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Dopamine D_1 and D_2 receptor selectivities of phenyl-benzazepines in rhesus monkey striata

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Abstract

Several phenyl-benzazepine compounds, putatively selective dopamine D_1 receptor agonists, have been used to study the effects of dopamine D_1 receptor stimulation in rodents and nonhuman primates. However, the dopamine receptor selectivities of these compounds have not been established in nonhuman primates. Accordingly, the relative selectivities of six phenyl-benzazepines for dopamine D_1-like and D_2-like receptors were assessed in rhesus monkey and, for comparison, rat striata. The compounds tested had higher affinity for D_1 than D_2 receptors in both species; however, their selectivity varied by up to three orders of magnitude. GTP (100 μM) reduced agonist binding at the high-affinity state of the dopamine D_1 receptor, but the magnitude of the effect of GTP did not reliably predict a compound's efficacy. Furthermore, a history of cocaine self-administration did not appear to influence dopamine receptor binding characteristics in the rhesus monkeys in this study. The present results will aid the comparison of dopamine receptor binding characteristics and behavioral effects of D_1 dopamine receptor agonists.

Keywords: Dopamine; Dopamine receptor agonist; Dopamine receptor antagonist; (Rhesus monkey); (Rat); Radioligand binding; SCH 23390; Spiperone; Phenyl-benzazepine

1. Introduction

Brain dopaminergic systems are implicated in a number of disorders, including psychomotor stimulant abuse, schizophrenia and Parkinson's disease. These disorders have often been studied using models developed in rodents. However, comparison of the anatomy of dopaminergic systems (Berger et al., 1991) and the effects of dopamine receptor stimulation (Pifl et al., 1991; Izenwasser and Katz, 1993; Vermeulen et al., 1994) have shown differences between rodents and primates that may have therapeutic importance. For instance, the prototypical selective dopamine D_1 receptor agonist SKF 38393 demonstrated higher efficacy to stimulate the production of cAMP in rat caudate than in rhesus monkey striata (Andersen and Jansen, 1990; Pifl et al., 1991; Weed et al., 1997). Dopaminergic drugs can also have different behavioral effects in rodents and primates. For instance, the dopamine D_1 receptor agonist SKF 77434, which has partial efficacy in both rats and rhesus monkeys (Weed et al., 1997), was shown to function as a positive reinforcer in rats (Self and Stein, 1992); however SKF 77434 failed to function as a reinforcer in rhesus monkeys (Weed and Woolverton, 1995) or squirrel monkeys (Grech et al., 1996). Therefore, evaluation of dopamine receptor binding characteristics using both rodent and nonhuman primate tissues may provide important information regarding the relationship between receptor stimulation and behavioral effects of dopaminergic compounds.

Central nervous system dopamine receptors are divided into two groups, the 'D_1-like' group: D_1/D_{1a}, D_5/D_{1b}, D_{1c} and D_{1d}; and the 'D_2-like' group: $D_{2long\ and\ short}$, D_3, D_4 or $D_{2al\ and\ s}$, D_{2b} and D_{2c} (Sibley and Monsma, 1992; Sugamori et al., 1994; Demchyshyn et al., 1995). D_1-like receptors are differentiated from D_2-like receptors by their structural homology and by their ability to stimulate production of cAMP (Kebabian and Calne, 1979; Sunahara et al., 1991; Tiberi et al., 1991). D_2-like receptors either inhibit or have no effect upon cAMP production (Stoof

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and Kebabian, 1981). D_{1a}/D_1 and D_{1b}/D_5 receptors are the best characterized of the D_1-like receptors, and to date the only D_1 subtypes demonstrated in mammalian brain (Sugamori et al., 1994; Demchyshyn et al., 1995). For convenience, the terms 'D_1' and 'D_2' will be used here to refer to any of the receptors in the respective groups and not to indicate the D_{1a} or D_{2a} receptors specifically.

A number of phenyl-benzazepine compounds are available that have been found to be selective for dopamine D_1 receptors in rodent tissue. These compounds have been used in a number of investigations of dopamine D_1 receptor function, including studies of the behavioral effects of dopamine D_1 receptor stimulation in nonhuman primates (Bergman et al., 1996; Grech et al., 1996; Weed et al., 1997). However, the selectivities of these compounds for subtypes of dopamine receptors have not been widely studied in primate tissue. Moreover, important species differences in the radioligand binding characteristics of previously identified phenyl-benzazepine dopamine D_1 receptor agonists have been reported in primates and rodents. For instance, SKF 38393 has been reported to have lower selectivity for dopamine D_1 receptors in human postmortem putamen than either SKF 82958 or SKF 77434 (O'Boyle and Waddington, 1987). In contrast, studies performed using rodent striata consistently report SKF 38393 as being more selective for dopamine D_1 receptors than either SKF 82958 or SKF 77434 (Arnt et al., 1988; Andersen and Jansen, 1990; Neumeyer et al., 1992). Interpretation of species differences such as these is confounded by variation in results due to the use of different assay systems by different laboratories. The primary goal of the present study was to assess the radioligand binding characteristics of a series of phenyl-benzazepine dopamine D_1 receptor agonists in rhesus monkey striata. An additional goal was to compare the radioligand binding characteristics of these compounds in primate and rodent striata. Although a substantial literature exists on the radioligand binding characteristics of these compounds in rodent tissue, these results were replicated in order to compare primate and rodent data from the same assay system.

The development of a radioligand binding assay to model the functional effects of dopamine D_1 receptor agonists would be beneficial to the further investigation of dopaminergic activity, especially in primate tissue. Such an assay would be advantageous if it could provide functional information similar to a cAMP assay while being less expensive, technically easier to perform, and more readily accommodating of frozen tissue. The effect of guanyl-nucleotides upon dopamine D_1 receptor agonist displacement of [^{3}H]SCH 23390 has been proposed as a model of the functional effects of dopamine D_1 receptor agonists which can be performed in a radioligand binding assay (Madras, 1993). Dopamine receptors belong to the 'super-family' of receptors coupled to GTP-binding proteins (G-proteins). Agonists generally displace antagonists bound to G-protein-coupled receptors in a biphasic fashion, and guanyl-nucleotides generally reduce the potency of agonists at G-protein-coupled receptors (Hess et al., 1986; Zahniser and Molinoff, 1978). Conversely, guanyl-nucleotides have little effect upon the binding of antagonists to G-protein-coupled receptors, because antagonists do not preferentially bind to either the coupled or uncoupled conformation of the receptor (Hess et al., 1986; Zahniser and Molinoff, 1978). Therefore, another goal of the present study was to study the effects of GTP on the displacement of [^{3}H]SCH 23390 by D_1 agonists to determine if the magnitude of the GTP effect is related to the traditional measure of intrinsic activity at dopamine D_1 receptors, stimulation of cAMP production. As with radioligand binding characteristics in rodents, effects of GTP on binding of some of these dopamine D_1 agonists to rodent dopamine D_1 receptors has been previously reported; however, to our knowledge this has yet to be studied in primate tissue. To facilitate inter-species comparisons, the rodent work was replicated in the same assay system used with the rhesus tissue.

Finally, although not a primary goal of the present study, brain tissue was available from monkeys with a variety of experimental histories. Roughly equal-sized groups of monkeys with histories of recent cocaine self-administration, cocaine self-administration at least two months prior to sacrifice, and cocaine-naïve monkeys, permitted the evaluation of different drug histories on long-term radioligand binding properties of striatal dopamine receptors.

2. Methods

2.1. Tissue collection

Due to factors not related to this project, brain tissue was available from a number of rhesus monkeys, *Macaca mulatta*. The 12 rhesus monkeys with histories of cocaine self-administration and/or discriminative-stimulus training with psychomotor stimulants included nine males (12278, 972, 8713, 8805, 8217, 9165, 8236, 8711, 9001) and three females (11083, 7976, 8619). The drug histories of the drug-experienced monkeys divided naturally into three groups: monkeys which had self-administered cocaine within two months of sacrifice (972, 8217, 8713, 8805, 8236), monkeys which had been drug-free for at least 2 months (7976, 8711, 9165, 9001, 8619) and monkeys with little or no history of dopaminergic drug administration (11083, 12278). No rhesus monkeys were sacrificed solely for use in the present study. In addition, tissue from four female monkeys with no history of dopaminergic drug exposure (11197, 12476, 14401, 14963) was acquired from the Oregon Regional Primate Center. This control tissue was included to allow for a determination of the effect of the monkeys' drug histories on the radioligand binding characteristics of their dopamine receptors.

Monkeys were sacrificed via exsanguination during deep pentobarbital anesthesia. Brains were collected immediately after sacrifice and the caudate nucleus and putamen were dissected from coronal sections according to the atlas of Snider and Lee (1961). Tissue was fast frozen on aluminum foil over solid CO_2 or in liquid nitrogen immediately after dissection with no additional preparation.

