PITUITARY PEPTIDES ON MOTIVATIONAL, LEARNING AND MEMORY PROCESSES

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Introduction

The studies of Selye (39) suggested a basic role of the adrenal cortex in adaptive mechanisms. His concept mainly concerned the non-specific bodily reactions to a wide variety of noxious stimuli. A great number of investigations subsequently evaluated the influence of a multitude of physical and pathological "stressors" on the pituitary adrenal system and the striking identical response under stress conditions reinforced the "non-specific" character of this system. Although Selye (39) acknowledged that even emotional stress results in an activation of the adrenal cortex, it took more than a decade to recognize that psychological stimuli elicit a similar "non-specific" activation of the pituitary-adrenal system. Observations in animal and man demonstrated that environmental stimuli which elicit psychological responses like fear or anxiety belong to the most potent activators of pituitary-adrenal function (31). Not only stimuli with an aversive content appeared to trigger pituitary-adrenal activity, but deviations from an expected reward appeared to be as potent in this respect (29). Mason (32) indicated the importance of emotional stimuli in stress-induced pituitary-adrenal activation. He suggested that "... the pituitary mediator underlying the pituitary-adreno-cortical response to the diverse stressors ... may simply be the psychological apparatus involved in emotional or arousal reactions to threatening or unpleasant factors in the life situation as a whole".

Clinical reports frequently commented on psychological changes in addition to electroencephalographic alterations in hyper- as well as hypocorticism (8, 12, 19) but these were not followed by systemic investigations on the role of these hormones on brain function. Nevertheless, scattered observations from the literature suggested a role of pituitary and adrenal hormone in nerve function. Torda and Wolff (45) reported an increase in electrical activity in the brain and in the sensitivity to convulsion inducing agents following ACTH (adrenocorticotrophic hormone) treatment. Krivoy and Guillemin (25) found that a related peptide, β-MSH (melanocyte stimulating hormone) stimulates evoked potentials of the dorsal root preparation of the cat spinal cord; an effect which this peptide shared with substance P

and bradykinin. ACTH was found to affect transmission in sympathetic ganglia of the cat (23), to stimulate inhibitory neurons in the rabbit spinal cord (24), to alter the threshold for electroshock seizures in young rats (49), to induce electroencephalographic changes (13, 33, 63) and more recently to increase the gastrocnemic action potential and contraction amplitude and the diurnal fatigue in hypophysectomized and adrenalectomized rats (41). These observations were better understood when it appeared that pituitary-adrenal system hormones influence behavioral adaptation to environmental changes (50). A great number of behavioral experiments mainly in rodents disclosed the implication of various pituitary and hypothalamic hormones and of steroids in motivational, learning and memory processes.

Hypophysectomy and avoidance behavior

This concept arose from the observation that the removal of parts of the pituitary gland leads to serious disturbances in acquisition and maintenance of conditioned avoidance behavior (50). Thus, lack of pituitary hormones is associated with a behavioral deficiency. This deficiency can be amended by treatment with ACTH, MSH or vasopressin but also with fragments of these hormones which in themselves have lost their classical, peripheral target effects, for example the production of corticosteroids in case of ACTH or antidiuretic, pressor and other endocrine effects in case of vasopressin. On the basis of these observations it was postulated that the pituitary manufactures peptides designated as "neuropeptides" which are involved in the formation and maintenance of new behavior patterns (51).

"Short term" and "long term" effects of neuropeptides

The seriously disturbed learning deficit of hypophysectomized rats can be restored by ACTH and ACTH analogues (α-MSH, β-MSH, ACTH 1-10, ACTH 4-10) or by vasopressin and vasopressin analogues. There exists however an essential difference between the behavioral effects of these two structurally unrelated classes of peptides. ACTH analogues exert a "short term" effect while the effect of vasopressin analogues is of a "long term" nature (3). Similar differences between these peptides are found in intact rats. A single injection of ACTH analogues delays extinction of a pole jumping avoidance response for several hours while a single injection of vasopressin analogues increases resistance to extinction of the avoidance response for days to weeks depending on the dose given (52, 53, 59), notwithstanding the fact that the half-life of these polypeptides amounts to minutes only. The same differential effects can be found on extinction of a shuttle box avoidance response (50) or on retention of passive avoidance behavior (1).

