Pharmacology Biochemistry and Behavior, Vol. 45, pp. 921–924, 1993
Printed in the U.S.A.

0091-3057/93 $6.00 + .00

A Subpopulation of Dopamine D_1 Receptors Mediate Repetitive Jaw Movements in Rats

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Received 19 October 1992

ROSENGARTEN, H., J. W. SCHWEITZER AND A. J. FRIEDHOFF. *A subpopulation of dopamine D_1 receptors mediate repetitive jaw movements in rats.* PHARMACOL BIOCHEM BEHAV 45(4) 921–924, 1993.—Repetitive jaw movements (RJM) in rats, a potentially useful animal model of tardive dyskinesia, appears to be mediated by the dopamine D_1 receptor as evidenced in part by their induction and inhibition with D_1 agonists and D_1 antagonists, respectively. Selective destruction of 60–90% of D_1 receptors by EEDQ, measured in several CNS dopaminergically innervated areas, preceded by protection of D_2, 5-HT_2, α_1 and α_2 receptors, however, failed to reduce D_1 agonist-augmentable RJM. Further, the affinity of dopamine toward displacement of ^{3}H-SCH-23390 binding from striatal D_1 receptors was significantly decreased by administered EEDQ, a counter-intuitive result in relation to D_1 responsitivity and RJM. Thus, at present it is suggested that an EEDQ-resistant D_1 receptor subpopulation may exist.

Dopamine D_1 receptors EEDQ inactivation Jaw movements

MATURE and aging rats exhibit repetitive jaw movements (RJM) characterized by bursts of seemingly purposeless repetitive opening and closing of the jaw with occasional tongue protrusions. This behavior is induced by the D_1 receptor agonist SK&F 38393 and can be attenuated by the D_2 agonist quinpirole or the D_1 antagonist SCH-23390 and facilitated by D_2 receptor blockade with such selective antagonists as sulpiride or eticlopride (4,10,15–19). Thus, activation of D_1 receptors or D_2 blockade produces RJM while, conversely, stimulation of D_2 or inhibition of D_1 receptors decreases the behavior.

Repetitive jaw movements in rats can also be evoked with electrical stimulation of the ventral area in the mid and posterior regions of the striatum and globus pallidus (21). A bilateral injection of SK&F 38393 into the ventral area of the striatum can produce similar jaw movements and these can be potentiated by injection of sulpiride into the dorsal striatum (1,11–13). Moreover, jaw movements are antagonized by SCH-23390 injected into the ventral striatum (12). Thus, ventral striatal D_1 receptors are involved in the manifestation of RJM and are inhibited by D_2 activity in the dorsal striatum (11–13).

To explore these relationships further, we reduced the number of functional D_1 receptors by 70–80% with the peptide coupling agent *N*-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ), a substance known to deplete selectively CNS biogenic amine receptors (7,9,14). Surprisingly, despite the irreversible inactivation of 70–80% of caudate D_1 receptors, D_1 agonist-stimulated behavior remained unchanged (20).

The present report deals with efforts to further understand this paradox. We have characterized the EEDQ resistant subpopulation of D_1 receptors by means of binding studies and have examined the effect of administered EEDQ on D_1 receptors in other dopaminergically innervated brain areas.

METHOD

Sprague-Dawley rats weighing 250–280 g were used for all studies. Animals were kept on a 12 h light–dark cycle with free access to food and water. *D_1* receptor Inactivation: D_1 receptors were inactivated, selectively, by first administering IP a mixture of receptor antagonists one hour prior to treatment with EEDQ, 6 mg/kg. The mixture consisted of eticlopride, 500 μg/kg; ketanserine, 10 mg/kg, prazosin, 5 mg/kg and iodoxan, 1.25 mg/kg, to protect D_2, S_2, α_1 and α_2 receptors, respectively, against inactivation by EEDQ (14,20). Rats were sacrificed for binding experiments or tested for RJM 24 h after treatment with EEDQ. In one experiment half of the rats given the above drugs were treated with a second dose of EEDQ, 6 mg/kg 24 h after the first. These rats were sacri-

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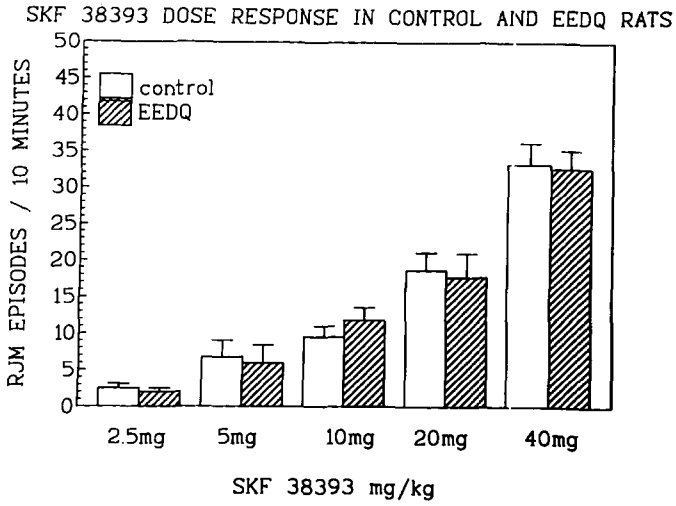

FIG. 1. Results were analyzed by two-way ANOVA. There were highly significant differences between doses but no differences between control (vehicle) and EEDQ-treated rats at any dose shown.

ficed 24 h after the second dose of EEDQ for use in binding studies.

