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Dopamine D₂ Receptors: A Potential Pharmacological Target for Nomifensine and Tranylcypromine But Not Other Antidepressant Treatments

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MARTIN, K. F., I. PHILLIPS, S. C. CHEETHAM AND D. J. HEAL. *Dopamine D₂ receptors: A potential pharmacological target for nomifensine and tranylcypromine but not other antidepressant treatments.* PHARMACOL BIOCHEM BEHAV 51(4) 565-569, 1995. — Treatment for 1 or 14 days by IP injection with the antidepressants, amitriptyline (10 mg/kg), bupropion (30 mg/kg), desipramine (10 mg/kg), GBR 12909 (10 mg/kg), sibutramine HCl (3 mg/kg), mianserin (5 mg/kg), and zimeldine (10 mg/kg), did not affect the number or affinity of dopamine D₂ receptors determined by [³H]raclopride binding to rat striatal membranes. Similarly, neither did a single, nor repeated (five times over 10 days), electroconvulsive shock, given under halothane anaesthesia, have any effect on [³H]raclopride binding parameters. By contrast, the noradrenaline and dopamine reuptake inhibitor, nomifensine (5 mg/kg), and the monoamine oxidase inhibitor, tranylcypromine (5 mg/kg), decreased the number of dopamine D₂ receptors by 12% and 11%, respectively, when given for 14 days. Administration of the D₂ receptor antagonist, haloperidol (1 mg/kg), for 14 days increased the number of [³H]raclopride binding sites by 17%. Thus, the data demonstrate that although nomifensine and tranylcypromine decrease D₂ receptor number after 14 days administration, this adaptive change is not observed with other antidepressant treatments. However, the findings do not preclude a contribution of altered dopamine D₂ receptor function to the efficacy of those drugs with potent effects on dopaminergic neuronal function.

Antidepressant drugs Electroconvulsive shock ECS Antipsychotic drugs Neuroleptic drugs
D₂ receptors

THE ROLE of dopaminergic systems in the etiology of affective disorders has received little attention until recently. This neglect has been because noradrenaline and 5-hydroxytryptamine (5-HT) were originally implicated in the biochemical basis of depression (7,16,28). However, the clinical efficacy of newer antidepressant drugs with pharmacological effects involving the inhibition of dopamine reuptake (e.g., nomifensine and bupropion) has aroused interest in the role of dopamine in the mechanism of action of antidepressants. In addition to antidepressant drugs, electroconvulsive shock (ECS) has also been shown to enhance dopamine receptor-mediated behaviors [for review, see (9)]. In the case of ECS, the results of biochemical and behavioral studies would suggest that these

changes result from increased D₁, but not D₂, function (25,30).

Willner (33) reviewed considerable evidence to indicate that chronic antidepressant treatments increased central dopaminergic function, although the site of these effects was unclear. Thus, apomorphine- and *d*-amphetamine-induced locomotor responses are reported to be increased following repeated administration of a range of antidepressant drugs (20-22,31).

Dopamine receptors have been divided into two major subtypes: D₁ receptors, which mediate stimulation of adenylate cyclase activity, and D₂ receptors, which can be negatively coupled to this enzyme (13). More recently, using molecular biological techniques, five dopamine receptors have been cloned. These can be assigned to one of the two classes de-

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scribed by Keabian and Calne (13). Thus, the D₁-like receptors include D₁ and D₅, while the D₂-like receptors include D₂, D₃, and D₄ [see (29) for review].

Limited receptor binding studies have suggested that antidepressant treatments do not alter dopamine D₂ receptor binding (2,14,17,26). By contrast, neuroleptic drugs such as haloperidol and sulpiride have been reported to produce reproducible increases in the number of D₂ binding sites (6,18). These studies, however, generally used [³H]spiperone to label dopamine D₂ receptors, and this approach is problematic because spiperone has affinity for other neurotransmitter receptors including 5-HT₁-like receptors (32). In addition, recent evidence has shown that dopamine D₂ receptor number is increased in the striatum of depressed suicide victims (3). Therefore, we have now used the more selective, high affinity D₂ receptor ligand, [³H]raclopride (15), to determine the effects of a comprehensive range of antidepressant treatments with diverse modes of action on striatal dopamine D₂ receptor binding in the rat. For comparison, we have also determined the effects of the antipsychotic haloperidol.

