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PHARMACOLOGY OF BINDING OF ^{3}H-SCH-23390 TO D-1 DOPAMINERGIC RECEPTOR SITES IN RAT STRIATAL TISSUE

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Abstract—^{3}H-SCH-23390, a selective antagonist of D-1 dopamine (DA) receptors, was used in a radioreceptor assay with rat brain striatal tissue, optimized biochemically, and extensively characterized pharmacologically with striatal membranes. Nonspecific binding, defined with excess *cis*(Z)-flupenthixol (300 nM), averaged 20–25% of total counts bound. Specific binding was linearly dependent on the amount of original striatal tissue (0–4 mg) or protein (0–250 μg), temperature dependent, saturable and reversible, and appeared to involve a single site at ligand concentrations limited to <10 nM. Binding in rat brain regions ranked as: striatum > accumbens > prefrontal cortex > posterior cerebral cortex > cerebellum. Association was virtually complete within 30 min at 30°, and the rate of dissociation at 30° was 0.0377 min^{-1} (half-time = 18.4 min). Affinity (K_a or K_d) determined from association and dissociation rate constants and from concentration isotherms averaged 0.349 and 0.340 nM respectively. Including Na^+ at 150 mM increased apparent maximum specific binding (B_{max}) by 19%, with a 29% increase in affinity; other monovalent cations alone had small effects on specific binding; Ca^{2+} and Mg^{2+} reduced binding by 30–40% at 5–10 mM, and a physiologic mixture of cations reduced binding by 42%. Agents (N = 85) were tested for potency (K_i or IC_{50}) in competition with the ligand (at 0.30 nM). Those known to have selective effects at D-1 receptors, generally, were most potent and stereoselective. Na^+ (150 mM) had little effect on the affinity of *cis*-thioxanthenes but decreased that of most other agents tested with high D-1 affinity. For antipsychotic agents, the correlation of typical clinical daily doses versus K_i at D-1 sites ($r = 0.06$) was much lower than at D-2 sites ($r = 0.94$). (−)Thioridazine was discovered to be D-1 selective, whereas the (+) enantiomer was selective for D-2 sites labeled with ^{3}H-spiperone. Relatively sedating antidepressants had greater D-1 affinity than their less-sedating, secondary amine congeners.

The proposal that there are two different types of cerebral dopamine (DA) receptors (D-1 and D-2) has been investigated since the 1970s [1, 2]. Knowledge of behavioral and neuroendocrine correlates of D-2 receptor agonism and availability of drugs and radioligands selective for D-2 sites encouraged characterization of this receptor type relatively early, whereas the lack of D-1 selective agents and limited knowledge of D-1 effects other than stimulation of DA-sensitive adenylate cyclase have delayed progress toward characterizing D-1 sites in the mammalian brain.

Initially, the D-1 receptor was characterized pharmacologically by use of the partially D-1 selective, preferentially active (Z[*cis*]) geometric isomers of thioxanthene neuroleptics as tritiated ligands, including ^{3}H-flupenthixol and ^{3}H-piflutixol (or pifluthixol) [3–5]. However, these DA antagonists are not highly selective for D-1 receptors and have some D-2 affinity and activity as well. Improvement in D-1 selectivity was reported by including saturating concentrations of a D-2 selective antagonist, such as spiperone, as a "masking" agent in such radioreceptor assays [6–9].

In the early 1980s, the first apparently highly selective D-1 receptor antagonist drug, the benzazepine derivative SCH-23390 (*R*[+]8-chloro-2,3,4,5-tetrahydro-3-methyl-5-phenyl-7-ol-benzazepine) was introduced [10–13]. Recently, this agent has been tritium-labeled and used in some biochemical and pharmacological characterizations of its binding [14–23]. In addition, there have been several studies of behavioral effects of unlabeled SCH-23390 [24–26]. The binding of ^{3}H-SCH-23390 also has been employed in preliminary assessments of cerebral DA receptor sites in normal aging (D-1 : D-2 ratio falls) [27] and in certain neuropsychiatric disorders such as Parkinson's disease (no change) [19] and schizophrenia (unchanged or reduced D-1 sites) [19, 28].

The aim of the present study was to clarify further some of the biochemical properties of the binding of ^{3}H-SCH-23390 to presumptive D-1 receptor sites in rat striatal tissue *in vitro*, to assess the effects of cations in particular, and to extend the pharmacological characterization of the affinity of a large number of psychotropic agents, many of which have not yet been evaluated in this way, particularly with simultaneous comparisons of D-2 receptor affinity.