Behavioral effects of ACTH analogues not restricted to avoidance behavior

The behavioral effects of pituitary peptides are not restricted to avoidance behavior. ACTH analogues delay extinction of approach behavior (food running response) (16, 22), facilitate reversal learning (42), increase resistance to a complex brightness discrimination task (42), facilitate reversal of CO_2-induced retrograde amnesia (36) and delay extinction of a sexually motivated approach response of male rats in a straight runway for a receptive female (4).

ACTH analogues and "arousal"

ACTH analogues have a central excitatory action in dogs (46). ACTH 4-10 induces a frequency shift in theta activity from 7.0 to 7.5 Hz in hippocampus and thalamus following stimulation of the reticular formation in free moving rats with implanted electrodes (47). Thus, ACTH 4-10 seems to facilitate transmission in midbrain limbic structures. This suggests that ACTH analogues increase the state of arousal in these structures. This may determine the motivational influence of environmental stimuli and thereby the probability of the generation of stimulus specific behavioral responses. Clinical studies are in keeping with this hypothesis (22).

ACTH analogues on [^{3}H] leucine incorporation into brain proteins

Neurochemical studies showed that hypophysectomy decreases the incorporation of [^{3}H]leucine into rapidly labelled proteins of brain stem origin *in vivo*, measured 5 min after injection of the precursor *directly* into the diencephalon. Treatment of hypophysectomized rats with ACTH 1-10 restored the incorporation of [^{3}H]leucine towards normal (37). Analysis of the labelled proteins by sequential extraction followed by polyacrylamide gelelectrophoresis revealed that ACTH 1-10 stimulated the incorporation of leucine into most proteins both from soluble and membrane origin (17, 35). Data on the effect of ACTH fragments on leucine incorporation in brain proteins *in vitro* (i.e. in slices from the posterior thalamus), again point to the rather general nature of the stimulatory effect of ACTH 1-10 (34). The stimulation of the rate of incorporation of radioactive leucine into these proteins by ACTH 1-10 seems related to the restoration of the behavioral deficiency of the hypophysectomized rat in the shuttle box. This is supported by experiments with 7-D-phe ACTH 1-10. This peptide which further deteriorates avoidance acquisition of hypophysectomized rats in the shuttle box at the same time lowers the already decreased incorporation of radioactive leucine into brain stem protein. Moreover, ACTH 11-24 which did not materially affect avoidance acquisition also failed to induce changes in [^{3}H] leucine incorporation. These results suggest that a disturbance in protein synthesis in

the brain stem is responsible for the deficient behavior of hypophysectomized rats. In view of the well documented effects of peptides on peripheral target cells (43) it seems conceivable to speculate that the observed biochemical effects of the ACTH analogues do not represent the first neurochemical events but rather an intermediate step in the train of events which ultimately lead to a functional response of the nerve cells involved. We have previously postulated that ACTH analogues would in fact regulate brain cellular metabolism in a similar manner as was proposed for their effects in the peripheral target cell i.e. through an effect on cellular cAMP content leading to a regulatory action at the translational level (15, 18, 38). If indeed brain cyclic nucleotides are mediators of the influence of ACTH on the nerve cell, it is conceivable that consequently changes in membrane permeability, enzyme activity and protein synthesis - responses commonly seen in a target cell depending on the protein kinase system of that cell - may lead to facilitation of synaptic activity of the neurons involved.

Structure activity studies with ACTH analogues

Structure activity studies with ACTH analogues were performed to determine the essential elements required for the behavioral effect of ACTH analogues. For these studies the pole jumping avoidance test was used (61). Peptides which delay extinction were measured in rats which were trained for three days. Extinction was studied on the fourth day. All animals which made eight or more positive responses were injected subcutaneously with peptide or saline. A second and third extinction of ten trials were run two and four hours later. The number of positive responses made during the last two extinction sessions served as an index of extinction. Peptides which facilitated extinction were assayed in rats which were trained for four days in order to increase resistance to extinction. On the fifth day an extinction session of ten trials was run and all rats which made eight or more avoidances were injected subcutaneously with the respective peptide. Extinction session was run four hours later. Peptides were administered in a "low" and a three times higher dose and compared with saline. The studies revealed that ACTH 4-7 contains the essential information for the behavioral effect of ACTH analogues (20). If the amino acid residue phenylalanine was replaced by its D-isomer in ACTH 1-10, ACTH 4-10 or ACTH 4-7, reversal of the behavioral effect was found in active avoidance behavior (table 1). Such peptides facilitate extinction of a shuttle box, pole jumping avoidance response and approach behavior (food running response) (5, 16, 20). This reversal is not found in passive avoidance behavior (53). Replacement of other amino acid residues in the D-configuration as in the hexapeptide [Lys^8]ACTH 4-9 failed to facilitate extinction of a pole jumping avoidance response. Such D-isomer substitutions delayed extinction as found with L-isomer amino acid ACTH sequences and often

acted stronger than the original molecule(Table 1).

