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Peptide-induced grooming behavior and caudate nucleus dopamine release

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We simultaneously measured the display of grooming behavior and, by monitoring the extracellular dopamine concentration via transversal microdialysis, the release of dopamine in the caudate nucleus in freely moving rats after i.c.v. administration of 1 μg adrenocorticotropic hormone-(1–24) (ACTH-(1–24)). During a period of 1 h after administration of the peptide, the incidence of excessive grooming behavior was increased. Concomitantly, the concentration of dopamine in the caudate nucleus dialysates was significantly increased (maximal effect 151% of basal release) whereas that of its metabolite DOPAC was unchanged. The potent α-melanocyte stimulating hormone (α-MSH) receptor agonist, [Nle^4,D-Phe^7]α-MSH, induced grooming behavior and stimulated caudate nucleus dopamine release (maximal effect 148% of basal release) whereas ACTH-(7–16)-NH_2 did neither induce grooming behavior nor cause an increase in caudate nucleus dopamine release. Single-dose tolerance was observed for ACTH-induced grooming but not for ACTH-induced dopamine release. These data are in support of the proposed involvement of brain dopamine systems in grooming behavior of the rat but at the same time suggest that the effect of ACTH/MSH-like peptides on dopaminergic transmission in the caudate nucleus is proximal to the final neural pathway involved in ACTH-induced grooming behavior.

The primary function of grooming behavior of the rat is concerned with the care of the body surface. However, Spruijt et al.[15] recently reviewed the evidence that the display of this behavior is often more related to the nature of the situation in which it occurs than to the condition of the fur. Thus, exposure to a novel environment or other stressors can elicit grooming behavior in the rat. It appears that in the rat grooming behavior coincides with the period after arousal and rather reflects the process of de-arousal due to the termination or habituation to a stressful situation than with enhanced fear[15]. It has long been recognized that after i.c.v. application of adrenocorticotropic hormone-(1–24) (ACTH-(1–24)) and its congeners excessive grooming behavior is observed which is phenotypically identical with that observed during de-arousal[7]. A series of experiments has been undertaken to identify the neural substrate underlying ACTH-induced excessive grooming. Local administration of ACTH-(1–24) into the substantia nigra, the periaquaductal gray or the paraventricular nucleus has been found to induce excessive grooming[12,17]. These sites are extensively innervated by ACTH/MSH fiber projections originating in the arcuate nucleus (for a review, see ref. 2).

Although the exact neural substrate is as yet not delineated, results of various studies have pointed to the important involvement of the nigrostriatal dopamine system. Indeed, intra-striatal pharmacological intervention at the level of dopamine D_2 receptors has been found to attenuate i.c.v. ACTH-induced excessive grooming (for a review, see ref. 15). We therefore decided, by monitoring the extracellular dopamine concentration in the caudate nucleus via transversal microdialysis during ACTH-induced grooming, to investigate whether the incidence of grooming behavior is associated with enhanced dopamine release in the caudate nucleus.

The preparation and implantation of the dialysis probe for transversal microdialysis was carried out ac-

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cording to Imperato and Di Chiara[8], with slight modifications[10]. Briefly, male Wistar rats (~250 g) were anesthetized with a 5% solution of chloral hydrate (400 mg/kg) and were fixed in a stereotaxic instrument (tooth bar set at −2.5 mm). The skull was exposed and holes were drilled in the temporal bones. The dialysis probe was implanted transversally through the caudate nucleus (coordinates 0.5 mm anterior and 5.5 mm ventral of the bregma). The dialysis device was fixed to the skull with dental cement. For i.c.v. injections, a plastic cannula was implanted into the foramen interventriculare according to Brakkee et al.[1]. After the operation, the rats were then housed individually in small cages and received water and food ad libitum.

One day after the operation, the inlet of the microdialysis probe was connected to a piece of flexible polyethylene tubing, which was attached to a syringe filled with Ringer solution (mM: NaCl 147, KCl 4 and $CaCl_2$ 2.3; pH 6), which was then placed in a CMA/100 microinjection pump (Carnegie Medicin, Stockholm, Sweden). The flow rate was set at 2 μl/min. The outlet of the probe was attached to a 35-cm piece of tubing allowing sampling from outside the cage. Samples were collected every 15 min in microvials containing 20 μl 0.025 mM acetic acid. During these 15-min periods, the time was measured during which a rat displayed elements of its grooming repertoir (see Ref. 7), providing a cumulative duration score per 15 min per rat. The concentration in the dialysates of dopamine and 3,4-dihydroxyphenylacetic acid (DOPAC) was measured using a HPLC apparatus (Hewlett Packard 1081 B) equipped with an electrochemical detector (Antec Instruments, Leiden, The Netherlands). The detection limit of dopamine was ~10 fmol/sample (signal-to-noise ratio of 3). Four 15-min fractions were collected before the first i.c.v. injection. The dopamine and DOPAC content of these four fractions was averaged to calculate basal release. The content of all fractions was expressed as percentage of basal release. For statistical analysis of the effects of saline and

