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Characterization of the Binding of SCH 39166 to the Five Cloned Dopamine Receptor Subtypes

M. A. B. TICE,[1] T. HASHEMI, L. A. TAYLOR, R. A. DUFFY AND R. D. McQUADE

Schering-Plough Research Institute, 2015 Galloping Hill Road, Kenilworth NJ 07033

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TICE, M. A. B., T. HASHEMI, L. A. TAYLOR, R. A. DUFFY AND R. D. McQUADE. *Characterization of the binding of SCH 39166 to the five cloned dopamine receptor subtypes.* PHARMACOL BIOCHEM BEHAV **49**(3) 567–571, 1994.—Characterization studies were conducted on the five cloned dopamine receptor subtypes (D_1–D_5) using the novel D_1-selective antagonist, SCH 39166, as well as other related benzazepines and dopaminergic agents. The results demonstrate that SCH 39166 exhibits saturable, high-affinity binding to the D_1 and D_5 receptors, but binds with low affinity to the D_2, D_3, and D_4 receptors. In contrast, the D_2 antagonist haloperidol showed low affinity for the "D_1-like" receptors and high affinity for the "D_2-like" receptors. A series of agonists was also evaluated and the D_5 receptor subtype displayed a two-site fit for the endogenous agonist dopamine, as well as for the agonist apomorphine. Differences in agonist binding among the D_1-like receptors reflect the importance of the nonconserved amino acid substitutions.

Dopamine receptors SCH 39166 Dopamine

HISTORICALLY, dopamine is believed to exert its physiological effects through interaction with two basic types of guanine nucleotide binding (G) protein-coupled receptors, the D_1 and D_2 dopamine receptors. These receptors are of interest because of their proposed involvement in neuropsychiatric disorders such as schizophrenia. Recently, molecular cloning and sequence homology studies have revealed the existence of at least five genes, each encoding a distinct dopamine receptor (5–8,15,19). The genes encoding the D_2, D_3, and D_4 receptors all contain introns, whereas the D_1 and D_5 genes are intronless. These five receptor subtypes have been subsequently grouped into two families of receptor proteins: those displaying "D_1-like" or "D_2-like" features.

The D_1 and D_5 receptors have been shown to stimulate adenylyl cyclase and to activate cyclic AMP-dependent protein kinases (5,16). In contrast, D_2 receptors, which differ greatly in their pharmacological and biochemical characteristics from D_1 receptors, inhibit adenylyl cyclase. It has been proposed that the lengths of the third cytoplasmic (i3) loop and the *C*-terminal tail determine the second messenger characteristics displayed by the dopamine receptors (13). The D_3 and D_4 receptors display D_2 pharmacology and structural characteristics of a long i3 loop and a short *C*-terminal tail. The D_4 receptor has been shown to inhibit adenylyl cyclase, whereas no conclusive data have demonstrated the second messenger selectivity of the D_3 subtype (13).

Currently available therapeutic agents used for the treatment of schizophrenia bind preferentially to the D_2-like dopamine receptors. However, treatment with these D_2-selective antagonists has long been associated with both short-term (i.e., extrapyramidal) side effects (EPS) and long-term (i.e., tardive dyskinesia) motor side effects due to their D_2 receptor blocking activity. Blockade of EPS has been accomplished in humans and monkeys by administration of anticholinergic drugs, suggesting the involvement of the cholinergic system in this movement disorder, and biochemical evidence has demonstrated that D_2 antagonists increase cholinergic neurotransmission.

The first specific D_1 receptor antagonist, SCH 23390, was reported not to evoke any abnormal movements in Cebus monkeys (1,3). In contrast, the D_2-selective neuroleptic, haloperidol, did produce abnormal movements that were qualitatively similar to those produced in humans (i.e., EPS). More recently, a novel benzonaphthazepine, SCH 39166 [(−)-*trans*-

[1] To whom requests for reprints should be addressed.

