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Dopamine receptor agonist potencies for inhibition of cell firing correlate with dopamine D_3 receptor binding affinities

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Abstract

The potencies for in vivo inhibition of substantia nigra pars compacta dopamine single cell firing were determined for apomorphine, BHT 920, N-0923, (±)-7-hydroxy-dipropylaminotetralin (7-OH-DPAT), (+)-3-(3-hydroxyphenyl)-*N*-propylpiperidine (3-PPP), pramipexole, quinelorane, quinpirole, RU 24926, U-86170, and U-91356. Significant correlation was obtained between the potencies of these 11 highly efficacious dopamine receptor agonists and the in vitro binding affinities at dopamine D_3 receptors, but not at dopamine D_{2L} receptors. These results support a functional role for the dopamine D_3 receptor subtype in the autoreceptor-mediated regulation of dopamine cell activity, while a role for dopamine D_2 receptors awaits further analysis. In addition, the results demonstrate the limitations of using currently available dopamine receptor agonists to delineate relative in vivo roles for the dopamine D_2 and D_3 receptor subtypes.

Keywords: Dopamine D_2 receptor; Dopamine D_3 receptor; Substantia nigra pars compacta; Autoreceptor; Single unit recording; 7-OH-DPAT ((±)-7-hydroxy-dipropylaminotetralin)

1. Introduction

It has been long appreciated that dopamine receptors exist on substantia nigra pars compacta dopamine cells which reduce cell firing rates when stimulated. When pharmacological evidence emerged supporting the existence of dopamine D_1 and D_2 receptor subtypes (Kebabian and Calne, 1979), a variety of neurophysiological studies utilizing dopamine D_1 or D_2 receptor selective drugs indicated that the dopamine autoreceptors were of the dopamine D_2 receptor subtype (Carlson et al., 1986, 1987; Mereu et al., 1985; Napier et al., 1986; Pinnock, 1983). This conclusion appeared confirmed when the dopamine D_2 receptor was cloned and mRNA encoding the dopamine D_2 receptor protein was identified in dopaminergic cells (Bunzow et al., 1988; Chen et al., 1991; Mengod et al., 1989). However, subsequent isolation of a related dopamine D_2-like receptor, termed the dopamine D_3 receptor, and identification of dopamine D_3 binding sites and mRNA in the substantia nigra pars compacta (Bouthenet et al., 1991; Levèsque et al., 1992; Sokoloff et al., 1990) suggest that the dopamine D_3 receptor subtype might also modulate substantia nigra pars compacta neuronal activity. Since drugs which act selectively at dopamine autoreceptors to regulate the activity of dopamine neurons would have therapeutic importance, there is considerable interest in determining the relative autoreceptor roles of the dopamine D_2 and D_3 receptor subtypes.

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Currently, there are no available drugs with sufficient dopamine D_3/D_2 receptor selectivity to permit definitive identification of the functional roles of the dopamine D_2 and D_3 receptor subtypes as autoreceptors. Therefore, in the present study, we investigated the relative autoreceptor roles of the dopamine D_2 and D_3 receptor subtypes by correlating the potencies of 11 highly efficacious dopamine receptor agonists for in vivo inhibition of substantia nigra pars compacta dopamine single cell firing with their affinities for dopamine D_{2L} and D_3 receptors as assessed by in vitro inhibition of [^{3}H]methylspiperone binding in Chinese hamster ovary cells transfected with rat dopamine D_{2L} or D_3 receptor cDNAs. Other studies using similar correlation analyses have proposed that dopamine D_3 receptors may mediate the inhibition of striatal dopamine synthesis (Meller et al., 1993) and cocaine self-administration (Caine and Koob, 1993).

