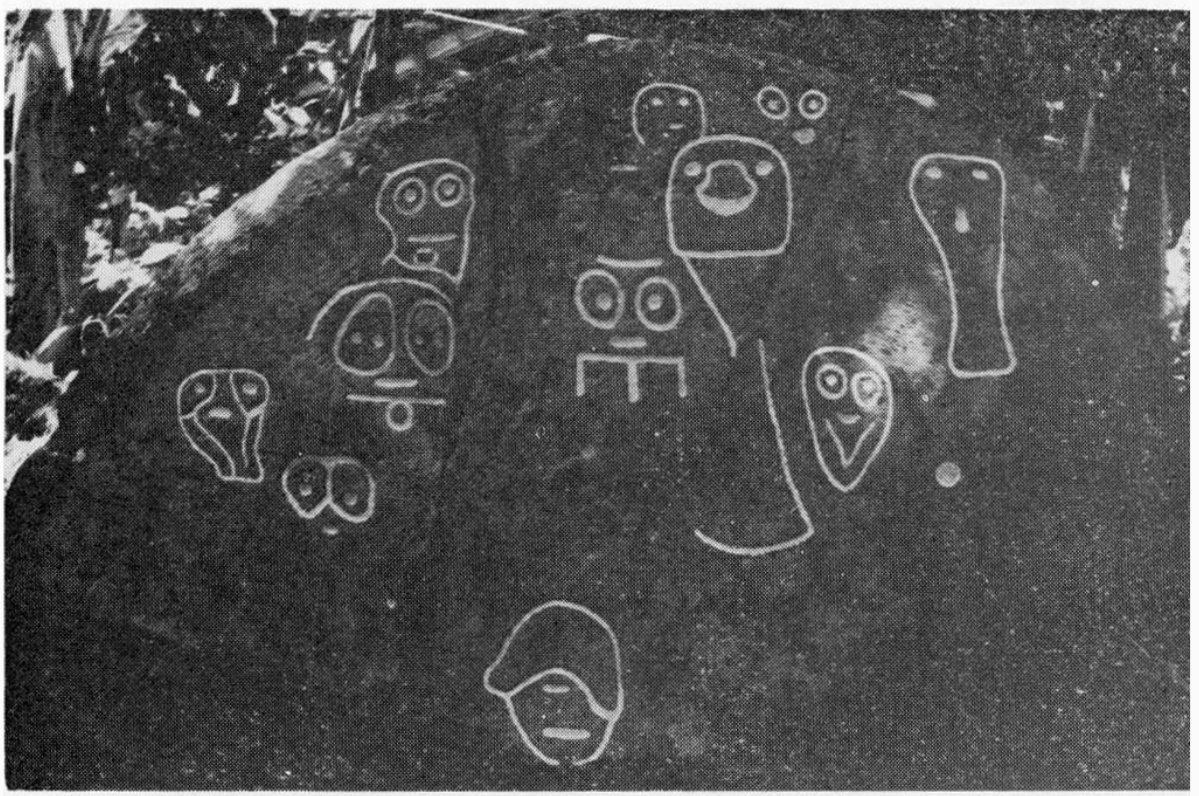


Fig. 6. — *Petroglyphs found in St. Luke (Montravail Forest) South Martinique.*

He founded the city of his Saint Patron, named it Saint Pierre, and built at Fort to defend himself from other European countries, eager to dispossess him and from the Caribean Indians against whom he fought so fiercely that they were expelled from the Island in 1658.

But there is a legend that says that the Caribeans, having lost all hope, went to a very high cliff, blinded themselves and threw themselves from it.

It is said that before going to the encounter of such terrible death, they cast a malediction on the French. "The Mountain of Fire will avenge us". The cliff is known as "The Caribean Tomb" until today.

As time passed by and the city prospered to the point that is was regarded as the most modern town in the Antilles West Indies, it flourished to such a degree that nobody even remembered the Caribean prophecy.

They had a fantastic Theater whose grandeur got to outshine others of its kind in Europe. La Martinique's Botanical Gardens supplied the Courts of Europe with exotic plants and flowers.

Then something happened that upset the gracious living of Saint Pierre.

More than a century after the last Caribean died, the Fire Mountain became restless and a violent carthquake was felt. Sulphorous emanations killed several wild animals But after that all regained the usual calm, nobody gave much attention to it. And that seemed to be justified since 500 *years slipped by until another carthquake indicated that the Fire Mountain was again awake, this time the noises were louder and frightened the menaced inhabitants of Saint-Pierre. From August to October the volcano kept its activity and shed ashes everywhere. The birds, due to the sulphur in the atmosphere, were choked to death. Everything looked muddy and dirty, a very disheartening view indeed.*

A scientific mission was sent to study the phenomena closely and to express their opinion about it. When they returned they gave a very scientific report of the Mont Pelé activity ending their findings with this curious word: "With fine weather the passengers aboard the ship arriving from France who can see this long wreath of white smoke rippling straight up into the sky must think that is a picturesque decoration added to the country, the final touch, the majesty of our old Mount Pelé lacked".

They seemed to be right, nothing harmful happened and half a century slipped by and Saint-Pierre was a wonderful city praised in the chronicles of travellers who were delighted at its sight.

So the people grew accustomed to the temperamental volcano without suspecting that in 1902 *the Mont Pelé tumultous activity would have a tragic end.*

So although the first warning was given in February with a strong sulphuric smell, they continued their routine live.

Toward the end of April, the things were turning worse and worse.

The sound of a great explosion was heard and noises muffled like rumbling thunder. The earth trembled.

The next day, it seemed that an eclipse had occurred; it was so dark. All of a sudden, with the sound of a cannon, the sky was set ablaze. Over the City fell a shower of ashes, and the situation became more critical.

But they seemed to be charmed. One was assuring the others that nothing would happen. They were just like the Caribeans blindly going to the encounter of their death.

Headcloth style informing that the wearer is a widow

A group of Folkloric Dancers dressed in colonial costumes

Besides, there were elections going on in the country, and the results were almost even for the parties. In order to obtain a majority that would give the victory, another ballot was going to take place. So both parties were fighting and influencing their adherents in order to obtain victory. That seemed to be also diabolically planned so that nobody would leave the place and suffer the Caribean malediction. The people were uneasy, the authorities of Saint-Pierre called Fort-de-France, telling the Governor about the situation. The answer was "not to encourage the people to leave the City".

Nobody can understand why they were so chained to that place that even in the newspaper appeared an announcement inviting people to go to Mont Pelé to take a close view of the phenomena ending with this paragraph:

"Weather permitting, the excursionists will spend a day which they will with pleasure remember for long".

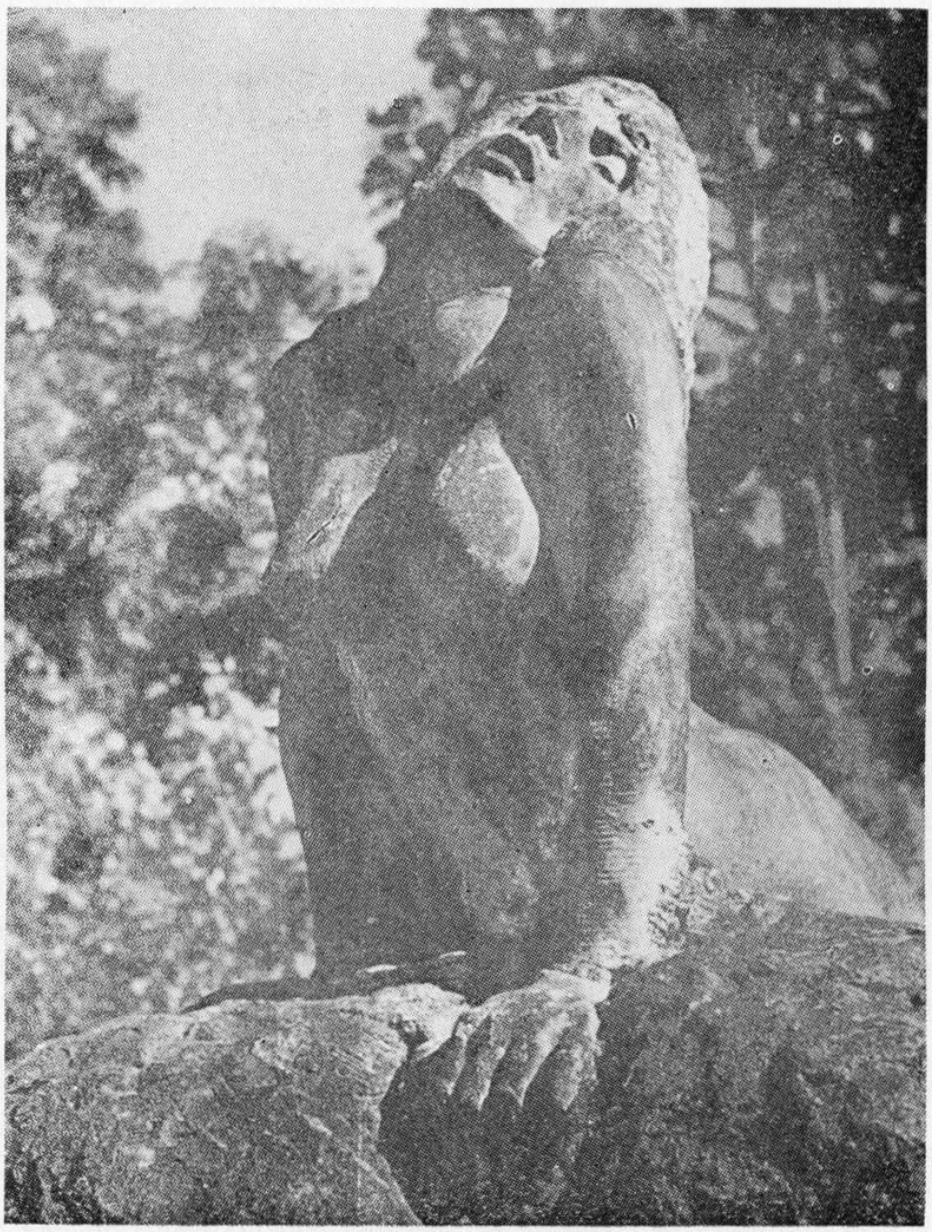


Fig. 7. — *Sculpture symbolizing St. Pierre rising from her ashes.*

But this nice prospect was annulled when the next day, at two o'clock, the volcano began vomiting flames and hurling stones miles away.

The schools were closed. It became difficult to breathe and everybody was

coughing. That was May 3 The bells were tolling and the population was ordered to throw water over the streets in order to get rid of the ashes.

There were no vegetables for eating and people got very much upset. As the days passed, the situation grew worse. The White River became a black torrent madly going down to the sea. And a mass of mud 18 feet high pushed the buildings of a big Company which were on its way to the sea, killing 23 people, the first victims of the Fire Mountain.

The eruption went on the next days. Nevertheless it is necessary to read the following notice on May 7 to realize how blind they were to the immediate danger.

"According to exterior signs the intensity of the eruption is decidedly declining. The height of the column of ashes which, last Sunday night, reached about 5.700 yards, only reached 2.600 yards this morning. The outflow of steaming mud in the valley of the white River no longer goes as far as the Sea. Many tourists have made for the crater".

A scientific mission sent to study the situation, headed by Lieutenant Colonel Gerbault, polytechnician and Artillery Director, with the participation of N.Mirville, a pharmacist-officer in the Colonial Army, M. Leonce a state civil engineer, M. Doze and M. Landes both teachers of Natural Sciences at Saint-Pierre, High-School.

In a telegram sent up, signed by Mr. Landes, is synthesized the judgement of several personalities who did not realize the danger hanging over Saint-Pierre.

"In my opinion, our Mont Pelé does not endanger the town of Saint-Pierre more than the Vesuvius endangers Naples".

By an irony of destiny he was, without knowing it telling the truth.

But then when the explosions began to be heard one after the other the people were panicked. The authorities of Saint-Pierre called the Governor at Fort-de-France telling him about the situation. The Governor said that he would go personally to pacify the people.

So the Governor and Colonel Gerbault with their wives and Mr. Husson, a private chancellor, arrived at Saint-Pierre on May 7 at four o'clock. Their presence brought some hope to the unhappy people and reassured them that nothing wrong would happen.

Only a few more sceptics ran away conscious of the imminent danger.

During the whole night, it seemed that the City was near the front line of a battlefield. Detonations were heard without pause. A column of dark smoke with gleaming flames were menacing the sky.

No one could sleep. A word began to run "This is an accursed place"; in their fright they were running madly from one place to the other, some to the church dressed in their best, others were feverishly trying to save their most precious possesions to be carried with them.

All this was in vain. The bell was Angelus tolling on that fatidic May 8, 1902 when suddenly a formidable explosion resounded and the "Fire Mountain" made true honour to its Caribean name, offering a Dantesque vision, blazing in flames. After two centuries and a half the Caribean prophesy:

"The Fire Mountain will avenge us" became true. It was 7.50 and life had stopped at Saint-Pierre.

Just after the tremendous blast a dark cloud crossed by lightnings emerged from the top of the Mountain and formed a colosal burning cloud, which with sinister glare, covered the mountain and fell over the City of Saint-Pierre, covered it and reached the sea in a terrible blazing whirl, shaking twisting, bur-

ning, destroying everything even some ships that were in the Port. The next day, Saint-Pierre the site of the "Creole" aristocracy, the mast famous city in the Caribean Islands, was just a big cementery for 30.000 people.

That was the end of that glorious city 200 years old, destroyed by a fiery furnace that twisted and melted everything.

In the middle of that desolation among 30.000 people dead, one man only was alive. After the danger passed many came back in despair to ee if any of their relatives were alive. But soon they realized that nobody could escape that tragic end. So when four days after the explosion three persons, coming down Victor Hugo Street were highly surprised and shocked when they heard a kind of low groan, they couldn't believe it. That sound came from behind the ruins of what had been the beautiful theater, the pride of Saint-Pierre.

Could anybody escape what would be compared to an atomic explosion?

In less than two minutes the fiery clouds, like a revengeful hand, came from the mountain to the sea with deafening fury destroying everything, shaking the boats and shedding death on the air, land and water.

The moanings they heard were coming from a prison that was behind the theater.

They came nearer walking over the still warm ashes. Twisted irons, and heaps of ruins. There, at the foot of Morne Arbel, inside a small cell, stonebuilt, where the light hardly showed the way through a little opening crossed by iron bars was a human being miraculously alive.

So, the only man that the Fire Mountain had spared was a convict, named Sykbaris, born in Le Precheur. He was charged and sentenced to prison for assault and battery and brawl. He had almost finished his sentence when he was taken to do some task. It came to his knowledge that in his native village was a nice festivity, so, he ran away in order not to miss it, after enjoying a great time, he gave himself up to finish his time. He was punished for his escapade with eigth days in the dungeon placed almost at the foot of Morne Abel, which saved his life.

This was the only safe shelter, a true atomic-bomb refuge.

Looking at it, a dark hole in the lowest part of the mountain, and thinking in the irony of destiny, we thought: "And after that they say that Crime doesn't pay".

After being rescued and suffering from horrible burns he was the only person able to tell what had happened that fatidic morning of May 8; "It was about eight, suddenly a tremendous noise burst out, everyone called for help, cried out "I am dying". Five minutes later, there were no more cries, except mine".

Those simple words eloquently told the magnitude of the Saint-Pierre tragedy.

Since then it has never recovered its magnificence; now it remains as a little village of fishermen all the activities have been centered in Forte-de-France which is the biggest and only city of La Martinique.

The convict saved by the Mountain owed to it a decent living.

He was under contract by the Barnum Circus to tell the world his incredible story and to show the numerous scars in his face and body, souvenirs of the "Fire Mountain".

His portrait can be seen at the Museum of Saint-Pierre along with many momentos of the terrible fate of an elegant creole elite tragically disappeared that 8 th of May, 1902.

Happily Dr. Garcín's family, who lived nearby in a place called Basse Pointe left the place in time to find refuge in Forte de France where they still live nowadays.

Dr. Garcín's birth-place was completely destroyed by the volcanic eruption that made history.

Dr. GARCIN'S FAMILY

We came to the Martinique in homage to the memory of our dear friend Prof. Raymond Garcin, who loved so much his birthplace to which he frequently referred to. He was so attached to his beautiful Island that he had pictures of it at his Office at the Salpetrière.

This offered us the pleasure of meeting his very charming family. When

Fig. 8. — *Profesor Raymond Garcin, famous French Neurologist, born at Martinique, arrives accompanied by the Uruguayan Professors Varela Fuentes and Mussio Fournier and is greeted by Dr. and Mrs Victor Soriano at a reception gaven in his honour, at their home in Montevideo.*

Dr. Daniel Garcin, also a successful practicing doctor, called on us at our place at Trois Ilets, inviting us to a cocktail, we had the surprise of having before us a person in many aspects different to our friend. He is a tall, blond and clear-eyed man, while Raymond as everybody remembers was short, a little stooped, as if for bending too much over books or over the patients beds.

But in which you learn unmistakenly the family ties, is in their sympathetic and kind smiles, and the real trade mark of the family, the nose.

Characteristics that we also found in an elder brother a banker, also tall but a stouter man.

The Garcins were very kind to invite the whole family in order to see us

including some couples of the younger generation. All of them were very charming people.

There were many kind of remembrances and anecdotes about our dear friend Raymond.

Their voices sounded proud when they referred to how brilliant he was in his youth, how he used to study in a very peculiar way placing several open books in line, reading a little of them at a time, going from one to another in a fabulous gymnastic of the intelligence, learning at the same time different subjects including some of spiritual guidance which always occupied a relevant place in his studies.

He left the Island under scholarship because of the modest means of a numerous family, to become afterwards one of the most brilliant Profesors of Neurology of France.

But he never forgot his little Island. Their brothers told us that when he returned after many years, he could barely disguise his emotion going from one place to another and standing in awe of reverence in front of the graves of his parents and the places of his childhood.

The people of the Martinique who went to Paris to be examined and cured knew that they had there a real friend, Dr. Garcin, who took special care of the patients placed them all together, so they would not feel lonesome and dejected in the big city and far from home. He kindly talked to them in creole, a special disfigurement of the French language, but with a musical ting agreeable to the ears.

The moments spent with the Garcins and their charming family made us at this and other occasions feel that we didn' lose one friend but that we have gained many like him at La Martinique.

We were also introduced to the special cuisine of the Island and many tropical fruits of delicious taste never seen before.

Clara got very fond of a delicious but strong cocktail made with rum of the Island. After bravely struggling with her French she was elated, because she said the cocktail improved her French, but I think that it only lowered her selferiticism.

When at the last meeting, Dr. Daniel Garcin took us to the small quay to take our boat to Trois Illets, we were for a long time talking on the seashore. The stars were brightly shining, some people were seated waiting for the little ship and we felt sorry when we grasped his friendly hand to say good bye.

Fig. 9. — *EMPRESS JOSEPHINE Fort de France. Monument erected to Josephine by her grandson Napoleon III.*

TROIS ILLETS

We were living at Trois Illets, a little village that owes its name to three little islands that dominate its landscape.

At a short walking distance from our bungalow was the house where Empress Josephine lived with her parents, as we were informed. And we began to walk, on the road which offered us a wonderful view as we were ascending the slope. We cauld say that they were of breathless beauty, because they were beautiful and we were really breathless with the ascending effort!

Luckily we had a lift from very kind people as we were using our thumbs for the first time in our lives as a means of transportation!

We found it very convenient. It served us as a psychological experience. We were happily surprised to find heart-warmed people and at the same time reassured that we impressed them.

We were left at the very entrance of the place which was amidst a luxuriant vegetation. There stood long ago a ranch named "La Petit Guinée" owned by the Tachers of la Pagerie and there was born Marie Joseph Rose Taschers who became Empress Josephine.

When we arrived at her parent's place we were a little diappointed; all that was left of the "Little Guinée" of 500 hectaries where 300 slaves worked was a kitchen miracously well preserved, which became "Le Musée de La Pagerie" of the ranch former construction were only left, some marks which show where the walls stood.

The presence of the past was felt invading our souls pounding at our hearts. As if under a magic spell we witnessed the past conquering the present, as in the vision of Ezequiel where the dried bones resurrected and became alive, absent walls began to rise. There, standing on a hill, the master house, was taking form a vast rectangle crowned by a flattened roof covered with flat scaled tiles, sustained by wooden columns and surrounded by a gallery.

Towards the north there was a gallery with wooden blinds, which permitted to profit the cooling effects of the winds, served as a dining room, then a big and solid construction where the lady of the house loved to be alone and rest. From it we could enjoy the sight of the colorful splashes of brilliant flowers, and smell their inebriating perfumes.

Nearby was a mill to crush the sugar-cane that rotated pulled by patient oxen.

Further away was the building where the sugar was procesed, with a square chimney, then the houses of the slaves and their Hospital.

There was a time when the "Petit Guinée" was considered one of the richest of the Island, its numerous slaves cultivated the sugar cane, the cacao's tree, the cotton. They also made a very much praised delicious rum.

The place of our evocation belonged to the Desverges, whose ancestor Dominique Desverges came from Annet-Sur-Marne and fought the English side by side with his brother in law Robert Lonvilliers de Poincy, Governor of the Island San Cristobal defending the French portion of it, and when this was lost Dominique leaving behind all his belongings, escaped to the Martinique.

In a short time the Desverges were counted among the most prominent families of the Island. Jean Desverges a member of the family married the granddaughter of the famous Guillaume d'Orange one of the pioneers of the colonization. His son Joseph Desverges married Marie Brown, less conspicuous, but who brought some economical advantages to the couple. Their house carried the name of Sanois, and they had a daughter whose name was Rose-Claire. When she

was twenty years old this girl married Joseph Tascher de la Pagerie whose family came from Saint-Mandé near Blois.

It was his pride to tell the children about the five years he spent in court serving as page of the Dauphine Marie-Joséphe de Saxe, Mother of King Louis XVI.

He also was sub-lieutenant in a company of the Marine's Artillery.

His father Gaspar Tascher de la Pagerie came to the Martinique in 1726 *and he married François Boureau de la Chevaliere, a direct descendent of the brother of Belain d'Estambuc a name venered in th Island as the one who gave Martinique to France.*

Fig. 10. — *Typical Martinique Hall. Furniture made from the Island's wood.*

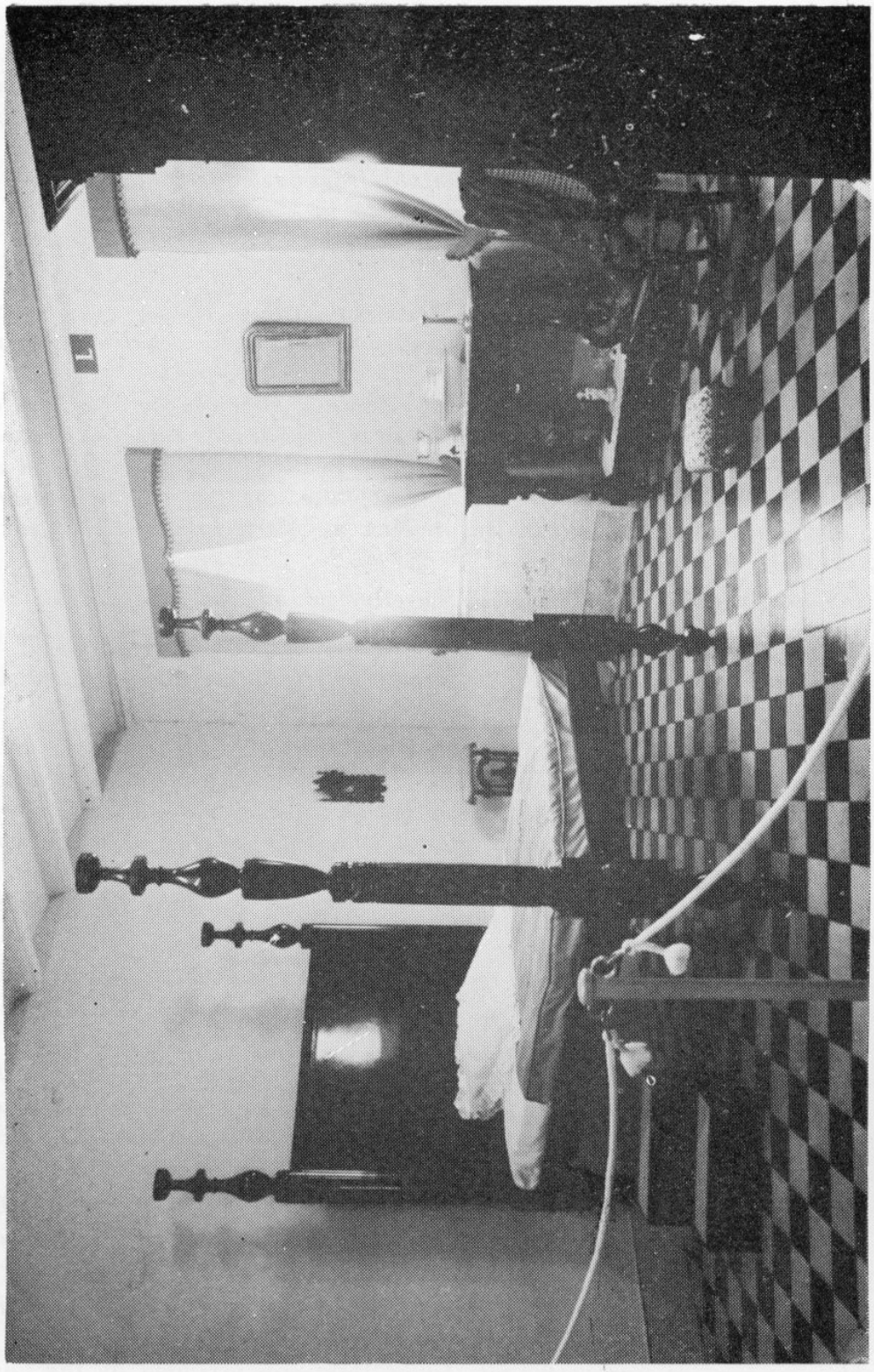


Fig. 11. — *Bedroom. Similar to that used by Josephine in her adolescence in Martinique.*

Gaspar proved not to be a good administrator and soon dilapidated his wife's fortune. His son Joseph Tascher de la Pagerie, as soon as he was married to Rose-Claire went to live with her parents.

