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Dopamine Neurotransmission in the Nucleus Accumbens may be Involved in Oxytocin-Enhanced Grooming Behavior of the Rat

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DRAGO, F., J. D. CALDWELL, C. A. PEDERSEN, G. CONTINELLA, U. SCAPAGNINI AND A. J. PRANGE, JR. *Dopamine neurotransmission in the nucleus accumbens may be involved in oxytocin-enhanced grooming behavior of the rat.* PHARMACOL BIOCHEM BEHAV 24(5) 1185–1188, 1986.—Intracerebroventricular (ICV) infusion of a low dose of oxytocin enhanced novelty-induced grooming in male rats. The present experiments were undertaken to investigate whether dopamine neurotransmission in the nucleus accumbens is involved in this effect. Bilateral lesions of the nucleus accumbens by microinjections of 6-hydroxydopamine (6-OHDA) totally prevented the enhancement of grooming behavior after subsequent ICV infusion of oxytocin. Furthermore, bilateral injections of the dopamine receptor antagonist, haloperidol, into the nucleus accumbens completely suppressed grooming behavior of rats infused ICV with oxytocin. These results suggest that dopamine neurotransmission in the nucleus accumbens is involved in the behavioral response enhanced by the peptide.

Oxytocin Grooming behavior Nucleus accumbens 6-hydroxydopamine Haloperidol Dopamine neurotransmission

IN a recent study we demonstrated that intracerebroventricular (ICV) infusion of low doses of oxytocin is followed by an enhancement of novelty-induced grooming in both male and female rats in a dose-dependent manner [5]. Oxytocin-enhanced grooming is characterized by an increased occurrence of genital grooming. Furthermore, peripheral administration of the dopamine receptor antagonist, haloperidol, or of the opiate receptor antagonist, naloxone, inhibited oxytocin-enhanced grooming.

Central dopamine neurotransmission has been implicated in grooming behavior enhanced by other peptides. In particular, the disruption of dopamine neurotransmission in the nucleus accumbens by microinjections of ergometrin produced an inhibition of ACTH-enhanced grooming in the rat [3]. Bilateral lesions of dopamine terminals in the nucleus accumbens by microinjections of 6-hydroxydopamine (6-OHDA) was followed by a suppression of prolactin enhanced grooming [7].

The present study was aimed at investigating the possible role of dopamine neurotransmission in the nucleus accumbens in oxytocin-enhanced grooming behavior of the rat. We have used two different experimental approaches for interfering with dopamine neurotransmission, i.e., the lesion of dopamine terminals by 6-OHDA or the blockade of dopamine receptors by haloperidol.

METHOD

Animals and Surgery

Male rats of Wistar strain (purchased from Charles River Breeders), weighing 180–190 g, were used. The animals were housed 4 per cage and kept at room temperature (21°C) under a constant light-dark cycle (lights on between 8.00 and 20.00). Seven days prior to the experimental session, all animals were subjected to the surgical manipulation. Rats were anaesthetized with sodium pentobarbital (Nembutal, Abbott, Switzerland, 40 mg/kg) and then fixed in a stereotaxic frame. A plastic cannula was inserted into the

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TABLE 1

EFFECTS OF BILATERAL LESIONS OF THE NUCLEUS ACCUMBENS BY 6-HYDROXYDOPAMINE (6-OHDA) ON OXYTOCIN-ENHANCED GROOMING IN THE RAT

Treatment Groups	(n)	Grooming Score
vehicle + ICV saline	(12)	15.3 ± 2.7
vehicle + ICV oxytocin	(12)	84.2 ± 7.6*
6-OHDA + ICV saline	(12)	14.9 ± 2.5
6-OHDA + ICV oxytocin	(12)	19.0 ± 2.8

Values are mean ± SEM. Injections of 6-OHDA (3 μg/1 μl) or its vehicle (1 μl) into the nucleus accumbens were performed 5 days prior to behavioral testing. Grooming observation was started 5 min after ICV infusions of oxytocin (3 μg/5 μl) or saline (5 μl). In parentheses the number of animals per each treatment group. *Significantly different as compared to control group injected into the nucleus accumbens with the vehicle and ICV with saline ($p<0.01$, Dunnett's test for multiple comparisons).

