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Neuropeptides and behavior: ACTH

W. H. GISPEN

University of Utrecht, The Netherlands

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In the last 25 years, enormous progress has been made in understanding the role that peptides play in regulating complex behaviors. Limiting the discussion to ACTH and congeners, it has been put forward that their effects on adaptive behaviors is due to their enhancement of the motivational or attentional properties of environmental stimuli. The multiplicity of the behavioral effects suggests that ACTH-like peptides contain more than one bit of behavioral information. It is believed that local, post-translational processing of neuropeptides is an important control mechanism in the availability and the identity of the active sequences. ACTH-induced excessive grooming in the rat is discussed in detail as it clearly illustrates some of the specific features of neuropeptide and behavior research, like, structure-activity, multiple information sites and receptors, restricted neural substrate, dose-response relation, etc. Furthermore, evidence is accumulating that peptide modulation of membrane properties (protein phosphorylation) in certain synapses may contribute to the behavioral activity of ACTH.

Willem Gispen, Division of Molecular Neurobiology, Institute of Molecular Biology and Rudolf Magnus Institute for Pharmacology, State University of Utrecht, Padualaan 8, 3508 TB Utrecht, The Netherlands.

THE MULTIPLE MESSAGE IN NEUROPEPTIDES: ACTH

The classical biological activity of ACTH is the growth-promoting (trophic) and steroidogenic action on the adrenal cortex. However, the N-terminal part of the ACTH molecule produces extra adrenal effects as well, including its action on nerve tissue. The study of structure-activity relationships using synthetic peptide fragments, has provided insight in the chemical organization of information in the ACTH molecule (Schwyzer, 1980). Schwyzer summarized his ideas about the molecular anatomy of ACTH as follows: (1) There are discrete (although sometimes overlapping) sequences of adjacent amino acids, responsible for the different components of the total biological action of ACTH; (2) different target cells or different receptors may be stimulated by different portions of the sequence; (3) partial sequences, obtained by synthesis, can produce effects similar to those elicited when contained in the complete molecule (Schulster & Schwyzer, 1980). The ACTH molecule, thus, can be divided into different regions with different functions (see Fig. 1). Considering the biological action on adrenal cortex cells, Schwyzer (1980) distinguishes the following sequences: $ACTH_{1-3}$, potentiator: enhances potency and efficacy; $ACTH_{4-9}$, message: triggers the receptor; $ACTH_{11-24}$, address: adds cell-specific affinity; $ACTH_{25-39}$, species label: antigenicity, transport, protection. Although the ultimate information for the receptor is encoded in the region (4–10), N- and C-terminal extension is necessary to obtain biological activity. It has been shown that some of the extra-adrenal effects are brought about by regions different from the message for the adrenal cortex receptor. Thus, a given peptide contains information that is encoded in multiple form and a given receptor may interact with one part of the molecule, whereas another, different receptor, even at the same cell, may react with another portion of the molecule. As different receptor-mediated responses may differ in effect (quality and quantity), one may expect a multiplicity of responses to a given peptide (in a heterogenous system like the nervous tissue). Indeed, the diversity of behavioral, neurophysiological and