METHOD

Male CD1 Sprague-Dawley-derived rats (Charles River, Margate), initially weighing 100–120 g, were used. They were housed in a temperature- and light-controlled room (21°C; 12L : 12D cycle, lights on 0800 h) and had free access to food and water.

Groups of 10 rats received either 1 or 14 daily injections (2 ml/kg, IP) of drugs or saline. In a second series of experiments, halothane-anesthetized rats received a single ECS (200 V, 2 s), or five shocks spread over 10 days via ear clip electrodes, as described by Heal et al. (12). Each shock resulted in a full tonic-clonic seizure. Control rats were anesthetized with halothane and had ear clip electrodes applied, but did not receive ECS treatment.

Between 23 and 25 h after the final treatments, animals were stunned and then killed by decapitation. The striata were removed, frozen in liquid nitrogen, and stored at –80°C until assayed. The number (B_{\max}) and affinity (K_d) of dopamine D₂ receptors was determined using six-point saturation binding analysis with [³H]raclopride on striatal membranes from individual rats, as described below. Tissue samples from control and treated rats were assayed in parallel.

The dopamine D₂ receptor binding assay was performed using [³H]raclopride (60 Ci/mmol, DuPont Ltd, UK) as previously described (23). On the day of analysis, the individual striata were homogenised in 40 vol of ice-cold 50 mM Tris-HCl (pH 7.4 at 25°C) using a Potter-S homogeniser (setting 5.5 for 10 strokes) and the homogenates centrifuged at 30,000 × g for 20 min at 4°C. The pellet was resuspended in 20 vol of 50 mM Tris HCl (pH 7.4 at 25°C) containing 120 mM NaCl, 5 mM KCl, 2 mM CaCl₂, 1 mM MgCl₂, and 10 μM pargyline for use in the binding assay.

Duplicate tubes containing 50 μl of membrane suspension, 50 μl of [³H]raclopride (0.3–10 μM final concentration), 50 μl of Tris-HCl buffer (as used in final membrane suspension) with or without (–)-sulpiride (1 μM final concentration to define specific binding), and 350 μl Tris-HCl buffer (as used in final membrane suspension) yielding a 500 μl final incubation volume were incubated at room temperature (~23°C) for 60 min. Membrane-bound radioactivity was recovered by filtration under vacuum through Skatron receptor binding filter mats (catalog No. 7031) using a Skatron cell harvester. Radioactivity was determined using a liquid scintillation counter.

Equilibrium dissociation constants (K_d) and maximum number of binding sites (B_{\max}) were determined by nonlinear regression analysis fitting to a one-site model (24). Scatchard plots were also made, and when the correlation coefficient was less than 0.95, the data were rejected (13% of all animals treated). These rejections are reflected in the n values in Tables 1 and 2. The method of Lowry et al. (19) was used to determine protein content of each incubation.

Data were analyzed using Student's unpaired t -test, and the null hypothesis was rejected when $p < 0.05$. However, when two treatment groups were compared with a single control group, the level of significance for rejection of the null hypothesis was set at $p < 0.01$.

The following drugs were used (source in brackets): zimeldine (HCl)₂ (Astra), sibutramine HCl (Boots Pharmaceuticals), bupropion (Burroughs Wellcome), nomifensine (Hoechst), mianserin (Organon), tranlycypromine SO₄ (SKB), haloperidol (Searle), amitriptyline HCl, desipramine HCl, sulpiride (Sigma), and 1,2(bis(4-fluorophenyl)-methoxy)-ethyl-4-(3-phenyl-propyl)piperazine (GBR 12909, R.B.I.). All drugs were dissolved in 0.9% saline and injected IP. Reagents were analytical grade and purchased from B.D.H., Fisons, or Sigma.

RESULTS

Effects of Antidepressant Drugs

None of the antidepressant drugs had any effect on the number (B_{\max}) or affinity (K_d) of dopamine D₂ receptors in the striatum after a single injection (Table 1).