One year before this wedding took place, in 1760, *the Marquis de Beauharnais colonial Governor of Martinique had at Fort Royal (to-day Fort de France) a son, who was baptized Alexander Vizcount de Beauharnais. His godmather was Mme. Renaudin, an aunt of Josephine, who in later years would play a decisive role on the first marriage of her niece, with her god-son.*

She was the Governors wife, accompanying lady, but she became the Governor's mistress and when this finished his term in the Island, Mme. Edmée

Renaudin left Martinique and sailed to Paris with the Governor's family.

She became prevalent in the Marquis'life, and Mme. de Beauharnais, feeling defeated left the home to live in retirement in one of her properties, a castle near Blois. Rose-Claire and Joseph de la Pagerie, had a child, and at a nearby small church there is an act of baptism that states "Today, July 27, 1763, I have baptized a girl of five weeks old from legitimate marriage of Mr. Joseph Gaspar de Taschers, Gentleman Lord de la Pagerie, Lieutenant of Reformed Artillery, and of Mrs Marie-Rose Desverges de Sanois· her father and mother. . . . . "

When they held the little body of Marie-Joseph-Rose their first offspring, they were far from thinking that this tender baby would become Josephine, Empress and Queen of France.

One year later, on December 11, 1764, another girl was born to the couple and received the name of Catherine-Desirée, and two years later another girl came to the de Tascher's home on September 3 1766, and was baptized with the name of Marie François. The name of Josephine was given by Napoleon to his love, and until that epoch she was known as Marie-Josephe-Rose, or Marie-Rose, or Rose as her mother.

During her infancy she was called Yeyette, as she was nicknamed.

Yeyette was a sensitive child that loved the Island's environment, the beauty of the flores, its dazzling birds, the rumor of the forest giving messages to the winds. . . . . .

Oh! Yeyette loved that place, and even in the more splendorous moments of her life was yearning for it. Thus, she tried to recreate it at her castle of Malmaison· importing rare and colourful birds, and exotic tropical plants, trying to recapture the atmosphere of her infancy, when she played with Genevieve and Mauricette, and enjoyed the placid environment of a home where her parents tenderly loved each other and grand-mother Marie Brown was ready to satisfy her wishes.

Her father was not a practical and active man, even if he was a valiant one, who· the same year that Yeyette was born, bravely fought the English, defending the Island.

Thus, the "Petit Guinée" wasn't as powerful as before. The Master's house was practically "gone with the wind", since a cyclone destroyed it, and it was never restored because of a lack of resources. Their temporary quarters at the sugar refinery became a permanent one.

Thus, Yeyette could not afford to attend an expensive school like the Convent of the Ursulines in Saint Pierre, where the girls of aritocratic rich families went.

Saint Pierre was at that time famous in the whole Antillas for the high standart of life of its inhabitants who used to enjoy fastuous reunions.

So, she was sent to a more modest school, that was attended by the daughters of good families who had not many economical resources, and at the age of nine, she became a boarder at the Convent of the Ladies of Providence at Fort Royal accross the bay, where she spent five years acquiring all the qualities a young lady ought to have to present herself in society.

She learned how to make a reverence gracefully, how to play the clavecin dance with elegance, and avoid making mistakes in her writings. Then she was qualified to leave the College.

Martinique, even today is permeated by Indian and African superstitions, many of "vaudou" origin.

Yeyette loved mystery and one day in 1777, she convinced her good wei-

Fig. 12. — *Martinique Costumes towards 1858.*

nurse, Gertrudis to take her to a nearby ravine called the "Croc-Souris" where a liberated slave Euphémie David delighted the attendants to her "sciences" making magic filters capable of doing wrong or right according to the client's wishes, and claimed that she was able to foresee the future.

We can imagine the girl trembling of emotion approaching the black witch to have her future predicted.

What the woman said would leave Yeyette perplexed, and it rung to her

ears all the time with the strangeness of her message "You will become more than a Queen".

What could a girl of fourteen think of this prediction?.

They carried a simple islander's life, enjoying the beauties of nature, playing at the river that sinously went through the valley under the acacia trees.

They were far away from the big metropolis, separated from France by a long trip crossing the Atlantic and depending on the quality of the Winds. Their only occassion for a real excitement was when they visualized on the horizon the approaching vessels with their inflated set of sails going towards Fort Royal.

They anticipated the pleasure of being invited to dazzling receptions given by her uncle, the Baron des Tascher, Commander of the port. Like fireworks that disrupted the rutinary spectacle of life, the balls given in honour of the

Fig. 13. — ***Martinique Woman. Sculpture by Malvina Hoffman. Museum of Natural History New York.***

visitors whether of oficial character, in Fort Royal, or more familiar at Trois Ilets, they seasoned her life and made her yearn for their return when the vessels were shrinking until they disappeared on the horizon.

There was a magic word that arose in her imagination the most cherished dreams and that word was "Paris" Paris! to go to Paris was her most sublime obsession· and that was granted to her in the most unsuspected way.

Mme Renaudin, aunt Edmée, reigned all powerful in the household of her lover the Marquis de Beauharnais, and in order to strengthen the bonds that attached her to this home, she thought that the best way was to marry his godson the Viscount Alexandre with some of her three nieces.

There was no doubt that he would marry one of them, the only question was which one.

The bridegroom was at that time seventeen, and Marie-Josephe-Rose was fourteen, and was considered too old, while Catherine Desirée of thirteen was considered a suitable match. Unfortunately when the letter arrived indicating Catherine-Desirée as the chosen bride, she was dead.

This left open for discussion which of the remaining daughters would be the one to take the place of the unfortunate Catherine Desirée.

It took a considerable length of time and of exchange of letters, to decide this question if the eldest Marie-Josephe then fifteen or Marie Françoise of eleven.

We can imagine the anxiety of Marie-Josephe-Rose, having this doubt pending over her destiny, and her wishes to visit Paris the city of her dreams.

Her father came to her aid, sending a letter in which he explained that the younger one, didn't like to leave her mother, and the eldest would be most pleased to go and continue exalting her virtues describing her as having an "exceptionally sweet disposition a fine complexion, and beautiful arms and eyes."

Aunt Edmée, and the father of the groom were ready to please him· and Mme Renaudin answered "We leave you to be guided by Providence. Bring either one of your daughters, but come, come, I conjure you". Marquis de Beauharnais answered in the same way accepting his decisión expressing "Bring us whichever daughter you consider more likely to suit my son".

It would horrify a girl of nowadays to become engaged in this way, without having seen the groom, even once, or being chosen by him as the best to share his life. Father Joseph Tascher received the marriage banns, sent by the Marquis of Beauharnais leaving in blank the name of the bride, to be filled in as he wished.

Even under these conditions, Marie-Josephe-Rose was elated. To go to Pa ris!. to be married to a young and handsome (so they said) viscount, would have seemed to her a fairy tale.

Her father ought to be greatly indebted to aunt Edmée, she not only provided an exceptionally good husband for his daughter, but gave him the 20.000 livres stipulated in the marriage contract.

Full of golden illusions, Marie-Josephe-Rose accompanied her father on a trip that ended in Brest on October 12, 1779.

Two weeks after Marie-Josephe-Rose's arrival to Brest accompanied by her father and her mulatto maid Euphémie, Alexandre came to greet them and to analize this provincial import who would become his wife.

Viscount Alexandre was a handsome young man, elegant and cultivated already an expert as a lover and conqueror of hearts of ladies older than himself.

What he really thought of this awkward and timid girl· whose charms we-

re still underdeveloped and didn't impress as beautiful, was softened in a letter commenting this encounter with his father, by telling "Mademoiselle de La Pagerie", may perhaps strike you as less pretty than might have been anticipated but I can assure you that her delightful manner and the sweetness of her nature exceed our fondest expectations", and in this he was truthful for these characteristics of her personality captivated everyone who knew her all along her life, and to which may be added a melodious voice, that was one of her most enga-

Fig. 14. — ***Josephine, when she was still called Marie-Josephe-Rose and nicknamed Yeyette.***

ging charms.

And what about Marie-Josephe-Rose? She must be dazzled at his sight, and surely ready to love him, and to bless her good fortune.

Soon fortune will seems not to be so bright as she thought.

After a short period of good behavior, restricted to the honey moon, Ale-

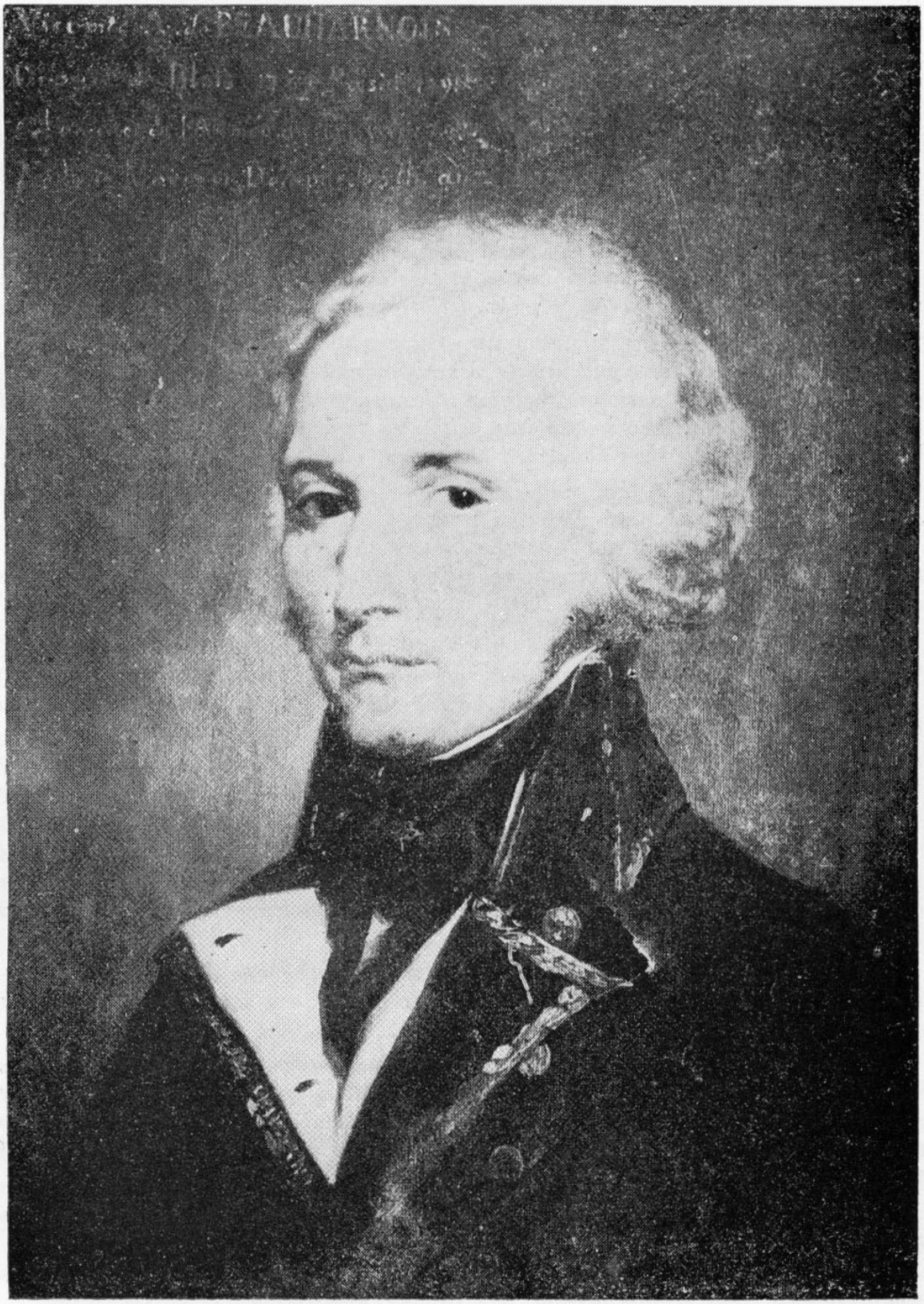


Fig. 15. — *Alexandre de Beauharnais, who under an agreable appearance hid a very cruel and pedantic nature.*

xandre began the ways of bachelor's life. His young wife was unsuitable to be introduced in the glitering social circle he used to mingle with. Surely they would laugh at her umpolished manners and aspect, the naivite of her remarks, which will stress her condition of a "Creole" as it was called those being born in far away colonial Island, this has nothing to do with any kind of mixture of black blood, only the fact that they were born in that midst, far away from France.

So, the poor girl lived secluded in the castle of her father in law, while her unfaithful dashing husband spent his time attending parties and involving himself in new amorous adventures.

But he promised himself to prepare his wife to meet the requirements of Paris social life, and fill the gaps of an inadecuate education, so he said in a letter addressed to his tutor Praticol "I promptly propose to compensate for the sad neglect of her fifteen years of life by divising a plan for her education" and it was his ambition that she took lessons in geography and history, reading aloud of verses by the best dramatists; to this ought to be added lessons of harp which would round a glittering profile of the personality of the young viscountess of Beauharnais.

This were his plans and young Marié-Josephe-Rose was left to meet this end alone in his father's home, because Alexandre spent the remaining part of his first year of marriage in garrison with his regiment, returning home for a brief period in which they conceived their first child.

To this followed a long and lonely time of pregnancy, during which she tried unsuccessfully to acomplish the program of education imposed upon her.

Her mood may have not been the best of the world. Her husband was distant and disdainful. Poor Marie-Josephe-Rose must have regarded her memories of Martinique, as the Paradise lost.

Mme Renaudin, her aunt, was distressed at the sight of a deteriorating relationship between the couple.

Finally Alexandre confessed that he felt that there was nothing in common between them He was a highly educated person that had spent years of preparation in the College of Plessis and the University of Heilderberg, while his wife was incapable to meet the plans of education he had traced for her. She was scorned as "indolent" and he left the project of educating his wife null.

She really became a kind of martyr in this her first marriage, she was blamed as "jelalous", when she had reasons for it, and she must have felt his despise in the bottom of her soul, that she felt grateful when he returned to Paris one year later.

Patient Marie-Josephe-Rose received him extreming her devotion and affection. But when her second child was born, his father was far away and couldn't come to reassure his wife of his love.

Seven months before the birth, the night of September 5, 1782, while his wife was asleep, he left the home leaving an explanatory and grandiloquent message in which he reported, that he had decided to join the French Army to the orders of Marquis Boulle to fight against the British, or in the alliance of the American, whether in the Atlantic or in the Caribean Sea.

His words were telling her that he was sacrifying his love and willingness to be near her for the glory of the country and the future of their children.

There is no doubt that Marie-Josephe-Rose loved her husband, but things would happen that would affront her beyond description.

Among the ladies in Paris who shared his gallantries was one older than him, Mme Longvre, a relative of his wife whose malice proved to be without

limits. She not only impudently maintained an amorous relationship with a young man married with a member of her own family, but even tried to cast shadows on Marie-Josephe-Rose's honesty, by telling him that she carried a dishonest life before her marriage.

This intrigant lady had already had a son from his lover, and having recently lost her husband, managed to go in the ship with Alexandre to Martinique.

While Marie-Josephe-Rose was expecting her second child, Alexandre was openly courting Mme. Longvre in her own Island, news of his misbehavior was sent to Paris, and Marie-Josephe-Rose distressed did not sent him the news of her daughter's arrival. Mme. Longvré was ready to inflict another blow on her reputation by telling her husband that according to the rules that prevailed at that time, the child came to this world before the prescribed nine months and was not his own. This made to his dignity flare and he launched against his wife the most infamous campaign Alexandre went to the limits of trying to bribe slaves from La Pagerie in order to obtain testimony of her depravity before her marriage.

Many like Brigette, refused with indignation to second his manouver to dirty Marie-Josephe-Rose honorability.

Only one slave named Maxime responded to bribery, and calumnied her, but paid his disloyalty by being chained· but the wrong deed had been consumed, and rendered bitter fruits.

In a letter highly insultant, the disolute husband, assumed a righteous wrath and these were Viscount de Beauharnais harsh words:

"If I had written you in the first hour of my rage, my pen would have scorched the paper... But now three weeks have passed, and, despite my despair and suffocating fury, I shall be able to control myself, to tell you in cold blood, that you are one of the vilest of creatures and that my sojourns in the Island had acquainted me with your abominable conduct here".

The letter continues pouring out diatribes against her honour pilling accusation upon accusation. He accused her of having an affair while accompanying her father to join him at Brest. "Any creature who would open her arms to a lover even as she makes ready to join the man to whom her throth is plight, has no soul; she is lower than the lowest strumpests of this earth... in the light of such excesses, what am I to think of the clouds and conflicts that have marred our marriage, what am I to think of this last child...?...I must accept it but I swear by heavens that it is the progeny of another, another's blood flows in its veins".

Thus, he, who carried a scandalous life injured an inocent soul by sending words that made her feel as though she were stabbed by a poisoned dart.

In conclusion, he ordered her to retire to a convent, or to return to Martinique· threatening her with taking more severe measures if she dare to defy his orders.

Such an unjust behavior towards his innocent wife didn't find echo in both families. Her parents at la Pagerie condemned his infamous conduct and proclaimed their daugter's honorability, corroborated by many of the people of the Island.

Alexandre's father who testified in favor of her daughter in law declared that her behavior was all the time without blemish.

This didnot change viscount de Beauharnais feelings toward his wife and he stated in another letter. "I urge you to warn my father and your aunt that their efforts to intervene will prove not only fruitless but even damaging your

cause. For six months now I have been hardening my heart against you. Resign yourself as I have a distasteful course of action, to a separation which must be an affliction above all to our children, and believe me, Madame, when I said that, of the two of us you are not the one to be more pitied" And expressed his disgust because she had neither gone to Martinique nor to the convent.

Being the things as it were Marie-Josephe-Rose, left his father in law's home taking refuge in the convent of Pedemont, taking with her, her son Eugene and leaving her daughter to be cared by a foster mother.

Aunt Renaudin chose for her a very fashionable convent, one of the best of Paris to which went the best ladies of France in distress to cure their souls from the misfortunes of life.

This was a new world of distinguished people to which she had access entering by the doors of sorrow, and the humiliation of rejection. In their company she learned to behave as the best ladies of the French Society at that time acquiring their actitudes and behavior, polishing her manners, incorporating their way of talking, their expressions, learning all the ways and doing of the people in the court life, she was like a chrysalis preparing herself in the convent to emerge years later as a dazzling social butterfly.

Marie-Josephe-Rose, reinforced with the testimony of her parents and father in law about the purity of her life· initiated a legal action demanding divorce from her husband, who at his turn could not prove all the charges of infidelity and misbehavior of which he accused his wife

So, she won the law suit, and Viscount Alexandre de Beauharnais had to apoligize for having falsely injured her good name and neglected his duties as a husband.

He consented in the payment of five thousand livres annually for her maintenance plus an additional one thousand livres, for the maintenance of his daughter, thus legally recognizing the daughter as his own child.

Marie-Josephe-Rose entered the convent at the age of twenty, mother of two children and lived there peacefully for two years, while her husband more liberated now than ever after legal separation continue his libertine way of life, begeting children out of wedlock, some of them generously protected by Josephine, when she was Empress of France. Still suffering from the impact of separation and the cruel experiences lived, after leaving the convent her first thought was to return to her beloved Island, Martinique, and on 1788 she sailed home, to stay there two years.

La Pagerie was not as gay as she knew it. Her beloved grandmother Marie Brown had already died and she found her father and her younger sister in a critical state of health.

There among her people she shed all the tender feelings that always filled her heart.

But her fatidic years did not end yet; the flame of the French Revolution reached the far away Island and disrupted its peaceful Caribean atmosphere, and started a civil war bringing panic to its inhabitants.

Josephine was terrified, and protected by her friends at Fort Royal, amid a heavy bombardement she crossed the Savana· and a cannon bullet almost killed her when it exploted dangerously near her in the same place where her imposing statue stands to-day. Gone were the peaceful days spent in her beloved Island, she would never come back to it she would never see her family again; her father died in 1790, when she reached France, and a year later, her younger sister of 25 year old succumbed to the scurvy.

Thus, without luggage, and without kissing goodby to the dear members

of her family, Marie-Josephe-Rose stepped into the frigate "La Sensible" which took her to Toulon.

Their dramatic scape is related by her daughter in her memories as follows:

"The revolution has begun in the colony; the Government forced us to flee precipitantly.

We were staying at the Government house in Fort Royal, when suddenly one evening my mother was advised that the town was under attack. She rushed out instantly to seek refuge on board of a vessel (The Sensible) which was commanded by an acquantance of hers. As she crossed the Savana (a public square) a cannon ball fell close beside us".

"The next day the mutineers, having overwhelmed the town, ordered the French ships to return to port, threatening them with fire from the port. Our ship's crew shouted out their determination to return to France and hurried to pull out of range of the shore batteries, just as the threat was put into execution; the cannors boomed but we escaped from their shots. Fate has spared us. And there we were unexpectedly on the high seas without so much as an adieu to anyone ashore".

In reaching Toulon on October 29, 1790, Marie-Josephe-Rose and Hortense learned that the turmoil that agitated France was like fliying from a danger to step into another greater one.

The tremendous change that France had experienced during her absence shook her with its menacing message of impending danger.

She was an aristocrat, and that was very dangerous in France in those days.

There were new three colored flags everywhere substituting the royal one Indicating that the Royal Government and every other power linked to it had collapsed. Everyone addressed each other as "Citizen" levelling and abolishing former ranks of distinction.

As Marie-Josephe-Rose advanced towards Paris, she grew more and more upset— Burning houses, blood— stained streets, etc. forced her to escape to Fontainebleau where her aunt Elmée and Marquis de Beauharnais lived quietly. There at least she felt fairly safe in a family reassuring atmosphere, enlightened by the encounter with his beloved son Eugene, then nine years old, who had been placed by his father in the College of Harcout.

Families became divided between royalists and revolutionaries and so were the children of Marquis de Beauharnais. while the eldest son remained loyal to the King and escaped into exile, the younger one, Alexandre de Beauharnais, was ready to proclaim himself a revolutionary, and joined them in persecuting the same privileged cast that he once belonged to.

Soon the name of Viscount de Beauharnais faded in the also pompous one General de Beauharnais. He paid few visits to his wife and children, and was remise in sending them economical support.

When things seemed settled, the French community ought to suffer another upheaval. Rumors had arisen that the Princes of Bourbon, seconded by the Royal dignities, were approaching France to impose their vengeance upon the rebels

This started a mass hysteria and reactivated dormant suspicions on all those aristocrats that had embrased the Revolution and may have been linked with the menacing invasion.

General de Beauharnais. disolute behavior and orgies had disgusted the Minister of War as well as his poor record as General carrying out his missions.

Abashed General de Beauharnais resigned pretending illness to avoid being

Fig. 16. — *Josephine matured into a beautiful lady whose charms captivated Napoleon.*

destituted on the grounds of incompetence. But more important than that, was the fact that he had the misfortune to arouse suspicion. And all his showmanship as a revolutionary, all the persecution of former friends in the name of Revolution were not enough to make him less vulnerable to the risk of being imprisoned under the claim of treason.

Marie-Josephe-Rose forgot all the bitterness that could have generated in her soul the past conduct of her husband, and risking her own safety wrote ma-

ny letters claiming her husbands innocence and loyalty to the cause, not to be confounded with that of his eldest brother a manifested Royalist.

All her pleading letters were useless and only led to attract the attention upon her and to be arrested and taken to prison.

At last both of them were under same roof, the tetric and unhospitable roof of the Carmes prison—

Men and women were in separate quarters· the condition of which were disastrous. Aristocratic ladies slept side by side in a humid room, feeling the abhorrent visits of rats, infested with vermins, having as its only way of airbarred slits. In that suffocationg unhealthy atmosphere. survival was a question of high spirits, and many of these well bred ladies had the resource of humor to lessen their penuries. At this, Lady de Beauharnais excelled, and her charms and tact were later described by some of the survivors.

The guillotin was having its daily quota of aristocratic necks to be beheaded by its sharp cutting edge.

Marie-Josephe-Rose tried to communicate with her children inventing new tricks when the correspondence of the prisioner to the members of their family was cut.

Every week a basket containing food and clean linnen were sent to the prisoner from their family and in order that her mother had the certainty that they were alive, they copied the list of the contents of the basket every time alternating Eugene and Hortense, so she may recognize their hand writing like a little voice telling, "Mother, rest peacefully, we are alive".

Another means of communication was Fortune, a little dog of Marie-Josephe-Rose, who knew how to pass like an exhalation through the guards to reach like a dart her owner and to come back in the same way carrying back and forth messages hidden in its collar.

Destiny was preparing for the self-reliant Viscount Alexandre de Beauharnais a dramatic end.

