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BRIEF COMMUNICATION

Potentiation of Spiperone-Induced Oral Activity in Rats After Neonatal 6-Hydroxydopamine

RICHARD M. KOSTRZEWA[1] AND ANWAR HAMDI

Department of Pharmacology, James H. Quillen College of Medicine
East Tennessee State University, Johnson City, TN 37614

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KOSTRZEWA, R. M. AND A. HAMDI. *Potentiation of spiperone-induced oral activity in rats after neonatal 6-hydroxydopamine.* PHARMACOL BIOCHEM BEHAV **38**(1) 215–218, 1991.—The influence of central dopaminergic fibers on drug-induced oral activity in rats has not been well studied. Rats were treated 3 days after birth with bilateral intracerebroventricular 6-hydroxydopamine (6-OHDA; 134 μg total, base form) to destroy dopaminergic fibers in the brain. Control rats received vehicle by the same route. At about 10 weeks of age, a challenge dose of the dopamine D2 receptor antagonist, spiperone (40 μg/kg, IP), produced an 8-fold increase in the number of oral movements during a 60-minute observation period, vs. the control group. SKF 38393 (3.0 mg/kg, IP), a D1 agonist, produced the same number of oral movements as spiperone in the 6-OHDA group, representing a 2.4-fold increase over the controls. The B_{max} and K_d for both D1 and D2 receptors was not changed in rat striatum by neonatal 6-OHDA treatment, even though dopamine content was reduced by 96%. These findings demonstrate that oral activity in rats can be greatly altered, even when there is no change in absolute numbers of D1 and D2 receptors and no change in the ratio of D1:D2 receptors.

Oral dyskinesia Dopamine D1 receptor D2 receptor 6-Hydroxydopamine Spiperone SKF 38393

NEUROLEPTIC drugs induce spontaneous oral activity in rats (6, 9, 26). Also, when neuroleptic-treated rats are challenged with apomorphine, a dopamine receptor agonist, there is an increase in the number of oral movements (25). Acute treatment of intact rats with a dopamine D2 receptor antagonist or D1 receptor agonist will likewise result in the induction of oral activity. The balance between D1 and D2 receptor responsivity has been suggested as an important factor in this event (20). This hypothesis is supported by the fact that there is a greater incidence of oral activity in rats when there is a functional or overt increase in the D1:D2 receptor ratio, as occurs (a) after treatment with a D2 receptor antagonist (1, 11, 20), (b) after treatment with a D1 receptor agonist (13–15, 20), (c) after prenatal neuroleptic treatment, to reduce D2 receptor number, (d) in certain strains of rats that have altered numbers of D2 receptors (22), or (e) as a consequence to aging (14,21). A reduction in oral activity following inactivation of D1 receptors in rats is compatible with this view (22).

The above associations between D1 and D2 receptors were obtained from rats that were not restrained. However, restraining of rats does not appear to alter the relationship between D1 and D2 receptor involvement in the genesis of oral activity in rats (10,12).

In the above studies the central dopaminergic system was intact. To assess whether dopaminergic fibers might have a role in the induction of oral activity by D1 agonists or D2 antagonists, we treated rats neonatally with the neurotoxin, 6-hydroxydopamine (6-OHDA), in order to destroy dopamine-containing neurons in the brain. The findings indicate that the incidence of oral activity can be greatly increased, even when the ratio of D1 to D2 receptors has not been changed.

METHOD

Subjects

Timed pregnant Sprague-Dawley albino rats were obtained from Charles River Laboratories (Research Triangle, NC). Animals were housed at 22 ± 1°C, under a 12-h–12-h light-dark cycle (on at 0700 h) and were allowed free access to food and

[1]Request for reprints should be addressed to Dr. Richard M. Kostrzewa, Department of Pharmacology, James H. Quillen College of Medicine, East Tennessee State University, P.O. Box 19810A, Johnson City, TN 37614.

water. At birth, litters were reassigned, so that each dam had rats from several litters. Different treatments were included with each reconstituted litter.

Treatment

At 3 days after birth, rats received a bilateral intracerebroventricular (ICV) injection of 6-OHDA HBr (66.7 μg, free base, on each side; Sigma Chemical Co., St. Louis, MO) or the vehicle, saline-ascorbic acid (0.1%). Rats were weaned at 28 days and were then group housed by sex in wire cages.