TABLE 2

EFFECTS OF BILATERAL MICROINJECTIONS OF HALOPERIDOL INTO THE NUCLEUS ACCUMBENS ON OXYTOCIN-ENHANCED GROOMING IN THE RAT

Treatment Groups	(n)	Grooming Score
vehicle + ICV saline	(12)	13.5 ± 2.8
vehicle + ICV oxytocin	(12)	83.8 ± 8.2*
haloperidol + ICV saline	(12)	15.8 ± 2.6
haloperidol + ICV oxytocin	(12)	17.8 ± 2.6

Values are mean ± SEM. Injections of haloperidol (1 μg/1 μl) or its vehicle (1 μl) into the nucleus accumbens were performed 30 min before ICV infusions of oxytocin (3 μl/5 μl) or saline (5 μl). Grooming observation was started 5 min after ICV infusions. In parentheses the number of animals per each treatment group. *Significantly different as compared to the control group injected into the nucleus accumbens with the vehicle and ICV with saline ($p<0.01$, Dunnett's test for multiple comparisons).

lateral ventricle (foramen interventriculare, Konig and Klippel A6360) according to the procedure described by Brakkee *et al.* [2]. A stainless steel guide cannula was also implanted bilaterally into the nucleus accumbens (Konig and Klippel coordinates A=9.4, L=1.2, D=0.6) according to the procedure described by Cools *et al.* [3]. The cannulae were cemented to the skull by dental acrylic cement. The animals were kept single-caged and allowed at least seven days to recover from surgery before behavioral testing.

After the behavioral experiments were completed the rats were sacrificed and the brains were fixed in a 10% formaldehyde solution. Histological sections of 300 μm were made and the localization of the stainless steel cannulae was controlled microscopically, followed by microphotography of the injected area.

Only data from animals with accurate localization of cannulae were considered.

Drugs

A highly purified synthetic preparation of oxytocin was kindly provided by Dr. Victor Hruby (Department of Chemistry, University of Arizona). Peptide solutions were freshly prepared before administration by dissolving it in a drop of acetic acid 0.02 M and adjusting the solution volume with 0.9% saline. The pH of the final solution was 7.0. The control solution was composed of saline with a drop of acetic acid (pH 7.0). 6-OHDA (Merck, USA) was dissolved in saline and ascorbic acid was added in equimolar concentration to avoid rapid oxidation of the drug. The pH of the final solution was 6.4. The control solution was composed of saline and ascorbic acid (pH 6.4). A commercial preparation of haloperidol hydrochloride dissolved in water (Serenase, Lusofarmaco, Italy) with pH 5.6, was also used. The control solution was composed of saline and the pH was adjusted at 5.6 by acetic acid 0.02 M.

Behavioral Tests

Grooming activity was scored as described by Gispen *et al.* [9]. Briefly, the rats were placed individually into transparent plastic cages (24×12×24 cm) in a low-noise room. The behavior of each rat was observed every 15 sec beginning immediately after the animals were placed into the boxes. The occurrence of the following activities was recorded: vibration of the forepaws, face washing, body grooming, scratching, paw licking, head shaking and genital grooming [8]. Behavioral observations lasted 30 min, with a maximum possible grooming score of 120. All experiments were performed blind to treatment between 9.00 and 14.00.

Spontaneous motor behavior was observed in a circular open field as described by Weijnen and Slangen [19]. Ambulation (number of floor units entered), rearing and defecation scores were recorded during a 3-min observation session.

