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Effects of ACTH and Related Peptides on Medial Septal Self-Stimulation

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GOWER, A. J., C. L. E. BROEKKAMP AND A. M. L. VAN DELFT. *Effects of ACTH and related peptides on medial septal self-stimulation.* PHARMACOL BIOCHEM BEHAV **24**(5) 1253–1256, 1986.—The effects of $ACTH_{1-24}$, $ACTH_{4-10}$, the $ACTH_{4-9}$ analog Org 2766 and [D-Phe^7] $ACTH_{4-10}$ on medial septal self-stimulation were determined in the rat following intracerebroventricular (ICV) injections. Self-stimulation rates were increased by 0.01–10 μg $ACTH_{1-24}$ or 0.1–10 μg $ACTH_{4-10}$, but not by Org 2766 or [D-Phe^7] $ACTH_{4-10}$. A dose of 1 μg $ACTH_{1-24}$ ICV did not affect open field behaviour. Subcutaneous administration of 1 μg $ACTH_{1-24}$ did not influence self-stimulation in the septum. Thus, the $ACTH_{1-24}$ effect appears to be a central effect and provides evidence for an influence of ACTH containing pathways on structures involved in maintaining self-stimulation behavior. A possible role of opiate receptors and dopaminergic neurons in this effect of $ACTH_{1-24}$ is also discussed.

$ACTH_{1-24}$ ACTH-releated peptides Self-stimulation Medial septum Open field

ADRENOCORTICOTROPHIC hormone (ACTH) occurs in the brain and possesses characteristics in common with classical neurotransmitters [8, 13, 19]. $ACTH_{1-24}$ and derivatives have behavioural effects after systemic administration and these effects are claimed to have a site of action in the central nervous system [2, 4, 19]. However few reports are available on central applications of this hormone fragment. One particular effect of $ACTH_{1-24}$ intracerebroventricularly (ICV) is elicitation of grooming, an effect which is suggested to be dopamine linked [2, 3, 9]. Dopamine is important in the mediation of self-stimulation [22]. We were therefore interested in the central effects of $ACTH_{1-24}$ and related peptide fragments on self-stimulation. We selected the medial septum as the target of self-stimulation since, relative to other brain areas, the medial septum sustains lower rates of self-stimulation which characteristically decline with time during a test session [1,7]. It was thought that such characteristics may be more sensitive to the effects of ACTH peptides which might be associated with stress and arousal changes. Also, Fekete *et al.* [5] reported effects on septal self-stimulation by systemic administration of $ACTH_{4-10}$ and a modified form of $ACTH_{4-9}$. We determined the effects of ICV injection of $ACTH_{1-24}$ and selected fragments on medial septal self-stimulation maintained by constant current stimulus intensity. Possible changes in general activity were assessed using an open field test.

METHOD

Animals

Male Wistar rats (Cpb:WU; TNO, Zeist, the Netherlands) were used which weighed 140–170 g at the time of surgery and 250–400 g at the time of testing. Following surgery, the rats were housed singly and maintained on a 12 hr light-dark schedule with ad lib access to food and water. Behavioural testing was done in the light period.

Surgery

Under pentobarbital anaesthesia (60 mg/kg; intraperitoneally), each rat was implanted with a single bipolar stimulating electrode (MS303, Plastic Products Co., USA) insulated except at the tip. The electrode was aimed at the medial septum, co-ordinates A 8.4, L 0.1, H 0.0 according to the König and Klippel rat brain atlas [16]; the electrode was directed at an angle of 7° to the midsagittal plane. Rats designated for ICV experiments were also implanted with a single guide cannula (external diameter 0.7 mm) with the tip located 1 mm above the lateral ventricle, co-ordinates A 5.8, L 1.7, H 3.1. At least 1 week post-operative recovery time was allowed before self-stimulation began.

