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BRIEF REPORT

ACTH-Induced Excessive Grooming in the Rat: Latent Activity of $ACTH_{4-10}$[1]

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Intraventricular injection of ACTH or peptides of the N-terminal part of ACTH in rats induces excessive grooming behavior. The present study deals with the grooming-induction potencies of short peptides sharing sequences with $ACTH_{4-10}$ (H-Met – Glu – His – Phe – Arg – Trp – Gly-OH). Although $ACTH_{4-10}$ itself is inactive, $ACTH_{4-7}$ and [D-Phe^7]$ACTH_{4-10}$ are capable of inducing excessive grooming behavior. From the ineffectiveness of a variety of other oligopeptides within the $ACTH_{4-10}$ sequence, it was concluded that $ACTH_{4-7}$ is the shortest sequence to induce this behavioral response as was found in studies on the effect of these peptides on the maintenance of a conditioned avoidance response. The present data underscore the need of detailed information on the configuration of peptides at the CNS receptor site.

Intracranial administration of ACTH or peptides of the N-terminal part of ACTH in rats induces excessive grooming behavior (Izumi, Donaldson, and Barbeau, 1973; Gispen, Wiegant, Greven, and de Wied, 1975), followed by a stretching and yawning syndrome (SYS) (Ferrari, Gessa, and Vargiu, 1963). The grooming response can be elicited in a dose-dependent manner and independent of the endocrine system. The latter was concluded from the results obtained using adrenalectomized, hypophysectomized, or castrated rats (Gispen *et al.*, 1975). Some authors suggest that intraventricular administration of ACTH in rodents in spite of the induction of SYS results in sexual excitement (Bertolini, Vergoni, Gessa, and Ferrari, 1968, 1969; Baldwin, Haun, and Sawyer, 1974), whereas in pigeons the behavioral response elicited by intraventricular ACTH was compared to displacement behavior shown in birds (Delius, Craig, and Chaudoir, 1976). One of the behaviors in rodents often interpreted as representing displacement activities is grooming (Fentress, 1968; Hinde, 1970). After a frightening stimulus voles flee and/or freeze,

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groom, and then walk around, or continue with maintenance behavior (Fentress, 1968). Thus the apparently irrelevant grooming occurs in the transitional state between freezing or fleeing and the other types of behavior. It is not clear, however, whether the grooming response induced by intraventricular-administered $ACTH_{1-24}$, which lasts for at least an hour after the injection and in most instances is only interrupted by stretching and yawning, in fact is related to the grooming seen in a transitional behavioral state as reported by Fentress (1968). Studies on the mechanism of ACTH-induced grooming are in progress, one of the possibilities being that intraventricular-injected ACTH-like peptides alter the rat's body temperazure and that excessive grooming then would reflect a thermoregulatory response. Preliminary data reveal, however, that $ACTH_{4-10}$ and [D-Phe^7]$ACTH_{4-10}$ when injected into the ventricles do not affect the rat's body temperature (Gispen, van Ree, and Ayre, unpublished observations). Thus, at present it seems that the biological significance of the grooming response is unclear. By using this behavioral response, however, information can be obtained on the interaction of ACTH-like peptides and the central nervous system.

Structure–activity studies on grooming-induction potencies of ACTH and congeners revealed that $ACTH_{1-24}$, α-MSH, and β_p-MSH are equipotent (Gispen *et al.*, 1975). These peptides share the sequence (4–10), i.e., H-Met–Glu–His–Phe–Arg–Trp–Gly-OH. Yet, after intraventricular administration of as much as 40 μg of $ACTH_{4-10}$ no excessive grooming was observed, whereas similar treatment with 0.3 μg of [D-Phe^7]$ACTH_{4-10}$ resulted in appreciable grooming activity (Gispen *et al.*, 1975). The present communication elaborates on the grooming-induction potency of peptides sharing sequences with $ACTH_{4-10}$.

The methodology involved has been described in detail elsewhere (Gispen *et al.*, 1975). Briefly, male rats, weighing 140–160 g, of an inbred Wistar strain (TNO, Zeist, the Netherlands) were used. Five days prior to the experimental session, a plastic cannula was implanted into the brain ventricle where the third and lateral ventricle merge. At the experimental day, the synthetic peptides (Organon International b.v., Oss, The Netherlands) were dissolved in saline and injected by free hand into the ventricle of the conscious rat (1 μl). After injection, the rats were placed individually into glass boxes (24 × 12.5 × 14 cm) in a low noise room, and recording of maintenance behavior began 15 min thereafter. The following behavioral elements were distinguished: vibrating, washing, grooming, scratching, paw licking, and tail licking (Gispen, Van der Poel, and Van Wimersma Greidanus, 1973). Recording of behavior was performed by a 15th-sec sampling procedure, i.e., the observer determined every 15th sec for 15 min whether or not the rat displayed one of the elements mentioned above. Since the predominant element appeared to be grooming, we prefer to refer to grooming in keeping with previous reports

(Ferrari *et al.*, 1963; Izumi *et al.*, 1973; Gispen *et al.*, 1975; Gispen and Wiegant, 1976). The validity of the sampling technique was established by simultaneous measurement of grooming duration and 15th-sec scores for one given rat (Gispen *et al.*, 1975). In the 50-min observation period a maximum of 200 positive 15th-sec grooming scores can be obtained.

