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Unilateral Inactivation of Dopamine Receptors After Intrastriatal Injection of N-Ethoxy-Carbonyl-2-Ethoxy-1,2-Dihydroquinoline (EEDQ): A Novel Rotational Model to Investigate Dopamine Receptor Interactions

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GIORGI, O. AND G. BIGGIO. *Unilateral inactivation of dopamine receptors after intrastriatal injection of N-ethoxy-carbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ): A novel rotational model to investigate dopamine receptor interactions.* PHARMACOL BIOCHEM BEHAV **35**(4) 877–884, 1990. —The interaction between D1 and D2 dopamine (DA) receptors was investigated in a novel rotational model. Rats were unilaterally injected into the striatum with the irreversible DA receptor blocker N-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ). This treatment induced a marked decrease in the density of D1 (−48%) and D2 (−45%) DA receptors available for binding to ^{3}H-SCH 23390 and ^{3}H-spiperone, respectively. Under these experimental conditions, the effect of DA receptor agonists were predominant on the intact side and resulted in rotations ipsilateral to the injected side. The effects of different agonists and antagonists for D1 and D2 DA receptors were evaluated 24 hr after EEDQ administration. The D2 agonist LY 171555 induced ipsilateral rotations in a dose-dependent manner (0.1–10.0 mg/kg, IP) in rats treated intrastriatally with EEDQ. In contrast, the D1 agonist SKF 38393 (1–20 mg/kg, IP) was unable to elicit circling behavior per se. However, SKF 38393 increased the number of rotations caused by LY 171555. The circling behavior induced by LY 171555 was blocked by the D2 antagonists (−)sulpiride and raclopride and by the D1 antagonist SCH 23390. Moreover, the inhibition of circling behavior induced by SCH 23390 was reversed by SKF 38393 in a dose-dependent manner. LY 171555 (1 mg/kg, IP) was unable to induce rotations in EEDQ-treated rats following DA depletion by α-methyl-p-tyrosine, whilst the combined administration of LY 171555 (1 mg/kg, IP) and SKF 38393 (10 mg/kg, IP) elicited intense circling behavior in DA depleted rats. Finally, the effect of LY 171555 plus SKF 38393 was completely antagonized by SCH 23390 (0.1 mg/kg, SC). These results indicate that the endogenous DAergic tone on D1 DA receptors plays a permissive role for the expression of the motor effects mediated via the stimulation of striatal D2 DA receptors.

D1 and D2 DA receptor interactions Circling behavior LY 171555 SKF 38393 SCH 23390 N-Ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ)

DOPAMINE (DA) receptors have been classified in two subtypes possessing unique pharmacological and biochemical properties [for reviews, see (7) and (8)]. D1 DA receptors mediate stimulation of adenylate cyclase activity, whereas D2 DA receptors mediate the inhibition of this enzyme (9, 23, 28, 29). The behavioral and motor effects of DA were previously believed to be predominantly mediated via D2 DA receptors (7,8). More recently, however, it has been shown that the stimulation of D1 DA receptors by the selective agonist SKF 38393 (27) results in forceful grooming of the flank and trunk with the snout and nonstereotyped sniffing behavior (5,20), suggesting a direct functional role of D1 DA receptors in motor control. There is also evidence that D1 DA receptor stimulation facilitates the behavioral (5,31) and electrophysiological (6,31) effects induced by selective D2 DA receptor agonists.

A similar synergistic interaction between LY 171555 and SKF 38393 has been described on the circling behavior elicited by stimulation of normosensitive DA receptors in rats with unilateral

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striatal lesions induced by quinolinic acid (4) or in rats submitted to a unilateral transection of the cerebral hemisphere (1).

In the present study, a novel rotational model was developed and used to investigate DA receptor interactions. This model is based on the induction of a unilateral inactivation of striatal DA receptors by locally injecting N-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ). Previous studies have shown that the systemic administration of EEDQ results in an irreversible blockade of a variety of neurotransmitter receptors, including D1 and D2 DA receptors (15,19). Here we report that the intrastriatal injection of EEDQ results in the inactivation of a significant fraction of D1 and D2 receptors as compared to the contralateral noninjected side. Under these experimental conditions, the effects of DA receptor agonists are predominant on the intact side and result in circling behavior towards the injected side (12). Therefore, we used this rotational model to evaluate the relative contribution of D1 and D2 DA receptors to the circling behavior elicited by DA agonists. The locomotor effects mediated by the stimulation of D1 and D2 DA receptors were also studied following the inhibition of DA synthesis with α-methyl-p-tyrosine (AMPT). In addition, the effects of the intrastriatal infusion of EEDQ on the binding parameters of muscarinic, benzodiazepine and serotonin ($5HT_2$) receptors were examined 1 day and 10 days after the injection.

Our results indicate that in this normosensitive DA receptor rotational model, the stimulation of D1 DA receptors either by endogenous DA or by selective agonists administered systemically, has a permissive role on the circling behavior induced by the activation of striatal D2 DA receptors.

