Intra-accumbens injections of ACTH induce excessive grooming in rats

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Rats with cannulas implanted into either the nucleus accumbens or the interventricular foramen were handled or handled and injected with 0, 20, 50, 80, or 1,000 ng of ACTH₁₋₂₄ and then were observed for 1 h afterwards in small testing chambers. Excessive grooming was displayed in animals that had received 80 and 1,000 ng into the nucleus accumbens, but only the 1,000-ng dose was effective in eliciting excessive grooming in animals with intraventricular injections. The excessive grooming found after the 80-ng dose of ACTH in the nucleus accumbens was similar in amount and time course to that observed when 1,000 ng was injected into the ventricular system. The results indicate that the nucleus accumbens is an effective site for ACTH₁₋₂₄ as it relates to excessive grooming.

Studies attempting to find sites of action of centrally applied ACTH in the induction of excessive grooming (see Gispen & Isaacson, 1981) have primarily involved the use of experimental lesions in different brain regions and subsequent observations of the behavioral effects of the peptide administration. Large bilateral lesions of the hippocampus reduce ACTH-induced excessive grooming (Colbern, Isaacson, Bohus, & Gispen, 1977), as do lesions of the substantia nigra (data from Gispen, reported in Gispen & Isaacson, 1981). However, electrolytic lesions within a large number of other brain regions do not interfere with the induced response (Colbern, Isaacson, Bohus, & Gispen, 1977).

Direct pharmacological manipulations with cannulated rats can provide suggestions as to possible sites of neuropeptide action related to excessive grooming. Microinjections of ACTH into the substantia nigra produce excessive grooming (Gispen, Ormond, Ten Haaf, & de Wied, 1980), but doses of up to 3 μ g into the caudate fail to induce the response (Wiegant, Cools, & Gispen, 1977). Cools, Wiegant, and Gispen (1978) reported similar negative results after ACTH injections into the caudate or nucleus accumbens. These latter authors stated that 1 μ g of ACTH injected bilaterally into the nucleus accumbens failed to induce excessive grooming. However, the effect of ACTH in the nucleus accumbens was mentioned only in passing in the study; consequently, the exact procedures and numerical data were not reported. The main objective in the Cools et al. study was to demonstrate the effect of dopamine (DA) agonists and antagonists placed into the

nucleus accumbens and neostriatum on ACTH-induced grooming rather than study the effects of placing the neuropeptide in these structures themselves.

Our interest in the role of the nucleus accumbens was stimulated by observations that 6-hydroxydopamine (6-OH-DA) lesions of that structure also reduced ACTH-induced excessive grooming (Springer, Isaacson, Ryan, & Hannigan, in press) in a manner similar to that of DA antagonists (Isaacson, Hannigan, Brakkee, & Gispen, Note 1). In addition, there is evidence that the reduction of excessive grooming produced by hippocampal lesions is secondary to DA changes in the nucleus accumbens, since the administration of a DA agonist to that area restores excessive grooming found after exposure to handling, transport, and a novel environment (Reinstein, Hannigan, & Isaacson, 1982) or to intracerebroventricular ACTH (Hannigan, Balaz, Springer, Ryan, & Isaacson, 1982). Furthermore, the reduced levels of excessive grooming found after hippocampal lesions could be disproportionately reduced in hippocampally damaged animals by low doses of haloperidol (Isaacson & Colbern, 1981).

In a pilot study (data not reported), we found that 1 μ g/ μ l ACTH injected unilaterally into the nucleus accumbens was effective in inducing excessive grooming. The amount and pattern of excessive grooming was similar to that observed after ACTH was injected into the interventricular foramen. However, it is possible that the excessive grooming observed may have resulted from leakage of the peptide into the ventricular system along the cannula guide. Therefore, we sought to find a way to rule out this possibility. We felt that diffusion from the injection site into the ventricular system from the nucleus accumbens would not be a likely explanation if ex-

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cessive grooming was produced at lower doses after nucleus accumbens injections than after ventricular injections. Accordingly, we undertook to determine the minimally effective doses of ACTH₁₋₂₄ required for the elicitation of excessive grooming after injection into the nucleus accumbens or the interventricular foramen.

