MOLECULAR PHARMACOLOGY, 28:391–399

Dopamine D_2 Receptor Binding Sites for Agonists

A Tetrahedral Model

PHILIP SEEMAN, MASAYUKI WATANABE,[1] DIMITRI GRIGORIADIS, JOSEPH L. TEDESCO, SUSAN R. GEORGE, U. SVENSSON, J. LARS G. NILSSON, AND JOHN L. NEUMEYER[2]

Departments of Pharmacology (P.S., M.W., D.G., J.L.T., S.R.G.) and Medicine (S.R.G.), Medical Sciences Building, University of Toronto, Toronto, Canada M5S 1A8; Graduate School of Pharmacy and Allied Health Professions (J.L.N.), Northeastern University, Boston, Massachusetts 02115; and Department of Organic Pharmaceutical Chemistry (U.S., J.L.G.N.), Biomedical Center, University of Uppsala, S-75 123 Uppsala, Sweden

Received April 16, 1985; Accepted September 3, 1985

SUMMARY

In order to develop a model for the putative binding sites between the D_2 dopamine receptor and many of its agonists, we obtained the dissociation constants of many dopaminergic agonists at the high affinity state, D_2^{high}, as well as at the low affinity state, D_2^{low}, of the receptor. [^{3}H]Spiperone was used to label the D_2 dopamine receptors in porcine anterior pituitary tissue. Agonists without any hydroxyl groups, such as 2-aminotetralin, effectively inhibited the binding of [^{3}H]spiperone; the addition of a hydroxyl group corresponding to the "*meta*" position in dopamine, however, enhanced the potency (in four series of agonists) by an order of magnitude. The R-(−)-enantiomers of the aporphines and 5,6,-dihydroxy-2-dipropylaminotetralin were more potent than the S-(+)-enantiomers. Although the 4-methoxy-2-dipropylaminoindans were potent, the R-(−)-11-methoxyaporphines were not. A tetrahedral model is proposed; this has two sites for agonist attachment, the extremities of the sites being separated by 8 Å, and their functional groups directed between 15° and 30° off the orthogonal from the receptor surface. Several steric obstacles are required to account for the inactivity of several congeners.

INTRODUCTION

The brain contains dopamine D_1 receptors, which stimulate adenylate cyclase, and D_2 receptors, which inhibit adenylate cyclase (1, 2). There is no generally accepted single model or pharmacophore for the D_2 receptor which precisely accommodates the many different types of dopamine congeners with agonist or antagonist activity (3).

Three types of D_2 receptor models have been proposed. (*1*) The *phenylethylamine* model proposes that the distance between the nitrogen atom and the center of the aromatic ring is 6 Å (4–6) for dopamine antagonists. (*2*) To explain the dopamine agonist actions of ergots, the *pyrroleethylamine* moiety (7–9) or the indoleethylamine moiety (10) has been proposed as the pharmacophore, where the weakly acidic indole NH group is bioisosteric with the "*meta*" OH of dopamine (10). (*3*) A third type of model requires a distance of approximately 7 Å between the nitrogen atom and the *meta* OH of dopamine or the corresponding OH of related congeners (3, 11–16).

There are at least two difficulties with these models. First, some 2-aminoindans are equipotent to (−)-apomorphine in causing rotation or inhibiting DOPA[3] synthesis, yet they do not readily fit any of the above models (17, 18). For example, the distance between the nitrogen atom and the oxygen atom in 4-hydroxy-2-dipropylaminoindan is 5.5 Å, which is not readily accommodated by any of the above models for the D_2 receptor.

Another reception is (±)-isoapomorphine [(±)-9,10-dihydroxyaporphine]. In order to account for the dopamine agonist inactivity (3, 19) of this molecule, Grol and Rollema (20) suggested that the A ring of isoapomorphine interacts with an obstacle near the receptor site. Cannon *et al.* (21), however, have prepared an analog of isoapomorphine without the interfering A ring, *i.e.*, derivatives of octahydrobenzo[*g*]quinoline, and yet these compounds were also inactive as dopamine agonists.

In order to develop a conceptual model to account for the putative binding sites for the D_2 receptor and its many agonists, we obtained agonist dissociation constants at the high affinity state of the D_2 receptor, D_2^{high},

This work was supported by the Medical Research Council of Canada and the Ontario Mental Health Foundation.

[1] Supported by a fellowship from the Canadian Friends of Schizophrenics.

[2] Supported by the National Institutes of Health, National Institute of Neurological and Communicative Disorders and Stroke.

[3] Abbreviation used is: DOPA, 6-dihydroxyphenylalanine.

0026-895X/85/050391-09$02.00/0

TABLE 1

Binding of [^{3}H]spiperone to D_2 receptors

The binding of [^{3}H]spiperone was done in the absence of 100 mM NaCl, unless indicated by (Na^+). The K_D of [^{3}H]spiperone was 64 pM in the presence of 100 mM NaCl and was 130 pM in the absence of NaCl. A single K value indicates that the compound only recognized a single population of [^{3}H]spiperone binding sites.

Agonist	K_D^{high}	K_D^{low}	Source
	nM		
Aminoindan-dipropyl·HCl [DR 4-7]	84.5	11,800	JC[a]
Aminoindan-4-OH-dipropyl-(±)·HBr [USDA 46]	27.3	2,220	LN
Aminoindan-4-OH-dipropyl-(−)·HBr [RD-219.1]	8.3	1,746	JC
Aminoindan-4-OH-dipropyl-(+)·HBr [RD-221.1]	106	18,519	JC
Aminoindan-4-OCH_3-dipropyl-R-(−)·HCl [RD-269.3]	63	3,977	JC
Aminoindan-4-OCH_3-dipropyl-S-(+)·HCl [RD-267.7]	20	1,609	JC
Aminoindan-4,5-diol-dipropyl·HBr [JPC 266]	60.9	6,400	JC
Aminoindan-4,6-diol-dipropyl·HBr [HA 97]	155	30,700	JC
Aminoindan-4,7-dimethoxy-dipropyl·HCl [RDS 127]	5.1	882	JC
Aminotetralin-(±)·HBr [JGC-127]	27,600		JC
Aminotetralin-5,6-diol-(±)·HBr	22.3	33,000	JC
Aminotetralin-6,7-diol-(±)·HBr [(±)-ADTN]	1.7	463	RB
Aminotetralin-dipropyl·HCl [TL 68; JGC 154]-(±)	42.6	9,160	JC
Aminotetralin-5-OH-dipropyl-(±)·HCl [JGC 174]	11.4	764	JM
Aminotetralin-5-OH-dipropyl-(−)·HCl	3.1	257	JM
Aminotetralin-5-OH-dipropyl-(+)·HCl	353	52,000	JM
Aminotetralin-5-OH-2-(N-*n*-propyl-N-thiophenethylamino)-(−)·HCl [N-0437]	0.14	38	AH
Aminotetralin-5-OH-2-(N-*n*-propyl-N-phenylethyl)-(±)·HCl [N-0434]	0.73	70	AH
Aminotetralin-6-OH-dipropyl-(±)·HI	57.3	14,340	JM
Aminotetralin-7-OH-dipropyl-(±)·HI	10.1	3,860	JM
Aminotetralin-7-OH-dipropyl-(−)·HCl	1,365	65,377	JM
Aminotetralin-7-OH-dipropyl-(+)·HCl	36.1	2,305	JM
Aminotetralin-5,6-diol-dipropyl-(±)·HBr	0.82	40	RB
Aminotetralin-5,6-diol-dipropyl-(−)·HBr	2.1	278	JM
Aminotetralin-5,6-diol-dipropyl-(+)·HBr	20.4	838	JM
Aminotetralin-6,7-diol-dipropyl-(±)·HBr [TL-232]	12	2,100	RB
Aminotetralin-6,7-diol-dimethyl-(±)·HBr [TL-99]	2.3	418	JC
Apomorphine-(−)·HCl	0.66	127	MF
Apomorphine-S-(+)·HCl	493	51,300	RB
Apomorphine-2-OH-(−)·HBr	1.1	139	JN
Apomorphine-2-OH-(+)·HBr	227	17,200	JN
Aporphine-N-propyl-11-methoxy-R-(−)·HCl	130	7,495	JN
Aporphine-N-propyl-11-OH-R-(−)·HCl	1.4	145	JN
Aporphine-N-Pr-10-hydroxy-(±)·HBr [WPD-IV-42][b]	7.3	3,560	JN
Aporphine-N-chlorethyl-10,11-diol-(−)·HCl	55.4	108,000	RB

