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Dopamine Involvement in ACTH-Induced Grooming Behavior

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GUILD, A. L. AND A. J. DUNN. *Dopamine involvement in ACTH-induced grooming behavior.* PHARMAC. BIOCHEM. BEHAV. 17(1) 31–36, 1982.—Low doses of dopamine agonists and antagonists were tested for their effects on the excessive grooming behavior induced by intracerebroventricular (ICV) injections of $ACTH_{1-24}$. Grooming scores were significantly depressed at doses of haloperidol, metoclopramide, pimozide, and butaclamol that did not decrease locomotor activity. In fact at two doses of haloperidol (0.067 and 0.10 mg/kg), grooming scores were decreased while locomotor activity was increased significantly. Metoclopramide increased grooming scores at a dose reported to block presynaptic dopamine receptors. Apomorphine potentiated the grooming induced by low doses of ACTH. These data support the hypothesis that dopaminergic neurotransmission is necessary for the display of ACTH-induced grooming behavior.

ACTH Dopamine Excessive grooming Apomorphine Haloperidol Metoclopramide Pimozide Butaclamol

GROOMING behavior may serve to clean the fur [33], promote [26] or prevent [37] heat loss, distribute pheromones from facial glands [36] or occupy time in conflict situations as a displacement behavior [28]. Increased grooming may be elicited by some moderately stressful procedures such as shaking and coating with charcoal [33], transport and placement in a novel environment [6,9], or immersion in water ([8], for a review see [20]).

Small amounts (0.2–2.0 μg) of $ACTH_{1-24}$ injected intracerebroventricularly (ICV) induce excessive grooming behavior in rats [21] and mice [32], as do β-endorphin [24] and morphine [3,40] but not the enkephalins [24,40]. These substances may act at high-affinity opiate receptors, since the opiate antagonists, naloxone and naloxazone, block ACTH- and β-endorphin-induced grooming [17, 22, 24]; and the potency of ACTH fragments in displacing [^{3}H]dihydromorphine from rat brain opiate receptors roughly parallels their potency in eliciting grooming behavior [23].

Many lines of evidence suggest that dopamine (DA) systems may be involved in the production of grooming behavior. Rohte and Muntzing [34] found that reserpine-treated mice (0.6 mg/kg) removed less charcoal dust from their fur than controls, but while the treatment depleted brain DA by 86%, it also depleted norepinephrine (NE) and serotonin by 73% and 71%, respectively. α-Methyl-p-tyrosine inhibited morphine-induced grooming, tyrosine hydroxylase, and the formation of all catecholamines [3]. 6-Hydroxydopamine (ICV) reduced naturally occurring self-grooming in macaques [31], and charcoal dust removal in mice [34], but in both studies a high dose was used without drug pretreatment to protect noradrenergic or serotonergic systems. In the latter study [34], NE was depleted more than DA.

Haloperidol reduced stress-induced grooming [8,33] but doses used were above 0.5 mg/kg, high enough to affect noradrenergic, serotonergic, and other systems. ACTH-induced grooming is also suppressed by haloperidol [39]. In addition, injection of 0.2 μg ACTH into the substantia nigra induced excessive grooming behavior [39].

DA antagonists can cause general behavioral depression. DA and dopaminergic brain systems are necessary for the initiation of most motor activities (see [27]); thus activity in DA systems may be a prerequisite for the display of grooming behavior only because grooming is a motor behavior. The doses of spiramide and pimozide used by Ayhan and Randrup [3] to suppress morphine-induced grooming suppressed the rats' locomotion and rearing in the open-field to an even greater extent. Most other experimenters did not report motor behavior. Wiegant *et al.* [39] stated that haloperidol or fluphenazine treatments "seemed not to affect" ongoing activity, but did not present relevant data.

METHOD

Subjects

Male CD-1 mice (Charles River, 20–35 g) were housed individually (Experiments 1–5, 7) or in groups of 2–4 (Experiment 6, 8–10) with free access to food and water, except during the behavioral observation and catalepsy test periods. Lights were on in the mouse colony room from 7:00 a.m. to

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7:00 p.m.; experiments were carried out between 9:00 a.m. and 4:00 p.m.