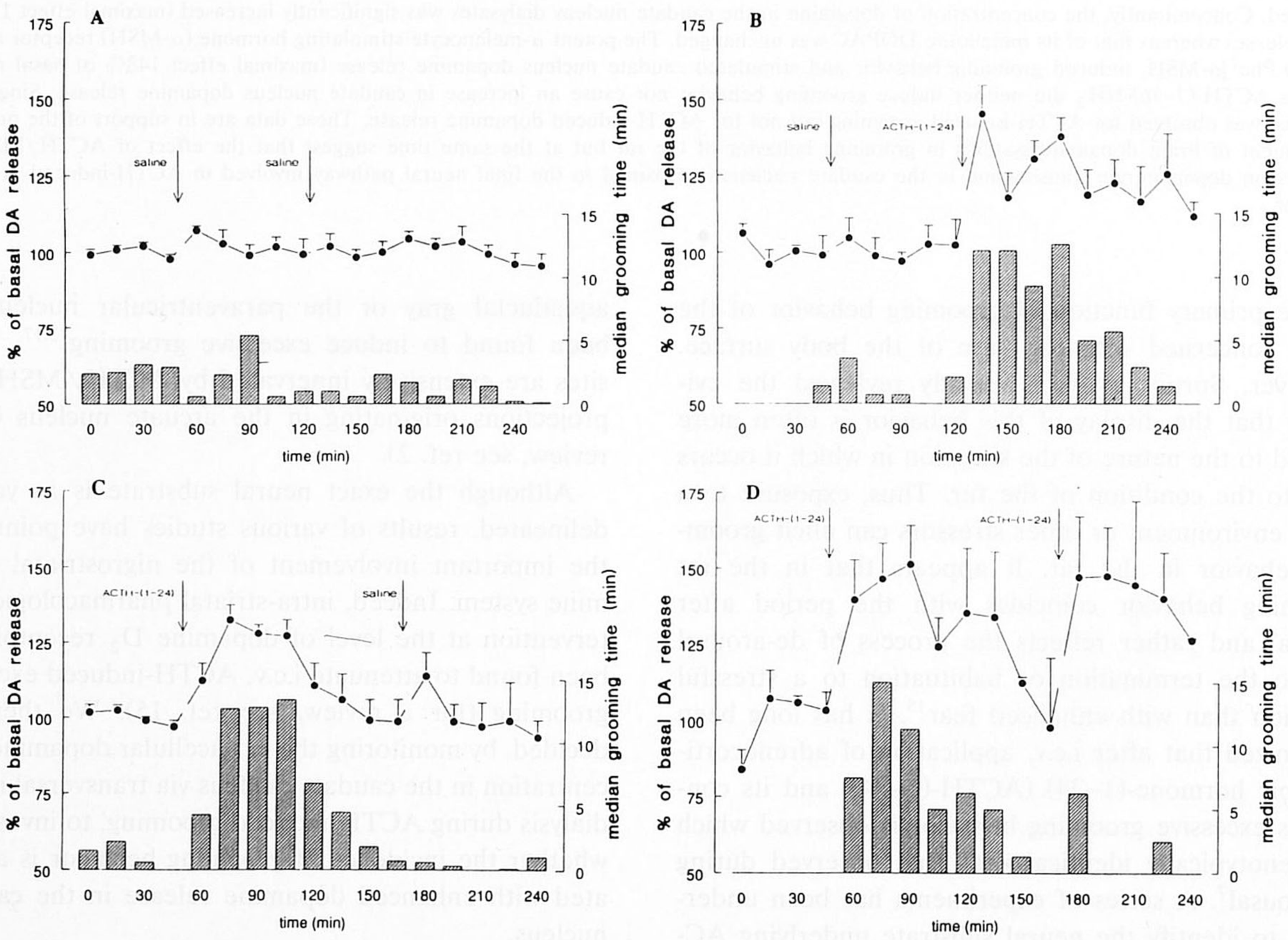

Fig. 1: effects of i.c.v. injection of saline (3 μl) or ACTH-(1–24) (1 μl in 3 μl saline) on dopamine concentrations in trans-striatal dialysates and on grooming behavior. Each curve consists of mean values ± S.E.M. ($n = 4–6$) of dopamine concentrations in 15-min fractions expressed as percentage of basal values (average of dopamine content in four fractions collected before first injection). Bars represent average time rats were displaying grooming behavior during 15-min sampling periods. Significant differences ($P < 0.05$) were found between saline-saline treated group and ACTH-(1–24)-saline and saline-ACTH treated groups (ANOVA, followed by Tukey B-tests). Within one group, treatment with ACTH-(1–24) significantly ($P < 0.05$, paired t-tests) enhanced dopamine release as compared with previous (B) or subsequent saline (C) treatment but not after previous ACTH-(1–24) treatment (D).

peptide injections, the values of the four 15-min fractions after an injection were averaged; subsequently, the difference between the average dopamine and DOPAC content in the dialysates after the first and second injection was used for statistical analysis. A similar procedure was used to compare grooming scores. Differences between groups were assessed using one-way ANOVA followed by Tukey B-tests. Differences between the effects after i.c.v. injections within one group were evaluated using paired Student's *t*-tests. Synthetic ACTH-(1–24), [Nle^4,D-Phe^7]α-MSH and ACTH-(7–16)-NH_2 (generous gifts from J. van Nispen, Organon International) or saline were administered i.c.v. in a volume of 3 μl with a 10-μl Hamilton microsyringe.