METHOD

Animals

Male Sprague-Dawley rats (Charles River, Como, Italy) weighing 280 to 320 g were used throughout. Animals were housed four to a cage, had free access to food and water and were maintained on a 12-hr light-dark cycle (lights on 8 a.m.–8 p.m.).

Surgical Procedures

Rats were anesthetized with chloral hydrate (400 mg/kg, IP) and placed in a stereotaxic frame. EEDQ (20 nmoles/2 μl) was injected unilaterally into the head and body of the striatum, according to coordinates derived from the atlas of Pellegrino *et al.* (24): head, 3.0 mm anterior, 2.8 mm lateral and 5.0 mm ventral; body, 1.0 mm anterior, 3.5 mm lateral and 4.7 mm ventral, using bregma and the dura surface as reference points. EEDQ was infused over 3 min and the cannula was left in place for 1 min after injection to allow diffusion away from the tip. The infusion rate was controlled by a Harvard pump which drove a 10 μl Hamilton syringe connected to the cannula with PE 10 polyethylene tubing. Control rats were treated in identical fashion, but infused with EEDQ vehicle (dimethyl sulphoxide: saline 1:9, v/v).

Circling Behavior in EEDQ-Treated Rats

Twenty-four hr after EEDQ administration, animals were placed individually in 45-cm diameter rotation chambers and allowed 2 hr to accomodate prior to testing. Beginning 5 min after drug injections, the number of complete rotations was counted by 2 observers unaware of the animal treatments in a series of 15-min intervals uniformly distributed throughout a total period of observation of 4 hr (see legends to figures and tables for further details). Animals were tested only once with a single dose of one drug or combination of drugs. Rotational data were analyzed using a one-way ANOVA and, where applicable (F value greater than 95% confidence level), post hoc comparisons of the means were made using the Duncan's test.

Pretreatment With α-Methyl-p-Tyrosine

In order to avoid stimulation of DA receptors by endogenous DA, groups of rats that had been intrastriatally injected with EEDQ were treated with the DA synthesis inhibitor α-methyl-p-tyrosine (AMPT). The first dose of AMPT (300 mg/kg, IP) was administered 18 hr after EEDQ and the second dose (200 mg/kg, IP) was given 2 hr later. Control rats received intrastriatal EEDQ followed by the equivalent volume of AMPT solvent (saline, 1 ml/kg, IP). Behavioral tests using DA receptor agonists and antagonists were started 2 hr after the second dose of AMPT. The behavioral observations were performed as described in the previous paragraph. Rats were sacrificed 4 hr after the second dose of AMPT and the striatal content of DA and dihydroxyphenyl acetic acid (DOPAC) was measured by HPLC-EC as previously described (11).

Receptor Binding Studies

General procedure. Incubation tubes received membrane suspension, different concentrations of ^{3}H-labeled radioligand and buffer (for determination of total binding) or buffer containing nonradioactive displacer (for determination of nonspecific binding). Specific binding was defined as the difference between total and nonspecific binding. After incubation, the reaction was stopped by adding 3.5 ml of ice-cold buffer, followed by filtration under vacuum through Whatman GF/B filters. The filters were washed twice with 3.5 ml of the ice-cold buffer, and placed into plastic minivials containing 3.5 ml of scintillation fluid (Atomlight, New England Nuclear). The radioactivity was measured in a scintillation spectrophotometer with an efficiency of 40%.

Dopamine Receptors

Tissue preparation. Rats were sacrificed by decapitation 24–30 hr or 10 days after EEDQ administration and the striata, n. accumbens and s. nigrae were dissected out and frozen at −80°C for up to 2 weeks before assay.

Tissues were homogenized in 100 volumes of 50 mM Tris-HCl buffer, pH 7.40 for 20 sec at a setting of 5 with a Brinkmann Polytron and centrifuged at 48,000 × g for 10 min. The pellet was washed once by resuspension and recentrifugation in 100 volumes of the same buffer. The final pellet was resuspended in 100 volumes of 50 mM Tris-HCl buffer, pH 7.40, containing 120 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$ and 1 mM $MgCl_2$ (Tris-salt buffer).

^{3}H-SCH 23390 binding assay (D1 DA receptors). Membrane suspensions (approximately 120 μg protein for the striatum and n. accumbens and 250 μg protein for the s. nigra) were added to ice-cold glass tubes containing 50 μl of 50 mM Tris-HCl buffer, pH 7.40, and incubated with ^{3}H-SCH 23390 (spec.act. 85 Ci/mmol, Amersham) in concentrations ranging from 0.098 to 3 nM, in a final volume of 0.5 ml Tris-salt buffer. Nonspecific binding was determined in the presence of 10^{-5} M cis-flupentixol. All tubes were incubated for 60 min at 37°C.