2. Materials and methods

2.1. Extracellular single unit recordings

Extracellular single unit activity of substantia nigra pars compacta neurons was monitored using standard recording techniques in male Sprague-Dawley rats (250–350 g) maintained under anesthesia (Carlson et al., 1986). Briefly, rats were anesthetized with chloral hydrate (400 mg/kg i.p.) and placed in a stereotaxic instrument. A recording electrode was lowered via a hydraulic drive through a small burr hole drilled in the skull to the substantia nigra pars compacta using the following stereotaxic coordinates: 3.0 mm anterior to the lambdoid suture, 2.0 mm lateral to lambda, and 6.5–7.2 mm ventral to the dura. All experiments were conducted in accordance with NIH's Guide for Care and Use of Laboratory Animals. Dopamine neurons were identified by stereotaxic location, by their distinctive extracellular action potentials, and by histological location of the blue spot resulting from iontophoresis of Pontamine Sky Blue at the end of each experiment. Following a 3–5 min baseline period, dopamine receptor agonists were administered through a lateral tail vein in exponentially increasing doses at 1 min intervals. Drug doses refer to the weight of the salts. Only one cell per animal was studied. Six to 16 animals were tested per experimental group. Mean baseline firing rates of experimental groups did not differ from one another.

2.2. Receptor binding assays

Chinese hamster ovary cells were stably transfected with cDNAs encoding rat dopamine D_{2L} and D_3 receptors (Zhang et al., 1994). Crude membranes were prepared from Chinese hamster ovary cells and resuspended in binding buffer containing 50 mM Tris HCl, 1 mM EDTA, 5 mM KCl, 1.5 mM $CaCl_2$, 4 mM $MgCl_2$, and 120 mM NaCl (pH = 7.4 at 22°C). Dopamine D_{2L} and D_3 receptor binding sites were assayed with [^{3}H]methylspiperone at 25°C for 60 min and stopped by filtration through GF/B filters coated with 0.5% polyethyleneimine and washed 3 times in ice-cold Tris HCl (pH = 7.4 at 4°C). Nonspecific binding was measured in the presence of 1 μM (+)-butaclamol for dopamine D_{2L} receptor binding and 10 μM (+)-butaclamol for dopamine D_3 receptor binding. Competition studies with dopamine receptor agonists ranging from 10^{-13} M to 10^{-4} M were performed with 0.3 nM [^{3}H]methylspiperone.

2.3. Drugs

Apomorphine was obtained from Sigma Company (St. Louis, MO, USA). BHT 920 (taxipexole) and pramipexole were obtained from Boehringer Ingelheim (Ridgefield, CT, USA). N-0923 was obtained from Whitby Research (Richmond, VA, USA). Quinpirole and quinelorane were obtained from Eli Lilly and Company (Indianapolis, IN, USA). RU 23926 was obtained from Roussel UCLA (Paris, France). U-86170F and U-91356A were obtained from The Upjohn Company (Kalamazoo, MI, USA). Haloperidol (injectable) was obtained from McNeil Pharmaceuticals (Spring House, PA, USA). YM-09151-2 was obtained from Yamanouchi Pharmaceutical (Tokyo, Japan). (+)-Butaclamol, (±)-7-hydroxy-dipropylaminotetralin (7-OH-DPAT), and (+)-3-(3-hydroxyphenyl)-*N*-propylpiperidine ((+)-3-PPP) were obtained from Research Biochemicals (Natick, MA, USA). [^{3}H]Methylspiperone (80–90 Ci/mmol) was purchased from NEN/Dupont (Boston, MA, USA). Cell culture media, transfection reagents, and fetal bovine serum were purchased from Gibco (Grand Island, NY, USA). All other chemicals were purchased from commercial suppliers.

2.4. Data analysis

Drug dose-response curves were analyzed using the ALLFIT computer program. ED_{50} values, the mean half-maximal dose for inhibition of dopamine cell firing, are the geometric means. The 95% confidence intervals of these ED_{50} values are reported. Correlation coefficients were determined by least squares linear regression and significance was tested using the null hypothesis (Instat).