Hortense recorded in her memories this curious episode in which God knows how some unknown friends managed so they could see their parents.

"An unknown woman came one day — Hortense refers — and mysteriously led us to a rear of the garden on the Rue de Sevres and up the stairs of a gardener's house, all the while cautioning us to keep silence. Accross from us we could see a huge building... and then a window opening, in which my mother and father appeared. In surprise and great emotion, I stretched out my arms to them and cried out. They made signs to me to be still but a sentinel at the foot of the wall had heard us, and he began to shout. The unknown woman hurried us out and home... We learned that the window of the prison has been sealed. This was the last time I saw my father; a few days later he was dead".

This unfortunate event came to happen around the middle of July when their father's name was included in the daily quota of fifty heads to be guillotined.

Some of her biographers said that the de Beauharnais were reconciled "in extremis".

A new days later the jailer came to seek Mme de Beauharnais's bed and when somebody asked him if they were going to give her a better one, he answered with a sinister smile that she would have the need of none, because she was going to be guillotined. The ladies began to cry and to lament, and Mme de Beauharnais trying not to lose her composture, assured them that that would

not happen, because a prophecy ought to be fulfilled and she would become more than a Queen in France.

According to Madmoiselle Ducrest's memories these were Mme de Beauharnais words.

"At which these ladies tears began to flow faster; they were afraid I had gone stark mad Actually I was not affecting a show of courage; I was at that moment convinced that the prediction made by my fortune teller would be realized.

With Madame d'Aguillon about to faint, I pulled her toward the window and was opening it to give her a breath of air, when suddenly I saw a woman below making vigorous and incomprehensible gestures in our direction. She kept plucking and pointing to her dress also could not imagine what that could signify. But when she persisted I called out Robe? To which she noded vigorously Yes. Then she picked up a stone, put it in her apron, took it out again and held up in her hands. "Pierre" (stone) I cried out, I cried, and this time to her obvious delight Next, holding up her dress and the stone together she began to go through the motions of slitting her throat, and then to dance about and to clap her hands. This singular charade filled us with an inexpressible emotion, for we dared to interpret that Robespierre had been guillotined, that we had a right to hope that France was saved. A few moments later, a crowd of our companions in misfortune rushed in and gave us the details of that memorable day...

They brought me back my bed of leather strips webbing on which I slept the soundest night's sleep of my life. Before I retired, I said to my friends "You see I have not been guillotined, I shall yet be crowned Queen of France".

She never doubted that prophecy made by Auphemie David, the old mulatto, in her adolescence in that far away Island of her birth, the Martinique.

How this would come to pass it was a mystery to her. Time will tell.

Drifting in the Revolution's turbulent waters Marie-Josephe-Rose experien ced the joy of liberation that induced the people into frenzied dancing in the streets, and the bitter taste of pertaining to the lot of the neediest who had to fight their place in the long line of people waiting for food.

The Martinique was under English occupation and no resources could be expected from it. Her husband's properties have been confiscated by the state.

Alone with two children to fight adversity, Marie-Josephe-Rose had to borrow from friends and to live thanks to the generosity of Madame Dumoulin, who provided her board and granted her daily bread.

A very dark outlook of life for one who expects to become Empress and Queen of France. Fighting tenaciously in order to recover her husband's and her own properties, aided by friends she could obtain the restitution of all her belongings left at her apartment in Paris when she was arrested, clothes, furniture, silverware etc. But regarding her other possessions she had to wait a few years more.

In the meantime visiting Goverment offices in order to obtain its devolution, became her daily routine. She became acquainted with important people and made very influential friends.

After having experienced a life in which she was not spared any kind of suffering and humilliation in her marriage, and having felt the frozen nearness of Death, and the pangs of hunger, Marie-Josephe-Rose was regaining the joy of life and at thirthy one she exhibited that femenine beauty whose softness was captured in numerous paintings and marbles. She became the most elegant lady of France.

She was approaching her destiny, but the last link in the chains of events ought to be her own son, Eugene.

How they met it is related in Napoleon's memories dictated in Saint Elene to the Count de las Casas, in third person as follows:

"Into his headquarters was ushered a boy of ten or twelve, who said, that he had come to entreat the commanding general to return the swords of his father, a former General of the Republic. This young child was Eugene de Beauharnais. the future Viceroy of Italy, Napoleon, responsive to the appeal of youth and sensitive to the nature of the request, granted it. Eugène burst into tears at the sight of his father sword.

The general was touched and manifested him such good favor that Madame de Beauharnais felt obliged to come, the following day, to express her thanks in person. Napoleon lost no time in returning the visit..."

"Her extraordinary grace and her irresistibly sweet manners" as Napoleon expressed in his memory, conquered his devotion and affection, and this reunion florished in ardent love and marriage was consumated on March 9, 1796. Hereafter she would be only known as Josephine, and few years later in solemn ceremony that David inmortalized in one of the Louvre's most visited painting, tre Dame Cathedral was resplendent of opulent gala the glitering light ilumi- Napoleon himself· Crowned Josephine Empress and Queen of France. The Nonated one of the most fascinatig pages of history. Thus, the profecy of Euphemie, the clarivident was fulfilled—

The MUSEUM OF LA PAGERIE

All that rest from "The Petit Guinée" Josephine's birthplace at the Martinique, is a kitchen, that huracans, and time has respected and become the Museum of the Pagerie.

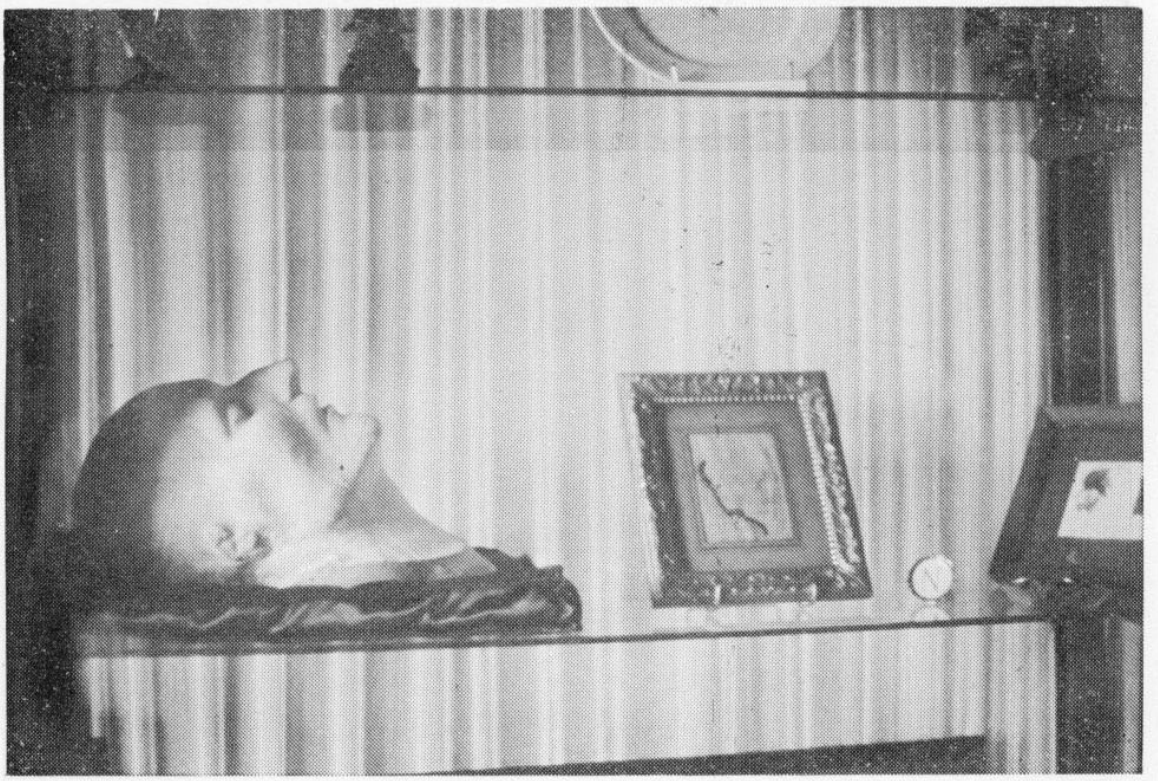


Fig. 18. — *Museum de la Pagerie. In a case Josephine's handkerchif, and beside it a very sad memento a piece of weed grown in Napoleon s tomb in St. Elene, and the emaciated death mask of the once Emperor of France.*

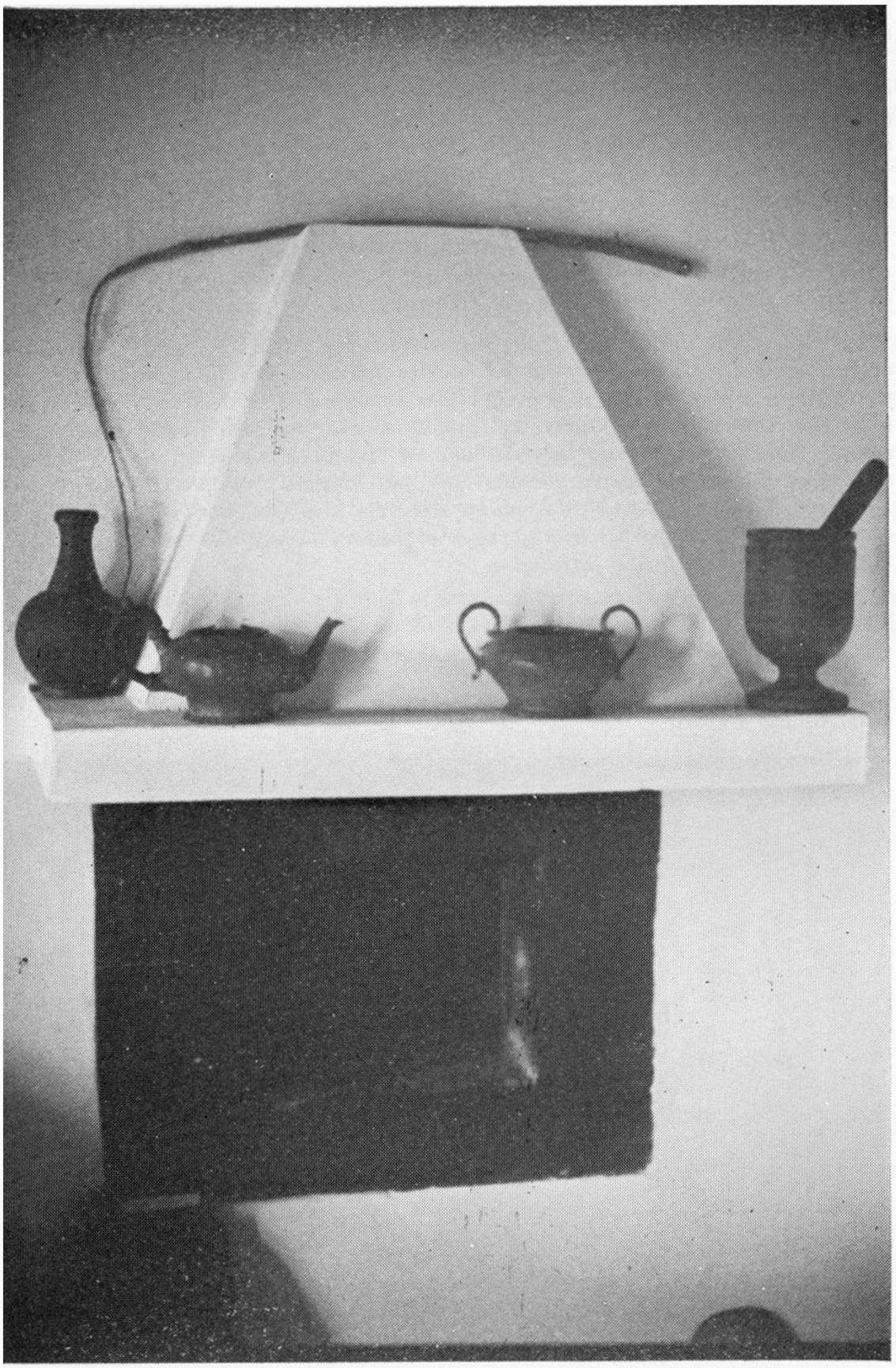


Fig. 17. — *Corner of the Kitchen-Museum de la Pagerie.*

There is like a summary of the two lives. Respetuosly we enter in this room filled with memories of the past.

We could admire Josephine's beauty from a painting of hers with her dark eyes wide open, her small mouth, and her curly air softly surrounding her soft complexion. Nearby her bed, of colonial style, in a case her delicate handkerchief, the minute partner of her coquettish movements, but beside it a very sad remembrance, a piece of weed grown on Napoleon's Tomb at Saint Elène, and beside it the emaciated death mask of the Emperor of France. More stimulating was to see two magnificent sculptures in Marble of the famous couple in their full brilliancy, when France and most of Europe were humble at their feet. In a small kitchen at La Martinique are many other mementos of those two singular lives. One from the Island of Corcica, the other from La Martinique, which made us think: Behave world of the Islanders! They have the conquerors soul.

Prof. Dr. VICTOR SORIANO

• News

NINCDS NOTES

LAZZARINI RECEIVES PHS HONOR

Dr. Robert A. Lazzarini, acting chief of the newly created NINCDS Laboratory of Molecular Genetics, has won the Public Health Service Superior Service Award for work on defective interfering virus particles. The Superior Service Award is the highest honor the Public Health Service can bestow on a civil service employee.

The award recognizes Dr. Lazzarini's discoveries about the origin of the particles and the mechanism by which they limit viral infections. Defective interfering particles are aberrant virus fragments which compete in an infected cell for enzymes needed by its destructive parent virus. The particles can prevent the infected cell from producing complete pathogenic viruses and may protect healthy cells from later virus infection.

Dr. Lazzarini, an NINCDS staff member since 1964, serves on the editorial boards of four virology journals, and in 1980 was a member of the Virology Study Section of NIH's Division of Research Grants. A University of California, Los Angeles graduate, he received his postdoctoral training at the Johns Hopkins University.

NINCDS SOLICITS ALZHEIMER'S DISEASE RESEARCH PROPOSALS

Innovative research projects involving Alzheimer's disease and related dementias are being sought by NINCDS and its sister organization, the National Institute on Aging.

Proposals are now being accepted for studies to determine the etiology or pathogenesis of Alzheimer's disease, to improve diagnosis, and to develop effective therapy. Areas of particular interest include cerebral circulation and metabolism, neurochemistry, immunology and virology, neuroendocrinology, genetics and epidemiology, animal models, and differential diagnosis. Both program (P01) and individual (R01) project applications are invited.

Proposals for both fundamental and clinical studies are welcome. Deadlines for receipt of P01 applications are October 1, February 1 and June 1; R01 proposals must be received by November 1, March 1 and July 1. The complete text of the proram announcement can be found in the *NIH Guide for Grants and Contracts*, vol. 10, no. 7, Maay 22, 1981.

Information about application procedures can be obtained from Dr. Eugene J. Oliver, Health Scientist Administrator, Neurological Disorders Program, NINCDS, Federal Bldg., Rm. 710, Bethesda, Md. 20205; tel: (301) 496-6541.

INSTITUTE SCIENTISTS AUTHOR BRAIN DYSFUNCTION BOOK

The collaborative efforts of NINCDS scientists Drs. Paul L. Nichols and Ta-Chuan Chen have resulted in a new book: *Minimal Brain Dysfunction: A Prospective Study.*

The book is one of a series of volumes dealing with specific developmental problems in children whose mothers participated in NINCDS's Collaborative Perinatal Project. The publication examines the relationships between minimal brain dysfunction symptoms and over 300 pre- and postnatal variables found in a group of nearly 30,000 children.

Among the many findings reported in the study are some with possible implications for public health policy. A strong relationship was discovered, for example, between maternal smoking and subsequent behavioral, learning, and neurological problems in children. In addition, many presumed perinatal hazards had less effect on children than did some social-class and demographic variables.

The 300-page book can be obtained from the publishers: Lawrence Erlbaum Associates, Inc., 365 Broadway, Hillsdale, N.J. 07642.

BOOKLET SERIES OUTLINES NIH TRAINING OPPORTUNITIES

A new series of pamphlets, *Research and Research-Related Manpower Development Programs Supported by the National Institutes of Health*, outlines postdoctoral and other training opportunities available in the biosciences through NINCDS and its sister organizations.

The four-part series of booklets examines NIH training programs for the following educational levels:

- postdoctoral
- postbaccalaureate
- college
- high school

Program summaries are provided in each pamphlet; included is information on eligibility, funding, points of contact, and application deadlines.

For copies of individual booklets or the complete four-part series, contact the Grants Inquiries Office, Division of Research Grants, Rm. 449, Westwood Bldg., 5333 Westbard Ave., Bethesda, Md. 20205 Tel: (301) 496-7441.

This information is prepared monthly by the Office of Scientific and Health Reports, National Institute of Neurological and Communicative Disorders and Stroke, National Institutes of Health, Bethesda, Md. 20205. Tel: (301) 496-5751.

REYE'S CONSENSUS STATEMENT RELEASED

Final recommendations have been issued by a 13-member panel of experts for the diagnosis and treatment of Reye's Syndrome, the subject of an NIH consensus development conference held in Bethesda, Md., in March.

The NINCDS was lead agency for the conference, which provided a forum for considering signs, symptoms, and laboratory findings associated with Reye's Syndrome. Participants at the conference, including research scientists, physicians, and parents, discussed the effectiveness of diferent treatments and the further studies needed to understand and treat the illness. The resulting consensus statement strongly recommended wide distribution of information about Reye's Syndrome.

Panel members urged prompt medical attention for children who "during or while recovering from a viral illness (most commonly chicken pox or influenza) unexpectedly develop repetitive vomiting and altered behavior such as lethargy, confusion, irritability, or aggressiveness". The consensus statement outlines laboratory tests helpful in diagnosing Reye's Syndrome, and advocates a uniform five-stage system for measuring the severity of the illness and for guiding treatment. In addition, the statement identifies a "lengthening list" of illnesses that mimic Reye's Syndrome and cause diagnostic problems for the physician.

The consensus panel also addressed the question of differing treatments at different stages of the illness. The administration of dextrose-containing fluids was regarded by panel members as a standard therapy for the earliest stage of the syndrome; osmolal therapy and hyperventilation were recognized as common treatments for controlling the intra-cranial pressure frequently found in later stages of the illness. Other pressure-control methods, including high-dose barbiturates, corticosetroids, cerebrospinal fluid withdrawal, and decompressive craniotomy, were judged to be still "experimental."

The usefulness of intracranial pressure monitoring generated some debate among conference participants. The consensus statement concludes: "Some physicians believe it improves their ability to manage patients; others do not."

The possible relationship between Reye's and the use of salicylates or acetaminophen was also explored. Panel members counseled physicians and parents to "be aware that most, if not all, medications have potential deleterious effects thus, caution in the use of salicylates in children with influenza and those with varicella is prudent." The panel agreed, however, that more data were needed before changes in current fever management practices could be recommended.

While emphasizing that complete recovery from Reye's Syndrome could be expected in the majority of cases, panelists also agreed that possible psychological after effects might be seen in some children who experience the more severe stages of the illness. Panel members offered useful suggestions to help ease the recovery period.

The consensus statement identifies several research areas for greater attention. The etiology and pathogenesis of Reye's Syndrome, with prevention the ultimate goal, were judged to be the most critical research concerns; epidemiologic. diagnostic, and management and outcome studies were also urged.

Copies of the final Reye's Syndrome con-

sensus statement can be obtained from Michael J. Bernstein, Director of Communications, Office for Medical Applications of Research, NIH, Bldg. 1, Rm. 216, Bethesda, MD 20205; tel: (301) 496-1143. Proceedings of the conference will be available later.

COMPUTER TOMOGRAPHY CONFERENCE SET FOR NOVEMBER

"Computer Tomography Scanning of the Brain" will be the subject of an upcoming consensus development conference sponsored by the National Institutes of Health.

The conference will be held November 4-6, 1981, in Masur Auditorium of the NIH Clinical Center (Bldg. 10).

The NINCDS is the lead Institute for this conference. Co-sponsor is the National Cancer Institute, in conjunction with the National Center for Health Care Technology. Assistance will be provided by the NIH Office for Medical Applications of Research.

The purpose of the conference is to reach agreement on issues involving computerized tomography (CT) scanning of the brain. Key questions include: What are the indications for employing CT scanning as a primary or secondary diagnostic tool for lesions of the brain? Are there any contraindications? How much radiation is delivered during use of current CT scan equipment, and how is this dosage commonly expressed? Has CT scanning influenced the management of intracranial disorders, such as malignancy, trauma, vascular anomalies, and cerebrovascular disease? Has the availability of CT brain scanning influenced the use of other methods of imaging the brain? What is the practical limit of definition and resolution in CT scanning that may preclude its value in the diagnosis of brain disease? What can be expected of future efforts in the development of CT scanning beyond its current diagnostic capabilities?

This consensus development conference will bring together biomedical research scientists, radiologists, radiation therapists, neurologists, neurosurgeons, other practicing physicians, consumers, and other persons from relevant fields. On the first two days, a series of experts will present their experiences and discuss the key issues with panel members responsible for developing a statement of consensus. On the third day, the panel, chaired by Dr. Fred Plum, chairman of the Department of Neurology at Cornell University Medical College, will present its preliminary report and invite comments from the audience.

Technical information on the conference can be obtained from Dr. Michael Walker, Director, Stroke and Trauma Program, NINCDS, Federal Bldg., Rm. 8A08, 7550 Wisconsin Ave., Bethesda, Md. 20205; tel: (301) 496-2581. Administrative arrangements are being handled by Ms. Yvonne Lewis, Prospect Associates, 11325 Seven Locks Rd., Suite 220, Potomac, Md. 20854; tel: (301) 983-0535.

HUNTINGTON'S DISEASE ROSTER A NEW "MATCHMAKER" FOR RESEARCH

The national Huntington's disease research roster is proving to be a natural "matchmaker" between scientists studyng the disease and HD patients and their families eager to facilitate research.

The NINCDS-supported roster, developed under the direction of Dr. Michael Conneally by Indiana University Medical Center's Department of Medical Genetics, offers HD families the chance to participate in research while supplying interested scientists with useful demographic or statistical data. The roster also allows investigators throughout the country to identify potential research subjects who are willing to be contacted and to locate individuals of particular research interest.

Huntington's disease families who agree to participate in the national roster are sent a family history questionnaire to complete and return, along with a consent form explaining the roster and its uses. The roster is volutary, and provisions have been made to maintain strict confidentiality. Completed questionnaires are checked by the roster staff, and may be followed up to confirm diagnoses and clarify inconsistencies or omissions. Pedigrees are linked where possible.

Scientists who ask for statistical data not involving names are sent the information. In cases where the names of specific families or individuals are needed (to obtain

tissue samples, for example), roster officials contact the family and explain the request. Roster personnel must obtain written permission from the family or the patient before releasing a name to an investigator.

Additional details about the HD roster are contained in a pamphlet available from Indiana University. To obtain the publication, or to participate in the project as a registrant or investigator, contact the HD Roster, Department of Medical Genetics, Indiana University School of Medicine, 1100 W. Michigan St., Indianapolis, Ind. 46223; or call collect (317) 264-2241.

NINCDS OFFERS REVISED SPECIAL REPORTS

Special reports on NINCDS research programs in multiple sclerosis, Huntington's disease, stroke, and spinal cord injury and nervous system trauma have been revised for 1981 and are now available in limited quantities.

These reports briefly describe the etiology, diagnosis, treatment, prevention, and ongoing research for specific disorders. The publications are prepared by the NINCDS Office of Scientific and Health Reports (OSHR) in connection with yearly Congressional appropriation hearings.

An additional revised special report on the NINCDS hearing, speech, and language program will be published soon. Still available are 1980 reports on epilepsy, muscular dystrophy and other neuromuscular disorders, spina bifida and neural tube defects, and amyotrophic lateral sclerosis.

Single copies of these reports may be obtained from the Office of Scientific and Health Reports, NINCDS, NIH, Bldg. 31, Rm 8A06, 9000 Rockville Pike, Bethesda, Md. 20205; tel: (301) 496-5751.

VOLUNTARY AGENCY PAMPHLET UPDATED

A revised and expanded edition of the NINCDS pamphlet *Voluntary Health Agencies Working to Combat Neurological and Communicative Disorders* is now available.

The pamphlet lists more than 50 voluntary health organizations alphabetically by disease category, and gives addresses, phone numbers, and key personnel for each organization.

Copies can be obtained from the NINCDS Office of Scientific and Health Reports, NINCDS, NIH, Bldg. 31, Rm 8A06, 9000 Rockville Pike, Bethesda, Md., 20205; tel: (301) 496-5751.

This information is prepared monthly by the Office of Scientific and Health Reports, National Institute of Neurological and Communicative Disorders and Stroke, National Institutes of Health, Bldg. 31, Rm. 8A06, Bethesda, Md. 20205.
Tel: (301) 496-5751.

INTERNATIONAL SYMPOSIUM

PSYCHOPHYSIOLOGICAL RISK FACTORS OF CARDIOVASCULAR DISEASES:

Psychosocial Stress, Personality and Occupational Specificity
with a Workshop on Emotional States, Methods of Analysis and Physiological Correlates
Karlovy Vary (Carlsbad), Czechoslovakia
September 7 — 11, 1981.
Site of the Symposium:
Hotel Moscow (Pupp)

ORGANIZED BY

Scientific Committee for Psychophysiology and Neurotoxicology
— of the Collegium Internationale Activitatis Nervosae Superioris (CIANS), Section of the World Psychiatric Association.
— and of the Permanent Commission and International Association of Occupational Health.