MATERIALS AND METHODS

Radiochemicals and drugs. The radioligands ^{3}H-SCH-23390 ([*N-methyl*-^{3}H]*R*[+]-8-chloro-2,3,4,5-tetrahydro-3-methyl-5-phenyl-7-ol-benzazepine; 70 Ci/mmol) and ^{3}H-spiperone ([*benzene* ring-

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^{3}H]spiroperidol; 26.8 Ci/mmol) were obtained from Dupont-NEN, Inc. (Boston, MA).

Drugs were provided as gifts or as purchases from the following corporations or individuals (numbers are used below to define sources of agents): (*1*) Abbott Laboratories, North Chicago, IL; (*2*) Aldrich Chemicals, Milwaukee, WI (purchases); (*3*) Astra Alab AB, Sweden; (*4*) Ayerst Laboratories, New York, NY; (*5*) Boehringer Laboratories, Ingelheim, F.R.G.; (*6*) Boehringer Laboratories, Mannheim, F.R.G.; (*7*) Burroughs-Wellcome Corp., Research Triangle, NC (purchase); (*8*) Chemical Services Co., East Hanover, NJ (purchase); (*9*) Ciba-Geigy Pharmaceuticals, Inc., Summit, NJ; (*10*) Endo Laboratories, Inc., Garden City, NY; (*11*) Ferrosan A/S, Sobarg, Denmark; (*12*) Geigy Research Laboratories, Ardsley, NY; (*13*) Gist-Brocades Laboratories, Delft, Netherlands; (*14*) Hoechst Pharmaceuticals, Inc., Cincinnati, OH; (*15*) Hoffmann-La Roche, Inc., Nutley, NJ; (*16*) Ives Laboratories, Inc., New York, NY; (*17*) Janssen Pharmaceutica, Beerse, Belgium; (*18*) Lederle Laboratories, Wayne, NJ; (*19*) Eli Lilly & Sons, Indianapolis, IN; (*20*) MacFarlan Smith Ltd., Edinburgh, Scotland (purchase); (*21*) McNeil Laboratories, Port Washington, PA: (*22*) Mead Johnson & Co., Evansville, IN; (*23*) Merck, Sharp & Dohme Research Laboratories, West Point, PA: (*24*) Organon Inc., West Orange, NJ; (*25*) Parke, Davis Research Laboratories, Ann Arbor, MI; (*26*) Charles Pfizer & Co., New York, NY; (*27*) Ravizza SpA, Milan, Italy; (*28*) Research Biochemicals Inc., Natick, MA (gifts and purchases); (*29*) Sandoz Ltd., Basel, Switzerland; (*30*) Schering Corp., Bloomfield, NJ; (*31*) Sigma Chemical Co., St. Louis, MO (purchases); (*32*) Smith, Kline, & French Laboratories, Philadelphia, PA; (*33*) E. R. Squibb Corp., New Brunswick, NJ; (*34*) USV Pharmaceutical Corp., New York, NY; (*35*) Wander-Sandoz Research Laboratories, Berne, Switzerland; (*36*) Wyeth Laboratories, Inc., Philadelphia, PA.

The *cis*-(Z) and *trans*-(E) isomers of flupenthixol and piflutixol were the gift of (*37*) Dr. John Hyttel, H. Lundbeck Laboratories, Copenhagen, Denmark; *N*-methyl-4-phenyl-1,2,3,6-tetrahydropyridine HCl (MPTP), 1-methyl-4-phenylpyridinium iodide (MPP^+), 5-methyl-MPTP HCl and *N*-methyl-4-phenyl-piperidine HCl, as well as *R*(−)-8-bromo-apomorphine (prepared by Dr. Francis Murphy) were the gifts of (*38*) Dr. J. L. Neumeyer, Northeastern University, Boston, MA; enantiomers of 3-(3-hydroxy-phenyl)-*N*-*n*-propylpiperidine HCl (3-PPP) were the gift of (*39*) Dr. P. Seeman, University of Toronto, Ontario, Canada.

Tissue preparation. Young adult (250–300 g), male Sprague–Dawley rats from Charles River Laboratories (Wilmington, MA) were adapted for at least a week before use, to housing in wire cages in groups of four to five under controlled conditions (20–22°, humidity at 40–50%, lights on from 7:00 a.m. to 7:00 p.m.). Animals were allowed free access to commercial food pellets and fresh tap water.

Tissue was obtained by decapitation and rapid removal and dissection of brains on ice. Brain regions (striatum and, in one experiment, nucleus accumbens septi, mesio-prefrontal cortex, parieto-temporal cortex, occipital cortex, and cerebellum) were dissected [29], pooled, and kept frozen at −70° until use (usually within 30 days).