After i.c.v. injection of saline little, if any, grooming behavior was observed whereas no significant changes were detected in the dopamine concentration in the caudate nucleus dialysates (Fig. 1A,B). In contrast, i.c.v. administration of 1 μg ACTH-(1–24) resulted in the expected enhanced display of grooming behavior in ACTH-naive rats (Fig. 1B–D)[6]. Although no detailed grooming pattern analysis was performed, the grooming bouts observed featured the normal cephalo-caudal direction of grooming, indicating that the constraint brought about by the implantation of the dialysis probe had no major influence on the execution of the behavioral response (see also Spruijt and Gispen[11]). Parallel with this increased grooming activity, there was a significant increase in the amount of dopamine (Fig. 1B–D) but not of DOPAC ($100.1 \pm 0.8\%$ of basal release ($n = 19$)) in the caudate nucleus dialysates. These data are in line with results of previous studies with classical neuropharmacological intervention techniques (see Spruijt et al.[15]), which suggest that dopamine systems are activated during peptide-induced excessive grooming.

Results of structure–activity studies support the notion that a MSH receptor is involved in the induction of grooming behavior (Spruijt et al.[12]). Indeed, i.c.v. administration of 1 μg of the potent α-MSH receptor agonist, [Nle^4,D-Phe^7]α-MSH, induced a marked display of grooming behavior as well as an elevation of caudate nucleus dopamine release, which reached a maximum of $148.2 \pm 10.0\%$ of basal level 30 min after peptide injection. Interestingly, the peptide fragment ACTH-(7–16)-NH_2, like ACTH-(1–24) an inhibitor of the in vitro binding of dopamine D_2 receptor ligands to the dopamine D_2 receptor[3,4], did neither induce grooming behavior[6] nor cause an enhanced release of dopamine above basal levels ($105.3 \pm 3.3\%$ of basal release ($n = 4$), at a dose of 1 μg). An obvious explanation for the absence of an effect of ACTH-(7–16)-NH_2, and of ACTH-(1–24) and [Nle^4,D-Phe^7]α-MSH for that matter, via a mechanism involving the release-modulating dopamine D_2 autoreceptor is that the intracerebral concentration of the peptides is too low to result in an appreciable displacement of endogenous dopamine from this receptor[3,4]. In fact, results of several studies have pointed to the significance of dopamine D_1 receptor activation in peptide-induced excessive grooming[5,15,16].

An intriguing feature of ACTH/MSH-induced excessive grooming is the occurrence of single-dose tolerance[9]: a second i.c.v. peptide injection given within a period of 2–16 h after the first injection is not or less effective in inducing excessive grooming. Neuropharmacological analysis of this phenomenon revealed that the reduced responsiveness was only observed when the first injection with the peptide did effectively interact with the neural substrate underlying grooming and, thus, led to the display of grooming behavior. If the induction of grooming after the first peptide injection was blocked by the simultaneous administration of naloxone, a second injection of the peptide, 16 h later, resulted in a normal display of grooming behavior as if the first administration had not taken place. Based on these and other results[9], it was concluded that a change in the responsiveness of a component of the neural substrate underlying grooming induced by the first exposure to the peptide leads to a reduced efficacy of the second exposure. It appeared that the crucial substrate component in this matter is close to the final efferent motor pathway executing the grooming repertoir[9,15]. Under the present experimental conditions, a second injection of 1 μg ACTH-(1–24), 2 h after the first injection, was hardly effective in inducing grooming activity. However, the effect of ACTH-(1–24) on the release of caudate nucleus dopamine after the second injection was of the same magnitude as that after the first injection (Fig. 1D). In each of the combinations studied, the i.c.v. injection of ACTH-(1–24) resulted in an increase in caudate nucleus dopamine release whereas the induction of grooming behavior was only observed after the first time that the neural substrate had responded to the peptide (Fig. 1D). Results of extensive neuropharmacological experiments and of lesion studies have revealed part of the neural substrate underlying ACTH-induced grooming. Peptide-induced grooming behavior is paralleled by changes in the activity of nigro-striatal and nigro-accumbens dopamine systems, a nigro-superior colliculus GABA-ergic system and a colliculus-periaquaductal grey opioid-containing system[14]. The present data are in line with the results of previous behavioral studies which suggested that the dopamine system is not in-

volved in the behavioral tolerance to a second injection of ACTH[13]. In fact, the present data provide the first biochemical evidence for the continuing responsiveness of the nigro-striatal dopamine system to i.c.v. ACTH administration. It remains to be elucidated where the MSH-like peptide receptor which is responsible for the activation of nigrostriatal neurons are localised. It seems unlikely that this receptor is located on the terminals of these neurons, since an intra-striatal injection of ACTH or α-MSH does not induce grooming[7].

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