Rat tissue was collected from male Sprague–Dawley rats (Harlan, Indianapolis, IN) immediately upon sacrifice by decapitation. The caudate-putamen was dissected by hand on ice and fast-frozen on aluminum foil resting on solid CO_2. Additionally, frozen, dissected Sprague–Dawley striata were purchased from Zivic-Miller (Zelienople, PA). Once it was determined that rat striatal tissue from either source had similar dopamine receptor binding characteristics, data from each tissue source was pooled (data not shown).

2.2. Tissue preparation

The assay system for radioligand binding studies was derived from Hyttel and Arnt (Hyttel and Arnt, 1987). Frozen rat caudate-putamen tissue or approximately equal proportions of frozen rhesus caudate and putamen tissue were thawed and homogenized in 100 vol of 50 mM potassium phosphate buffer (dibasic + monobasic, pH of 7.4) and centrifuged at 25,000 × *g* at 4°C for 10 min. The resulting pellet was suspended in fresh buffer and recentrifuged after which the pellet was suspended at 1.0 mg/ml for [^{3}H]SCH 23390 (dopamine D_1 receptor ligand) displacement assays and 2.0 mg/ml (original wet weight) in fresh buffer for [^{3}H]spiperone (dopamine D_2 receptor ligand) displacement assays.

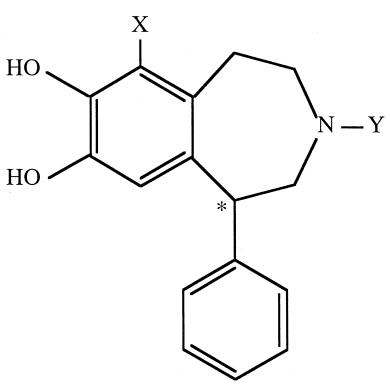
2.3. Radioligand binding assays

Displacement assays consisted of 16 concentrations of dopamine agonists (or eight concentrations of dopamine antagonists) or buffer; $MgCl_2$ ([Final] = 4 mM); GTP ([Final] = 100 mM) or buffer; 0.25 ± 0.02 nM [^{3}H]SCH 23390 (Dupont-NEN, Boston, MA) and 500 μl of tissue homogenate. The final assay volume for all tubes was brought to 5.0 ml, with the 50 mM potassium phosphate buffer. Nonspecific binding of [^{3}H]SCH 23390 was defined with 100 μM *cis*-flupentixol. Assays were initiated by transfer from an ice-bath to a water bath at 37°C and were incubated for 60 min. All assays were terminated by rapid vacuum filtration in a 24 well Brandel cell harvester using Whatman GF/C filters (Brandel, Gaithersburg, MD). The filters were rinsed 2 × with 5.0 ml of ice-cold buffer, and deposited into Packard Top Count deep well plates. 500 μl of Microscint-20 cocktail (Packard Instruments, Downers Grove, IL) was added to each well. The filters soaked for a minimum of 4 h to allow for sufficient tritium extraction prior to determination of bound radioactivity using a Packard Top Count scintillation counter.

For displacement of [^{3}H]spiperone binding, the methods were as described above for [^{3}H]SCH 23390 displacement with the following exceptions: eight concentrations of each compound were employed, specific binding of 0.07 ± 0.002 nM [^{3}H]spiperone (Dupont-NEN, Boston, MA) was defined as that displaced by 100 μM *S*-butaclamol (RBI, Natick, MA), and each tube contained 0.1 μM mianserin to mask the binding of spiperone to serotonin receptors. The effect of GTP upon displacement of [^{3}H]spiperone was not investigated.

Saturation studies were performed in both rat and monkey striata as described above for displacement assays. However, samples were incubated with varying concentrations of [^{3}H]SCH 23390 [0.45–5.2 nM] or [^{3}H]spiperone [0.013–1.44 nM] Analysis of Scatchard transformation of the saturation isotherms for [^{3}H]SCH 23390 revealed that ligand concentrations > 2.5 nM exceeded the 95% confidence intervals around the linear regression line, which is consistent with binding to a low affinity site (data not shown). Since this site did not appear to be saturable, data from concentrations of [^{3}H]SCH 23390 > 2.5 nM were omitted. Omission of these concentrations significantly increased the goodness-of-fit of the rectangular hyperbolic function ($P < 0.05$).

Determination of protein levels in tissue homogenate samples was performed using the bicinchoninic acid method (Smith et al., 1985; kits from Pierce, Rockford, IL). Absorbance (at 560 nm) was measured on a Beckman spectrophotometer (Beckman, Palo Alto, CA).



Compound	X	Y
SKF 38393	H	H
SKF 81297	Cl	H
SKF 77434	H	CH_2CHCH_2
SKF 82958	Cl	CH_2CHCH_2
6-Br APB	Br	CH_2CHCH_2

* 6-Br APB enantiomers differ here

Fig. 1. Chemical structures of substituted 1-phenyl-3-benzazepine dopamine D_1 receptor agonists. Unsubstituted compound is SKF 38393. Substitutions at the *X* and *Y* positions yield the compounds as indicated. The *R*(+) and *S*(−) enantiomers of 6-Br APB differ at the carbon indicated with an asterisk.

2.4. Drugs

Dopamine HCl (Sigma, St. Louis, MO), mianserin and *cis*-flupentixol (RBI, Natick, MA) were dissolved in buffer. *S*-Butaclamol HCl, SKF 38393 (±7,8-Dihydroxy-1-phenyl-2,3,4,5-tetrahydro-1*H*-3-benzazepine) HCl, SKF 77434 (±3-allyl-7,8-dihydroxy-1-phenyl-2,3,4,5-tetrahydro-1*H*-3-benzazepine) HBr, SKF 82958 (±3-allyl-6-chloro-7,8-dihydroxy-1-phenyl-2,3,4,5-tetrahydro-1*H*-3-benzazepine) HBr, *R*(+) and *S*(−) 6-BrAPB (3-allyl-6-bromo-7,8-dihydroxy-1-phenyl-2,3,4,5-tetrahydro-1*H*-3-benzazepine) HBr, SKF 81297 (±6-chloro-7,8-dihydroxy-1-phenyl-2,3,4,5-tetrahydro-1*H*-3-benzazepine) HBr, SCH 23390 (*R*(+)-7-chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1*H*-3-benzazepine) HCl, spiperone HCl (all RBI, Natick, MA), and SCH 39166 ((−)-*trans*-6,7,7a,8,9,13*b*-hexahydro-3-chloro-2-hydroxy-*N*-methyl-5-*H*-benzo(*d*)naphtho-(2,1-b)azepine) HCl (Schering-Plough, Kenilworth, NJ) were dissolved at 1.0 mM in 10% ethanol and ultra-pure water and diluted in buffer. Dopamine,