SPONSORED BY

WHO Psychosocial Centre, Stockholm
European Brain and Behaviour Society
European Society of Cardiology
Charles University Medical Faculty of Higiene, Prague
Institute of Clinical and Experimental Medicine, Prague
Institute of Hygiene and Ejidemiology, Prague.

LOCAL ORGANIZERS

Czechoslovak Medical Society J. E. Purkyné
Czechoslovak Society for the Study of Higher Nervous Functions

Czechoslovak Society of Cardiology.

The Symposium has been convened in honour of the 90th birthday of Academician Vilém Laufberger, founder of Czechoslovak psychophysiology and active research worker in electrophysiology.

SCIENTIFIC PROGRAMME

MAIN TOPICS

Methodical discussions on psychophysiological pathogenetic mechanisms of cardiovascular diseases and on intervention procedures of their risk factors will continue on the line started by the L. Levi's WHO Symposia Series "Society, Stress and Disease".

The Workshop on Emotional States, Methods of Analysis and Physiological Correlates will discuss the topics of negative emotions which are supposed to play an important role in the etiology of psychophysiological disorders.

One session on "Psychosomatic Risk Factors: An Indication for Specific Preventive Intervention" (co-organized by CIBA-GEIGY Basel) will be oriented toward the position of pharmacotherapy in the complex treatment of risk factors of ischaemic heart disease especially of hypertension, in the context of individual behavioral and psychophysiological characteristics.

NATIONAL MIGRAINE FOUNDATION

LECTURES

The Spring issue of the Newsletter presented abstracts of several of the lectures delivered at the Sixth Annual Continuing Education Course: The Diagnosis and Treatment of Headache, convened in Scottsdale, Arizona, January 9 - 11, 1981. Several more are reviewed here:

Dr. Robert Kunkel noted that *cluster headache* was a particular type of headache which was first described years ago but which is still not well recognized by many persons and physicians. Initially, sensitivity to histamine was thought to be the cause of this headache but this is no longer true.

Cluster headache presents with such striking signs and symptoms that one wonders why it is often not recognized and why more is not known about it. It occurs predominantly in males with the average age of onset the early forties. With rare exceptions, the painful episode is short, sometimes less than 30 minutes and no more than three hours. Attacks may occur several times in a 24 hour period, often with striking regularity at the same time day after day, with the early morning hours, one to two hours after retiring, the most common time of attack.

The pain of cluster headache is intense, so much so that the sufferer can rarely lie still, like the migraineur. In the midst of an attack, the patient will jump out of bed and pace the floor, bang his head on the wall, hit his head or undertake some other form of vigorous exercise. The pain is more often a steady, boring or burning pain rather than the pulsating throbbing headache that is migraine.

Cluster headache is almost always unilateral and is usually located behind the eye. There is rarely a warning or aura, with the pain usually reaching its peak intensity very quickly once the attack begins. The pain is often accompanied by a variety of symptoms, notably tearing of the eyee, nasal stuffiness and congestion on the same side as the head pain, followed by rhinorrhea.

As the name implies, cluster headache is typically a periodic condition where attacks occur daily for several weeks or even several months following which there is a remission with pain-free intervals of varying duration. Some 10 % of patients with this type of headache do not experience remissions and this is said to be *chronic cluster headache.*

Patients with cluster headache exhibit certain personality characteristics; they are more likely to experience ulcer disease, the majority smoke more and drink more than the average population, and they demonstrate high scores in such traits as conscientiousness, responsibility, self-sufficiency, resourcefulness, tension and anxiety, frustration and aggression.

A distinctive facial appearance appears to be typical of many patients with cluster headache: —a ruddy complexion with the skin thickened, coarse and deeply pitted; prominent furrows on the forehead with vertical creases between the eyebrows; the chin often jutting and square cut with dimpling and possibly a cleft in the chin.

It is generally agreed now that while

both migraine and cluster headache are vascular and therefore related, they are not variants of the same syndrome, but exhibit sufficient differences to label them as separate entities. Differences include sexual incidence, duration of attacks, character of pain as well as some biochemical alterations during the course of an attack.

The treatment for cluster headache is essentially pharmaceutical, with the three most useful drugs being methysergide, ergotamine tartrate and corticosteroids. Most clusters can be shortened or controlled by using one or a combination of these drugs. Lithium has been found quite useful recently in controlling attacks, with its prime use being in chronic cluster headache.

Treatment of the acute attack is difficult because of the brief duration and sudden onset, but ergotamine tartrate or oxygen inhalation can be useful in shortening or aborting the individual attack.

In describing *migraine, Dr. Seymour Diamond* emphasized that vascular dilatation was common to the four types, classic, common, hemiplegic and ophthalmoplegic migraine. In classic migraine, this vascular dilatation follows an initial phase of vasoconstriction in which preheadache experiences are described, most often of a visual nature. These do not occur or are not recognized by the patient suffering common migraine and this serves as the main difference between it and classic migraine. Hemiplegic migraine and ophthalmoplegic migraine are considered to be more severe forms of classic migraine.

Migraine afflicts more women than men and usually begins in the second and third decades of life, though it may appear in childhood. A positive family history is obtained in about 70 % of those experiencing migraine. The headache is described as a dull ache, progressively worsening and developing into a throbbing or pulsating pain. About 70 % of migraine is one-sided, frequently affecting the frontal or temporal regions, but it may settle behind the eye. While attack may vary from one every few days to one or two a year, most frequently patients report two to four attacks a month. Most migraine episodes last longer than four hours and usually for a day or two. Onset can occur at any time of the day, not uncommonly on waking.

An inability to adapt to stressful situations creates anger and resentment and has been described as the repressed hostility of the migraine patient. Migraine patients as a whole tend to build for themselves lives with too many environmental demands. They are extremely sensitive to overload, so it is not surprising that attacks occur more frequently when the patient is excessively tired.

Various misconceptions exist about the influence of diet, in part because of the folklore and lack of adequate scientific exploration. As a result, many migraineurs are apt to have constructed elaborate diets in the hope of escaping recurrent migraine attacks. Occasionally, these diets assume ridiculous extremes for example, one may find that a patient is living primarily on scallions or bananas.

It is true that some ingested substances will provoke vascular headache in susceptible individuals. Alcohol produces a vascular headache in migraine patients, especially if they are in a migraine phase, and it is a prime catalyst in patients with cluster headache, who are highly sensitive to even small amounts of alcohol.

Foods which contain tyramine, a vasoactive amine, may precipitate headache in migraineurs, possibly because they appear to metabolize it in a manner different from normal controls. Foods rich in tyramine include strong or aged cheeses, pickled herring, chicken livers, canned figs and the pods of broad beans. This is an example of a vasoactive substance producing headaches and is not to be considered a real allergic reaction. So too is moderately severe headache after the ingestion of cured meat products such as hot dogs, bacom, ham and salami. These contain sodium nitrite which maintains their red coloring and which acts as the trigger in susceptible patients.

Approximately 70 % of the women with migraine say that some of their attacks occur prior to, during, or at the end of their menstruation. By the third month of pregnancy, most women are free of their migraine attacks except for a very small number who get their first attack with pregnancy. The oral contraceptives and post-menopausal hormone therapies usually increase the severity and frequency of migraine attacks.

Treatment of migraine may be abortive for headaches that occur occasionally and prophylactic for headaches that occur frequently. For abortive or symptomatic relief of the acute attack, drugs used include ergotamine tartrate, dihydroergotamine and isometheptene mucate. For prophylaxis, methysergide, propranolol, MAO inhibitors and platelet inhibitors as well as amitriptyline are the drugs most often employed.

Dr. William Speed emphasized that the diagnosis of *muscle contraction headache* depends on the characteristics and pattern of headache, and that it was imperative for the physician to obtain a thorough and detailed history and to undertake a physical examination with particular emphasis to the head, neck and neurologic system. Special attention must be focused on emotional factors since they appear in many patients with headache.

A distinction must be made between the two types o muscle contraction headache, episodic and chronic. The *episodic type* is quite common, with the headaches being usually mild to moderate in intensity and described as tight, pressing, squeezing, or aching sensations. They may be triggered by fatigue, an acute family crisis, peaking of stressful work loads or any temporary stressful situation. They usually subside following the cessation of the offending stimulus or may be relieved by over-the-counter analgesics.

Chronic muscle contraction headache is constant and unremitting and may be present for weeks, months or years. While these resemble the episodic type in the character of the pain, they also give rise to descriptions such as soreness, weight-like sensations, tight bands, a feeling like that of a tight skull cap, or crawling sensations. At times there may be intermittent jabbing, stabbing or piercing components.

Some patients with muscle contraction headache may also exhibit an associated vascular headache, described as throbbing and pounding. The intensity of the latter is aggravated by jarring the head, bending over, coughing, sneezing, straining, by exposure to bright lights, loud noises and physical exertion, but these stimuli do not affect the intensity of the muscle contraction headache.

Muscle contraction headaches may be secondary to certain underlying disorders such as eye disturbances, nasal and paranasal inflammation, temporomandibular joint dysfunction and viral infections, but it is far more likely that they are associated with tension, anxiety, depression, repressed hostility, unresolved dependency needs and psychosexual conflicts.

It is futile to try to treat chronic muscle contraction headaches with the over-the counter analgesics used for the acute type for they are inadequate for the purpose. Too many patients tend to use ever-increasing amounts with little benefit but with the increasing likelihood of habituation or perpetuation of the chronicity of the pain.

The tricyclic antidepressants are the most useful agents for the treatment of chronic muscle contraction headaches. It is not clear whether the relief obtained is due to an antidepressant effect or to an analgesic effect mediated by interaction with brain neurotransmitters.

Biofeedback is considered of value in many such patients, but it is important to realize that this is most helpful in patients who are motivated to succeed in such training and who have the ability to concentrate.

It is highly important that a strong effort be made to give patients an understanding of their problem and to help them realize that neither physician nor patient working alone can resolve the headache complaint. Good physician/patient dialogue is important and repeated office interviews may prove rewarding.

ACUPUNCTURE:

AN APPRAISAL

Excerpts are presented here of an article by George A. Ulett, M.D., Ph. D, St. Louis. Mo., which appears in the Journal of the American Medical Association. It provides a current and much-needed assessment of acupunture for the relief of pain.

ACUPUNCTURE is the most controversial of nondrug pain control methods in contemporary US medicine. This form of treatment originated in China some 4,000 years ago. It has been of interest in the United States for scarcely a decade although widely used in Europe since early in this century and universally acclaimed for its

pain-relieving qualities.

TRADITIONAL ACUPUNCTURE

United States physicians have failed to embrace acupuncture for several reasons, but mainly because, as it is usually presented to them, it has lacked documented scientific validity and has been taught as a practice based on ancient Taoist philosophy passed down through the centuries with relatively little change.

Because fanciful inferential theorizing is customary for unorthodox practitioners, chiropractors and other non-MD's have dominated the practice of acupuncture, influenced state legislatures for licensing, established courses for teaching traditional acupuncture, and opened "acupuncture-mill" —type clinics nationwide. As their diagnostic and treatment methods have not always been acceptable and they have treated all types of illness without discrimination, this, too, has served to alienate practicing physicians.

Notwithstanding the foregoing, a small but increasing number of US physicians have found acupuncture to be a useful part of their practice, despite the inability to explain in terms acceptable to their colleagues how they obtained favorable results by this method of treatment. Thus, every day in the United States and elsewhere, thousands of patients are being treated with acupuncture and reporting favorable results to their friends and neighbors. Family physicians are with increasing frequency being asked: "As your treatments have not relieved my pain should I now try acupuncture?"

MODERN ACUPUNCTURE

Alternative, scientific explanations for the action of acupuncture are now appearing from many laboratories, including some in Sweden, Austria, and Canada, as well as the United States and the People's Republic of China. For those physicians faced with the necessity to treat patients with pain that is chronic and nonresponsive to the usual methods of chemical analgesia, acupuncture now seems a reasonable alternative.

While traditional acupuncturists select from among some 400 or more points located on hypothetical meridians, the modern acupuncturist uses a smaller number of points. Work from Albert Einstein Medical School has pointed out that many of the most effective acupuncture points coincide with the motor points commonly used in electromyography, and, indeed, it has seemed that these may be the only points that need stimulation. In the treatment of pain, motor points are selected from within the same dermatome or a neural segment adjacent to the area of pain. Other points are selected from the extremities where there are located the largest number of muscles. It is perhaps more than coincidental that one of the most effective and widely used acupuncture points, Ho Ku, is located at the base of the thumb. The thumb, of all the digits and limb segments, has the largest cortical representation as demonstrated by both sensory and motor homunculi.

Rapidly gaining scientific support is the theory that, at least in part, acupuncture works through the release of brain neurotransmitters. While there is as yet a dearth of direct evidence that acupuncture induces a release of endorphins in the brain of humans, yet in animals electroacupuncture can slow the reaction to noxious stimuli, and naloxone blocks the effect of acupuncture stimulation.

Electrical stimulation has been found more effective than needle twirling, an observation that is substantiated by reports from our own laboratory. Stimulation must be sufficiently above the threshold of medium-sized fibers. Too strong a stimulus, however, activates the finer C fibers and serves to intensify pain.

The hypothesis that one kind of sensory input may be inhibited by another kind is within everyday experience, whereby rubbing, massage, or pressing of a body part may somewhat relieve pain. Also severe paain may initiate reflex acts of jaw and fist clenching and muscle tightening, which tend to make the pain more bearable.

Stimulation by needles inserted deeply into muscle, as with electroacupuncture, produces not only skin stimulation but also stimulation of medium-size afferents from the muscle tissue as well. With this the changes produced in the CNS* seem to be of a magnitude sufficient to continue the

beneficial effect long after the stimulation has ceased. In some instances permanent relief occurs.

CONCLUSION

While a sizeable number of experiments and clinical observations have been reported, much more work is needed to answer the many questions that still exist about acupuncture treatment. The evidence now available, however, is sufficient to place this age-ald Chinese healing art, modernized to US standards, on a solid scientific base. There seems little doubt but that a more physiologically determined acupuncture-type stimulation can and will play an increasingly important role in the relief of pain.

TOURETTE SYNDROME ASSOCIATION

The First International Gilles de la Tourette Syndrome Symposium took place in New York City on May 27, 28 and 29, 1981. Co-chairmen were Dr. Thomas N. Chase, Director of Intramural Research at NINCDS, and Dr. Arnold J. Friedhoff, Professor of Psychiatry and Director of the Millhauser Laboratories at the New York University School of Medicine. This Symposium was sponsored by NINCDS, The Gateposts Foundation, Inc. of New York, and the Tourette Syndrome Association.

Almost three hundred physicians and scientists from around the world participated in this meeting. They came from China, Japan, India, England, Belgium, France, Denmark, Germany, and Canada, as well as every section of the United States. The topics covered were:

Clinical Aspects
Basic Structural and Functional Aspects
Animal Models
Clinical Pathology and Chemistry
Genetics
Epidemiology
Neuropsychology
Clinical Pharmacology

As the Chairman of the Gateposts Foundation, H.B. Pearl, wrote in the Preface to the Program, this meeting offered a unique opportunity for scientists to meet and exchange views with colleagues from around the world, and thereby lay the foundation for a clearer understanding of, and more effective treatment for, this disabling disorder.

Raven Press expects to publish the proceedings in the early part of 1982.

BOARD OF DIRECTORS HONORS VOLUNTEERS AT BANQUET

The Symposium banquet, held on May 28, 1981, provided an opportunity for the T.S.A. Board of Directors to publicly honor some individuals who have made an important contribution to this Association's work.

Those honored were:

Dick Gavett, who has been our first Honorary National Chairman and has donated his time and talent to the creation of public service radio and televisión commercials which are enabling thousands of people with Tourette Syndrome to be diagnosed.

Erica Feinholtz, Director of Scientific Projects, who has worked untiringly to expand our understanding of T.S.

Orrin Palmer, a courageous young man who has been an inspiration to all those with Tourette's.

Eleanor Pearl, whose selfless dedication and compassionate devotion and generosity have helped to ease the burden of those suffering from T.S.

H.B. Pearl, in gratitude for over ten years of devoted service, dedicated commitment, and generous support on behalf of Tourette Syndrome victims.

Ogilvie and Mather, the advertising agency, was honored for its contribution of talent and effort in the production of our Public Service announcements. Diane Courtney accepted the award for Ogilvie and Mather as well as for Dick Cavett, who was unable to attend.

Judy Wertheim, President of the Tourette Syndrome Association, had the pleasure of presenting plaques to the above individuals. In addition, Dr. Sheldon Novick, Medical Director of the T.S.A., was honored for his contributions, and received a newly reprinted edition of Samuel Johnson's original dictionary in gratitude for his efforts to make the medical community aware of this illness.

TOURETTE SYNDROME WEEK
May 24, 1981

Both New York City and New York Sta-

te proclaimed the week of May 24 to be "Tourette Syndrome Week" in honor of the First International Symposium on Tourette Syndrome.

T.S.A. MEDICAL DIRECTOR ADDRESSES BANQUET

Dr. Sheldon Novick spoke on the role of the Medical Director of the T.S.A., and shared with the audience some of his early efforts at stimulating interest in the illness among the medical community.

He recalled how, in 1974, very few physicians were knowledgeable about Tourette Syndrome. Our T.S.A. membership, at the time, numbered about 100 individuals. It was clear that progress in this disorder would not come until more physicians became interested in T.S., and patients began to be properly diagnosed. Dr. Novik contacted those physicians who had written on T.S. as well as those working in related areas, both in this country and abroad. As a direct result of these contacts, our Newsletter began to list research centers interested in T.S. across the country. Other Association members actively pursued publicity in the media, and the growing number of diagnosed patients demonstrated that Tourette's was not rare, and this was itself an encouragement to research. So, too, was our members' willingness to participate in research projects. As Dr. Novick concluded, the Symposium, with its large attendance of some of the world's most prominent clinicians and researchers, "vividly demonstrates the growth of interest in Tourette Syndrome, and gives us hope that the accumulating knowledge will benefit all who suffer from this disorder."

FIRST POSTDOCTORAL RESEARCH FELLOWSHIP IN TOURETTE SYNDROME

Two one-year Postdoctoral Fellowships will be awarded in 1982 to selected applicants for research related to Tourette Syndrome. The Fellowships will be between $ 17,000 and $ 20,000 each, commensurate with experience, and may be done at any institution with adequate facilities. Candidates who have completed their doctoral training (M.D., or Ph.D., or equivalent) and desire postodctoral research experience are eligible. Previous research experience in movement disorders is desirable but not essential. Apply by letter, sending 12 copies of the following:

curriculum vitae, list of publications, current sources of reseaarch support, at least two letters of recommendation, and an abstract (not to exceed four pages) of proposed research.

The latter, focused on any aspect of relevance to Tourette Syndrome, should contain information with respect to:

1. candidate's qualifications and objectives;
2. scientific background for project;
3. methods;
4. significance;
5. percentage of time to be devoted to project;
6. sponsor; and
7. research facilities and support available for project.

Candidates are welcome whose field of interest is one of the following; all areas of basic sciences including neuroscience, pharmacology, biochemistry, and molecular biology, or from areas of clinical sciences including neurology, psychiatry, immunology, and human genetics.

Applications should be submitted to:

Sheldon Novick, M.D.
Medical Director, Tourette Syndrome Association 40-08 Corporal Kennedy Street, Bayside, N.Y. 11361 Telephone: (212) 224-2999

Candidates will receive notification by January 15, 1982 for award effective July 1, 1982.

SUMMARY OF SCIENTIFIC HIGHLIGHTS

Editor's Note: We asked the chairpersons of each section to summarize the highlights of their section for our readers.

Clinical Overview *Thomas N. Chase*

Leading of the Clinical Overview session, Dr. Arthur Shapiro (Mt. Sinai School of Medicine) reviewed the history and current status of TS based on 16 years experience with over 1,000 patients. Emphasizing both the fact and fiction of TS, Dr. Shapiro placed in clear perspective the neurologic, be-

havioral, genetic, and therapeutic aspects of this disorder. Dr. Stanley Fahn (Columbia University) then analyzed the clinical spectrum of motor tics. He noted that many extrapyramidal movement disorders such as tremor, chorea, and athetosis are reasonably well defined, have a limited spectrum, and usually consist of simple movement patterns. By contrast, motor tics are complex coordinated movements, noted for their variability and migration in the individual patient. Most commonly involving the face, head or neck, tics usually lack rhythmicity and vary in intensity. Dr. Donald Cohen (Yale University) next presented his views on the interaction of biological and psychological factors in the natural history of TS. He considers TS as a familial, neuropsychiatric disorder, affecting mechanisms of psychomotor inhibition. Biological vulnerability may be expressed in attentional, learning, and behavioral problems before onset of tics. Progression of the motor disorder may be accompanied by persistent Attention Deficit Disorder, compulsions, obsessions, and impulsivity. The range of symptoms and their severity— from chronic multiple tics in family members to incapacitating, complex tics and compulsions— may reflect a genetic heterogeneity of biological and experientially modified patterns of response to the underlying diathesis. The heterogeneity of TS was further discussed by Dr. Eric Caine (University of Rochester) based on clinical observations during the administration of d-amphetamine, l-amphetamine, and haloperidol. In these studies vocal and motor tics did not respond to stimulation by these drugs in a uniform fashion, thus suggesting that they reflect more than one neurochemical abnormality.

Dr. Harold Klawans (Rush University) reviewed Gilles de la Tourette's original description of the syndrome that now bears his name. It had never before been published in English. Tourette is now remembered chiefly for having described this condition, but his view as to its differential diagnosis, pathophysiology, and treatment are now largely forgotten. Based on a careful translation and reexamination of Tourette's treatise, Dr. Klawans was able to make numerous observations of historical interest and of relevance to modern neurology. In the Session's final presentation, Dr. T.J. Murray (Dalhousie University) discussed Dr. Samuel Johnson's movement disorder. Dr. Johnson was noted by friends to have almost constant tics and gesticulations and to make various noises and whistling sounds Furthermore, he often displayed compulsive acts such as touching posts, measuring his footsteps on leaving a room, and gesturing in a complex manner before crossing a threshold. Thus his symptoms of involuntary jerking movements, vocalisations, and compulsive actions constitute the syndrome complex of Gilles de la Tourette from which Johnson suffered most of his life.

Clinical Pharmacology *Dr. Stanley Fahn*

Dr. Van Woert presented an overview on pharmacological studies in T.S. He discussed the dopamine hypothesis and pointed out that the evidence for it is not strong.

Dr. Leckman discussed the results with clonidine and reported that some patients can do well with this drug. There is no correlation between motor and vocal tics in their response.

Dr. Moldofsky studied pimozide in T.S. He found this drug to be as effective as haloperidol. Pimozide produced a rise in serum prolactin levels in all patients, regardless of their response clinically.

Dr. Novick, in the discussion, reported that a woman with T.S. had a marked lessening of symptoms during pregnancy. Dr. Fahn polled the audience and found four other clinician who had similar experience in one patient each. This made a total of five women in whom pregnancy ameliorated tics.

Drs. E. and A. Shapiro discussed the types of dyskinesias that are seen following haloperidol treatment. Withdrawal dyskinesias, akathesia and dystonia have been seen. They feel that T.S. patients are less likely than psychiatric patients to develop dyskinesias. In the discussion that followed, various cases of haloperidol-induced dyskinesias were mentioned. Dr. Fahn pointed out that children are more likely to develop the dystonic form of tardive dyskinesia rather than the choreatic form.

Dr. Borison presented four studies. He reported that 1) lithium was rather ineffectual, 2) clonidine is as effective as haloperidol and usually in the same patients, 3) phenothiazines are as effective as halo-

peridol, and 4) amantadine can overcome some of the neuroleptic-induced adverse effects.

Dr. K. Davis reviewed cholinergic drug studies in various movement disorders including T.S. Although such drugs have to been effective so far in T.S. patients, newer drugs should be studied. Dr. Davis plans to evaluate oral physostigmine in T.S.

Dr· Stahl pointed out the evidence for both excesive dopamine activity and for a lessened cholinergic activity in T.S. patients. He described the entity of tardive Tourette Syndrome, having seen some cases himself and mentioning others reported in the literature. He speculates that treatment of T.S. with haloperidol may only give shortterm relief, and instead may, in fact, be creating long-term or tardive Tourette Syndrome.

Dr. Rapoport studied the coincidence of both hyperactivity in children and tic syndromes. About an equal number of children received stimulants for treatment of hyperactivity before the onset of tics as those receiving them after the onset of tics. In some patients, the stimulants did not seem to aggravate tics.

Genetics *Dr. Xandra Breakefield*

Three papers were presented on the inheritance pattern of the GT syndrome in family pedigrees and on attempts to find biochemical markers which correlate with this syndrome. Dr. Kenneth Kidd from Yale University reported on the familial association of the GT syndrome and multiple tics. Males are more frequently affected with these traits as compared to females. and affected females show a larger proportion of affected family members as compared to affected males. These findings suggest that a larger number of genetic and environmental factors are necessary for expression of this syndrome in females. Since monozygotic twins which share identical genes do not necessarily both express the GT syndrome, it is clear that environmental factors have a critical role in the manifestation of the disease. The mode of inheritance of predisposing genes in families is not clear. The data is compatible with either many genes or a single gene determining susceptibility, and in fact the responsible gene(s) may be different in different families. Studies by Dr. Israel Hanin from the Western Psychiatric Institute show that GT patients have higher levels of choline in their red blood cells as compared to controls, but unaffected family members of these patients also have high levels. Thus this biochemical marker, which may reflect one aspect of the metabolism of the neurotransmitter acetylcholine in these individuals, is not associated with expression of the disease, but may represent a modifying factor. Dr. David Comings of the City of Hope presented evidence that gene(s) involved in expression of the GT syndrome are not linked to genes at the histocompatibility locus.