Following termination of the experiment the position of the electrodes was confirmed histologically in a random

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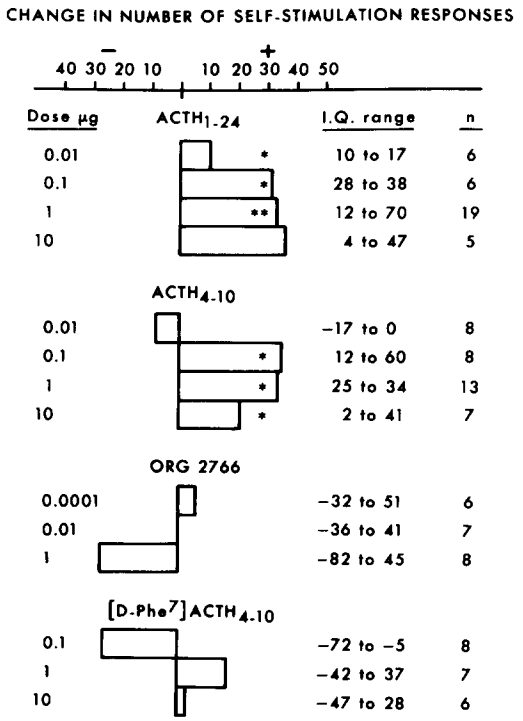

FIG. 1. Effects of ICV injected peptides on self-stimulation. Change in median number of responses in post-injection half hour are presented with interquartile range. *$p<0.05$, **$p<0.001$.

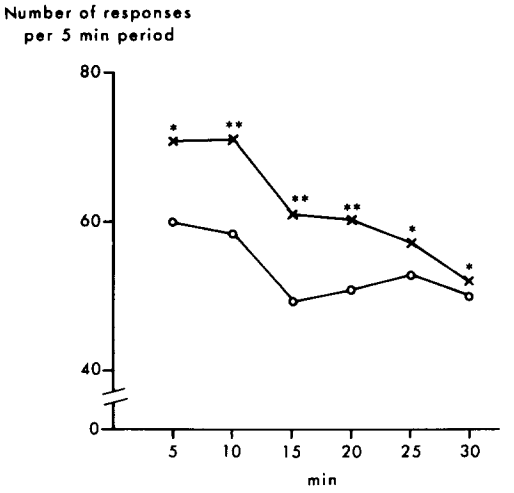

FIG. 2. Effect of ICV $ACTH_{1-24}$ on septal self-stimulation followed over time. Data are based on median number of lever presses per 5 min (n=19). ○ ICV saline, χ ICV $ACTH_{1-24}$. *$p<0.05$; **0.01 with respect to controls, tested with matched pairs Wilcoxon test.

TABLE 1

EFFECT OF $ACTH_{1-24}$, ICV, ON OPEN FIELD BEHAVIOUR

	$ACTH_{1-4}$ 1 μg/rat	Saline 5 μl/rat
	mean (s.e.m.)	
Number of entries	64 (11)	82 (13)
Number of rearings	43 (10)	48 (7)
Number of starts	71 (13)	96 (11)
Total moving time (sec)	169 (30)	207 (24)
Total distance run (cm)	2647 (498)	3355 (527)
Average speed (cm/sec)	16 (1)	16 (1)

sample of 10 rats; thereafter positive responding was taken as sufficient evidence of accurate placement (see also [7]). The positions of the guide cannulae were verified in all animals by injecting 5 μl Evans blue dye immediately after killing the rats and examining the distribution of the dye in freshly removed brain. A total of 35 rats displaying septal self-stimulation and with correct cannula placements were used in these experiments.

