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COMMENTARY

BEHAVIOURALLY ACTIVE ACTH ANALOGUES

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Pituitary peptides are implicated in the formation and maintenance of learned behaviour. This concept originated from the observation that removal of the pituitary gland leads to serious disturbances in acquisition of conditioned avoidance behaviour. The deficient behaviour of the hypophysectomized rat could be readily amended by treatment with ACTH [1] (adrenocorticotrophic hormone). This appeared to be an extra-adrenal effect of ACTH since peptides related to ACTH, but which in themselves showed marginal corticotrophic activities, also exhibit potent behavioural effects. Thus, both α- and β-MSH (melanocyte stimulating hormone) affected behaviour in the same way as ACTH. Since ACTH, α- and β-MSH share the sequence ACTH 4–10 as a common core, this was considered an indication that the minimal requirements essential for such an activity were located within this heptapeptide. This assumption received support from the finding that ACTH 1–10 was as active as ACTH, whereas ACTH 11–24 was inactive [2]. These peptides not only substituted for a behavioural deficiency in hypophysectomized rats, but they also appeared to affect the maintenance of previously acquired conditioned avoidance behaviour in intact rats [1]. On the basis of these and other findings, it was postulated [1] that the pituitary manufactures peptides, designated as 'neuropeptides', which are physiologically involved in acquisition and maintenance of new behaviour patterns.

Since then, a number of other behavioural effects of ACTH analogues have been reported. These concern delay of extinction of approach behaviour (food running response) [3,4], modulation of reversal learning of a complex brightness discrimination task [5, 6, 7], facilitation of memory retrieval after retrograde amnesia induced by CO_2 or ECT (electroconvulsive treatment) [8] and facilitation of sexually motivated behaviour [9].

Initially, normalization of an impaired acquisition of a shuttle box avoidance response in hypophysectomized rats served as the parameter of the behavioural action of ACTH analogues [1]. This rather time consuming procedure was replaced for routine purposes by a pole jumping avoidance test [1].

RELATIONSHIP BETWEEN CHAIN LENGTH AND BEHAVIOURAL ACTIVITY

ACTH 4–10 was the shortest peptide with a behavioural potency comparable with that of the parent molecule [2]. Shortening of the sequence ACTH 4–10 step by step from the carboxyl end revealed that the tetrapeptide ACTH 4–7 contains the essential elements required for the behavioural effect of ACTH analogues (Table 1). The tryptophan and arginine residues could be removed without appreciable loss of activity. This is indicative for a dissociation regarding the structural requirements for behavioural and MSH-activity. It was found that tryptophan is essential for MSH-activity, since ACTH 1–8 without this amino acid in position 9 had lost MSH-activity [12]. These results therefore demonstrate differences in structural requirements for behavioural activity (ACTH 4–7) and MSH-activity (ACTH 6–9) [13].

D-ISOMER SUBSTITUTIONS IN ACTH ANALOGUES

The decapeptide ACTH 1–10 [14] in which the phenylalanine in position 7 was replaced by its D-

Table 1. Effect of progressive shortening of the sequence ACTH 1–10 from the amino- and the carboxyl end on inhibition of extinction of a pole jumping avoidance response in rats

	1	2	3	4	5	6	7	8	9	10	Approximated potency
ACTH 1–10	H—Ser—	Tyr—	Ser—	Met—	Glu—	His—	Phe—	Arg—	Trp—	Gly—OH	1
ACTH 2–10		H—Tyr—	Ser—	Met—	Glu—	His—	Phe—	Arg—	Trp—	Gly—OH	1
ACTH 3–10			H—Ser—	Met—	Glu—	His—	Phe—	Arg—	Trp—	Gly—OH	1
ACTH 4–10				H—Met—	Glu—	His—	Phe—	Arg—	Trp—	Gly—OH	1
ACTH 5–10					H—Glu—	His—	Phe—	Arg—	Trp—	Gly—OH	0·5
ACTH 6–10						H—His—	Phe—	Arg—	Trp—	Gly—OH	0·1
ACTH 7–10							H—Phe—	Arg—	Trp—	Gly—OH	0·1
ACTH 4–9				H—Met—	Glu—	His—	Phe—	Arg—	Trp—OH		1
ACTH 4–8				H—Met—	Glu—	His—	Phe—	Arg—OH			1
ACTH 4–7				H—Met—	Glu—	His—	Phe—OH				1
ACTH 4–6				H—Met—	Glu—	His—OH					0·3