Behavioral Testing

Twenty-four hours after a single dose of EEDQ or vehicle (ethanol, propylene glycol, water v/v/v ratio of 1 : 1 : 2 (14), 10 rats from each group were placed randomly in separate cages 7″ × 7″ × 10″ deep. Groups of two animals, whose prior treatment was not made known to the rater, were injected with one of five doses (see Fig. 1) of SK&F 38393 (not made known to the rater) every minute. Fifteen minutes after the first pair was injected, that pair was observed for RJM for 1 min. The rater then moved to the second pair and, in this manner, all pairs of rats were rated. This 10-pair rating system was repeated 9 times to obtain RJM/10 min/rat. The entire routine, repeated four times, amassed RJM/10-min scores for 10 rats per dose of SK&F 38393 for both vehicle and EEDQ treated rats. These rats were used only once for behavioral testing.

TABLE 1

EEDQ INACTIVATION OF D_1 RECEPTORS IN VARIOUS DOPAMINERGIC BRAIN AREAS

Area	% of Control ± SEM
Substantia nigra	33 ± 7.8*
Striatum:	
Dorsomedial	19 ± 7*
Ventromedial	21 ± 5.7*
Dorsolateral	19 ± 7*
Ventrolateral	11 ± 1.4*
Nucleus accumbens	28 ± 7.3*
Amygdala	23 ± 5.2*

Two-way ANOVA followed by Student's *t*-test revealed a $p \leq 0.001$ vs. appropriate control (control data not shown) for each brain area.

*N = 6 for each group.

TABLE 2

INACTIVATION OF D_1 DOPAMINE RECEPTORS BY EEDQ: EFFECT OF A SECOND DOSE OF EEDQ

Treatment	N	% of Maximal Binding ± SEM
Vehicle	6	100 ± 4
EEDQ 6 mg/kg single injection	6	22.9 ± 6*
EEDQ 6 mg/kg second injection 24 h following first injection	6	20.5 ± 9

*$p < 0.001$ vs.vehicle (ANOVA followed by Neuman-Kuel's analysis).

Binding Studies

In a different group of rats treated with EEDQ but not used in behavioral studies, animals were sacrificed by decapitation 24 h following a single or double EEDQ administration; brains were quickly removed and dopaminergic areas dissected according to Heffner et al. (8) and stored at −80° until assay. For the binding assay striatal tissue was homogenized with a Brinkman Polytron for 5 s at setting 6, in 0.05 M Tris-HCl buffer containing 1 mM EDTA, 5 mM kCl, 1.5 mM $CaCl_2$, 4 mM $MgCl_2$, and 12 mM NaCl, pH 7.4 (2). Homogenates were centrifuged at 20,000 × g for 10 min and the membrane pellets were resuspended in same buffer to yield the final tissue concentration of 4 mg/ml. For saturation analysis, 0.5 ml of ^{3}H-SCH 23390 (S.A. 70.3 Ci/mmol, Amersham) was used in the range of 0.05–2.0 nM. Nonspecific binding was defined in the presence of 10 μmolar (+)butaclamol. Tubes were incubated for 30 min at 37° and filtered through Whatman GF/B filters, which were washed three times with the same buffer, using a Brandel Cell Harvester. For competition binding studies membranes (0.5 ml) were incubated in 1 nM ^{3}H-SCH 23390 with dopamine as the displacing agent in the range of 10^{-3} to 10^{-11} M in sodium-free 0.05 M Tris buffer containing 0.1% ascorbate and 12 μm nialamide (3). The mixtures were incubated for 30 min at 37° and filtered as described above. Radioactivity in the filters was estimated by scintillation spectroscopy.

Statistical Analysis

In binding studies statistical analysis was carried out by two-way analysis of variance (ANOVA) followed by Newman-Kuel's or Student's *t*-test. In the competition binding

TABLE 3

SCATCHARD ANALYSIS OF STRIATAL D_1 RECEPTORS*

	Vehicle	EEDQ
B_{max}†	111.4* ± 3.5	31.2‡ ± 2.1
K_d	0.69 ± 0.01 nM	0.68 ± 0.01 nM

*N = 6 for each group.
†pmol/g wet wt ± SEM.
‡$p < 0.001$ compared with vehicle (Student's *t*-test).