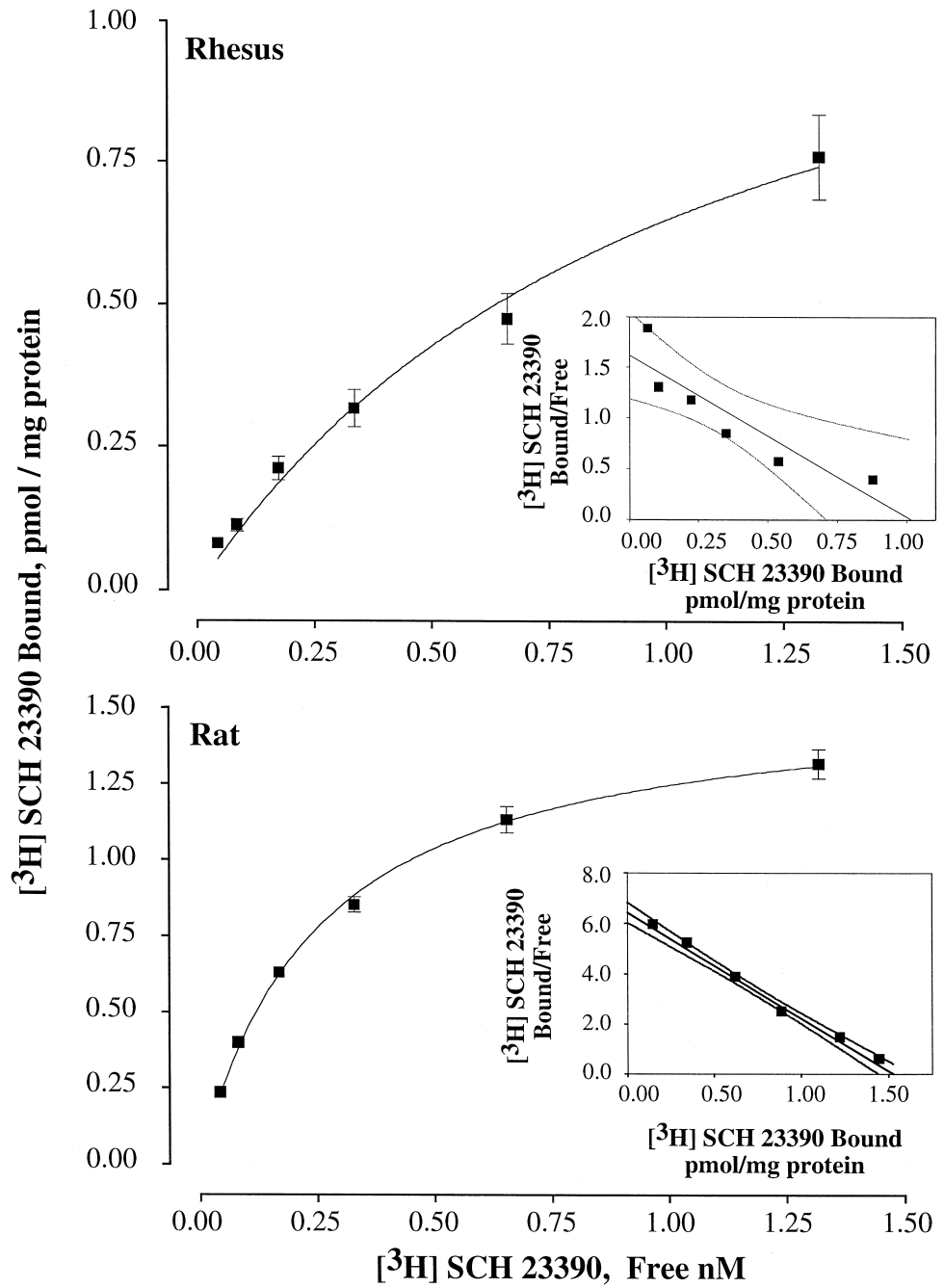


Fig. 2. [^{3}H]SCH 23390 saturation experiments in rhesus and rat striata. Top panels represent data from rhesus monkey striata. Bottom panels represent data from rat striata. Main graphs: *Y*-axis represents bound [^{3}H]SCH 23390 in pmol/mg protein, *X*-axis represents free [^{3}H]SCH 23390 in nM. Inset graphs are the same data transformed into Scatchard analyses with dotted lines representing the 95% confidence intervals of the linear regression.

S-butaclamol and *cis*-flupentixol were mixed fresh for each assay. Approximately half of the assays were conducted using freshly mixed dopamine D_1 receptor agonists. Once it was determined that using frozen drugs had no effect on the displacement curves of either [^{3}H] ligand, assays were conducted using frozen drug solutions.

The chemical structures of the six 1-phenyl-3-benzazepine dopamine D_1 receptor agonists used in these studies are described in Fig. 1. These dopamine D_1 receptor agonists are all modifications of the parent compound, SKF 38393. Modifications are *N*-allyl substitution (SKF 77434, SKF 82958 and 6-Br APB) and/or 6-halogenation

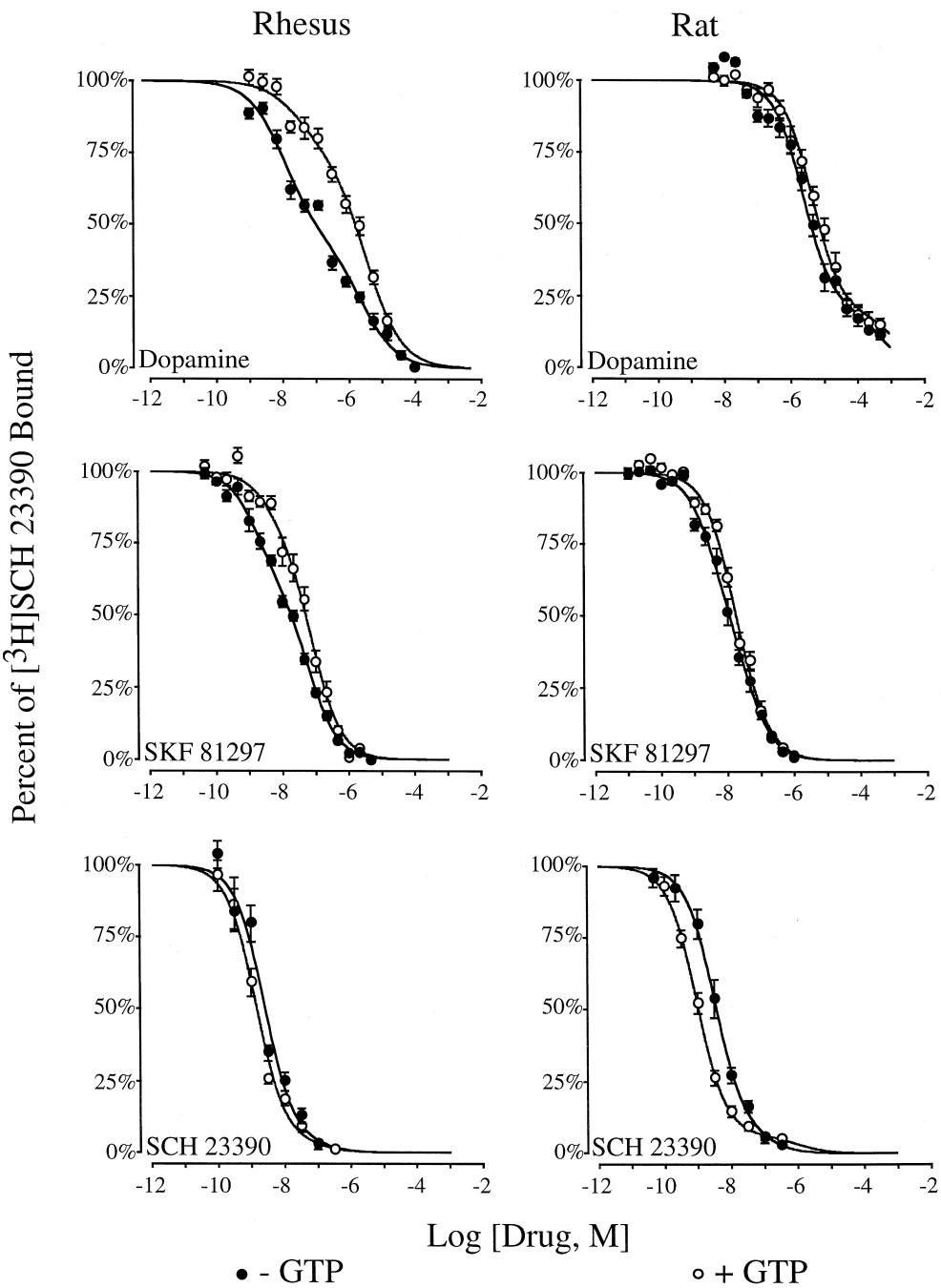


Fig. 3. Effects of GTP upon displacement of [^{3}H]SCH 23390. Left panels represent displacement of [^{3}H]SCH 23390 in rhesus striata. Right panels represent displacement of [^{3}H]SCH 23390 in rat striata. *Y*-axis represents percent of [^{3}H]SCH 23390 bound. *X*-axis represents concentration of drug in log molar units. $N = 8$ for rhesus striata, and $N = 6$ for rat striata. Filled symbols represent displacement in the absence of 100 μM GTP. Open symbols represent displacement in the presence of 100 μM GTP. Data for dopamine, SKF 81297 and SCH 23390 are presented as representative of agonists with differing D_1:D_2 selectivities and a selective antagonist, respectively. Since radioligand concentrations varied by less than 15% of their overall means, data were normalized as percent of specific binding for all displacement assays.

Table 1
Effects of 100 μM GTP on displacement of [^{3}H] SCH 23390 in rhesus striata

Agonists	−GTP			+GTP		
Two-site model	K_i high (nM)	K_i low (nM)	% High	K_i high (nM)	K_i low (nM)	% High
Dopamine	31.8 (16.8 to 60.3)	1,960 (998 to 3,850)	55.1% (47.3 to 62.9)	50.1 (14.8 to 170)	2,190 (1,600 to 2,990)	20.0%[a] (9.2 to 30.8)
SKF 38393	10.6 (5.29 to 21.1)	300 (217 to 415)	32.2% (21.2 to 43.2)	10.9 (1.19 to 100)	341 (274 to 424)	9.6%[a] (0.48 to 18.8)
SKF 77434	1.21 (0.46 to 3.18)	79.3 (60.8 to 103)	21.6% (13.6 to 29.7)		*87.8 (82.3 to 94.8)*	
SKF 81297	1.38 (0.83 to 2.31)	48.1 (35.0 to 66.0)	39.3% (30.2 to 48.4)	1.34 (0.13 to 14.0)	53.9 (40.4 to 71.8)	11.6%[a] (0.0 to 23.2)
SKF 82958	1.53 (0.33 to 7.10)	29.8 (18.4 to 48.1)	24.7% (2.9 to 46.5)		*62.4 (49.1 to 79.4)*	
R(+)6-BrAPB	2.45 (1.10 to 5.46)	72 (34.7 to 149)	47.9% (28.9 to 66.9)	2.43 (0.64 to 9.21)	83.2 (53.5 to 129)	25.7%[a] (10.3 to 41.0)
S(−)6-BrAPB		*91.0 (78.1 to 106.1)*		1.7 (0.046 to 63.4)	185.4 (150 to 230)	6.2% (0.7 to 13.1)

Numbers in parentheses are 95% CIs.
Italics indicate that the K_i from a one-site model was the most appropriate affinity estimate.
[a]Significant decrease in the percentage of high affinity binding sites ($P < 0.05$). $N = 6–8$.