6,7,7*a*,8,9,13*b*-hexahydro-3-chloro-2-hydroxy-*N*-methyl-5*H*-benzo-[*d*]-azepine] (Fig. 1), was also shown to be a potent and selective dopamine D_1 antagonist (2). Coffin et al. reported that SCH 39166 administered to Cebus monkeys did not produce any abnormal movements and possessed a longer duration of action than SCH 23390 in primates (4). Repeated administration of SCH 39166 has also been capable of reversing abnormal movements in haloperidol-sensitized Cebus monkeys (9). These results continue to support the theory that D_1 receptors are not involved in the extrapyramidal side effects associated with administration of dopaminergic antagonists. Pharmacological characterization of SCH 23390, a potent D_1 antagonist, revealed affinity for both 5-HT_{1C} (17) and 5-HT_2 receptors (12), whereas SCH 39166 exhibited significantly less affinity for these serotonin receptor subtypes. This observation was especially relevant in vivo, where SCH 39166 was demonstrated to exhibit a selectivity greater than 100-fold in terms of its binding to D_1 and 5-HT_2 receptors (11). The low EPS liability, duration of action, and selectivity of SCH 39166 for D_1 binding sites suggest that it displays a unique pharmacological profile.

In the current report, pharmacological characterization studies were conducted to determine the selectivity of SCH 39166 for the cloned dopamine receptor subtypes as well as the affinities of various other dopaminergic agonists and antagonists.

METHOD

Materials

[*N*-methyl-^{3}H]SCH 23390 (85.6 Ci/mmol) and [^{3}H]spiperone (24 Ci/mmol) were purchased from New England Nuclear/Dupont (Boston, MA). All tissue culture reagents were obtained from Gibco BRL (Grand Island, NY) and all other chemical reagents were purchased from Sigma Chemical Co. (St. Louis, MO). All SCH compounds were synthesized under the direction of Dr. J. Berger. Membranes containing D_3 dopamine receptors were purchased from Research Biochemicals Incorporated (Natick, MA).

Cell Culture

Stable cell lines expressing the D_1, D_2, and D_5 receptors and transiently transfected cell membranes containing the D_4 receptor were obtained from Drs. O. Civelli and D. Grandy (Vollum Institute, Portland, OR). The human dopamine D_1 and D_5 receptors were subcloned into the pCD-PS expression vector and stably expressed in Ltk$^-$ and GH_4Cl cells, respectively (5,7,16). The human D_2 (long form) receptor was subcloned into the pZem228 expression vector and stably expressed in Ltk$^-$ cells (6). The D_2 cell line was grown in the presence of 500 nM sulpiride, a dopamine antagonist, to prevent growth inhibition by endogenous catecholamines (personal communication, O. Civelli). These cells were maintained in appropriate growth media detailed by American Tissue Culture Collection (Rockville, MD) with 400 μg/ml of Geneticin (G418, Schering-Plough).

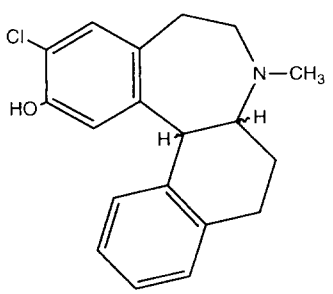

FIG. 1. Structure of SCH 39166.

Membrane Preparation and Radioligand Binding Assay

Membranes were prepared with minor modifications according to the method described by Zhou et al. (20). The cells expressing the cloned dopamine receptors were homogenized in 10 volumes of 50 mM Tris-HCl, 6 mM $MgCl_2$, and 1 mM EDTA, pH 7.4, with a Dounce homogenizer. The homogenates were centrifuged at 40,000 × *g* for 1 h, at 4°C. All membrane pellets were resuspended in the homogenization buffer. Protein assays were performed using the BCA protein assay reagent from Pierce (Rockford, IL).

Saturation studies were performed on the D_1 and D_5 receptors by incubating 7.5 and 15 μg, respectively, of membrane protein with increasing concentrations of [^{3}H]SCH 23390 in a total volume of 1 ml at 37°C for 1 h. In competition experiments, various dopamine agonists and antagonists were incubated with 0.7 nM [^{3}H]SCH 23390 and the same membrane protein concentrations as above. Nonspecific binding was defined in the presence of 10 μM SCH 23390. Radioligand binding to D_2 receptors was conducted at 37°C for 1 h, whereas receptor binding to D_3 and D_4 membranes was determined at 37°C for 20 min, all in a final volume of 1.0 ml. For all three receptors, saturation studies were carried out by incubating 20–30 μg of membrane protein in the presence of increasing concentrations of [^{3}H]spiperone (0.02–2.0 nM). Competition studies were conducted using a concentration of 0.3 nM [^{3}H] spiperone. The binding buffer utilized for all studies was 50 mM Tris-HCl, pH 7.4, containing 0.9% NaCl, 0.001% BSA, and 0.25% ascorbic acid. Nonspecific binding was determined in the presence of 10 μM (+)butaclamol. Prior to filtration, Whatman GF/B filters were soaked in the binding buffer for 10 min.

