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Neurologically Active Peptides

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BARBEAU, A., M. GONCE AND A. J. KASTIN. *Neurologically active peptides.* PHARMAC. BIOCHEM. BEHAV. 5: SUPPL. 1, 159–163, 1976. – This paper reviews recent evidence that a number of small peptides found in the brain are active in the central nervous system and behaviorally. Attention is focused on MSH/ACTH 4-10, α- and β-MSH, and the prohormone β-LPH, as they produce a syndrome of yawning and stretching. Studies with substance P and mainly with MIF-1 are also reviewed. It is shown that substance P is an excitatory transmitter or modulator in the dorsal spinal cord and that MIF-1 has antiparkinson properties. It is concluded that many polypeptides have direct actions on the central nervous system independent of their neuroendocrine properties.

Polypeptides Substance P MSH-ACTH 4-10 β-MSH β-LPH MIF-1

THE LAST 15 years have seen the extraordinary growth of knowledge in the physiology, pharmacology and biochemistry of monoamines, particularly spurred on by the findings of abnormal dopamine metabolism in Parkinson's disease and the encouraging success of levodopa therapy in that illness [1]. Recently there has been renewed interest in the role of amino acids in the brain, such as gamma-aminobutyric acid (GABA) [56] and the more neglected taurine [31].

In parallel with this activity, the field of neuroendocrinology has been steadily growing, leading to exciting new findings. One of the products of these studies is undoubtedly the recognition that a number of peptide hormones act directly upon the brain to affect learning and behavior. Prominent among these active hormones are substances isolated from the hypothalamus which act, on the one hand, as releasing or release-inhibiting factors and, on the other hand, possess independent behavior modifying properties. The present paper will review some examples of these recent findings, with particular emphasis upon our own experiments. A thorough study of the role of peptides in neurology would have to consider the following three main facets: (1) Behavioral and neurological effects of pituitary hormones particularly ACTH and β-MSH. (2) Behavioral and neurological effects of intracerebral peptides: In this category are found angiotensin, lysine vasopressin, the sleep inducing peptides, the dark avoidance peptide, scotophobin, ameletin and finally substance P. (3) Behavioral and neurological effects of hypothalamic peptides. At present there is evidence of activity for TRH, LHRH, somatostatin and MSH-release inhibiting hormone (MIF). A more complete and detailed review is in preparation for publication elsewhere [3].

BEHAVIORAL AND NEUROLOGICAL EFFECTS OF PEPTIDE HORMONES

A number of well known, and important, functions of the organism are dependent upon the action of peptide hormones: sexual behavior, including female receptivity state and mating, are influenced by gonadotropins; eating can be induced by insulin; thirst and the consequent drinking are inhibited by vasopressin, while motivated drinking is induced by angiotensin [59]; parturition can be triggered or accelerated by oxytocin; it is also well known, particularly in frogs, that melanocyte stimulating hormone (MSH) can induce skin color changes; lipolysis can be induced by both MSH and β-lipotropic hormone (β-LPH) [19]. Finally the best studied phenomenon is that of neurosecretion in the hypothalamus-pituitary interphase [24].

Most of the active peptides studied are not synthesized *de novo*. Rather they originate from the enzymatic breakdown of much larger molecules. This discovery was made simultaneously for insulin, which derives from proinsulin, by Steiner and Oyer [62] and for β-MSH, which originates from lipotropin, by Chrétien and Li [14]. It has led to important advances in the chemistry of many hormones. For example, it is now known that β-LPH, a peptide with 91 amino acids, is the precursor of β-MSH (sequences 37 or 41 to 58) which may not even be present as such in humans, and of γ-LPH (sequence 1–58) [12,13]. A recent study [28] indicates that residues 61 to 65 form the structure of methionine-enkephalin, a pentapeptide which may be the natural ligand for opiate receptors. Celis *et al.* [9] have also demonstrated that the tripeptide MIF resulted from the splitting of oxytocin. Such breakdown is carried out by a number of peptidases which are being isolated in the brain. It may well be that the regional concentrations of such peptidases could be the local factor responsible for specificity of peptide mapping.

Effects of ACTH

Some of the early studies on the behavioral effects of peptide hormones were carried out in hypophysectomized animals by De Wied and his colleagues in Utrecht [20]. These animals had decreased extinction of conditioned

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TABLE 1

INCIDENCE, FREQUENCY AND LATENCY OF YAWNING STRETCHING BEHAVIOR INDUCED BY POLYPEPTIDES AND AMINO ACIDS GIVEN INTRAVENTRICULARLY IN RATS