Just prior to use in the D-1 assay, tissue was weighed, thawed, and hand-homogenized (14 up-and-down strokes) with a Potter–Elvehjem Teflon-on-glass tissue homogenizer (Kontes, Vineland, NJ) in approximately 100 vol. (per frozen wt) of ice-cold hypotonic (50 mM) Tris buffer, pH 7.4. The homogenate was centrifuged at 20,000 *g* for 10 min at 4°. The pellet obtained was washed twice more by resuspending in fresh ice-cold buffer by vigorous vortexing and then recentrifuging under the same conditions. The final pellet was resuspended in 200 vol. (per initial wt) of the same buffer. Binding of ^{3}H-SCH-23390 obtained using this standard tissue preparation did not differ substantially from that obtained using preparations with more or fewer (one to five) washes or an unwashed crude homogenate, by adding Polytron homogenization, or by including an inhibitor of monoamine oxidase (pargyline, 10 mM, donated by Abbott Laboratories).

For comparison studies, ^{3}H-spiperone was used to label D-2 sites in fresh pooled rat striatal tissue homogenized in Tris buffer (50 mM, pH 7.7) as described previously [30].

Radioreceptor assays. For binding to D-1 receptors with ^{3}H-SCH-23390, glass tubes (triplicate) received 100 μl of test agents in 50 mM Tris buffer (pH 7.4, with or without NaCl, 150 mM, or other cations as chloride salts), 100 μl of ^{3}H-SCH-23390 (typical final concentration, 0.3 nM), and 200 μl of striatal tissue preparation yielding, 1.0 mg of original tissue or 61.4 ± 1.6 μg (mean ± SEM) of protein, per assay tube—all at a final volume of 1.0 ml.

To determine non-specific binding ("blank condition"), *cis*(Z)-flupenthixol (donated by Lundbeck Laboratories) was included (in triplicate) at 300 nM (shown to be in excess in a preliminary experiment) in each testing condition. Tubes, kept on ice until assay, were incubated in a shaking water bath for 30 min at 30° (standard conditions). Samples were filtered in a Cell Harvester (Brandel Corp., Gaithersburg, MD) under −15 mm Hg vacuum through glass fiber (GF) filter sheets (Type 32, Schleicher & Schuell, Keene, NH), which were washed twice with 5 ml of ice-cold 50 mM Tris buffer (pH 7.4), punched into 3 cm circles, and counted for tritium at *ca.* 50% efficiency in 8 ml of Liquiscint (National Diagnostics, Highland Park, NJ).

For the assay of binding to D-2 sites, ^{3}H-spiperone was added in 50 mM Tris buffer containing 5 mM Na_2EDTA and 1 mM L-ascorbic acid, all at pH 7.7, to provide a final ligand concentration of 0.15 nM. Rat striatal tissue was added at an average of 1.6 mg of original wet wt (98.2 μg protein); (+)butaclamol (300 nM; donated by Ayerst Laboratories) was used to define non-specific binding. The final volume was 1.8 ml, and tubes were incubated for 15 min at 37°, filtered, and counted under conditions already described. This method has been described in detail previously [30, 31].

Data analysis. Receptor-specific binding in the D-1 assay was taken as the difference between total tritium counts bound after incubation with ^{3}H-SCH-23390 alone and that remaining in the presence of

excess (300 nM) *cis*(Z)-flupenthixol under optimized standard conditions (30°, 30 min, 150 mM Na^+, pH 7.4); analogous blank and standard assay conditions already defined were used in the D-2 assay. In some experiments, ligand concentration isotherms were determined using six to eight concentrations (0.05 to 10 nM) of free ligand [*F*], ^{3}H-SCH-23390. Results were plotted in linearized form as specific binding (*B*) versus the ratio of bound:free ligand (*B*/*F*) to provide computed values of apparent K_d (negative slope) and B_{max} (y-intercept) [32, 33].

In other experiments, the affinity constant K_a was determined as the ratio of the association and dissociation rate constants ($K_a = k_{-1}/k_{+1}$). In pharmacological experiments, the affinity of each test agent was determined by the use of four to eight concentrations, above and below the approximate IC_{50}, in triplicate. Resulting inhibition data were fit to optimized curves by a microcomputer method that yields $IC_{50} \pm$ SEM and a slope function, and permits *t*-testing for significance of differences (at N = number of drug concentrations) [34, 35]. In the case of agents with relatively high D-1 affinity and for direct comparisons of D-1 and D-2 affinities, these values were converted to K_i from the equation: $K_i = IC_{50}/[1 + (F/K_d)]$ [36]. Data are presented as means ± SEM unless stated otherwise. Correlations were by linear regression (to obtain Pearson's *r*).