All binding studies were terminated by rapid filtration using a Brandel cell harvester. The filters were washed with 10-ml ice-cold binding buffer. Bound radioligand was determined by liquid scintillation spectrometry. Affinity constants for all tested compounds were determined by the EBDA computer program (10).

RESULTS

D_1 and D_5 Receptor Binding

Specific binding of [^{3}H]SCH 23390 to transfected Ltk$^-$ cells expressing the D_1 receptor was found to exhibit a K_d of 1.5 nM with a receptor density (B_{max}) of 3200 fmol/mg of protein. The D_5 receptor expressed in GH_4Cl cells bound [^{3}H]SCH 23390 with similar high affinity, saturable binding, exhibiting a K_d of 1.5 nM and a B_{max} of 1700 fmol/mg of protein.

To establish the pharmacological profile of the D_1 and D_5 receptors, experiments were performed with agonists and antagonists in competition with [^{3}H]SCH 23390. The novel D_1-selective antagonist, SCH 39166, bound to both receptor subtypes with high affinity, yielding K_i values of 5.0 and 4.4 nM and Hill coefficients of 0.95 ± 0.11 and 0.95 ± 0.15,

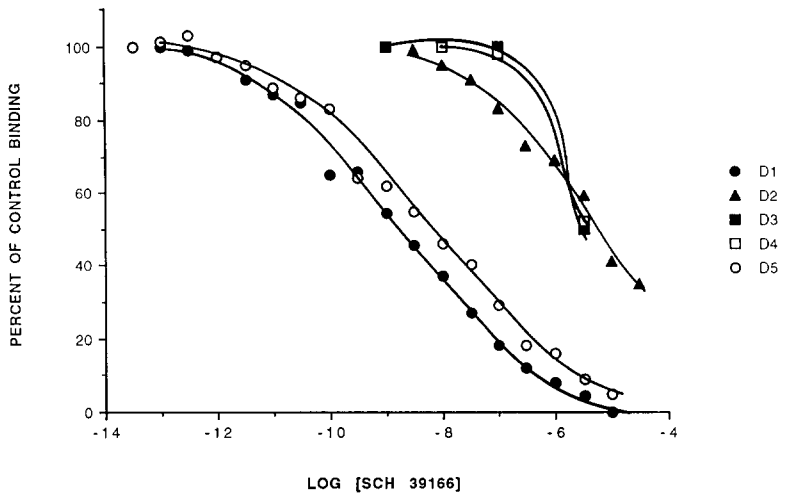

FIG. 2. Binding of SCH 39166 to cell membranes expressing the cloned dopamine receptors. Competition curves show the inhibition of [^{3}H]SCH 23390 binding to the D_1 and D_5 receptors and inhibition of [^{3}]spiperone to the D_2, D_3, and D_4 receptors by SCH 39166. Data are representative of at least three experiments.

respectively. SCH 39166 displayed similar potency for both receptors with a K_i ratio of 0.88 between D_1 and D_5 (Fig. 2). In contrast, the D_1 and D_5 receptors exhibited low-affinity binding for SCH 39165, the (+) enantiomer of SCH 39166. These results revealed the stereoselective nature of ligand–receptor binding associated with the D_1 and D_5 receptors. The effect of subtle sequence differences between the D_1 and D_5 receptors was also reflected in SCH 39165 displacement, with D_1 exhibiting a fourfold higher affinity for the compound. The removal of the *N*-methyl group found on SCH 39166, yielding the compound SCH 40853, resulted in a threefold increase in K_i values for both receptor subtypes.

Another series of related benzazepines, SCH 23388, SCH 23389, and SCH 23390 (1), reflected the importance of stereoselective binding of the D_1 and D_5 receptors as well as the effect of ring substitutions on affinity. SCH 23390, [(+)-7-chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1*H*-3-benzazepine], has been shown to be a selective D_1 and D_5 antagonist with high affinity for both receptors. However, the (−) enantiomer of SCH 23390, SCH 23388, has low affinity for both receptors. The other analogue of this benzazepine series, SCH 23389, is a positive isomer with a methyl group replacing the chloride atom at the seventh carbon position on the aromatic ring. The subsitution of a methyl group for a chloride atom caused a small change in affinity for the D_1 receptor and a sixfold change for the D_5 receptor. The replacement of the chloride atom with an iodine atom (SCH 38840) increased the K_i values for both receptor subtypes approximately five- to sixfold. However, the binding affinities associated with the ring substitutions were still in the low nanomolar range. Therefore, ring substitutions of an electronegative atom with a neutral group, such as CH_3, or with a less electronegative atom, such as iodine, caused only small changes in affinities. Another D_1-selective antagonist, NO-687 [(+)-5(2,3-dihydro-7-benzofuranyl)-7-hydroxy-3-methyl-8-nitro-2,3,4,5-tetrahydro-1*H*-3-benzazepine, generously provided by Novo Nordisk, Denmark], displayed relatively high affinity for both the D_1 and D_5 receptors with K_i values of 19.8 and 8.6 nM, respectively. The D_2-selective antagonist haloperidol, however, inhibited binding at the D_1 and D_5 receptors only at very high concentrations. Competiton experiments involving the agonist, apomorphine, resulted in displacement curves revealing the presence of high- and low-affinity sites for both the D_1 and D_5 receptors (Table 1). Previously, only single-site affinity for apomorphine had been reported for the D_1 and D_5