Drugs

Haloperidol (McNeil Laboratories) was dissolved in 1–2 drops of glacial acetic acid, diluted with 0.9% saline and the pH adjusted to about 5.5 with NaOH; a vehicle solution was made similarly. Pimozide (Janssen Pharmaceutica) was dissolved in 0.1 M acetic acid (pH 3.0). Butaclamol (HCl; Ayerst Laboratories) was dissolved in 0.9% saline with one drop of Tween 80. A 5.0 mg/ml metoclopramide HCl solution (Reglan Injectable, Robins) was diluted with distilled water; the 0.0 mg/kg dose was diluted in the same way from the equivalent drug-free 7.8 mM sodium metabisulfite-0.12 M saline vehicle. Apomorphine (HCl; Sigma) was dissolved in a 0.1% sodium metabisulfite-0.9% saline vehicle. $ACTH_{1-24}$ (Organon) was dissolved in 0.14 M NaCl containing 10^{-3} M HCl. Drugs were injected intraperitoneally (IP) in 5 ml/kg, immediately prior to ICV ACTH injections and 15 minutes before behavioral rating. Coded labels identified the solutions so that the experimenter was unaware of the treatment group of any subject.

Cannulation

The mouse cannulation procedure was adapted from that described by Brakkee *et al.* [7] for rats. To make the cannulae, a length of PE-50 tubing (Clay-Adams) was heated and twisted with a 27-gauge needle inserted so that a small thickened button formed transversely at the middle. The tubing at one end was trimmed and beveled to 2.5 mm, and the other end was trimmed for a total cannula length of 9.0 mm. Mice were anesthetized with Nembutal (65 mg/kg). Atropine (1 mg/kg, IP), and Combiotic, a penicillin/streptomycin combination (20 U/12.5 mg/kg IM), were also injected at this time. Cannulae were implanted bilaterally at 0.6 mm P, 1.6 mm L (estimated) from bregma. The cannulae, inserted through drilled holes (1.0 mm diameter), reached 2.5 mm below the skull surface. A 16 mm wound clip (points removed) was clamped across the skull just caudal to the cannulae, so that a portion of the button was secured beneath the clip. A headcap of dental cement was molded around the cannulae and beneath and above the wound clip to fix the cannulae to the skull. Dye injections showed good filling of the lateral cerebral ventricles with these coordinates.

A 4 to 5 day postoperative recovery period was allowed before beginning ICV injections. The mice received prophylactic injections of Combiotic daily (Experiments 1 and 2) or every other day (Experiments 3–10).

ICV ACTH Injections

Conscious mice were hand-restrained and the 10 mm 27-gauge needle of a 10 μl Unimetrics microsyringe was inserted into the 9 mm cannula. 0.5 μg (Experiments 1–9) or 0.15 μg (Experiment 10) $ACTH_{1-24}$ in 1.0 μl saline (each side) was injected into each cannula over a 5-sec period, allowing 15 sec for diffusion.

Behavioral Observation

Eight to twelve mice were observed per session in individual transparent 16×30×15 cm mouse cages. The mice were placed in the observation cages immediately after the ACTH injections; behavioral rating began 15 min later. A time-sampling method was used [5,21] to rate each mouse's behavior once each 30 seconds as either moving, quiet, grooming, or stretching/yawning behavior. Thus in the 45-minute observation period 90 scores were recorded. The experimenter was blind as to the treatment group of any mouse.

Catalepsy Test

Immediately after scoring grooming and other behaviors (60 to 75 minutes after ACTH and drug injections), catalepsy was measured as an estimate of gross motor impairment. Animals were placed with their forepaws on the edge of a wooden box 3.5 cm high and released [38]. The latency to move from this position was measured in seconds, and the median score of three trials taken for each mouse.

Procedure

Each mouse was tested with the 1.0 μg dose of ACTH at least once, 1–7 days before, and once, 7–10 days after the series of drug injections; no IP injections were given during these ACTH pretests and post-tests. In addition, the mice of Experiment 10 were given an ACTH pretest and post-test with 0.3 μg ACTH.