TABLE 1

Amino acid sequences of various ACTH analogues

		4	5	6	7	8	9	10		
ACTH 4-10	H	Met	Glu	His	Phe	Arg	Trp	Gly	OH	+
ACTH 4-7	H	Met	Glu	His	Phe	OH				+
ACTH 6-9			H	His	Phe	Arg	Trp	Gly	OH	θ
7 ^{D}Phe-ACTH 4-10	H	Met	Glu	His	^{D}Phe	Arg	Trp	Gly	OH	-
4 ^{D}Met-8-Lys-ACTH 4-9	H	^{D}Met	Glu	His	Phe	Lys	Trp	OH		++
5 ^{D}Glu-8-Lys-ACTH 4-9	H	Met	^{D}Glu	His	Phe	Lys	Trp	OH		++
6 ^{D}His-8-Lys-ACTH 4-9	H	Met	Glu	^{D}His	Phe	Lys	Trp	OH		++
8 ^{D}Lys-ACTH 4-9	H	Met	Glu	His	Phe	^{D}Lys	Trp	OH		+++
9 ^{D}Trp-8-Lys-ACTH 4-9	H	Met	Glu	His	Phe	Lys	^{D}Trp	OH		++
4 Met(O)-8-Lys-ACTH 4-9	H	Met(O)	Glu	His	Phe	Lys	Trp	OH		+
4 Met(O)-8^{D}Lys-9 Phe-ACTH 4-9	H	Met(O)	Glu	His	Phe	^{D}Lys	Phe	OH		+++++

+ inhibition of extinction
- facilitation of extinction
θ essential for MSH activity

These results indicate a dissociation between the requirements for steroidogenic and behavioral activity of ACTH analogues. Substitution of histidin or arginine by D-isomers decreases MSH-activity and substitution of arginine by lysine in position 8 is accompanied by loss of steroidogenic activity in ACTH 1-24 (44) and MSH-activity in ACTH 1-17 (7). When tryptophan in position 9 is replaced by phenylalanine, a marked decrease in steroidogenic potency is found (21) but in the presence of D-lysine in position 8 the behavioral activity rises a hundredfold. It was further found that oxidation of methionine to the sulfoxide level (11, 30) also decreases steroidogenic and MSH-activity but increases the behavioral potency. Combination of the respective substitutions in the same molecule i.e. methionine sulfoxide for methionine, D-lysine for arginine and phenylalanine for tryptophan yields a peptide which acts a thousand times stronger than ACTH 4-10 as measured in the pole jumping avoidance response and at the same time contains a thousand times less MSH-activity (20). A partial explanation

for this potentiating effect on behavior may be found in protection against enzymatic degradation. The in vitro half-life of the various substituted analogues of [Lys^8]ACTH 4-9 correlated with the behavioral potency (62).

Consolidation defects in diabetes insipidus rats

Vasopressin and analogues have a "long term" effect on active and passive avoidance behavior (1,52, 55). These neuropeptides affect the consolidation of learned behavior (3). Vasopressin is physiologically involved in memory processes since rats with a hereditary hypothalamic diabetes insipidus (DI) which lack the ability to synthetize vasopressin (48) are inferior in acquiring a shuttle box avoidance response and lack the ability to maintain the response as compared to heterozygous littermates or homozygous normal rats (4). Memory impairment is readily detected in a simple one-trial passive avoidance procedure (57). Homozygous DI rats fail to exhibit avoidance behavior in this situation when tested twenty-four, forty-eight or seventy-two hours after shock exposure. Treatment immediately after the shock trial with a vasopressin analogue restores the behavior of these animals. Homozygous rats do avoid without vasopressin treatment, if tested immediately after the shock trial, indicating that "memory" rather than "learning" processes are disrupted in the absence of vasopressin. Similar effects were found in intact rats when serum containing antibodies against vasopressin were administered into one of the lateral ventricles, in contrast to intravenous administration of a hundred times as much of the same serum. Intraventricular administration of anti-oxytocin serum is inactive in this respect (60).