RESULTS

In preliminary experiments, the binding of ^{3}H-SCH-23390 to rat striatal membranes was assayed at different incubation times and temperatures (0–90 min at 22°, 30°, 37°, and up to 360 min at 4°). Specific binding reached a maximum within the first 20–30 min at 30° or 37°, while at 4° binding was slower and required 180 min to reach apparent equilibrium. Binding was linearly dependent on the amount of striatal tissue between the equivalent of 0 and 4 mg of original fresh tissue per assay (0 to *ca.* 250 μg of protein per assay). The range of specific binding (under standard conditions, at the K_d concentration of the ligand with striatal tissue) varied little with pH between 6.5 and 8.0 ($< \pm 10\%$) but the approximate optimum (pH 7.4) was used routinely thereafter.

The association rate of ^{3}H-SCH-23390 with membranes prepared from rat striatum was calculated from the relationship of specific binding (*B*) versus time (*t*) up to 90 min at 30° (equilibrium was attained by *ca.* 60 min), and the calculated association rate constant (k_{+1}) was 0.108 min^{-1}. The dissociation rate was determined by adding a large excess of unlabeled *cis*(Z)-flupenthixol (10 μM) after binding had approached equilibrium (60 min), and *B* versus *t* was evaluated for another 60 min, to yield k_{-1} = 0.0377 min^{-1} and a half-life of dissociation = 18.4 min, leading to a computed K_a = 0.349 nM. The mean apparent K_d values calculated from linearized plots of *B* versus *B*/*F* in three independent concentration isotherm experiments (*F* = 0.05 to 10 nM) were similar, at 0.340 nM. Also, the latter function was highly linear ($r > 0.98$), suggesting a single phase or site of labeling.

The estimated rank-order of abundance of apparent D-1 receptor sites in regions of rat brain was evaluated in one experiment using the standard assay at $F = K_d$, and at two tissue concentrations (2 and 4 mg of original tissue, or *ca.* 125 and 250 μg of protein, per assay) to confirm a linear dependency on tissue. Nucleus accumbens showed binding that averaged 50–60% of that of corpus striatum, and binding was lower (15–20% of striatum) in prefrontal cortex, very low in parietal and occipital cortex (5–10% of striatum) and virtually undetectable and not linearly protein dependent in the cerebellum. Due to these relatively low levels of apparent D-1 binding in other regions, only striatal tissue was used in subsequent experiments.

The effects of cations on D-1 receptor binding included a small enhancing effect of sodium: at 150 mM, Na^+ increased both maximum apparent specific binding (19% higher B_{max}) and affinity (29% lower K_d), as well as reducing the apparent K_i for unlabeled SCH-23390 by 20 ± 3%, as is reported below. In contrast, Li^+ had even less effect (less than 10% change in specific binding at [Li^+] = 1–150 mM); K^+ had a small inhibitory effect (<10% lower B_{max} with an insignificant effect on K_d at 1–10 mM). The divalent cations Ca^{2+} (1–5 mM) and Mg^{2+} (1–10 mM) reduced binding at $F = K_d$ by 30–40%. A physiological mixture of cations (NaCl, 120; KCl, 5; $CaCl_2$, 2; and $MgCl_2$, 1 mM) in 50 mM Tris buffer decreased binding markedly by 42.0 ± 0.4%.

To evaluate the possible nonspecific interactions of ^{3}H-SCH-23390 with serotonin (5HT-2 or S-2) receptor sites, as can be a problem with radioreceptor assays for D-2 sites in some cerebral tissues [21, 23], we included ketanserin (30 nM; donated by Janssen Laboratories), a potent and selective 5HT-2 antagonist, in assays with ^{3}H-SCH-23390 and striatal tissue. The results indicated that neither the apparent affinity (K_d) of ^{3}H-SCH-23390, the blank defined with 300 nM *cis*-flupenthixol, nor the IC_{50} of unlabeled SCH-23390 or of *cis*-flupenthixol changed when ketanserin was included ($< \pm 5\%$ changes). Accordingly, it was considered unnecessary routinely to include ketanserin to "mask" 5HT-2 sites in striatum. These parameters were not evaluated with nonstriatal tissues since there was much less apparent D-1 binding in nonstriatal tissues.