Saline-treated rats, placed in a novel glass box usually display exploratory and grooming behavior, which may last for some 15–20 min after the intraventricular injection, but they then invariably fall asleep and thus show little grooming activity during the observation period. In contrast, the intraventricular injection of $ACTH_{1-24}$ (3 $\mu g/\mu l$) elicits nearly maximal grooming activity (Table 1). Injection of an equimolar dose of $ACTH_{4-10}$ is ineffective whereas treatment with the analogue [D-Phe^7]$ACTH_{4-10}$ generates excessive grooming. Similar injection of $ACTH_{4-7}$ results in grooming activity as seen after [D-Phe^7]$ACTH_{4-10}$. However, rats treated with $ACTH_{7-10}$ displayed a behavior similar to that of saline treated subjects. The sequences (5–9), (4–8), (4–6), and (5–7) were ineffective in inducing excessive grooming. Introduction of a D-phenylalanine into $ACTH_{4-7}$ and $ACTH_{7-10}$ at the seventh position did not modify the activity of these peptides (Table 1).

TABLE 1

$ACTH_{4-10}$ and Excessive Grooming in the Rat

Treatment	Dose (μg)	n	15th sec grooming scores (mean ± SEM)	Amino acid sequence
Saline	1 μl	6	30 ± 5	
$ACTH_{1-24}$	3.0	4	170 ± 8	
$ACTH_{4-10}$	1.0	4	27 ± 5	H–Met–Glu–His–Phe–Arg–Trp–Gly–OH
$ACTH_{4-9}$	0.8	4	36 ± 10	H–Met–Glu–His–Phe–Arg–Trp–OH
$ACTH_{4-8}$	0.6	4	30 ± 10	H–Met–Glu–His–Phe–Arg–OH
$ACTH_{4-7}$	0.6	8	71 ± 7	H–Met–Glu–His–Phe–OH
$ACTH_{4-6}$	0.4	4	27 ± 7	H–Met–Glu–His–OH
$ACTH_{5-7}$	0.4	4	27 ± 12	H–Glu–His–Phe–OH
$ACTH_{7-10}$	0.6	8	30 ± 5	H–Phe–Arg–Trp–Gly–OH
[D-Phe^7]$ACTH_{4-10}$	1.0	4	89 ± 15	
[D-Phe^7]$ACTH_{4-7}$	0.6	8	68 ± 5	
[D-Phe^7]$ACTH_{7-10}$	0.6	8	21 ± 4	

In view of the common sequence (4–10) in ACTH and α- and β-MSH, similarities in their action on behavior have been ascribed to the presence of that sequence (de Wied, 1974). With respect to the effects of these peptides on avoidance behavior, $ACTH_{4-10}$ does indeed have a behavioral potency comparable with that of the parent molecule.

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 (de Wied, Witter and Greven, 1975). With respect to induction of excessive grooming and SYS, Ferrari *et al.* (1963) reported that at high doses $ACTH_{4-10}$ induced SYS in dogs; Baldwin *et al.* (1974) using rabbits concluded that this sequence may have some activity in inducing SYS and sexual excitement but is much weaker than $ACTH_{1-24}$. In rats, the sequence (4–10) is devoid of grooming inducing activity (Table 1; Gispen *et al.*, 1975). This finding is supported by recent observations of Rees, Dunn and Iuvone (1976), using mice. Inversion of the configuration of the phenylalanine residue at the seventh position caused a remarkable potentiation of the tetrapeptide $ACTH_{4-10}$ (Table 1) which could not be achieved by introduction of a D-arginine residue in position 8 (Gispen *et al.*, 1975). This observation points to a latent activity of sequence (4–10) with respect to grooming and underscores the importance of the seventh amino acid residue in ACTH–CNS interactions (see also de Wied *et al.*, 1975). The latent activity of $ACTH_{4-10}$ also becomes apparent when the peptide is elongated at the C-terminal part, since in order of decreasing potency [Lys^{17}, Lys^{18}] $ACTH_{5-18}$-NH_2, $ACTH_{5-16}$-NH_2, and $ACTH_{5-14}$ were able to induce excessive grooming (Gispen *et al.*, 1975). Combining these data with those from the present study, one is tempted to conclude that the latent activity of $ACTH_{4-10}$ resides in the sequence (4–7), and in addition, that the presence of (5–7) is essential. It seems that the C-terminal part of $ACTH_{4-10}$ is not involved since (7–10) and (7–16) are devoid of grooming induction activity (Table 1) (Gispen *et al.*, 1975). Thus, the present study points to (4–7) as being the shortest peptide to induce the behavioral response, as was also concluded from the structure-activity studies with ACTH peptides and avoidance behavior (de Wied *et al.*, 1975). However, it should be kept in mind that the structural requirements for expression of activity in the two behaviors are not identical since in avoidance behavior $ACTH_{7-16}$ is as potent as $ACTH_{4-10}$ and the sequences 4–9 and 4–8 are as potent as 4–7 (de Wied *et al.*, 1975). Thus it would appear that the central nervous system contains more than one type of ACTH-sensitive site (receptor) involved in more than one function. It may be that for all ACTH–CNS interactions the crucial information resides within the sequence 4–7, with a key role for phenylalanine in the seventh position (see de Wied *et al.*, 1975) and that different interaction of the adjacent amino acids with different ACTH-sensitive sites in the brain is responsible for the observed discrepancies in structure–activity relationships for ACTH–CNS effects (de Wied *et al.*, 1975; Gispen *et al.*, 1975, 1976). Such reasoning underscores the need of detailed information on the configuration of peptides of the N-terminal part of ACTH in solution or even at the receptor site in the CNS.

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