Table 2
Effects of 100 μM GTP on displacement of [^{3}H]SCH 23390 in rat striata

Agonists	−GTP			+GTP		
Two-site model	K_i high (nM)	K_i low (nM)	% High	K_i high (nM)	K_i low (nM)	% High
Dopamine	14.9 (3.18 to 69.8)	2,260 (1,570 to 3,260)	25.9% (18.4 to 33.4)	55.2 (5.63 to 541)	3,950 (2,470 to 6,300)	17.2% (6.7 to 27.8)
SKF 38393	1.9 (0.71 to 5.1)	116 (89.8 to 150)	19.3% (13.5 to 25.1)		*89.6 (79.6 to 101)*	
SKF 77434		*146 (118 to 181)*			*111 (97.2 to 126)*	
SKF 81297	1.17 (0.42 to 3.25)	17.7 (7.56 to 41.3)	43.0% (17.2 to 68.7)		*5.2 (4.4 to 6.2)*	
SKF 82958	0.75 (0.23 to 2.48)	27.2 (7.66 to 96.7)	37.1% (20.0 to 54.6)	1.63 (0.51 to 5.19)	47.5 (5.65 to 400)	44.3% (23.7 to 65.0)
R(+) 6-BrAPB	0.25 (0.04 to 1.5)	9.81 (6.12 to 15.7)	23.0% (10.1 to 35.6)		*10.2 (8.0 to 13.1)*	
S(−) 6-BrAPB	33.1 (12.7 to 86.1)	642 (357 to 1,150)	30.8% (14.4 to 47.2)		333 (275 to 403)	

Numbers in parentheses are 95% CIs.
Italics indicate that the K_i from a one-site model was the most appropriate affinity estimate.
$N = 6$.

Table 3
Effects of 100 μM GTP on displacment of [^{3}H]SCH 23390 in rhesus and rat striata: fold-shifts

Agonists	Rhesus striata			Rat striata		
One-site model	−GTP K_i (nM)	+GTP K_i (nM)	Fold shift + GTP/−GTP	−GTP K_i (nM)	+GTP K_i (nM)	Fold shift + GTP/−GTP
Dopamine	173 (148 to 202)	1,230[a] (1,080 to 1,400)	7.11	1,306 (1,040 to 1,650)	2,467[a] (1,930 to 3,160)	1.89
SKF 38393	113 (101 to 126)	265[a] (241 to 291)	2.35	62.6 (54.4 to 72.0)	89.6[a] (79.6 to 101)	1.43
SKF 77434	40.3 (35.5 to 45.8)	87.8[a] (82.3 to 94.8)	2.18	146 (118 to 181)	111 (97.2 to 126)	0.76
SKF 81297	13 (11.6 to 14.6)	39.3[a] (34.8 to 44.2)	3.02	5.2 (4.4 to 6.2)	8.5[a] (7.5 to 9.7)	1.63
SKF 82958	16.3 (14.4 to 18.4)	62.4[a] (49.1 to 79.4)	3.83	4.8 (3.5 to 6.5)	5.5 (4.0 to 7.5)	1.15
R(+) 6-BrAPB	14.3 (12.0 to 17.1)	44.1[a] (34.7 to 56.1)	3.08	5.3 (4.2 to 6.6)	10.2[a] (8.0 to 13.1)	1.92
S(−) 6-BrAPB	91.0 (78.1 to 106.1)	156.2[a] (138.3 to 176.6)	1.72	218.0 (185 to 257)	333[a] (275 to 403)	1.53
SCH 23390	1.60 (1.09 to 2.34)	1.03 (0.83 to 1.28)	0.64	1.81 (1.29 to 2.52)	0.56[a] (0.45 to 0.69)	0.31
SCH 39166	3.74 (3.16 to 4.43)	2.54[a] (2.24 to 2.87)	0.68	0.27 (0.20 to 0.36)	0.39 (0.27 to 0.59)	1.44

Table 4
DA receptor binding and selectivity of D1 agonists in rhesus and rat striata

Agonists	Rhesus striata			Rat striata		
One-site model	[^{3}H]SCH 23390 K_i (nM)	[^{3}H]Spiperone K_i (nM)	Selectivity D1 vs D2	[^{3}H]SCH 23390 K_i (nM)	[^{3}H]Spiperone K_i (nM)	Selectivity D1 vs D2
Dopamine	173 (148 to 202)	134 (118 to 152)	0.77	1,306 (1,040 to 1,650)	337 (243 to 466)	0.26[a]
SKF 38393	113 (101 to 126)	66,100 (55,800 to 78,400)	585[a]	62.6 (54.4 to 72.0)	150,000 (46,100 to 257,000)	2,400[a]
SKF 77434	40.3 (35.5 to 45.8)	557 (450 to 691)	13.8[a]	146 (118 to 181)	245 (185 to 324)	1.68[a]
SKF 81297	13 (11.6 to 14.6)	14,400 (12,500 to 16,500)	1,100[a]	5.2 (4.4 to 6.2)	4,940 (3,380 to 7,220)	950[a]
SKF 82958	16.3 (14.4 to 18.4)	310 (254 to 380)	19.0[a]	4.8 (3.5 to 6.5)	144 (101 to 204)	30.0[a]
R(+)6-BrAPB	14.3 (12.0 to 17.1)	152 (125 to 184)	10.6[a]	5.3 (4.2 to 6.6)	298 (209 to 426)	56.2[a]
S(−)6-BrAPB	91.0 (78.1 to 106.1)	667 (516 to 863)	7.33[a]	218.0 (185 to 257)	303 (227 to 404)	1.39[a]
SCH 23390	1.95 (1.54 to 2.47)	2,820 (2,190 to 3,630)	1,450[a]	1.81 (1.29 to 2.52)	3,410 (2,700 to 4,310)	1,880[a]
SCH 39166	3.74 (3.16 to 4.43)	1220 (1,010 to 1,470)	330[a]	0.27 (0.20 to 0.36)	2,970 (2,640 to 3,330)	11,000[a]

(Cl: SKF 81297 and SKF 82958; Br: $R(+)$ and $S(-)$ 6-BrAPB).

2.5. Data analysis

Radioligand binding data was initially reduced and analyzed using iterative curve fitting (Prism, Graphpad, San Diego, CA). Scatchard transformations of saturation data were used to aid the evaluation the number of binding sites displayed by saturation data. To compare the goodness-of-fit of one-site models to two-site models, one-site and two-site models with all Hill coefficients fixed to -1 were fit to displacement data. The one-site model was assumed unless the mean square error was significantly reduced by using a two-site model ($P < 0.05$ using a univariate F-test). K_i values and their 95% confidence intervals were calculated for each compound using K_d values derived from saturation studies of the radioligand and the method of Cheng and Prusoff (1973). Most of the compounds displaced [^{3}H]SCH 23390 binding in a biphasic manner, and the assay conditions included 16 concentrations in order to examine the characteristics of each site in the presence of GTP. As the effects of GTP on the displacement of [^{3}H]spiperone binding were not studied, [^{3}H]spiperone binding was examined using eight concentrations in order to provide a single estimate of K_i. Therefore, comparisons of ligand selectivity for D_1 and D_2 dopamine receptors were made using the K_i value for monophasic displacement of [^{3}H]SCH 23390 and the K_i value for monophasic displacement of [^{3}H]spiperone.

The K_i values for monophasic displacement of [^{3}H]SCH 23390 in the presence and absence of GTP were used to calculate the 'fold-shift' induced by GTP. The K_i in the presence of GTP was divided by the K_i in the absence of GTP and the resulting number was reported as the fold-shift. A fold-shift greater than one represents a rightward shift in the displacement curve induced by the presence of GTP. A fold-shift less than one represents a leftward shift in the displacement curve induced by the presence of GTP. The fold-shift measure has been reported as being a useful measure of the effect of GTP (Madras, 1993). The effects of 100 μM GTP upon the displacement of [^{3}H]SCH 23390 are reported in terms of one-site and two-site models as described above. Comparisons of the rank orders of the fold-shifts in rhesus and rat striata were done using Pearson's Rho procedure (GB-STAT, Dynamic Microsystems, Silver Spring, MD). Similar comparisons were made between fold-shift and efficacy to stimulate cAMP production in both species (data from Weed et al., 1997). The null hypothesis of Pearson's Rho is that rank orders differ.

The specific binding determined in the displacement assays was used to calculate the B_{max} of tissue samples using the K_d from the saturation experiments and the measured concentration of [^{3}H]ligand from each assay. The monkeys were divided into groups according to their drug history and the average B_{max} and its 95% confidence interval were calculated for each group. The three groupings were: little or no exposure to dopaminergic drugs (control), drug-free for more than two months prior to sacrifice and drug-free for less than two months prior to sacrifice.