Based on these preliminary optimizing experiments, the standard conditions of D-1 binding assay were taken as follows: rat striatal tissue, Na^+ 150 mM, pH 7.4, incubation at 30° for 30 min, usually at $F = K_d = 0.3$ nM. The ability of a large number of test agents to compete with ^{3}H-SCH-23390 binding was then evaluated under these standard conditions. Agents included isomers of D-1 and D-2 antagonists and agonists and aporphines (Tables 1 and 2), antidepressants (Table 3), and other miscellaneous compounds (Table 4). In addition, the affinities of ten representative agents with relatively high D-1 affinities ($K_i < 500$ nM) were evaluated further with and without added Na^+ (150 mM) (Table 2). SCH-23390 itself and the *cis*-thioxanthenes piflutixol and flupenthixol showed slight *increases* in apparent affinity with Na^+ added (K_i = 0.15 vs 0.12 nM, 0.70 vs 0.55 nM, and 1.49 vs 1.33 nM at 0 and 150 mM Na^+, respectively, for these three compounds). This effect was, in part, accounted for by the ability of Na^+ to decrease K_d of the labeled ligand as already

Table 1. Affinity (K_i) of DA antagonists and agonists at D-1 and D-2 receptor sites in rat striatal tissue

Compound (*source*)	K_i (nM) D-1	D-2	D-1:D-2 Potency ratio
(+)SCH-23390 (*30*)	0.12	1,210	10,083
cis-Piflutixol (*37*)	0.55	0.79	1.44
cis-Flupenthixol (*37*)	1.33	2.10	1.58
Fluphenazine (*33*)	5.00	4.80	0.96
(+)Butaclamol (*4*)	5.96	2.80	0.47
(−)Thioridazine (*29*)	6.90	22.6	3.28
Loxapine (*18*)	15.0	0.95	0.063
trans-Piflutixol (*37*)	18.1	10.3	0.57
Chlorpromazine (*32*)	21.5	30.0	1.43
SKF-38393 (*32*)	26.6	>10,000	>376
trans-Flupenthixol (*37*)	39.0	42.0	1.08
Haloperidol (*21*)	50.1	13.4	0.27
(+)Thioridazine (*29*)	75.0	9.30	0.12
Spiroperidol (*17*)	143	0.33	0.0023
Clozapine (*35*)	171	64.0	0.37
Fluperlapine (*29*)	205	306	1.49
(−)Apomorphine (*20*)	236	45.0	0.19
(−)NPA (*28*)	340	4.10	0.012
(±)6,7-ADTN (*7*)	428	172	0.40
(−)8-Bromo-apomorphine (*38*)	612	4,330	7.07
(−)10,11-dimethyl-NPA (*38*)	687	861	1.25
Promazine (*36*)	790	31.5	0.040
Dopamine (*31*)	860	7,200	8.37
(−)3-PPP (*39*)	970	1,300	1.34
(+)NPA (*38*)	1,100	153	0.139
(+)Apomorphine (*28*)	1,660	235	0.142
(−)Apocodeine (*38*)	1,690	3,470	2.05
Epinine (*31*)	1,800	1,520	0.84
(−)10,11-Methylenedioxy-NPA (*38*)	1,910	1,670	0.87
FG-5803 (*11*)	*ca.*2,000	—	—
Ampcrozide (*11*)	*ca.*2,000	—	—
Melperone (*11*)	*ca.*2,000	—	—
(−)Butaclamol (*4*)	*ca.*5,000	333	*ca.*0.067
(−)Sulpiride (*27*)	*ca.*5,000	80.0	*ca.*0.016
(+)Sulpiride (*27*)	*ca.*5,000	*ca.*5,000	*ca.*1.00
Domperidone (*17*)	*ca.*10,000	—	—
(+)3-PPP (*39*)	>10,000	*ca.*5,000	*ca.*0.50
Bromocriptine (*29*)	>10,000	—	—
Molindone (*10*)	>10,000	—	—
Raclopride (*3*)	>10,000	—	—
(±)5,6-ADTN (*7*)	>50,000	—	—
BHT-920 (*6*)	>50,000	*ca.*1,000	*ca.*0.02
(±)Quinpirole (*19*)	>100,000	—	—