TABLE 1—*continued*

Agonist	K_D^{high}	K_D^{low}	Source
	nM		
Aporphine-N-propyl-10,11-diol-(−)·HCl ["NPA"]	0.4	23	RB; SW[c]
Aporphine-N-propyl-10,11-diol-(+)·HCl	3.5	872	RB
Aporphine-9,10-diol-(±)·HBr [(±)-isoapomorphine]	18,700		JN
Benzazepine-7,8-diol-1-Ph-(±) [SKF 38393][b]	157	8,800	SK
Benzo[*f*]quinoline-7,8-diol-*cis*·HBr [TL-224]	98	330,000	JC
Benzo[*f*]quinoline-7,8-diol-*trans*·HBr [TL-137]	1.8	680	JC
Benzo[*f*]quinoline-N-Me-7-ol-*cis*·HBr [GJH 173][b]	276	5,600	JC
Benzo[*f*]quinoline-N-Me-8-ol-*cis*·HCl [GJH 175][b]	72,000		JC
Benzo[*f*]quinoline-N-Et-7,8-diol-*trans*·HBr [TL 121][b]	4	459	JC
Benzo[*f*]quinoline-N-Pr-*trans*·HCl [CS 265][b]	105	23,500	JC
Benzo[*g*]quinoline-N-Me-7,8-diol-*trans*·HBr [TL 302][b]	33,700		JC
Benzo[*g*]quinoline-N-Pr-6,8-diol-*trans*·HBr [Ha 103][b]	354	22,000	JC
Benzo[*g*]quinoline-N-Pr-7,8-diol-*trans*·HBr [TL 304][b]	17,100		JC
Benzo[*f*]quinoline-N-Pr-8,9-diol-*trans*·HBr [TL 308][b]	5.4	223	JC
Benzo[*f*]quinoline-N-Pr-8,9-diol-*cis*·HBr [TL 312][b]	1,070	74,500	JC
BHT 920·Cl_2 Azepine	84.2	4,710	KT
Dopamine·HCl	7.5[d]	4,300	Si
Dopamine-dimethyl·HBr	20	10,200	JC
Dopamine [sulfonium analog]-dimethyl iodide	1,200		DM
Dopamine-dipropyl·HBr [JGC 24]	5.4	1,550	JC
Epinephrine-(−)·bitartrate	1,020	128,000	SW
Epinephrine-(+)·bitartrate	1,380	839,600	SW
Epinine·HCl	10.4	3,430	Si
Ergots & related congeners:			
Bromocriptine mesylate	4.8		SZ
Bromocriptine-8-iso	46		SZ
Ergocriptine-α	2.9		Si
Ergocriptine-α-dihydro	3.1		SZ
Ergoline-8-amino [CU 32-085CH]	3.9	202	SZ
Ergoline-6-Pr-9-Oxa-*trans*-(±)·HCl [RU 29717][b]	1.3	146	RU
Lergotrile mesylate	5.5	547	LY
(±)-LY 141865[e]	20.5	5,160	LY
(−)-LY 171555.HCl [active enantiomer of LY 141865)][e]	4.8	3,680	LY
(−)-LY 156525·tartrate [active enantiomer of LY 141865]	8	2,805	LY
(+)-LY 156525·tartrate [inactive enantiomer of LY 141865]	89	154,000	LY
Pergolide mesylate [LY 127809]			
Without Na^+	0.75	60	LY
With Na^+	0.14	109	
Indolamine-benz-diPr-(±)·oxalate [RU 28251][b]	63	4,600	RU
Indole-benz[*cd*]-4-dipropyl-amino oxalate [LY 92151]	12	2,111	LY
Indole-4-(dipropylaminoethyl)·fumarate [BD 179]	417	33,600	JC

TABLE 1—*continued*

Agonist	K_D^{high}	K_D^{low}	Source
	nM		
Indole-4-piperidinyl-(±)·HCl [RU 27251]	72	11,600	RU
Indole-4-piperidinyl-N-Pr-(+)·maleate [RU 28952][b]	717	14,400	RU
Indole-4-(N-Pr-pyrrolidine)-(±) [RU 29898]	1,400	37,900	RU
Indole(iso)-5-(dipropylamino)·maleate [LY 127798]	78	36,100	LY
Isoproterenol-(−)·D-bitartrate	81,000		Si
Norepinephrine-(−)·HCl	51.5	12,600	Si
Norepinephrine-(+)·bitartrate	568	540,000	SW
Phenylethylamine·HCl	1,750	7,300	Si
Phenylethylamine-dipropyl·HCl [HF-I-22-5]	650	90,900	JC
Phenylethylamine-dipropyl-3-OH·HBr [VI-182]	105	23,500	JC
Phenylethylamine-3-OH-N-Ph-Et-N-Pr [RU 24213][b]	15.8	1,260	RU
Phenylethylamine-3-OH-N-(3-OH-Ph-Et)-N-Pr [RU 24926][b]	10.8	531	RU
3-PPP-(−)·HBr[f]	15.8	1,090	HL
3-PPP-(+)·HBr[f]	161	40,500	HL
Serotonin·HCl	6,074	183,000	Si
Troponylpiperazine-R-(+); [AY 27109]	2,010		AY
Troponylpiperazine-S-(−); [AY 27110]	1,120		AY
Tyramine-*meta*	84.3	1,920	BC
Tyramine-*para*·HCl	1,980	352,000	Si

[a] The sources of drugs are abbreviated as follows: AH, Professor Alan Horn, University of Groningen, Groningen, The Netherlands; AY, Ayerst Research Laboratories, Montreal, Canada; BC, Dr. B. Costall, University of Bradford, Bradford, U.K.; DM, Professor Duane D. Miller, The Ohio State University, Columbus, OH; HL, H. Lundbeck & Co., A/S, Copenhagen-Valby, Denmark; JC, Professor J. G. Cannon, University of Iowa, Iowa City, IA; JM, Dr. J. McDermed, The Wellcome Research Laboratories, Research Triangle Park, NC; JN, Professor J. L. Neumeyer, Northeastern University, Boston, MA; KT, Dr. Karl Thomae, GmbH, Biberach an der Riss, West Germany; LN, Professor J. L. G. Nilsson, Apoteksbolaget, Stockholm, Sweden; LY, Lilly Research Laboratories, Indianapolis, IN; MF, Merck Frosst Laboratories, Montreal, Canada; RB, Research Biochemicals, Inc., Wayland, MA; RU, Centre de Recherches Roussel UCLAF, Romainville, France; Si, Sigma Chemical Co., St. Louis, MO; SK, Smith Kline & French Laboratories, Philadelphia, PA; SW, Sterling-Winthrop Research Institute, Rensselaer, NY; SZ, Sandoz, A. G., Basel, Switzerland.