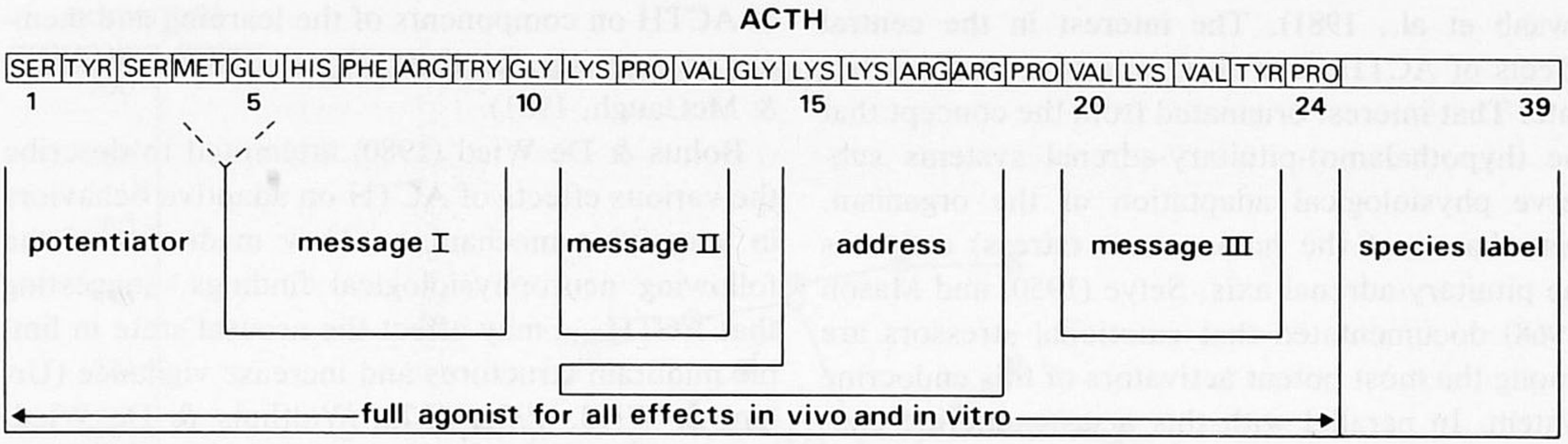

Fig. 1. Multiple information sites in ACTH according to Schwyzer (1980). The messages I, II and III activate different receptors. The potentiator adds to the intrinsic activity and the address part enhances receptor specificity.

neurochemical effects of ACTH is compatible with such a view. This multiplicity of information in neuropeptides makes the study of their neurochemical mechanism of action extremely difficult. Based on their assumed neurophysiological and neurochemical action, neuropeptides can be classified as follows: (1) putative neurotransmitters; (2) neurohormones, and (3) neuromodulators. The value of such a distinction is still to be proven as a given peptide seems to act via all three mechanisms (Barker & Smith, 1979).

GENERATION OF NEUROPEPTIDES

Proteolysis of polypeptides serves a two-fold function. It is a process of elimination that, by degrading the biologically active peptides, terminates their action. On the other hand, it is also a process of generation that, by restricted proteolysis of (inactive) precursor molecules, generates biologically active oligopeptides and thus initiates their action.

Although the proteolytic enzymes also in brain have a rather general action, there is evidence for some selective degradation. The presence in brain of the precursor peptide pro-opiomelanocortin (POMC), β-endorphin, $ACTH_{1-39}$ and α-MSH suggests that the peptides β-endorphin, ACTH and MSH are generated by specific proteolysis of POMC (see Swaab, Achterberg, Boer, Dogterom & van Leeuwen, 1981).

Knowledge of the regulation of such selective proteolysis is extremely important. For, specific proteolysis of neuropeptides containing multiple information sites may give rise to a local supply of biologically active entities with similar or opposing action. Previously, we have suggested that the simultaneous release of opposing biologically active peptides from β-LPH (ACTH/MSH versus endorphin) could serve as a precise modulatory mechanism in the control of certain behaviors (Gispen, Van Ree & De Wied, 1977). The work of De Wied (1978) on the different biological actions of α- and γ-type endorphins implies that under physiological conditions the balance between the generation of α- and γ-type endorphins from β-LPH is critical to maintain brain and behavior homeostasis. Therefore, irregular post-translational processing of β-LPH may well be the cause of a number of psychopathological states (De Wied, 1978).

With respect to specific proteolysis of ACTH, it has been shown that in the intermediate lobe of the pituitary, $ACTH_{1-39}$ is cleaved into $ACTH_{1-16}$ and $ACTH_{17-39}$ (Scott, Bloomfield, Lowry, Gilke, London & Rees, 1976). In the brain a similar processing of ACTH derived from POMC is likely to occur (Watson & Akil, 1980). Interestingly, $ACTH_{1-16}$ contains the information for all hitherto known CNS actions of ACTH.

In addition to selective proteolysis, more and more evidence has accumulated that other post-translational structural modifications of peptides may be of crucial importance to their biological activity. N-acetylation, C-amidation, glycosylation are known examples. Currently, much research is conducted to unravel the regulation of the enzyme activities involved in post-translational processing of neuropeptides.