3. Results

3.1. Analysis of [^{3}H]SCH 23390 radioligand binding characteristics in rhesus and rat striata

Results of four saturation experiments using [^{3}H]SCH 23390 in rhesus monkey and rat striata are presented in Fig. 2. Two of the rhesus monkeys had no history of dopaminergic drug administration and two monkeys had histories of cocaine self-administration. Based on overlapping 95% confidence intervals, no differences due to drug history were seen between these two groups of monkeys and the data were pooled for further analysis. The affinity of [^{3}H]SCH 23390 was 1.02 nM (95% CI: 0.65 to 1.36) for rhesus striata and 0.26 nM (0.21 to 0.30) for rat striata. The B_{max} was 1.35 pmol/mg protein (0.89 to 1.80) for rhesus striata and 1.56 pmol/mg protein (1.47 to 1.65) for rat striata.

Specific binding values from the displacement assays allowed for additional estimations of B_{max} for both species. These estimates were calculated using the K_d from the saturation studies, the specific binding values, and the measured concentration of [^{3}H]ligand from displacement assays. Group means ($N = 3$ per group) for the B_{max} of [^{3}H]SCH 23390 in control monkeys, the < 2 months drug-free group, and the > 2 months drug-free group were 1.57 (95% CI: 1.33 to 1.80), 1.74 (1.33 to 2.15) and 1.54 (1.34 to 1.73) pmol/mg protein, respectively. Given that drug history did not affect B_{max}, the B_{max} estimates of both the [^{3}H]SCH 23390 saturation and displacement assays for all animals were combined to yield an overall estimate of 1.63 pmol/mg protein (95% CI: 1.32 to 1.94; $N = 13$).

Specific binding accounted for 90–95% of total binding of [^{3}H]SCH 23390. Fig. 3 presents the effects of 100 μM GTP on [^{3}H]SCH 23390 for representative displacement curves of dopamine, SKF 81297 and SCH 23390 from rhesus and rat striata. In rhesus striata (Table 1), a two-site model of the displacement of [^{3}H]SCH 23390 by dopamine D_1 receptor agonists produced a significantly better fit than a one-site model in both the presence and absence of GTP with three exceptions: $S(-)$ 6-BrAPB in the absence of GTP and SKF 77434 and SKF 82958 in the presence of GTP. The percentage of [^{3}H]SCH 23390 binding at the higher-affinity site was reduced by coincubation with GTP ($P < 0.05$, based upon 95% CI's) for all agonists to which a two-site model produced a significantly better fit than a one-site model. In general, the presence of GTP reduced the percentage of higher-affinity sites without affecting the

K_i for either site in rhesus striata. Inclusion of GTP produced a small but significant decrease in the K_i of the lower affinity site for SKF 82958-displaceable [^{3}H]SCH 23390 binding.

In rat striata displacement of [^{3}H]SCH 23390 binding in the absence of GTP was best described by a two-site model for six of the seven compounds tested (Table 2). In contrast, displacement of [^{3}H]SCH 23390 by SKF 77434 was best fit to a one-site model. However, in the presence of GTP the displacement of [^{3}H]SCH 23390 was best fit to a two site model for only two of seven agonists (dopamine and SKF 82958). The percentage of receptors in the high-affinity state was not significantly affected in the presence of GTP for dopamine or SKF 89258. The conversion of a two-site model to a one-site model by the presence of GTP for the compounds other than dopamine or SKF 82958 is consistent with a nearly complete loss of one of the binding sites in the presence of GTP (Table 2). For four of the five compounds, the K_i of the single site in the presence of GTP was not significantly different from the K_i of the low-affinity site in the absence of GTP, suggesting that the site that was lost was the high affinity site.

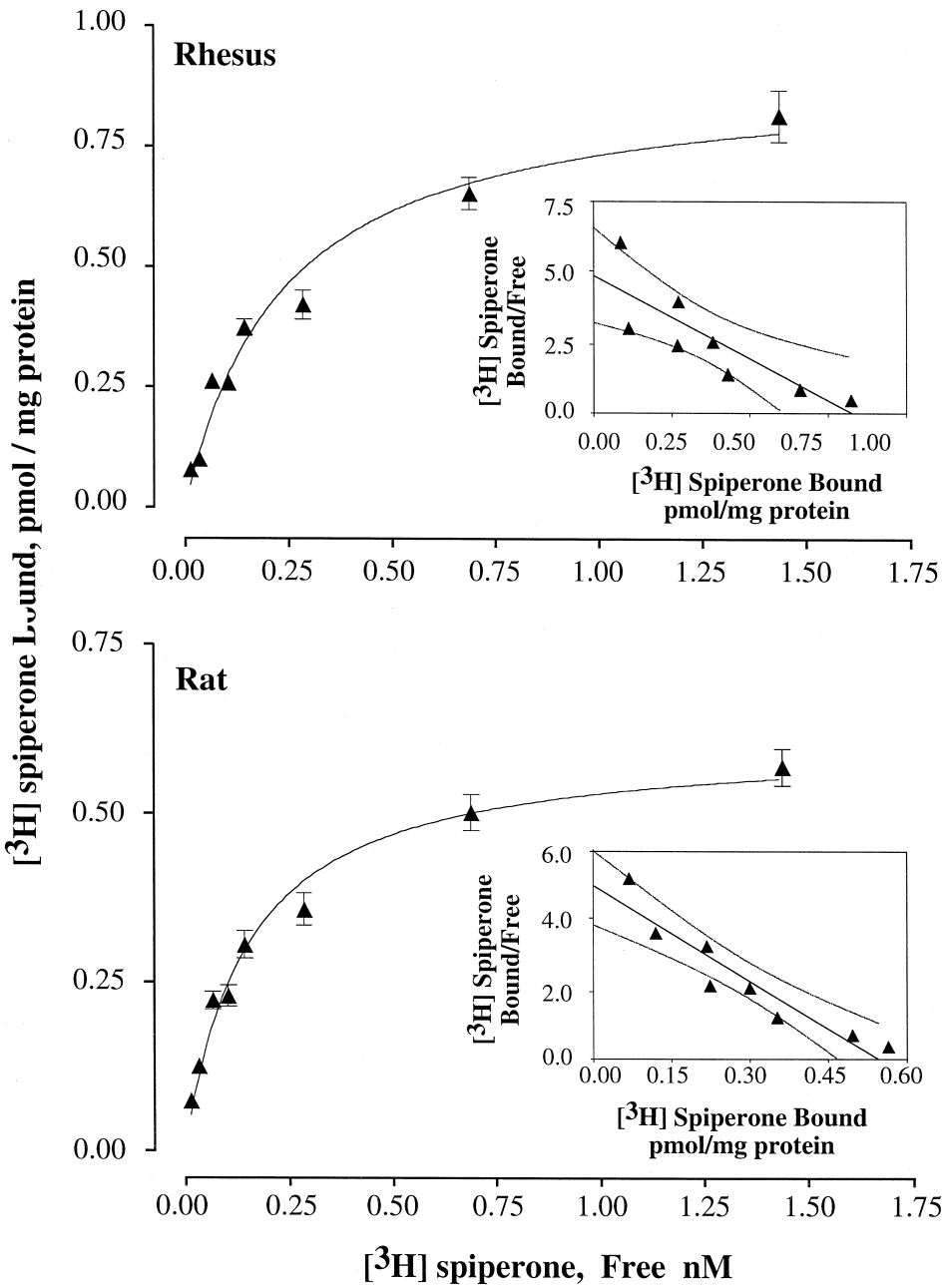


Fig. 4. [^{3}H]spiperone saturation experiments in rhesus and rat striata. Top panels represent data from rhesus monkey striata. Bottom panels represent data from rat striata. Main graphs: *Y*-axis represents bound [^{3}H]spiperone in pmol/mg protein, *X*-axis represents free [^{3}H]spiperone in nM. Inset graphs are the same data transformed into Scatchard analyses with dotted lines representing the 95% confidence intervals of the linear regression.