Administration of the selective noradrenaline reuptake inhibitor, desipramine (10 mg/kg), the selective 5-HT reuptake inhibitor, zimeldine (10 mg/kg), or the noradrenaline and 5-HT reuptake inhibitors, amitriptyline (10 mg/kg) and sibutramine (3 mg/kg), for 14 days did not alter the B_{\max} or K_d for [³H]raclopride binding to dopamine D₂ receptors (Table 1). By contrast, nomifensine (5 mg/kg), a noradrenaline and dopamine reuptake inhibitor, decreased the number of striatal D₂ receptors by approximately 12% (control $B_{\max} = 243 \pm 7$ fmol/mg protein, $n = 10$; $p < 0.01$, Table 1). This treatment did not alter the K_d value for [³H]raclopride binding. Surprisingly, the selective dopamine reuptake inhibitors, bupropion (30 mg/kg) and GBR 12909 (10 mg/kg) had no significant effects on D₂ receptor binding (Table 1). The atypical antidepressant, mianserin (5 mg/kg), when administered for 14 days, was also without effect on [³H]raclopride binding (Table 1). Tranlycypromine (5 mg/kg), an irreversible inhibitor of monoamine oxidase (MAO), significantly ($p < 0.01$) decreased the number of D₂ binding sites by 11% (control $B_{\max} = 256 \pm 8$ fmol/mg protein, $n = 10$; Table 1), but had no effect on the K_d value (Table 1) when given once daily for 14 days.

Effects of Haloperidol

Haloperidol (1 mg/kg) is an antagonist at dopamine D₂ receptors. Following repeated daily doses (14) of haloperidol, the number of dopamine D₂ receptors was increased by 17% (control $B_{\max} = 230 \pm 4$ fmol/mg protein, $n = 7$, $p < 0.05$; Table 1). This drug did not alter the K_d value (Table 1).

Effect of Electroconvulsive Shock

The binding of [³H]raclopride to striatal membranes was measured 24 h after a single ECS or five shocks spread over a 10-day period. No changes in either the B_{\max} or K_d were found in comparison with halothane-anesthetized controls (Table 2).

TABLE 1
EFFECT OF ANTIDEPRESSANT AND ANTIPSYCHOTIC DRUG TREATMENT ON THE B_{max} AND K_d VALUES OF [³H]RACLOPRIDE BINDING TO RAT STRIATAL MEMBRANES

Treatment (Dose, mg/kg IP)	1 Day			14 Days		
	B_{max}	K_d	n	B_{max}	K_d	n
Saline (pooled control)	229 ± 1	1.68 ± 0.05	48	249 ± 3	1.66 ± 0.02	48
Desipramine (10)	228 ± 16	1.83 ± 0.08	8	245 ± 10	1.78 ± 0.09	10
Zimeldine (10)	244 ± 6	2.00 ± 0.11	7	231 ± 9	1.53 ± 0.09	10
Amitriptyline (10)	248 ± 15	1.72 ± 0.14	8	233 ± 17	1.46 ± 0.05	8
Sibutramine (3)	243 ± 12	1.93 ± 0.08	8	226 ± 7	1.64 ± 0.08	10
Nomifensine (5)	248 ± 7	1.69 ± 0.03	10	209 ± 5**	1.31 ± 0.04	10
Bupropion (30)	227 ± 10	1.37 ± 0.08	9	264 ± 8	1.63 ± 0.11	4
GBR 12909 (10)	220 ± 10	1.80 ± 0.06	10	231 ± 22	1.88 ± 0.11	3
Mianserin (5)	228 ± 8	1.96 ± 0.15	9	245 ± 8	1.73 ± 0.08	9
Tranlycypromine (5)	228 ± 10	1.95 ± 0.13	8	202 ± 8**	1.50 ± 0.13	6
Haloperidol (1)		ND		277 ± 12*	1.72 ± 0.12	9

Each value is the mean ± SE mean; B_{max} = fmol/mg protein, K_d = nM, ND = not determined.
* p < 0.05, † p < 0.01, vs. own control, Student's t -test. Control values have been pooled for clarity.