ACTH AND ADAPTIVE BEHAVIORS IN THE ANIMAL

It was not until 1977 that immunochemical studies revealed the presence of ACTH in brain cells (see

Swaab et al., 1981). The interest in the central effects of ACTH, however, was of a much earlier date. That interest originated from the concept that the (hypothalamo)-pituitary-adrenal systems subserve physiological adaptation of the organism. Disturbance of the homeostasis (stress) activates the pituitary-adrenal axis. Selye (1950) and Mason (1968) documentated that emotional stressors are among the most potent activators of this endocrine system. In parallel with this neuroendocrine connection it was recognized that conversely the brain could be a target for hormone action. It emerged that ACTH was able to affect brain function independently of its action on the adrenal cortex (see for a review Bohus & De Wied, 1980). For, although ACTH and glucocorticoids both have pronounced effects on certain behaviors, the effect of ACTH is direct and not elicited via steroid mediation. The fact that ACTH displays similar activity in intact/adrenalectomized or hypophysectomized animals and that fragments of the N-terminus which lack endocrine activity have full behavioral activity is in line with this notion. Given a role of ACTH, separate of its hormonal action in the general adaptation syndrome, it has been questioned whether pituitary ACTH was at all important for the central effects. It seems, however, that presently, one is unable to favor either one possible source, i.e. pituitary or brain ACTH. Firstly, there is no question that systemic treatment with peptides, thus mimicking circulating peptides, elicits centrally behavioral effects. Secondly, a unique ACTH-transport system from the adenohypophysis towards the septal complex has been described (see Witter, Gispen & De Wied, 1981). Furthermore, little or no information is available on the function of peptidergic neuronal networks containing ACTH.

Murphy & Miller (1955) were the first to show that injection of ACTH during shuttlebox training delays subsequent extinction of conditioned avoidance behavior. De Wied (1966) found that α-MSH, $ACTH_{1-10}$ and $ACTH_{4-10}$ were as effective as $ACTH_{1-24}$ in this respect. The behavioral effects of ACTH are certainly not limited to conditioned avoidance behavior. For, ACTH and congeners improve maze performance, facilitate passive avoidance behavior, delay extinction of a food rewarded behavior in rats and delay extinction of conditioned taste aversion and sexually motivated behavior (see Bohus & De Wied, 1980).

In addition, there is vast literature on the effects of ACTH on components of the learning and memory process (see Martinez, Rigter, Jensen, Vasquez & McGaugh, 1981).

Bohus & De Wied (1980) attempted to describe the various effects of ACTH on adaptive behaviors in a unifying mechanism. They made use of the following neurophysiological findings, suggesting that $ACTH_{4-10}$ may affect the arousal state in limbic midbrain structures and increase vigilance (Urban & Wied, 1976, 1978; Wolthuis & De Wied, 1976). These observations led to the hypothesis that ACTH and related peptides, by temporarily increasing the state of arousal in the limbic brain, enhance the motivational value of environmental stimuli. In this manner the peptides may increase the probability of generating stimulus-specific responses (Bohus & De Wied, 1980; De Wied, 1974).

Over the years, Sandman & Kastin (1981) have carried out many experiments in animal and man, studying the behavioral effects of α-$MSH/ACTH_{4-10}$. Their view is that ACTH will affect learning processes by an increased level of attention. The subject would be more apt to make use of the clues in learning a new task. Whether or not the two views on the behavioral mechanism of action of ACTH are more similar than different, in this limited review, is of less importance. What seems more of interest is that a variety of laboratories have shown behavioral effects of extremely small quantities of systemically administered non-toxic peptides of the MSH/ACTH family. Structure-activity studies led to the development of an extremely potent $ACTH_{4-9}$-analog, which was protected against proteolytic degradation (Bohus & De Wied, 1980). Numerous studies have recently been or are carried out to measure the behavioral profile of this peptide in healthy and diseased volunteers (Gaillard, 1981).

NOVELTY-INDUCED GROOMING IN THE RAT

Grooming behavior is a common species-characteristic movement pattern with readily definable components (Fentress, 1973). Constituting components are vibration, face washing, body grooming, scratching, paw licking, head and body shaking, and genital grooming (Gispen & Isaacson, 1981). In general, the grooming behavior in rodents is interpreted to represent maintenance of the fur, and is therefore extremely important to the survival of the

