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A Full Repetitive Jaw Movement Response After 70% Depletion of Caudate D_1 Receptors

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ROSENGARTEN, H., J. W. SCHWEITZER AND A. J. FRIEDHOFF. *A full repetitive jaw movement response after 70% depletion of caudate D_1 receptors.* PHARMACOL BIOCHEM BEHAV **34**(4) 895–897, 1989.—Repetitive jaw movements (RJM) in the rat can be produced in a dose-dependent manner with the selective D_1 agonist, SKF 38393. Administration of the protein coupling agent, N-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ) to rats pretreated with a D_2 receptor blocker resulted in a 70–80% reduction of D_1 dopamine receptors, but only a 10% reduction of D_2 receptors in the rat caudate. Twenty-four hours following EEDQ, the RJM response to SKF 38393 was assessed. The massive selective reduction of the D_1 receptor density was found not to modify the rate of RJM induced by SKF 38393 in that dose response curves in control and EEDQ-treated rats were essentially identical. These data provide evidence to indicate that there is a functional D_1 receptor reserve for D_1-mediated RJM behavior.

SKF 38393-inducible RJM in control and EEDQ-treated rats

WE have previously demonstrated that repetitive jaw movements (RJM) in rats can be produced in a dose-dependent manner through activation of D_1 receptors with a selective D_1 agonist, SKF 38393. The characteristic features of this behavior in rats are bursts of purposeless repetitive opening and closing of the rat jaw and tongue protrusion, while stereotyped behavior manifested by goal directed licking and biting is absent (9). RJM can be facilitated through D_2 receptor blockade with a selective antagonist such as sulpiride or eticlopride, and antagonized by a selective D_1 receptor antagonist, SCH 23390 (10–12).

D_1 and D_2 receptors in the brain interact in various ways and antagonistic, as well as synergistic interactions have been described for different dopaminergic behaviors (1, 2, 4, 7). In the present study we have examined the quantitative relationship between D_1 activation and RJM response induced by the selective D_1 agonist SKF 38393 in control and experimental rats with 70–80% depletion of D_1 dopamine (DA) receptors.

In these studies the D_1 receptors were depleted with the peptide coupling agent N-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ) which is known to produce irreversible inactivation of DA receptors (9,10). Administration of EEDQ in rats resulted in a reduction of D_1 and D_2 DA receptor density (B_{max}) on the order of 70–80% with no change of affinity (5,8).

METHOD

For all studies male Sprague-Dawley rats, weighing 250–280 g, were used. Rats were housed 4 to a cage in an animal facility at $21 \pm 1°C$ with a relative humidity of $55 \pm 5°C$, under a 12-hour light-dark cycle, and with free access to commercial food pellets and tap water. Irreversible D_1 receptor blockade was carried out by administering either 6 or 20 mg of EEDQ (Aldrich Chem. Co.) IP. In the present study we selectively decreased the density of D_1 receptors to 20–30% of their baseline value and studied the effect of the selective D_1 agonist SKF 38393 in 6 different doses ranging from 2.5–60 mg/kg, and that of the selective D_1 antagonist SCH 23390, 3 or 30 μg/kg. A mixture of an S_2 antagonist, ketanserine, 5 mg/kg, α_1 antagonist, prazosin, 5 mg/kg, α_2 antagonist, idazoxan, 1.25 mg/kg, and D_2 antagonist, eticlopride, 500 μg/kg was administered SC to protect S_2, α_1 and α_2 adrenoreceptors and D_2 receptors, respectively. The selective inactivation of D_1 receptors permitted us to study the RJM response to SKF 38393 stimulation.

Behavioral Testing

Twenty-four hours following EEDQ administration, individual rats were transferred, each to a wire mesh cage, $7 \times 7 \times 10$ inches, for one hour habituation prior to RJM assessment. Behavioral testing was carried out for 10 minutes, 45 minutes following subcutaneous administration of SKF 38393 to groups of 10 rats/dose, and the number of RJM episodes registered by an observer unaware of the treatment paradigm. The effect of SCH 23390 inhibition on SKF 38393-inducible behavior was also tested in rats with depleted D_1 receptors.