^{3}H-Spiperone binding assay (D2 DA receptors). Incubation tubes contained striatal membrane suspension (equivalent to 100–120 μg protein), 40 nM ketanserin (to occlude striatal 5 HT_2 sites) and ^{3}H-spiperone (spec.act. 71.5 Ci/mmol, Amersham) in con-

TABLE 1

IRREVERSIBLE BLOCKADE OF ^{3}H-SCH 23390 AND ^{3}H-SPIPERONE BINDING SITES IN THE RAT STRIATUM AFTER THE LOCAL INJECTION OF EEDQ

Experimental Group	One Day After Injection K_D (nM)	One Day After Injection B_{max} (fmoles/mg prot.)	Ten Days After Injection K_D (nM)	Ten Days After Injection B_{max} (fmoles/mg prot.)
		^{3}H-SCH 23390 Binding		
Solvent (I)	0.62 ± 0.03	1320 ± 110	0.46 ± 0.09	1121 ± 117
Solvent (C)	0.70 ± 0.04	1370 ± 140	0.51 ± 0.07	1213 ± 96
EEDQ (I)	0.63 ± 0.05	724 ± 82*	0.49 ± 0.08	1194 ± 109
EEDQ (C)	0.79 ± 0.07	1388 ± 163	0.53 ± 0.05	1098 ± 121
		^{3}H-Spiperone Binding		
Solvent (I)	0.06 ± 0.01	283 ± 36	0.05 ± 0.01	309 ± 41
Solvent (C)	0.05 ± 0.01	306 ± 24	0.07 ± 0.01	297 ± 29
EEDQ (I)	0.05 ± 0.01	173 ± 37*	0.06 ± 0.02	308 ± 35
EEDQ (C)	0.06 ± 0.01	312 ± 29	0.05 ± 0.01	321 ± 37

Animals were sacrificed 1 day or 10 days after the injection of EEDQ or its solvent and ^{3}H-SCH 23390 and ^{3}H-spiperone binding isotherms were determined in pooled striata from 3 rats. B_{max} and K_D values were determined as described in the Method section. The results are expressed as the mean ± S.E.M. of three independent experiments carried out in triplicate. *$p<0.05$ vs. the contralateral noninjected side. (I): injected side; (C): contralateral side.

centrations ranging from 0.04 to 2 nM in a final volume of 1 ml Tris-salt buffer. Nonspecific binding was determined in the presence of 1 μM (+)butaclamol. Incubations were carried out for 60 min at 37°C.

Muscarinic, Serotonin ($5HT_2$) and Benzodiazepine Receptors

^{3}H-Quinuclidinyl benzilate (^{3}H-QNB) binding assay (muscarinic receptors). A modification of the method of Yamamura and Snyder (32) was employed in these experiments as previously described (10). Striatal membrane preparations (approximately 100 μg of protein) were incubated for 60 min at 37°C in a final volume of 2 ml of 50 mM Na-K phosphate buffer (pH 7.4) containing different concentrations (0.01–1 nM) of ^{3}H-QNB (spec.act. 44 Ci/mmol, Amersham). Nonspecific binding was defined as that occurring in the presence of 10 μM atropine.

^{3}H-Ketanserin binding assay ($5HT_2$ receptors). The procedure of Leysen *et al.* (17) was followed. Striatal tissue suspensions (approximately 100 μg protein) were incubated (15 min at 37°C) in a final volume of 2 ml of 50 mM Tris-HCl buffer (pH 7.7) containing different concentrations (0.1–4 nM) of ^{3}H-ketanserin (spec.act. 95 Ci/mmol, New England Nuclear Corporation). Nonspecific binding was determined in the presence of 10 μM methysergide.

^{3}H-Flunitrazepam (^{3}H-FNT) binding assay (benzodiazepine receptors). Assays were carried out as described previously (13). Membrane preparations (200–250 μg protein) were incubated for 60 min at 4°C in a final volume of 0.5 ml of 50 mM Tris-HCl buffer (pH 7.4) in the presence of 0.12–8 nM ^{3}H-FNT (spec.act. 79 Ci/mmol, New England Nuclear). Nonspecific binding was determined in the presence of 5 μM clonazepam (kindly provided by Hoffmann-La Roche, Basel, Switzerland).

The maximum number of binding sites (B_{max}) and the apparent dissociation constant (K_D) were calculated by linear regression analysis of Scatchard plots obtained from ^{3}H-SCH 23390, ^{3}H-spiperone, ^{3}H-QNB, ^{3}H-ketanserin and ^{3}H-FNT binding isotherms. Protein was measured according to Lowry *et al.* (18) using bovine serum albumin as a standard.

Drugs

SKF 38393 hydrobromide (Smith, Kline and French, USA), LY 171555 hydrochloride (Eli Lilly, USA), (−)sulpiride hydrochloride (Ravizza, Italy), raclopride tartrate (Astra, Sweden), prazosin hydrochloride (Sigma, USA) and AMPT (Sigma, USA) were dissolved in saline. Yohimbine hydrochloride (Sigma, USA) and ketanserin (Janssen, Belgium) were dissolved in a few drops of acetic acid and then diluted with saline. EEDQ (Aldrich Chemical Co., USA) and SCH 23390 (Schering-Plough Corp., USA) were dissolved in dimethyl sulphoxide:saline 1:9 (v/v). All the drugs were administered IP in a volume of 1 ml/kg except SCH 23390 which was given SC and EEDQ which was injected intrastriatally.