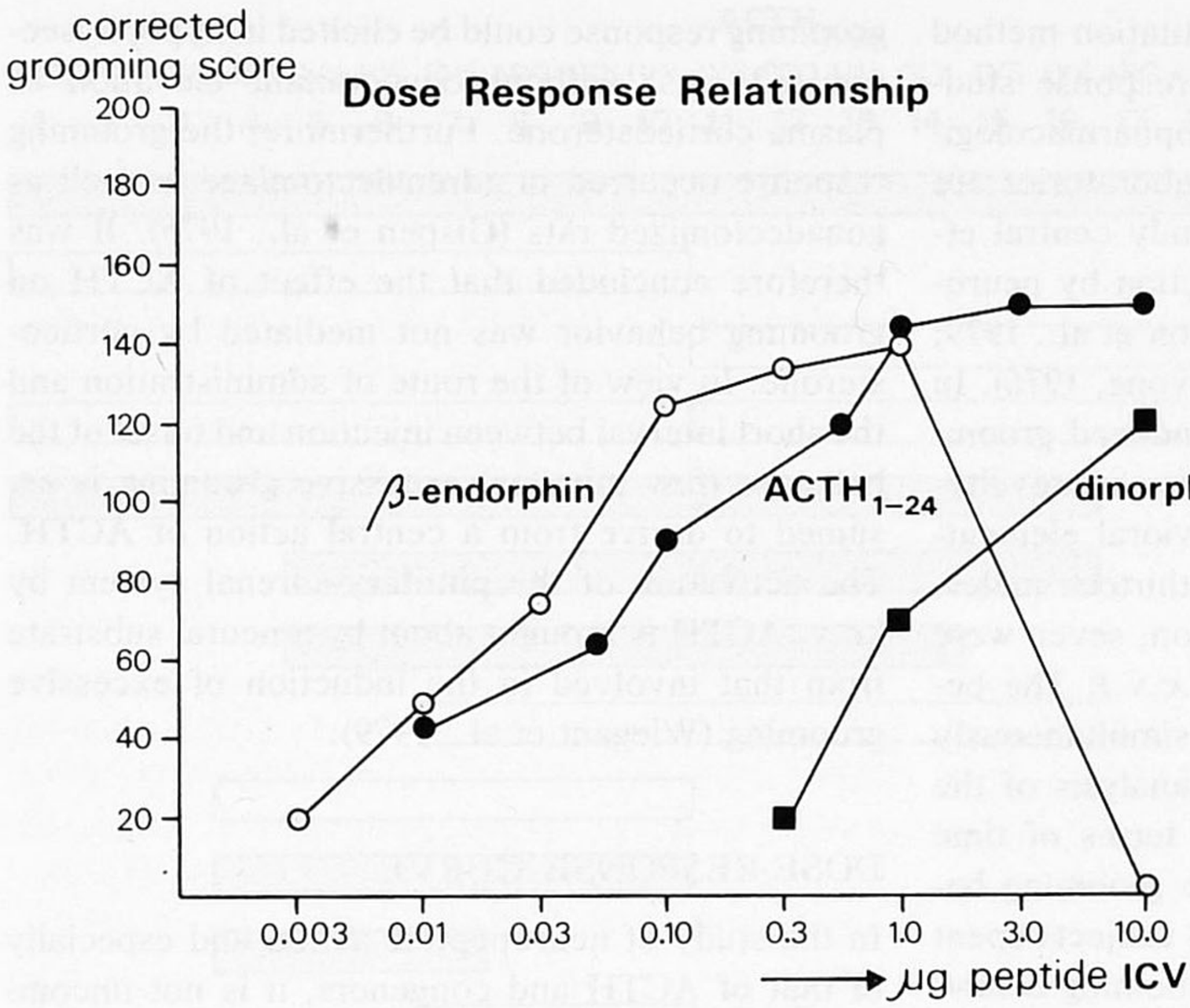

Fig. 2. Dose response curves for peptide-induced excessive grooming. The scores are corrected for the amount seen in saline treated rats.

individual. However, we and many others have noted that elements of the grooming behavior may also be seen in environmental situations which provide the rat with potentially stressing or novel stimuli or stimuli that bear conflicting information (Cohen & Price, 1979; Colbern, Isaacson & Hannigan, 1981; Fentress, 1968*a*, *b*; Jolles, Rompa-Barendregt & Gispen, 1979). The function of this stress or novelty grooming response is not clear. Previously, we considered the possibility that it represented displacement activity, a de-arousal mechanism serving homeostasis (Jolles et al., 1979). At present, experiments are in progress to test this possibility.