D_1 and D_2 Receptor Saturation Analysis

For the determination of D_1 and D_2 receptor density in the rat striatum, animals were decapitated 24 hours after treatment.

TABLE 1

EFFECT OF EEDQ TREATMENT ON D_1 AND D_2 RECEPTOR DENSITIES IN RAT STRIATUM

EEDQ Dose mg/kg	D_1 B_{max} pmol/g* ± S.E.M.	D_1 K_d nmol ± S.E.M.	D_2 B_{max} pmol/g* ± S.E.M.	D_2 K_d nmol ± S.E.M.
0	116 ± 5.5	0.64 ± 0.05	38.9 ± 1.2	0.05 ± 0.003
6	28.4 ± 2.3†	0.62 ± 0.02	34.1 ± 1.8	0.047 ± 0.002
20	22.1 ± 4.0†	0.64 ± 0.03	—	—

*g tissue, original tissue wet weight.

†$p<0.005$ as compared to controls. Student's t-test used for statistical analysis. Data are means ± S.E.M. from four rats for each EEDQ dose and controls.

Brains were removed, dissected over ice and stored at −80° until assay.

The tissue was homogenized in 10 volumes of Tris-HCl buffer at pH 7.7, resuspended in 100 volumes of the same buffer and centrifuged at 20,000 × g for 10 minutes. The sediment was resuspended in 100 volumes of the same buffer and recentrifuged again for 10 minutes at 20,000 × g. The final sediment was resuspended in Tris-HCl buffer, pH 7.7, in a final concentration of 10 mg of original wet weight per ml. The equivalent of 2 mg of tissue homogenate was used in the binding assay.

For the determination of D_2 receptor density, ^{3}H-spiroperidol spec.act. 24 Ci/mmol (NEN) at a final concentration of 0.8 nmolar was used as the radioligand, R43448 at a final concentration of 0.1 μmolar served to occlude the S_2 binding and 1 μmolar (+) butaclamol for the nonspecific binding. Samples were incubated for 30 minutes and terminated by rapid filtration under vacuum through Whatman GF/B filters and washed 3 times with 5 ml of Tris-HCl buffer. The labelled material retained on the filter was counted by liquid scintillation spectrometry (6).

Binding of ^{3}H-SCH 23390 spec.act. 80 Ci/mmol (Amersham) to D_1 receptors was carried out by saturation analysis according to the method of Billard *et al.* (3). For the determination of nonspecific binding, 1 μM cis-flupenthixol was included for D_1 receptors. All tubes for D_1 binding contained 0.1 μM R43448 to occlude serotonin binding. Tubes were incubated for 30 minutes at 37°C and then returned to the ice bath and filtered through S&S #32 glass fiber filters. Filters were washed three times with 5 ml of ice-cold 50 mM Tris buffer and counted in 10 ml Liquiscint (National Diagnostic) by liquid scintillation spectrometry at 35% counting efficiency. B_{max} and K_d values were estimated by Scatchard analysis.

RESULTS

EEDQ administration to rats in a dose of 6 and 20 mg/kg resulted, 24 hours later, in 70–80% selective depletion of D_1 receptors in the striatum, with no change in receptor affinity. Eticlopride administration prior to EEDQ protected D_2 receptors from EEDQ inactivation (Table 1). Selective reduction of D_1 receptor density by EEDQ to 20–30% of the control value did not modify the rate of RJM induced by SKF 38393 in a dose range from 2.5–60 mg/kg. The dose-response curves in control and EEDQ-treated rats were essentially identical (Fig. 1). SCH 23390 was capable of inhibiting the SKF 38393-inducible RJM response in control and EEDQ-treated rats with the same IC_{50}, suggesting