One measure of the effect of GTP upon displacement of [^{3}H]SCH 23390 is to calculate a ratio of the K_i with and without GTP. The higher the 'fold-shift' ratio, the larger the effect of the GTP. Should the K_i be doubled in the presence of GTP this would be shown as a 'fold-shift' of 2 (or a two-fold shift in the K_i). In rhesus monkey striata, dopamine had the largest fold-shift of 7.11, and the antagonist SCH 23390 had the smallest 0.64 (Table 3). In rat striata $R(+)$ 6-BrAPB had the highest fold shift of 1.92 and SCH 23390 had the lowest of 0.31 (Table 3). Rank order comparisons between the fold-shift induced by GTP in rat and rhesus striata indicated that these orders differed between the species (Rho = 0.43, $P > 0.05$). Comparisons between the fold-shifts and efficacy to stimulate cAMP

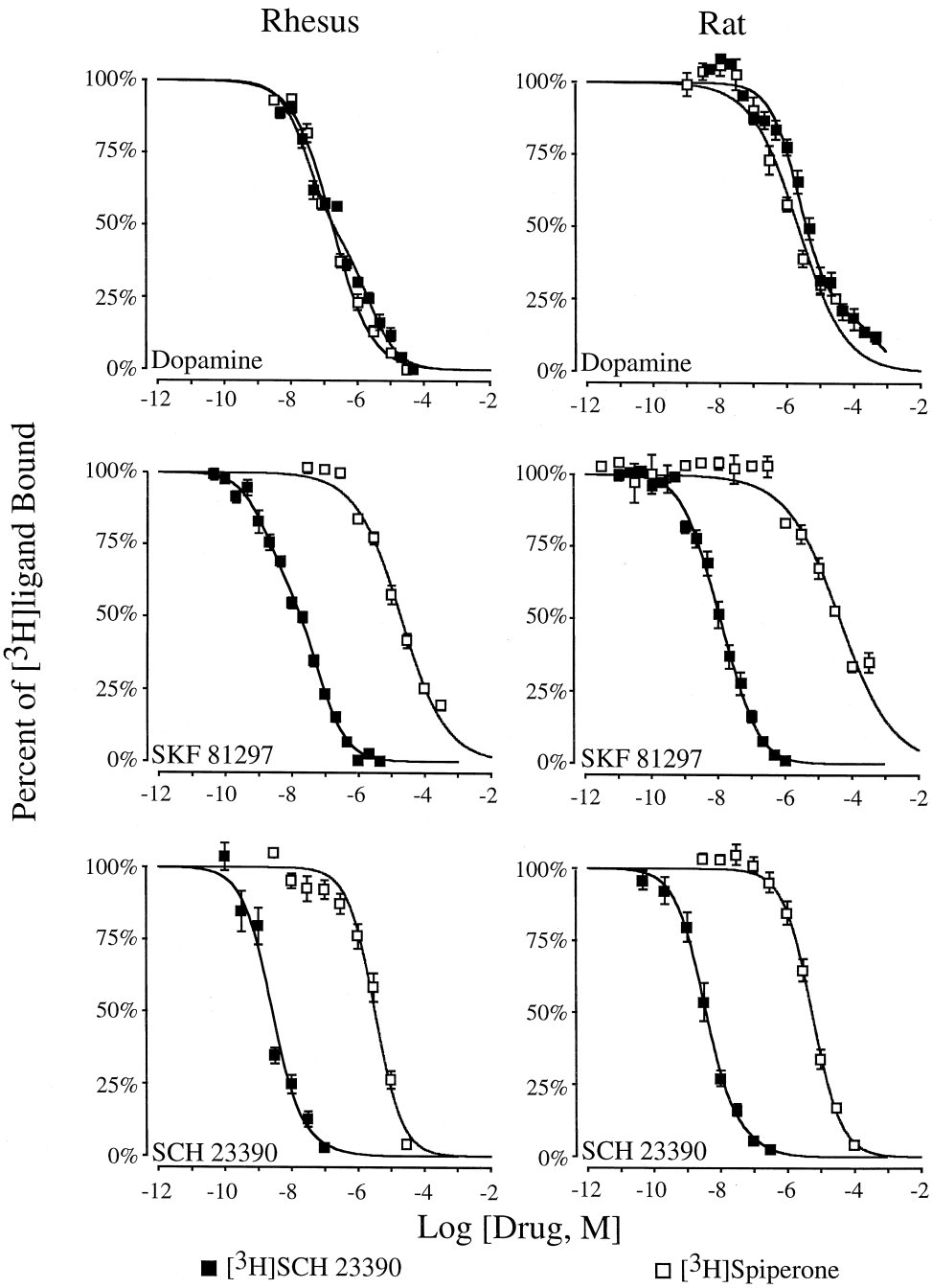


Fig. 5. Displacement of [^{3}H]SCH 23390 and [^{3}H]spiperone. Left panels represent displacement of [^{3}H]SCH 23390 and [^{3}H]spiperone in rhesus striata. Right panels represent displacement of [^{3}H]SCH 23390 and [^{3}H]spiperone in rat striata. *Y*-axis represents percent of [^{3}H]ligand bound. *X*-axis represents concentration of drug in log molar units. $N = 8$ for rhesus striata, and $N = 6$ for rat striata. Filled symbols represent displacement of [^{3}H]SCH 23390. Open symbols represent displacement of [^{3}H]spiperone. Data for dopamine, SKF 81297 and SCH 23390 are presented as representative of agonists with differing D_1:D_2 selectivities and a selective antagonist, respectively.

indicated that these rank orders were the same in rhesus tissue (Rho = 0.89; $P < 0.01$) but differed in rat tissue (Rho = 0.29, $P > 0.05$).

3.2. Analysis of [^{3}H]spiperone radioligand binding characteristics in rhesus and rat striata

Results of four saturation experiments using [^{3}H]spiperone in rhesus monkey and rat striata are presented in Fig. 4. As with [^{3}H]SCH 23390, no differences in the K_d values were seen between the control and drug experienced monkeys, and the data were pooled for further analysis. The K_d of [^{3}H]spiperone was 0.24 nM (95% CI: 0.18 to 0.3) in rhesus striata and 0.15 nM (0.12 to 0.18) in rat striata. The B_{max} was 0.91 (0.83 to 0.99) pmol/mg protein in rhesus striata and 0.60 (0.56 to 0.65) pmol/protein in rat striata, suggesting somewhat higher densities of dopamine D_2 receptors in rhesus monkeys than rats.

Using specific binding values from [^{3}H]spiperone displacement assays, no significant differences were seen in the B_{max} of [^{3}H]spiperone between control monkeys and monkeys with drug histories. Group means ($N = 3$) for the B_{max} of [^{3}H]spiperone in control monkeys, the < 2 months drug-free group, and the > 2 months drug-free group were 1.59 (95% CI: 1.36 to 1.82), 1.46 (1.26 to 1.66) and 1.63 (1.31 to 1.95) pmol/mg protein, respectively. Given that drug history did not affect B_{max}, the B_{max} estimates of both the [^{3}H]spiperone saturation and displacement assays for all animals were combined to yield an overall estimate of 1.38 pmol/mg protein (95% CI: 1.06 to 1.70)

As with [^{3}H]SCH 23390, specific binding accounted for approximately 90–95% of total [^{3}H]spiperone binding. The one-site K_i values from the displacement of [^{3}H]spiperone were compared to the one-site K_i values from the displacement of [^{3}H]SCH 23390 and presented in terms of D_1:D_2 selectivity (Table 4). Fig. 5 presents representative [^{3}*H*]*SCH* 23390 and [^{3}H]spiperone displacement curves in the presence of dopamine, SKF 81297 and SCH 23390 for rhesus and rat striata. All of the dopamine D_1 receptor agonists and antagonists examined in the present study displayed higher affinities for the dopamine D_1 receptor than the dopamine D_2 receptor. However, SKF 81297 and SKF 38393 were the only agonists to display D_1:D_2 selectivities greater than 100-fold in either species.

4. Discussion

The primary goal of the present study was to assess the radioligand binding characteristics of dopamine D_1 receptor agonists in rhesus monkey striata. The dopamine D_1 receptor agonists studied here all had greater affinity for D_1 than for D_2 sites; however dopamine D_1:D_2 receptor selectivities ranged widely. The rank order for dopamine D_1:D_2 receptor selectivity in rhesus striata was: SKF 81297 > SKF 38393 > > SKF 82958 > SKF 77434 > *R*(+) 6-BrAPB > *S*(−) 6-BrAPB > dopamine. The rank order for dopamine D_1:D_2 receptor selectivity in rat striata was: SKF 38393 > SKF 81297 > > *R*(+) 6-BrAPB > SKF 82958 > > SKF 77434 ≥ *S*(−) 6-BrAPB > dopamine. Although there were some species differences that led to a change in the precise rank order of D_1:D_2 selectivity, (e.g., *R*(+) 6-BrAPB displayed 10.6-fold selectivity in rhesus striata and 56.2-fold D_1:D_2 selectivity in rat striata) the same agonists that were highly selective in rhesus striata were highly selective in rat striata (i.e., SKF 81297 and SKF 38393).

For both species, binding of dopamine D_1 receptor agonists to the dopamine D_1 receptor occurred at two sites. This is consistent with previous reports in primates and rodents: macaques (Madras et al., 1988), humans (Gilmore et al., 1995), and rats (Hess et al., 1986; Zahniser and Molinoff, 1978). Similarly, the K_i values of SKF 38393 tested in the present study in rhesus monkeys agreed reasonably well with previous reports from cynomolgus monkeys: 10.6 high, 300 nM low affinity here; 1.8 high, 184 nM low affinity (Madras et al., 1988). Furthermore, displacement of [^{3}H]SCH 23390 in rat striata by agonists such as SKF 81297 produced K_i values similar to those reported elsewhere: 1.2 high, 17.7 low, present study; 1.4 high, 23 low (Andersen and Jansen, 1990). However, binding at two sites for SKF 82958 in the present study is in contrast to its apparent one-site binding reported in human striata (Gilmore et al., 1995). The use of 16 agonist concentrations in the present study allowed for higher resolution of two-site binding for compounds that have relatively similar affinities for the two sites, such as SKF 82958.