METHOD

Subjects

Twenty-four male and female Long-Evans hooded rats weighing between 250 and 400 g at the start of the experiment were used. The animals were housed in single cages with ad-lib food and water. The colony room was maintained on a 12-h-on, 12-h-off light cycle, with lights turned on at 0800 h.

Surgical Procedures

Two surgical procedures were used, depending on the intended location of the cannulas. In both procedures, the rats were deprived of food and water for 15 h prior to surgery. The animals were anesthetized by a mixture of chloral hydrate (90 mg/kg), nembutal (56 mg/kg), and atropine sulfate (.75 mg/kg). Each animal was placed in a stereotaxic instrument, the scalp was incised, and two stainless steel screws were attached to the skull. Two holes were then drilled at appropriate rostral-caudal coordinates and stainless steel cannulas were implanted bilaterally into the nucleus accumbens. The two skull screws and the cannulas were cemented into place with dental acrylic. Stainless steel wire stylets were placed into each cannula. In other animals, polyethylene cannulas were implanted into the interventricular foramen following the procedure described by Brakkee, Wiegant, and Gispen (1979).

Procedure

The apparatus used for observing the animals and the procedures other than surgical procedures were described by Colbern, Isaacson, Hannigan, and Gispen (1981). Between 1200 and 1500 h, the animals received intracranial injections using a 10- μ l syringe. The wire stylet plug in the cannula was removed, and a needle attached to the syringe was inserted into the cannula. One microliter of either physiological saline (0.9%) or ACTH₁₋₂₄ (Organon, Ltd.) dissolved in saline and made into 20-ng, 50-ng, 80-ng, or 1- μ g doses was injected unilaterally. Following the microinjections, each animal was placed in the observation chamber for 60 min. Approximately 1 to 2 min elapsed between the time of injection and the placement of the animal into the observation chamber. The 15-sec sampling technique described by Gispen, Wiegant, Greven, and de Wied (1975) was used to score the following behaviors: grooming (face washing, head and forearm shaking, body and genital licking, tail preening), rearing (body extended with both forepaws off the floor), locomotion (bodily movement of three or more paws), stretching (body extended in stretched, arched, or crouched positions), yawning, simultaneous stretching and yawning, wet-dog shakes (vigorous and rapid bodily shaking beginning with the head and progressing to the tail), and coprophagia. At the end of the session, the animals were removed from the testing chambers, placed into their home cages, and returned to the vivarium.

Eight animals with cannulas directed to the nucleus accumbens were tested on 8 consecutive days. After being brought to the holding/injection room, the animals were placed in the observation chamber after the following treatments: Day 1—direct placement into the observation chamber, with no injection; Day 2—20 ng/ μ l ACTH; Days 3 and 4—50 ng/ μ l ACTH; Days 5 and 6—80 ng/ μ l ACTH; Day 7—1 μ l saline; Day 8—1 μ g/ μ l ACTH. All injections were unilateral. Another 16 animals with cannulas directed into the interventricular foramen (ICV) were tested on 6 consecutive days with the same general procedure described above.

Treatments occurred in the following manner: Day 1—direct placement into the observation chamber, with no injection; Day 2—20 ng/ μ l ACTH; Day 3—50 ng/ μ l ACTH; Day 4—80 ng/ μ l ACTH; Day 5—1 μ l saline; Day 6—1 μ g/ μ l ACTH.

The group receiving injections into the nucleus accumbens was tested for 2 consecutive days at the same (50- and 80-ng) doses, while a single day of testing at each dose was given to the group receiving ventricular injections. The 2nd day of testing for the nucleus accumbens group was conducted to insure that satisfactory injections of ACTH₁₋₂₄ had been achieved. Occasionally during repeated injections, especially into parenchyma, a transient clogging of the implanted cannula or a temporary malfunction of the microsyringe may occur. The problem is often alleviated by 2 days' testing at the same dose (Colbern, Isaacson, Green, & Gispen, 1978). Despite the additional days of testing, only scores obtained from the first test at each dose were used in analyses of the data.