Experimental Procedure

In the first experiment, we studied the effects of bilateral 6-OHDA-induced lesions of the nucleus accumbens on oxytocin-enhanced grooming. Some rats were injected bilaterally with 6-OHDA (3 μg/1 μl) into the nucleus accumbens, while other rats received similar microinjections of the vehicle alone (1 μl). Five days later, oxytocin (3 μg/5 μl) or its vehicle (5 μl) were infused ICV into the animals. Grooming observation started 5 min after oxytocin or saline infusion.

The second experiment was designed to study the effects of bilateral microinjections of haloperidol into the nucleus accumbens on oxytocin-enhanced grooming. Haloperidol (1 μg/1 μl) or its vehicle (1 μl) were injected bilaterally into the nucleus accumbens of freely-moving rats. Thirty min later, some rats received an ICV infusion of oxytocin (3 μg/5 μl), while other rats were given a similar ICV infusion of the vehicle alone (5 μl). After 5 min, grooming observation was initiated.

Because of the extra-pyramidal effects of haloperidol, we also intended to evaluate whether the influence of microinjections of this drug in the nucleus accumbens on oxytocin-enhanced grooming was specific to this behavioral response or dependent on an overall motor action. Haloperidol (1 μg/1

TABLE 3

EFFECTS OF BILATERAL MICROINJECTIONS OF HALOPERIDOL INTO THE NUCLEUS ACCUMBENS ON OPEN FIELD BEHAVIOR IN OXYTOCIN- AND VEHICLE-INJECTED RATS

Treatment Groups	(n)	Ambulation	Rearing	Defecation
vehicle + ICV saline	(10)	82.2 ± 7.8	12.6 ± 1.8	4.7 ± 1.8
vehicle + ICV oxytocin	(10)	96.8 ± 9.2	15.6 ± 2.4	3.5 ± 1.2
haloperidol + ICV saline	(10)	78.8 ± 8.6	11.8 ± 1.4	3.9 ± 1.6
haloperidol + ICV oxytocin	(10)	77.8 ± 9.6	12.0 ± 2.0	4.0 ± 1.5

Values are mean ± SEM. Injections of haloperidol (1 μg/1 μl) or its vehicle (1 μl) into the nucleus accumbens were performed 30 min before ICV infusions of oxytocin (3 μl/5 μl) or saline (5 μl). Open field test was started 5 min after ICV infusions. In parentheses the number of animals per each treatment group.

μl) or its vehicle (1 μl) were injected bilaterally into the nucleus accumbens of freely moving rats. Thirty min later, some rats received an ICV infusion of oxytocin (3 μg/5 μl), while other rats were given a similar ICV infusion of the vehicle alone (5 μl). After 5 min, the open field test was initiated.

Statistical Analysis

Dunnett's test for multiple comparisons was used to compare grooming scores from different experimental groups. A p-value of 0.05 or less was accepted as indicative of a significant difference.

RESULTS

The histological localization of the injection cannulae revealed that points of drug deposition were included bilaterally within the nucleus accumbens and surrounding regions did not show any damage due to the injection procedure.

In animals with sham lesions of the nucleus accumbens ICV infusion of oxytocin resulted in a sustained increase in grooming activity over a 30-min observation period (Tables 1 and 2). However, animals with previous bilateral 6-OHDA-induced lesions of the nucleus accumbens did not show more grooming activity than control rats (Table 1).

Bilateral microinjections of haloperidol into the nucleus accumbens did not affect the level of grooming in rats given ICV infusion of the vehicle alone. However, haloperidol pre-treatment prevented any increase of grooming activity of rats which were given ICV infusion of oxytocin (Table 2).

The open field test revealed that ambulation and rearing appeared to be slightly (not significantly) increased in oxytocin-treated rats as compared to animals injected with the vehicle alone (Table 3). Haloperidol pre-treatment did not affect the open field behavior of rats given ICV infusion either of oxytocin or of the vehicle.