The dopamine D_1:D_2 receptor binding characteristics reported here are necessary to evaluate the receptor mechanisms underlying the behavioral effects of these compounds. Unfortunately, binding affinities are not sufficient to predict functional or behavioral effects. For instance, although SKF 38393 and SKF 81297 are similarly in their high D_1:D_2 selectivity, SKF 38393 is a low-efficacy dopamine D_1 receptor agonist while SKF 81297 is a high efficacy agonist in rhesus striata [stimulation of cAMP production (Weed et al., 1997)]. Furthermore, SKF 81297 functioned as a positive reinforcer in rhesus monkeys while, under the same conditions, SKF 38393 did not (Weed and Woolverton, 1995). The shift in dopamine D_1 receptor agonist displacement of [^{3}H]SCH 23390 induced by GTP was evaluated as a predictor of the functional or behavioral effects of dopamine D_1 receptor agonists that might supplement the D_1:D_2 selectivity data. This model would have an advantage over cAMP assays in that it could be performed in a radioligand binding assay. The presence of 100 μM GTP reduced the amount of high-affinity dopamine D_1 receptor agonist binding in both species. The rank order for fold-shift induced by GTP rhesus striata was: dopamine > SKF 82958 > *R*(+) 6-BrAPB > SKF 81297 > SKF 38393 > SKF 77434 > *S*(−)

6-BrAPB. Recently, the ability of each of these compounds to stimulate the production of cAMP was analyzed in both rat and rhesus monkey striata (Weed et al., 1997). The rank order of efficacy to stimulate the production of cAMP in rhesus striata was: dopamine > SKF 81297 > SKF 82958 > *R*(+) 6-BrAPB > SKF 38393 > SKF 77434 > *S*(−) 6-BrAPB. Although the precise rank orders for GTP fold-shifts and efficacy were not identical, statistical analysis (Spearman's Rho) indicated they were not significantly different.

By comparison, the rank order of efficacy to stimulate the production of cAMP in rat striata was: SKF 81297 > dopamine > SKF 82958 > SKF 38393 > *R*(+) 6-BrAPB > SKF 77434 > *S*(−) 6-BrAPB. The rank order for fold-shift induced by GTP in rat striata was: *R*(+) 6-BrAPB > dopamine > SKF 81297 > *S*(−) 6-BrAPB > SKF 38393 > SKF 82958 > SKF 77434. The difference in the two rank orders was statistically significant. The rank ordering of the fold-shift measure in rats does not appear to correspond to these or any published values for the stimulation of the production of cAMP. Therefore, this particular assay may not useful as a predictor of functional effects of dopamine D_1 receptor agonists. However, other binding assays involving guanyl nucleotides that may predict functional effects of dopamine agonists have been reported. Another assay that appears promising as a predictor of the functional effects of agonists at G-protein coupled receptors, such as dopamine receptors, uses binding of the activated G-protein GTP-gamma S (Chabert et al., 1994; Gardner et al., 1996). Additionally, the stable GTP analog Gpp(NH)p was reported to produce rightward shifts in the displacement of SCH 23390 by dopamine D_1 receptor agonists in a manner related to the efficacy of the dopamine D_1 receptor agonist (Madras, 1993). That study used substantially different assay conditions in addition to a different guanyl nucleotide, and this may have contributed to the differences between their findings and those of the present study.

The K_d of [^{3}H]SCH 23390 in rhesus striata was slightly higher than has been reported in a related monkey, the cynomolgus monkey (Madras et al., 1988). Interestingly, based on 95% confidence intervals, the dissociation constant for [^{3}H]SCH 23390 was significantly higher in the rhesus monkey than in the rat, as found in the present study and as previously reported (Hyttel and Arnt, 1987; Andersen and Jansen, 1990). Similarly, the K_d of [^{3}H]spiperone was somewhat higher in rhesus monkeys than in cynomolgus monkeys (Madras et al., 1988) and [^{3}H]spiperone displayed sub-nanomolar dissociation constants in rhesus monkey striata that were slightly but significantly lower than those seen in rats both herein and previously (Hyttel and Arnt, 1987; Andersen and Jansen, 1990). Since, as noted, variation in assay conditions make direct comparisons across experiments difficult, the conservative conclusion is that rhesus and cynomolgus monkeys are similar in D_1 and D_2 binding. Although these differences in K_d may represent an actual affinity difference between dopamine receptors in rhesus and rat, the differences are small and K_d estimates from the displacement studies suggest that affinities are similar across species. It seems unlikely that these represent real species differences in D_1 and D_2 affinity.

For the same reasons, absolute B_{max} values are difficult to compare across studies. Slightly higher densities of dopamine D_1 and D_2 receptors in rhesus striata were found in the present study (1.2 times higher dopamine D_1 than D_2 receptor density). Although this difference was not statistically significant, it was consistent with the somewhat higher density of dopamine D_1 receptors reported for cynomolgus monkeys (1.4 times higher dopamine D_1 than D_2 receptor density; Madras et al., 1988). However, these results are in contrast to those of Farfel et al. (1992) reporting that dopamine D_2 receptor density that was 3 times higher than that of dopamine D_1 receptors in control rhesus monkeys. Estimates using rodent striata usually indicate a two to three-fold higher density of dopamine D_1 receptors than D_2 receptors. For instance, the present study found 2.6 times higher density of D_1 than D_2, and Hyttel and Arnt (1987) found 2.8 times more D_1 than D_2. Although the primate literature is somewhat discordant, a conservative interpretation is that macaque striata does not display the overabundance of dopamine D_1 receptors relative to D_2 receptors seen in rodent striata.

A few studies have addressed the effects of cocaine history on dopamine receptor density in rhesus monkeys. Decreases in dopamine D_1 and D_2 receptor density have been demonstrated in rhesus monkeys with at least 18-month histories of i.v. cocaine self-administration (Moore et al., 1998a,b). In the Moore et al. studies, autoradiographic binding procedures were begun after the last cocaine self-administration session; therefore, these changes represent receptor adaptations during chronic cocaine exposure. Farfel et al. (1992) reported a reduction in dopamine D_1 but not D_2 receptor density two weeks after the last dose of a two-week treatment with 12–16 mg/kg per day non-contingent cocaine in rhesus monkeys. Therefore, cocaine administration in macaques can produce reductions in dopamine D_1 receptors lasting at least two weeks after exposure to cocaine. In the present study neither group of monkeys with histories of cocaine self-administration (<2 months or >2 months between cocaine administration and sacrifice) differed from control for either dopamine D_1 or D_2 receptor density. The results from the present study are consistent with recovery of any cocaine-induced dopamine receptor changes within months of the last cocaine exposure. Typically, the costs of procuring and training monkeys used in behavioral studies limits the use of experimentally naïve animals. Often, naïve animals are included with animals that have a significant pharmacological history. The similarity of dopamine receptor K_i and B_{max} values between naïve and cocaine-experienced animals suggests that the animals typically used in

self-administration studies do not have grossly abnormal dopamine receptors as a consequence of their cocaine exposure. However, there has been a report of decreases in dopamine D_2 receptor density lasting at least 8 months following chronic self-administration of cocaine in rhesus monkeys (Nader et al., 1997). Additional and more controlled investigations of the long-term effects of cocaine self-administration in primates will be needed to clarify this issue.

The present study provides documentation of the dopamine D_1/D_2 receptor selectivities in primate tissue of a series of putatively dopamine D_1 receptor selective agonists. These agonists are being used more and more frequently to investigate the behavioral effects of dopamine D_1 receptor stimulation in both rodents and primates. The results of the present study provide important information necessary to determine which receptors mediate the behavioral effects of these compounds. For highly selective ligands, such as the agonists SKF 38393 and SKF 81297, or the antagonists SCH 23390 and SCH 39166, binding selectivities suggest that dopamine D_1 receptors mediate their effects. However, as discussed above, the receptor binding selectivities are necessary but not sufficient information to predict which receptors mediate the effects of dopamine agonists with lower D_1/D_2 selectivities. Although the intrinsic efficacy at dopamine D_1 receptors has been studied, the intrinsic efficacy at dopamine D_2 receptors is needed to determine receptor mechanisms of compounds with low dopamine D_1/D_2 receptor binding selectivities, such as SKF 82958 or *R*(+) 6-Br APB. It is possible that these compounds have little activity at dopamine D_2 receptors and therefore are functionally selective dopamine D_1 receptor agonists; however it is also possible that they function as mixed dopamine D_1/D_2 receptor agonists. Nonetheless, the present results will be useful for further comparison of dopamine receptor binding characteristics and behavioral effects of phenyl-benzazepine dopamine D_1-like receptor agonists.

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References

Andersen, P.H., Jansen, J.A., 1990. Dopamine receptors: selectivity and dopamine D_1 receptor efficacy. Eur. J. Pharmacol. 188, 335–347.