Substance	Dose ($\mu g/50\ \mu l$)	Excessive Grooming Incidence	Yawning Stretching Incidence	Frequency	Latency (min)
β^{1-24}ACTH	25.0	2/2	2/2	++++	41 42
α-MSH (Synthetic)	25.0	2/2	2/2	++++	45 49
β-h-MSH (Synthetic)	15.0	2/2	2/2	++++	61 62
β-LPH (Sheep)	2500.0	2/2	2/2	++++	39 42
β-LPH (Sheep)	25.0	0/2	0/2		
Acetate buffer in saline	(1.0 M)	0/2	0/2		

Incidence: Number of animals where behavior occurred/Total number of animals
Frequency: ++++ (very marked), +++ (Marked), ++ (moderate), + (mild)

avoidance responses (CAR) which were corrected by the administration of ACTH, MSH or vasopressin. These effects were not dependent on the presence of adrenals. De Wied is of the opinion that ACTH facilitates learning CAR by affecting motivational processes. He and his colleagues have studied many fractions of the ACTH molecule to find that the most active is composed of a 7 amino acid sequence situated between amino acids 4 and 10 (ACTH 4-10): this sequence is Met-Gly-His-Phe-Arg-Tr-Gly. Modifications and substitutions of the sequence have been attempted, but most are inactive except when the methionine is oxidised to the sulfoxide, arginine is replaced by D-Lysine and tryptophan by phenylalanine. The resulting peptide is 1000 times more active than MSH/ACTH 4-10, probably because the substitutions increase resistance to degradation by enzymes. There is evidence that MSH/ACTH 4-10 can increase visual memory on the Benton retention test in man and that the peptide prolongs the pattern of mental alertness on the EEG, by decreasing the duration of α pattern. This finding, duplicated by MSH, led Kastin, Miller and Sandman [39] to postulate that both peptides facilitate CAR by increasing the ability of the animal to pay attention to the task.

A very strange neurological phenomenon consisting of yawning and stretching crises of muscular hypertonus has been observed in dogs and rats after the intracerebral injection of MSH/ACTH 4-10, MSH and β-LPH [32] (Table 1). Since yawning and stretching can be considered as an effort of the body to delay the onset of sleep or to reinforce wakefulness after sleep, this neurological observation is of importance in understanding the behavioral effects of the peptides.

It is also noteworthy that the 7 amino acid sequence (Met-Glu-His-Phe-Arg-Try-Gly) is common to all 3 peptides that were active intraventricularly in our studies. The fact that it takes a much higher dose of β-LPH to produce the syndrome, is in accord with its function as pro-hormone.

Effects of MSH

Melanocyte stimulating hormone (MSH) possesses the same 7 amino acid sequence as MSH/ACTH 4-10 and it has essentially the same behavioral action. In addition it has been demonstrated that, *in vitro*, it can facilitate spinal cord transmission in cats. This property is antagonized by chlorpromazine. MSH can also produce stretching and yawning when injected intracerebrally [32]. It is also known to produce alterations in learning behavior and EEG changes similar to limbic system electrical activity [58]. Stratton and Kastin [64] suggest that MSH produces increased motivational arousal.

BEHAVIORAL AND NEUROLOGICAL EFFECTS OF INTRACEREBRAL PEPTIDES

We will discuss in this chapter only one such peptide: Substance P, discovered in 1931 by von Euler and by Gaddum [68], and characterized as an undecapeptide by Chang and collaborators [10]. In human brain highest concentrations of the substance are found in the substantia nigra and hypothalamus [21,45]. In the spinal cord, it was found highly concentrated in the dorsal horn, where the highest level was in the dorsal part [65]. After the unilateral ligation and/or section of the dorsal roots, the level of substance P in the dorsal horn, particularly in the dorsal part, was markedly lowered [65]. In the ligated and sectioned dorsal root, substance P was highly accumulated on the ganglion side, whereas its level was lowered on the central side of the ligature. Spinal cord substance P was found to be identical with hypothalamic substance P [66]. This and other arguments led to the hypothesis that substance P may be a sensory neurotransmitter [15, 44, 45, 63]. This has been confirmed by neurophysiological studies. Substance P is indeed excitatory at spinal cord [40], cuneate [41] and cortical [49] levels. It is antagonistic to the effect of Lioresal (β-(4-chlorophenyl)-γ-aminobutyric acid) [57], a substance useful in the treatment of some forms of spasticity. Substance P has also been shown to stimulate adenylate cyclase in brain [22].

BEHAVIORAL AND NEUROLOGICAL EFFECTS OF HYPOTHALAMIC PEPTIDES

TRH (TSH Releasing Hormone)

This small tripeptide (L-pyroglutamyl-L-histidyl-L-prolineamide) was first shown in 1972 to be behaviorally active in animals [53] and in man [54]. The subsequent experimental observations include: (1) Increased motor activity [7,26]; (2) EEG activation and desynchronization [42]; (3) Antagonism of phenobarbital induced and reserpine induced hypothermia [54,61]; (4) Antagonism of sleeping-time induced by phenobarbital and reversal of the phenobarbital sedation [8, 16, 54]; (5) Antagonism of the depressant effect of α-methyl-p-tyrosine on motor activity [43]; and (6) Potentiation of Dopa-induced hypermotility and of the tremor produced by 5HTP [53].