All binding parameters were obtained by weighted non-linear least square analysis for one-site model using the program INPLOT4. Coanalysis of three different experiments, performed in triplicate, was used to determine IC_{50} values. Inhibition constants (K_i) were

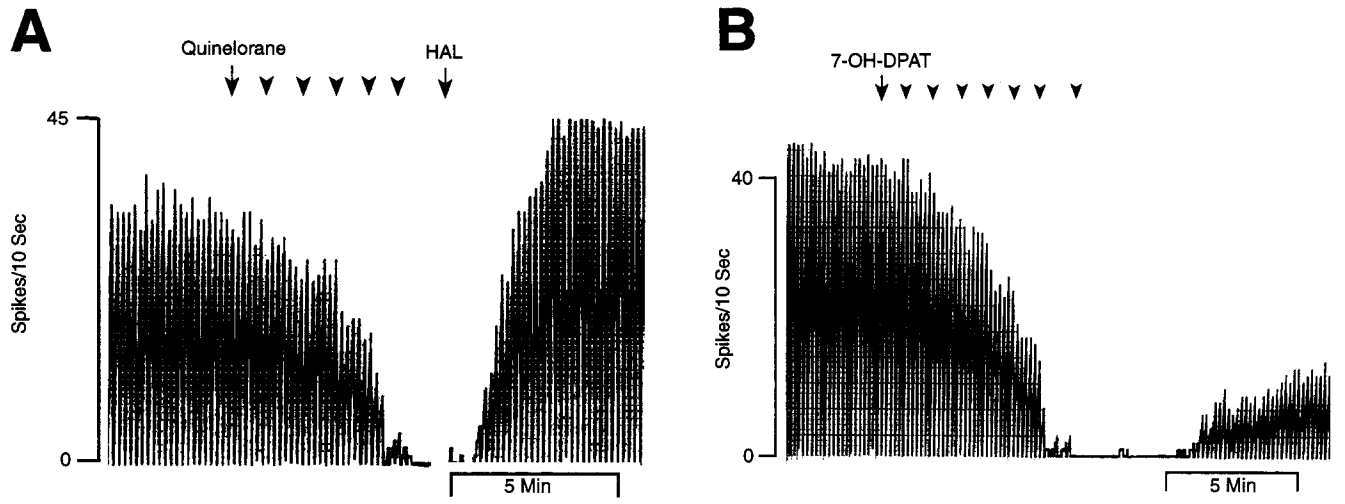

Fig. 1. A: Histogram depicting inhibition of dopamine cell firing by quinelorane. Quinelorane was given i.v. at doses of 0.15, 0.15, 0.3, 0.6, 1.25 and 2.5 μg/kg (total dose, 4.95 μg/kg) at times indicated by the arrows. Haloperidol (HAL; 0.05 mg/kg) reversed the inhibition. B: Histogram depicting inhibition of dopamine cell firing by 7-OH-DPAT. 7-OH-DPAT was given i.v. at doses of 0.15, 0.15, 0.3, 0.6, 1.25, 2.5, 5 and 10 μg/kg (total dose, 19.95 μg/kg) at times indicated by the arrows. A spontaneous recovery of firing was observed.

estimated according to the equation $K_i = IC_{50}/(1 + S/K_d)$ where S represents the ligand concentration.

3. Results

All dopamine receptor agonists selected for this study were effective inhibitors of in vivo dopamine single cell activity. Inhibitions of cell activity were typically reversed with the dopamine receptor antagonists haloperidol or YM-09151-2 (0.025–0.2 mg/kg). The most potent dopamine receptor agonist in the series was quinelorane, with an ED_{50} value of 0.8 μg/kg (95% confidence limit: 0.5–1.1 μg/kg, $n = 9$; Figs. 1A and 2A). 7-OH-DPAT, a dopamine receptor agonist frequently cited as being relatively selective for the dopamine D_3 receptor subtype, was the second most potent ($ED_{50} = 1.3$ μg/kg, 0.8–2.0 μg/kg, $n = 6$; Figs. 1B and 2A). N-0923 ($ED_{50} = 1.5$ μg/kg, 0.7–3.1 μg/kg, $n = 8$), and BHT 920 ($ED_{50} = 1.7$ μg/kg, 1.2–2.5 μg/kg, $n = 16$), had potencies similar to that of 7-OH-DPAT. The least potent agents were pramipexole ($ED_{50} = 20.2$ μg/kg, 12.3–28 μg/kg, $n = 9$) and (+)-3-PPP ($ED_{50} = 63.0$ μg/kg, 36–110 μg/kg, $n = 10$). Overall there was a 79-fold difference in the range of ED_{50} values for inhibition of dopamine cell firing within this series of drugs, as shown in Table 1.