The limited availability of information on the genetic basis of the GT syndrome rests in large part on the limited number of linkage markers currently available on the human genome, and on the small number of biochemical markers that can be assessed in tissues which can be readily obtained from humans· Dr. Frank Ruddle presented exciting new approaches in molecular genetics which will expand our ability to identify the abnormal genes and gene products responsible for inherited human diseases of unknown etiology. By taking advantage of normally occurring variations in the structure of DNA itself it will be possible to construct a set of linkage markers which cover the entire human genome. Thus in a pedigree in which a discrete set of genes determine expression of the GT syndrome, it will be possible to locate and isolate the responsible portion(s) of the genome using DNA from white blood cells. This portion can be replicated (cloned) in a bacterial host by recombinant DNA technology and used to establish the nature of gene products for which it codes. Further, by gene transfer of this DNA clone to mouse embryos it may be possible to establish animal models of the disease. Although these approaches are a number of years away, they are feasible and will eventually provide the information necessary to unravel the molecular basis of this syndrome.

Epidemiology *Dr. Rasmus Fog*

There are no reliable incidence or prevalence rates for Tourette Syndrome and only estimates have been made.

Neuroepidemiology gives rise to many methodological problems. Dr. Bruce S. Sch-

oenberg from NINCDS gave an excelent review of how epidemiological research should be designed. Dr. A.R. Lucas from the Mayo Clinic tried to estimate the frequency of the disorder based upon an investigation in Rochester from 1976-1981· What he called a ' risky extrapolation" was an expectancy of 4.6 new cases per million inhabitants per year in the U.S.

Dr. K. Kondo from the Brain Research Institute of the Nigata University in Japan gave a survey of 80 patients with Tourette Syndrome and concluded that no clear-cut Mendelian transmission of the disorder is tenable.

Clinical Studies

Bennett A. Shaywitz, Donald J. Cohen

The session on clinical studies covered a range of methods and populations. Dr. Marsden from London presented an elegant study of electroencephalographic (EEG) correlates of motor tics and voluntary movements. A negative. upgoing EEG potential precedes voluntary movements but not tics. This neurophysiological distinction suggests the view that tics are not generated in the same manner as the voluntary acts which use the same musculature. Using surface electrodes, it is difficult to define the structures subserving tic movments· Somatosensory evoked potentials may aid in clarifying aspects of TS neurophysiology, as described by Dr. Desmedt of Belgium. Little research has been done using somatosensory potentials in TS but that which has been done has shown them to be normal. Dr. Ludlow and her collaborators from the NIMH described some of the major features of language disorders in TS, and, in extremely careful studies, have laid the groundwork for further delineation of subgroups of patients.

The most widely publicized language feature of TS is coprolalia, and Dr. Nuwer from UCLA investigated the nature of this symptom using computer methodologies of language analysis. In ordinary, "normal" language, there are two common classes of swear words —those referring to the body and bodily acts (fuck, shit, ass, prick, etc.) and those with theological connotation (God, God-damn, Christ, etc.) In TS, body curse terms are by far the most frequent coprolalic words, and there is a paucity of theological terms. Computer generated language which starts from random phonemes generates surprisingly many common swear words of the body function, body-part nature, and not theological curses. Body swears are, thus, phonetically common. This model in part explains one aspect of the origin of common curses— they are simple, frequent, pronemic constructs. The study does not address the release of curse terms in TS, the frequent curses in ordinary language that relate to taboo relations (bastard, mother-fucker), or the curse terms which are highly individualized and situation specific which may be most embarassing to a TS sufferer (e.g· the sudden exclamation which describes an individual's race or a physical anomaly).

Dr. Nomura and her colleagues from Japan and Dr. Lieh Mak from Hong Kong presented important information on TS in Oriental populations. TS in the Orient is strikingly similar to the syndrome described in Western populations: the natural history, major symptom clusters, and associated learning and behavioral difficulties follow patterns well described in the Western literature. There are two differences. First, there is a relative infrequency of coprolalia, perhaps related to cultural factors. And, second, the Oriental studies do not confirm an over-representation of Ashkenazi (or even Sephardi) Jews as has beem observed in studies based in New York.

Dr. Asam's studies of German TS patients again revealed the consistency of the syndrome in this population. Dr. Asam emphasized the spectrum of psychological difficulties which are found in TS and an approach to their treatment. The relation of TS to other forms of behavioral and neuropsychiatric disturbance was discussed in various presentations in the Symposium. Dr. P· Clayton from Minneapolis showed how family studies may help illuminate this area. Her pilot study found a high incidence of obsessive compulsive difficulties in relatives of patients with TS; this work complements the studies from the United States and Japan that have shown a clustering of TS and multiple tics in families.

Basic Structural and Functional Aspects

Dr. Edward Bird

This session dealt with a review of the relationship between the various regions of the

human brain with particular attention being given to those areas that are thought to be altered in Gilles de la Tourette Syndrome.

Since the neurons that connect one area of the brain to another area contain specific chemical substances, these neurons can be traced with new techniques. Prof. Hassler showed how a surgical lesion placed in critical areas of the brain could interfere with the message that is being transmitted along certain nerve pathways, and how this was helping some people with Gilles de la Tourette Syndrome.

Dr. Nauta presented very elegant studies that he and his collaborators have done on animals by placing chemical substances in one area of the brain where they are taken up by the neurons and tracing these neurons to their termination in another area of the brain.

Dr. Susan Iversen pointed out from their studies that the cortex of the brain (the outer area of the brain) had very important direct connections with the deeper nuclei in the brain stem that produces Dopamine, an important chemical substance that plays a role in the production of abnormal movements.

Dr. Bunney studied the effect of some of the drugs used in Tourette patients by injecting these into animals and recording their effects on the neurons that produce Dopamine.

Dr. Pickel showed another new technique to trace Dopamine neurons in the brain by using special antibodies produced in animals against the chemical substances that are involved in the production of Dopamine.

Most of these studies have been carried out in animals, and researchers have reached the stage that they are now ready to apply these techniques to the human brain to determine the lesion in Tourette Syndrome. For this, post-mortem studies will be required, much as has been done for Parkinson's Disease and Huntington's Disease.

Clinical Pathology and Chemistry

Yves Agid

The purpose of the session on "Clinical Pathology and Chemistry was mainly to establish whether a biochemical analysis performed on patients with Gilles de la Tourette Syndrome (GTS) could give heuristic information concerning the pathophysiology of symptoms. Information of different biochemical parameters, particularly neurotransmitters, can be made either in patients by taking samples of cerebrospinal fluid (CSF) or in post-mortem material. The latter study is problematical because brains of patients with GTS are difficult to obtain. Dr. Richardson (Massachusetts General Hospital, Boston) showed that there were only two reports of morphological study in GTS. An arrested development of the striatum was suspected in one case and new neuropathological approaches are necessary to confirm and/or extend this result.

As sthown by Drs. Bird (Harvard Medical School, Belmont) and Yamamura (University of Arizona, Tucson), examination of different neurotransmitters (such as dopamine) neuropeptides (i.e., substance P) or the corresponding receptors in the brains of subjects with Huntington's Disease gave interesting information concerning the nature and the location of selective biochemical defects in the basal ganglia of these patients. Dr. Slevin (Johns Hopkins University) emphasized that glutamate receptors which are abundant in the striatum can now be easily measured.

Since an excessive activity of dopaminergic neurons has been postulated in the brains of patients with GTS, examination of dopamine metabolites in the CSF has been made by the groups at Johns Hopkins Hospital (Dr. Singer and co-workers) and of the Biological Research Unit, NIMH, Rockville, Maryland (Dr. Koslow). The reduced levels of the dopamine metabolite homovanillic acid (HVA) observed in the CSF of patients is consistent with the hypothesis of an inadequate dopaminergic function in GTS.

SPRING MEMBERSHIP MEETING HELD IN NEW YORK CITY, MAY 16, 1981.

A team of researchers from the Yale Child Study Center addressed our membership meeting at Mt. Sinai Hospital on May 16. They were:

Donald Cohen. M.D. Prof. of Pediatrics, Psychiatry and Psychology
Jill Detlor, R.N., M.S.
David Pauls, Ph.D., Research Associate

in Genetics

Kenneth Kidd, Ph.D., Associate Professor of Human Genetics and Psychiatry.

Dr. Cohen spoke of the general orientation of his group, what they have learned about Tourette Syndrome in the past years, what they hope to study in the future, and their approach to treatment. Tourette Syndrome appears to have a broad range of symptoms and a broad range of outcomes. It involves a dysfunction in the central nervous system's ability to respond to stimuli. Therefore, patients with Tourette's have problems in organization, attention, inhibition, and modulation of sensory input.

Some patients show signs of hyperactivity, learning disability, and attention deficit disorder prior to the first T.S. symptoms. These are obvious signs of CNS dysfunction. Others are bright, energetic, and thoughtful until the first symptoms appear. The rapid onset of motor and phonic tics in these children is very frightening to the child and his family. Moreover, since it often takes a long period of time between onset of symptoms and diagnosis, the child may find himself in a terrible state of anxiety.

Although no one can predict if symptoms will get better or worse as years pass, Dr. Cohen believes that after a person has T.S. for a few years, the severity of the disorder is announced. The attentional and behavioral symptoms associated with T.S. deserve further investigation. When children have had T.S. for four or five years and have difficulties with peers, and school problems, total intervention is warranted. Medication is not enough to treat the whole person.

At each stage of development, T.S. has a different impact. When a child has problems paying attention, is impulsive and hyperactive, he needs to find a way to cope with these problems. Some patients do not learn how to cope and the problems continue into early adulthood. The clinician must help the patient and family to understand what it means to experience T.S. throughout the course of development.

The Yale studies on the possible cause of T.S. have included biochemical research. Because haloperidol can be a successful therapy for some, the assumption has been that there is too much dopamine production in patients with Tourette's and haloperidol is effective because it inhibits dopamine. However, after years of study, it now appears that other neurotransmitters such as norepinephrine and serotonin may be involved in T.S. As more neurotransmitters are studied, they too may be found to be implicated in T.S. The systems are all intimately related, and changes in one effects changes in the others. It is possible that T.S. may be caused by a dysfunction of two or three chemical systems. Clonidine has been found to affect both the dopamine and norepinephrine systems. Haloperidol affects the dopaminergic and serotonergic systems. This new understanding will probably lead to new pharmacologic approaches.

EEG tests are also being studied to try to understand why 50 % of patients have abnormal EEG's, what causes this, and what differentiates these patients from those with normal EEG's.

Haloperidol can be remarkably successful therapy for some patients, but it is not a panacea for everyone. The dose required for symptom control may result in side effects, such as cognitive dulling or depression. Dr. Cohen feels any impairment of intellectual function is too high a price to pay for symptom relief.

Clonidine has been tried for about two-and-a-half years. It has been effective on behavioral problems such as attention deficit, hyperactivity, inhibition, and compulsive behaviors. It has also been helpful for motor and phonic symptoms. Some physicians have reported successful treatment using a combination of haloperidol and clonidine.

Dr. Cohen cautioned on the use of stimulant medication in families of patients with T.S. Giving such medication to a hyperactive sibling of a T.S. patient may bring on T.S. in that child.

Dr. Cohen and his colleagues view Tourette Syndrome as a serious, chronic disorder which should be studied in all its aspects —neurochemical, clinical, psychological, and pharmacological— just as other serious diseases of childhood are studied. He praised the Tourette Syndrome Association for mobilizing families to publicize the existence of Tourette Syndrome, and for encouraging quality research.

Jill Detlor, R.N., has just begun to study the educational problems associated with T.S. She noted that research in the past

emphasized motor and phonic tics but not the broad impact upon the life of the patient and family· School problems arise not only because of tics and noises, but also due to behavioral problems, the impact of haloperidol on learning, and the attitudes of other children as well as teachers.

A survey of the literature on T.S., going back to the last century, has shown that the obstacles to learning in the classroom have affected more than half of the T.S., patients.

It is hoped that, from this consideration of the problems, will come suggestions for helping the child with T.S. to function better in the classroom situation.

Dr. David Pauls spoke about the genetics o T.S. He and Dr. Kidd are trying to define the patterns of transmission in T.S. families, and to recognize and identify milder forms of T.S· in families where the full-blown syndrome is not present in relatives. Chronic multiple tics appear to represent a milder form of T.S., and large numbers of relatives in many T.S. families have tics. Pauls and colleagues have confirmed the sex difference reported by Shapiro in the past: more males than females are afflicted, but believe there is a greater likelihood of females with T.S. transmitting it.

Currently, in-depth genetic studies on a large number of T.S. families are being conducted under a grant from the National Institutes of Health. The result of these studies should, in the future, provide more conclusive statistics on the genetic aspects of this illness

Dr. Kenneth Kidd spoke about "Genetic Models and Genetic Counseling for T.S·" The Yale team, which interpreted available data before they began their present study, is convinced that there is a very real genetic component in T.S., but they cannot yet prove it. They know tics and T.S. cluster in families, but they don't know the underlying genetic defect. When the genetic component can be identified, that will enhance our understanding of what can be done to prevent the illness or to improve treatment of it. Whereas Dr. Cohen is trying to understand what goes wrong biochemically, Dr. Kidd is trying to understand what goes wrong genetically. According to Dr. Kidd, we are presently undergoing a "revolution" in the human genetics field because we now have the ability and techniques to study molecular levels of DNA (genetic materials). Although their T.S. study is just beginning, laboratory methods are being developed to study blood samples of whole families with a T·S. member. The goal is to be able to pin down the gene or genes responsible for susceptibility to T.S. or multiple tics. The work is expensive and time consuming so that only limited numbers of people can be studied. Dr. Kidd credited the T.S.A. for focusing attention on this disorder, making the government aware that research is necessary, and in that way aiding researchers such as himself to receive federal funds for their studies.

During the question-and-answer period, a woman asked about the advisability of taking Haldol while pregnant, and remarked that her symptoms had gotten better while she was pregnant and off Haldol. Dr. Cohen replied that the role of hormones in T.S. is not understood at present. He thought it was wise to go off medication during pregnancy, but when and how would have to be discussed with each individual's physician.

Asked if there was any evidence of immunological defect or environmental factors causing T·S., Dr. Cohen responded that none has been identified as yet.

In the future, the Yale team will be studying other drugs which affect the noradrenergic and dopaminergic systems. Dr. Cohen reported a 50 % to 60 % success rate on patients whom he has treated with clonidine. He noted that the major improvements were seen in areas of irritability, compulsions, attention problems, motor control and vocal symptoms. Efforts are being made to undestand why clonidine helps a certain group of patients, and doesn't help others. He noted that patients who have discontinued haloperidol feel brighter and perform better in school. Thus he feels that haloperidol should be used at very low dosages and should be carefully monitored

PRESIDENT'S MESSAGE

Dear Friends,

It is with extreme pride that I share with you here a personal account of the events of the three days that encompassed the

First International Symposium on Tourette Syndrome, held in New York, May 27-29. Those of us in attendance, both Association members as well as professionals who came from far and wide, agree that the meeting was an overwhelming success. The reality of the event actually surpassed even the highest expectations that were reserved for this occasion.

All the presenters, as well as the participants that I spoke with, had nothing but praise and appreciation to express for the opportunity afforded them to participate in this meeting. A great deal of mutual respect and admiration was flowing back and forth between the various physicians, researchers, clinicians, etc. This was particularly gratifying to see because the group included so many distinguished and respected individuals in various fields.

One sentiment I heard expressed over and over was how far we have come in such a short time. Ten years ago hardly anyone knew or cared about Tourette Syndrome. Today, prominent scientists have come from around the world—Germany, France, England, Denmark, Japan, etc— to discuss their work and gain greater knowledge and understanding from interaction with others, like themselves, eager to expand their enlightenment and look for new answers to old questions. I accepted numerous compliments on behalf of the National Tourette Syndrome Association for the outstanding work that we have done and the level of achievement we have been able to reach. While we can all be justly proud, we also realize how much work lies ahead still to be done.

This monumental accomplishment of an International Symposium could never have taken place without the generous support of all its sponsors. First is the Gateposts Foundation, to whom we are extremely grateful. The valuable contributions of the various representatives of NINCDS as well as the Symposium Committee of the National Tourette Syndrome Association must also be cited as factors that have led to the success of this historical event. It is gratifying to know that there are so many dedicated individuals working on behalf of those suffering from Tourette Syndrome. The beneficial impact of this meeting will continue to be felt for a long time. As thrilled as we all are with its success, I know that time will increase the beneficial effects even more.

We can all be proud of the strides we are making. I want to take this opportunity to also thank all those members who have contributed to our Fellowship Research Fund. Whether your contribution was $ 3 or $ 3,000, it was greatly appreciated. As a result of a very successful response, we are proud to announce that we will be able to award two Fellowships next year to deserving investigators. To date we have raised almost $ 30,000 and money is still coming in. (We go to press considerably earlier than you receive this newsletter).

Thank you one and all. If we all continue to place our efforts behind the same goals, we must assuredly be victorious in our endeavors.

Judy Wertheim, President

CONTRIBUTORS TO SPECIAL RESEARCH FUND

We are proud to have had such a wonderfully generous response from our membership and their friends and are sincerely grateful to all who contributed. As of June 1 we have raised $ 30,000 and donations are still coming in. This will allow us to award two research fellowships next year. Thank you all for your generosity and involvement.

GENERAL CONTRIBUTIONS

Special thanks to Sherman and Doris Given for their exceptionally generous gift.

The Given's donation will help to support our Educational and Fellowship programs, and will enable us to continue to identify undiagnosed patients and bring hope to all our families.

And our sincere gratitude to:
Grenoble Knitwear, Inc.
Robert and Marie Maizenegger
U.S. Tobacco Co.
for their benevolent support of this Association's activities.

ORPHAN DRUG UPDATE

To date, more than 65 Congressmen haphan Drug Bill is still in the Subcommittee ve co-signed HR 1663. However, the Oron Health and the Environment and we

have no word as to when it will be reporte to the full Committee on Energy and Commerce. Additionally, no Senator has yet offered to introduce the Bill in the Senate.

TSA AWARDED GRANT

The TSA has been awarded a much needed grant from the American Legion Child Welfare Foundation which will allow us to purchase and distribute copies of our radio and TV Public Service Announcements. In addition, the grant will enable us to create a Lending Library for copies of the award winning film, "Tourette Syndrome The Sudden Intruder."

We ask all persons. ESPECIALLY those who are not involved with local chapters, to contact their local radio and TV stations to ask if they would be willing to place our radio or TV ads. If you live in an area with an active TSA chapter, please contact your local chapter Chairperson to coordinate efforts and avoid duplications of requests to each station. If you receive a positive response from a station, please write or call Mrs. Lily Frandzel, Project Director, 4320 Enfield Ave., Skokie, Ill. 60076. Phone (312) 675-2121. Scripts are available if the stations require them for review. Please emphasize that this campaign does NOT seek funds, but rather seeks to identify undiagnosed patients.

KNOW YOUR RIGHTS

Question: How will President Reagan's budget cuts and "Block Grants" affect people with TS?

Answer: The president has proposed grouping numbers of programs into "Block Grants." This means that a "social services block grant" will contain many services which were geared towards the poor, the elderly and the handicapped. States will not have to spend money on all of the programs. Rather, they will be able to pick the ones they wish to support, and ignore those they wish to stop funding.

The social services block grant will include ' Vocational Rehabilitation" services. Some states may eliminate this program which is geared toward training the handicapped for employment and placing them in jobs. The "Education Block Grant" will combine the "Education for All Handicapped Children Act" (PL94-142) with educational services for the poor, bi-lingual, etc. Some states may choose to drop their "Special Education and Related Services" for the handicapped and target educational funds toward other populations. No matter what each state chooses to spend its money on, they will have 25 % less dollars to spend.

Question: What can I do to insure that my state continues to provide individualized educational programs for handicapped children as well as Vocational Rehabilitation and Developmental Disability Services?

Answer: Each governor will have to decide how to spend the monies a state receives from the federal government. It is imperative that people with TS join with other organizations representing the handicapped to influence their State Governor and Legislature, demanding that funds continue to be used to benefit handicapped children and adults. If we don't do this in EVERY state, programs benefitting people with TS might be eliminated.

INTERNATIONAL YEAR OF DISABLED PERSONS—

Life with TS in Australia

by Patricia Kleiman

Watching our son battle with T.S. has been a shattering experience affecting the whole family. From the age of 9, he has lived a life that one should not be expected to tolerate. We had the usual nightmarish run-around in the early days, and we went through the emotional scale of worry, frustration, anger and despair.

Due to the negative opinions of the many physicians we visited, I wrote in desperation to a friend who was then living in Connecticut. She had witnessed a TV program about Tourette Syndrome only a few weeks previously. There are still few doctors in Australia today who are familiar with T·S. We were very fortunate to find Professor Richard Ball. He has fostered media coverage and urged us to get involved whit the Tourette Syndrome Association. Thus far, T.S. stories have appeared on three TV channels, two newspapers, and one talk show as well as the episode from *Quincy*.

Prof. Ball has set up a research clinic at the St. Vine Hospital in Melbourne. Through media exposure, we are finding many T.S. sufferers. Each letter we recive from these people has a similar heart-wrenching story. It is difficult to understand how so many people have lived through so much without help, and how they have remained sane over the years. One elderly gentleman, over whose letter I shed more than the usual tears, was 75 years old and finally found the answer. He learned that his condition had a name, and that he wasn't insane. Another letter from the outback reported great difficulty by the mother in communicating with others about her son who was now 21 years old. This young man had given up hope because if his distrust of the medical profession.

These people are searching for answers and slowly, the message is getting through. Unfortunately, our Australia TSA is not very large and not yet well-organized.

I try to remember back to what our life was like six years ago, and it is hard to believe that we had a life very different from the one we have now. Those dreadful early stages, how we would hate to relieve them all again, although for us it wasn't nearly as traumatic as some of the stories I heard. Sometimes I wish a camera were going 24 hours a day because no one would believe haw many situations arise, some hilarious, some crazy, some so frustrating... Some days I kid myself that I have accepted T.S. and I am coping beautifully, then along comes one of those bummers of a day and I am gone! Some days my son is restless, bored, and unhappy. The worst part about it is that you can't do anything to help the situation. He becomes upset about not being able to write properly, or not having any friends. Some days, everything seems to go fine. Some children can be unkind. Unfortunately, unkindness is not only a disease of the young, it afflicts adults, too. Sometimes he gets negative comments from his teachers, even though they have enough literature explaining the disorder. Homework is the blight of his life.

Schools here in Australia do not appear to be equipped to handle anything but the norm. One can't live on the doorstep of the school, trying to educate those who think their education is complete, or to explain to those who will listen, but have still made up their minds on the subject. They believe only what they wish to believe.

Life with T.S. would, I imagine, have the same heartbreak and frustrations as it has in the United States or any other country. We all know the feelings of despair. We live each day as it comes. And we all have that incredible safety valve that keeps us from going berserk at different times. The same old hassles with doctors, teachers peer groups, and ahe general public. We survive because we have the right to survive.

PUBLICITY

Publicity about Tourette Syndrome is usually the direct result of regional members' efforts at educating their communities about this disorder. It is an important facet of this Association's work, promoting appropriate diagnosis and treatment for those suffering from this illness. Although the T.S.A. has been vigorously publicizing Tourette Syndrome since 1975, the necessity for continuing these efforts is frequently pointed out to us by newly diagnosed patients, and by material we come across which will discuss tics and never mention Tourette's. Such an item appeared in The Harvard Medical School Health Letter, March 1981. The "Comment on Tics" briefly describes tics but makes no mention of T.S. The item concludes: "Whatever the cause, most children outgrow their tics within a few years. Some will need emotional support or psychological therapy, but drugs are seldom indicated. "We have written to the Editors, provided them with information on Tourette Syndrome, and asked them to inform their readers about this illness and the T.S.A.

Other publicity follows, and it is a pleasure to note that most of these articles are informative and sensitive:

Newark Star Ledger (N.J.), May 10, 1981. "Victims struggle against rare brain disorder".

The Phoenix Gazette (Arizona), April 6, 1981. "Tourette's: never a calm moment".

Syracuse Herald-American (N.Y.), Feb. 15, 1981. "Boy fights neurological disorder"

The Muncie Star (Indiana), March 6, 1981. "Tourette Syndrome, a bizarre disorder, often misdiagnosed"

The Reader's Digest, April, 1981. "The brain slowly yields its secret" (Reprinted from Geo Magazine, October, 1980).

The Sydney Sun-Herald (Australia), December 14, 1980. "It helps that he's not alone"

The Age (Sydney, Australia), October 7, 1980. "Strange sounds denote an illness".

The Sydney Sun-Herald (Australia), Dec. 14, 1980. "Disease with an odd name".

Congressional Record, March 26, 1981. Orphan Drug Legislation HR 1663.

Dr. Oliver Sacks was interviewed about Tourette's on the Dick Cavett Show, April 23, 1981.

Abbey Meyers was on WABC-TV's "Good Morning New York" show, April 9, 1981

Bayside Times, April 23, 1981. "TS fighters receive helping hand".