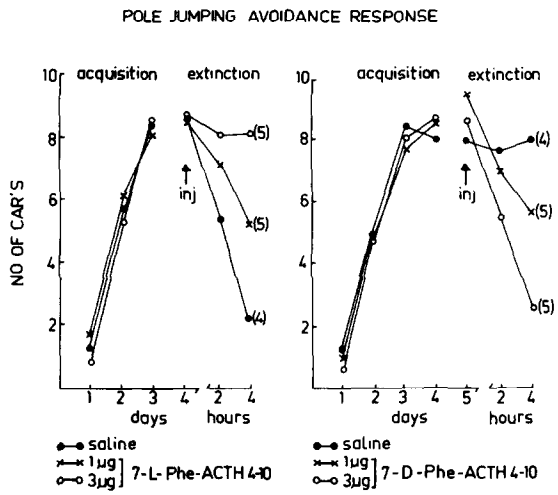

Fig. 1. Left. Effect of 7-L-Phe-ACTH 4–10 on extinction of a pole jumping avoidance response. The peptide was administered subcutaneously immediately following the first extinction session (↑). Inhibition of extinction is apparent at 2 and 4 hr after injection. The effect is dose dependent. Rats were trained for 3 days. Right. Effect of 7-D-Phe-ACTH 4–10 on extinction of a pole jumping avoidance response. The peptide was administered immediately following the first extinction session (↑). Facilitation of extinction is apparent at 2 and 4 hr after injection. The effect is dose dependent. Rats were trained for 4 days. The number of animals is given in parentheses. The results presented in this review were obtained with a pole jumping avoidance test [10]. Intact Wistar strain rats were trained to jump onto a pole placed in the middle of a box within 5 sec after presentation of the conditioned stimulus (CS), which was a light mounted on the top of the transparent upper side of the box. Rats which failed to jump within 5 sec received the unconditioned stimulus (US), a shock to the feet of the animal via the grid floor of the box. Ten trials were given each day with an average interval of 60 sec. After acquisition of the avoidance response, extinction was studied. In this situation, the CS is presented but if the animal fails to jump within 5 sec, the CS is not followed by the US of shock. Peptide effects were determined on the rate of extinction of the avoidance response. Peptides which delay extinction were measured in rats which were trained for 3 days. On the 4th day an extinction session was run and all rats which made 8 or more avoidances (jumped within 5 sec) were injected subcutaneously with the respective peptide. Extinction sessions were run again 2 and 4 hr later. Peptides which facilitate extinction, were assayed in rats which were trained for 4 days. This made the animals more resistant to extinction. On the 5th day an extinction session was run and all rats which made 8 or more avoidances were injected subcutaneously with the respective peptide. Extinction sessions were run again 2 and 4 hr later. Peptides were administered in a low dose and a 3 times higher dose and their effect compared with that of saline [11].