TABLE 1

PHARMACOLOGICAL PROFILE OF THE D_1 AND D_5 DOPAMINE RECEPTORS

	K_i Values (nM)	
	D_1	D_5
Agonists		
Dopamine	429.5 ± 110.8	34.2 ± 9.1
		4.9 ± 2.7 μM
Apomorphine	1.3 ± 0.2	2.3 ± 0.5
	612.9 ± 219.8	5.0 ± 1.8 μM
SKF 38393	227.0 ± 50.1	21.8 ± 6.0
Antagonists		
SCH 23390	2.4 ± 0.7	2.8 ± 1.0
SCH 23388	351.9 ± 32.1	496.8 ± 175.2
SCH 39166	5.0 ± 1.1	4.4 ± 2.1
SCH 39165	175.1 ± 34.0	799.7 ± 40.3
SCH 22389	3.5 ± 0.8	18.5 ± 0.4
SCH 38840	10.8 ± 2.2	17.3 ± 3.4
SCH 40853	7.2 ± 3.5	8.0 ± 3.1
NO-687	19.8 ± 3.5	8.6 ± 2.7
Haloperidol	337.3 ± 43.8	322.1 ± 114.1

K_i values represent the mean values ± SEM of at least three experiments.

receptors, with K_i values of 680 and 363 nM (16). The endogenous ligand, dopamine, displayed a biphasic dose-response curve to only the D_5 receptor; no significant two-site fit was found for the D_1 receptor. The agonist, SKF 38393, exhibited a 13-fold higher affinity for the D_5 receptor than for the D_1 receptor, with both receptors exhibiting a single-site fit.

D_2 Receptor Binding

The specific binding of [^{3}H]spiperone to the D_2 receptor expressed in Ltk$^-$ cells was found to be saturable and of high affinity, with a K_d of 0.15 nM and a B_{max} of 1414 fmol/mg of protein. Competition experiments showed that haloperidol, a D_2-selective antagonist, exhibited high affinity for the D_2 receptor, with a K_i of 6.3 nM. In contrast, the D_1 selective antagonist, SCH 39166, was virtually inactive, with a K_i of 3751.8 nM, resulting in a $D_2 : D_1$ K_i ratio of 750 (Fig. 2). The slope of the indirect Hill plot was 0.78 ± 0.18. Similar results were obtained using the other D_1-selective antagonist, SCH 23390. The atypical antipsychotic clozapine displayed a reasonably high affinity for the D_2 receptor, with a K_i of 81.0 nM. However, clozapine was 11-fold less potent than haloperidol.

The competition experiments evaluating dopamine agonists demonstrated that apomorphine displaced [^{3}H]spiperone from D_2 receptors with a K_i of 275.9 nM. The D_2 receptor has been shown to demonstrate high- and low-affinity states for apomorphine (14). The endogenous agonist dopamine was approximately 30-fold less potent, with a K_i of 7061.0 nM (Table 2).