RESULTS

As shown in Table 1, the density of striatal D1 DA receptors available for binding to ^{3}H-SCH 23390 was markedly decreased 24 hr after the local injection of EEDQ (−48% as compared with the contralateral noninjected striatum), whereas no significant alterations were observed in the apparent dissociation constant (K_D). A similar decline in the B_{max} of striatal D2 receptors labelled with ^{3}H-spiperone (−45% vs. the contralateral side) with no change in the K_D was observed 24 hr after the intrastriatal injection of EEDQ (Table 1).

The effects of the local infusion of EEDQ on serotonin ($5HT_2$), muscarinic and benzodiazepine receptors in the rat striatum were investigated 1 day and 10 days after the injection (Table 2). No significant changes were observed in the B_{max} and K_D values for muscarinic and benzodiazepine receptors 24 hr after EEDQ. On

TABLE 2

EFFECT OF THE LOCAL INJECTION OF EEDQ ON THE BINDING PARAMETERS OF SEROTONERGIC (5-HT_2), MUSCARINIC AND BENZODIAZEPINE RECEPTORS IN THE RAT STRIATUM

Experimental Group	One Day After Injection		Ten Days After Injection	
	K_D (nM)	B_{max} (fmoles/mg prot.)	K_D (nM)	B_{max} (fmoles/mg prot.)
		^{3}H-Ketanserin Binding		
Solvent (I)	0.63 ± 0.07	230 ± 37	0.57 ± 0.04	250 ± 45
Solvent (C)	0.74 ± 0.09	210 ± 55	0.71 ± 0.10	226 ± 43
EEDQ (I)	0.68 ± 0.12	182 ± 10*	0.48 ± 0.12	230 ± 28
EEDQ (C)	0.57 ± 0.08	260 ± 25	0.67 ± 0.08	240 ± 30
		^{3}H-QNB Binding		
Solvent (I)	0.06 ± 0.01	898 ± 118	0.08 ± 0.01	788 ± 97
Solvent (C)	0.07 ± 0.02	946 ± 79	0.07 ± 0.02	896 ± 108
EEDQ (I)	0.08 ± 0.01	910 ± 106	0.05 ± 0.02	852 ± 87
EEDQ (C)	0.06 ± 0.01	876 ± 97	0.06 ± 0.01	921 ± 74
		^{3}H-Flunitrazepam Binding		
Solvent (I)	1.33 ± 0.31	850 ± 90	1.90 ± 0.37	903 ± 74
Solvent (C)	1.76 ± 0.40	840 ± 81	1.51 ± 0.40	861 ± 49
EEDQ (I)	1.80 ± 0.26	820 ± 59	1.89 ± 0.34	893 ± 62
EEDQ (C)	1.90 ± 0.33	920 ± 83	1.42 ± 0.29	857 ± 45

Animals were sacrificed 1 day or 10 days after the intrastriatal injection of EEDQ or its solvent and ^{3}H-ketanserin, ^{3}H-QNB and ^{3}H-flunitrazepam binding isotherms were determined as described in the Method section. The results are expressed as the mean ± S.E.M. of three independent experiments carried out in triplicate. *$p<0.05$ vs. the contralateral noninjected side. (I): injected side; (C): contralateral side.

the other hand, a significant decrease in the density of $5HT_2$ receptors labelled with ^{3}H-ketanserin was observed 24 hr after EEDQ (Table 2), although this effect was not so pronounced as that obtained with D1 and D2 DA receptors (Table 1).

It is important to note that no alterations in the binding parameters of the different neurotransmitter receptors investigated were detected 10 days after EEDQ infusion, suggesting that this treatment does not cause any relevant neuronal damage (Table 1 and Table 2). Similarly, the local infusion of the EEDQ solvent (DMSO: saline, 1:9, v/v) failed to modify the binding parameters of any of the radioligands investigated either 1 or 10 days after injection (Table 1 and Table 2).

In addition, the intrastriatal infusion of EEDQ or its vehicle failed to induce any significant alteration in the binding parameters of D1 DA receptors in the n. accumbens and in the s. nigra (Table 3).

The motor effects induced by the systemic administration of different agonists and antagonists for D1 and D2 DA receptors were evaluated 24 hr after EEDQ. The selective D2 agonist LY 171555 (29) induced ipsilateral rotation in a dose-dependent manner in rats treated intrastriatally with EEDQ (Fig. 1A), but not in controls injected with the EEDQ vehicle (not shown). As shown in Fig. 1B, the IP administration of LY 171555 at the doses of 0.5 mg/kg or 1 mg/kg, induced intensive circling behavior that had a rapid onset and persisted for more than 4 hr. In contrast, the D1 agonist SKF 38393 (1–20 mg/kg, IP) was unable to elicit rotation by itself (Fig. 1A), but increased the number of turns induced by LY 171555 at a fixed dose of 0.5 mg/kg, IP (Fig. 2). As shown in Table 4, the selective D2 antagonists (−)sulpiride (50 and 100 mg/kg, IP) (21) and raclopride (2 mg/kg, IP) (22) completely prevented the circling behavior induced by LY 171555 (1 mg/kg, IP).