isomer appeared to exhibit an effect on extinction which was opposite to that of the 'all L-' ACTH 1–10 i.e. facilitation of extinction of a shuttle box avoidance response (Fig. 1). This effect was found in intact as well as in hypophysectomized rats. Thus, inversion of configuration of the phenylalanine residue reversed the behavioural effect of the original molecule. Although 7-D-Phe-ACTH 1–18 is a strong competitive inhibitor of ACTH stimulated adenylcyclase activity [15], it is unlikely that the reversal effect of 7-D-Phe-ACTH analogues on behaviour is due to similar competitive inhibition. 7-D-Phe-ACTH 1–10 is active in the absence of ACTH and MSH i.e. in the hypophysectomized rat. In addition, it exhibits a similar effect as 7-L-Phe-ACTH analogues on passive avoidance behaviour in intact rats [16], i.e. facilitation of passive avoidance behaviour. Thus, in both active and passive avoidance behaviour, 7-D-Phe-ACTH analogues inhibit an active response. This suggests that the 7-D-Phe-ACTH analogues contain an intrinsic new activity on extinction. The heptapeptide 7-D-Phe-ACTH 4–10 and the tetrapeptide 7-D-Phe-ACTH 4–7 appeared to be as active as the decapeptide analogue (Table 2).

The reversal of action on active avoidance behaviour was found only for analogues with the 7-phenylalanine residue in the D-configuration. Successive replacement of each of the other amino acid residues in the hexapeptide 8-Lys-ACTH 4–9 by D-isomers failed to facilitate extinction of the avoidance response. Thus, the reversal is an exception and a privilege of 7-D-Phe-ACTH analogues. All other D-isomer substitutions delayed extinction of the avoidance response as found with the 'all-L-' ACTH analogues. Generally, such substitutions caused potentiation of the effect; this was strongest when lysine in position 8 was replaced by its D-isomer. These results again indicate a dissociation between requirements for behavioural and MSH-activity, since it has been found [17, 18] that in the sequence ACTH 6–10, MSH-activity increased when the aromatic residues phenylalanine or tryptophan were replaced by their D-isomers, whereas this activity was lost when the ionisable residues i.e. the basic histidyl or arginyl residues were converted to their D-isomers.

Surprisingly, the combination of D-methionine in position 4 with D-lysine in position 8 resulted in a decrease instead of an increase in potentiation. A similar combination of D-methionine in position 4 with D-phenylalanine in position 7 prevented the reversal of the behavioural effect caused by the introduction of the 7-D-phenylalanine residue. Thus, 4-D-Met, 7-D-Phe-ACTH 4–10 appeared to delay extinction of a pole jumping avoidance response. Obviously, the configuration of the *N*-terminal meth-

Table 2. D-Isomer substitution in position 7 of several ACTH analogues causes reversal of the behavioural effect and leads to facilitation of extinction

	1	2	3	4	5	6	7	8	9	10	Approximated potency
7-D-Phe-ACTH 1–10	H—Ser—	Tyr—	Ser—	Met—	Glu—	His—	D-Phe—	Arg—	Trp—	Gly—OH	1
7-D-Phe-ACTH 4–10			H—	Met—	Glu—	His—	D-Phe—	Arg—	Trp—	Gly—OH	1
7-D-Phe-ACTH 4–7			H—	Met—	Glu—	His—	D-Phe—OH				1
7-D-Phe-ACTH 7–10							H—	D-Phe—	Arg—	Trp—Gly—OH	0·1

Results obtained in the pole jumping avoidance test.

Table 3. Substitution by L- and D-isomers in position 7 of various ACTH analogues. The effect of L-isomer substitution on inhibition of extinction, that of D-isomer substitution on facilitation of extinction of a pole jumping avoidance response

	4 5 6 7 8 9 10	Approximated potency
ACTH 4–10	H—Met—Glu—His—Phe—Arg—Trp—Gly—OH	1
7-Leu,8-Lys-ACTH 4–9	H—Met—Glu—His—Leu—Lys—Trp—OH	1
7-Trp-ACTH 4–10	H—Met—Glu—His—Trp—Arg—Trp—Gly—OH	1·5
7-Pmphe-ACTH 4–10	H—Met—Glu—His—Pmphe—Arg—Trp—Gly—OH	3
7-D-Phe-ACTH 4–10	H—Met—Glu—His—D-Phe—Arg—Trp—Gly—OH	1
7-D-Leu,8-Lys-ACTH 4–9	H—Met—Glu—His—D-Leu—Lys—Trp—OH	0·3
7-D-Trp-ACTH 4–10	H—Met—Glu—His—D-Trp—Arg—Trp—Gly—OH	0·3
7-D-Pmphe-ACTH 4–10	H—Met—Glu—His—D-Pmphe—Arg—Trp—Gly—OH	1·5

ionine, in itself not strictly essential, modulates the behavioural activity pattern in two-fold substituted analogues.