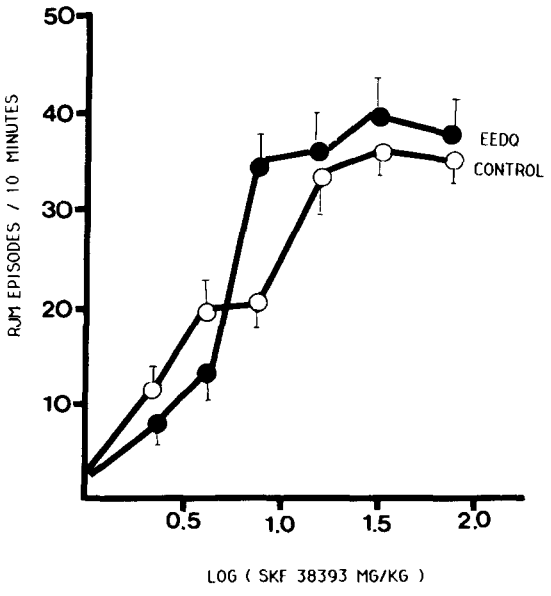

FIG. 1. Dose-response curves comparing the potency of SKF 38393 to induce RJM in vehicle and EEDQ-exposed rats. Vertical bars represent S.E.M.

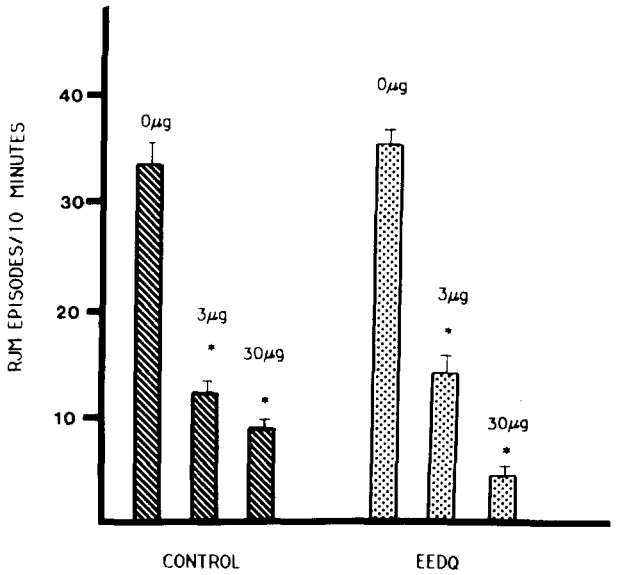

FIG. 2. The effect of two doses of SCH 23390 on the mean (±S.E.M.) frequency of SKF 38393-inducible RJM episodes in vehicle and EEDQ-pretreated rats.

that this response is mediated by the same population of D_1 receptors (Fig. 2).

DISCUSSION

A D_1 receptor population of only 20–30% of control rats is sufficient to mediate a full RJM response to selective D_1 agonists and antagonists. These results were surprising because SKF 38393, a partial agonist, usually needs full receptor occupancy to induce a biological response. From our data it seems that the RJM response is mediated by a subpopulation of D_1 receptors or, that in the expression of RJM, 20% of D_1 receptors are sufficient, suggesting the presence of a functional D_1 receptor reserve for RJM behavior, although we have not been able to show a classical shift in the dose response.

This mechanism may be of particular interest in RJM that appear during neuroleptic treatment or during withdrawal (11). Neuroleptics have a higher affinity for D_2 receptors than for D_1 receptors and, thus, a faster washout from D_1 receptors; therefore, as was demonstrated earlier (10,12), partial blockade of the D_2 system will facilitate the appearance of RJM, while 20–30% of available D_1 receptors are sufficient for the full RJM response to agonist stimulation. If this behavior is analogous to the oral movements in human tardive dyskinesia, it is plausible that similar mechanisms may be responsible for involuntary oral behavior during chronic neuroleptic treatment or during neuroleptic withdrawal.

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