[b] Chemical abbreviations are: Pr, propyl; Me, methyl; ol, hydroxy; Ph, phenyl; Et, ethyl.

[c] Average of three experiments, where the SW source of NPA·HCl was confirmed by Prof. J. Neumeyer to be (−) [−70°].

[d] Dopamine is the average of four experiments.

[e] Pyrazolo[3,4-*g*]quinoline-5-Pr-2*H*-*trans*-(−)·HCl.

[f] 3-(3-Hydroxyphenyl)-N-*n*-propylpiperidine.

as well as at the low affinity state of the receptor, D_2^{low}. These two states of the D_2 receptor have been described by Sibley *et al.* (22) and De Lean *et al.* (23). We had previously referred to D_2^{High} as "D_4," defining D_4 as those sites which could not be converted into D_2^{Low} by guanine nucleotide (3). Recently, however, we have found that guanine nucleotide and sodium ions can *completely* convert all of the D_2 receptors from their D_2^{High} state to their D_2^{Low} state, thus obviating the need for the term D_4 (see

TABLE 2

Binding of [^{3}H]spiperone to D_2 receptors[a]

Antagonists and Miscellaneous	K_D^{high}	K_D^{low}	Source
	nM		
Benperidol	0.24		JP[b]
Benzazepine-8-Cl-3-Me-5-Ph-7-ol-R-(+) [SCH 23390][c]	1,690		SC
Bulbocapnine-(+)	27,600		RB
Bulbocapnine-N-Pr-nor-Me-(±)·HCl [DRE 76][c]	67	3,800	JN
Butaclamol-(+)·HCl	0.88		AY
Chlorpromazine·HCl	3.0		PO
Clebopride			
Without Na^+	116		AL
With Na^+	4.6		
Clonidine	79,000		
Clozapine	86.2		SZ
Domperidone	0.6		JP
Flupenthixol-α·diHCl	0.88		HL
Flupenthixol-β·diHCl	76		HL
Fluphenazine·diHCl	0.49		SQ
Haloperidol			
Without Na^+	1.17		JP
With Na^+	1.48		
Metoclopramide·HCl			
Without Na^+	463		DI
With Na^+	24		
Molindone·HCl			
Without Na^+	544		EL
With Na^+	15		
Nomifensine hydrogen maleate	20,500		HO
Nomifensine-8-desamino-3′,4′-diol·HBr	500	11,500	DN
Pimozide	4		JP
Pipamperone·2HCl	123		JP
Prochlorperazine ethanedisulfonate	7.9		SK
Promazine·HCl	71.6		WY
Promethazine·HCl	186		WY
Propranolol-(−)·HCl	80,400		IC
Spiperone			
Without Na^+	0.26		JP
With Na^+	0.11		
Spiperone-*para*-fluoro	0.59		JP
Sulpiride-(±)			
Without Na^+	3,232		DI
With Na^+	53		
Sulpiride-(−)			
Without Na^+	1,374		RA
With Na^+	18.2		
Sulpiride-(+)			
Without Na^+	94,000		RA
With Na^+	868		
Thioproperazine	0.52		SK
Thioridazine·HCl	5.5		SZ
Thiothixene-*cis*	0.44		PF
Thiothixene-*trans*	52		PF
Trifluoperazine·2HCl	1.2		SK
Trifluperidol	0.98		JP
YM-09151-2			
Without Na^+	0.95		YM
With Na^+	0.07		

[a] See legend to Table 1.

[b] The sources of drugs are abbreviated as follows: AL, Laboratorios Almirall, Barcelona, Spain; AY, Ayerst Research Laboratories, Montreal, Canada; DI, Delagrange International, Paris, France, and Nordic Pharmaceuticals, Laval, Quebec, Canada; DN, Professor D. E. Nichols, Purdue University, West Lafayette, IN; EL, Endo Laboratories, Inc.,

Ref. 24). A similar situation had occurred for the D_1 receptor, where we now find that D_1^{High} (formerly "D_3") can be *completely* converted into D_1^{Low} (25). [^{3}H]Spiperone was used in the present study to label the D_2 dopamine receptors in anterior pituitary tissue, since this tissue has no serotonin receptors to which [^{3}H]spiperone can bind. In this communication we wish to describe a possible model to account for the binding constants obtained.

MATERIALS AND METHODS

The dissociation constants (K_D values) for various agonist congeners at the high and low affinity states of the dopamine D_2 receptor were obtained using [^{3}H]spiperone as follows (26, 27).

Pig anterior pituitaries (Bocknek Organic Material, Rexdale, Ontario, Canada) were stored at −70°. After thawing, the pituitary tissue was dissected free of neurointermediate lobe and the attached hypophyseal stalk. The tissues were minced and homogenized (Brinkmann Polytron, 25 sec, setting 7, full power being 10) in 20 vol of buffer. The buffer contained 50 mM Tris-HCl (pH 7.4 at 20°), 5 mM KCl, 1.5 mM $CaCl_2$, 4 mM $MgCl_2 \cdot 6H_2O$, 1 mM EDTA, and 12 μM nialamide. NaCl was omitted in order to permit the [^{3}H]spiperone to bind to the high affinity state of D_2; it is known that 100 mM NaCl assists in converting high affinity D_2 receptors (D_2^{high}) into D_2^{low} receptors which have a low affinity for dopamine (26, 27). The pituitary homogenate was passed through cheesecloth and centrifuged at 480 × *g* for 5 min at 0°. The supernatant was centrifuged at 49,500 × *g* for 30 min at 0° and the pellet was resuspended in buffer. This suspension was rehomogenized by Polytron for 10 sec, preincubated for 10 min at 37°, and then put on ice for 45 min. The binding of [^{3}H]spiperone (22 to 30 Ci/mmol; New England Nuclear, Boston, MA) to the homogenate was done at a final concentration of 200 pM in buffer containing 0.1% ascorbic acid. The incubation was started by adding 100 μl of homogenate into tubes containing the test drug and [^{3}H]spiperone; the final volume was 5 ml (4 mg of original wet tissue/final ml). The tubes were incubated for 75 min at 20°.

The suspensions were then filtered (12 tubes simultaneously) by a cell harvester (Skatron, Lier, Norway), using two glass fiber filter mats stapled together (Skatron no. 7031, Sterling, VA) and a vacuum of 400 to 500 mm Hg. The filter mat was rinsed for 15 sec with 7 ml of 50 mM Tris-HCl (pH 7.4 at 20°). The filter circles were placed in liquid scintillation mini-vials along with 4 ml of scintillation fluid (Beckman Ready Solv EP). After 12 hr of shaking (100 rpm at 4°), the vials were monitored for tritium in a refrigerated Packard 460C liquid scintillation spectrometer at 35% efficiency. Specific binding of [^{3}H]spiperone was defined as that binding which was inhibited by the presence of 1 μM (+)-butaclamol (Research Biochemicals Inc., Wayland, MA). The K_D of [^{3}H]spiperone was 130 pM in the absence of NaCl and 64 pM in the presence of 100 mM NaCl. The competition data were analyzed using the LIGAND program (28). The program provided two statistical criteria to judge whether a two-site fit was better than a one-site fit, or whether a three-site fit was better than a two-site fit.