The "Parents and Children" column of the New York Daily News on June 1 and 4, 1981, contained a discussion of tics and mention of Tourette Syndrome by Saul Kapel, M.D. The information appeared to be drawn from "The Pedriatrician's Guide to Tics, Tourette Syndrome and Other Movement Disorders" published by the T.S.A.

The Quincy, M.E. TV program on Tourette Syndrome (March 4, 1981) produced much newspaper publicity across the country, both prior to the showing and subsequently. Jack Klugman's testimony in Washington on behalf of Orphan Drugs in the week that followed the Quincy show also received widespread coverage in the media The following is a list of some of this publicity which our office is aware of.

Tulsa (Oklahoma) World, February 22, 1981.

Medical World News, March 30, 1981. "FDA may ease approval rules for orphan drugs"

Variety, March 11, 1981. "Quincy to D.C. for real hearing on orphan drugs".

The Milwaukee Journal, March 10, 1981. "Star of TV's Quincy makes a house call"

Dallas Times Herald, March 4, 1981. TV column discussed Quincy and Tourette Syndrome.

Norwalk, CT newspaper, March 10, 1981 "Klugman supports use of "Orphan Drugs" in Congress testimony"

Morris Sun (MN), February 24, 1981. "Early diagnosis helps child adjust to Tourette Syndrome".

Fort Worth Star-Telegram, March 4, 1981. "Focus on a little-known ailment".

Cedar Rapids Gazette (Iowa), "Will Quincy generate help for Tourette Syndrome?

People Magazine, April 16, 1981. "Dr. Arthur Shapiro's battle against Tourette Syndrome gets a boost from TV's Quincy.

The New York Times, March 10, 1981. "House told of need for Orphan Drugs".

Sunday Patriot News (Harrisburg, PA), March 8, 1981. "Television diagnosed his illness"

The New York Times Science Times, March 17, 1981. "Drugs that promise help but not profit reside in limbo".

The Washington Post, March 10, 1981. "TV's Quincy tells Hill about rare diseases"

The Post (West Palm Beach, FL), March 3, 1981. "Couple seeks to educate others about little-known syndrome"

Chicago Sun-Times, March 10, 1981 "Jack Klugman takes Quincy drug crusade to Washington"

Sun-Tattler (Hollywood, FL), March 10, 1981. "Klugman (Quincy) pushes rare disease drugs"

The Herald (Melbourne, Australia), March 18, 1981. "Life is hell in the grip of the Syndrome"

NCR REPORT

On Hay 21, 1981, NCR hosted a luncheon for key professional aides to the members of the Senate and House Appropriations Subcommittees, those individuals who have a voice in government expenditures for health research. The luncheon served to introduce these Senate and House staff members to volnteers and professionals who have devoted their lives to promoting research on neurological disorders.

The principal speakers were Jennifer Jones Simon and Sally Kellerman. Mrs. Simon is a member of the National Advisory Council of NINCDS. She expressed her total commitment to furthering the advance-

ment of research on all aspects of neurological and related conditions.

Ms. Kellerman first became involved in support of research through the Hereditary Disease Foundation of Dr. Milton Wexler in Beverly Hills, California. In her luncheon address, she stated that a member of her family had Tourette Syndrome, and she made a plea for more funding for Tourette's and other neurological conditions.

Dr. Katherine Bick, Deputy Director of NINCDS, gave a stirring speech about the spectacular advances in knowledge of the brain, which now seem possible through the use of the PETT (Positron emission transverse tomography) Scan.

NCR's primary concern is that neurological research receives adequate funding. It is hoped that this meeting, and similar ones, will promote support for this essential research among the members of the Congress.

LETTER TO THE EDITOR:

At our recent membership meeting a young mother who has Tourette Syndrome told the audience that she experienced amelioration of symptoms during pregnancy. I would appreciate hearing from Tourette patients about the effect of pregnancy on their symptoms, and whether medication was used during that time (type of medication used and amount taken). I would also be interested in hearing whether symptoms were affected during nursing.

If symptoms are indeed affected by pregnancy and nursing, it may mean that hormones have an influence on Tourette symptoms and this kind of information could provide a lead for future research

Thank you,
Sheldon Novick, M.D.
Medical Director, T.S.A.

VOLUNTERS FOR A PIMOZIDE TREATMENT STUDY

Dr. Arthur K. Shapiro will be conducting a double-blind cross-over treatment and study comparing pimozide and placebo.

Pimozide has been reported to be efective in suppressing the symptoms of Tourette Syndrome and to have fewer side effects than haloperidol. Some patients report that pimozide has been more effective for them.

Treatment will include an initial diagnostic evaluation and visits every two weeks over a 16 week period. Specially developed tests will be used to monitor side effects and determine appropriate dosages.

There will be no charge for treatment or medication. If you are interested in either participating in this study or in obtaining additional details about the study, please call Dr. Elaine Shapiro, Tuesday, Wednesday, Thursday or Friday at (212) 650-5946

SCIENTIFIC PAPERS

TOURETTE SYNDROME IN MENTALLY RETARDED CHILDREN

Authors: Gerald S. Golden and Lawrence Greenhill

Journal: Mental Retardation, Vol. 19, Nº 1, February 1981.

"This report concerns six children who are mentally retarded and have abnormal vocalizations and motor tics. Their symptoms fit all of the diagnostic features of Tourette Syndrome and treatment produced favorable results in four of the five children for whom therapy was begun. Although the vast majority of patients with Tourette Syndrome have normal intelligence, the recognition of this condition in mentally retarded individuals and application of effective treatment can reduce the social incapacitation in this already stigmatized group."

The authors urge that treatment proceed cautiously because there is a high incidence of extrapyramidal reactions in these patients.

THE TREATMENT AND ETIOLOGY OF TICS AND TOURETTE SYNDROME

Authors: Arthur K. Shapiro and Elaine Shapiro

Journal: Comprehensive Psychiatry, Vol. 22, Nº 2, 1981.

A comprehensive paper which defines tics and reviews chemotherapeutic approaches to treatment of Tourette Slndrome.

OCULAR MOVEMENT ABNORMALITIES IN GILLES DE LA TOURETTE'S

SYNDROME (T.S.)—IN CONTRAST TO CHILDHOOD DYSTONIA

Authors: Yoshiko Nomura, Junko Kato, Kei Hachimori, Masaaki Fujimoto, and Masaya Segawa

Journal: Brain & Development, Nº 3, 1978

The authors state: "Our present studies on ocular movement combined whit responsiveness to Haloperidol or Pimozide and urinary catecholamine metabolites suggest that multiple transmitter systems might be involved in the pathophysiology of T.S. Similar points are suggested regarding dystonia, also. Furthermore, clinical observation of posture abnormalities in T.S., dystonic spasm in dystonia and patterns of neck involvement in both, point to a possible partially common pathway in both disorders. Future evaluation on these points might lead to further understanding of the disorder and treatment for Haloperidol or Pimozide unresponsive cases."

FAMILIAL GILLES DE LA TOURETTE'S SYNDROME ASSOCIATED WITH TUBEROUS SCLEROSIS

Authors: Kenneth Lee Mathews, M.D.

Journal: Texas Medicine, Vol. 77, April 1981.

ABSTRACT: "Two brothers were diagnosed with Gilles de la Tourette's Syndrome and successfully treated with haloperidol. Evidence of tuberous sclerosis was present in both brothers and the father. The associaation of Gilles de la Tourette's Syndrome and tuberous sclerosis supports the postulate of a genetically determined neuroanatomical lesion causing Gilles de la Tourette's Syndrome. A review of the literature regarding possible organic etiologies is included."

GILLES DE LA TOURETTE'S SYNDROME

Author: Gerald S. Golden

Journal: Texas Medicine, Vol. 77, April 1981.

This letter to the editor summarizes in concise fashion what is currently known about Tourette Syndrome: the criteria for diagnosis, the treatment of choice, the possible deleterious effect of stimulant drugs on some patients, and the genetic data available at the present time.

EARLY DIAGNOSIS OF THE TOURETTE SYNDROME

Author: P.V. Melnichuk

Journal: Zhurnal Nevropatologii i Psikiatiee, Vol· 80, 1980

SUMMARY: "A test for diagnosing rapid hyperkinesias based on determining phenylacetoglutamine (PAG), an end product of phenylalanine metabolism, is offered. It was found that in patients with Tourette's syndrome PAG excretion with the urine was lowered, the degree of this lowering being independent of the patient's age, sex, and nourishment. An increase in PAG excretion was observed in patients with the asthenoneurotic syndrome. In patients with a sharp increase in PAG excretion, the treatment with haloperidol appeared to be ineffective. The data obtained allow a conclusion that the lowered PAG excretion is an early prognostic sign of a number of nervous system diseases accompanied with hyperkinesias. The level of PAG excretion with the urine can also serve as a criterion determining advisability of haloperidol administration."

ROLE OF CATECHOLAMINE EXCRETION EXAMINATIONS IN FORECASTING THE EFFECTIVENESS OF TREATING TICTYPE HYPERKINESIAS IN CHILDREN

Author: E.L. Dadali.

Journal: Pediatrika, Oct. 1980.

SUMMARY: "In 35 children with tictype hyperkinesias the excretion of free catecholamines and DOPA was examined. As a result of the treatment given, all the children showed a considerable improvement of their state that manifested itself in a pronunced relief or complete disappearance of the hyperkinesias. This was accompanied with normalization of the catecholamine metabolism. The data obtained give one grounds to believe that disturbance of the catecholamine metabolism play an important role in the development of tictype hyperkinesias in children."

PIMOZIDE-INDUCED ENURESIS

Author. Arthur K. Shapiro

Journal: American Journal of Psychiatry, Vol. 138, January 1981

In a Letter to the Editor, Dr. Shapiro suggests "that some antipsychotic medication can induce enuresis in susceptible young individuals." Shifting administration of the medication from bedtime to morning made a difference in one case treated by the author.

GILLES DE LA TOURETTE'S SYNDROME: CASE REPORT

Author: L.I. Shenken

Journal: New Zealand Medical Journal, Sep. 24, 1980

This is a report of a 24 year-old young man who had a severe case of Tourette's since the age of six. Although his family had taken him to numerous physicians, he had never received a diagnosis or appropriate treatment. One of the psychiatrists consulted by the parents had suggested that the child "be put away." But the parents never gave up hope of finding help. "The effect on family life was shattering." The family had no social life because the symptoms were so disruptive.

At the age of 24, the patient was seen by a psychiatrist who was baffled by the symptoms. However, shortly thereafter the doctor came upon an article on Samuel Johnson and T.S. in a British medical journal. The patient was started on haloperidol and, after six months, was leading a reasonably normal social life for the first time in his life on a very low dose of medicine - 1.5 mg, two or three times a week.

TOURETTE SYNDROME, AN ACQUIRED ENCEPHALOPATHY? A REPORT OF TWO CASES WITH EPILEPSIFORM DYSRHYTHMIA

Authors: Thomas R. Marra, Norman C. Reynolds, Jr., and David S. Dahl.

Journal: Clinical Electroencephalography, Vol. 11, Nº 3, July 1980.

Summary: "The electroencephalogram, although providing no specific diagnostic information for Tourette Syndrome, may be useful in evaluating the pathology in which the syndrome occurs, since it appears that at least some cases may occur in the setting of an underlying acquired encephalopathy of diverse etiology. A neurochemical basis for the Tourette Syndrome expressed as an extrapyramidal circuit imbalance may also relate to a discharge threshhold for epileptiform activity. A more detailed electroencephalographic evaluation of such patients using nasopharyngeal or sphenoidal recording may be useful in this regard."

LES TICS CHEZ L'ENFANT

Authors: J. Dalery, J. Maillet, and R. de Villard.

Journal: Neuropsychiatrie de L'Enfance et de L'Adolescence, Vol. 28, Nº 12, 1980.

This paper considers tics and Tourette Syndrome and suggests that some tics are psychologically determined and require psychotherapy, whereas others may have a biochemical basis and be amenable to treatment with Haldol or similar medication.

BOOK REVIEW: Gilles de la Tourette Syndrome, by Arthur Shapiro, et al., recieved a favorable review in the Journal of the American Academy of Child Psychiatry, Vol. 20, Nº 1, 1981.

IRMA NEWS AND VIEWS

International Rehabilitation Medicine Association

Published by EULAR Publishers, POB 146, CH-4011 Basel 1980 — Nº 2.

Minutes of the IRMA Council Meeting held on Thursday, 28 August 1980 at 16.00 in Room E at the Convention Centre, Folkets Huset, Stockholm

Before the meeting was called to order, the President announced with regret *the deaths of Sidney Licht and Philip Nichols.* Dr. Erdman then paid tribute to the work of Dr. Licht in recognition of all that he had done to found the Association and Dr. Wynn-Parry following, re-emphasized and also paid tribute to Dr. Philip Nichols. The meeting stood in silence to respect the memory of these two distinguished former members.

1.—3 The meeting opened with the introduction of the councillors and observers present: a quorum was established and the

agenda as distributed was agreed

4. Correction and approval of minutes of last meeting

The minutes of the previous meeting had been previously circulated and were accepted as a true record.

5. President's report

The president's report was given by Dr Wilhelm M. Zinn.

6. Secretary's report

The secretary's report was given by Dr. Christopher Evans.

7. Treasurer's report

The treasurer's report was given by Dr. William J. Erdman II.

8. IRMA IV report.

Dr. Herman J. Flax reported on the forthcoming meeting IRMA IV at Puerto Rico and circulated a preliminary programme which will be published in the next issue of the Journal IRM.

9. Reports of liaison officers to international organizations

a) *Dr. Hachen*, as the IRMA delegate to the CIOMS, reported that IRMA was now a full member of this institution and had been elected as a member of the Executive Committee. President and secretary also attended two of the CIOMS symposia which dealt with the organization of international medical congresses, medical ethics and the economics of national health services.

b) *Dr. Herman J. Flax* then reported on the international assembly of the International Federation of Physical Medicine and Rehabilitation under whose auspices the Stockholm meeting was being held, and informed the meeting that the next venue would be in 1984 in Jerusalem.

c) *Dr. Wilhelm M. Zinn* told the council that he had been elected an individual member of the Medical Commission of Rehabilitation International at a meeting of that commission in March 1979 in Geneva. He also attended an international symposium organized by Dr. David Symington in Kingston, Canada of that commission in June 1980, which firmly strengthened the ties of contact and collaboration with the international medical rehabilitation institutions represented by their delegates.

10. Journal International Rehabilitation Medicine, Editor's report

The editor of the Journal of International Rehabilitation Medicine was unable to be present but had sent a report which was read by Dr. C. B. Wynn-Parry.

11/13/14. Aims of IRMA/Establishment of an administrative secretariat/Amendments to IRMA statutes including membership dues

Dr. Wilhelm M. Zinn declared he had now served as president for two years. During this time it had become clear to the Executive Committee that the present financial structure was inadequate. There were insufficient funds even to produce and send out NEWS & VIEWS, and it was impossible to attempt the realization of the other aims of IRMA. To send out NEWS & VIEWS separately costs exactly the same as to send out the whole Journal. In order that IRMA fulfils the requirements of the existing statutes, which include good contact between members and the Executive Committee, the Executive Committee discussed the problem on Tuesday, August 26, 1980 and unanimously recommended the following measures:

From January 1, 1981 the dues will be raised to Swiss francs 56.- per annum. This will include the privileges of membership of IRMA, such as reduced congress fees. In addition, members will now receive our official publication, the Journal IRM, incorporating or replacing NEWS & VIEWS, which will not be published separately. By arrangement with the publisher, this will allow the establishment of an administrative secretariat based in Basle while the honorary secretary and treasurer will remain as before but without the imposible administrative burden.

12. Role and election of councillors

The role of councillors was further discussed and it was stressed that it was most important that individual member countries should nominate their councillors at least three months in advance of the next meeting in Puerto Rico. This point was reemphasized by Dr. Herman J. Flax from his past experience as secretary and supported by Dr. Hachen, who suggested that the role of IRMA councillors in the future should be more precisely defined. The president was most anxious to see the role of the councillors strengthened, and to this end will contact them more regularly and work out with them a more substantial role for the future.

15. Place and date for IRMA V

It was proposed by Dr. Hachen and seconded by Dr. Flax that Professor Burniston's offer to hold IRMA V in Australia should be accepted. This was carried unanimously.

16. Nomination of officers of the Executive Committee

Dr. Zinn nominated Dr. Wood in place of Dr. Nichols as an ex officio member of the Executive Committee and pointed out that it was not possible at this meeting to elect, but that it was the president's responsibility to nominate any necessary replacement.

17. Future activities

It was agreed that the contribution that IRMA should make to the International Year of Disabled Persons was a topic which should be left to each individual country to undertake. Dr. Zinn pointed out that there would be local meetings, probably in Switzerland, which were being planned and which would be prepared in conjunction with IYDP; one possibly to be held in Switzerland and the other, on sexual problems, to be held most probably in Israel.

There was no other business and it was proposed by Dr. Wynn-Parry and seconded by Professor Burniston that the meeting should be adjourned, the next to be held in Puerto Rico in 1982. Dr. Wynn-Parry concluded by paying tribute to the outstanding work done by the president, whoch was warmly supported by all members present.

The meeting closed at 17.40. *Dr. Christopher D. Evans.*
Honorary Secretary

President's Report

Following IRM III, there were many tasks: thanking all the contributors, collecting manuscripts, selecting those for publication, and settling a number of residual problems. We were pleased to receive approximately 200 more or less enthusiastic letters from contributors, committee members and customers.

On September 29/30, 1978 the first Editorial Meeting of our new Journal was held in Bad Ragaz. It was an excellent experience. Subsequently the painstaking selection of what should be published from the material presented at IRMA III in the Journal or in the form of monographs or otherwise took us almost a year of discussion and correspondence. Apart from the papers published in the Journal INTERNATIONAL REHABILITATION MEDICINE the following monographs have been published or are in preparation:

Balder Gloor and Roland Brueckner — Rehabilitation of the visually disabled and the blind at different ages
Hans Huber Publishers, CH-3000 Bern 9/Switzerland

Hans Wider — Körperbehinderte als Motorfahrzeuglenker, Strassenverkehrsamt des Kantons Zürich, Uetlibergstrasse 301, 8045 Zürich/Switzerland

Y. Rideau — "Enquête économique à propos de la réadaptation"
E. Helander — "Coût-bénéfice" et "Coût-efficacité"
B. Primeau et al — in Réadaptation, N° 264, 1980, p. 3-37
Adresse de la rédaction:
10, Rue de Sèvres, 75007 PARIS, Tél. 222-22-73

Paul Bach-y-Rita — Rehabilitation of Patients with Brain Damage. Recovery of Function: Theoretical Considerations. Hans Huber Publishers, CH-3000 Bern 9/Switzerland

Wing/Kielholz — Rehabilitation of Patients with Schizophrenia and with Depressions
Hans Hubers Publishers, CH-3000 Bern 9/Switzerland

F. Affolter and E. Stricker — Perceptual Processes as Prerequisites for Complex Human Behavior

Hans Huber Publishers, CH-3000 Bern 9/Switzerland

Inger Nordqvist und P.O. Lundberg — Sexual Problems in Rehabilitation: Analyses and Solutions.

Analyses and Solutions Eular Publishers. P.O. Box 146, CH-4011 Basel/Switzerland.

On November 30 and December 1, 1978, Dr. Hachen and the president attended the XIIth Round Table Conference of the Council for International Organizations of Medical Sciences (CIOMS) in Estoril, Portugal. Our delegate to CIOMS, Dr. Hachen, was asked to write a separate report on the two symposia held on this occasion.

In the beginning of January 1979 our secretary, Dr. Chris D. Evans, spent two days at Bad Ragaz to work out guidelines for the activities of the secretariat.

On January 19-20, 1979, I had a business meeting with Dr. Philip Nichols and Leslie Nicklin, again concerning our Journal.

In March 1979 our secretary, Dr. Chris D. Evans, Dr. Hachen and the president attended an important meeting of the Medical Commission of Rehabilitation International in Geneva. The president was elected as an individual member of this commission. At the business meeting there was a thorough discussion of educational problems. Other subjects were the International Year of Disabled Persons 1981, WHO policies, programmes and activities relating to prevention of disability and rehabilitation, etc.

In May 1979 I visited our secretariat in London, which at that time seemed to be developing in a promising way.

On May 25-26, 1979 a further editorial meeting was held in Basle, at which Dr. Langton-Hewer was present for the first time. A programme was prepared for the subsequent three or four numbers of the Journal. Philip Nichols was so interested and so pleased with his work for the Journal that it was a great joy to collaborate with him. All who knew this energetic and apparently flourishing man were shocked by the news of his sudden and fatal illness. Certainly, his loss was the most severe blow to our Journal, its second year of publication having just begun. Dr. Nichols will be greatly missed, both by IRMA and the Journal. In September 1979, at the European Rheumatology Congress, our co-editor, Dr. Philip H. N. Wood, of Manchester, agreed to take over and to serve as temporary acting editor.

In November 1979, Dr. Chris D. Evans, Dr. Hachen and the president again attended a CIOMS Round Table Conference in Geneva on "Health problems and services: the economic context". Although there were not many "hard" facts presented, there was a good discussion on recommendations and requirements for change. On this occasion, our delegate, Dr. Hachen, was elected member of the Executive Committee of CIOMS. IRMA is now as fully represented as possible in this important institution.

Professor Brodin and his colleagues gave the president the honour of advising the organizing committee of the 8th World Congress of the International Federation of Physical Medicine and Rehabilitation. The contacts were excellent and we are looking forward to attending a highly attractive scientific programme.

In the beginning of January 1980, the president attended another fruitful editorial meeting of our Journal in London. On this occasion, an evening was spent with Dr. C. B. Wynn Parry and Dr. Chris D. Evans, in assessment and evaluation of IRMA's functioning and further policies. Subsequently, the president contacted the members of the Executive Committee to suggest certain changes which, hopefully, will strengthen our institution and facilitate our work.

In February 1980, Dr. Chris D. Evans worked for several weeks at Valens and it became clear that in his new job in London he would need some time to be able to serve IRMA as successfully as originally envisaged. Fortunately, he is now settled in his new position, and we can anticipate receiving his full support once again.

In June 1980 the president attended a seminar of the Medical Commission of Rehabilitation International in Kingston (Ontario, Canada), which was extremely well organized by Dr. David Symington.

Excellent papers and demonstrations were presented on var ious subjects, such as amputee management, clinical mechanics, computer programming as a career for high level quadriplegics, driver training programme, EMG biofeedback research LAB, and others.

During the following week the president attended Rehabilitation International's World Congress in Winnipeg, Canada. IRMA had been invited to contribute to the programme and to organize a symposium on Sexual Problems in Rehabilitation.

With the help of Dr. Inger Nordqvist, Ms Sandra Cole and Professor Ted Cole, and a number of excellent speakers who were experts in various fields, as well as talks from disabled persons, this symposium was a great success. We enjoyed a large audience and it seemed to be just the right programme for the occasion. Unfortunately, a second IRMA symposium on Communication, which had also generated much interest, had to be cancelled.

At the end of July 1980, another edito-

rial meeting of the Journal IRM was held in London, under the chairmanship of Dr. Philip Wood. Due to the enormous problems arising after the death of our first chief editor, there was a regrettable but unavoilable delay in the issue of Nos. 1 and 2, Vol. 2.

IRMA's other activities included collaboration and advice for the 5th World Congress of Sexology in Jerusalem (June, 1981), and the Organization of a special two-day conference on "Sexology and Disability", as a pre-congress symposium.

The president was pleased to be kept fully informed by our vice-president, Dr. Herman J. Flax, of his constant efforts and extensive correspondence regarding the preparation of IRMA IV. Dr. Flax will personally report on his congress.

W. M. Zinn, MD

IRMA President

Secretary's Report

The task of the secretary of IRMA over the last two years has been in three main areas:

1. To establish contact with other IRMA members, especially those abroad,
2. to maintain a record of members,
3. to produce and distribute NEWS & VIEWS.

1. Liaison

During the last two years your secretary has made five visits overseas. The first was to Switzerland to visit Rehabilitation centres, and six such centres were seen. He attended the conference of the MEDICAL COMMISSION of Rehabilitation International in Geneva, and later the CIOMS conference. On each occasion he was one of the official representatives of IRMA. He was invited to advise the Sultanate of Oman on the development of Rehabilitation Centres there, and most recently has been to the German Democratic Republic to see the rehabilitation facilities there, and to set up a link between the GDR and the UK services. He was able to contact Professor Renker and Professor Presber who were the official hosts.

The president Dr. W. M. Zinn from Switzerland. the vice-president Professor Burniston from Australia, Dr. Morris from New Zealand, and the late Dr. Sydney Licht visited the UK and were welcome visitors to the Royal Hospital and Home for Incurables when your secretary was the Director there.

2. Membership

This task has been most difficult. Initially, on receipt of the index from Dr. Herman J. Flax all the addresses were checked, and plates made so that automatic labelling could be used for mailing, but this facility was withdrawn, so that only one mailing shot has been possible, and two issues of NEWS & VIEWS were sent to all known members. In addition to the size of the problem there remained the difficulty in knowing which members were in arrears with their dues. As Dr. Erdman has pointed out, the present level of subscription is so low that it does not permit extensive pursuit of outstanding dues. So, although there are some 1,700 members in theory your secretary has no way of knowing the real figure of those who wish to retain membership. The use of automated equipment for the future seems essential. During the last two years there have been about 40 new members who have paid their dues to me direct, and there have been more who have applied direct to Dr. Flax or Dr. Erdman.