The number of D₂ sites did, however, appear to be higher in membranes prepared from rats receiving repeated halothane and halothane/ECS when compared to those receiving a single treatment. It is possible that this may be related to repeated exposure to halothane anesthesia, but it is more likely to be due to the use of a different batch of radioligand for these experiments.

DISCUSSION

The primary aim of the present study was to determine the effects of a range of typical and atypical antidepressant treatments, including ECS, on [³H]raclopride binding to dopamine D₂ receptors. Our second aim was, using the novel D₂ ligand, [³H]raclopride, to confirm the well-documented increases in D₂ receptor binding following repeated haloperidol treatment. The results showed that this antipsychotic drug increased dopamine D₂ receptor number as predicted from studies using other D₂ receptor ligands. The magnitude of this increase was similar to that previously reported Liskowsky and Potter (19).

With respect to antidepressants, however, our results generally confirm the finding of previous studies (2,14,17,26) that antidepressant treatments do not alter dopamine D₂ receptor binding. Furthermore, the present data also agree with Klimek and Nielsen (14), who showed that bupropion, a dopamine reuptake inhibitor, had no effect on dopamine D₂ receptors. We have also found that GBR 12909, a more potent, selective

dopamine reuptake inhibitor (1), had no significant effects on dopamine D₂ receptor binding. In contrast, we have found that other drugs that are capable of enhancing dopamine function such as the noradrenaline plus dopamine reuptake inhibitor, nomifensine, and the MAO inhibitor, tranlycypromine, significantly decreased the number of [³H]raclopride binding sites. Dopaminergic function determined by measurement of tissue 3-methoxytyramine (3-MT) is markedly enhanced after inhibition of MAO by tranlycypromine (10). Bupropion, on the other hand, has no significant effects on striatal 3-MT levels (10) or dopamine release in vitro (11). Microdialysis studies have shown that bupropion (5) and GBR 12909 (33) only increase extracellular dopamine levels. Several studies have shown nomifensine to have some direct dopamine agonist properties in addition to its well-known ability to inhibit dopamine reuptake and release dopamine from nerve terminals (4,8,27). It is possible, therefore, that the decreased dopamine D₂ receptor binding following repeated administration of nomifensine and tranlycypromine antidepressants is due to their enhanced ability to stimulate D₂ receptors compared with bupropion and GBR 12909. Such an effect is analogous to the increase in binding observed after chronic treatment with the D₂ receptor antagonist, haloperidol [this study, (6,17)].

Although the results presented here are different from those reported in depressed suicide victims (3), it is noteworthy that these authors did not distinguish between different antidepressant drugs in the treated group. The affinity of dopamine D₂ receptors in antidepressant-treated suicide victims

TABLE 2
EFFECT OF SINGLE AND REPEATED ECS ON B_{max} AND K_d OF [³H]RACLOPRIDE BINDING TO RAT STRIATAL MEMBRANES

Treatment	Single Treatment			Repeated Treatment		
	B_{max}	K_d	n	B_{max}	K_d	n
Halothane	204 ± 11	1.35 ± 0.06	9	286 ± 6	1.21 ± 0.02	12
ECS	227 ± 9	1.33 ± 0.05	10	294 ± 8	1.23 ± 0.04	12

Each value is the mean ± SE mean; B_{max} = fmol/mg protein, K_d = nM.

was also higher than in controls, and this may have contributed to the change in receptor number. In addition, it is possible that an interaction between the pathology of the disease and the antidepressant drugs used may have resulted in increased dopamine D₂ receptor number. This possibility has not been addressed by the experiments reported here.

The present data showing that repeated ECS did not alter dopamine D₂ receptor number are not only in agreement with other binding studies (2), but also agree with the results from behavioral studies (30). These authors found that the behavioral response to the selective dopamine D₂ receptor agonist, RU 24213, was not altered following repeated ECS. However, they did find that D₁ receptor-mediated behaviors were enhanced. Biochemical studies, which have determined the effects of repeated ECS on the modulation of adenylate cyclase by D₂ receptor stimulation, have also shown that D₂ receptor function is not altered after repeated ECS (25). These results, therefore, are in accord with our findings, reported here, that D₂ receptor binding is not altered by ECS.