Garden City, NY; HL, H. Lundbeck & Co., A/S, Copenhagen-Valby, Denmark; HO, Hoechst Aktiengesellschaft, Frankfurt am Main, West Germany; IC, Imperial Chemical Industries, Ltd., Macclesfield, U.K.; JN, Professor J. L. Neumeyer, Northeastern University, Boston, MA; JP, Janssen Pharmaceutica, Beerse, Belgium; PF, Pfizer Inc., Groton, CT; PO, Poulenc Ltd., Montreal, Canada; RA, Ravizza S.P.A., Milan, Italy; RB, Research Biochemicals, Inc., Wayland, MA; SC, Schering Corporation, Bloomfield, NJ; SK, Smith Kline & French Laboratories, Philadelphia, PA; SQ, The Squibb Institute for Medical Research, Princeton, NJ; SZ, Sandoz A. G., Basel, Switzerland; WY, Wyeth Laboratories, Philadelphia, PA; YM, Yamanouchi Pharmaceutical Co., Ltd., Tokyo, Japan.

[c] Chemical abbreviations are: Pr, propyl; Me, methyl; ol, hydroxy; Ph, phenyl.

RESULTS

Except for four ergots (bromocriptine, isobromocriptine, α-ergocriptine, and α-dihydroergocriptine), the dopamine agonists inhibited the binding of [^{3}H]spiperone to D_2 receptors in two phases, as indicated in Table 1. The agonist dissociation constants (K_D values) at these two high and low affinity phases or states of the D_2 receptor (24, 25) were calculated using LIGAND; the values are listed in Tables 1 and 2. The two most potent agonists were pergolide (140 pM at D_2^{High} in the presence of Na^+) and the 5-hydroxytetralin, N-0437 (Ref. 29), the K_D of which was 146 pM and which was not significantly affected by the addition of 100 mM NaCl.

Role of the hydroxyl group. Several compounds (Table 1), including 2-aminotetralin, had no free hydroxyl groups, yet they could inhibit the binding of [^{3}H]spiperone, albeit at rather high concentrations in some cases (*e.g.*, 27,600 nM for the unsubstituted 2-aminotetralin, JGC-127, but 42.6 nM for 2-dipropyl-aminotetralin [TL-68] at the D_2^{High} state) (Table 1). The addition of a hydroxyl group corresponding to the *meta* position in dopamine, however, enhanced the potency by an order of magnitude (Table 1). This is illustrated in Figs. 1, A, B, and 2A.

A single free hydroxyl group in the "*para*" position, however, did not enhance potency. This is shown in Fig. 1A for *para*-tyramine, and in Fig. 1, E and F, for (±)-10-hydroxy-N-propylnoraporphine. Blocking the free hydroxyl group with a methyl ether, as in (−)-11-methoxy-N--propylnoraporphine, reduces potency, even though the oxygen atom is at the *meta* position (Fig. 1F). These effects have previously been observed (13).

The ergots, which contain no hydroxyl groups, were potent (Fig. 1H, Table 1), suggesting that the pyrrole nitrogen may serve as a binding site isosteric with the *meta* hydroxyl group in dopamine, 2-aminotetralin, and aporphines.

Role of the N-propyl groups. In general, but not always, the N-propyl substituents enhanced the potency of the agonist. For example, the dissociation constant of 2-aminotetralin [JGC-127] was 27,600 nM, whereas that for 2-dipropylaminotetralin [TL-68] was 42.6 nM. The dissociation constant of (±)-5,6,-dihydroxy-2-aminotetralin was 22.3 nM, whereas that for (±)-5,6-dihydroxy-2-dipropylaminotetralin was 0.82 nM at D_2^{High}. (−)-Apomorphine had a dissociation constant of 0.66 nM at D_2^{High}, whereas (−)-N-propyl-norapomorphine had just under twice the potency with a K_D of 0.4 nM.

In other instances, however, the N-propyl substitution reduced or did not enhance potency appreciably. For example, (±)-6,7-dihydroxy-2-aminotetralin [(±)-ADTN] had a dissociation constant of 1.7 nM at D_2^{High}, whereas the K_D value for (±)-6,7-dihydroxy-2-dipropylaminotetralin [TL-232] was 12 nM. Dopamine had a K_D of 7.5 nM whereas that for dipropyldopamine [JGC-24] was not significantly different (5.4 nM).

Stereochemistry. Although the R-(−)-enantiomers of aporphines and certain 2-aminotetralins (*i.e.*, 5-hydroxy or 5,6-dihydroxy) were more potent than the S-(+)-isomers (Table 1), (+)-7-hydroxy-2-dipropylaminotetralin (K_D = 36 nM) was more potent than the (−)-enantiomer (K_D = 1365 nM).