Novel as well as stressful stimuli are known to activate the pituitary-adrenal system (Mason, 1968). However, evidence was presented to suggest that the novelty-induced grooming and pituitary-adrenal function are dissociated (Jolles et al., 1979). Yet, the pituitary peptide hormone ACTH may mediate the novelty-induced grooming response (see also below). Dunn, Green & Isaacson (1979) reported that the intraventricular administration of anti-ACTH antiserum suppressed novelty-induced grooming in the rat. Whether this ACTH in the cerebrospinal fluid derived from pituitary origin or from peptidergic neurons synthesizing and releasing ACTH remains unclear.

Given the possibility that similar brain mechanisms play a role in adaptive and grooming behavior, i.e. arousal, it was worthwhile to study more fully the effect of ACTH on grooming in the rat. Therefore, we developed a simple behavioral test which allows the screening of relatively large groups of rats within short time as compared to the experimental design of adaptive behavioral tests (Gispen, Wiegant, Greven & De Wied, 1975).

ACTH-INDUCED EXCESSIVE GROOMING IN THE RAT

The first reported behavioral stereotopy to occur after injection of ACTH into mammals is the so-called stretching and yawning syndrome (Ferrari, 1958). This response could only be observed after intracranial injection and was often preceded by excessive grooming (Ferrari, Gessa & Vargiu, 1963; Izumi, Donaldson & Barbeau, 1973). Excessive grooming is only seen after central application of the peptide and is interrupted by short episodes of the stretching and yawning syndrome (Gispen et al., 1975). Routinely, the peptide is injected into the foramen of Monrou through pre-implanted plastic cannulae. The rats are then placed into novel observation boxes and behavioral analysis by means of 15 sec time sampling procedure begins 15 min later. This observation period usually lasts 50 min and thus a maximum of 200 positive grooming scores

can be observed. This simple quantitation method has enabled us to carry out dose-response studies, structure-activity studies, neuropharmacological experiments, etc. A variety of laboratories are now using similar procedures to study central effects and especially grooming induction by neuropeptides (Drago & Bohus, 1981; Dunn et al., 1979; O'Donohue, 1982; Rees, Dunn & Iuvone, 1976). In an attempt to see whether ACTH-induced grooming differed in composition from saline or novelty-induced grooming, a detailed behavioral element-duration analysis was made, using thirteen males. Six of them received a saline injection, seven were treated with $ACTH_{1-24}$ (1 μg/3 μl i.c.v.). The behavior of the subject was recorded simultaneously by closed-current TV followed by analysis of the tape of each subject's behavior in terms of time spent in various components of the grooming behavior. As expected, ACTH-treated subjects spent much more time in the display of grooming behavior. If the data were converted to proportion of time spent per grooming element as a percentage of the total time spent grooming, no significant changes could be detailed between the two groups of rats. Thus, it appears that ACTH enhances the display of grooming without changing the composition of the behavioral response relative to saline or non-injected control rats (Gispen & Isaacson, 1981).

INDEPENDENCE OF THE PERIPHERAL ENDOCRINE ORGANS

As $ACTH_{1-24}$ has complete classical hormonal activity, it was pertinent to show that the peptide-elicited grooming is the result of a direct effect of these peptides on the central nervous system and not the result of an indirect action mediated by peripheral endocrine glands. Other authors have reported that steroid hormones play a permissive or mediating role in the sexual responses in mammals (Bertolini, Gessa & Ferrari, 1975) or yawning in guinea pigs (Rodriguez-Sierra, Terasawa, Goldfoot & De Wied, 1981) elicited by ACTH.

Wiegant, Jolles, Colbern, Zimmerman & Gispen (1979) demonstrated that intracerebroventricular injection of $ACTH_{1-24}$ and $ACTH_{1-16}$-NH_2 not only induced excessive grooming, but also elevated the circulating levels of plasma corticosterone. Systemic administration of $ACTH_{1-24}$, but not of $ACTH_{1-16}$-NH_2, increased the steroid level, whereas no excessive grooming was observed. The grooming response could be elicited in hypophysectomized rats, without concomitant elevation of plasma corticosterone. Furthermore, the grooming response occurred in adrenalectomized as well as gonadectomized rats (Gispen et al., 1975). It was therefore concluded that the effect of ACTH on grooming behavior was not mediated by corticosterone. In view of the route of administration and the short interval between injection and onset of the behavior (few minutes) excessive grooming is assumed to derive from a central action of ACTH. The activation of the pituitary-adrenal system by i.c.v. ACTH is brought about by a neural substrate from that involved in the induction of excessive grooming (Wiegant et al., 1979).