D_3 and D_4 Receptor Binding

Scatchard analysis of saturation studies using [^{3}H]spiperone with the D_3 and D_4 receptors yielded K_d values of 0.32 nM for the D_3 receptor and 0.56 nM for the D_4 receptor. Competition studies using the D_3 and D_4 receptor showed that haloperidol potently displaced [^{3}H]spiperone, with K_i values of 6.1 nM and 10.0 nM, respectively. In contrast, clozapine exhibited significantly higher affinity for the D_4 receptor than for the D_3 receptor, with K_i values of 60.6 nM and 472.0 nM, respectively. SCH 23390 and SCH 39166 were both weak displacers of [^{3}H]spiperone from D_3 and D_4 receptors. SCH 23390 exhibited a K_i of 837.5 nM for the D_3 receptor and a K_i of 6054.9 nM for the D_4 receptor. SCH 39166 had low micromolar K_i values for both D_3 and D_4 receptors, yielding D_3/D_1 and D_4 / D_1 K_i ratios of > 200 and 1187, respectively (Fig. 2). Due to the limited quantity of D_3 and D_4 receptors, full dose-response curves could not be constructed to satisfy the curve-fitting requirements for indirect Hill plots. The endogenous agonist, dopamine showed relatively high affinity for the D_3 receptor, with a K_i of 129.5 nM. In comparison to the the D_2 receptor affinity, dopamine was 40-fold more potent at the D_3 receptor (Table 2). This similar trend in potency of dopamine was seen between the D_1 and D_5 receptors.

TABLE 2

PHARMACOLOGICAL PROFILE OF THE D_2, D_3, AND D_4 DOPAMINE RECEPTORS

	K_i Values (nM)		
	D_2	D_3	D_4
Haloperidol	6.3 ± 0.9	6.1 ± 1.9	10.0
Clozapine	81.0 ± 10.5	472.0 ± 133.0	60.6
SCH 23390	2751.7 ± 782.6	837.5 ± 46.0	6054.9
SCH 39166	3751.8 ± 141.4	>1000	5934.1
Dopamine	7061.0 ± 477.0	129.5 ± 39.6	ND
Apomorphine	275.9 ± 31.2	ND	ND

K_i values for D_2 and D_3 represent the mean of at least three experiments. K_i values for D_4 represent the average of two experiments, due to limited quantity of receptors.

DISCUSSION

Biochemical and behavorial studies have demonstrated that SCH 39166 is a potent and selective D_1 antagonist (11,17,18). In the present study, SCH 39166 displaced [^{3}H]SCH 23390 from both the cloned D_1 and D_5 receptors in a dose-dependent, high-affinity manner. In contrast, SCH 39166 was a weak displacer of [^{3}H]spiperone from the D_2, D_3, and D_4 receptors. The D_1 and D_5 receptors responded similarly to the D_1-selective antagonists SCH 23390 and SCH 40853. The result of competition studies with SCH 39166, the active benzonaphthazepine (−) enantiomer, indicated a stereoselective profile for D_1 and D_5 receptors when compared to SCH 39165, the less active (+) enantiomer. This stereoselectivity was also seen among a series of benzazepines, SCH 23388, SCH 23389, and SCH 23390. In this case, the (+) enantiomers, SCH 23390 and SCH 23389, were much more potent than the (−) enantiomer, SCH 23388.

In contrast, haloperidol, the D_2-selective antagonist, was a poor displacer of [^{3}H]SCH 23390 from the D_1 and D_5 receptors, but was a potent displacer of [^{3}H]spiperone from the D_2, D_3, and D_4 receptors. Clozapine, an atypical antipsychotic drug, had a range of potencies among the D_2-like receptors from 60–470 nM, with the D_2 and D_4 receptors displaying a similar high affinity.

It is interesting to see how similar the pharmacological antagonist binding profile is for the D_1 and D_5 receptors. However, the rank order of potency exhibited by these receptors for agonists is very different. Only the D_5 receptor displayed the presence of both high- and low-affinity binding for the endogenous agonist, dopamine, with K_i values of 34.2 nM and 4.9 μM, suggesting that the D_5 receptor may be more efficient in coupling to the G_s-protein than the D_1 receptor. Another agonist, SKF 38393, exhibited a 13-fold greater affinity for the D_5 receptor compared to the D_1 receptor, inferring that nonconserved amino acids in the agonist binding region of the D_1-like receptors may account for these differences. Dopamine also displayed greater potency at the D_3 receptor than the D_2 receptor. Therefore, variations in gene sequence between the D_1 and D_5 receptors and the D_2 and D_3 receptors must play a critical role in agonist binding.

The cloning of multiple dopamine receptor genes has lead to the need to determine the individual pharmacological and biochemical profiles of the receptor proteins. It is crucial to determine the location of these receptors in brain regions affected by neurological disorders, to determine the appropriate pharmacotherapy. Therefore, SCH 39166, a selective D_1 antagonist with minimal EPS liability and high affinity for both the D_1 and D_5 receptors, could possibly prove to be a novel therapeutic for the treatment of such neurological disorders as schizoprenia and tardive dyskenesia without the side effects of the currently available drugs.

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