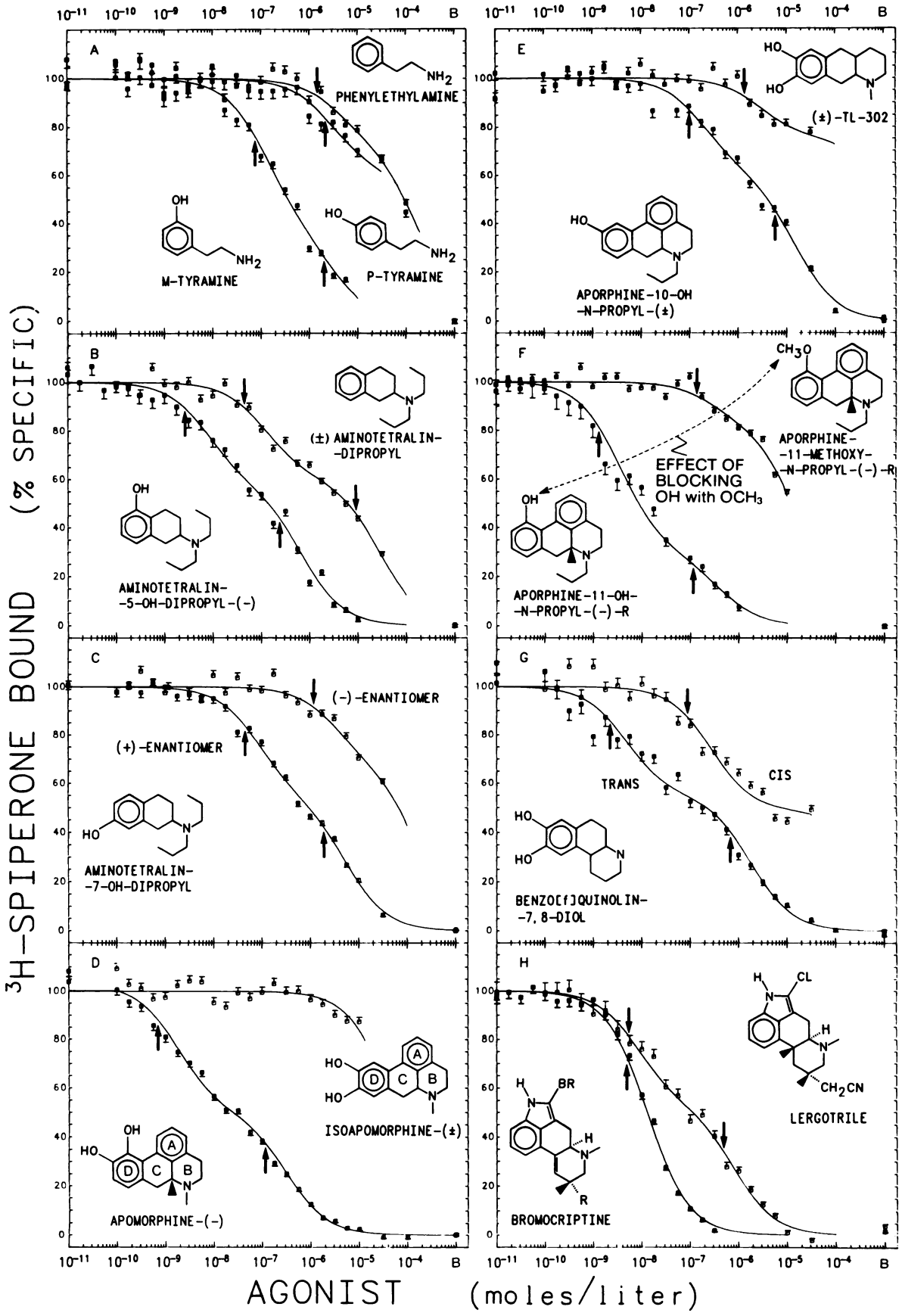

FIG. 1. *Competition between [^{3}H]spiperone and dopamine agonists at D_2 dopamine receptors in pig anterior pituitary membrane homogenate*
Specific binding was defined as that inhibited by 1 μM (+)-butaclamol. Total binding was generally between 1000 and 1700 dpm per filter. The *arrows* indicate the dissociation constants (K_D) at D_2^{High} and D_2^{Low}, as determined by LIGAND (28), using a K_D for [^{3}H]spiperone of 130 pM under these conditions. *Vertical bars* indicate SE for triplicate determinations.

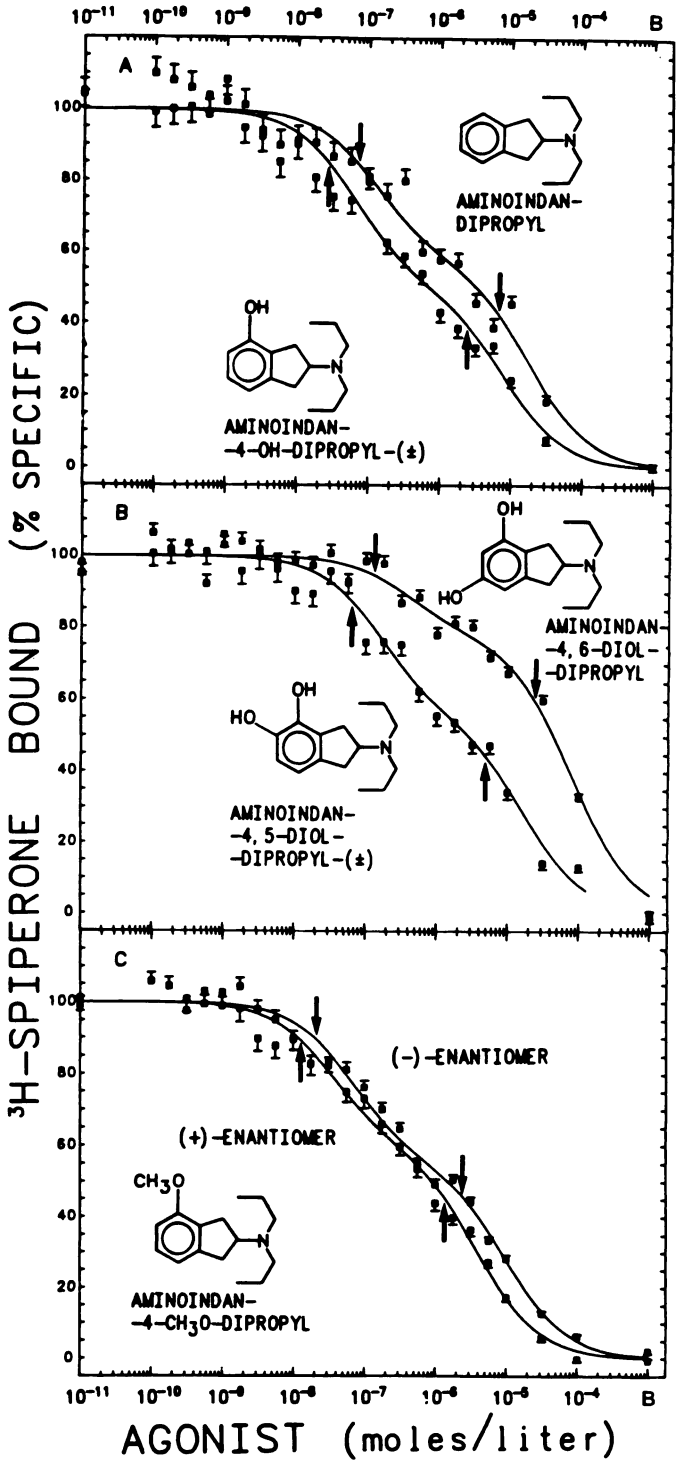

FIG. 2. *Competition between [^{3}H]spiperone and several aminoindans at D_2 dopamine receptors in pig anterior pituitary membrane homogenate*

See legend to Fig. 1.

The 4-hydroxy-2-aminoindan enantiomers differed 13-fold in potency, with R-(−)- being more potent than S-(+)- (Table 1). The 4-methoxy-2-aminoindans, however, differed by about 3-fold in potency, with S-(+)-4-methoxy-2-dipropylaminoindan having a K_D of 20 nM at D_2^{High} and R-(−)-methoxy-2-dipropylaminoindan having a K_D of 63 nM (see Ref. 18 for stereochemistry).

Steric factors. Although the methoxy-2-aminoindans

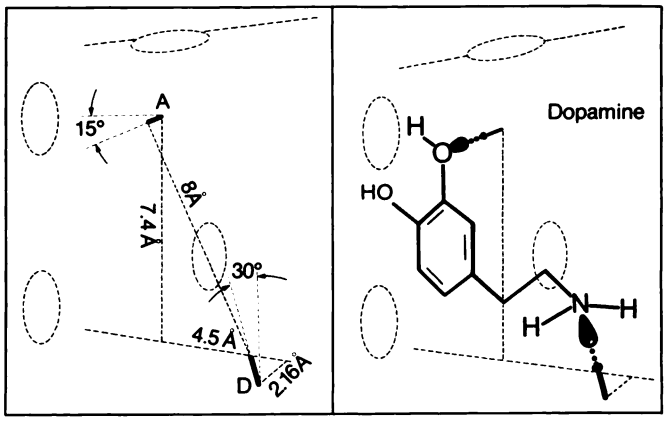

FIG. 3. *Proposed D_2 receptor pharmacophore*

The receptor atom *A* forms a hydrogen bond with the —OH or the —NH of the dopamine agonist. The receptor atom *D* forms a hydrogen bond with the tertiary amine of the dopamine agonist. The direct interatomic distance between atoms A and D is 8 Å. The direction of the A hydrogen bond is about 15° off the orthogonal from the receptor surface. The direction of the D hydrogen bond is about 30° off the orthogonal. The regions at "7, 10, and 1 o'clock" are steric obstacles on the receptor. The back wall and the floor of the receptor are assumed to be hydrophobic surfaces. Thus, norepinephrine (or epinephrine) does not make a suitable fit because its β-hydroxyl group meets the hydrophobic floor. The Dreiding model is dopamine, hydrogen-bonded to the receptor. The spheroids (*dashed lines*) indicate steric obstacles (in the receptor) to account for the inactivity of certain congeners.

were potent [the S-(+)-4-methoxy-2-dipropylaminoindan K_D at D_2^{High} was 20 nM, comparatively the same as (−)-4-hydroxy-2-dipropylaminoindan, with a K_D value of 8.3 nM; Table 1], the methoxy-aporphines were not (Fig. 1F), despite the fact that the methoxy group is in the corresponding *meta* position in both cases (see Discussion).