DOSE-RESPONSE CURVE

In the study of neuropeptide action and especially of that of ACTH and congeners, it is not uncommon to obtain inverted U-shaped dose-response curves. The phenomenon has been observed in a number of neurochemical and behavioral studies. The multiplicity of information sites and peptide receptors might be responsible for this biphasic dose-response curve. With respect to ACTH-induced excessive grooming, a more common dose-response relationship was observed (see Fig. 2). In contrast, β-endorphin at low doses is a very potent grooming inducer, but at high doses the induction of catalepsy is the predominant behavioral sign, leading to a marked drop in grooming activity (Fig. 2). $Dynorphin_{1-13}$, in the doses used, seems more to act like $ACTH_{1-24}$. The figure indicates further that the time sampling analysis is a suitable method to quantify a central action of certain neuropeptides.

STRUCTURE ACTIVITY

Some of the complicated manners in which information is encoded in the primary structure of ACTH, can be readily demonstrated using grooming as the behavioral response to ACTH. As is shown in Fig. 3, $ACTH_{1-24}$, but neither its composing sequences (1–10) or (11–24), nor their equimolar combination, will elicit excessive grooming in the rat. A possible explanation could be that a message site in one sequence needs the presence of an address site in the other sequence for expression of its biological activity. In addition, it may be that