Repeated injections in the same animals were used to reduce variability and show that the drug effects were reversible. Two or three doses of a drug, including a zero or vehicle dose, were tested on any one group (n=7–12) of cannulated mice. The order of the doses was counterbalanced across animals in a Latin Square design, allowing 7 days between injections for drug metabolism.

Statistics

Many of the data failed Cochran's test for homogeneity of variance; therefore differences between groups were tested with the Wilcoxon matched-pair signed-ranks test [35]. This test compares scores within individual animals and is thus more sensitive than tests comparing population medians or means.

RESULTS

Saline-injected animals exhibited only low grooming scores (15%) under the testing conditions used. In no case was there any effect of order or day of treatment, nor were there any significant differences between ACTH pretest, ACTH post-test, and vehicle-plus-ACTH scores. The results of all the behavioral tests are summarized in Table 1.

Haloperidol (Experiments 1 and 2, Fig. 1)

At the highest dose tested (0.20 mg/kg), haloperidol significantly depressed both moving and grooming scores, while quiet scores were increased. However, at both 0.10 and 0.067 mg/kg grooming scores were depressed while moving scores increased. Thus at these doses there was a selective depression of grooming at the expense of locomotion, quiet scores being significantly increased only by 0.10 mg/kg. None of the behaviors scored was significantly affected by 0.033 mg/kg.

Catalepsy

Catalepsy was observed only at the 0.20 mg/kg dose of haloperidol (median latency: 12 sec). No catalepsy was ob-

TABLE 1

BEHAVIORAL SCORES FOR MICE RECEIVING ICV ACTH AND DRUGS ACTIVE ON DOPAMINE RECEPTORS

Experiment	N	Drug Treatment 1	Dose (mg/kg)	G	M	Q
1	12	Haloperidol	0.00	66	17	5
			0.10	35‡	35‡	12*
			0.20	0‡	10‡	79‡
2	11	Haloperidol	0.000	67	18	3
			0.033	65	19	4
			0.067	60‡	22†	6
3	12	Metoclopramide	0.0	68	18	0
			4.0	60‡	20	10‡
			8.0	52‡	12	23‡
4	10	Metoclopramide	0.0	66	15	2
			1.0	79*	12	0
			2.0	70	17	3
5	9	Pimozide	0.00	57	19	12
			0.10	47*	22	18
			0.15	45‡	18	26‡
6	11	Pimozide	0.000	66	20	5
			0.050	63	21	5
			0.075	58*	20	10
7	11	Butaclamol	0.0	56	15	19
			2.0	59	21	10
			4.0	60	22*	8‡
8	10	Apomorphine	0.00	60	19	13
			0.50	62	24‡	4‡
			1.00	59	28*	3‡
9	11	Apomorphine (+0.3 μg ACTH)	0.00	35	38	17
			0.50	36	49*	7†
			1.00	39*	46	6‡

$ACTH_{1-24}$ (1 μg, ICV) was injected bilaterally (0.5 μg each side) and drugs with activity on dopaminergic systems at the stated dose (IP). Fifteen minutes later behaviors were scored each 30 sec as grooming (G), moving (M) or quiet (Q) (see text).

*$p<0.05$, †$p< 0.02$, ‡$p<0.01$, Wilcoxon matched-pairs signed ranks test.

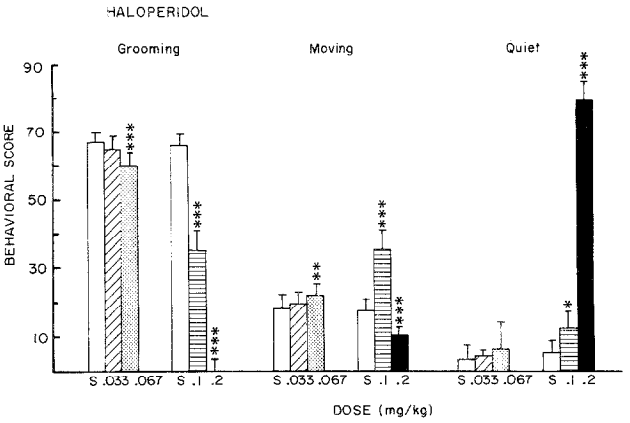

FIG. 1. Effects of haloperidol on behaviors induced by ICV ACTH. Mice were treated with the stated dose of haloperidol (IP) simultaneously with $ACTH_{1-24}$ (1 μg, ICV). Behaviors were scored from 15 to 60 min following these injections. Bars represent medians plus interquartile ranges. Experiment 1 (n=12) tested 0.1 and 0.2 mg/kg; Experiment 2 (n=11) tested 0.033 and 0.067 mg/kg haloperidol. S=saline vehicle. *Significantly different from vehicle, $p<0.05$; **$p<0.02$; ***$p<0.01$.