MODIFICATION OF ACTH-ANALOGUES BY SUBSTITUTION OF THE 7-PHENYLALANINE RESIDUE

Substitution by L-leucine in 7-Leu, 8-Lys-ACTH 4–9 did not impair the behavioural activity and led to delay of extinction of the pole jumping avoidance response in amounts comparable with that found for ACTH 4–10. Substitution of 7-phenylalanine by L-tryptophan in ACTH 4–10 slightly increased the potency and substitution by L-pentamethyl-phenylalanine (Pmp)* even more so (Table 3). These results suggest that the electron donor properties of the amino acid residue in position 7 correlate to some extent with behavioural potency.

Introduction of the D-isomers of these amino acid residues caused reversal of the behavioural effect as found with 7-D-phenylalanine (Table 3). However, this activity was reduced by substitution with D-leucine or D-tryptophan. Introduction of pentamethyl-phenylalanine in the D-configuration* somewhat augmented the activity of the molecule.

MODIFICATIONS OF ACTH ANALOGUES BY SUBSTITUTIONS IN OTHER POSITIONS

The substitution of arginine by lysine in position 8, which is accompanied by loss of steroidogenic activity in 8-Lys-ACTH 1–24 [19], loss of steroidogenic and MSH-activity in 8-Lys-ACTH 1–17-NH_2 and of MSH-activity in 8-Lys-ACTH 6–10 [20], did not reduce behavioural activity (Table 4). Substitution of L-lysine by D-lysine even increased the behavioural potency by a factor of thirty. Replacement of phenylalanine by tryptophan in position 9, which causes a marked decrease in steroidogenic potency in 5-Gln, 9-Phe-ACTH 1–20-NH_2 [21], induced a three-fold potentiation of behavioural activity and in the presence of 8-D-Lys, a hundred-fold increase (Table 4). Another change in the molecule which decreases the steroidogenic activity of ACTH [22] and the melanocyte-stimulating activity of MSH [23], i.e. oxidation of the -SCH_3 of the methionine residue to -S=O, also gave rise to an increase in behavioural potency (Table 4). The introduction of three of these modifications led to a thousand-fold potentiation (Table 4). Thus, subcutaneously administered H-Met(O)-Glu-His-Phe-D-Lys-Phe-OH in nanogram quantities appeared to inhibit extinction of a pole jumping avoidance response in intact rats. The same modifications led to a thousand-fold decrease in MSH-activity [11]. However, preliminary observations indicate that the steroidogenic activity was less reduced than was predicted from the various substitutions.

A partial explanation for the effectiveness of the various substitutions may be found in their protection against enzymatic degradation. Incubation of ^{14}C-labelled ACTH 4–9 analogues with plasma or brain extracts revealed that the *in vitro* half life of various substituted analogues of ACTH 4–9 correlated with their behavioural potency [11]. In addition, all metabolic fragments contained less than 5 per cent biological activity in comparison with H-Met(O)-Glu-His-Phe-D-Lys-Phe-OH. In D-lysine substituted peptides, Phe-D-Lys-Phe (Table 4) was the main metabolite found. This peptide, although much less active than H-Met(O)-Glu-His-Phe-D-Lys-Phe-OH, still contained approximately 10 per cent of the activity of ACTH 4–10.