RESULTS

Histology

Following the completion of testing, the animals were anesthetized with chloroform and intracardially perfused with saline followed by a 10% Formalin solution. The brains of all animals were sectioned at 80 μ , saving every section through the region of the cannulas' tracks. All sections were stained with formol thionin. The location of the nucleus accumbens and ICV cannulas were schematically reconstructed. The animals with misplaced cannulas or extensive tissue damage were withdrawn from the analysis. Histological examination of the cannulas directed toward the nucleus accumbens indicated general placement at the dorsal nucleus accumbens, medial to the anterior commissure (see Figure 1). Five subjects had acceptable cannula placements; of the rest, two rats with intended nucleus accumbens placements had misplaced cannulas and one animal died prior to treatment. Seven of the 16 ICV animals were included in the behavioral analysis. Nine animals in the ICV group were withdrawn from the

Figure 1. Schematic representation of cannula placements in the nucleus accumbens.

analysis due to incidental cannula damage to the thalamus or caudate. Histological examination of the cannula placements in acceptable animals indicated accurate implantation into the interventricular foramen and relatively minor damage to the fornix and dorsal hippocampus.

Behavior

Analysis of the behavioral data was performed using a mixed-design analysis of variance. A priori comparisons were made using the within-group variability of the data entering into the calculations (Keppel, 1973). Since there was no difference between the ICV or nucleus accumbens groups on the two control days (no injection and saline injection), comparisons of each site of the injection group with its control group were made using the combined means of these two scores.

The total amount of grooming, the number of grooming bouts initiated (starts), and the percent of grooming episodes per interval range (0-2.5 min, 2.75-5 min, >5.25 min) for animals receiving injections of various ACTH doses in the nucleus accumbens or interventricular foramen are presented in Table 1.

Enhanced total grooming scores relative to control days were found in animals receiving 80 and 1,000 ng of ACTH₁₋₂₄ in the nucleus accumbens [$F(1,10) = 11.18$, $p < .003$, and $F(1,10) = 8.92$, $p < .007$, respectively]. Evaluated on a similar basis, in the ICV group, enhanced grooming was found only after the 1,000-ng dose [$F(1,10) = 51.29$, $p < .0001$]. In the overall statistical analysis, the effects of intracerebral injections of ACTH₁₋₂₄ depended on both the placement of injection and the dose used [$F(5,50) = 3.468$, $p < .01$]. The nucleus accumbens group exhibited a dose-dependent difference, relative to the ICV group, in the total amount of grooming, the percentage of different durations, and the time course of the grooming (Table 1 and Figure 2). In addition, the 80-ng dose yielded a greater amount of grooming in

the nucleus accumbens group [$F(1,10) = 15.67$, $p < .003$] than in the animals receiving the 80-ng ICV injection. Although the number of grooming bouts initiated (starts) was similar in both groups, the nucleus accumbens group exhibited longer duration grooming episodes than did the ICV group. A significant difference was observed for duration intervals ranging between 2.75 and 5 min [$\chi^2(3) = 8.55$, $p < .05$] and intervals greater than 5.25 min [$\chi^2(3) = 14.03$, $p < .005$]. The pattern of grooming during the 60-min session (see Figure 2) also differed between groups. Excessive grooming after the 80-ng dose in the nucleus accumbens group exhibited a temporal pattern virtually identical to the grooming after the 1,000-ng dose in ICV (and nucleus accumbens) groups. There were no differences between the 20-, 50-, or 1,000-ng doses across the two groups as measured by total grooming, percentage of grooming episodes of different durations, and grooming over time.

The nucleus accumbens group exhibited a decrease in locomotor activity relative to the ICV group after the 20-ng dose [$F(1,10) = 12.97$, $p < .005$] and after the 50-ng dose [$F(1,10) = 5.82$, $p < .04$]. Rearing totals were similar in both groups at all doses.

DISCUSSION

These results show that a low dose of ACTH placed into the nucleus accumbens induces excessive grooming that is similar in amount and time course to that observed when 1,000 ng is injected into the ventricular system. The 80-ng dose of ACTH was ineffective in inducing excessive grooming when placed in the interventricular foramen. This finding is consistent with results of Gispén et al. (1975), who found that .01- and .1- μ g ICV doses of ACTH₁₋₂₄ failed to induce excessive grooming. However, their results are at odds with more recent data of Brakkee and Gispén (reported in Isaacson, Hannigan, Brakkee, &

Table 1
The Effect of Intracerebral Injections of ACTH₁₋₂₄ on the Display of Grooming in the Rat