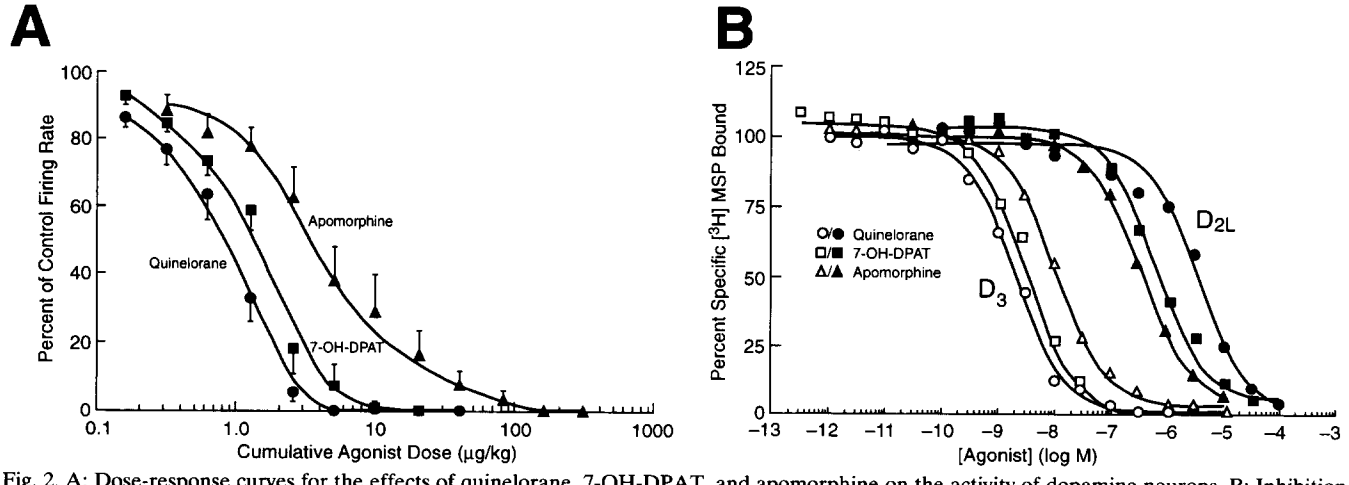

Fig. 2. A: Dose-response curves for the effects of quinelorane, 7-OH-DPAT, and apomorphine on the activity of dopamine neurons. B: Inhibition of [^{3}H]methylspiperone binding by quinelorane, 7-OH-DPAT, and apomorphine in Chinese hamster ovary cells transfected with rat dopamine D_3 or D_{2L} receptor cDNA.

Table 1
Measures of in vivo inhibition of dopamine neuronal activity and in vitro inhibition of [^{3}H]methylspiperone binding to Chinese hamster ovary cells transfected with cDNA for the dopamine D_3 or D_{2L} receptor

Dopamine agonists	ED_{50} (μg/kg) for inhibition of dopamine neuronal activity	K_i values, nM	
		D_3	D_{2L}
Quinelorane	0.8	4.7	972
7-OH-DPAT	1.3	4.0	237
N-0923	1.5	0.9	11
BHT 920	1.7	35.4	796
U-86170	2.7	51.1	205
Quinpirole	3.4	14.0	1,919
Apomorphine	3.9	31.5	73
RU 24926	6.8	8.8	86
U-91356	13.2	63.8	875
Pramipexole	20.2	13.6	1,890
(+)-3-PPP	63.0	335.0	6,126

When the inhibition constant (K_i) values for in vitro binding affinity at the dopamine D_3 receptors in transfected Chinese hamster ovary cells were examined, the three drugs with the lowest K_i values, N-0923, 7-OH-DPAT, and quinelorane, were also the most potent receptor agonists at inhibiting in vivo dopamine neuronal activity (Fig. 2B and Table 1). BHT 920, on the other hand, although very similar in potency to these drugs with respect to dopamine cell inhibition in vivo, had an 8- to 40-fold higher K_i for dopamine D_3 receptor binding. The drugs with the highest K_i values were U-91356 and (+)-3-PPP. Overall, for this series of dopamine receptor agonists, there was a 372-fold range in K_i values (Table 1).