Twelve additional rats, for use in the open field experiment, were each prepared with a single ICV guide cannula, co-ordinates given above. All placements were confirmed as correct by injecting Evans blue dye immediately post-mortem

Self-Stimulation Experiments

Experiments were carried out in grey perspex Skinner boxes (30×18×41 cm) equipped with a single lever. Depression of the lever produced a 0.3 sec train of negative pulses (100 pps; 0.2 msec pulse duration). One pole of the electrode was connected to the zero of the instruments, which was not grounded. The cage and lever were isolated from instrument zero and from ground. The rats were trained using standard operant procedures; rats failing to acquire the response after two 30 min sessions were rejected. Training was continued for at least 8 more sessions until consistent day to day responding was achieved at a submaximal rate of at least 300 responses per hour, maintained by a current intensity of 150–300 μA. Before testing the peptides, each rat was well accustomed to handling and injection procedures by training sessions preceded by saline injections. For peptide testing, each dose of each peptide was investigated separately in a group of 6–8 rats using a cross-over procedure, over 2 test days, usually consecutive. Under this procedure, half of the group received peptide on test day 1 and saline on test day 2; the remaining rats received saline on test day 1 and peptide on test day 2. In this way each rat served as his own control. Self-stimulation was recorded for 30 min immediately following injection. Differences between peptide and saline sessions are presented as medians with interquartile ranges (iqr) and were assessed using the non-parametric Wilcoxon matched pairs signed-ranks test for related data [20].

Open Field Experiments

The effects of 1 μg/rat $ACTH_{1-24}$ ICV on general activity were determined using a TV-based automated open field. The open field itself consisted of a plywood box, 100×100×20 cm high, painted black which was divided into several areas, viz. corners, edges and inner field. The system is described in detail elsewhere [17] and permits measurement of several behavioural parameters. These include entries from one are to another; rearings; number of starts; total time spent moving; total distance run and the average speed. Separate groups of 6 rats were used for ACTH and

saline control respectively. The open field behaviours were recorded over 20 min period starting 10 min after ICV injection.

Peptide Treatment

The following peptides were used: $ACTH_{1-24}$, $ACTH_{4-10}$, Org 2766 ([$Met(O_2)^4$, D-Lys^8, Phe^9]-$ACTH_{4-9}$) and [D-Phe^7] $ACTH_{4-10}$. The peptides were dissolved in saline immediately before use, in a volume of 2 ml/kg for SC injection or 5 μl/rat for ICV injection. During control sessions each rat received an equivalent volume of saline. ICV injections were made by inserting an injection needle which protruded 1 mm beyond the end of the guide cannula. Plastic containers and syringes were used throughout.

The peptides were all synthetized in the chemical R and D Laboratories at Organon, Oss, the Netherlands.

RESULTS

The effects on self-stimulation of ICV-administered peptides are given in Fig. 1. Median baseline response rates for the groups were between 319 and 487 in 30 min. Examination of the baseline responding at 5 min intervals showed a gradual decline in responding with time by about 20% over the 30 min session. Increments due to peptide treatments were observed from the beginning of the self-stimulation session and were maintained during the 30 minute session. The time course of the effects of 1 μg $ACTH_{1-24}$ is illustrated in Fig. 2.

$ACTH_{1-24}$ enhanced self-stimulation responding at all doses tested. The fragment $ACTH_{4-10}$ had about the same activity and increased self-stimulation at 0.1, 1 and 10 μg. Org 2766 and [D-Phe^7] $ACTH_{4-10}$ were unable to enhance self-stimulation at the doses tested. The latter peptide was tested in the same dose range as $ACTH_{1-24}$ and Org 2766 was tested over a wide dose range in view of the low doses at which behavioural effects are described for this analogue [6]. It was experienced that the enhancement might not occur in rats which are not well accustomed to the handling and injection procedure. This was noticed with a group of rats prepared for a replication experiment in which we habituated the rats only once to the injection procedure and in which we did not see an enhancement of self-stimulation after $ACTH_{1-24}$ (1 μg: +1.5 response; n=6). However the enhancement was observed with a second injection of $ACTH_{1-24}$ after a period of extra handling (1 μg: +22 responses; $p<0.05$; n=6). With this precaution the effect of $ACTH_{1-24}$ (or $ACTH_{4-10}$) was then reproduced in two independent groups of rats (data combined in Figs. 1 and 2).

Subcutaneous injection of $ACTH_{1-24}$ did not enhance self-stimulation. On a median baseline of 394 responses in half an hour the treatment of 1 μg $ACTH_{1-24}$ had no effect or a tendency to reduce responding. The treated rats had a median change of −23 (iqr −35 to +34; n=5).

