Affinity for Dopamine D_2, D_3, and D_4 Receptors of 2-Aminotetralins. Relevance of D_2 Agonist Binding for Determination of Receptor Subtype Selectivity†

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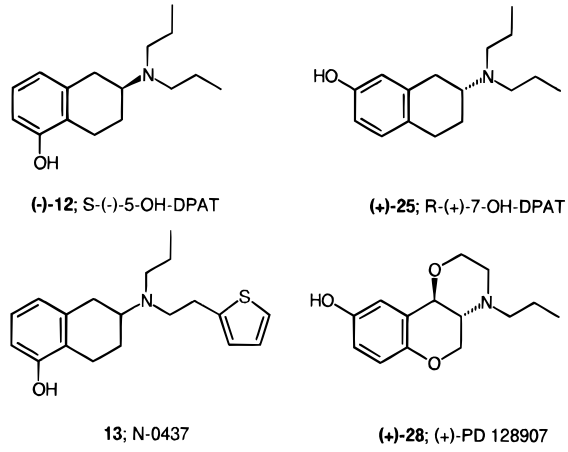
A series of 2-aminotetralins, substituted with a methoxy or a hydroxy group on the 5- or 7-position, and with varying *N*-alkyl or *N*-arylalkyl substituents, were prepared and evaluated in binding assays for human dopamine (DA) D_2, D_3, and D_4 receptors. Some members of this series were prepared in former studies, but were never tested *in vitro* with single receptor subtypes, and these were examined again. None of the tested 2-aminotetralins showed high affinity for the dopamine D_4 receptor. However, a number of the 2-aminotetralins showed high affinity for both the D_2 and the D_3 DA receptors, as exemplified by compounds **11**−**15** and **21**−**26**, while some had a reasonable selectivity for the DA D_3 receptors. The affinities of the 2-aminotetralins for the D_{2L} receptor depended on the type of radioligand (agonist or antagonist) used. The agonist affinity data, obtained by using the agonist ligand [^{3}H]N-0437, are thought to be more relevant for calculating DA receptor subtype selectivity.

Introduction

The pharmacological importance of the 2-aminotetralin (2-amino-1,2,3,4-tetrahydronaphthalene) structure has been known for a long time. Initially, the aminotetralins were characterized by their sympathomimetic action (causing mydriasis, contraction of the uterus, changes in blood pressure and respiration, and increased intestinal motility in test animals).[1] During the late 1960s the dopaminergic activity of 2-aminotetralins was identified which lead to an active synthesis program.[2] The development of dopamine (DA) receptor agonists has proceeded along two main routes, i.e. rigidification of the neurotransmitter DA and dissection of the semisynthetic agonist apomorphine. The aminotetralins can be regarded as a combination of both approaches. The 2-aminotetralin system has proved to be a valuable structural base not only for dopaminergic, but also for the development of serotonergic and adrenergic ligands, as well as compounds that interact with melatonin receptors. For example, the prototypic aminotetralin with serotonergic activity has the hydroxyl group in the 8-position,[3] while affinity for the melatonin receptor needs a 8-methoxy group and an amide instead of an amine moiety.[4]

Several research groups have studied these compounds to elucidate their structure activity relationship for DA receptors.[5−12] It was concluded that one aromatic hydroxyl group in the 5- or 7- position of the 2-aminotetralin is sufficient for potent dopaminergic activity (Chart 1).[5,7] In addition, one of the *N*-alkyl substituents should preferably be a *n*-propyl group (but not larger), while the size of the second *N*-alkyl group is a less stringent factor.[8,9] Initially, these studies

Chart 1



(-)-12; S-(-)-5-OH-DPAT **(+)-25**; R-(+)-7-OH-DPAT

13; N-0437 **(+)-28**; (+)-PD 128907

selected (*S*)-(−)-5-hydroxy-2-(*N*,*N*-di-*n*-propylamino)-tetralin [(*S*)-(−)-5-OH-DPAT, **(−)-12**, Chart 1] as the most potent 2-aminotetralin.[5,7,12] Later 5-hydroxy-2-(*N*-*n*-propyl-*N*-(2-thienylethyl)amino)tetralin (N-0437, **13**) was found to be an even more potent dopamine (DA) agonist.[11] Moreover, (*R*)-(+)-7-hydroxy-2-(*N*,*N*-di-*n*-propylamino)tetralin [(*R*)-(+)-7-OH-DPAT, **(+)-25**] was prepared and later shown to have preference for the D_3 DA receptor subtype.[13]