3. NEWS & VIEWS

Ideally the contents of the Journal should be made up from items sent to the secretary by members, but because of the change of address most members have had no definite one to write to, and it is suspected that a lot of correspondence will have gone astray. To fill the four issues so far published copy has had to be written by the secretary, drawing on the visits already referred to. To save costs of postage the first two issues were sent together, and so will the next two.

General

During the time that your secretary was working as the Director of the RHHI research department, the hospital supported IRMA considerably by providing secretarial and financial help. In September 1979 this support was withdrawn, so that it has been possible to achieve little since then, and more important, there has not been a settled address for the secretariat. Some of the cost of setting up an office was defrayed by RHHI, but it did not provide running expenses.

It will be seen from the attached financial report that there has been extensive financial support of IRMA from various sources and that the income from non-IRMA funds far exceeds that from subscriptions. Despite the heavy cost of mailing the first issues, and the cost of setting up the office, it will be seen that there is still over £ 300 in the bank. Most of the extra money has been provided by private sources, and many of the visits made have been sponsored by other organizations.

Support from officers and councillors during the difficult last two years has been most welcome, and particular thanks are due to Dr. Flax, Dr. Erdman, and especially to Dr. Zinn.

Dr. Christopher D. Evans
Honorary Secretary

IRMA

Financial Report

Expenditure of Secretariats (only IRMA)	£ p.
Printing & Stationery	505 30
Postage	277 30
Secretarial Salary	265 23
Bank Charges	15 00
Cash at Bank	322 84
	1685 67

Expenditure (Shared)	
Telephone	
Answering Machine	
Office Equipment	
Provision of Office and heating & c.	
Travel to Switzerland on three occasions, Hotel bills and fares	
Travel to DDR	
Travel to Oman	
Income (only IRMA)	
Subscriptions	207 11
Gift from Dr. Licht	20 00
Transfer from Dr. Erdmann	752 67
	979 78

It will be seen, only taking direct expenditure into account, that IRMA has apparently spent 705.89 pounds sterling more than it has received. On top of this the cost of travel, equipping and maintaining the secretariat together with all the other items in the brackets have been subsidized by other sources, these include the RHHI, the Overseas Development Association, RADAR and the DDR. Many of the costs of the office have been shared with my own practice, and I would estimate the sum to be well over 1500 pounds. The president has been instrumental in making this task possible.

4th Congress of the International

REHABILITATION MEDICINE ASSOCIATION SAN JUAN, PUERTO RICO

18—24 APRIL 1982

The Fourth World Congress of the International Rehabilitation Medicine Association will meet in San Juan, Puerto Rico, April 18—24, 1982.

The Congress theme is "New Findings That Influence Disease and Rehabilitation", which is also the title of a 36-hour, AMA CME Category I, accredited course, divided into twenty, two-hour seminars during the mornings and two, eighthour sessions during the afternoons. The speakers will be distinguished investigators in the medical sciences. In addition, there is provision for individual papers and scientific films relating to the congress theme. IRMA is a society of physicians from all the fields of medicine and surgery interested in rehabilitation medicine. At present, there are about 1800 members from 70 countries. President is Dr. Wilhelm M. Zinn, Switzerland and Secretary, Dr. Christopher Evans, United Kingdom. For information address: Dr. Herman J. Flax, Chairman IRMA IV, P. O. Box 11696, Caparra Station, Puerto Rico 00922 USA.

Research Group in Neurological

STATEMENT OF THE REHABILITATION OF THE WORLD FEDERATION OF NEUROLOGY

In the International Year of Disabled Persons 1981 attention should be drawn to the serious disability suffered by those affected by disorders and injuries of the nervous system due to deficiencies in rehabilitation services and sometimes due to lack of interest in their rehabilitation needs.

In common with other forms of disabi-

lity those with disturbance of neurological function suffer grave limitation of mobility, pain and discomfort but in addition they suffer the problems of incontinence of bladder and bowel. Above all they are frequently robbed of the power of communication with their friends and families. All of this is compounded by the social stigma aroused by their strangeness of utterance and sometimes by distortion of their features.

Their number is legion and the failure to exploit their potential to its full by early rehabilitation using modern techniques causes them to be a burden on society, their family and their nation. This is particularly true of those with head injury, stroke and spinal cord injury for whom a particular plea is made in the International Year of the Disabled.

John B. Cook MD FRCP
Honorary Secretary

CALENDAR

16—18 February 1981

10th Annual Congress of the Society for Rehabilitation in the German Democratic Republic, with international participation.
Enquiries: Society for Rehabilitation in the GDR, Harz 42—44, DDR-402 Halle.

18—20 February 1981

4th Annual Conference of the Canadian Orthopaedic Nurses Association to be held at the Sheraton Centre, Toronto, Ontario.

All nurses and allied health personnel with an interest in orthopedics are cordially invited.

Enquiries: Ellen Hill, Co-Chairperson, Publicity, Canadian Orthopaedic Nurses Association, 43 Wellesley St. E., Toronto, Ontario, M4Y 1H1, Canada.

6—10 April 1981

Third European Regional Conference of Rehabilitation International to be held in the Congress Centre in the Vienna Imperial Palace, Austria.
Main theme: The Handicapped Person in Society.

The Congress will comprise plenary sessions, poster session, free papers, arts exhibition commercial exhibition, film and video presentations. The programme includes the following daily topics: prevention and medical rehabilitation as a task of social medicine; situation of disabled persons in the framework of education and vocational training systems; social rehabilitation integration.
Plenary sessions: German and English will be official congress languages (simultaneous translation). There is no simultaneous translation for the workshops which will be held in English.
A comprehensive social programme will include visits to Vienna Imperial Castle, Schönbrunn Castle, Belvedere Castle, Spanish Riding School, Museum of Fine Arts. Albertina Gallery, etc.
Registration Fees: before 15 January 1981: participants S 2,250. — accompanying persons S 800.
Reisebüro Mondia, A-1010 Vienna, Börsendorferstrasse 4, Austria, is providing special IT-flight arrangements in cooperation with Austrian Airlines and other IATA airlines from major European cities.
Enquiries: Interconvention Kongressorganisationsges. m. b. H., Hofgurg-Heidenplatz, Postfach 105, A-1014 Vienna, Austria.

10—22 May 1981

British Medical Course on the Management of Locomotor Disorders to be held in Edinburgh, Scotland.

The aim of this course is to show the current British approach to the rehabilitation of patients who have a variety of disorders which affect locomotion. It will be of particular interest to members experienced in one aspect of this problem who wish to learn about the treatment of other conditions with which they deal only occasionally. The Directors of Studies will be Professor Cairns Aitken and Dr. John Hunter, Consultants in Rehabilitation Medicine at the Rehabilitation Studies Unit at the University of Edinburgh. The course will be based at the Pfizer Foundation, headquarters of the Edinburgh Post-Graduate Board of Medicine, which is constituted by the University of Edinburgh, Royal College of Physicians of Edinburgh and the Royal College of Surgeons in Edinburgh. Members will visit four hospitals in Edinburgh for clinical teaching which will be through multi-professional case conferences and there will be four evenings devoted to small group discussions in the hotel. The course is intended for those holding positions of authority in the various professions contributing to rehabilitation. Half the members

will be drawn from medical and surgical specialities and others will come from nursing, social work, physiotherapy, occupational therapy, prosthetics/orthotics, clinical psychology and vocational counselling.
There are vacancies for 36 members. Fee to be announced. Application forms to be received in London by 2 January 1981.
Enquiries: Mrs. Y. Keeler, Publicity and Recruitment Section, Courses Dept., The British Council, 65 Davies Street, London W1Y 2AA, Great Britain.

14—19 June 1981

International Symposium on Blind Infants and Young Children to be held in Tel Aviv, Israel.
Organized within the framework of the International Year of Disabled Persons by the Israel Society for Rehabilitation of the Dihabled and the International Institute for Visually Impaired Birth to Seven. It is open to physicians, nurses, teachers, psychologists, rehabilitation workers and especially parents of blind children.
Enquiries: Dr. E. Chigier, Secretariat, International Symposium on Blind Infants and Children, P.O. Box 394, Tel Aviv, Israel.

21—26 June 1981

5*th World Congress of Sexology*, in Jerusalem.
Main theme: Applied Sexology.
A pre-congress symposium on "Applied Sexology and Disability" will be held in Haifa from 18—19 June 1981 to mark the International Year for Disabled Persons 1981.
Enquiries: KENES, P.O. Box 16271, Tel Aviv, Israel.

4—11 September 1981

3*rd World Congress on Pain* to be held in Edinburgh, Scotland, organized by the International Association for the Study of Pain.
Plenary session themes: Pain and inflammation, Deafferentiation pain, Psychological methods in chronic pain, neurochemistry of nociception.
Plenary lectures: on: Electrostimulation procedures, development of a universal language of pain syndromes, endorphins and pain - an update. Language: English. A full social programme is also planned. Accommodation can be reserved either in the University Halls of Residence or in hotels.
Enquiries and registration packages from: Pain Congress Secretariat, the University of Edinburgh, Centre for Industrial Consultancy & Liaison, 16 George Square, Edinburgh EH8 9LD, Scotland, UK.

1—6 November 1981

IX Latin Medical Congress and the IV National Medical Congress of Rehabilitation, both organized by the American Medical Association of Rehabilitation (AMLAR) and the Ecuadorian Society of Physical Medicine and Rehabilitation, to be held in Quito, Ecuador.

18—24 April 1982

4*th World Congress of The International Rehabilitation Medicine Association* to be held at the San Juan Resort Center, Puerto Rico, sponsored by the International Rehabilitation Medicine Association and organized by the section of Physical Medicine and Rehabilitation of the Puerto Rico Medical Association.
Congress theme: New findings that influence disease and rehabilitation. A comprehensive programme includes 20 twohour seminars and 2 eight-hour courses plus individual paper essions, films and exhibits. In addition, there is provision for individual papers and scientific films relating to the congress theme.
A full social programme is also planned.
Registration Fee: before 15 January 1982 for IRMA members US$ 275.-, nonmembers US$ 325, allied health personnel US$ 125.-.
Deadline for submission of abstracts: 15 October 1981.
Simultaneous interpretation in French and Spanish if at least 75 members requiring this service in each language register by 15 January 1982.
Enquiries: Herman J. Flax, M.D., Chairman IRMA IV, PO Box 11696, Caparra Hts. Station, Puerto Rico, 00922 USA.

XII CONGRESO INTERNACIONAL DE PSICOTERAPIA

XII INTERNATIONAL CONGRESS OF PSYCHOTHERAPY
XII INTERNATIONALE KONGRESS FÜR PSYCHOTHERAPIE
XII CONGRÉS INTERNATIONAL DE PSYCHOTHÉRAPIE

XII CONGRESO INTERNACIONAL DE DE PSICOTERAPIA
RIO DE JANEIRO
DE 19 A 25 DE AGOSTO DE 1982
PATROCINIO: INTERNATIONAL FEDERATION FOR MEDICAL PSYCHOTHERAPY (IFMP)
ASSOCIAÇÃO BRASILEIRA DE PSIQUIATRIA (ABP)

Río de Janeiro —
de 19 a 25 de Agosto de 1982
Patrocinado por la Federación Internacional de Psicoterapia Médica y Associação Brasileira de Psiquiatria

PSICOTERAPIA Y CULTURA

Psychotherapy and Culture
Psychothérapie et Culture
Psychotherapie und Kultur
Psicoterapia e Cultura

El Comité Ejecutivo del XII Congreso Internacional de Psicoterapia, en el transcurso de las reuniones para la organización del evento y tras analizar las diversas sugerencias propuestas sobre el tema principal "PSICOTERAPIA Y CULTURA", decidió estructurar el Congreso en la forma siguiente:

CEREMONIAS DE APERTURA Y CLAUSURA:

La ceremonia formal de apertura se realizará el jueves 19 de agosto al anochecer Una breve ceremonia de clausura tendrá lugar el miércoles 25 de agosto. por la mañana.

SESIONES PLENARIAS:

Las principales exposiciones, dedicadas a los asuntos de mayor interés general. serán presentadas por 2 "Key-speakers", y se realizará un Panel obre cada tema, participando 5 "Key-speakers" por cada asunto.

GRUPOS DE TRABAJO:

Serán organizados de acuerdo con las necesidades de cada participante.

CONFERENCIAS CLINICAS Y DISERTACIONES ESPECIALES:

Abordarán asuntos del más alto interés común, principalmente referentes al tema central del Congreso.

PANELES O SIMPOSIOS:

Contarán con la participación de 5 "Key-speakers" por cada asunto.

MESAS REDONDAS:

Se organizarán varias Mesas Redondas, en las que tomarán parte 5 "Key-speakers" para discutir sus respectivos temas.

TEMAS LIBRES:

Dedicaremos 4 días para la presentación de Temas Libres, organizados en 3 sesiones por día. Los respectivos formularios deberán enviarse dentro del plazo estipulado (hasta el 20 de marzo de 1982).

EXHIBICIONES:

Organizaremos exhibiciones para todos los días.

SESIONES DE POSTERS:

Serán organizadas de acuerdo con las necesidades. Los congresistas que deseen presentar resúmenes y gráficas de sus trabajos. que se fijarán en tableros adecuados. y someterlos a consideración de los asistentes. deberán consignar en el formulario adjunto un resumen de su contribución, haciéndolo llegar a más tardar el 20 de marzo de 1982.

CURSOS:

Serán ofrecidos cerca de 12 Cursos, dictados por Profesores invitados, con participación máxima de 50 congresistas. La inscripción previa podrá efectuarse hasta la fecha de apertura del Congreso (19 de agosto).

TRABAJOS CIENTIFICOS:

Los interesados en presentar un trabajo científico deberán llenar el formulario de resúmenes anexo y enviarlo directamente a la Secretaría General.

El XII Congreso Internacional de Psicoterapia celebrará sus sesiones en la Universidad del Estado de Río de Janeiro - Rua São Francisco Xavier, 524, en el período del 19 al 25 de agosto de 1982.

Tema Principal:

PSICOTERAPIA Y CULTURA

Durante el Congreso serán discutidas las implicaciones de la psicoterapia y cultura.

El tema principal fue dividido en varios subtemas, a saber:

SESIONES PLENARIAS:

Contarán con dos "Key-speakers" por cada uno de los siguientes subtemas:

1 — Psicoterapia como un proceso libertador.
2 — Contribuciones antropológicas a la psicoterapia.
3 — Psicoterapia para las clases económicamente menos favorecidas.
4 — La dependencia de la psicoterapia en los factores culturales.
5 — La contribución de la psicoterapia para la higiene mental.

Después de las Sesiones Plenarias estos mismos subtemas serán discutidos en Paneles con la participación de 5 "Key-speakers".
Paralelamente se debatirán en Mesas Redondas los siguientes temas:

1 — Psicoterapia y ética.
2 — Psicoterapia y lingüística.
3 — El futuro de la psicoterapia (necesidad de cambiar el modelo médico).
4 — ¿Psicoterapia individual o de grupo?
5 — Psicoterapia y violencia.
6 — Terapia familiar.
7 — Psicoterapia breve.
8 — Psicoterapia alternativa.
9 — Ansiolíticos y psicoterapia.
10 — Neurolépticos y psicoterapia.
11 — Psicoterapia en las instituciones.
12 — Antidepresivos y psicoterapia.
13 — Psicoterapia de los ancianos.
14 — Historia de la psicoterapia.
15 — Psicoterapia y riesgo de suicidio.
16 — La visión de los jóvenes psiquiatras sobre la psicoterapia.

El XII Congreso Internacional de Psicoterapia procurará facilitar la discusión y el esclarecimiento de todas las cuestiones antes mencionadas. Con este propóito se planeará una serie de "Work-shops", de preferencia con un número reducido de disertaciones y un mayor énfasis en el trabajo de grupo.

IVA SYMPOSIUM

Premiere annonce
Appel aux Communications
First Announcement
Call for Papers
Symposium International sur l'Angiographie intraveineuse
15 - 18 Septembre 1982
International Symposium on Intravenous Angiography
September 15 - 18 1982
DGRST Délégation à la Recherche Scientifique et Technique
SEE Ingenierie Biomédicale (Section 27 de la SEE)
UPS Université Paul Sabatier
Toulouse - France

IVA SIMPOSIUM

COMITE DE PATRONNAGE/PONSORS

J. ESCAT. Président de la Commission Médicale consultative du C.H.R. de Toulouse
R. GUIRAUD. Président du Pôle Régional G.B.M. de Toulouse
B. GUIRAUD-CHAUMEIL. Doyen de la Faculté de Médecine Toulouse-Purpan
Y. LEMARIE. Directeur Général du C.H.R. de Toulouse
J. NOUGARO. Président de l'Institut National Polytechnique de Toulouse
P. PUEL. Doyen de la Faculté de Médecine de Toulouse-Rangueil

COMITE SCIENTIFIQUE/PROGRAMME COMMITTEE

A. BRUEL (Toulouse - France)
M. DUCOS DE LAHITTE (Toulouse - France)
J.W. LUDWIG (Utrecht - Netherlands)
M. LAVAL-JEANTET (Paris - France)
C. MANELFE (Toulouse - France)
J.P. MARC-VERGNES (Toulouse - France)
T.F. MEANEY (Cleveland - Ohio - U.S.A.)
C.A. MISTRETTA (Madison - Wisconsin - U.S.A.)
S. NUDELMAN (Tucson - Arizona - U.S.A.)
A. RASCOL (Toulouse - France)
C.M. STROTHER (Madison - Wisconsin - U.S.A.)

COMITE D'ORGANISATION/ORGANISING COMMITTEE

J.P. MARC-VERGNES (Chairman)
M. DUCOS DE LAHITTE
C. MANELFE

SUJETS/TOPICS

1. Techniques radiologiques: Procédés d'injection - Produits de contraste - Positionnement - Immobilisation - Technique de soustraction de film.
2. Techniques informatiques: Principes et modatiltés de l'acquisition des images imagerie numérique (traitement d'image, visualisation, stockage, hard copy...).
3. Indications et résultats (y compris évaluation des résultats de la chirurgie vasculaire, informations physiologiques) dans le territoire cérébro-vasculaire.
4. Indications et résultats (y compris évaluation des résultats de la chirurgie vasculaire, informations physiologiques) dans le domai ne thoracique: cœur, coronaires, artères pulmonaires, évaluation des masses médiastinales...
5. Indications et résultats (y compris évaluation des résultats de la chirurgie vasculaire, informations physiologiques) dans le territoire de l'aorte abdominale, et des artères rénales et périphériques.
6. Aspects économiques de l'Angiographie intraveineuse.
   Présentations orales de produits et d'appareils par des représentats d'entreprises industrielles et commerciales.

1. General technical aspects: Injection techniques - Contrast media - Tolerance - Positionning - Immobilisation - Film substraction technique.
2. Digital technical aspects: Design considerations for image acquisition - Digital imaging including processing, display, archival storage and hard copy...
3. Indication and results (including evaluation of the results of vascular reconstructive surgery, physiologic informations...) in the cerebro-vascular field
4. Indication and results (including evaluation of the results of vascular reconstructive surgery, physiologic informations...) in the thoracic field: heart, coronary and pulmonary arteries, evaluation of mediastinal masses...
5. Indications and results (including evaluation of the results of vascular reconstructive surgery, physiologic informations...) in the field of abdominal aorta, renal and peripheral arteries.
6. Economic aspects of IVA.
   Oral presentations of products and devices by representative of companies.

INSTRUCTIONS AUX AUTEURS/INSTRUCTIONS TO AUTHORS

II est rappelé aux auteurs de communications qu'ils doivent soumettre un résumé d'une page dactylographiée environ, en français ou en anglais, au plus tard le 15 mars 1982. Les références bibliographiques les plus importantes seront jointes à ce résumé.
Authors are reminded to submit an abstract in English or French no later than March 15th, 1982. The major references should be attached to this abstract limited to about one typed page.

AFFICHES/POSTERS

Les futurs participants sont invités à soumettre des affiches.
Ces affiches seront exposées près des salles de conférences. A heures fixes leurs auteurs se tiendront à la disposition des congressistes pour répondre aux questions éventuelles.
Intending participants are invited to submit posters.
The posters will be displayed next to the conference room. At scheduled times authors will be present to discuss their contribution.

EXPOSITIONS-DEMOSTRATIONS/
EXHIBITION-DEMONSTRATIONS

Divers équipements pourront être présentés durant le Symposium. Dés facilités seront accordées aux participants pour leur permettre de faire des démonstrations à condition d'indiquer à l'avance la nature de l'équipement nécessaire.
Various equipments can be presented during the Symposium. Facilities will be provided to enable participants to demonstrate. The necessary facilities must be requested in advance.

LIEU/LOCATION

Université Paul Sabatier
118, Route de Narbonne - TOULOUSE

LANGUES OFFICIELLES/
OFFICIAL LANGUAGES

Français - Anglais
French - English

DROITS D'INSCRIPTION/

FEES: 800 FF
Cette inscription donne droit à un exemplaire des Actes du Symposium et aux repas de midi.
These fees include one copy of the Symposium Proceedings and lunches.

DEPARTMENT OF HEALTH & HUMAN SERVICES

National Institutes of Health
Bethesda, Maryland 20205
Federal Building
Room 904
(301) 496 - 1714
May 14, 1982

To: Members of the World Federation of Neurology Research Committee on Neuroepidemiology

Dear Colleague:

The second meeting of the year will be held on Thursday, June 17, in Cincinnati, Ohio in conjunction with the Society for Epidemiologic Research.

Business Meeting: 1:00 - 1:45 p.m., Thursday, June 17, 1982, Cincinnati Convention and Exposition Center. The room will be posted on the day of the meeting, and information will be available at the meeting registration desk.

Due to prior commitments, I shall be unable to attend this year's session; however, Dr. Gary B. Beringer will preside over the meeting on my behalf.

Agenda for Business Meeting

I. Standardization of classification
II. Standardization of methodology for neuroepidemiologic studies in developing countries
III. Educational programs.

A. Courses
   1. Advanced Course on Neuroepidemiology, San Miniato, Italy, 1981
   2. Controlled Clinical Trials in Neurology, Washington, D.C., 1982.
   3. Occupational Neurology, Finland, 1982.
   4. Neuroepidemiology, Quito, Ecuador, 1982.
   5. Advances in Neuroepidemiology, Caracus, Venezuela, 1982.

B. Fellowship programs
   . World Health Organization
   2. National Institutes of Health.

IV. Journal
V. Scientific programs
   1. Society for Epidemiologic Research, Snowbird, Utah, June, 1981.
   2. International Epidemiologic Association, Edinburgh, Scotland, August, 1981.
   3. Satellite Symposium, World Congress of Neurology, Kyoto, Japan, September, 1981.
   4. American Public Health Association, Los Angeles, California, November, 1981.
   5. Italian Society of Neurology, Neuroepidemiology Section, Milan, Italy, February, 1982.
   6. American Academy of Neurology, Washington, D.C., April, 1982.
VI. Research Centers
VII. Miscellaneous

We are indebted to Dr. B. Specker for the local arrangements in Cincinnati. I hope many of you will be able to attend.

Sincerely yours,
Bruce S. Schoenberg, M.D., Dr. P.H.
Chairman, World Federation of Neurology Research Committee on Neuroepidemiology

2nd INTERNATIONAL SYMPOSIUM ON BRAIN - HEART RELATIONSHIP

Jerusalem, Israel, June 5—8, 1983
Devoted to the memory of the late Professor Sylvan Lavy.

LOCAL ORGANIZING COMMITTEE:
SHLOMO STERN, M.D., CHAIRMAN
Professor of Medicine. Bikur Cholim Hospital and Hebrew University-Hadassah Medical School, Jerusalem

SHAUL FELDMAN, M.D.
Professor of Neurology. Hebrew University-Hadassah Medical School, Jerusalem.

HENRY N. NEUFELD, M.D.
Professor of Medicine. Chaim Sheba Medical Center. Sackler School of Medicine, Tel Aviv University.

RAMI RAHAMIMOFF, M.D.
Professor of Physiology. Dean, Hebrew University-Hadassah Medical School, Jerusalem.