DISCUSSION

Central dopamine neurotransmission seems to be involved in peptide-enhanced grooming behavior of the rat. In particular, the dopaminergic nigro-striatal and meso-limbic systems have been implicated in ACTH- and prolactin-enhanced grooming. In fact, microinjections of the dopamine receptor antagonist, haloperidol, into the neostriatum totally suppresses ACTH- and prolactin-enhanced grooming [6,20]. Furthermore, microinjections of haloperidol or ergometrine into the nucleus accumbens [3] or chemical lesions of this nucleus [7] inhibit grooming behavior enhanced by ACTH or prolactin. Oxytocin-enhanced grooming also seems to depend upon dopamine receptor activation, as peripheral administration of haloperidol suppresses it [5]. The present results show that bilateral lesions of the nucleus accumbens by 6-OHDA lead to an inhibition of oxytocin-enhanced grooming of the rat. Also, a suppression of oxytocin-enhanced grooming was seen after bilateral microinjections of haloperidol into the nucleus accumbens. No change of spontaneous motor behavior was observed in oxytocin-treated rats after bilateral microinjection of haloperidol in the nucleus accumbens, suggesting that the inhibiting effect of this drug on oxytocin-enhanced grooming is specific to this behavioral change and is not the result of whole motor activity being depressed. These data suggest that dopamine neurotransmission in the nucleus accumbens is involved in the behavioral effect of oxytocin. However, the possibility cannot be ruled out that other neurotransmitters, e.g., noradrenaline and serotonin, are also involved. In fact, 6-OHDA may lead to a degeneration of noradrenergic nerve terminals [17] and haloperidol can interfere with noradrenergic [1] as well as with serotonergic receptors [12]. Since the nucleus accumbens is very rich in noradrenergic fibers [11], a reasonable argument could be made that oxytocin-enhanced grooming may also involve noradrenergic neurotransmission in this area.

Dopamine neurotransmission in the nucleus accumbens appears to play a somewhat different role in the mediation of grooming behavior induced by different neuropeptides. Microinjections of haloperidol into the nucleus accumbens attenuates but not completely suppresses ACTH-enhanced grooming, while microinjections of ergometrine were followed by a total inhibition of this behavior [3]. In another study, microinjections into the nucleus accumbens of des-tyrosine-γ-endorphin, a non-opiate analogue of γ-endorphin which is believed to possess neuroleptic-like activity, were followed by a total suppression of ACTH-enhanced grooming [10]. The different responses resulting from the pharmacological manipulation of the nucleus accumbens suggest that this nucleus plays only a modulatory role in ACTH-enhanced grooming [8]. In contrast, microinjections of 6-OHDA or haloperidol completely abolish oxytocin- and prolactin-enhanced grooming [7], which suggest that dopamine neurotransmission in the nucleus accumbens is essential to the mediation of these behavioral effects.

Bilateral microinjections of ACTH into the nucleus accumbens fail to enhance grooming behavior in rats, while local application of the peptide into the substantia nigra induced the behavioral response [8]. This suggests that ACTH may act at the level of cell bodies rather than on the termi-

nals of the dopaminergic meso-limbic system. The present results do not allow any conclusions about the site of action of oxytocin in enhancing grooming behavior. However, oxytocin has been reported to affect central neurotransmission, namely monoamine neurotransmission [18]. Both dopamine and noradrenaline turnover rates are influenced by oxytocin in various brain areas [16]. Though the significance of these changes is not well understood, it is possible that these neurochemical modifications are involved in oxytocin enhanced grooming behavior. Also, it is not known whether oxytocin can act on specific receptors located in the nucleus accumbens. It is worth mentioning in this respect that binding sites for oxytocin have recently been described in limbic-midbrain regions of the rat [4].

It is of interest to note that another behavior elicited by oxytocin, i.e., maternal behavior [13,14], seems to involve the nucleus accumbens. In fact, lesions of the posterior and dorsal part of this nucleus resulted in reduction and impairment of maternal behavior in female rats [15]. Thus, the nucleus accumbens may be a site of action of behavioral changes induced by oxytocin.

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