Behavioral Observation

At 10 or 11 weeks after birth, between 1000 and 1600 h, each rat was given, respectively, a single intraperitoneal (IP) challenge dose of vehicle, spiperone (40 μg/kg; Research Biochemicals Inc., Natick, MA) or (±)SKF 38393 HCl [1-phenyl-2,3,4,5-tetrahydro-(1H)-3-benzazepine-7,8-diol hydrochloride]; 3.0 mg/kg, salt form; Smith Kline & French Laboratories, Philadelphia, PA). Rats were then placed in clear plastic cages (48 × 26 × 18 cm) in a quiet, well-ventilated and well-lighted room, and were observed one at a time. Numbers of rapid jaw movements were determined for one minute every ten minutes over a 60-minute period, beginning 60 minutes after spiperone or 10 minutes after SKF 38393. Because of markings on the rats the observer was aware of the treatment group of each rat during this test session.

Assessment of Dopamine D1 and D2 Receptor Binding

Rats were decapitated at 12 weeks after birth. Brains were removed and the striata were dissected free, and frozen on dry ice. Tissues were stored at −60°C. Striata were assessed for dopamine receptor binding activity, using the method of Schulz et al. (23) for D1 receptors and the method of Creese and Snyder (7) for D2 receptors. Briefly, striata were homogenized with a Teflon on glass mortar and pestle, in 100 volumes of 50 mM Tris buffer (pH 7.4). This procedure was used to avoid destruction of D1 and D2 receptors during this tissue preparation state (17). Homogenates were then centrifuged at 35,100 × g for 10 min at 4°C using a Beckman J2-21M centrifuge and JA-20.1 rotor. After one wash and a second centrifugation, tissue pellets were resuspended in 200 volumes of fresh buffer.

For dopamine D1 receptor binding, aliquots (0.4 ml) of homogenates were added to [^{3}H]SCH 23390 (Amersham, Arlington Heights, IL) in Tris buffer containing 120 mM NaCl, 5 mM KCl and 2 mM $CaCl_2$. Eight different concentrations of ligand were used (50 to 2500 pM, final conc.). Samples (2.5 ml incubation mix) were incubated for 40 min at 37°C in a shaking water bath, and then were rapidly filtered under partial vacuum on Whatman GF/F glass fiber filters using a Millipore filtration unit. Filters were washed 3 times with ice-cold Tris-salt solution. After drying, filters were placed in 9 ml of fluor, and tritium activity was determined in a Beckman LS 9800 liquid scintillation spectrometer. A GraphPAD program (ISI Software, Philadelphia, PA) using a double rectangular hyperbola equation calculated the B_{max} and K_d values for each sample.

For dopamine D2 receptor binding, a procedure identical to that for D1 binding was used, except that the incubate contained 1 mM $MgCl_2$ in place of $CaCl_2$ and 40 nM ketanserin to inhibit binding of the D2 radioligand, [^{3}H]spiperone, to serotonin type 2 receptors. Eight concentrations of [^{3}H]spiperone (25 to 1500 pM; Amersham) were used.

Determination of Striatal Dopamine Content

A radiometric method, employing CAT-A-KIT (Amersham), was used to determine the dopamine content of rat striatum. This kit is a modified version of the method of Passon and Peuler (19) and is sensitive to less than 100 pg of dopamine. Briefly, striata were homogenized in 0.1 M perchloric acid, and an aliquot was incubated in the presence of catechol-O-methyltransferase with [^{3}H]S-adenosyl-methionine. After several extractions, the [^{3}H]methoxytyramine product was separated from the substrate and other catecholamine products. Tritium activity was determined as above.

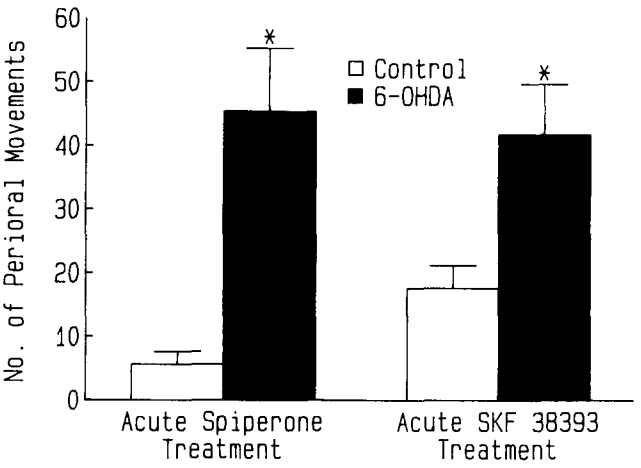

FIG. 1. Effects of neonatal 6-OHDA treatment (134 μg ICV, 3 days after birth) on spiperone- and SKF 38393-induced oral activity in rats. The numbers of oral movements were determined for one minute every ten minutes over a 60-minute period, beginning 60 minutes after spiperone or 10 minutes after SKF 38393. Rats were 10 weeks of age at the time of spiperone treatment and 11 weeks of age at the time of SKF 38393 treatment. Each group is the mean of 5 rats. *Indicates $p<0.001$, vs. the vehicle control group.