On the other hand, the α_1 adrenoceptor antagonist prazosin (5 mg/kg, IP), the α_2 adrenoceptor antagonist yohimbine (10 mg/kg, IP) and the $5HT_2$ serotonin receptor antagonist ketanserin (5 mg/kg, IP) failed to prevent the rotation induced by LY 171555 (Table 4).

As shown in Fig. 2, the selective D1 DA receptor antagonist SCH 23390 (16), when injected at the doses of 0.1 or 0.5 mg/kg, SC at the same time as LY 171555 (at the fixed dose of 0.5 mg/kg, IP) completely prevented the circling behavior elicited by the D2 agonist. Moreover, the inhibitory effect of SCH 23390, at the dose of 0.1 mg/kg, SC, was reversed in a dose-dependent manner by the simultaneous administration of SKF 38393 (Fig. 2). In contrast, when rats were treated with LY 171555 (0.5 mg/kg, IP) plus SCH 23390 (0.5 mg/kg, SC), the concurrent administration of SKF 38393 (1, 5, or 20 mg/kg, IP) was unable to restore the circling behavior observed in rats treated with LY 171555 alone (Fig. 2).

Taken together, the above results suggest that the activation of D1 DA receptors by endogenous DA or by selective agonists administered systemically plays a facilitatory role on the expres-

TABLE 3

BINDING PARAMETERS OF D1 DOPAMINERGIC RECEPTORS IN THE N. ACCUMBENS AND S. NIGRA 24 HR AFTER THE INTRASTRIATAL INJECTION OF EEDQ

	Specific ^{3}H-SCH 23390 Binding			
	N. Accumbens		S. Nigra	
Experimental Group	K_D (nM)	B_{max} (fmoles/mg prot.)	K_D (nM)	B_{max} (fmoles/mg prot.)
Solvent (I)	0.42 ± 0.07	726 ± 94	0.36 ± 0.02	410 ± 32
Solvent (C)	0.48 ± 0.05	745 ± 109	0.34 ± 0.05	435 ± 57
EEDQ (I)	0.50 ± 0.09	802 ± 74	0.41 ± 0.07	427 ± 28
EEDQ (C)	0.46 ± 0.07	779 ± 121	0.39 ± 0.04	396 ± 29

Animals were sacrificed 24 hr after the intrastriatal injection of EEDQ or its solvent and ^{3}H-SCH 23390 binding isotherms were determined in pooled n. accumbens and s. nigrae from 5 rats. Shown are the mean ± S.E.M. of 3 independent experiments. (I): injected side; (C): contralateral side.

sion of motor effects elicited by the stimulation of striatal D2 DA receptors. In order to test this possibility, we investigated the effects of LY 171555 and SKF 38393, given separately or in combination, following DA depletion by AMPT in rats injected intrastriatally with EEDQ. As shown in Table 5, the local infusion of EEDQ induced a two-fold increase in the content of DOPAC in the injected striatum, whereas the injection of EEDQ solvent had no effect; however, the concentrations of DOPAC and DA were decreased to the same values in the EEDQ-injected striatum and in the contralateral side after AMPT administration (Table 5). The depletion of endogenous DA by AMPT was associated with a dramatic decrease in the number of rotations elicited by LY 171555 in EEDQ-treated rats (Table 6). Moreover, the combined administration of SKF 38393 and LY 171555 rapidly induced intense circling behavior and this effect was completely antagonized by SCH 23390 at the dose of 0.1 mg/kg, SC (Table 6).

DISCUSSION

The present results indicate that the intrastriatal injection of EEDQ induces a significant decrease in the densities of D1 and D2 DA receptors with no change in K_D, supporting a nonequilibrium rather than a competitive antagonism by this compound. This is in line with previous reports on the effects of the systemic administration of EEDQ on DA receptors in the rat brain (15,19).

The unilateral inactivation of a significant fraction (45% to 48%) of striatal DA receptors determines a predominant stimulation of the output mechanisms "downstream" from DA receptors in the noninjected striatum following the systemic administration of DA receptor agonists. This in turn results in circling behavior