Grol and Rollema (20) had proposed that (±)-isoapomorphine [(±)-9,10-dihydroxyaporphine] was inactive because of steric factors caused by the A ring. The comparison between (±)-isoapomorphine and (−)-apomorphine is shown in Fig. 1D, and that for an A ring-deleted aporphine, the benzo[*g*]quinoline [(±)-TL-302], is shown in Fig. 1E.

DISCUSSION

In order to account for the active and inactive congeners in Table 1, the following model is proposed (Fig. 3). (*1*) There are two binding sites for hydrogen bonds, the extremities being separated by approximately 8 Å. (*2*) The hydrogen bonding receptor groups are directed between 15° and 30° off orthogonal to the surface of the receptor, as illustrated in Fig. 3. (*3*) To account for the lower activity of serotonin, norepinephrine, octahydrobenz[*h*]isoquinoline, 1-(aminomethyl)-6,7-dihydroxy-tetralin, and S-(+)-4-hydroxy-2-aminoindan, steric obstacles are placed at "7," "10," and "1 o'clock" (Fig. 3), and at the "back wall" and "bottom" of the receptor (see later).

As illustrated in Fig. 4, this model accommodates the active congeners related to dopamine, *i.e.*, (−)-apomorphine, (+)-6,7-dihydroxy-2-aminotetralin, (+)-4-methoxy-2-dipropylaminoindan, and bromocriptine. The binding is probably by hydrogen bonds with either an —OH group or, as in the case of the ergots, by the pyrrole

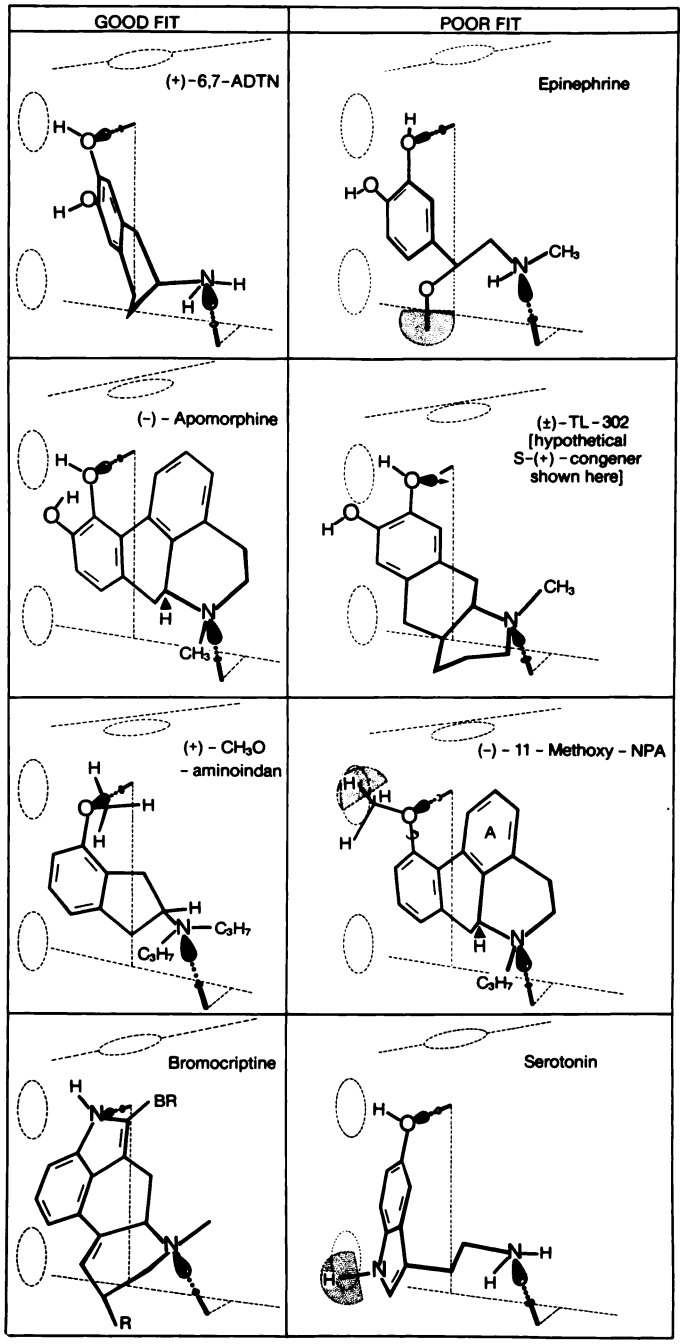

FIG. 4. *Illustration of the good fit (left) of four dopaminergic agonists to the dopamine receptor model, and the poor fit (right) of less active or inactive dopamine congeners*

The Dreiding stereomodels were redrawn from photographs taken at slightly different angles in order to see the ring structure clearly; thus, these photographic angles resulted in slight differences in the drawn positions of the steric obstacles. The active congeners (*left*) avoid the obstacles. Although the methoxy group in R-(−)-methoxy-NPA can freely rotate (as indicated by the *arrow*), there are only two positions which would permit the direction of the lone pair of electrons to be directed toward the receptor; both of these positions are sterically hindered by either the obstacle at 10 o'clock or the A ring, thus resulting in a poor fit of this molecule with the receptor. In the case of the (−)-N-propyl-norapomorphine, there is sufficient room for the propyl group between the N atom and the "floor" of the receptor. The *arrow* in (±)-*TL-302* indicates that the direction of the lone pair electrons is considerably out of line with the proposed direction of the receptor binding site.

—NH group, as proposed by Camerman and Camerman (30). The major advantage of the present proposed tetrahedral model for D_2 is that it accommodates the 2-aminoindans, whereas previous models (see Introduction) do not.

Thus, the lone electron pair of the nitrogen in all of the congeners may be considered as potonated, since it has been suggested that the nitrogen binds via Coulombic attraction to an electronegative site on the receptor (11). The N—H bond orientation is critical for optimum binding. This has been discussed previously by Nichols (9). Under physiological conditions, it is expected that both the protonated and neutral forms of these congeners would be present, but possibly only the protonated form reacts with the receptor. It is important to note that the dissociation constant of the positively charged sulfonium analog of dimethyldopamine was much higher (*i.e.*, much less potent) than that for dimethyldopamine itself (Table 1). We are presently doing experiments to examine the relative potencies of charged and neutral forms of apomorphine.

Steric obstacles at 1 and 7 o'clock were added to account for the inactivity of serotonin at the D_2 receptor (Fig. 4, Table 1). Steric obstacles at 1 and 10 o'clock were added to account for the inactivity of congeners of octahydrobenz[*h*]isoquinoline (31) and 1-(aminomethyl)-6,7-dihydroxy-tetralin (Fig. 5; Ref. 32) derivatives.