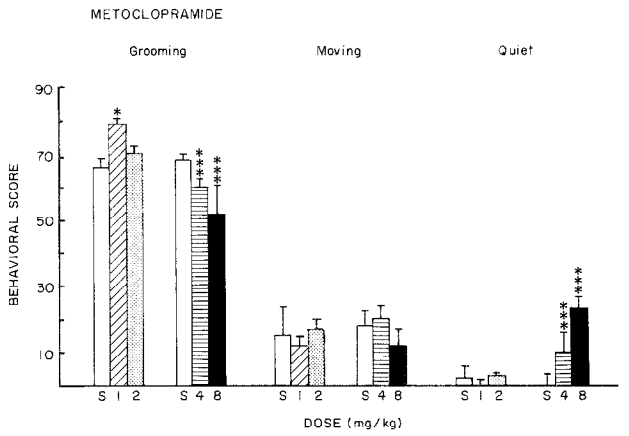

FIG. 2. Effects of metoclopramide on behaviors induced by ICV ACTH. As Fig. 1, except that metoclopramide replaced haloperidol. Experiment 3 (n=12) tested 4.0 and 8.0 mg/kg; Experiment 4 (n=10) tested 1.0 and 2.0 mg/kg metoclopramide.

served at lower doses of haloperidol or with any other drug (median latencies in all cases <1sec).

Metoclopramide (Experiment 3 and 4, Fig. 2)

At the two higher doses of metoclopramide (4.0 and 8.0 mg/kg) grooming scores were significantly decreased and quiet scores increased. However, at the lowest dose tested (1.0 mg/kg) grooming scores were significantly increased. Locomotor scores were not affected at any dose tested. Inhibition of grooming was thus observed at doses previously reported to affect postsynaptic dopaminergic receptors, whereas potentiation was observed at a dose previously reported to affect preferentially presynaptic receptors [1].

Pimozide (Experiments 5 and 6, Fig. 3)

Like haloperidol and metoclopramide, pimozide inhibited ACTH-induced grooming. The effect was significant only at the three higher doses tested (0.075, 0.10 and 0.15 mg/kg). Unlike one previous study [30], moving scores were not significantly affected, and quiet scores were elevated only by the highest dose (0.15 mg/kg).

Butaclamol (Experiment 7 and 8, Fig. 4)

In a pilot study using five mice, grooming scores were increased by both 2.0 and 4.0 mg/kg of butaclamol ($0.1>p>0.05$). In Experiment 7, no significant effects on grooming were observed, while 4.0 mg/kg butaclamol elevated moving scores and depressed quiet scores. Ten mg/kg

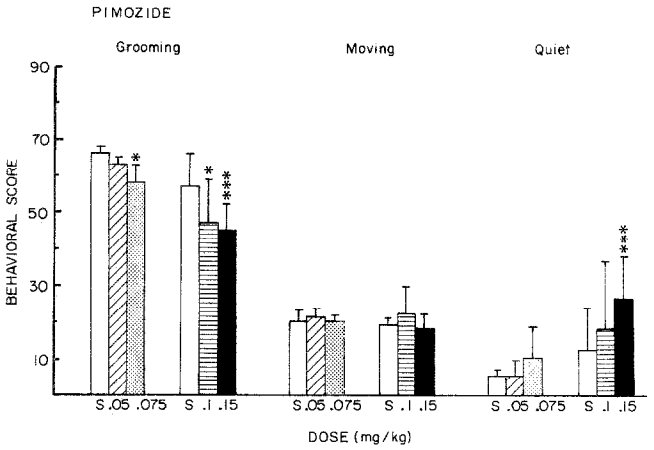

FIG. 3. Effects of pimozide on behaviors induced by ICV ACTH. As Fig. 1, except that pimozide replaced haloperidol. Experiment 5 (n=9) tested 0.10 and 0.15 mg/kg; Experiment 6 (n=11) tested 0.05 and 0.075 mg/kg pimozide.