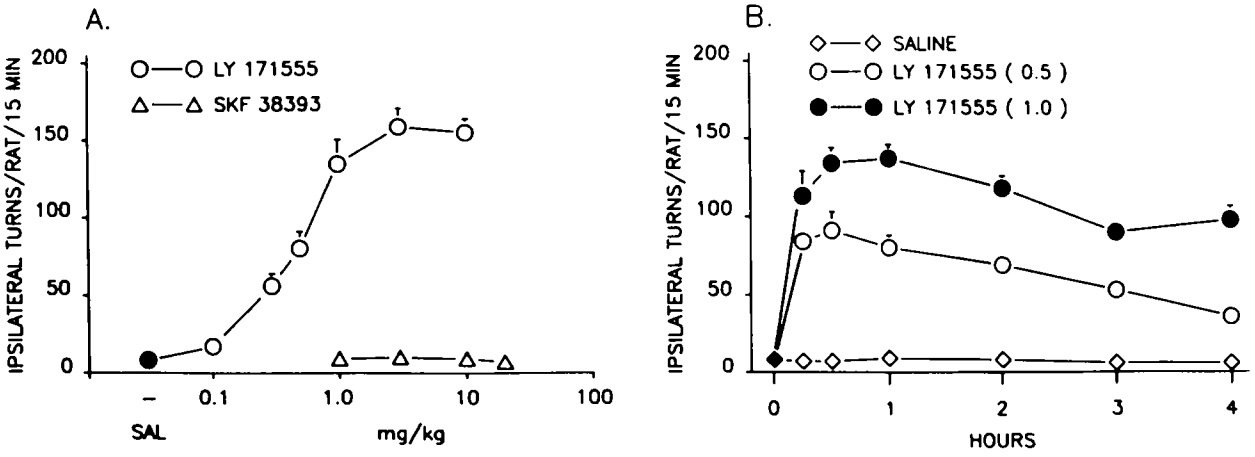

FIG. 1. Ipsilateral rotation induced by LY 171555, but not by SKF 38393 in rats intrastriatally treated with EEDQ. (A) Dose-response curve: animals were treated IP with saline (●), SKF 38393 (Δ), or LY 171555 (○) 24 hr after the intrastriatal injection of EEDQ. Shown are the mean ± S.E.M. net ipsilateral turns per rat counted over a 15-min period beginning 30 min after drug administration (n = 3 to 6 rats for each data point). (B) Time course of the rotation induced by LY 171555: 24 hr after the intrastriatal infusion of EEDQ, different groups of rats were treated with saline (◇) or LY 171555 at the doses of 0.5 mg/kg, IP (○) or 1 mg/kg, IP (●). Each value indicates the mean ± S.E.M. net ipsilateral turns per rat counted over a series of 15-min periods beginning 30 min before (◆, time zero control) and 15 min, 30 min, 1, 2, 3, and 4 hr after injection (n = 6 rats in each group).

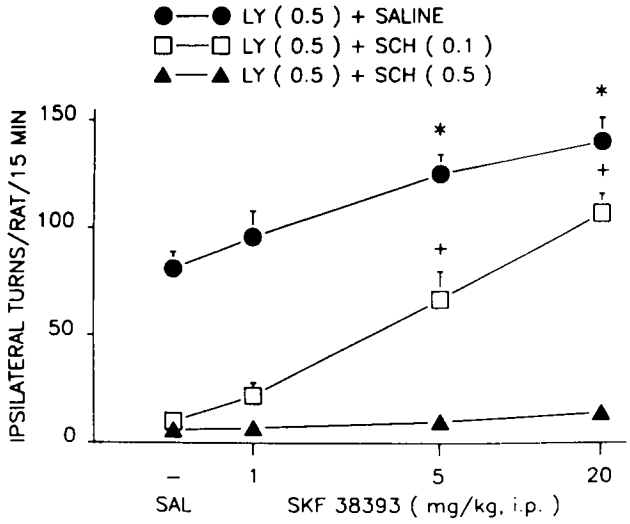

FIG. 2. Ipsilateral rotation induced by LY 171555: potentiation by SKF 38393 and antagonism by SCH 23390. SKF 38393 (1, 5, or 20 mg/kg, IP) or saline were administered to different groups of rats receiving one of the following treatments: LY 171555 plus saline (●, 0.5 mg/kg, IP), or LY 171555 plus SCH 23390 (□, 0.5 mg/kg IP + 0.1 mg/kg, SC) or LY 171555 plus SCH 23390 (▲, 0.5 mg/kg, IP + 0.5 mg/kg, SC). LY 171555, SCH 23390 and SKF 38393 were injected simultaneously. Each value is the mean ± S.E.M. net ipsilateral turns per rat counted over a 15-min period beginning 30 min after drug administration (n = 4 rats for each data point). *$p<0.05$ vs. LY 171555 alone; +$p<0.05$ vs. LY 171555 plus SCH 23390 (0.1 mg/kg, SC).

towards the injected side mediated by the intact hemisphere (12).

Our results also demonstrate that this novel rotational model provides a useful experimental tool to investigate the interactions between DA receptor subtypes. Thus, the selective stimulation of D2 receptors by LY 171555 induced circling behavior and this effect was blocked by the D2 antagonists (−)sulpiride and raclopride. On the other hand, the D1 agonist SKF 38393 failed to elicit rotations when given alone, but potentiated the circling behavior induced by LY 171555.

In addition, the selective D1 antagonist SCH 23390 blocked the rotations induced by LY 171555, whereas SKF 38393 restored circling behavior in a fashion depending on the dose of SCH 23390. It appears unlikely that the antagonism by SCH 23390 of LY 171555-induced rotations is mediated via D2 DA receptor blockade since SCH 23390 binds with very low affinity to D2 DA receptors both in vitro (16) and in vivo (19). In fact, high doses of SCH 23390 (up to 3 mg/kg, IP) fail to prevent the irreversible inactivation of striatal D2 DA receptors induced by the systemic administration of EEDQ (19).