In contrast to the results obtained for dopamine D_3 receptor binding, only one of the four most potent drugs at inhibiting in vivo dopamine cell firing, N-0923, was among those with the greatest affinity for in vitro dopamine D_{2L} receptor binding (Table 1). Overall, the series of dopamine receptor agonists had a 557-fold range of affinities for dopamine D_{2L} receptor binding, with N-0923, apomorphine, and RU 24926 having the highest affinities and pramipexole, quinpirole, and (+)-3-PPP having the lowest affinities (Table 1).

The ED_{50} values for inhibition of single unit activity of substantia nigra pars compacta dopamine neurons were compared with the K_i values for inhibition of [^{3}H]methylspiperone binding to Chinese hamster ovary cells transfected with cDNA for the dopamine D_3 (Fig. 3A) or dopamine D_{2L} receptors (Fig. 3B). The potencies of the 11 dopamine receptor agonists for in vivo inhibition of dopamine single cell firing correlated significantly with in vitro binding affinities at dopamine D_3 receptors ($r = 0.69$; $F(1,9) = 8.21$, $P = 0.02$), but not with affinities at dopamine D_{2L} receptors ($r = 0.51$; $F(1,9) = 3.13$, $P = 0.11$).

4. Discussion

The present study examined 11 highly efficacious dopamine receptor agonists (apomorphine, BHT 920, N-0923, 7-OH-DPAT, (+)-3-PPP, pramipexole, quinelorane, quinpirole, RU 24926, U-86170, and U-91356) with respect to their relative in vitro dopamine D_{2L} and D_3 receptor binding affinities and in vivo ED_{50} values for inhibition of dopamine cell firing. A significant correlation was obtained betweeen the receptor agonists' ED_{50} values and the affinities for dopamine D_3 receptors, thus lending support to the hypothesis that the dopamine D_3 receptor subtype plays a functional role in the autoreceptor-mediated regulation of dopamine cell activity. In contrast, the correlation between the dopamine receptor agonists' ED_{50} values and the affinities for dopamine D_{2L} recep-