ACTH Dose	Grooming Total		Grooming Starts		Percent of Grooming Episodes per Interval		
	Mean	SD	Mean	SD	0-2.5 Min	2.75-5 Min	5.25 Min
Location Injected: Nucleus Accumbens							
0	38.40	18.53	12.60	4.02	.88	.12	.00
20	58.20	32.44	14.4	7.91	.92	.07	.01
50	80.4	58.77	13.8	9.36	.86	.10	.04
80	146.00	47.50	15.6	6.09	.66	.19	.15
1000	136.4	29.01	11.4	3.9	.76	.13	.11
Location Injected: Interventricular Foramen							
0	51.57	18.48	15.14	4.67	.94	.06	.00
20	52.57	18.02	17.0	6.3	.94	.04	.02
50	66.0	22.69	18.14	3.52	.94	.04	.02
80	56.43	22.84	17.71	5.62	.94	.05	.01
1000	142.8	43.15	18.0	3.02	.77	.13	.10

Note—ACTH doses are given in nanograms per microliter.

Figure 2. The effect of nucleus accumbens and intraventricular injections at varying doses of ACTH₁₋₂₄ on the amount of grooming during the 60-min session. The mean scores for the amount of grooming were calculated at 5-min intervals.

Gispén, Note 1). In this more recent report, very low doses of ACTH₁₋₂₄ were found to induce some excessive grooming. Nevertheless, the excessive grooming we found after the 80-ng nucleus accumbens injection and the failure to find it after the 80-ng ICV injection suggest that the nucleus accumbens is an ACTH-sensitive site for the excessive grooming response. In addition, doses of 50, 80, and 1,000 ng of ACTH were found to be ineffective in inducing excessive grooming when injected into the caudate (unpublished observations). This is consistent with results of similar work by Wiegant et al. (1977).

The overall amounts of excessive grooming following injections of 20 and 50 ng of ACTH into the accumbens do not differ from each other or from that found after saline administration. However, a closer examination of the time courses of grooming (Figure 2) after these ACTH doses reveals that although grooming in the first 30 min after injection (Intervals 1-6) is not changed, the 50-ng dose into the nucleus accumbens produces a significant elevation in grooming [$t(5)=6.578$, $p < .01$] during the final 30 min. This is the naloxone- and dopamine-sensitive period of excessive grooming reported by Isaacson et al. (Note 1). This suggests that although the 50-ng dose may not produce an elevation of excessive grooming measured over the entire period, it still may exert a subtle facilitation of the response.

It is impossible to reconcile our positive results with intra-accumbens injections of ACTH with previous reports that failed to find such an effect (e.g., Cools et al., 1978). Possible differences in the sites of injection could be involved, since the nucleus accumbens does not appear to be homogeneous with regard to sources of innervation (e.g., Beckstead, 1979). It is possible that unknown procedural factors may also be involved. However, the repeated-injection procedure used in this study is not likely to be influential, since animals given repeated ICV injections of ACTH₁₋₂₄ do not exhibit adaptation or tolerance when the injections are given daily (Colbern, Isaacson, Green, & Gispén, 1978). Similarly, we have found that intra-accumbens injections of ACTH₁₋₂₄ administered daily over 4 consecutive days (unpublished data) produce equivalent results on all days. In any case, positive effects are likely to be of greater value than are negative ones, provided that alternative explanations of the results are not available.

The animals that received ACTH into the nucleus accumbens exhibited extremely low levels of activity after all doses of the neuropeptide, including the 20- and 50-ng doses, which failed to elicit excessive grooming. This does not occur in the ICV group. The animals in that group had a reduction in activity only after the 1,000-ng dose. Therefore, although difference in activity between the 1,000-ng ICV injection and the 80-ng nucleus accumbens injection conceivably could be explained by a competition of grooming and activity, this would not explain the results found after the 20- and 50-ng nucleus accumbens injections.

In the overall view, the results of the present study suggest that the nucleus accumbens is an effective site of action for centrally administered ACTH. This is not to say that the nucleus accumbens is the only site or even the primary site of central action. The sensitivity of the nucleus accumbens to intracerebral ACTH and the effectiveness of intracerebral ACTH in inducing excessive grooming must be considered with regard to the entire neural network. Its role and function in this capacity is yet to be determined.

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