Behavioral effects of vasopressin analogues not restricted to avoidance behavior

Vasopressin and desglycinamide lysine-8-vasopressin (DG-LVP) reverse retrograde amnesia (36) but these peptides do not seem to affect extinction of a food running response (16). However, vasopressin analogues are active in approach behavior. Male rats in a T-maze trained to run for a receptive female when treated with DG-LVP after each acquisition session, choose the correct arm of the T-maze in a significantly higher percentage. This effect was even stronger during extinction sessions after discontinuation of the treatment. Here again, the copulation reward appeared to be essential for the behavioral effect of the peptide. DG-LVP also delays the disappearance of intromission and ejaculating behavior of male rats following castration (2) when given before or immediately after copulation. Thus, vasopressin not only affects the maintenance of learned approach behavior but also a genetically determined sexual behavioral repertoire of the male rat.

DG-LVP protects against puromycin induced memory blockade in mice (27). This suggests that vasopressin affects memory through protein synthesis. DG-LVP also facilitates

the development of resistance to the analgesic action of morphine in mice (26). Conversely, in the absence of vasopressin i.e. homozygous DI rats, the development of resistance to the analgesic action of morphine as measured on the hot plate is severely disturbed (58). This disturbance can be restored by treatment with vasopressin analogues. Development of resistance to morphine analgesia may be regarded as a learning process (9). This view is corroborated by observations showing that protein synthesis inhibitors which impair memory also prevent the development of resistance to the analgesic action of morphine (10).

Vasopressin analogues and paradoxical sleep

Electrophysiological studies have recently revealed that rhythmic slow activity (RSA) during paradoxical sleep (PS) episodes contains substantially lower hippocampal theta frequencies in homozygous DI rats as compared to heterozygous DI and homozygous normal animals. This can be restored by treatment with desglycinamide arginine-8-vasopressin (DG-AVP) (47). Interestingly, PS deprivation leads to consolidation deficits (14, 28, 40). It might be therefore that the impaired memory of diabetes insipidus rats is due to the low quality of RSA during PS.

Structure activity studies with vasopressin analogues

Attempts to determine the active core of the vasopressin molecule which contains the requirements for the behavioral effect were only partially successful due to the variation in purity of available compounds. Their effect was tested in the pole jumping test as described previously (52). Arginine-8-vasopressin (AVP) appeared to be the most potent peptide followed by LVP (Table 2). Removal of the glycinamide (DG-LVP and DG-AVP) decreased the potency to approximately 50 percent. Oxytocin and vasotocin were equally potent and possess circa 20 percent of the activity of AVP and LVP. Pressinamide had retained only 10 percent of the behavioral potency. Since oxytocin is as active as vasotocin, the ring structure seems to be more important than the tail for the behavioral effect of vasopressin. Nevertheless, removal of the C-terminal part of the molecule led to a drastic decrease in potency. It may be that it protects against metabolic degradation en route to the central nervous system (CNS). In fact, when pressinamide was administered via one of the lateral ventricles, only twice as much AVP was needed to induce an equipotent resistance to extinction of the pole jumping avoidance response. In addition, both peptides were hundreds of times more active when given through this route than following subcutaneous administration (54).

In conclusion, the pituitary contains various neuropeptides which exert a "short term" effect like ACTH analogues or a "long term" effect like vasopressin analogues on acquisition and maintenance of new behavior. The pituitary through these neuropeptides affects motivational, learning

and memory processes, which enable the organism to cope adequately with environmental changes.

TABLE 2

Effect of various related peptides on resistance to extinction of a pole jumping avoidance response

Arginine 8-vasopressin	H Cys Tyr Phe Gln Asn Cys Pro Arg Gly NH_2
Lysine 8-vasopressin	H Cys Tyr Phe Gln Asn Cys Pro Lys Gly NH_2
Desglycinamide lysine 8-vasopressin	H Cys Tyr Phe Gln Asn Cys Pro Lys OH
Desglycinamide arginine 8-vasopressin	H Cys Tyr Phe Gln Asn Cys Pro Arg OH
Oxytocin	H Cys Tyr Ile Gln Asn Cys Pro Leu Gly NH_2
Arginine 8-vasotocin	H Cys Tyr Ile Gln Asn Cys Pro Arg Gly NH_2
Pressinamide	H Cys Tyr Phe Gln Asn Cys NH_2

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