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Table 4. Potency of structurally modified ACTH analogues as determined on the rate of extinction of a pole jumping avoidance response

	4 5 6 7 8 9 10	Approximated potency
ACTH 4–10	H—Met—Glu—His—Phe—Arg—Trp—Gly—OH	1
8-Lys-ACTH 4–9	H—Met—Glu—His—Phe—Lys—Trp—OH	1
8-Lys,9-Phe-ACTH 4–9	H—Met—Glu—His—Phe—Lys—Phe—OH	1
4-Met(O)-ACTH 4–10	H—Met(O)—Glu—His—Phe—Arg—Trp—Gly—OH	10
8-D-Lys-ACTH 4–9	H—Met—Glu—His—Phe-D-Lys—Trp—OH	30
8-D-Lys,9-Phe-ACTH 4–9	H—Met—Glu—His—Phe-D-Lys—Phe—OH	100
4-Met(O),8-D-Lys,9-Phe-ACTH 4–9	H—Met(O)—Glu—His—Phe-D-Lys—Phe—OH	1000
8-D-Lys,9-Phe-ACTH 7–9	H—Phe-D-Lys—Phe—OH	0·1

STRUCTURAL RELATIONS BETWEEN ACTH-ANALOGUES AND RELEASING HORMONES

The structure of thyrotropin releasing hormone (TRH) is related to that of the tetrapeptide ACTH 4–7 (Table 5). In fact, TRH also delays extinction of the pole jumping avoidance response, albeit that its potency is only one third of that of the tetrapeptide (Table 5). It has been shown that minor changes in the TRH molecule dramatically alter the intrinsic TSH (thyrotrophin stimulating hormone)-releasing activity [24]. In contrast, the behavioural effect is not affected by such measures since substitution of proline amide by phenylalanine amide or tryptophan amide did not damage the effect on extinction of the pole jumping avoidance response (Table 5). The tryptophan substituted tripeptide acts even somewhat stronger than the other tripeptides (Table 5). It is possible that some of the recently reported behavioural influences of TRH [25] have certain central effects in common with ACTH analogues. Such effects may be shared by those hypothalamic and pituitary peptides which contain related amino acid sequences. Indeed, luteinizing hormone-releasing hormone (LH-RH) appeared to be as potent as ACTH 4–7 in delaying extinction of a pole jumping avoidance response. LH-RH contains the *N*-terminal sequence pGlu-His-Trp-, a tripeptide which is slightly more active on extinction than TRH and approximately half as potent as ACTH 4–7 (Table 5).

STRUCTURE–ACTIVITY RELATIONSHIP BETWEEN ACTH-ANALOGUES AND DOGFISH β-MSH

Earlier observations indicated that the sequence 7-D-Phe-ACTH 7–10 delayed extinction of a shuttle box avoidance response [1], but more recent experiments in the pole jumping avoidance test with newly synthetized peptide demonstrated facilitation of extinction. In both tests, the effect was markedly less than that of 7-D-Phe-ACTH 4–10. The major breakdown product of the substituted hexapeptide H-Met(O)-Glu-His-Phe-D-Lys-Phe-OH, i.e. Phe-D-Lys-Phe, still possesses behavioural activity although much smaller than that of ACTH 4–10 (Table 4). This suggests that essential features of behavioural activity are not exclusively restricted to the locus ACTH 4–7, but are present in the area 7–9 as well. This latter sequence may contain information for behavioural activity in a dormant form which needs potentiating modifications, e.g. chain extension, to become expressed. This is demonstrated in recent findings which showed that dogfish α-MSH is behaviourally as active as dogfish β-MSH [26]. These peptides share the sequence H-His-Phe-Arg-Trp-OH as a common characteristic. This sequence in itself has only minor behavioural effects [2]. Thus, the essential requirements for the behavioural effect of ACTH analogues may not be restricted to a single locus or active core but may be present in at least two regions of the molecule, which show *per se* only marginal behavioural activities. These can be potentiated by chain elongation or by the introduction of modifications that are believed to make the molecule more resistant to metabolic degradation.