These neuropharmacological findings soon led to trials in man in various forms of unipolar or bipolar depressions and in schizophrenia, with widely divergent results [17, 47, 53]. At the present time the efficacy of TRH as an antidepressant still has to be demonstrated [36].

LHRH (Luteinizing Hormone Releasing Hormone)

As is well known, LHRH triggers ovulation, it has also been shown that LHRH can increase sexual receptivity in female animals and that it induces mating behavior, as long as the animals are given a priming dose of testosterone or estrogen according to their sex [34,43].

MIF-I (MSH-Release Inhibiting Hormone)

In mammals the predominant control over melanocyte-stimulating hormone (MSH) release from the pituitary is exerted by a hypothalamic factor, MIF-I or M.R.I.H. (MSH release-inhibiting hormone) [37], whose structure was shown to be that of a tripeptide: L-prolyl-L-leucyl-glycine amide (P-L-G-NH_2) [48]. In fact, two such tripeptides have been identified in hypothalamic tissues, but MIF-I appears to be the compound more worthy of study. Celis, Taleisnik and Walter [9] have indeed shown that this tripeptide can be formed, through the action of peptidases, from oxytocin.

Numerous pharmaceutical studies have indicated that MIF-I can potentiate the behavioral effects of Levodopa and oxotremorine in both intact and hypophysectomised animals [29, 30, 35, 50, 51, 52]. On the basis of these observations, MIF-I was tried in man. The rationale for this approach was further strengthened by the findings of Cotzias *et al.* [18] that MSH injections aggravated the symptoms of Parkinson's disease and by the fact that MSH levels were usually, but not always, elevated in Parkinson's disease [38,60].

Based on these premises, Kastin and Barbeau [33] carried out a number of experiments in Montreal. The antiparkinsonian activity of slow intravenous infusions of 20 to 40 mg MIF-I was clearly demonstrated. These results were soon partially confirmed by Chase and collaborators [11,69], who found some antiparkinson activity with small infusions of the drug. More recently, Fischer and associates [23] in Germany made similar positive observations in 10 patients, again after intravenous treatment. They observed simultaneous mood brightening and thought that the effect of MIF-I was mostly upon mood and motivation.

Moving to oral preparations of MIF-I proved to be disappointing in the hands of Barbeau and collaborators [5]. A double-blind study in 20 patients failed to show any improvement with doses as high as 1.5 g/day. However, intravenous injections of 200 mg of MIF-I [4,5] were clearly beneficial upon rigidity and akinesia in 6 patients. This was confirmed in independent studies by Gerstenbrand *et al.* [25] who used doses as high as 400 mg IV per day.

A number of pharmacological studies demonstrated that MIF-I potentiated apomorphine-induced hypermotility in rats rendered akinetic through a bilateral hypothalamic lesion with 6-hydroxydopamine [4,6]. Coupled with the L-Dopa potentiation observed by Plotnikoff *et al.* [52] these data justified the use of MIF-I in conjunction with Levodopa in man. Barbeau reported findings in 6 patients [2]. When MIF-I was injected intravenously as a bolus of 200 mg, it greatly potentiated the effect of an oral levodopa dose upon motor performance objectively measured by a battery of tests. This amelioration in motility was accompanied by improvement in intellectual functioning.

These experiments suggest that MIF-I is an active tripeptide in the central nervous system and that it can offer therapeutic advantages. However much work still remains to be done to prove this latter affirmation.

ROLE AND MODE OF ACTION OF CNS PEPTIDES

There is still much controversy, and few facts, bearing on the question of the mode of action of CNS peptides. The observations that they are mainly localized in synaptosomes and even along certain pathways outside the hypothalamus [27,46] militate in favor of a possible neurotransmitter or neuromodulator role. Our previously mentioned finding [4] that MIF-I potentiates the action of apomorphine, a dopamine receptor agonist, can be interpreted to mean that this modulating action is situated at the receptor site. This would be compatible with some peptide-induced changes in the concentration of cAMP and the morphine activity antagonism observed with MSH and ACTH. Finally Ungar [67] has hypothesized that some peptides may play a role in neural coding. He postulates that peptides are the code designation of each innate pathway. There is now accumulating evidence that some peptides increase the excitability of nerve cells [55], possibly through an effect on transport of calcium ions.

DISCUSSION

Whatever their true role as neuro-humoral transducers in the brain, it is becoming increasingly evident that CNS peptides, in particular those from the hypothalamus, have major extraendocrine functions [36] which can perhaps be used in the future for new therapeutic approaches in mental and neurological disorders. Such tentative steps have already been taken.

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