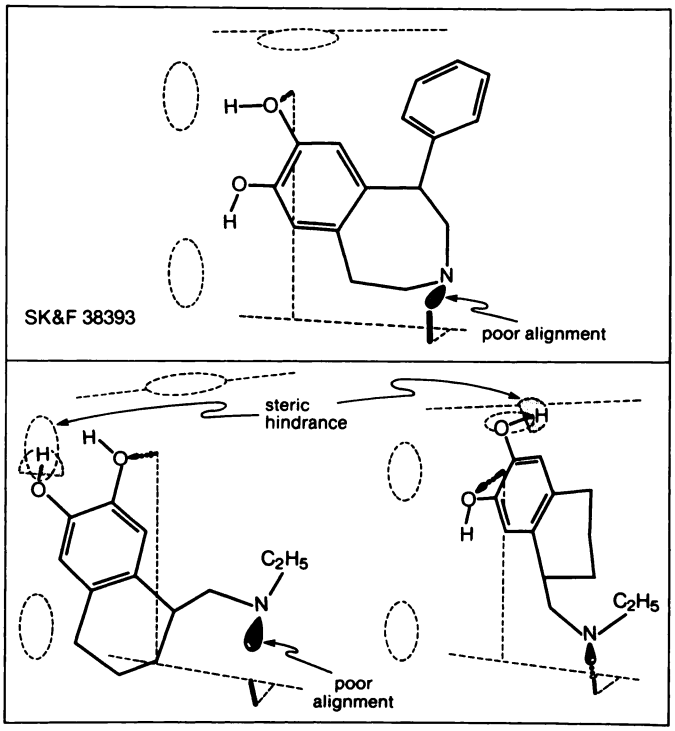

FIG. 5. *Poor fit of the D_1 dopamine agonist SK&F 38393 and an aminotetralin*

The D_1 dopamine receptor agonist SK&F 38393 (*top*) does not fit the proposed tetrahedral model for the D_2 receptor. At best, the orientation of the lone pair of electrons (on the N atom) is considerably out of line with that required by the model for the receptor. Steric obstacles at 1 and 10 o'clock were added to account for the inactivity of 1-(aminomethyl)-6,7-dihydroxy-tetralin derivatives (*bottom*). The Dreiding stereomodels were redrawn from photographs taken at different positions in order to see the chemical structures more clearly.

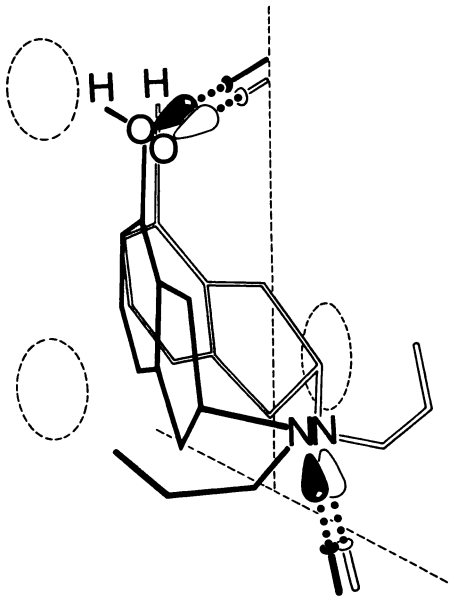

FIG. 6. *R-(−)-4-hydroxy-2-dipropylaminoindan (left) with the S-(+)-enantiomer (right)*

Only one propyl group is shown, for the sake of clarity. To account for the observed different potencies of these enantiomers on dopamine receptors, there may be a steric obstacle at the "back" (right side) of the receptor, so as to hinder the S-enantiomer from fitting properly.

These obstacles are avoided by all of the active dopamine congeners. The obstacle at 10 o'clock is particularly interesting, since it accounts for the inactivity of R-(−)-11-methoxy-N-propyl-norapomorphine. The methyl group interacts with the 10 o'clock obstacle since the methyl group is sterically hindered from rotating toward the A ring. In the case of the (−)- and (+)-4-methoxy-2-aminoindans, however, the methyl group is free to rotate, since there is no A ring to impede it; thus, the (−)- and (+)-4-methoxy-amino-2-indans can avoid the obstacle at 10 o'clock.

The stereoselective difference of 13-fold between the enantiomers of 4-hydroxy-2-aminoindan agrees with the 4- to 100-fold difference in potency observed for rat rotation or on cardiac presynaptic dopamine receptors (18), with the R-(−)- enantiomer always being more potent than the S-(+)-enantiomer. With the 4-methoxy-2-aminoindans, however, the (+)- compound was about 3-fold more potent than the (−)- compound.

To account for the lower potency of the S-(+)-4-hydroxy-2-aminoindan, it is possible that there may be an obstacle at the "back" of the receptor (Fig. 6, *right*). Such an obstacle, however, should result in a consistent difference between the R- and S-enantiomer potencies for the 4-hydroxy pair and the 4-methoxy pair of 2-aminoindans.

The lower potency of S-(+)-apomorphine (compared to R-(−)-apomorphine) may result from either steric interaction between the A ring and the floor of the receptor, or, as illustrated in Fig. 4 for S-(+)-TL-302, the orientation of the electron lone pair from the —OH group is not aligned with the corresponding binding site of the receptor.

In those instances when the N-propyl substitution enhanced the potency of the agonist, the enhancement appeared to correspond to the increased hydrophobicity of the congener. For example, the K_D^{High} of (±)-5-hydroxy-2-dipropylaminotetralin was 11.4 nM. Its congener, N-0434 (Ref. 29), is approximately 35 times more lipophilic (using Hansch analysis), leading one to expect a K_D^{High} of about 0.33 nM for N-0434; the observed value was 0.73 nM, not appreciably different from 0.33 nM.

As illustrated in Fig. 5, the D_1 dopamine receptor agonist SK&F 38393 (and the related congeners) does not fit the tetrahedral model here proposed for D_2.

In addition to examining whether the active form of apomorphine is protonated or neutral, future work should also consider whether the agonist —OH group attaches to the receptor by means of donating the proton or by accepting a proton (from the receptor) which attaches to one of the two pairs of unshared electrons of the oxygen atom. Such attachment via oxygen's unshared electrons has previously been implied for D_1 agonists (33), but such a proposal is considered difficult to test experimentally (34).

ACKNOWLEDGMENTS

We thank Professor John P. Long and Jan Flynn, University of Iowa, for their generous assistance in donating compounds. We also thank the individuals and pharmaceutical companies listed in Table 1, Footnote a and Table 2, Footnote b, for generously donating their compounds.

REFERENCES

1. Onali, P., M. C. Olianas, and G. L. Gessa. Selective blockade of dopamine D_1 receptors by SCH-23390 discloses striatal dopamine D_2 receptors mediating the inhibition of adenylate cyclase in rats. *Eur. J. Pharmacol.* **99**:127–128 (1984).
2. Kebabian, J. W., and D. Calne. Multiple receptors for dopamine. *Nature* **277**:93–96 (1979).
3. Seeman, P. Brain dopamine receptors. *Pharmacol. Rev.* **32**:229–313 (1980).
4. Horn, A. S., M. L. Post, and O. Kennard. Dopamine receptor blockade and the neuroleptics, a crystallographic study. *J. Pharm. Pharmacol.* **27**:553–563 (1975).
5. Humber, L. G., F. T. Bruderlein, and K. Voith. Neuroleptic agents of the benzo-cycloheptapyridoisoquinoline series: a hypothesis on their mode of interaction with the central dopamine receptor. *Mol. Pharmacol.* **11**:833–840 (1975).
6. Olson, G. L., H.-C. Cheung, K. D. Morgan, J. F. Blount, L. Todaro, and L. Berger. A dopamine receptor model and its application in the design of a new class of rigid pyrrolo[2,3-*g*]isoquinoline antipsychotics. *J. Med. Chem.* **24**:1026–1034 (1981).
7. Bach, N. J., E. C. Kornfeld, J. A. Clemens, and E. B. Smalstig. Conversion of ergolines to hexahydro- and octahydrobenzo[*f*]quinolines (depyrrolo-ergolines). *J. Med. Chem.* **23**:812–814 (1980).
8. Bach, N. J., E. C. Kornfeld, N. D. Jones, M. O. Chaney, D. E. Dorman, J. W. Paschal, J. A. Clemens, and E. B. Smalstig. Bicyclic tricyclic ergoline partial structures. Rigid 3-(2-aminoethyl)pyrroles and 3- and 4-(2-aminoethyl)pyrazoles as dopamine agonists. *J. Med. Chem.* **23**:481–491 (1980).
9. Nichols, D. E. The development of novel dopamine agonists, in *Dopamine Receptors (American Chemical Society Symposium Series 224)* C. Kaiser and J. W. Kebabian, eds.). American Chemical Society, Washington, D.C., 201–218 (1983).
10. Cannon, J. G., B. J. Demopoulos, J. P. Long, J. R. Flynn, and F. M. Sharabi. Proposed dopaminergic pharmacophore of lergotrile, pergolide, and related ergot alkaloid derivatives. *J. Med. Chem.* **24**:238–240 (1981).
11. Neumeyer, J. L. S. J. Law, and J. S. Lamont. Apomorphine and related aporphines as probes of the dopamine receptor, in Gessa G. L. and Corsini G. U. Eds. *Apomorphine and Other Dopaminomimetics.* Vol. 1: *Basic Pharmacology* (G. L. Gessa, and G. U. Corsini, eds.). Raven Press, New York, 209–218 (1981).
12. Kaiser, C. Structure-activity relationships of dopamine receptor agonists, in *Dopamine Receptor Agonists* (G. Poste and S. Crooke, eds.). Plenum Press, New York, 87–137 (1984).
13. Neumeyer, J. L., D. Reischig, G. W. Arana, A. Campbell, R. J. Baldessarini, N. S. Kula, and K. J. Watling. Aporphines. 48. Enantioselectivity of (R)-(−)- and (S)-(+)-N-*n*-propylnorapomorphine on dopamine receptors. *J. Med. Chem.* **26**:516–521 (1983).