With the new developments of molecular biology, several novel DA receptors have been identified. It is generally accepted that the D_2, D_3, and D_4 receptors all belong to the D_2-like family of receptors, while the D_5 is more related to the D_1 receptor. Although the precise functions of these newly cloned DA receptors still remain to be discovered, their selective brain distribution, particularly for D_3 and D_4, makes them potential targets for development of novel antipsychotic drugs. This is illustrated by the fact that the D_3 and D_4 receptors are predominantly located in the limbic system,[14,15] a brain area which is known to be associated with cognitive and emotional functions and thought to be altered in psychiatric disorders such as schizophre-

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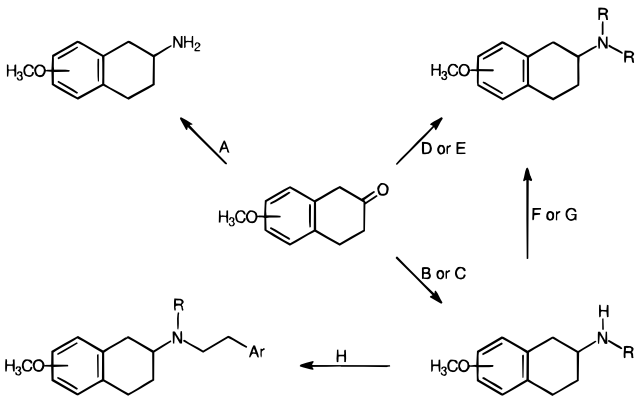
Scheme 1[a]



[a] Reagents: (A) (1) $PhCH_2NH_2$, *p*-TsOH−H_2O, toluene; (2) PtO_2, H_2, MeOH; (3) Pd−C (10%), H_2, MeOH; (B) RNH_2, $NaBH_3CN$, AcOH, MeOH; (C) RNH_2, $NaB(OAc)_3H$, HOAc, 1,2-dichloroethane; (D) R_2NH, $NaBH_3CN$, AcOH, MeOH; (E) R_2NH, $NaB(OAc)_3H$, HOAc, 1,2-dichloroethane; (F) RX, K_2CO_3, CH_3CN; (G) (1) RC(O)X, Et_3N, CH_2Cl_2; (2) $LiAlH_4$, Et_2O; (H) $ArCH_2COOH$, $(CH_3)_3NBH_3$, xylene.

nia. Recently, Seeman et al. found an elevated number of D_4 binding sites in postmortem brain tissues of schizophrenic patients.[16] However, this finding has been challenged by others.[17] The pharmacological investigation of the DA D_3 and D_4 receptors has generated a need for pharmacological tools that exhibit a high degree of selectivity for these receptor subtypes.

The aminotetralins prepared in earlier studies were generally tested in behavioral studies or in biochemical and radioligand binding assays using striatal brain tissue. With the successful cloning of different DA receptor subtypes, it is now possible to determine the affinity and the selectivity of a ligand for a single receptor subtype. The availability of these receptor subtypes encouraged us to test a homologous series of 2-aminotetralins including known compounds like (*R*)-(+)-7-OH-DPAT, (*S*)-(−)-5-OH-DPAT, and N-0437 (Chart 1) for their affinity for cloned human D_{2L}, D_3, and $D_{4.2}$ receptors. In this study, we report the synthesis of a series of 2-aminotetralins substituted with a methoxy or a hydroxy group in the 5- or 7-position and their affinity for cloned human D_{2L}, D_3, and $D_{4.2}$ receptors. PD 128907 [(4a*R*,10b*R*)-(+)-*trans*-3,4,4a,10b-tetrahydro-4-*n*-propyl-2*H*,5*H*-[1]benzopyrano[4,3-*b*]-1,4-oxazin-9-ol, Chart 1, **(+)-28**], the most DA D_3 selective agonist to date,[18,19] was included in the assays as a reference compound.