The effects of $ACTH_{1-24}$ 1 μg/rat ICV on open-field behaviour are given in Table 1. The peptide produced no significant changes in any of the parameters measured.

DISCUSSION

In the present experiment we find rate-enhancing effect on medial septal self-stimulation of $ACTH_{1-24}$ and $ACTH_{4-10}$. The effects of $ACTH_{1-24}$ were obtained following central but not peripheral administration, implicating a central site of action. As our injections were intraventricular we cannot specify a particular structure on which the ACTH fragments are active. Possible brain structures are the septum, periaqueductal gray area and other areas in which ACTH-like immunoreactivity has been identified [13] and which are located near the ventricular system.

The lack of effect of Org 2766 in our experiments seems to contrast with the reported effects of this putative ACTH analogue on self-stimulation [5,15]. Both Katz [15] and Fekete *et al.* [4] reported enhanced self-stimulation after Org 2766. However, the use of systemic injections in these studies makes a comparison with our results difficult. In addition, there are other procedural differences which may account for the contrasting results. Katz used medial forebrain bundle stimulation and reported peptide-induced increases in the total number of responses elicited over a 24 hr session. Also, although in common with the present study Fekete *et al.* used medial septal self-stimulation, the enhancement was obtained at low rates of responding elicited at low stimulus intensities when an ascending series of stimulus intensities were presented but not when a descending series was used.

We found no effect of $ACTH_{1-24}$ ICV on open field activity. Parameters reflecting motor activity such as number of rearings, total moving time or total distance run were unchanged. This makes it unlikely that the enhancement on self-stimulation responding is a consequence of enhanced motor activity. It was unexpected that $ACTH_{1-24}$ did not change the open field parameters because, if grooming was induced, this would have interfered with activity parameters. Apparently no enhanced grooming occurred in the open field which was in accordance with non-quantified observation via the TV monitor. In a later test the same animals responded to $ACTH_{1-24}$ with almost continuous grooming when placed in a smaller cage (data not reported). In the self-stimulation cage no grooming was observed. Isaacson and Green [12] observed grooming after ICV $ACTH_{1-24}$ in a smaller environment than our environment and this grooming interfered with exploratory behaviour. These data indicate that grooming in response to $ACTH_{1-24}$ can be suppressed by stronger tendencies for other behaviour such as searching or self-stimulation, depending on the environmental stimuli. Dopamine is implicated in brain reward [10,22] and is also implicated in ACTH-induced grooming [2, 3, 11]. However there is a dissociation between grooming induction and self-stimulation enhancement: [D-Phe^7] $ACTH_{4-10}$ is reported to induce grooming but does not enhance self-stimulation whereas $ACTH_{4-10}$ increases self-stimulation but does not cause grooming [9]. This suggests that different receptors are involved in ACTH-induced grooming and ACTH-induced self-stimulation enhancement and that different brain pathways are important for these behavioural effects of ACTH.

Although it is not possible to pinpoint processes responsible for $ACTH_{1-24}$ mediated self-stimulation enhancement the data provide further characterisation of central effects of $ACTH_{1-24}$. The small magnitude of the effect and the importance of proper handling for the effect may point to effects on arousal or modultory effects on motivation and reward. It is tempting to relate our findings to the finding of Jouhaneau-Bowers and Le Magnen [14] who described self-administration maintained by $ACTH_{1-24}$. However, it remains questionable whether the same parts of the central nervous system are influenced by peripheral injections as are influenced by intraventricular injections.

A relation might exist between the enhancing effects of $ACTH_{1-24}$ and $ACTH_{4-10}$ on self-stimulation and affinity for

opiate receptors. Unlike the other peptides in this study, these two show affinity for opiate receptors in the brain [21]. In line with this possibility, naloxone 0.3 and 1.0 mg/kg SC decreased self-stimulation (data not shown) which precludes its use to investigate possible blockade of ACTH peptide effects.

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