The above results suggest that in this experimental model in which rotations are mediated via normosensitive receptors, some degree of stimulation of D1 receptors by endogenous DA is required for the expression of D2 receptor-mediated effects. This hypothesis is supported by the finding that LY 171555 elicits a very weak circling behavior in EEDQ-treated rats after the inhibition of DA synthesis by AMPT, whereas the combined administration of LY 171555 and SKF 38393 results in intense rotational behavior in DA-depleted animals; in addition, this effect is also antagonized by SCH 23390. It is worth noting that the local infusion of EEDQ does not change the striatal concentration of DA, but produces a two-fold increase in the content of DOPAC in this brain area, an effect similar to that of neuroleptic agents (26). The latter results indicate that EEDQ does not affect DA synthesis and storage. This lack of effect of EEDQ on DAergic nerve endings is important for the interpretation of the behavioral actions mediated via stimulation of D2 DA receptors that are strongly influenced by the endogenous DAergic tone. In this context, it is important to note that, in rats treated with AMPT, the concentrations of DA and DOPAC were very similar in the EEDQ-injected striatum as compared to the contralateral, noninjected side (Table 5).

Therefore, our results are in line with those previously reported using different experimental models in which circling behavior is mediated via stimulation of normosensitive DA receptors in rats with a unilateral striatal lesion induced by quinolinic acid (4) or in rats submitted to a unilateral transection of a cerebral hemisphere (1). The experimental procedure used in this study has some advantages when compared to other methods in which rats undergo permanent lesions following hemispheric hemitransection or intracerebral injections of neurotoxic agents.

Firstly, the intrastriatal injection of EEDQ does not appear to induce any relevant cellular damage as indicated by the finding that the densities of muscarinic, benzodiazepine, $5HT_2$ serotonin and D1 and D2 DA receptors are unaltered by 10 days after EEDQ.

Accordingly, this lack of biochemical alterations is associated with a complete functional recovery. In fact, rotations are not observed when rats are challenged with LY 171555 (1 mg/kg, IP), 10 days after EEDQ (3 ± 1 turns/rat/15 min; n = 4).

Secondly, the irreversible blockade induced by EEDQ seems to be restricted to a few classes of neurotransmitter receptors. Thus, striatal muscarinic receptors and benzodiazepine recognition sites are not affected by the local injection of EEDQ (Table 2); in addition, the systemic administration of EEDQ has no effect on the binding properties of cerebral opiate receptors and β-adrenergic receptors (19). It should be noted, however, that EEDQ inactivates α-adrenergic receptors and serotonergic receptors, in addition to DA receptors [(19); Table 2]. One possible way to circumvent this problem is to protect receptor systems sensitive to EEDQ by

TABLE 4

IPSILATERAL ROTATION INDUCED BY LY 171555: ANTAGONISM BY (−)SULPIRIDE AND RACLOPRIDE, BUT NOT BY KETANSERIN, PRAZOSIN AND YOHIMBINE

Treatment	Dose (mg/kg)	Turns/15 Min/Rat
Solvent	—	137 ± 11
(−)Sulpiride	10	112 ± 14
(−)Sulpiride	50	41 ± 6*
(−)Sulpiride	100	13 ± 5*
Raclopride	2	9 ± 3*
Prazosin	5	124 ± 16
Yohimbine	10	118 ± 13
Ketanserin	5	142 ± 9

Different groups of rats were treated IP with one of the antagonists or saline (1 ml/kg, IP) 15 min before LY 171555 (1 mg/kg, IP). Shown are the mean ± S.E.M. ipsilateral rotations per rat counted over 15-min observational periods beginning 30 min after the administration of LY 171555 (n = 3 to 9 rats in each experimental group). *$p<0.01$ vs. the control group.

TABLE 5

DOPAMINE AND DOPAC CONTENT IN THE STRIATUM OF RATS TREATED WITH AMPT

		Injected Striatum		Contralateral Striatum	
Intrastriatal Treatment	Systemic Treatment	DA (ng/g)	DOPAC (ng/g)	DA (ng/g)	DOPAC (ng/g)
Solvent	Solvent	11506 ± 675	1235 ± 104	11822 ± 571	1157 ± 86
Solvent	AMPT	3124 ± 306*	347 ± 89*	2986 ± 257*	309 ± 60*
EEDQ	Solvent	11127 ± 983	2564 ± 197†	12139 ± 612	1198 ± 72
EEDQ	AMPT	2931 ± 332*	356 ± 71*	2793 ± 270*	341 ± 39*

Rats were treated with EEDQ (IS) and AMPT (IP) or their respective vehicles as described in the Method section. Shown are the means ± S.E.M. of 5 animals in each experimental group. *$p<0.01$ vs. the respective AMPT vehicle-treated group; †$p<0.01$ vs. the respective contralateral side.