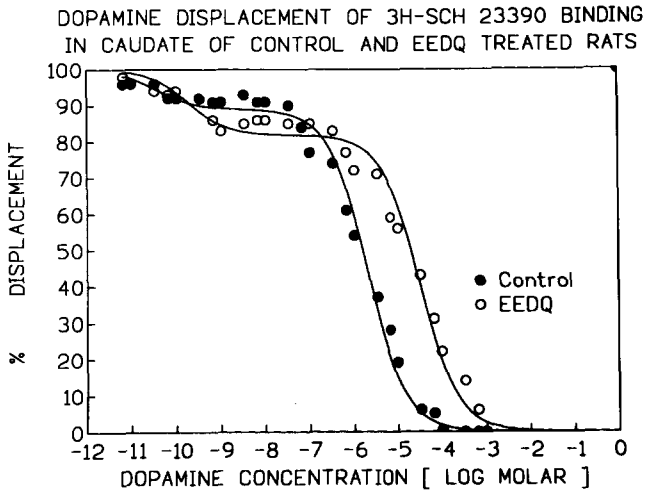

FIG. 2. Curves were fitted by Graphpad-In Plot analysis. A significant sixfold decrease (see text) in low affinity dopamine displacement of ^{3}H-SCH-23390 binding is evident in EEDQ-treated rats.

studies Graphpad In-Plot analysis was used. This method permits the analysis of two site competitive binding curves assuming that the two sites have equal affinity for the radioligand, but may have different affinities for the competing ligand (Graphpad Software, San Diego, CA 92121).

RESULTS

SK&F 38393, a specific D_1 receptor agonist, induced RJM in a dose-dependent manner in vehicle and EEDQ treated rats (Fig. 1). Results were analyzed by two-way ANOVA. There was a significant dose effect of EEDQ on D_1 receptor number, $F(1, 70) = 134.9, p < 0.001$. There were no significant group differences between control and EEDQ treated rats, at all doses of SK&F 38393 tested, $F(1, 70) = 0.51, p > 0.05$.

EEDQ administration resulted in a 65–90% inactivation of D_1 dopamine receptors in the striatum, amygdala, nucleus accumbens, and substantia nigra (Table 1). A second administration of EEDQ, 24 h later did not further reduce the D_1 receptor number (Table 2). Results were analyzed by two-way ANOVA followed by Newman-Keul's analysis. EEDQ produced a significant decrease D_1 receptor number in the striatum following a double administration of EEDQ. Scatchard analysis of ^{3}H-SCH-23390 binding to D_1 dopamine receptors in the caudate of vehicle and EEDQ treated rats displaced with (+)butaclamol revealed a single binding site in each group and a 73% decrease in B_{max} without change in affinity after EEDQ (Table 3). Graphpad In-Plot analysis of dopamine displacement of ^{3}H-SCH 23390 binding to striatal membranes revealed two populations of D_1 dopamine receptors with similar ratios in vehicle and EEDQ treated rats. However, the affinity of the major portion of the D_1 receptors (90% of the total specific binding) decreased by about sixfold after EEDQ administration (Fig. 2) (K_i: control, 785 nM; EEDQ, 4510 nM). These differences were statistically significant when analyzed with Student's t-test. The IC_{50} values of high and low affinities for the D_1 receptors were individually obtained for each displacement curve in groups of 8–10 curves and analyzed by Student's t-test. Changes, if any, in the high affinity site, which represented about 10% of the total population, were difficult to assess or interpret.

DISCUSSION

Of major interest in the present study is the finding that EEDQ pretreatment resulted in a 65–90% drop in D_1 receptor number in caudate and other dopaminergically innervated CNS areas without change in the maximum response or ED_{50} for SK&F 38393-inducible RJM. From saturation binding studies it was found that the population of D_1 receptors remaining after EEDQ was identical to that of controls as regards K_d, and the EEDQ-resistant population behaved similarly toward displacement of ^{3}H-SCH 23390 by (+)butaclamol. However, the affinity for dopamine in displacement of ^{3}H-SCH 23390 dropped by approximately a half order magnitude in the EEDQ group.

Strong and substantial evidence links RJM with D_1 receptor activity (5,6,17–20). Likewise, there is abundant evidence for the reciprocal relationship between D_2 activity and RJM, which, moreover, has a sound biochemical basis in that D_2 stimulation by quinpirole in the caudate can reduce the production of c-AMP inducible by D_1 stimulation (21). Therefore, the fact that the population of receptors remaining after EEDQ is sufficient to promote a full RJM response is compatible with the proposal that there is a D_1 receptor reserve for this behavior; however it is known that the maximum production of c-AMP by caudate homogenates is directly related to the number of D_1 receptors; that is EEDQ, in fact, lowers dopamine stimulated c-AMP production (9). Thus, on the basis of present information it appears that the RJM response is not linked to the production of D_1 inducible c-AMP or that there is a subpopulation of EEDQ resistant D_1 dopamine receptors that is responsible for this D_1 mediated behavior. Additional evidence for the presence of more than one D_1 receptor stems from a recent observation (15) that the D_1 agonist, A-68930, induces oral behaviors *not* readily blocked by SCH 23390.

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