Chemistry

All compounds were prepared from 5- or 7-methoxy-2-tetralones, which in turn were prepared from 1,6- or 2,7-dihydroxynaphthalene, respectively, according to known procedures.[20] The 2-tetralones were subsequently converted to amines by various synthetic methods (Scheme 1). The primary amines were formed by condensation with benzylamine, followed by catalytic debenzylation (route A). Secondary amines and symmetrical tertiary amines were prepared by reductive amination with the appropriate (di)alkylamine (route B or C and D or E, respectively). Other tertiary amines were prepared by N-alkylation, or by N-acylation followed by $LiAlH_4$ reduction, of a secondary amine (route F or G). Phenylethyl or thiophenethyl groups were introduced by reductive alkylation of the appropriate secondary amine with phenylacetic acid or 2-thienylacetic acid, respectively, in the presence of trimethylaminoborane (route H).[11] Finally, demethylation with 48% HBr or BBr_3 afforded the phenols. The physical properties of the 2-aminotetralins are collected in Table 1.

Pharmacology

The 2-aminotetralins were tested for their *in vitro* binding affinity for human dopamine (DA) D_{2L}, D_3, or $D_{4.2}$ receptors, expressed in Chinese hamster ovary (CHO) K-1 cells. In the antagonist binding studies, the affinity of the compounds was determined by their ability to displace [^{3}H]spiperone from D_{2L}, D_3, or $D_{4.2}$ DA receptors. In the agonist binding studies, the affinity for the D_{2L} DA receptor was determined using [^{3}H]N-0437 as the radioligand. Receptor affinities are presented in Table 2.

Results and Discussion

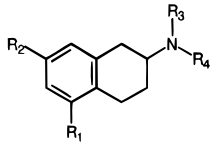
The D_2 receptor can exist in a high- or low-affinity state for agonists, depending on the degree of G-protein coupling.[21] This contrasts with the D_3 receptor, which is reported to exist mainly in a high-affinity state.[14] Initial evaluation of compounds for D_{2L} receptors in CHO K-1 cells revealed compounds like PD 128907 [**(+)-28**] with low affinity (K_i = 1183 nM), as compared to D_3 receptors expressed in same cells, where PD 128907 had a K_i value of 1.1 nM, indicating good selectivity for D_3 versus D_{2L}. However, due to the measurement of both high- and low-affinity D_{2L} sites in CHO K-1 cells, binding affinity was further evaluated at D_{2L} using the agonist ligand [^{3}H]N-0437 which measures only the high-affinity state of the receptor. Using the latter ligand to measure the high-affinity state, the affinity of PD 128907 was 42 nM. The difference between the binding selectivity of high-affinity states of the D_{2L} and D_3 receptor was considerably decreased in agreement with previous studies.[22] To achieve a proper comparison of D_{2L} and D_3 affinity data for the compounds in this study, we have also measured the affinities for the high-affinity state of the D_{2L} DA receptor labeled by [^{3}H]N-0437. We believe that these agonist binding data are more relevant in assessing D_{2L}/D_3 receptor selectivity, than the antagonist binding data obtained with [^{3}H]-spiperone.[19]

The aminotetralins in this study show no selectivity, and only weak to moderate affinity for the D_4 receptor. Although D_2, D_3, and D_4 receptors are all classified as D_2-like receptors, the binding data in this study illustrate the closer relationship between D_3 and D_2 receptors; the affinity in [^{3}H]spiperone binding (Table 2) of the compounds tested for D_{2L} and D_3 receptors is in general a factor 10−100 higher than the affinity for D_4, with the exception of compound **(+)-19**.