9th WORLD CONGRESS OF SOCIAL PSYCHIATRY

Preliminary Program
Porte Maillot

WORLD ASSOCIATION FOR SOCIAL PSYCHIATRY

Honorary Life President
J.H. MASSERMAN, USA

Honorary President
V. HUDOLIN, YU

Founder
J. BIERER, UK

Preident
G. VASSILIOU, GR

Chairman
V. HUDOLIN, YU

Secretary-General and President Elect
J.L. CARLETON, USA

Treasurer
U. MALENDORF, USA

Legal Counsel and Permanent Representative
K. MAYER, CH

Executive Council
P. ADAMS, USA - A. KASHA, DZ
Z. BIZON, PL - R. MACIAS, MEX
I. BLUMBERG, USA - L. MILLER, IL
J.A. BUSTAMANTE, C - S. NAKA, J
A.G. FERREIRA, P - S. ROST, S
G. FERSCHTUT, RA
W. SCHINDLER, UK
P. JEAN, F - C. VEIL, F
V. JELIC, YU

International Secretariat Committee

President
V. HUDOLIN, YU

Members
J.L. CARLETON, USA
G. FERREIRA, P
P. JEAN, F
R. MACIAS, MEX
P.O. DONNELL, E
S. ROST, S

FRENCH ASSOCIATION FOR SOCIAL PSYCHIATRY AND PSYCHOPATHOLOGY

President
P. JEAN

Vice-Presidents
Y. PELICIER
P. CHANOIT

Secretary-General
Y. THOBIE

Assistant Secretary General
J. de VERBIZIER

Treasurer
S. BAUMÉ

LL *Assistant Treasurer*
G. COURS-MACH

Members
R. AMIEL
M. BAUDRY
Y. CASTELLAN
F. CLOUTIER
L. CROCQ
M. HUGUET
C. IZARD
J.C. SCOTTO

LOCAL ORGANIZING COMMITTEE OF THE 9th WORLD CONGRESS

President
P. JEAN

Members
R. AMIEL - L. CROCQ
J.C. ARCHAMBAULT - M. HUGUET
M. BAUDRY - C. IZARD
S. BAUHÉ - C. KOUPERNIK
B. BOISSET - Y. PELICIER
Y. CASTELLAN - M. PHIBERT
P. CHANOIT - J.C. SCOTTO
F. CLOUTIER - Y. THOBIE
G. COURS-MACH - J. de VERBIZIER

President of the Scientific Council
P. SIVADON

with the cooperation of a great number of French and foreign colleagues

SYNOPSIS

- Collecting of the Congress documents
- Late registrations
(Palais des Congrès)
- Opening Session
- Psychopathological problems associated with underemployment

In the evening:

- Welcome Reception
(Hotel Concorde Lafayette)

● Attitudes of the general public towards Health and Prevention measures

In the evening:

● Concert
(Cathedrale Notre-Dame de Paris)

Morning:

● Selected topics in Social Psychiatry

Afternoon:

● Excursions

● *Free Evening*

● Social indicators and mental health indicators

In the evening:

● Evening Party
(Jardin d'Acclimatation)

● Psychopathological aspects of the consumption of drugs.

● Collecting of the Congress documents

● Late registrations

The Organizing Committee strongly advises all Congressists to come and collect their documents *the day before* the official opening of the Congress, from 9 h 00 until 13 h 00 and from 14 h 00 until 18 h 00, on *Sunday, July* 4 at the Palais des Congrès

● Opening session

● Psychopathological problems associated with underemployment

Opening Session of the Congress *
It will be held in the Main Auditorium of "la Sorbonne", seat of the oldest university in Paris, founded in 1257

Lectures

● Aspects of underemployment

Speaker:
not yet confirmed

● Psychosociological aspects of unemployment

Sjeaker:

Professeur A. TOURAINE (F) Director of the Centre d'Etudes des Mouvements Sociaux.

Ecole des Hautes Etudes
en Sciences Sociales.

Invitations cards will be sent before the Congress to all advance registered persons.

● Psychopathological problems associated with underemployment

Palais des Congrès
Porte Maillot

Symposia and workings groups

● Mental pathology and unemployment

● Labour mobility as related to underemployment

● Underemployment and demography

● Underemployment as related to technological development

● Psychological attitudes and social behaviour of young people in search of their first joh

● Difficulties of the ageing worker

● Psycho-sociological consequences to the dismissal

● Alterning unemployment

Frees papers and posters

● Attitudes of the general public towards health and prevention measures

Morning
Lecture
Speaker:

Professeur R. SENAULT (F) Chairman of the Comité Français d'Education pour la Santé

Round Table

Criteria

for selecting a therme for a Health and Prevention campaign.

Chairman:

Professor E.A. SAND (B) Université libre de Bruxelles

Afternoon

Symposia and working groups

● Behaviour of the teenagers in front of hygienic and preventive measures concerning contraception

● The experience of the "corner-street-groups"

● Danger of the failures and reasons of the success of prevention campaigns

● Public's behaviour towards mental illness

● Alternatives to medical care

Free papers and posters

● **Selected topics in social psychiatry**

Morning

Symposia and working groups Free papers Posters

Afternoon

Choice of excursions

● **Social indicators and mental health indicators**

Morning

Lectures

● The concept of the social indicator

Speaker:
not yet confirmed

● The concept of the health indicator

Speaker:
Professor P. RECHT
Commission of the European Communities (Belgium)

Round table

● Are social indicators good mental health indicators?

Chairman:
not yet confirmed

Afternoon

Symposia and working groups

● Attempted suicid

● Suicides, problem behaviour (possibly to be subdivided into adolescent and adult suicide).

● Fatigue, subjective dimension and objective value as a health indicator

● Delinquency and criminality (as a social indicator)

Free papers and posters

● **Psychopathological aspects of the consumption of drugs**

Morning

Lecture

How medical practice has evolved in different economic, political and cultural contexts

Speaker:
Professor T. A. LAMBO,
Deputy Director General of W.H.O. (Nigeria)

Round table

Traditional medicines and scientific medicine

Moderator:
Doctor T.A. BAASHER
E.M.R.O. (Alexandria, Egypt)

Afternoon

Symposia and working groups

● Iatrogenic processes

● Use and misuse of convenience drugs

● Prescription and consumption of drugs

● Drug addiction as related to drug consumption in developped societies

● How patients'attitudes have changed towards the medical profession (with particular reference to the increasing number of malpractice suits)

● Free health care and its impact on the consumption of drugs

● Self-medication

Free papers and posters

INTERNATIONAL REHABILITATION MEDICINE ASSOCIATION

I N V I T A T I O N

to the IRMA Council Assembly on Tuesday, April 20, 1982 at 17.00 at the Convention Center San Juan - Puerto Rico.

A G E N D A

1. Call to order
2. Introduction of Councillors
3. Establish quorum and adoption of the Agenda
4. Correction and approval of minutes of last meeting as published in NEWS & VIEWS 1980, 2 (ref. B) August 1980 Stockholm
5. Obituatry Pierre LAMBERT
   — Professor J.M. ANDRE - W.M. ZINN, M.D.
   Obituary Marian WEISS
   — Dr. K. MILANOWSKA
   Obituary Victor SANTANA-CARLOS
   — Rrs. M. SANTANA-CARLOS - Chris D. EVANS, M.D.
6. President's report
   — W.M. ZINN, M.D.
7. Secretary's report
   — Christopher D. EVANS M.D.
8. Treasurer's report
   — William J. ERDMAN II, M.D.
9. IRMA IV report
   — Herman J. FLAX, M.D.
10. Report and future activities of liaison officers to international organizations
    a) Council of International Organisations of Medical Sciences (C.I.O M.S.)
    — Hans-Jürg HACHEN, M.D.

b) International Federation of Physical Medicine and Rehabilitation (I.F.P.M.R.)
— Herman J. FLAX, M.D.
c) Medical Commission of Rehabilitation International
— Herman J. FLAX, M.D.
d) Organisation Mondiale de Cooperation Diplomatique (O.M.C.D.)
— Christopher D. EVANS, M.D.

11. Journal INTERNATIONAL REHABILITATION MEDICINE
Editor's report
— Philip H.N. WOOD, M.D.
12. To discuss aims of IRMA and its future structure
13. Election of Councillors
14. Establishment of Administrative Secretariat
progress report
— Mrs. D. LANG
15. 1981 UN International Year of Disabled Persons short reports from councillors expected
16. Election of officers of the Executive Committee
17. Future activities
a) Committee on measurement
— J.J. GERHARDT, M.D. - J. RIPPSTEIN, M.D.
b) IRMA V
— George G. BURNISTON, M.D.
c) Educational problems
d) Probable regional meeting
e) Other activities
18. New business
19. Resolutions
20. Adjournment

NINCDS - Notes

New Name, Addittion, for NIH Clinical Center

The NIH Clinical Center in Bethesda. Md., formally received a new name, the Warren Grant Fagnuson Clinical Center. and a new wing, the Ambulatory Care Research Facility· in October in ceremonies attended by HHS Secretary Richard S Schweiker.

In renaming the Clinical Center, NIH recognized the longtime support of Federal biomedical research given by the former senator from Washington, who for many years chaired the Senate Subcommittee on health appropriations. As a freshman congressman in 1937, Senator Magnuson sponsored legislation creating the National Cancer Institute, the first of the National Institutes of Health.

The dedication of the Ambulatory Care Research Facility, a modern $100 million wing of the Clinical Center devoted to outpatient clinics and laboratories, was of special interest to NINCDS scientists who plan to occupy facilities there devoted to research on communicative and nervous system disorders. The nearly completed fifth-floor NINCDS clinic will include movement disorder, neuromuscular disease, pharmacology, and ear, nose, and throat laboratories. and a supporting "medical procedures" area where patients can be screened. treated. and monitored. The Institute's PET scanner will also be located in the new ACRF, and plans are under way to build a cyclotron nearby to supply the scanner with isotopes.

It has been estimated that the Magnuson Clinical Center, including the ACRF wing, will now accommodate 7·600 hospitalized patients and 100,000 outpatient visits a year.

Epilepsy Explained in New NINCDS Pamphlet

Epilepsy: *Hope Through Research*, the latest in a series of NINCDS pamphlets designed to provide the public with information about research on neurological and communicative disorders, is now available through the Institute's Office of Scientific and Health Reports.

The 28-page booklet describes the symptoms, diagnosis, and causes of different types of epileptic seizures, and reports on the various treatments now in use. The publication also explores the research approaches being used to understand and combat the disorder, and offers advice to patients, family members, and those who may find themselves in a position to give first aid during a seizure. The final pages list several voluntary health organizations especially concerned about epilepsy, and the location of human tissue banks which support epilepsy research.

Single copies of *Epilepsy*: *Hope Through Research* can be obtained from the Office of Scientific and Health Reports, NINCDS, Bldg. 31, Rm. 8A06, 9000 Rockville Pike,

Bethesda, Md. 20205; Tel.: (301) 496-5751.

Head and Spinal Cord Injury Statistics Summarized

Major findings and highlights of the NINCDS-sponsored National Head and Spinal Cordd Injury Survey have now been published in condensed form by the Institute's Office of Scientific and Health Reports. The condensed survey report addresses the rates, costs and circumstances of a problem that affects 430,000 Americans a year.

The complete survey report, which was published as a supplement to the *Journal of Neurosurgery* in November 1980, presents the first reliable statistical picture of head and spinal cord injury problems in the United States. The survey concentrated on injuries which ocurred between 1970 and 1974, and which resulted in admissions to hospitals in the Nation's 48 contiguous states.

The condensed summary, *The National Head and Spinal Cord Survey* (Stock no 017-049-00122-1), is available at $2.50 a copy from the Superintendent of Documents, U.S. Government Printing Office. Washington, D.C. 20402.

VI MEETING OF THE EUROPEAN SOCIETY FOR STEREOTACTIC AND FUNCTIONAL NEUROSURGERY

Roma (Italy): June 2 - 4, 1983

MAIN TOPICS:

1) EPILEPSY
2) CEREBRAL TUMORS
3) PAIN

For Informations write to:

Prof. Gian Franco Rossi
Istituto di Neurochirurgia
Università Cattolica
Largo A. Gemelli, 8
00168 Roma (Italia).

• Book Reviews

MODERN PRACTICAL NEUROLOGY

PERITZ SCHEINBERG

1981 - Raven Press - New York
352 pages.

The Preface of the author explains very well the orientation of the book.

"The second edition of Modern Practical Neurology has been completely revised and details added to make it more responsive to the needs of medical students, house officers, and physicians who undertake to manage patients with neurological disorders. Its format is different from most standard texts of neurology in that historical, neuroanatomical, neurophysiologic and neuropathological descriptions are not extensive but are limited to what is required to clarify the mechanisms of the disorder being described. Although the organization of the book is disease-oriented· a special effort has been made to explain symptoms and signs in the cóntext of the mechanism of their causation.

Many medical students and physicians regard clinical neurology as a specialty concerned with the differential diagnosis of complex, degenerative disorders for which there is no useful treatment. It is true that many neurologic diseases, even some common ones, cannot be successfully treated. but the same problem exists in most of medicine, with the physician offering amelioration rather than cure. It is also true-and in this regard the public relations of neurology have not been good-that neurologists have a great deal to offer the majority of their patients. To that end, one of the major objectives of this book is to emphasize tha practical problems of diagnosis and management. Liberal use has been made of computed tomography and other radiographic techniques in discussing diagnosis and when indicated, specific details of therapy are included.

This volume, then, is less concerned with theories of mechanisms that with a concise review of the diagnosis and management of those neurological disorders most commonly seen in practice. It is not intended to be encyclopedic. Numerous monographs and multiple volume neurology text are available to the reader who wishes more information. To assist students, residents, and others so interested in a review of pertinent literature, extensive bibliographies have been compiled to accompany each chapter.

The views expressed herein are the construct of personal experience, literature search, and much opinion-seeking others. The reader should be reminded that, like all single-author texts, these opinions may not be shared by all other authors, or clinicians."
This is the contents:

1 — The neurologic Exmination
2 — Cerebrovascular Diseases
3 — Dementia
4 — Disorders of Movement· Tone, and Coordination
5 — Brain and Spinal cord Ináuries.
6 — Dizziness
7 — Infections of the Nervous System
8 — Multiple Sclerosis
9 — Headache
10 — Epilepsy
11 — Management of the Unconscious Patient
12 — Syndromes of the Neck and Low Back
13 — Neuromuscular Disorders
1b — Gait Disorders
15 — Intracranial and Spinal Tumore
16 — Neurologic Aspects of Medical Diseases.

This is a book written in a precise and consise way, aiming to orientate the reader in a brief lapse of time in the patient's management, in all the aspects related to diagnosis and treatment, offering to the reader all the technical and scientific advances.

The author in his role of Professor and clinician has accumulated knowledge and experiences that he expounds with great aptitude as a teacher, in order that it may eassily reach all kinds of readers, whether they are specialists interested in being up to date with the care of neurological patcients or general practitioners that wish to be able to manage all kinds of patients that way demand his attention in any kind of ailment.

Prof. Dr. Víctor Soriano

CLINICAL NEUROANATOMY FOR MEDICAL STUDENTS

RICHARD S. SNELL M.D., Ph.D.

559 pages - Firsts - Edition - 1980
Little, Brown and Company - Inc.

We will reproduce parts of the Preface of the authour.

"While it is fascinating to learn about the ultramicroscopic structure of synapses and the detailed connections of the hypothalamus, it is first essential that a student understand such phenomena as tha pupillary light reflex, be able to explain why a patiente with cerebellar disease tends to fall to the same side as the lesion, and wnow the level at which the spinal cord terminates within the vertebral canal".

"The purpose of this book is not to replace the larger reference texbooks of neuroanatomy, but, rather, to offer the practical aspects of neuroanatomy, in a simplified manner. Clinical problems requiring a knowledge of anatomy for solution are presented at the end of each section. References to neurcanatomical literature are included so that the student can acquire a deeper knowledge of an area of interest, should he so desire".

CONTENTS:

1 — Organization of the Nervous System.
2 — The Neuron.
3 — Neuroglia.
4 — Nerve Fibers and Peripheral Nerves.
5 — Receptor and Effector Endings.
6 — Dermatomes and Muscular Activity.
7 — The Spinal Cord.
8 — The Medulla Oblongata.
9 — The Pons.
10 — The Cerebellum.
11 — The Fourth Ventricle.
12 — The Midbrain (Mesencephalon).
13 — The Cerebrum.
14 — The Cerebral Hemispheres.
15 — The Structure and Functional Localization of the Cerebral Cortex.
16 — The Limbic System.
17 — The Ventricular System and the Formation and Fate of the Cerebrospinal Fluid.
18 — Blood-Brain and Blood-Cerebrospinal Fluid Barriers.
19 — The Ascending Tracts of the Spinal Cord.
20 — The Descending Tracts of the Spinal Cord and Skeletal Muscle Activity.
21 — Connections of the Cerebellum.
22 — The Cranial Nerve Nuclei and Their Central Connections.
23 — The Thalamus and its Connectiones
24 — The Hypothalamus and Its Connections.
25 — The Autonomic Nervous System.
26 — The Meninges of the Brain.
27 — The Blood Supply of the Brain.
Answers to Clinical Problems.

This book is profusely illustrated. Many of its illustrations are schematic and beautifully designed which makes them very explicit.

Because of the simplicity and clearness of its descriptions, it is very easy to read, even thovg neuroanatomy is a difficult subject to learn.

Besides its attractive presentation, it conducts the reader to the consideration of the clinical topics related with every aspect of neuroanatomy. In this way each chapter has a sector belonging to clinical notes, where the pathology related to it is described. After this comes a chapter dealing with Clinical Problems in which there are brief clinical histories connected to the ubject.

At the end of the book there are answers to the medical problems posed in the different chapters of the book.

In this way the author has fulfilled his aim of reaching the medical students with this excellent book on Clinical Neuroanatomy.

Prof. Dr. Víctor Soriano

THE DIAGNOSIS OF STUPOR AND COMA

FRED PLUM M.D. and JERONE B. POSNER, M.D.

Edition 3 — F.A. DAVIS COMPANY PHILADELPHIA — 1980 vol. 373 pages.

We reproduce the preface to the Third Edition, to the First Edition and "a final word" of the book.

"During the eight years since the second edition of "The Diagnosis of Stupor and Coma" the development of computerized transaxial tomography has transformed the process of diagnosis clinical neurology. Where CT units are available. technology has replaced clinical deduction in the ca-

pacity to identify and localize many intra cranial mass or destructive lesions.

But the art of diagnosis is to comprehend the whole picture: where the lesion is. what it comprises, and above all, what it is doing to the patient. CT scanning cannot answer the sometimes difficult question of whether a visualized lesion is of sufficient size and location to explain the associated symptoms, nor does it provide an answer to the metabolic or diffuse disturbances that cause or worsen severe disability of the brain. These steps require the thought of an informed doctor. Accordingly, we have kept our original goal for Stupor and Coma: to provide a clinically slated volume that will help the reader understand and diagnose severe brain dysfunction both as it exists and as it evolves in the seriously ill. The book remains a treatise on pathophysiology because radiologic, electrophysiologic, and chemical technology by themselves are insufficient substitutes for the physician's educated mind in the management of patients.

Advances in knowledge or at least in our understanding of our subject have led to substantial changes in this edition. Chapter 1 has been largely rewritten and extenive additions and revisions have been made in Chapters 2 through 5. Chapter 6, on prognosis, has been substantially lengthened to reflect the results of recent studies on outcome from head trauma and medical coma. Finally, since outcome from coma often depends to a very great extent on the general physician treats the patient during the first hours after onset and even before the diagnosis is reached, we have added a chapter outlining principles of early management. Many illustrations have been either redrawn or added, thanks to the skill of the medical illustator, Hugh Thomas.

Stupor and coma are such common clinical states that it is unusual for a busy hospital emergency room to pass a day without facing a diagnostic problem involving one or the other. Depite this ubiquity of the problem however, no current volume has directed itself at the clinical approach to diagnosis of states of impaired consciousness. and Cheyne's monograph of 150 years ago, from which we have appropriated the charming frontispiece, is now medically somewhat out of date. It has been our good fortune to be associated with hospitals and colleagues serving wide segments of the community and receiving many diagnostic from other physicians.

The demands of patients for care forced us to develop for ourselves a systematic approach in diagnosing coma, and the lively interest among our associates in the whys and hows of unconsciousness prompted us to explain our approach and summarize some of the lessons learned. This book is the result."

A FINAL WORD

"This boock has presented a physiology approach to the differential diagnosis and the emergency management of the stuporous and comatose patient. The approach is based on the belief that after a history and a general physical and neurologic examination, the informed physician can with resonable confidence, place the patient into one of four major groups of illnesses that cause coma. The specific group into which the patients is placed directs the rest of the diagnostic evaluation and treatment. At times, however, the diagnosis is uncertain even after the examination is completed and it is necessary to defer even the preliminary categorization of patients until the CT scan or metabolic test are carried out and the most serious infections or metabolic abnormalities have been considered. No patient should be denied a CT scan if there is any suspicion of a mass lesion, despite the absence of focal signs. No patient should be denied glucose if there is any suspicion of hypoglycemia, despite the presence of hemiplegia or other focal signs. At all times during the diagnostic evaluation and the treatment of a patient who is stuporous or comatose, the physician must ask himself whether his diagnosis could possibly be needs to seek consultation or undertake other diagnostic or therapeutic measures. Fortunately, with constant attention to the changing state of consciusness and a willingness to reconsider the situation minute by minute, few mistakes should be made. Good luck!"

CONTENTS

1.— The Pathologic of signs and Symptoms of Coma
2.— Supratentorial Lesions Causing Coma
3.— Subtentorial Lesions Causing Coma
4.— Multifocal, Diffuse, and Metabolic Brain Diseases Causing Stupor or Coma
5.— Psychogenic Unresponsiveness
6.— Brain Death
7.— Prognosis in Coma
8.— Approach to the Unconscious patient.

This book is an extraordinary contribution for a complete information about the subject and for the attention of the different types of patients that in emergency require the care as a consequence of a deficiency of the conciousness.

The authors have not lessened efforts no matter the extent of the book to explain in an extensive, accurate and concise way all the processes that in stupor and coma may be involved, profiting from all the progresses in science for an uptodate explanation of every subject and to elaborate for the study and management of the patients all the most adjusted techniques, taking into consideration, diagnosis, pronostic and treatment.

Evidently it is a very important book for specialists in diseases of the Nervous System and for all the physicians that are involved in the management of emergencies.

Prof. Dr. Víctor Soriano

"THE PRIMARY ACOUSTIC NUCLEI"
RAFAEL LORENTE DE NO—1981

RAVEN PRESS: NEW YORK-177 pages.
We reproduce part of the Foreword of Prof. Víctor Goodhill:

"This extraordinary work on the central auditory neural structures comes at an auspicious time. Audiologic attempts to deal with central pathway diagnoses have escalated sharply in the past decade".

"Rarely is reference made to any of Dr. Lorente's classic obervations. Thus, the exquisite role of the acoustic tubercle (tuberculum acousticum (t.ac.) has not been adequately considered in basic conceptions necessary for anatomico-physiologic interpretations."

"The t.ac. has been defined by Dr. Lorente as the cerebellum of the acoustic system. In the course of phylogenesis the cochlea appeared late after the structure and connections of the cerebellum had developed to deal with body posture and motion. In the later phylogenetic appearance of the cochlea and acoustic system, there was need for a new" acoustic cerebellum"—the t.ac. which has a far more elaborate structure than the motor cerebellum."

"In December 1923, Professor Robert Bárány came to lecture in Zaragoza and observed Dr. Lorente's nystagnus experiments. Professor Bárány expressed great interest in the work and with the approval of Cajal he invited Dr. Lorente to move to Sweden. After obtaining his M.D. degree from the University of Madrid in 1923, Dr. Lorente joined Bárány's department in Uppsala, where he began work in 1924 doing both anatomical research on the labyrinth and its centers and physiological studies of labyrinthine reflexes of eye muscles both in normal rabbits and in rabbits with experimental lesions of the vestibular pathways or nuclei (1933c.")

"Through the advice of Dr. Alan Gregg of the Rockefeller Foundation, Dr. Lorente accepted a research position at the Central Institute for the Deaf in St. Louis, which had been founded by Dr. Max Goldstein.

In April 1931, Dr. Lorente and his wife came to St. Louis Missouri.

In St. Louis Dr. Lorente continued his experiments producing discrete lesions to modify vestibulo-ocular reflexes and published his classic papper entitled "Vestibulo-Ocular Reflex Arc" (1933c). In that paper he introduced the concept that the nervous system is made up of combinations of chains of neurons of two types which he designated as multiple and closed chains. Since both types of chains are always interlaced, nerve impulses may circulate through the chains. Consequently, the nervous system could no longer be considered to consist of neurons of fiexed order, such as first order neurons, second order neurons, third order neurons, etc. He also explained that (a) vesticular reflexes can be established through the reticular substance after all direct pathways have been severed, and (b) after certain lesions in the reticular

system, the direct pathways are insufficient to establish the rapid phase of nystagmus."

"After some preliminary work on auditory neuroanatomy in Uppsala, he continued his investigations in St. Louis resulting in the now classic paper (1933b) on the general plan of structure of the prima ry cochlear nuclei.

A very important concept appeared for the first time in that paper, namely that since there are internal paths connecting the neurons of the various parcels of the acoust nuclei, the discharge of nerve impulses by a given acoustic neuron must depend upon the impulses brought in by the cochlear nerve as well as upon the discharges of other acoustic neurons."

"In 1933 Dr. Lorente had made this essential statement The whole vestibular system has revealed itself as constituted by numerous chains of neurons, reciprocally connected in many ways and having their links in various anatomic nuclei. All the chains work in intimate collaboration and all are necessary for the production of the normal reflex reactions."

"For a number of years I felt very strongly that Dr. Lorente's classic work on the primary acoustic nuclei should be made available to the otologic and the neuroscience communities, and I therefore suggested to the editors of Raven Press that such a monograph by Dr. Lorente would be of grest interest. I am delighted that both Dr. Lorente and Raven Press have collaborated so fruitfully in the publication of this classic volume, which clearly delineates the morphologic substtrate of auditory processing. Future investigations in the fields of audiology, auditory neurophysiology, and otology will depend greatly upon this fundamental historical contribution."

CONTENTS

This is an exceptional book in which is embodied a monumental labor of investigation of the author following the lines established by his ancient teacher, Prof. Ramon y Cajal.

It is evident that many of the functional aspects of the Nervous System can be only understood if the anatomical basis can be analized in its microscopical aspect of cells and pathways of the sector under study and in this sense the contribution of Dr. Lorente de Nó to the better knowledge of the anatomical integration of the primary accoustic nuclei is extraordinary.

It may be assured that this book, due to its extraordinary neurohistological studies, will become a historical one in the investigation of the nervous system, because due to the actual orientation of the neurosciences one of this kind will not be repeated.

Prof. Dr. Víctor Soriano