Abbreviations: NPA, *N-n*-propylnorapomorphine (10,11-dihydroxynoraporphine); ADTN, 5,6- or 6,7-dihydroxy-2-aminotetralin; and 3-PPP, 3-(3-hydroxyphenyl-*N-n*-propylpiperidine). The computed SEM averaged 11.1 ± 0.7% of each value of K_i; only mean values of K_i (nM) are shown for simplicity for each ligand, following optimized assay conditions described in Materials and Methods, using tritiated SCH-23390 (with Na^+ present) and spiperone, respectively, as ligands for D-1 and D-2 sites in rat striatal tissue. The relative D-1 versus D-2 affinity of each agent also is shown as the ratio of the respective K_i values (D-2/D-1). Sources of compounds cited as (*n*) are keyed to the source list in Materials and Methods. Hill slopes for compounds with relatively high affinity at D-1 sites (apparent $K_i < 2000$ nM) were computed for DA antagonists (0.91 ± 0.04; N = 16) and agonists or aporphines (0.80 ± 0.05, N = 12).

described, since IC_{50} values were virtually unchanged with Na^+ added (averaging 100.3% of IC_{50} without Na^+ added). The other seven agents tested showed substantially *decreased* affinity (increased K_i) by an average of 1.87 ± 0.32-fold (with corresponding increases of IC_{50} values as well, by 2.28 ± 0.38-fold; not shown), under conditions (Na^+ = 150 mM) that also optimized the binding of the ligand itself (Table 2). For all ten compounds tested with high D-1 affinity, values of K_i with versus without Na^+ included correlated closely ($r = 0.885$, slope = 0.517; Table 2), as did values of IC_{50} ($r = 0.886$, slope = 0.424; not shown).

When antidepressant agents were evaluated for their D-1 affinity, the imipramine analogs with tertiary amine alkyl side chains had higher affinity than those with secondary amine side chains. Other antidepressant agents structurally dissimilar to imi-

Table 2. Affinity (K_i) of DA agonists and antagonists at rat striatal D-1 sites with and without sodium (150 mM)

Compound (*source*)	K_i (nM) With Na^+	No Na^+	Ratio
SCH-23390 (*30*)	0.12 ± 0.02	0.15 ± 0.02	0.80
cis-Piflutixol (*37*)	0.55 ± 0.07	0.70 ± 0.07	0.79
cis-Flupenthixol (*37*)	1.33 ± 0.27	1.49 ± 0.17	0.89
(+)Butaclamol (*4*)	3.05 ± 0.14	3.44 ± 0.60	0.89
Fluphenazine (*33*)	5.00 ± 0.64	3.58 ± 0.36	1.40
Haloperidol (*21*)	50.1 ± 3.5	17.2 ± 1.2*	2.91
Spiroperidol (*17*)	143 ± 18	90.4 ± 9.8*	1.58
Clozapine (*35*)	171 ± 13	60.8 ± 5.9*	2.81
(−)Apomorphine (*20*)	236 ± 10	226 ± 11	1.04
Dopamine (*31*)	411 ± 84	171 ± 31*	2.40

Competition for binding versus ^{3}H-SCH-23390 (D-1 assay) was conducted with no sodium, or 150 mM NaCl added to the assay buffer using methods described in Materials and Methods, and selecting agents with relatively high D-1 affinity ($K_i < 500$ nM). The mean apparent increase in affinity without Na^+ was 1.55 ± 0.27 times, and the K_i values were correlated ($r = 0.885$, slope = 0.517). Values are means ± SEM, N = 6.

* Indicates significantly different from the standard assay condition (with Na^+ present) by *t*-test, at $P < 0.05$, or less.

Table 3. Affinity (IC_{50}) of antidepressants at D-1 sites in rat striatal tissue

Compound (*source*)	IC_{50} (nM)
Amoxapine (*18*)	154
Amitriptyline (*23*)	167
Doxepin (*26*)	226
Clomipramine (*9*)	320
Trimipramine (*16*)	357
Maprotiline (*9*)	451
Nortriptyline (*19*)	508
Mianserin (*24*)	526
Imipramine (*9*)	3,950
Protriptyline (*23*)	5,830
Trazodone (*22*)	9,960
Desipramine (*34*)	*ca.* 10,000
Fluoxetine (*19*)	>10,000
Nomifensine (*14*)	>10,000
Zimelidine (*3*)	>10,000
Tranylcypromine (*32*)	>10,000

Experimental conditions were as for Table 1, except that only the D-1 assay was used.

pramine had very weak interactions (Table 3). Among other miscellaneous agents, included mainly to test the specificity of the D-1 assay method, almost all had relatively low affinity ($IC_{50} > 1$ μM), with the exception of cyproheptadine ($IC_{50} = 227$ nM) and two selective blockers of DA transport, GBR-13069 and GBR-12909 ($IC_{50} = 640$ and 1320 nM respectively) with moderate affinity. Other blockers of DA uptake (benztropine, cocaine, and mazindol) had low affinity at the D-1 site labeled with ^{3}H-SCH-23390. Furthermore, agents with selective actions at adrenergic receptors (such as norepinephrine, yohimbine, clonidine, prazosin, and propranolol), serotonin receptors (serotonin, cinanserin, ketanserin), cholinergic receptors (atropine, benztropine, scopolamine), or other sites also had very low D-1 affinity (Table 4).