Hydroxy compounds have an increased affinity for D_3 and D_{2L} receptors, as compared to their corresponding methoxy compounds.[8] This trend seems to extend for D_4 receptors, although less pronounced for the 7-substituted compounds, which is illustrated by comparing the affinities of compounds **4** and **11** versus **17** and **21**. Possibly, hydrogen bond donation plays a less important role in the binding of 7-hydroxy-2-aminotetralins to the $D_{4.2}$ receptor.

The lipophilic character of the nitrogen substituents seems to have a marked effect on affinity toward the

Table 1. Physical Properties of the 2-Aminotetralins



compd	R_1	R_2	R_3	R_4	mp, °C (salt)
1	OCH_3	H	H	H	224−228 (HCl)[a]
2	OCH_3	H	CH_3	H	208−209 (HCl)[b]
3	OCH_3	H	CH_2CH_3	H	251 (HCl)[c]
4	OCH_3	H	$(CH_2)_2CH_3$	H	277−279 (HCl)[d]
5	OCH_3	H	$(CH_2)_3CH_3$	H	211−212 (HCl)[e]
6	OCH_3	H	CH_3	CH_3	214−215 (HCl)[f]
7	OCH_3	H	CH_2CH_3	CH_2CH_3	226−227 (HCl)[g]
8	OCH_3	H	$(CH_2)_2CH_3$	$(CH_2)_2CH_3$	205−207 (HCl)[h]
9	O-c-C_5H_9[i]	H	$(CH_2)_2CH_3$	$(CH_2)_2$-2-Th[j]	144−147 (HCl)[k]
10	OH	H	H	H	257 (HBr)[l]
11	OH	H	$(CH_2)_2CH_3$	H	248−251 (HBr)[m]
(+)-12	OH	H	$(CH_2)_2CH_3$	$(CH_2)_2CH_3$	208−210 (HBr)[n,o]
(−)-12	OH	H	$(CH_2)_2CH_3$	$(CH_2)_2CH_3$	208−210 (HBr)[n,p]
13	OH	H	$(CH_2)_2CH_3$	$(CH_2)_2$-2-Th[j]	185−188 (HCl)[q]
14	OH	H	$(CH_2)_2CH_3$	$(CH_2)_2$-3-Th[r]	184−187 (HCl)[s]
15	OH	H	$(CH_2)_2CH_3$	$(CH_2)_2$-Ph	190−193 (HCl)[t]
16	H	OCH_3	H	H	208−210 (HCl)[u]
(+)-17	H	OCH_3	$(CH_2)_2CH_3$	H	262−265.5 (HCl)[v]
(−)-17	H	OCH_3	$(CH_2)_2CH_3$	H	257−260.5 (HCl)[w]
(+)-18	H	OCH_3	$(CH_2)_2CH_3$	$(CH_2)_2CH_3$	261−262 (HCl)[x]
(−)-18	H	OCH_3	$(CH_2)_2CH_3$	$(CH_2)_2CH_3$	262−263 (HCl)[y]
(+)-19	H	OCH_3	$(CH_2)_2CH_3$	$(CH_2)_2$-2-Th[j]	159−160.5 (HCl)[n,z]
(−)-19	H	OCH_3	$(CH_2)_2CH_3$	$(CH_2)_2$-2-Th[j]	159−160.5 (HCl)[n,aa]
20	H	OH	H	H	182−183 (HBr)[bb]
21	H	OH	$(CH_2)_2CH_3$	H	196−198 (HBr)[cc]
22	H	OH	CH_3	CH_3	172−175 (HBr)[dd]
23	H	OH	CH_3	CH_2CH_3	210−212 ($^1/_2C_2H_2O_4$)[ee]
24	H	OH	CH_3	$(CH_2)_2CH_3$	206−207 ($^1/_2C_2H_2O_4{}^1/_2H_2O$)[ff]
(+)-25	H	OH	$(CH_2)_2CH_3$	$(CH_2)_2CH_3$	167−169 (HCl)[gg]
(−)-25	H	OH	$(CH_2)_2CH_3$	$(CH_2)_2CH_3$	166−168 (HCl)[hh]
26	H	OH	$(CH_2)_2CH_3$	$(CH_2)_2$-2-Th[j]	162−165 (HCl)[ii]
27	H	OH	$(CH_2)_2CH_3$	$(CH_2)_2$-Ph	ND[jj]