14. Wikstrom, H. Centrally acting dopamine receptor stimulants with special reference to dopamine autoreceptors and stereoselectivity. *Acta Univ. Ups. Abstr. Upps. Diss. Fac. Pharm.* **84:** 1–50.
15. McDermed, J., and R. J. Miller. Antipsychotic agents and dopamine agonists. *Annu. Rep. Med. Chem.* **14:**12–21 (1979).
16. McDermed, J. D., H. S. Freeman, and R. Ferris. Enantioselectivity in the binding of (+)- and (−)-2-amino-6,7-dihydroxy-1,2,3,4-tetrahydronaphthalene and related agonists to dopamine receptors, in *Catecholamines: Basic and Clinical Frontiers*, vol. 1 (E. Usdin, I. Kopin, and J. Barchas, eds.). Pergamon Press, New York, 568–570 (1979).
17. Hacksell, U., L.-E. Arvidsson, U. Svensson, J.L.G. Nilsson, H. Wikstrom, P. Lindberg, D. Sanchez, S. Hjorth, A. Carlsson, and L. Paalzow. Monophenolic 2-(dipropylamino)indans and related compounds: central dopamine-receptor stimulating activity. *J. Med. Chem.* **24:**429–434 (1981).
18. Cannon, J. G., R. G. Dushin, J. P. Long, M. Ilhan, N. D. Jones, and J. K. Swartzendruber. Synthesis and dopaminergic activity of (R)- and (S)-4-hydroxy-2-di-*n*-propylaminoindan. *J. Med. Chem.* **28:**515–518 (1985).
19. Neumeyer, J. L., M. McCarthy, S. P. Battista, F. J. Rosenberg, and D. G. Teiger. Aporphines 9. Synthesis and pharmacological evaluation of (±)-9,10-dihydroxyaporphine, (±)-isoapomorphine (+)-, (−)- and (±)-1,2-dihydroxyaporphine and (+)-2,9,10-tetrahydroxyaporphine. *J. Med. Chem.* **16:**1228–1233 (1973).
20. Grol, C. J., and H. Rollema. Conformational analysis of dopamine by the INDO molecular orbital method. *J. Pharm. Pharmacol.* **29:**153–156 (1977).
21. Cannon, J. G., T. Lee, H. D. Goldman, J. P. Long, J. R. Flynn, T. Verimer, B. Costall, and R. J. Naylor. Congeners of the B conformer of dopamine derived from *cis*- and *trans*-octahydrobenzo[*f*]quinoline and *trans*-octahydrobenzo[*g*]quinoline. *J. Med. Chem.* **23:**1–5 (1980).
22. Sibley, D. R., A. De Lean, and I. Creese. Anterior pituitary dopamine receptors: demonstration and interconvertible high and low affinity states of the D-2 dopamine receptor. *J. Biol. Chem.* **257:**6351–6361 (1982).
23. De Lean, A., B. F. Kilpatrick, and M. Caron. Dopamine receptor of the porcine anterior pituitary gland: evidence for two affinity states of the receptor discriminated by both agonists and antagonists. *Mol. Pharmacol.* **22:**290–297 (1982).
24. Grigoriadis, D., and P. Seeman. Complete conversion of brain D_2 dopamine receptors from the high- to the low-affinity state for dopamine agonists, using sodium ions and guanine nucleotide. *J. Neurochem.* **44:**1925–1935 (1985).
25. Seeman, P., C. Ulpian, D. Grigoriadis, I. Pri-Bar, and O. Buchman. Conversion of dopamine D_1 receptors from high to low affinity for dopamine. *Biochem. Pharmacol.* **34:**151–154 (1985).
26. George, S. R., M. Watanabe, and P. Seeman. Dopamine D_2 receptors in pituitary: a single population without reciprocal agonist/antagonist states. *J. Neurochem.* **44:**1168–1177 (1985).
27. Watanabe, N., S. R. George, and P. Seeman. Dopamine receptor conversion from agonist high- to low-affinity state is dependent on temperature and sodium ions. *Biochem. Pharmacol.* **34:**2459–2463 (1985).
28. Munson, P., and D. Rodbard. "Ligand": A versatile computerized approach for characterization of ligand-binding systems. *Anal. Biochem.* **107:**220–239 (1980).
29. Beaulieu, M., Y. Itoh, P. Tepper, A. S. Horn, and J. W. Kebabian. N,N-disubstituted 2-aminotetralins are potent D-2 dopamine receptor agonists. *Eur. J. Pharmacol.* **105:**15–21 (1984).
30. Camerman, N., and A. Camerman. On the stereochemistry of dopaminergic ergoline derivatives. *Mol. Pharmacol.* **19:**517–519 (1981).
31. Cannon, J. G., T. Lee, F.-L. Hsu, J. P. Long, and J. R. Flynn. Congeners of the conformer of dopamine derived from octahydrobenz[*h*]isoquinoline. *J. Med. Chem.* **23:**502–505 (1980).
32. Cannon, J. G., Z. Perez, J. P. Long, and M. Ilhan. 1-(Aminomethyl)-6,7-dihydroxytetralin derivatives: synthesis and assessment of dopamine-like effects. *J. Med. Chem.* **26:**813–816 (1983).
33. Kaiser, C. Stereoisomeric probes of the dopamine receptor, in *Dopamine Receptors (American Chemical Society Symposium 224)* (C. Kaiser and J. W. Kebabian, eds.). American Chemical Society, Washington, D.C., 223–246 (1983).
34. McDermed, J. Commentary: stereoisomeric probes of the dopamine receptor, in *Dopamine Receptors, (American Chemical Society Symposium 224)* (C. Kaiser and J. W. Kebabian, eds.). American Chemical Society, Washington, D.C., 247–250 (1983).

Send reprint requests to: Philip Seeman, Department of Pharmacology, Medical Sciences Building, University of Toronto, Toronto, Canada M5S 1A8.