Statistics

Behavioral and biochemical data of treated and control groups were compared by an analysis of variance (ANOVA), followed by the post-ANOVA test of Newman-Keuls.

RESULTS AND DISCUSSION

When rats were treated with vehicle at 10 weeks after birth and observed for 1 hour afterwards, there was a mean incidence of 3.2 ± 1.6 and 3.8 ± 2.1 oral movements in the control and 6-OHDA groups, respectively. A challenge dose of spiperone (40 μg/kg, IP) produced a slight increase in oral activity in the control group, to 5.6 ± 2.0 oral movements (Fig. 1). However, in the group of rats that received 6-OHDA (ICV, 66.7 μg on each side) at 3 days after birth, the mean number of spiperone-induced oral movements was increased to 45.4 ± 9.9 ($p<0.001$). An acute dose of SKF 38393 (3.0 mg/kg, IP) at 11 weeks of age also increased the number of oral movements in the 6-OHDA group, to 41.8 ± 7.9. This was also different from the number of oral movements induced by SKF 38393 in the control group ($p<0.001$). In these studies the observer was aware of the treatment groups of rats because of markings on each animal. In subsequent studies, with the observer being blinded to the treatment group of each rat, a virtually identical result has been obtained.

Neonatal treatment with 6-OHDA resulted in a 96% reduction in striatal dopamine content ($p<0.001$), indicating that 6-OHDA effectively destroyed the majority of dopamine-containing fibers.

TABLE 1

EFFECT OF NEONATAL 6-OHDA TREATMENT[1] ON MEAN (±SEM) DOPAMINE CONTENT AND DOPAMINE D1 AND D2 RECEPTOR BINDING IN RAT STRIATUM[2]

		D1 Receptor Binding		D2 Receptor Binding	
Treatment	Dopamine Content (ng/g tissue)	B_{max} (pmol/μg protein)	K_d (pM)	B_{max} (pmol/μg protein)	K_d (pM)
Vehicle	8760 (1320)	1254 (49)	371 (12)	505 (43)	88 (6)
6-OHDA	348 (84)*	1203 (69)	359 (15)	517 (64)	86 (9)

[1]6-OHDA (134 μg) was administered bilaterally ICV, half in each ventricle, at 3 days after birth.

[2]Each group is the mean of 4 to 6 rats.

*Indicates $p<0.001$, compared to the 'vehicle' group.

This finding is consistent with reports by Breese et al. (2–5) and Zigmond and co-workers (16, 18, 24). The B_{max} and K_d for D1 and D2 binding in rat striatum was not changed by 6-OHDA treatment (Table 1). This finding is also compatible with studies by Breese et al. (4).

The major finding in the present report is that a D1 agonist and D2 antagonist can increase the incidence or oral movements, above that in control rats, even when D1 and D2 receptor number is not changed in the striatum. It is felt that the relationship between D1 and D2 receptors is an important determinant in the production of oral movements in rats. However, we propose that D1 and D2 receptor number per se is not so important as the sensitivity of the respective receptors. In a long series of studies, Breese and co-workers have shown that the sensitivity of neonatal 6-OHDA-lesioned rats is often increased, with the animals becoming particularly sensitive to D1 agonists [(3–5, 8); see also (27)]. The present results seem to be in conformity with the proposed supersensitization of D1 receptors, since SKF 38393, a D1 receptor agonist, produces enhanced oral responses in the rats with neonatal 6-OHDA treatment. The induction of oral activity by spiperone, a D2 receptor antagonist, is not so readily explained. However, in the presence of spiperone, synaptic dopamine from residual dopaminergic neurons, would act preferentially on D1 receptors, resulting in a greater imbalance in D1:D2 receptor activation. This mechanism would be compatible with other reports on the influence of D1:D2 ratios on the induction or oral activity in rats (20,23).

This initial finding of a greatly enhanced oral response in neonatal 6-OHDA-lesioned rats is one that should facilitate studies on the association of dopamine receptors with this behavior.

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