[a] Lit.[8] mp 266−267 °C. [b] Lit.[8] mp 210 °C. [c] Lit.[8] mp 247−248 °C. [d] Lit.[8] mp 260−261 °C. [e] Lit.[8] mp 210.5−211.5 °C. [f] Lit.[8] mp 214−216 °C. [g] Lit.[8] mp 217−219 °C. [h] Lit.[8] mp 171 °C. [i] Cyclopentyloxy. [j] 2-thienylethyl. [k] Lit.[27] no mp reported. Anal. ($C_{24}H_{33}NOS\cdot HCl$) C, H, N. [l] Lit.[8] mp 252−253 °C. [m] Lit.[8] mp 250−252 °C. [n] Melting point of racemate. [o] Lit.[8] mp 188−190 °C. [p] Lit.[8] mp 188−190 °C. [q] Lit.[11] mp 185−188 °C. [r] 3-Thienylethyl. [s] Lit.[28] mp 184−187 °C. [t] Lit.[8] mp 205−206 °C, lit.[9] mp 178−181 °C. [u] Lit.[29] mp 213−214 °C. [v] Lit.[10] mp 260−262 °C. [w] Lit.[10] mp 260−261 °C. [x] New compound. Anal. ($C_{17}H_{27}NO\cdot HCl$) C, H, N. [y] New compound. Anal. ($C_{17}H_{27}NO\cdot HCl$) C, H, N. [z] Lit.[28] mp 159−160 °C. [aa] Lit.[28] mp 159−160 °C. [bb] Lit.[7,12] no mp reported, lit.[30] mp 166.5 °C (HCl salt). Anal. ($C_{10}H_{13}NO\cdot HBr\cdot{}^1/_2H_2O$) C, H, N. [cc] New compound. Anal. ($C_{13}H_{19}NO\cdot HBr$) C, H, N. [dd] New compound. Anal. ($C_{12}H_{17}NO\cdot HBr\cdot H_2O$) C, H, N. [ee] New compound. Anal. ($C_{13}H_{19}NO\cdot{}^1/_2C_2H_2O_4$) H, N; C: calcd, 67.18; found, 66.32. [ff] New compound. Anal. ($C_{14}H_{21}NO\cdot{}^1/_2C_2H_2O_4\cdot{}^1/_2H_2O$) C, H, N. [gg] Lit.[10] mp 165−168 °C. [hh] Lit.[10] mp 169−171 °C. [ii] Lit.[28,31] no mp reported. Anal. ($C_{19}H_{25}NOS\cdot HCl$) C, H, N. [jj] Not determined; compound failed to crystallize. Anal. ($C_{21}H_{27}NO\cdot HCl\cdot{}^3/_4H_2O$) C, H, N.

D_4 receptor. The aminotetralins that have a primary or secondary amine group on the 2-position show weak affinities. However, compounds with a lipophilic moiety, such as a phenethyl or thiophenethyl substituent attached to the nitrogen, display higher affinities toward the D_4 receptor. This is illustrated by comparing the affinities of **(+)-17** and **(−)-17** (*N*-monopropyl) with **(+)-18** and **(−)-18** (*N*,*N*-dipropyl), and with **(+)-19** and **(−)-19** (*N*-propyl-*N*-thiophenethyl), respectively. However, steric effects may be important as well to explain the increasing affinity for the $D_{4.2}$ receptor within this subset of compounds.

The DA moiety can be incorporated in these structures in two ways, the so-called α- and *β*-rotameric form.[6] Structures are designated α- or *β*-conformers if they have their OH-group in the 5- or 7-position, respectively. According to the McDermed receptor model,[5] in the case of α-conformers the *S*-enantiomer is the most active, whereas for *β*-conformers the *R*-enantiomer is the most active enantiomer, findings that were also observed in this study. Table 2 shows that both conformers have affinity for the D_{2L} and D_3 receptor. However, α-conformers display less preference for the D_3 receptor (taking the D_{2L} agonist binding into account) compared to *β*-conformers. For example, (−)-5-OH-DPAT (**(−)-12**) binds merely with a 26-fold selectivity, whereas (+)-7-OH-DPAT (**(+)-25**) binds with a 60-fold selectivity for D_3 over D_2 receptors (taking D_{2L} agonist binding into account). The $D_{4.2}$ receptor shows no α- or *β*-conformer preference.