CONCLUDING REMARKS

The foregoing considerations reveal the difficulty of assigning a specific amino acid sequence to the effect of pituitary hormones on learned behaviour. Although position 7 in the *N*-terminal part of ACTH contains highly specific information (since substitution by D-isomer amino acid residues reverses the behavioural effect) none of the amino acids in other positions seems to be particularly important. It is possible that classical structure–activity considerations are not sufficient to elucidate the active core of neuropeptides which occupies receptors in the brain involved in the expression of learned behaviour. In an excellent discussion on the principles of hormone actions, Hechter [27] stressed that most of the residues of polypeptide hormones appear to be involved in high affinity binding, whereas only a limited number of residues constitute the 'active' site [27]. ACTH 4–10 has been considered as the active 'core' involved in receptor activation of ACTH for steroid production while other parts of the molecule serve affinity [28]. The amino acid sequence ACTH 5–10 was regarded as the active 'core' of MSH for the expansion of melanophores and the rest of the hormone was considered to serve functions of transport, species specification, etc. [29]. These functions are not directly related to the interaction between hormone and receptor.

The sequence ACTH 4–10 was termed the 'acton' of ACTH, the clause for: 'go into action'. In the 'acton' of the ACTH-MSH-LPH family, only a few critical residues or active sites represent the topochemical unit which corresponds to the word for initiating action designated as 'transducon'. ACTH 5–25, ACTH 6–24 and ACTH 7–23 have 100%, 50% and no activity respectively in the isolated rat adrenal cell preparation [30]. Thus, His^6 may be regarded as an element of the 'transducon' for ACTH. Met^4, Glu^5 and Gly^{10} can be eliminated as active sites in the core of MSH since ACTH 6–9 is the minimal fragment with MSH-activity. Modification of Trp^9 with *O*-nitrophenyl-sulfonylchloride reduces ACTH activity on adrenocortical receptors [31] and substitution

Table 5. Comparison between effects of peptides related to ACTH and releasing hormones on extinction of a pole jumping avoidance response

		Approximated potency
ACTH 4–7	H—Met—Glu—His—Phe—OH	1
	p.Glu—His—Phe—NH_2	0·3
TRH	p.Glu—His—Pro—NH_2	0·3
	p.Glu—His—Trp—NH_2	0·5
LH-RH	p.Glu—His—Trp—Ser—Tyr—Gly—Leu—Arg—Pro—Gly—NH_2	1

of Trp^9 by Phe in ACTH 1–20 amide almost completely reduces steroidogenic activity. These findings indicate a possible role of Trp^9 as an element of the 'transducon' or a critical 'binding site' for ACTH. Most of the information conveyed in ACTH 1–24, α- and β-MSH is therefore involved in addressing the message (peptide) from the sender (the endocrine sending cell) to the right receiver (the target cell; receptor) and only a few elements are involved in activating the receptor.

With regard to behavioural activity, the hormonal message for 'go into action' seems to be embodied in the sequence 4–7, which therefore may be considered an 'acton'. However, since the sequences Phe-D-Lys-Phe and D-Phe-Arg-Trp-Gly were also found to exhibit significant behavioural activity, the same message appears to be conveyed by the sequence 7–9. Since both clauses for 'go into action' share the residue Phe^7 as a common word, this residue may be considered a keyword or 'transducon'. The observed specificity for the reversed behavioural action of D-Phe^7 substitution confirms this idea. The finding that Phe^7 can be replaced by Leu or Trp without much alteration of activity cannot be explained other than by assuming that these amino acids fulfill similar topochemical requirements necessary for receptor activation. These requirements might be provided by a peptide backbone pattern rather than by a particular amino acid side chain [27]. However, these considerations should be regarded with care. The structure–activity studies with ACTH analogues in the present experiments were performed *in vivo*, using behaviour as a measure of activity. This measure does not necessarily reflect receptor interactions *per se*, but includes the availability of the various peptides for the receptor. This availability could differ considerably for the various analogues, because of individual differences in absorption, elimination and passage in the CNS. Alternatively, the analogues might interfere in various ways with the behavioural activity of naturally occurring peptides. Experiments *in vitro* on peptide–receptor interaction are therefore needed before definite conclusions regarding the topochemical requirements can be drawn.

