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Induction of excessive grooming in the rat by fragments of lipotropin

CURRENT research reveals the existence of endogenous peptides in brain[1,2] and pituitary tissue[3,4], which are presumably derived from lipotropin (LPH) and which have opiate like effects and affinity for opiate receptors (enkephalin[1], endorphins[5], C-Fragment[6]). N-terminal peptides of ACTH also have measurable, although much lower, affinity for rat brain receptors *in vitro*[7,8]. ACTH and congeners are known to play a crucial role in the acquisition and maintenance of a variety of behaviours in animals and man[9,10]. Intraventricular but not systematic administration of these peptides elicits a stretching and yawning syndrome[11,12]. In rats this syndrome is preceded by a display of excessive grooming[13,14] and this grooming response can be suppressed by peripheral administration of specific opiate antagonists (naloxone, naltrexone)[15]. Morphine also induces grooming[15] and this observation prompted us to study the effect of LPH fragments on excessive grooming in the rat in the absence and presence of an opiate antagonist.

The methodology has been described in detail elsewhere[14,15]. Briefly, female rats of an inbred Wistar strain (TNO, Zeist, The Netherlands) had plastic canulae implanted in the 3rd cerebral ventricle, 1 week before the observation session. The behavioural procedure consisted of a 15th-s sampling technique, the validity of which has already been established[14]. During a 50-min period the observer determined every 15th s whether the rat displayed an element of the maintenance repertoire (that is vibrating, washing, grooming, scratching, licking paw, licking tail)[16]. Since the predominant element recorded seemed to be grooming, we prefer to refer to grooming in keeping with previous reports[11,13,14]. Immediately after intraventricular injections into conscious rats (1 μl), the rats were placed individually into glass boxes (24 × 12.5 × 14 cm) in a low noise room and 15 min later the behavioural analysis began. The effect of naloxone (Endo, Ny, 0.5 ml 100 g^{-1} subcutaneous 1 mg kg^{-1}) on peptide-induced excessive grooming was studied 25 min after the intraventricular injection of the peptide. Therefore the scoring was interrupted for 5 min to allow for administration of the opiate antagonist; subsequently the behavioural analysis was continued for another 40 min. The following synthetic peptides were tested: $ACTH_{1-24}$, β_p-MSH (LPH_{41-58}), $ACTH_{4-10}$ (LPH_{47-53}), Met-enkephalin (LPH_{61-65}), Leu-enkephalin ([Leu^{65}]LPH_{61-65}), LPH_{61-69}, LPH_{65-69}, α-endorphin (LPH_{61-76}). C-Fragment (LPH_{61-91}) was isolated from porcine pituitary glands[3].

Saline treated rats, placed in a novel glass box usually display exploratory and grooming behaviour which may last for some 15–20 min after intraventricular injection but then invariably fall asleep and thus show little grooming activity during the observation period, which starts 15 min after the injection. In contrast, the intraventricular injection of 0.3 μg of LPH_{61-91} elicited nearly maximal grooming activity (165 out of 200 possible positive scores). The effect appeared to be dose-dependent. A dose of as little as 10 ng significantly induced grooming activity (Table 1). LPH_{61-91} therefore has a higher potency than $ACTH_{1-24}$[14]. It should be noted, however, that in contrast to ACTH-induced grooming, administration of LPH_{61-91} in low doses such as those used here, not only elicited grooming but also excitation in some rats typified by quick movements of body and head, jumping, gnawing and body shakes. In a subsequent experiment the effect of subcutaneously administered naloxone on LPH_{61-91}-induced excessive grooming was studied. Rats treated with 0.1 μg LPH_{61-91} displayed excessive grooming before naloxone (Table 2). However, after subcutaneous administration of 1 mg kg^{-1} naloxone, excessive grooming was markedly suppressed. Interestingly, no overall behavioural differences were noted between saline/saline and saline/naloxone treated rats, suggesting that naloxone itself did not affect ongoing behaviour. Similar results were obtained using 0.1 mg naloxone and 0.1 μg LPH_{61-91}. Structure activity studies were carried out to identify the active amino acid sequence responsible for excessive grooming. It has been found previously that intraventricular injection of sheep β-LPH does not elicit grooming in the rat unless an extreme dose (2.5 mg) is used[13]. Apparently β-LPH itself is practically devoid of activity in this respect. LPH_{61-76} (α-endorphin) was much less active than LPH_{61-91}. The sequence LPH_{61-69} seemed slightly less active than LPH_{61-76} (Table 1). LPH_{61-65} and [Leu^{65}]LPH_{61-65} induced hardly any grooming activity over the wide dose range tested (0.1–29 μg). Similarly, LPH_{65-69} was found to be inactive (Table 1). LPH_{47-53} which is common to LPH, MSH and ACTH has latent grooming activity[17]. Administration of LPH_{47-53} in doses up to 40 μg does not induce excessive grooming (Table 1) whereas administration of LPH_{47-50} or [D-Phe^{50}]LPH_{47-53} (= [D-Phe^{7}]$ACTH_{4-10}$) elicits significant grooming at a dose of 1 μg[14,15]. As reported previously the sequence LPH_{41-58} (= β-MSH) is equipotent to $ACTH_{1-24}$ in inducing the grooming response (Table 1)[14]. Excessive grooming was interrupted by short episodes of stretching and yawning only in the case of $ACTH_{1-24}$, LPH_{41-58} and LPH_{61-76}, again suggesting that excessive grooming behaviour and stretching/yawning may result from different mechanisms[14]. The results indicate that within the C-fragment LPH_{61-69} contains the essential sequence for excessive grooming. Elongation of this sequence, however, markedly enhances the response.