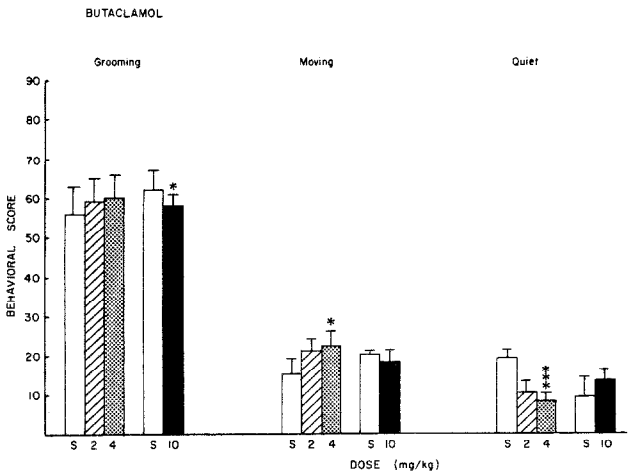

FIG. 4. Effects of butaclamol on behaviors induced by ICV ACTH. As Fig. 1, except that butaclamol replaced haloperidol. Experiment 7 (n=11) tested 2.0 and 4.0 mg/kg; Experiment 8 (n=7) tested 10.0 mg/kg butaclamol.

butaclamol decreased grooming scores in Experiment 8, while moving and quiet scores were not significantly affected (Fig. 4).

Apomorphine (Experiments 9 and 10, Fig. 5)

At doses of 0.5 and 1.0 mg/kg apomorphine stimulated moving scores and depressed quiet scores but did not affect grooming scores. Because this might have been a "ceiling effect" on high grooming scores, we also tested a lower dose of $ACTH_{1-24}$ (0.3 μg total). In this case 1.0 mg/kg apomorphine significantly elevated grooming scores, while moving scores were only elevated significantly by 0.5 mg/kg. Both doses depressed quiet scores.

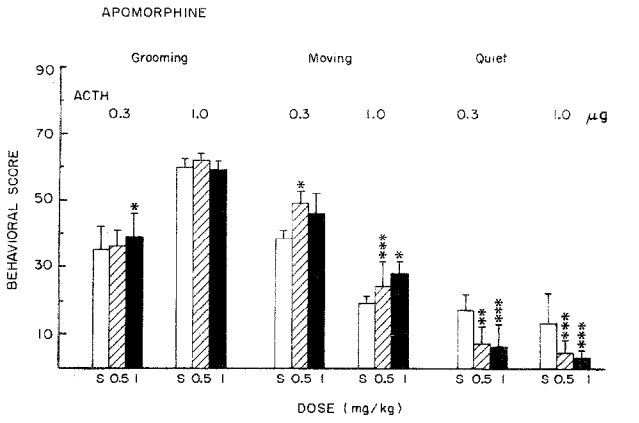

FIG. 5. Effects of apomorphine on behaviors induced by ICV ACTH. As Fig. 1, except that apomorphine replaced haloperidol. Experiment 9 (n=10) tested apomorphine at 0.5 and 1.0 mg/kg and 1 μg $ACTH_{1-24}$; Experiment 10 (n=11) used the same doses of apomorphine, but only 0.3 μg of $ACTH_{1-24}$.

Stretching and Yawning

Stretching and yawning did not occur at high enough frequency to be adequately tested with a 30-second time-sampling technique; median stretching/yawning scores ranged from 0–2 in all experiments. They were rated separately to avoid biasing moving or grooming scores. None of the drug treatments significantly altered the stretching and yawning scores, but a different set of experiments would be necessary to test a dopaminergic involvement in stretching and yawning.