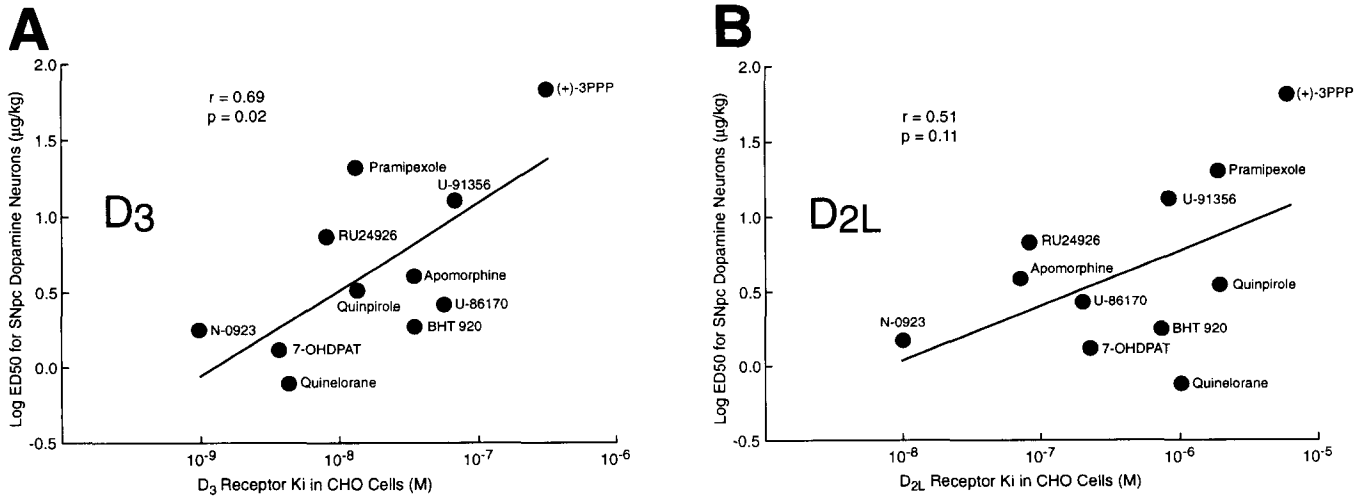

Fig. 3. Correlation between ED_{50} values and K_i values for the dopamine D_3 receptor ($r = 0.69$, $P = 0.02$; panel A) and dopamine D_{2L} receptor ($r = 0.51$, $P = 0.11$; panel B).

tors was not statistically significant, leaving the autoreceptor role of the dopamine D_2 receptor in question. These results are somewhat surprising in view of the fact that the level of dopamine D_2 receptor mRNA in the substantia nigra pars compacta is much higher than that of dopamine D_3 receptor mRNA (Bouthenet et al., 1991).

This study also demonstrates the potential limitations of using only a few drugs to draw conclusions about in vivo sites of action based on in vitro receptor affinities, as can be illustrated by selected comparisons from the full series of receptor agonists. For example, quinelorane has a 7-fold greater affinity for dopamine D_3 receptor binding and a 13-fold lower affinity for dopamine D_{2L} receptor binding than does apomorphine. Since quinelorane is 5 times more potent than apomorphine at inhibiting dopamine cell firing in vivo, this selected comparison would suggest an autoreceptor role for the dopamine D_3 receptor (see Fig. 2). In a second comparison, apomorphine has a 2-fold lower affinity for dopamine D_3 receptor binding and a 26-fold greater affinity for dopamine D_{2L} receptor binding than does pramipexole. Since apomorphine is 5 times more potent at inhibiting dopamine cell firing than pramipexole, this selected comparison supports an autoreceptor role for the dopamine D_2 receptor subtype. In a third comparison, the similar in vitro binding affinities of quinpirole and pramipexole for dopamine D_3 and D_{2L} receptors would lead to the prediction of similar in vivo potencies. However, quinpirole demonstrates a 6-fold higher potency than pramipexole for the inhibition of dopamine cell firing.

Overall, the series of dopamine receptor agonists in this study demonstrated a wider range of affinities for in vitro binding to dopamine D_3 and D_{2L} receptors, 372-fold and 557-fold respectively, than was demonstrated for in vivo effects, where only a 78-fold range of potencies was observed. These data suggest that there are variables which differentially affect binding to dopamine receptor subtypes expressed in Chinese hamster ovary cells and efficacy at reducing dopamine cell firing rate. First, the affinity state (high versus low) of the receptor may have varied between conditions used for in vitro binding measures and actual binding conditions in vivo. Secondly, the amount of drug at the site of action in vivo (dopamine autoreceptors located on dopamine cell bodies) may have differed between drugs as drug metabolism, degree of passage across the blood-brain barrier, and rate of diffusion all affect drug access to receptor sites. Thirdly, in the present study the relative in vitro affinities at dopamine D_{2L} and D_3 receptors were assessed by competition with a radiolabelled antagonist, methylspiperone, rather than with a radiolabelled agonist. However, while receptor agonist and antagonist binding studies may provide different absolute affinities, especially for the D_2 receptor as compared to the D_3 (Gonzalez and Sibley, 1995, M.F. Piercy, personal communication), they should provide similar relative potencies for efficacious drugs, such as the receptor agonists chosen for examination in this study.

In summary, the present study demonstrates that for a series of dopamine receptor agonists, in vivo effects on dopamine cell firing correlate with in vitro binding at dopamine D_3 receptors, but not with binding at dopamine D_2 receptors. While these results are inconclusive with respect to the role of dopamine D_2 receptors, they suggest a functional autoreceptor role for the dopamine D_3 receptor subtype. Further evaluation of the relative roles of the dopamine D_2 and D_3 receptor subtypes in vivo is currently limited by the variables that differentially affect in vitro and in vivo phenomena and the lack of selectivity of available receptor agonists.