The available data on the affinity of LPH fragments for opiate receptors in rat brain membrane fractions indicate that LPH_{61-91} has the highest affinity (IC_{50} for DHM 2.2×10^{-9} M, for etorphin 3×10^{-7} M[5,6]) followed by (61–65), (61–69) and (61–76) in that order. The affinity of fragments of ACTH and LPH_{47-53} ($ACTH_{4-10}$) studied with labelled DHM is several orders of magnitude lower than those peptides mentioned above and the sequence α-MSH[Ac-Ser^{3}]$ACTH_{1-13}$-NH_2—which is as active as $ACTH_{1-24}$ in inducing excessive grooming[14]—is virtually inactive[7,8].

Recently, we have reported that subcutaneously administered fragments of ACTH which have some affinity for the brain opiate receptors and which are devoid of *in vivo* corticotropic activity counteract morphine-induced analgesia in the rat as tested on the hot plate[18,19].

The biological significance of induction of excessive grooming is unclear. Some authors have interpreted the behavioural signs

Table 1 LPH fragments and excessive grooming

Peptide sequence	Lowest dose at which excessive grooming was observed* (μg)
LPH_{61-91}	0.01
LPH_{61-76}	1.7
LPH_{61-69}	5.5
LPH_{61-65}	>29†
$[Leu^{6}]_{61-65}$	>29†
LPH_{65-69}	>20†
LPH_{41-58}(=β-MSH)	0.1
LPH_{48-53}(=$ACTH_{4-10}$)	>40†
$ACTH_{1-24}$	0.1

*Grooming was scored using a 15th-s sampling technique (observation session of 50 min, beginning 15 min after intraventricular injection. For all peptides at least 5 dosages were tested with at least 4 rats per group. Saline-treated rats as average reach a score of 20–30 (out of maximum of 200). Significant excessive grooming is observed when scores are above 55.
†No activity observed over a dose range of 0.1 μg to indicated dose.

Table 2 Effect of naloxone on LPH_{61-91} induced excessive grooming in the rat

Treatment				Number of rats	Amount of grooming	
$t=0$ min IIIrd ventricle		$t=25$ min subcutaneous			15–25 min	30–70 min
LPH_{61-91}	0.1 μg	saline	0.5 ml	6	32 ± 1*	87 ± 12
LPH_{61-91}	0.1 μg	naloxone	1 mg/kg	6	29 ± 2	6 ± 3
saline	1 μl	naloxone	1 mg/kg	3	14 ± 8	12 ± 7
saline	1 μl	saline	0.5 ml	3	16 ± 6	9 ± 5

*Mean ± s.e.m.

induced by intracranial administration of ACTH-like peptides in terms of sexual excitement[20,21]. The behavioural response in pigeons has been likened to displacement behaviour[22]. Both electrical stimulation of limbic structures[23,24] and central application of Zn^{2+} (ref. 12) may induce grooming activity. The latter observation is of interest in view of the known importance of bivalent cations to the mechanism of action of ACTH[25,26].

The present study was aimed at investigating the nature of the peptide-CNS interaction underlying behavioural effects. Since opiate antagonists suppress ACTH and LPH-induced behaviour, one is tempted to conclude that the neural substrate for this behaviour is sensitive to ACTH fragments, LPH fragments and opiates. Interestingly, intraventricular administration of low doses of morphine also induces excessive grooming to the same extent as LPH_{61-76}[15].

In view of the behavioural effects produced by intracerebral injection of low doses of LPH fragments, the question whether the "opiate-like" activity of these neuropeptides is their most important physiological effect remains to be elucidated.

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Neonatal progesterone and feminine sexual development

IT has been generally concluded that the inherent programme of sexual differentiation in both sexes of mammals is female. If androgens are present during the critical periods of sexual differentiation, then both genetic males and females will be organised for masculine reproductive organs[1], hepatic steroidogenic enzymes[2], hypothalamic control of gonadotropin secretion (tonic)[3] and sexual behaviour[4]; whereas an absence of either gonad during the critical developmental periods allows for the expression of the inborn female programme[1–3,5]. These results have led to the generally held concept that feminine differentiation requires no hormonal imprinting and will occur normally as long as androgens are not present during the critical periods of sexual embryogenesis. We have reported, however, that interference with perinatal pituitary or adrenal function in female rats causes defects in normal pubertal feminine development which suggests that endogenous hormones may be essential for feminine organisation[6]. Unlike oestradiol and testosterone which both masculinise the female rat[7], progesterone treatment antagonises these effects and protects the developing female from exogenous oestrogens and androgens[8]. In fact, serum progesterone levels in the foetal monkey have been shown to be significantly higher in the female than in the male[9] and we have recently postulated that perinatal progesterone may be required for feminine neural differentiation[10]. We present here evidence demonstrating that neonatal female rats have a markedly higher level of serum and adrenal progesterone than do neonatal males, and since serum progesterone levels can be further increased by exogenous gonadotropins–adrenal (progesterone) axis may be required for normal feminine sexual differentiation.

At 3 d of age the serum progesterone concentration was ten times greater in female pups than in males (Table 1) and the adrenal progesterone concentration was also significantly greater in the neonatal females. Administration of pregnant mare serum gonadotropin (PMSG, which has both follicle stimulating hormone (FSH) and luteinising hormone activities, with FSH predominant[11]) elevated serum progesterone levels on both 3-d-old male and female rats.

Our progesterone results in neonatal rats are in agreement with findings in adult animals, in that rat adrenals