DISCUSSION

The present results show that four separate dopaminergic receptor antagonists, haloperidol, metaclopramide, pimozide, and butaclamol at appropriate doses all decreased ACTH-induced grooming scores. Haloperidol, pimozide, butaclamol, and metaclopramide have been reported to show very little binding to serotonergic [29], noradrenergic [2], or muscarinic acetylcholine [19] receptors *in vitro* and *in vivo*. Four different dopamine receptor blockers are unlikely to share the same side-effects. Therefore, these data strongly suggest that their inhibitory effects on grooming are due to their dopaminergic receptor antagonist properties, and support the hypothesis of a dopaminergic involvement in grooming.

Because doses of each of the drugs exist for which grooming behavior was depressed while moving scores were unaffected, the effect on grooming does not appear to be due to a nonspecific depression of motor activity. Indeed at two doses of haloperidol (0.067 and 0.10 mg/kg), locomotor activity was significantly elevated while grooming scores were decreased. This might be explained simply by a shift from grooming to locomotor activity as also observed following chlordiazepoxide [18]. Nevertheless, the possibility must be considered that the dopaminergic involvement in ACTH-induced grooming represents a nonspecific role of DA in motor activity, but that grooming is more sensitive locomotor activity. A further possibility is that different types of populations of DA-receptor are involved (see, for example, [30]).

Additional evidence for an involvement of DA in grooming behavior was the finding that apomorphine, a DA-receptor agonist, potentiated grooming.

A problem with the use of dopaminergic agonists and antagonists is that they may act on either pre- or postsynaptic receptors, actions which should produce opposite effects. In general, the evidence is consistent with lower doses of haloperidol or metoclopramide affecting primarily presynaptic receptors, and higher doses affecting postsynaptic receptors [1,30]. (If both receptors are affected, the effects on the postsynaptic receptor will predominate). This is clearly seen by the effect of haloperidol on locomotor activity, which is inhibitory at high doses and facilitatory at low ones (Fig 1). Metoclopromide shows a similar effect on grooming scores (Fig. 2). Nevertheless, such interpretations must be treated very cautiously in the absence of direct evidence for action on the various receptors, evidence which is currently very difficult to obtain. It is not clear why we did not observe increased grooming at low doses of haloperidol or pimozide, or increased moving at low doses of metoclopramide or pimozide. Most probably it is a question of finding exactly the right dose to observe the selective effects.

Our results confirm those of Wiegant *et al.* [39] who found a barely significant ($p=0.05$) suppression of ACTH-induced grooming in their rats at 0.1 mg/kg haloperidol. Also, while the excessive grooming induced by ICV prolactin may differ from that induced by ACTH [13], it was inhibited by 0.5 mg/kg haloperidol IP [14] and with intraneostriatal but not intranigral application of haloperidol [15]. Furthermore, Green *et al.* [25] found that 0.2 mg/kg haloperidol reduced novelty-induced grooming in the rat. No depression of activity at this dose was observed in an open-field hole-board.

Which dopamine systems are necessary for the display of grooming behavior cannot be determined by the present experiments. Other studies have shown that haloperidol injected into the neostriatum was more potent in reducing ACTH-induced grooming than when it was injected into nucleus accumbens, while ergometrine was more potent in nucleus accumbens than in neostriatum [10]. This suggests that both brain regions are important for ACTH-induced grooming. Cools *et al.* [10] suggest that the drugs act at different receptors, the DA_e and DA_i receptors. Ergometrine, their DA_i antagonist, and (3,4-dihydroxyphenolamino)-2-imidazoline (DPI), a putative DA_i agonist, both reduced grooming when injected into the accumbens. However, DPI is a potent α-adrenergic agonist [4] and ergometrine may have α-adrenergic activity [11], therefore one of the receptors may be an α-adrenergic one.

Independent evidence that ICV ACTH may activate dopaminergic systems is provided by the results of Delanoy *et al.* [12]. They found that following ICV ACTH, slices of frontal cortex showed increased DA but not NE synthesis from [^{3}H]tyrosine. This effect was specific for frontal cortex, because in the striatum a decrease in DA synthesis (not statistically significant) was found. Studies using the deoxyglucose procedure have indicated a decrease in glucose uptake in pyriform cortex significantly correlated with the grooming induced by ACTH [16].

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