In conclusion, therefore, the present data are generally in agreement with previous animal studies carried out using ligands less selective than [³H]raclopride for D₂ receptors, and which concluded that antidepressant treatments do not alter D₂ receptors. However, we have found that some drugs, such as nomifensine and tranylcypromine, which cause marked increases in dopaminergic function, lead to a decrease in the density of striatal D₂ sites in the rat brain. This suggests that although induction of changes in dopamine D₂ receptors is not an essential property of antidepressant treatments, alterations in D₂ receptors cannot be precluded from a contribution to the efficacy of drugs with specific, potent effects on dopaminergic neuronal function.

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REFERENCES

- Andersen, P. H. The dopamine uptake inhibitor GBR 12909: Selectivity and molecular mechanism of action. *Eur. J. Pharmacol.* 166:493-504; 1989.
- Bergstrom, D. A.; Kellar, K. J. Effect of electroconvulsive shock on monoaminergic receptor binding sites in rat brain. *Nature* 278: 464-466; 1979.
- Bowden, C.; Cheetham, S. C.; Crompton, M. R.; Katona, C. L. E.; DePaermentier, F.; Theodorou, A. E.; Horton, R. W. Dopamine D₁ and D₂ receptor binding sites in depressed suicide victims. *Br. J. Pharmacol.* 111:2P; 1994.
- Braestrup, L. Biochemical differentiation of amphetamine vs. methylphenidate and nomifensine in rats. *J. Pharmacol.* 29:463-470; 1977.
- Brown, E. E.; Damsma, G.; Cumming, P.; Fibiger, H. C. Interstitial 3-methoxytyramine reflects striatal dopamine release: An in vivo microdialysis study. *J. Neurochem.* 57:701-707; 1991.
- Burt, D. R.; Creese, I.; Snyder, S. H. Antischizophrenic drugs: Chronic treatment elevates dopamine receptor binding in brain. *Science* 196:326-328; 1977.
- Curzon, G. Tryptophan pyrrolase—A biochemical factor in depressive illness. *Br. J. Psychiatry* 115:1367-1374; 1969.
- Gianutsos, G.; Morrow, G.; Light, S.; Sweeney, M. I. Dopaminergic properties of nomifensine. *Pharmacol. Biochem. Behav.* 17: 957-964; 1982.
- Gleiter, C. H.; Nutt, D. J. Chronic electroconvulsive shock and neurotransmitter receptors. An update. *Life Sci.* 44:985-1006; 1989.
- Heal, D. J.; Frankland, A. T. J.; Buckett, W. R. A new and highly sensitive method for measuring 3-methoxytyramine using HPLC with electrochemical detection. Studies with drugs which alter dopamine metabolism in the brain. *Neuropharmacology* 29: 1141-1150; 1990.
- Heal, D. J.; Frankland, A. T. J.; Gosden, J.; Hutchins, L. J.; Prow, M. R.; Luscombe, G. P.; Buckett, W. R. A comparison of the effects of sibutramine hydrochloride, bupropion and methamphetamine on dopaminergic function: Evidence that dopamine is not a pharmacological target for sibutramine. *Psychopharmacology (Berlin)* 107:303-309; 1992.
- Heal, D. J.; Prow, M. R.; Buckett, W. R. Effects of antidepressant drugs and electroconvulsive shock on pre- and postsynaptic α_2 -adrenoceptor function in the brain: Rapid down-regulation by sibutramine hydrochloride. *Psychopharmacology (Berlin)* 103: 251-257; 1991.
- Kebabian, J. W.; Calne, D. B. Multiple receptors for dopamine. *Nature* 227:93-96; 1979.
- Klimek, V.; Nielsen, M. Chronic treatment with antidepressants decreases the number of [³H]SCH 23390 binding sites in the rat striatum and limbic system. *Eur. J. Pharmacol.* 139:163-169; 1987.
- Köhler, C.; Hall, H.; Ögren, S.-V.; Gawell, L. Specific in vitro and in vivo binding of [³H]raclopride. A potent substituted benzamide drug with high affinity for dopamine D₂ receptors in the rat brain. *Biochem. Pharmacol.* 34:2251-2259; 1985.
- Lapin, I. P.; Oxenkrug, G. F. Intensification of central serotonergic processes as a possible determinant of thymoleptic effort. *Lancet* i:132-136; 1969.
- Lerer, B.; Jabotinsky-Rubin, K.; Bannet, J.; Ebstein, R. P.; Belmaker, R. H. Electroconvulsive shock prevents dopamine receptor supersensitivity. *Eur. J. Pharmacol.* 80:131-134; 1982.
- Liskowsky, D. R.; Potter, L. T. Dopamine D₂ receptors in the striatum and frontal cortex following chronic administration of haloperidol. *Neuropharmacology* 26:481-483; 1987.
- Lowry, O. H.; Rosebrough, N. J.; Farr, A. L.; Randall, R. J. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* 193:265-275; 1951.
- Maj, J.; Papp, M.; Skuza, G.; Bigajska, K.; Zazula, M. The influence of repeated treatment with imipramine, (+)- and (-)-oxaprotiline on behavioural effects of dopamine D₁ and D₂ agonists. *J. Neural Transm.* 76:29-38; 1989.
- Maj, J.; Rogoz, Z.; Skuza, G.; Sowinska, H. Repeated treatment with antidepressant drugs increases the behavioural response to apomorphine. *J. Neural Transm.* 60:273-282; 1984.
- Maj, J.; Wedzony, K. Repeated treatment with imipramine or amitriptyline increases the locomotor response of rats to (+)-amphetamine given into the nucleus accumbens. *J. Pharm. Pharmacol.* 37:362-364; 1985.
- Martin, K. F.; Phillips, I.; Cheetham, S. C.; Buckett, W. R. Sodium dependent binding of [³H]raclopride to rat striatal membranes. *Br. J. Pharmacol.* 100:405P; 1990.
- Munson, D. J.; Rodbard, D. LIGAND: A versatile computerized approach for characterization of ligand-binding systems. *Anal. Biochem.* 107:220-239; 1980.
- Newman, M. E.; Lerer, B. Effects of chronic electroconvulsive shock on D₁ and D₂ dopamine receptor-mediated activity of adenylate cyclase in homogenates of striatum and limbic forebrain of rat. *Neuropharmacology* 28:787-790; 1989.
- Peroutka, S. J.; Snyder, S. H. Chronic antidepressant treatment decreases spiperidol-labelled serotonin receptor binding. *Science* 210:88-90; 1980.
- Scheinin, M.; Lindberg, R.; Syvalathi, E.; Hietala, J.; Pihlajamacki, K.; Scheinin, H. Noradrenergic and dopaminergic effects of nomifensine in healthy volunteers. *Clin. Pharmacol. Ther.* 40: 88-96; 1987.