Table 4. Affinity (IC_{50}) of miscellaneous agents in rat striatal D-1 sites

Compound (*source*)	IC_{50} (nM)
Cyproheptadine (*23*)	227
GBR-13069 (*13*)	640
GBR-12909 (*13*)	1,320
Ketanserin (*17*)	1,400
Cinanserin (*33*)	5,580
Yohimbine (*31*)	*ca.* 10,000
Indoramine (*36*)	*ca.* 10,000
Atropine (*2*)	>10,000
Benztropine (*23*)	>10,000
Buspirone (*22*)	>10,000
Carbamazepine (*12*)	>10,000
Clonidine (*5*)	>10,000
(−)Cocaine (*8*)	>10,000
Diazepam (*15*)	>10,000
Diphenhydramine (*25*)	>10,000
Histamine (*31*)	>10,000
MPTP (*38*)	>10,000
(−)Norepinephrine (*31*)	>10,000
Prazosin (*26*)	>10,000
(±)Propranolol (*4*)	>10,000
Scopolamine (*2*)	>10,000
Serotonin (*31*)	>50,000
Mazindol (*29*)	>100,000

Methods are as for Table 1, except that only D-1 affinity was evaluated. MPTP is *N*-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; similarly low affinity ($IC_{50} > 10{,}000$ nM) also was found with its congeners: 1-methyl-4-phenylpyridinium, 5-methyl-MPTP, and *N*-methyl-4-phenyl-piperidine (all from *38*), omitted for simplicity.

DISCUSSION

The present results support and extend other recent reports reviewed in the introduction concerning the use of ^{3}H-SCH-23390 as a selective radioligand with which to label D-1 dopaminergic receptor sites in mammalian brain tissue. The binding of this ligand was found to be avid, reversible, and pharmacologically as well as stereochemically selective in striatal tissue, and apparently selective for the regional anatomical distribution of DA-rich sites in rat brain tissue.

The effects of cations on the D-1 binding assay generally were small, but Na^+ had consistent effects indicating small increases of affinity of the ligand itself and of the pharmacologically active *cis*-isomers of the thioxanthenes, but moderate decreases in affinity of several other DA agonists and antagonists with relatively high affinity for D-1 sites. For example, in agreement with other recent observations [9, 21], there was a moderate decrease in affinity of DA itself (2.40-fold increase in K_i, Table 2; 2.94-fold increase in IC_{50}, not shown), similar to their findings when sodium and guanosine nucleotides were included in a similar assay system. This change was interpreted as suggesting a shift in binding sites toward a lower-affinity state for the

endogenous agonist by sodium and GTP, as had been proposed previously also to occur at D-2 sites [9].

Pharmacological selectivity was supported by the relatively high affinity of DA agonists or antagonists with known selectivity for D-1 sites (e.g. unlabeled SCH-23390 itself; SKF-38393 ≫ quinpirole: respectively D-1 and D-2 selective agonists; and the active *cis-* > *trans*-isomers of the thioxanthene neuroleptic agents piflutixol and flupenthixol; Table 1). Similar to other reports of a high selectivity by (+)SCH-23390 for D-1 over D-2 sites, of 2,500- [14] to 6,400-fold [15], we also found a very high degree of preference for D-1 sites under the optimized conditions of the present assays (10,000-fold, Table 1).

In addition, there was a high degree of stereochemical selectivity among enantiomeric pairs that included the more actively dopaminergic (−) antipodes of the apomorphines: (−) > (+)apomorphine, (−) > (+)*N*-propylnorapomorphine, as well as (+) ≫ (−) butaclamol, and (−) ≫ (+)3-PPP (in agreement with the D-2 selectivity of all of these isomer pairs) (Table 1). Also, the highly D-2 selective benzamide DA antagonists domperidone, raclopride and sulpiride showed very low affinity in the present D-1 assay, and the antidopaminergic (−) antipode of sulpiride, while weak against D-1 sites, showed a higher affinity and selectivity for D-2 sites labeled with ^{3}H-spiperone, as expected (Table 1). Another phenothiazine enantiomeric pair, (−) and (+) methotrimeprazine, also were evaluated (G. Faedda, M. Froimowitz, and R. J. Baldessarini, unpublished observations with agents provided by Smith, Kline & French Laboratories). Similar to a reported 45-fold preference for the (−) enantiomer (levomepromazine) at D-2 sites [37], there was a 14-fold preference versus D-1 binding (K_i values = 35.1 ± 4.1 and 505 ± 78 nM for (−) and (+)methotrimeprazine respectively).