Arnt, J., Bogeso, K.P., Hyttel, J., Meier, E., 1988. Relative dopamine D_1 and D_2 receptor affinity and efficacy determine whether dopamine agonists induce hyperactivity or oral stereotypy in rats. Pharmacol. Toxicol. 62, 121–130.

Berger, B., Gaspar, P., Verney, C., 1991. Dopaminergic innervation of the cerebral cortex: unexpected differences between rodents and primates . Trends Neurosci. 14, 21–27, published erratum appears in Trends Neurosci 1991 Mar;14(3):119.

Bergman, J., Spealman, R.D., Madras, B.K., Rosenzweig-Lipson, S., 1996. Agonist efficacy and the behavioral effects of dopamine D_1 receptor ligands: drug interaction studies in squirrel monkeys. J. Pharmacol. Exp. Ther. 276, 942–950.

Chabert, C., Cavegn, C., Bernard, A., Mills, A., 1994. Characterization of the functional activity of dopamine ligands at human recombinant dopamine D_4 receptors. J. Neurochem. 63, 62–65.

Cheng, Y., Prusoff, W.H., 1973. Relationship between the inhibition constant (Ki) and the concentration of inhibitor which causes 50 percent inhibition (I50) of an enzymatic reaction. Biochem. Pharmacol. 22, 3099–3108.

Demchyshyn, L.L., Sugamori, K.S., Lee, F.J., Hamadanizadeh, S.A., Niznik, H.B., 1995. The dopamine D_1 receptor. Cloning and characterization of three pharmacologically distinct D_1-like receptors from *Gallus domesticus*. J. Biol. Chem. 270, 4005–4012.

Farfel, G.M., Kleven, M.S., Woolverton, W.L., Seiden, L.S., Perry, B.D., 1992. Effects of repeated injections of cocaine on catecholamine receptor binding sites, dopamine transporter binding sites and behavior in rhesus monkeys. Brain. Res. 578, 235–243.

Gardner, B., Hall, D.A., Strange, P.G., 1996. Pharmacological analysis of dopamine stimulation of [35S]-GTP gamma S binding via human D_2short and D_2long dopamine receptors expressed in recombinant cells. Br. J. Pharmacol. 118, 1544–1550.

Gilmore, J.H., Watts, V.J., Lawler, C.P., Noll, E.P., Nichols, D.E., Mailman, R.B., 1995. 'Full' dopamine D_1 agonists in human caudate: biochemical properties and therapeutic implications. Neuropharmacology 34, 481–488.

Grech, D.M., Spealman, R.D., Bergman, J., 1996. Self-administration of D_1 receptor agonists by squirrel monkeys. Psychopharmacology (Berl) 125, 97–104.

Hess, E.J., Battaglia, G., Norman, A.B., Iorio, L.C., Creese, I., 1986. Guanine nucleotide regulation of agonist interactions at [3*H*]SCH23390-labeled D_1 dopamine receptors in rat striatum. Eur. J. Pharmacol. 121, 31–38.

Hyttel, J., Arnt, J., 1987. Characterization of binding of 3*H*-SCH 23390 to dopamine D-1 receptors. Correlation to other D-1 and D-2 measures and effect of selective lesions. J. Neural Tran. 68, 171–189.

Izenwasser, S., Katz, J.L., 1993. Differential efficacies of dopamine D_1 receptor agonists for stimulating adenylyl cyclase in squirrel monkey and rat. Eur. J. Pharmacol. 246, 39–44.

Kebabian, J.W., Calne, D.B., 1979. Multiple receptors for dopamine. Nature 277, 93–96.

Madras, B.K., 1993. Dopamine and D_1 dopamine receptor agonists: dopamine receptor selectivity and response to guanine nucleotides in nonhuman primate brain. Soc. Neurosci. Abstr. 19, 1063.

Madras, B.K., Fahey, M.A., Canfield, D.R., Spealman, R.D., 1988. D_1 and D_2 dopamine receptors in caudate-putamen of nonhuman primates (*Macaca fascicularis*). J. Neurochem. 51, 934–943.

Moore, R.J., Vinsant, S.L., Nader, M.A., Porrino, L.J., Friedman, D.P., 1998a. Effect of cocaine self-administration on dopmaine D_1 receptors in rhesus monkeys. Synapse 28, 1–9.

Moore, R.J., Vinsant, S.L., Nader, M.A., Porrino, L.J., Friedman, D.P., 1998b. Effect of cocaine self-administration on dopmaine D_2 receptors in rhesus monkeys. Synapse 30, 88–90.

Nader, M., Mach, R., Ehrenkaufer, R., Gage, H., Morton, T., 1997. Long-term down-regulation of dopamine D_2 receptors following cocaine self-administration in monkeys. In: Harris, L.S. (Ed.), Problems of Drug Dependence, 1997: Proceedings of the 59th Annual Scientific Meeting, The College on Problems of Drug Dependence, NIDA Res.

Mono. 1997, Vol. 178, US Dept. of Health and Human Services, NIH, NIDA, Rockville, MD, p. 154.

Neumeyer, J.L., Kula, N.S., Baldessarini, R.J., Baindur, N., 1992. Stereoisomeric probes for the D_1 dopamine receptor: synthesis and characterization of *R*-(+) and *S*-(−) enantiomers of 3-allyl-7,8-dihydroxy-1-phenyl-2,3,4,5-tetrahydro-1*H*-3-benzazepine and its 6-bromo analogue. J. Med. Chem. 35, 1466–1471.

O'Boyle, K.M., Waddington, J.L., 1987. New substituted 1-phenyl-3-benzazepine analogues of SK and F 38393 and *N*-methyl-thienopyridine analogues of dihydroxynomifensine with selective affinity for the D-1 dopamine receptor in human post-mortem brain. Neuropharmacology 26, 1807–1810.

Pifl, C., Reither, H., Hornykiewicz, O., 1991. Lower efficacy of the dopamine D_1 agonist, SKF 38393, to stimulate adenylyl cyclase activity in primate than in rodent striatum. Eur. J. Pharmacol. 202, 273–276.

Self, D.W., Stein, L., 1992. The D_1 agonists SKF 82958 and SKF 77434 are self-administered by rats. Brain Res. 582, 349–352, published erratum appears in Brain Res 1992 Sep 25;591(2):359.

Sibley, D.R., Monsma, F.J. Jr., 1992. Molecular biology of dopamine receptors. Trends Pharmacol. Sci. 13, 61–69.

Smith, P.K., Krohn, R.I., Hermanson, G.T., Mallia, A.K., Gartner, F.H., Provenzano, M.D., Fujimoto, E.K., Goeke, N.M., Olson, B.J., Klenk, D.C., 1985. Measurement of protein using bicinchoninic acid. Anal. Biochem. 150, 76–85, published erratum appears in Anal Biochem 1987 May 15;163(1):279].

Snider, R.S., Lee, J.C., 1961. A Sterotaxic Atlas of the Monkey Brain. University of Chicago Press, Chicago.

Stoof, J.C., Kebabian, J.W., 1981. Opposing roles for D-1 and D-2 dopamine receptors in efflux of cyclic AMP from rat neostriatum. Nature 294, 366–368.

Sugamori, K.S., Demchyshyn, L.L., Chung, M., Niznik, H.B., 1994. D_{1A}, D_{1B}, and D_{1C} dopamine receptors from *Xenopus laevis*. Proc. Natl. Acad. Sci. U.S.A. A 91, 10536–10540.

Sunahara, R.K., Guan, H.C., BF, O.D., Seeman, P., Laurier, L.G., Ng, G., George, S.R., Torchia, J., Van Tol, H.H., Niznik, H.B., 1991. Cloning of the gene for a human dopamine D5 receptor with higher affinity for dopamine than D_1. Nature 350, 614–619.

Tiberi, M., Jarvie, K.R., Silvia, C., Falardeau, P., Gingrich, J.A., Godinot, N., Bertrand, L., Yang-Feng, T.L., Fremeau, R.T. Jr., Caron, M.G., 1991. Cloning, molecular characterization, and chromosomal assignment of a gene encoding a second D_1 dopamine receptor subtype: differential expression pattern in rat brain compared with the D_1A receptor. Proc. Natl. Acad. Sci. U.S.A. 88, 7491–7495.

Vermeulen, R.J., Jongenelen, C.A., Langeveld, C.H., Wolters, E.C., Stoof, J.C., Drukarch, B., 1994. Dopamine D_1 receptor agonists display a different intrinsic activity in rat, monkey and human astrocytes. Eur. J. Pharmacol. 269, 121–125.

Weed, M.R., Woolverton, W.L., 1995. The reinforcing effects of dopamine D_1 receptor agonists in rhesus monkeys. J. Pharmacol. Exp. Ther. 275, 1367–1374.

Weed, M.R., Paul, I.A., Dwoskin, L.P., Moore, S.E., Woolverton, W.L., 1997. The relationship between reinforcing effects and in vitro effects of D_1 agonists in monkeys. J. Pharmacol. Exp. Ther. 283, 29–38.

Zahniser, N.R., Molinoff, P.B., 1978. Effect of guanine nucleotides on striatal dopamine receptors. Nature 275, 453–455.