28. Schildkraut, J.; Kety, S. Biogenic amines and emotion. *Science* 156:21-30; 1967.
29. Seeman, P.; Van Tol, H. M. Dopamine receptor pharmacology. *Trends Pharmacol. Sci.* 15:264-270; 1994.
30. Sharp, T.; Kingston, J.; Grahame-Smith, D. G. Repeated ECS enhances dopamine D₁ but not D₂ agonist-induced behavioural responses in rats. *Psychopharmacology (Berlin)* 100:110-114; 1990.
31. Spyrali, C.; Fibiger, H. C. Behavioural evidence for supersensitivity of postsynaptic dopamine receptors in the mesolimbic system after chronic administration of desipramine. *Eur. J. Pharmacol.* 74:195-206; 1981.
32. Strange, P. G. Isolation and characterisation of dopamine receptors. In: Creese, I.; Fraser, C. M., eds. *Receptor biochemistry and methodology*, vol. 8: Dopamine receptors. New York: Alan R. Liss, Inc.; 1987:29-45.
33. Westerink, B. H. C.; Damsma, G.; DeVries, J. B.; Koning, H. Dopamine reuptake inhibitors show inconsistent effects on the in vivo release of dopamine as measured by intracerebral dialysis in the rat. *Eur. J. Pharmacol.* 135:123-128; 1987.
34. Willner, P. Dopamine and depression: a review of recent evidence. III. The effects of antidepressant treatments. *Brain Res. Rev.* 6:237-246; 1983.