TABLE 6

ROTATIONS INDUCED BY THE COMBINED ADMINISTRATION OF LY 171555 AND SKF 38393 IN AMPT-TREATED RATS: ANTAGONISM BY SCH 23390

Pretreatment	Treatment	Turns/15 Min/Rat
EEDQ (IS) + Saline (IP)	LY 171555 (1.0)	102 ± 9
EEDQ (IS) + AMPT (IP)	LY 171555 (1.0)	22 ± 7*
EEDQ (IS) + AMPT (IP)	SKF 38393 (10.0)	3 ± 1*
EEDQ (IS) + AMPT (IP)	LY 171555 (1.0) + SKF 38393 (10.0)	98 ± 6
EEDQ (IS) + AMPT (IP)	LY 171555 (1.0) + SKF 38393 (10.0) + SCH 23390 (0.1)	15 ± 6*

AMPT or its vehicle was administered to EEDQ-treated rats as described in the Method section. LY 171555 and SKF 38393 were given IP, separately or in combination, 2 hr after the second dose of AMPT. SCH 23390 was injected SC 60 min after the combined administration of LY 171555 plus SKF 38393. Numbers in parentheses indicate the doses in mg/kg. Shown are the means ± S.E.M. ipsilateral rotations per rat counted over a 15-min period starting 35 min after the injection of LY 171555, SKF 38393 and SCH 23390 (n = 5 rats in each experimental group). The number of rotations/15 min/rat in the group treated with LY 171555 + SKF 38393 + SCH 23390 before the administration of the antagonist was 106 ± 11 (not significant vs. the group treated with LY 171555 + SKF 38393). *$p<0.01$ vs. LY 171555 plus SKF 38393 and vs. the control group pretreated with saline and treated with LY 171555 alone.

pretreatment with agents selective at those receptors (3, 14, 19, 25). In this context, preliminary results from our laboratory indicate that the protection of $5HT_2$, α_1 and α_2 receptors by the combined IP administration of ketanserin (5 mg/kg), prazosin (5 mg/kg) and yohimbine (10 mg/kg) 30 min before the intrastriatal injection of EEDQ does not prevent the rotations elicited by a challenge dose of 1 mg/kg, IP of LY 171555 administered 24 hr after EEDQ (EEDQ alone: 125 ± 7 turns/rat/15 min, n = 4; EEDQ + protection: 141 ± 13 turns/rat/15 min, n = 4). This finding suggests that the inactivation of α-adrenergic and serotonin receptors does not play a relevant role in the motor effects of DA receptor agonists in rats injected intrastriatally with EEDQ. Accordingly, ketanserin, prazosin and yohimbine failed to antagonize the rotation induced by LY 171555 in rats treated with EEDQ alone (Table 4).

Thirdly, the inactivation of DA receptors induced by the local infusion of EEDQ provides a potentially useful experimental tool to study the effects mediated via the activation of DA receptors and the functional interactions between DA receptor subtypes not only in the striatum, but also in other areas of the brain. In fact, the intrastriatal injection of EEDQ does not affect the binding parameters of D1 DA receptors in the n. accumbens and in the s. nigra. These findings are in line with the possibility that the receptor inactivation induced by EEDQ is restricted to the injection site. Further studies using selective ligands for different neurotransmitter receptors are required to test this possibility.

The synergistic interactions between normosensitive DA receptor subtypes reported herein have also been observed in previous electrophysiological and behavioral studies. Thus, D1 and D2 DA receptors exert synergistic effects on the firing rates of basal ganglia neurons and on the expression of stereotyped behavior in rats (5, 6, 31).

Another experimental model that has been extensively used to investigate the effects of DA on motor function is turning behavior in rats with unilateral 6-hydroxydopamine-induced lesions of the nigrostriatal pathway (30). In this model, both D1 and D2 DA receptor agonists produce contralateral rotational behavior (2) as a result of the activation of supersensitive striatal DA receptors (30). Moreover, SCH 23390 is much more potent at antagonizing the rotations induced by SKF 38393 than those elicited by a D2 agonist, whereas the selective D2 antagonist spiroperidol has the reverse selectivity (2). The molecular mechanisms underlying the differences in the interactions between normosensitive and supersensitive DA receptor subtypes are still unknown.

In conclusion, we have developed a rotational model that has potential utility as a new approach to investigate the interactions between normosensitive DA receptor subtypes in the expression of DA-mediated behaviors. However, the results obtained using this model should be interpreted with caution because the effects of EEDQ on neurotransmitter systems that play a modulatory role on DAergic behaviors are incompletely characterized at present. This limitation becomes especially important when the interactions between DA receptor subtypes are investigated using a single dose of each drug instead of dose-response curves.

The results obtained so far are consistent with the view that endogenous DA may induce its behavioral actions in the intact

animal by concurrent stimulation of both D1 and D2 DA receptors. Therefore, a better understanding of the functional interactions between D1 and D2 DA receptors may provide a basis to improve the therapeutic management of clinical disorders associated with alterations in DAergic function, such as schizophrenia and Parkinson's disease.

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