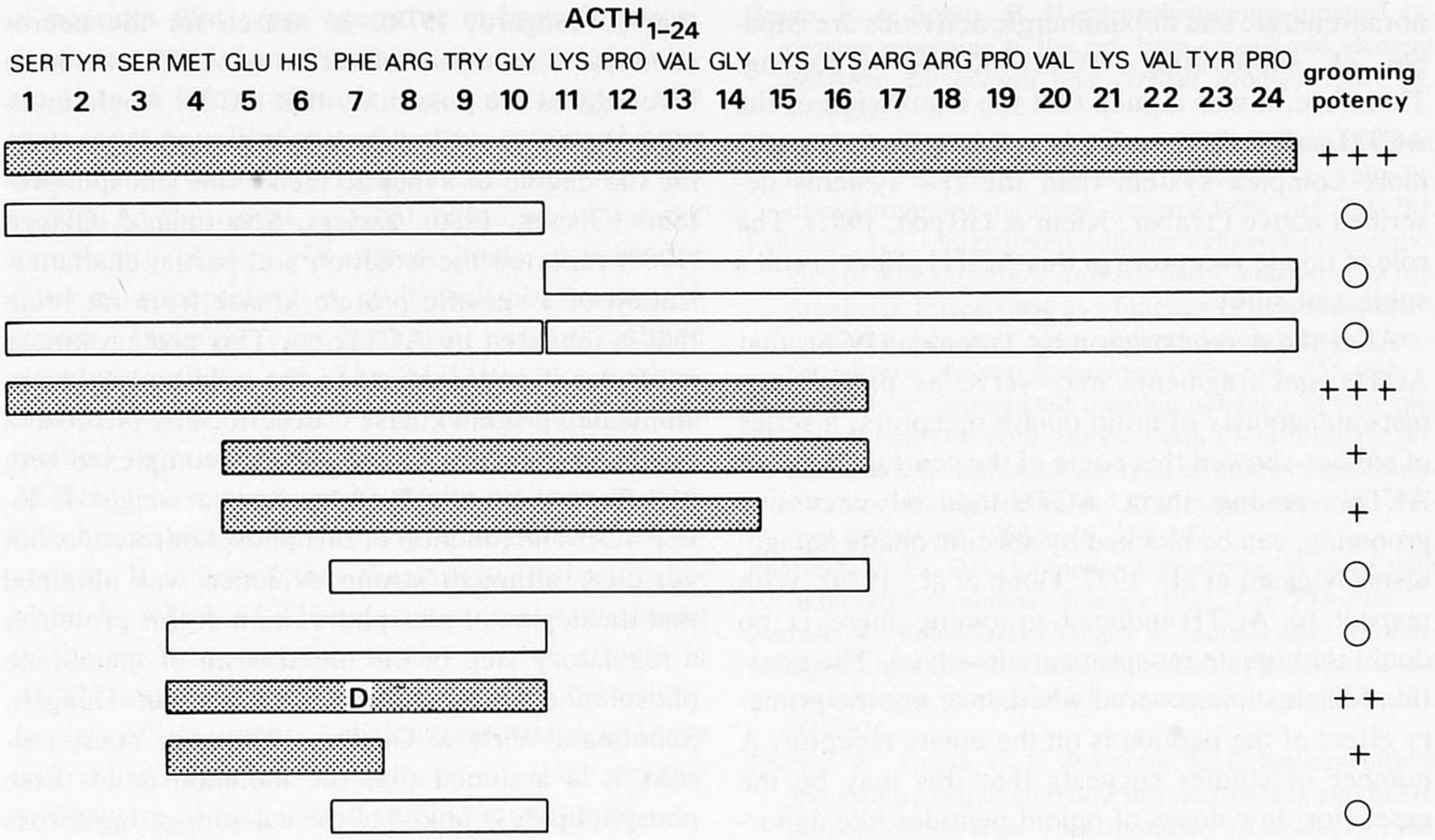

Fig. 3. Structure activity of ACTH-induced excessive grooming. + denotes grooming activity. 0=inactive. D=D-phenylalanine.

the stereoconformation of the molecule $ACTH_{1-24}$ at the receptor site favors the activation of the receptor by the message. The message site is somewhere in the region (5–13) and may be present in the sequence (5–7). The induction of activity by D-substitution of the amino acid phenylalanine in (4–10), the inactivity of (4–10) and (7–10), but the activity of (4–7) are in support of this view. For full expression of activity elongation especially at the C-terminus seems necessary.

NEUROPHARMACOLOGY

A variety of studies have addressed the question what neurotransmitter system is involved in the grooming response. The use of relatively specific agonists and antagonists suggested that the grooming response depends on the integrity of neural pathways with dopaminergic and opiate receptors (see Gispen & Isaacson, 1981). Based on a variety of arguments, Cools (1977) proposed that at least two distinct dopaminergic (DA) systems seem to exist in the brain: an excitation-mediating DA system and an inhibition-mediating DA system. Hence it was concluded that the ACTH-induced excessive grooming is, in particular, sensitive to changes in the balance of these two DA systems (Cools, Wiegant & Gispen, 1978; Wiegant, Cools & Gispen, 1977). Local manipulation of these systems in the n. accumbens and the n. caudatus affect the ACTH-induced grooming (Cools et al., 1978; Gispen, Ormond, Ten Haaf & De Wied, 1980). Evidence is accumulating that the substantia nigra is a structure of special importance to the expression of the ACTH-induced grooming. Destruction of the substantia nigra but not of other brain areas suppresses ACTH-induced grooming, whereas local injection of ACTH into this region elicits the response (Gispen & Isaacson, 1981). The only other known brain lesion that interferes with ACTH-induced grooming is the destruction of the majority of the hippocampus (Colbern, Isaacson, Bohus & Gispen, 1977). Recent studies, however, seem to indicate that the hippocampus is not the target for ACTH in inducing grooming, but that it modulates some properties of the neural substrate underlying this ACTH-induced response (Isaacson, Hannigan, Springer, Ryan & Poplawsky, 1982). However, a number of psychoactive drugs with proposed mechanisms of action ranging from serotonergic,

noradrenergic and dopaminergic activities are capable of suppressing ACTH-induced grooming. Therefore, it was argued that the expression of the ACTH-induced grooming is resulting from a much more complex system than the DA systems described above (Traber, Klein & Gispen, 1982). The role of opiate receptors in this ACTH effect is still a subject of study.

After the demonstration by Terenius (1976), that ACTH and fragments may serve as partial agonists/antagonists of brain opiate receptors, a series of studies showed that some of the central effects of ACTH, among them ACTH-induced excessive grooming, can be blocked by specific opiate antagonists (Wiegant et al., 1977; Dunn et al., 1979). With respect to ACTH-induced grooming there is no doubt that opiate receptors are involved. The question remains unanswered whether or not the primary effect of the peptide is on the opiate receptor. A number of studies suggests that this may be the case. For, low doses of opioid peptides like dynorphin and β-endorphin are potent elicitors of grooming and morphin itself has some activity. Although no tolerance develops when the peptide is given daily, there is a peculiar short time tolerance which lasts for hours and which is reminiscent of that seen for opiates in thermoregulation (Gispen & Isaacson, 1981).