Introducing two *n*-propyl groups on the nitrogen of the two most simple aminotetralins of the series has a much more pronounced effect on the affinities of the 5-hydroxy-substituted analogue **10** than on the 7-hydroxy-substituted analogue **20**. Compound **(−)-12** has about a 250-fold increased affinity for the D_2 receptor, and about a 600-fold increased affinity for the D_3 receptor, compared to **10**. This increase in affinity is almost absent for the 7-OH series, illustrated by the comparison of **(+)-25** with compound **20**; D_2 DA binding hardly changes, and D_3 DA binding increases only about 9-fold. This difference in increase was also found by Seiler et al., although they measured receptor binding in homogenate from calf caudate nucleus.[12]

Table 2. Receptor Affinities of the 2-Aminotetralins

	K_i, nM[a]			
	competition for [^{3}H]spiperone binding			competition for [^{3}H]N-0437 binding
compound	D_{2L}	D_3	$D_{4.2}$	D_{2L}
1	334	683	>3333	ND[b]
2	131	409	603	ND
3	180	501	1519	ND
4	380	136	485	ND
5	694	603	>3333	ND
6	181	361	734	ND
7	120	857	964	ND
8	416	529	634	ND
9	1046	211	324	ND
10	1492	343	>3333	ND
11	285	0.75	76	0.50
(*R*)-(+)-12	90	76	182	55.1
(*S*)-(−)-12[c]	6	0.54	47	14.0
13[d]	20	3.99	55	0.06
14	24	1.82	72	0.14
15	19	3.37	54	0.50
16	5825	2220	>3333	ND
(*R*)-(+)-17	5882	1372	>3333	ND
(*S*)-(−)-17	3206	3055	>3333	ND
(*R*)-(+)-18	5882	648	1504	ND
(*S*)-(−)-18	3351	266	335	ND
(*R*)-(+)-19	550	113	23	15.9
(*R*)-(−)-19	1241	154	134	250
20	78	5.12	>3333	3.3
21	836	0.66	269	3.8
22	55	1.41	729	5.4
23	173	0.71	1033	2.5
24	190	0.74	32	6.7
(*R*)-(+)-25[e]	56	0.57	110	34
(*S*)-(−)-25	4777	58	>3333	275
26	59	0.75	22	0.76
27	524	1.98	54	ND
(+)-28, PD 128907	1183	1.1	7000	42

[a] K_i values are means of two to six separate experiments the results of which did not vary more than 25%. [b] ND, not determined. [c] (*S*)-(−)-5-OH-DPAT. [d] N-0437. [e] (*R*)-(+)-7-OH-DPAT.

In conclusion it is essential to use the agonist data for D_2 binding when determining D_2/D_3 receptor selectivity. If not, one would erroneously conclude that compounds **21**, **23**, and **24** are highly selective for the D_3 receptor. Compound **(+)-25** is the only aminotetralin that has a reasonable selectivity for the D_3 receptor (ca. 60-fold), which is in accordance with other studies.[13]

Experimental Section

Chemistry. Melting points were determined in open glass capillaries on an Electrothermal digital melting-point apparatus and are uncorrected. Elemental analyses by Robertson Microlit Laboratories Inc., Madison, NJ, or by the Department of Chemistry, University of Groningen, were performed for new compounds and for compounds that were previously published without a melting point. Where elemental analyses are indicated, results obtained were within 0.4% of the theoretical values, except where noted.