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References

Bouthenet, M.L., E. Souil, M.P. Martres, P. Sokoloff, B. Giros and J.C. Schwartz, 1991, Localization of dopamine D_3 receptor mRNA in the rat brain using in situ hybridization histochemistry: comparison with dopamine D_2 receptor mRNA, Brain Res. 564, 203.

Bunzow, J.R., H.H. Van Tol, H.M. Grandy, D.K. Albert, J. Salon, M. Christie, C.A. Machda, K.A. Neve and O. Civelli, 1988, Cloning and expression of a rat D_2 dopamine receptor cDNA, Nature 336, 783.

Caine, S.B. and G.F. Koob, 1993, Modulation of cocaine self-administration in the rat through D-3 dopamine receptors, Science 260, 1814.

Carlson, J.H., D.A. Bergstrom and J.R. Walters, 1986, Neurophysiological evidence that D_1 receptor blockade attenuates postsynaptic but not autoreceptor-mediated effects of dopamine agonists, Eur. J. Pharmacol. 123, 237.

Carlson, J.H., D.A. Bergstrom, B.G. Weick and J.R. Walters, 1987, Neurophysiological investigation of the effects of the D-1 agonist SKF 38393 on tonic activity of substantia nigra dopamine neurons, Synapse 1, 411.

Chen, J.F., Z.H. Qin, F. Szele, G. Bai and B. Weiss, 1991, Neuronal localization and modulation of the D_2 receptor mRNA in brain of normal mice and mice lesioned with 6-hydroxydopamine, Neuropharmacology 30, 927.

Gonzalez, A.M. and D.R. Sibley, 1995, [^{3}H]7-OH-DPAT is capable of labeling dopamine D_2 as well as D_3 receptors, Eur. J. Pharmacol. 272, R1.

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

Levèsque, D., J. Diaz, C. Pilon, M.P. Martres, B. Giros, E. Souil, D. Schott, J.L. Morgat, J.C. Schwartz and P. Sokoloff, 1992, Identification, characterization, and localization of the dopamine D_3 receptor in rat brain using 7-[^{3}H]hydroxy-*N*,*N*-di-*n*-propyl-2-aminotetralin, Proc. Natl. Acad. Sci. USA 89, 8155.

Meller, E., K. Bohmaker, M. Goldstein and D.A. Basham, 1993, Evidence that striatal synthesis-inhibiting autoreceptors are dopamine D_3 receptors, Eur. J. Pharmacol. 249, R5.

Mengod, G., M.I. Martinez-Mer, M.T. Vilaro and J.M. Palacios, 1989, Localization of the mRNA for the dopamine D_2 receptor in rat brain by in situ hybridization histochemistry, Proc. Natl. Acad. Sci. USA 86, 8560.

Mereu, G., M. Collu, E. Ongini, G. Biggio and G.L. Gessa, 1985, SCH 23390, a selective dopamine D_1 antagonist, activates dopamine neurons but fails to prevent their inhibition by apomorphine, Eur. J. Pharmacol. 111, 393.

Napier, T.C., B.S. Givens, D.W. Schulz, B.S. Bunney, G.R. Breese and R.B. Mailman, 1986, SCH 23390 effects on apomorphine-induced responses of nigral dopaminergic neurons, J. Pharmacol. Exp. Ther. 236, 838.

Pinnock, R.D. 1983, Sensitivity of compacta neurones in the rat substantia nigra slice to dopamine agonists, Eur. J. Pharmacol. 96, 269.

Sokoloff, P., B. Giros, M.P. Martres, M.L. Bouthenet and J.C. Schwartz, 1990, Molecular cloning and characterization of a novel dopamine receptor (D_3) as a target for neuroleptics, Nature 347, 146.

Zhang, L.J., J.E. Lachowicz and D.R. Sibley, 1994, The D_{2S} and D_{2L} dopamine receptor isoforms are differentially regulated in Chinese hamster ovary cells, Mol. Pharmacol. 45, 878.