Recent data of Aloyo, Zwiers & Gispen (1982) indicate, however, that the opiate receptor is not the primary site of action of the peptide. For, opioid peptides which lack the N-terminal tyrosine residue and have no affinity for the opiate receptor are still potent grooming inducers. Thus, it would appear that opiate receptors are involved in the neural substrate at a site different from the primary target of the peptides.

MEMBRANE PROTEIN PHOSPHORYLATION AS A POSSIBLE CORRELATE OF ACTH-INDUCED GROOMING

Over the years, there has been specific interest in the role of brain membrane phosphoproteins in chemical neurotransmission. The rationale being that changes in protein structure as brought about by cyclic phosphorylation and dephosphorylation are potentially important for the function of these proteins in the membranes. Especially the regulation of ion permeability of the membrane and hence the excitability of the neuron involved are thought to be regulated by membrane protein phosphorylation (Greengard, 1978). In search for the neurochemical mechanism of action of ACTH we have investigated the possibility that ACTH would modulate the transmission in certain synapses by altering the degree of synaptic membrane phosphoproteins (Gispen, 1980). Zwiers, Schotman & Gispen (1980) reported the isolation and partial characterization of a specific protein kinase from rat brain that is inhibited by $ACTH_{1-24}$. This protein kinase is similar if not identical to the lipid and calcium-stimulated protein kinase C described by Nishizuka (1982). In brain this kinase is partly complexed with its substrate protein B-50 (molecular weight 48 K, IEP 4.5). The function of this phosphoprotein is not yet clear although strong evidence was obtained that the degree of phosphorylation of this protein is a regulatory step in the metabolism of membrane phosphoinositides (Jolles, Zwiers, Van Dongen, Schotman, Wirtz & Gispen, 1980). For many reasons it is assumed that the metabolism of these phospholipids is linked to the calcium gating across the membrane (Michell, Kirk, Jones, Downes & Creba, 1981). As the B-50 protein seems to be localized predominantly in presynaptic membranes, our present working hypothesis is that ACTH may modulate neurotransmission by a direct effect on the function of the presynaptic membrane (Zwiers, Jolles, Aloyo, Oestreicher & Gispen, 1982).

Structure-activity studies with the B-50 protein kinase *in vitro* revealed that only those fragments of ACTH that induce excessive grooming *in vivo*, inhibit this protein kinase. Furthermore, this inhibition could not be blocked by co-incubation with naloxone (Gispen, Zwiers, Wiegant, Schotman & Wilson, 1979). Moreover, the *in vitro* administration of $ACTH_{1-24}$ resulting in excessive grooming at the same time affected synaptic membrane phosphorylation as well (Zwiers, Wiegant, Schotman & Gispen, 1977). Further work is in progress to more fully elucidate the role of the brain-specific phosphoprotein B-50 (Kristjansson, Zwiers, Oestreicher & Gispen, 1982). One of the issues that should be addressed is the specificity and causality of the possible relationship between ACTH effects on grooming and phosphorylation of membrane proteins.

CONCLUDING REMARKS

The field of neuropeptides and behavior is in rapid development and one is amazed by the vast number

of peptides that seem operative in brain function. As neuropeptides in principle are bearers of multiple sites of information, it is not unexpected that they exert a multiplicity of central actions. I have used the peptide ACTH as an example, but I am convinced that most of what is known about the complexity of its CNS effects holds likewise for other neuropeptides, i.e. multiplicity of neuro-substrates, biphasic dose-response curves, defined structure activity, etc. The ACTH-induced excessive grooming behavior is a reliable and simple method to study some of the central actions of ACTH. I have discussed various features of peptide–brain interactions using this behavioral response. It is hoped that the insight in the mechanisms by which ACTH induces excessive grooming in the rat, may aid the studies on the effects of ACTH and congeners on more complex behaviors. In view of the potential clinical importance of these and other peptides, knowledge of their precise mechanism of action is of utmost importance.

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