Although these views seek to reconcile the results obtained, we are also confronted with the problem that peptides structurally unrelated to ACTH-analogues, exert behaviourally similar effects. For example, vasopressin analogues [32], scotophobin-like peptides [33] and several releasing hormones appeared to inhibit extinction of the pole jumping avoidance response. On the other hand, oxytocin, angiotensin II, insulin and growth hormone in amounts in which ACTH 4–10 exerts behavioural activity, were ineffective [34].

Further studies with vasopressin analogues revealed that the effect of these peptides on conditioned avoidance behaviour was of a long term nature in contrast to that of ACTH analogues which exert a short term effect. Evidence was obtained that vasopressin analogues affect the consolidation of learned behaviour [32], whereas ACTH analogues probably increase the state of arousal in midbrain limbic structures which may increase the motivational value of environmental stimuli [16]. Such influences are reflected in identical behavioural effects, i.e. acceleration of acquisition and inhibition of extinction. However, more extensive investigations using a multitude of tests may reveal more specific differences in the behavioural effects of chemically unrelated peptides than the exclusive use of conditioned avoidance tests as employed in the present experiments.

It is conceivable that a great variety of sequences which in some way show a relationship with *N*-terminal ACTH analogues occur in pituitary hormones. These may all be involved in the formation and maintenance of new behaviour. They may be degradation products of pituitary peptides which originate in the blood, and, after entering the brain, affect their central target structures. They may also be precursors of pituitary hormones or entities produced as such in the gland, being released in response to the stress with which the formation of new behaviour is associated. Such peptides again may enter the brain via the circulation or via discharge into the cerebrospinal fluid (CSF), either by retrograde transport along the pituitary stalk or via the basilar cysterns which seem to connect the hormone producing cells of the pituitary gland with the liquor [35]. Similar entities may originate from hypothalamic and other brain structures, like the releasing hormones or other brain oligopeptides [36]. However, the impaired avoidance acquisition in the hypophysectomized rat indicates that pituitary peptides are more essential for the formation of new behaviour than brain oligopeptides.

The site of the behavioural action of ACTH analogues is in midbrain limbic structures as was derived from lesion and implantation studies [16]. The mesodiencephalic area and more specific the nuclei parafascicularis appeared to be essential for the behavioural effect of ACTH analogues. Lesions in these nuclei prevent the inhibitory effect of α-MSH or ACTH 4–10 on extinction of a shuttle box or pole jumping avoidance response. Electrophysiological studies suggest that ACTH and ACTH analogues exert an excitatory action [16]. It was shown recently that ACTH 4–10 induces a frequency shift in theta activity, evoked by stimulation of the reticular formation, from 7·0 to 7·5 Hz in the hippocampus and thalamus of rats [37]. Since similar shifts can be obtained after increasing the stimulus intensity, it is possible that ACTH analogues facilitate transmission in midbrain limbic structures. This suggests that neuropeptides increase the state of arousal in these structures. This may determine the motivational influence of environmental stimuli which in turn may result in an increase in the probability of generating stimulus specific responses.

As revealed from biochemical studies, the mechanism of action probably is located in the cell membranes of midbrain limbic structures. Treatment of hypophysectomized rats with ACTH 1–10 enhanced, with 7-D-Phe-ACTH 1–10 impaired, and with ACTH 11–24 did not affect the incorporation of [^{3}H]leucine into brain stem cytoplasmic proteins [38]. The biochemical effects therefore run parallel with the behavioural effects of these three analogues. It is possible that interaction of neuropeptides with membranes of specific target cells in midbrain limbic structures results in a similar effect as the influence of ACTH analogues on isolated adrenal cells [30], i.e. by inducing conformational changes which stimulate cyclic AMP production. This increased synthesis of certain proteins might facilitate

the transmission in these structures [39] and form new behavioural patterns.

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