Additional clues to structure–activity requirements for interaction at D-1 sites included the marked decrease in affinity in *R*(−)apomorphine analogs with occluded catechol groups (Table 1). That is, D-1 affinity ranked as: (−)apomorphine ≥ (−)*N*-propylnorapomorphine > (−)10,11-dimethyl-*N*-propylnorapomorphine > (−)10,11-methylenedioxy-*N*-propylnorapomorphine. Also, halogenation of apomorphine (8-bromo analog) reduced D-1 affinity markedly. The importance of the positioning of the catechol moiety of DA analogs also is suggested by the D-1 preference of racemic 6,7-dihydroxy-ADTN > 5,6-dihydroxy-ADTN. The presence of an *N*-methyl group in epinine lowered affinity compared to DA itself, similar to the slight decrease in D-1 affinity for *N*-propyl versus *N*-methyl analogs of (−) or (+)apomorphine (Table 1).

Overall, the correlations between D-1 and D-2 site affinities were weak ($r = 0.40$; N = 25 agents with K_i at D-1 sites < 2000 nM; Table 1) and not instructive, perhaps in part due to the heterogeneous collection of agents tested in the two DA receptor assays.

The observation of D-1 selectivity for (−)thioridazine is a new finding that is particularly interesting in that the (+) antipode appeared to be selective for D-2 sites (Table 1). The preferential pharmacologic effects of (+)thioridazine as a typical D-2 antagonist in behavioral and neurochemical testing procedures also have been observed recently [38]. Our findings also supported the relative D-2 over D-1 selectivity of the butyrophenone analogs haloperidol and spiroperidol.

Some atypical neuroleptic agents, including clozapine and its congener fluperlapine, showed moderate affinity at D-1 sites, as well as moderate affinity at D-2 sites (Table 1), and have been reported recently to have relatively high affinity to D-1 sites linked to adenylate cyclase [16]. Their interactions with D-1 sites may contribute to their unusual clinical activity, which includes antipsychotic effects but very little of the acute extrapyramidal effects associated with typical neuroleptics of the phenothiazine, thioxanthene, and butyrophenone types [39]. Relatively high affinity for D-1 sites was not, however, characteristic of other atypical or experimental antipsychotic agents, including melperone, amperozide or its congener FG-5803, molindone, and BHT-920 [40–43]. Moreover, when twelve antipsychotic agents having some affinity at D-1 sites ($K_i \leq 5000$ nM in Table 1) were evaluated by correlating K_i versus typical clinical daily doses [39, 44], the correlation was very weak ($r = -0.060$); in contrast, a similar correlation with D-2 affinity was very high ($r = 0.935$, $P < 0.001$). This close association of clinical potency of antipsychotic agents with D-2 affinity accords well with previous work [44] and may reflect the historical development of neuroleptic agents by screening methods which probably reflect D-2 antagonism [39].

The observation that tertiary amine tricyclic antidepressant analogs of imipramine have moderate affinity at D-1 receptor sites in rat striatal tissue (Table 3) was predicted in an earlier study by Karobath [45] using inhibition of DA-sensitive adenylate cyclase as a functional index of interactions of antidepressants with cerebral D-1 receptors. He too found a much higher affinity for tertiary amine than for secondary amine antidepressants (e.g. amitriptyline > nortriptyline; imipramine > desipramine). Indeed, the seven compounds evaluated in both studies (Table 3, [45]) show a strong correlation of affinities in the ^{3}H-SCH-23390 assay and DA-sensitive adenylate cyclase assay ($r = 0.966$, N = 7, $P < 0.001$). This effect appears to be one more aspect of the complex pharmacology of agents such as amitriptyline, doxepin, and other tertiary amine dibenzazepine antidepressants, which also have relatively high affinity at central α-1 adrenergic, muscarinic acetylcholine, and H-1 histaminergic sites—effects which, together, may contribute to the relative tendency of these antidepressants to induce sedation as well as hypotension and other autonomic side effects [39].

Overall, the present results provide strong additional support to the impression that ^{3}H-SCH-23390 is a useful and selective radioligand with which to study the characteristics of the D-1 receptor sites in mammalian cerebral tissues.

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