Pharmacology. Cell Lines Expressing Dopamine (DA) Receptor Isoforms. A cell line expressing the human DA D_{2L} was purchased from Dr. O. Civelli, Oregon Health Sciences University. The D_{2L} receptor cDNA was subcloned into the expression vector, pRc/CMV. The plasmids were transfected by electroporation into CHO K-1 cells. A single stable transfectant, resistant to the antibiotic G418, was isolated and selected for use in the binding studies. The human DA D_3 receptor cDNA cloned in the pcDNAIneo plasmid was obtained from Dr. K. O'Malley and stably transfected into CHO K-1 cells by a modified calcium phosphate precipitation technique,[23] and transfectants were selected in G418, isolated, and screened for expression of human D_3 receptors by radioligand binding as previously described.[24] For D_4 binding, CHO K-1 cells stably transfected to express the human recombinant DA $D_{4.2}$ receptor subtype were used.[25]

Cell Culture and Preparation of Cell Membranes. CHO K-1 cells expressing human DA D_{2L}, D_3, and $D_{4.2}$ receptors were grown in 162 cm^2 culture flasks in F12 medium (Gibco Laboratories, Grand Island, NY) supplemented with 10% fetal bovine serum (FBS, Hyclone, Logan, UT) in an atmosphere of 5% CO_2/ 95% air at 37 °C. Cells were grown until confluent, after which growth medium was removed and replaced with 0.02% EDTA in a phosphate-buffered saline solution (Sigma Chemical Co. St. Louis, MO) and scraped from the flasks. The cells were centrifuged at about 1000*g* for 10 min at 4 °C and then resuspended in TEM buffer (25 mM Tris-HCl, pH 7.4 at 37 °C, 1 mM EDTA, and 6 mM $CaCl_2$) for D_{2L} and D_3 or the $D_{4.2}$ buffer (50 mM Tris-HCl, pH 7.4, 5 mM EDTA, 1.5 mM $CaCl_2$, 5 mM KCl, and 120 mM NaCl) and homogenized with a Brinkman Polytron homogenizer at setting 5 for 10 s. The membranes were pelleted by centrifugation at 20000*g* at 4 °C for 20 min, and then the pellets were resuspended in appropriate buffer at 1 mL/flask and stored at −70 °C until used in the receptor binding assay.

Receptor Binding Assays: D_2, D_3, and $D_{4.2}$ Dopamine Receptors. A cell membrane preparation (400 μL) was incubated in triplicate with 50 μL [^{3}H]N-0437 (2 nM for D_{2L}) or [^{3}H]spiperone (0.2 nM for D_{2L}, 0.5 nM for D_3, 0.2 nM for $D_{4.2}$), 50 μL buffer, or competing drugs where appropriate to give a final volume of 0.5 mL. After a 60 min incubation at 25 °C, the incubations were terminated by rapid filtration through Whatmann GF/B glass fiber filters (soaked for 1 h in 0.5% polyethylenimine) on a Brandel MB-48R cell harvester, with three washes of 1 mL ice-cold buffer. Individual filter disks containing the bound ligand were placed in counting vials with 4 mL of scintillation fluid (Ready Gel, Beckman Instrument Inc, Fullerton, CA) and then counted in a Beckman LS-6800 liquid scintillation counter at an efficiency of 45%. Nonspecific binding was defined in presence of 1 μM of haloperidol.

Data Calculation. Saturation and competition binding data were analyzed using the iterative nonlinear least-squares curve-fitting Ligand program. In competition experiments, apparent K_i values were calculated from IC_{50} values by method of Cheng and Prusoff.[26] Experimental compounds were made up as stock solutions in dimethyl sulfoxide (DMSO). The final concentration of 0.1% DMSO used in the incubation mixture had no effect on the specific binding. Each observation was carried out in triplicate. To allow these calculations, K_d values were measured for the interaction of various ligands with the receptor. These were as follows: [^{3}H]spiperone binding, human D_3, 0.15 ± 0.02, human D_{2L}, 0.12 ± 0.01 and human $D_{4.2}$, 0.093 ± 0.005 nM (n = 3); [^{3}H]N-0437 binding, human D_{2L}, 2.24 ± 0.05, human D_3, 1.77 ± 0.05 nM (n = 3).

References

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