MOLECULAR PHARMACOLOGY, 22:281–289

Further Characterization of Structural Requirements for Agonists at the Striatal Dopamine D-1 Receptor

Studies with a Series of Monohydroxyaminotetralins on Dopamine-Sensitive Adenylate Cyclase and a Comparison with Dopamine Receptor Binding

M. P. SEILER AND R. MARKSTEIN
Preclinical Research, Sandoz Ltd., CH-4002 Basel, Switzerland

Received December 10, 1981; Accepted April 13, 1982

SUMMARY

A series of monohydroxy-2-aminotetralins with either a primary or a tertiary (*N*,*N*-di-*n*-propylated) amino group was investigated on dopamine-sensitive adenylate cyclase from rat striatum in order to study the influence of the position of the phenolic hydroxy group in combination with the substitution pattern of the amino group in these semirigid dopamine (DA) analogues. The monohydroxyaminotetralins stimulated adenylate cyclase, indicating that a catechol group is not essential for D-1 receptor activation. 7-Hydroxy-2-aminotetralin (7-OH-AT) was found to be the most active compound among the primary amines, and 5-hydroxy-2-(*N*,*N*-dipropylamino)tetralin (5-OH-DPAT) was the most potent compound among the tertiary amines; in the 7-OH series, the activity resided in the (+)-(2R)-enantiomers, whereas in the 5-OH series the (−)-(2*S*)-enantiomers represented the more potent enantiomers. These results, together with a lack of synergism observed with the 5,7-dihydroxyaminotetralins, can best be explained with a D-1 receptor model containing two major binding sites and, among others, an accessory binding site to which the *N*,*N*-di-*n*-propyl substituents of the 5-OH series but not those of the 7-OH series have access. According to this model, DA would interact with its receptor in the β-rotamer conformation of the aminoethyl side chain; however, *N*,*N*-di-*n*-propylation would cause a change in preferred conformation at the receptor toward the α-rotamer form. The potency at adenylate cyclase correlated excellently with [^{3}H]DA binding (calf caudate nucleus) and poorly with [^{3}H]spiroperidol binding, suggesting that [^{3}H]DA binding in striatum involves binding to D-1 receptors.

INTRODUCTION

There have been considerable efforts to elucidate the structural requirements for DA[1] receptor activation in different parts of the central nervous system and in the periphery using various dopamine derivatives or analogues (1, 2). However, many of these studies measure behavioral effects or make use of animal models which involve interactions with different subtypes of dopamine receptors or even other neurotransmitter systems. This renders an interpretation of the results for the purpose of receptor characterization rather difficult. In addition, *in vivo* data are influenced by differences in metabolism and in pharmacokinetics. More reliable results in this respect may be obtained by the use of suitable *in vitro* models.

DA-sensitive adenylate cyclase, an enzyme widely distributed in animals and man, represents a frequently used *in vitro* functional model for dopamine receptors (3). In the periphery, it has been linked to physiological responses such as release of parathyroid hormone in bovine parathyroid (4), vasodilation in dog renal arteries (5), or electrophysiological events in mammalian superior ganglia (6). Its exact role within the different areas of the brain remains to be clarified (7). The receptor activating this enzyme has recently been classified as a DA D-1 receptor (7).

---

[1] The abbreviations used are: DA, dopamine; 6,7-$(OH)_2$-AT, 6,7-dihydroxy-2-aminotetralin; 5,6-$(OH)_2$-AT, 5,6-dihydroxy-2-aminotetralin; 5,7-$(OH)_2$-AT, 5,7-dihydroxy-2-aminotetralin; 5-OH-AT, 5-hydroxy-2-aminotetralin; 6-OH-AT, 6-hydroxy-2-aminotetralin; 7-OH-AT, 7-hydroxy-2-aminotetralin; 8-OH-AT, 8-hydroxy-2-aminotetralin; 6,7-$(OH)_2$-DPAT, 6,7-dihydroxy-2-(*N*,*N*-di-*n*-propylamino)tetralin; 5,6-$(OH)_2$-DPAT, 5,6-dihydroxy-2-(*N*,*N*-di-*n*-propylamino)tetralin; 5,7-$(OH)_2$-DPAT, 5,7-dihydroxy-2-(*N*,*N*-di-*n*-propylamino)tetralin; 5-OH-DPAT, 5-hydroxy-2-(*N*,*N*-di-*n*-propylamino)tetralin; 6-OH-DPAT, 6-hydroxy-2-(*N*,*N*-di-*n*-propylamino)tetralin; 7-OH-DPAT, 7-hydroxy-2-(*N*,*N*-di-*n*-propylamino)tetralin; 8-OH-DPAT, 8-hydroxy-2-(*N*,*N*-di-*n*-propylamino)tetralin.

0026-895X/82/050281-09$02.00/0

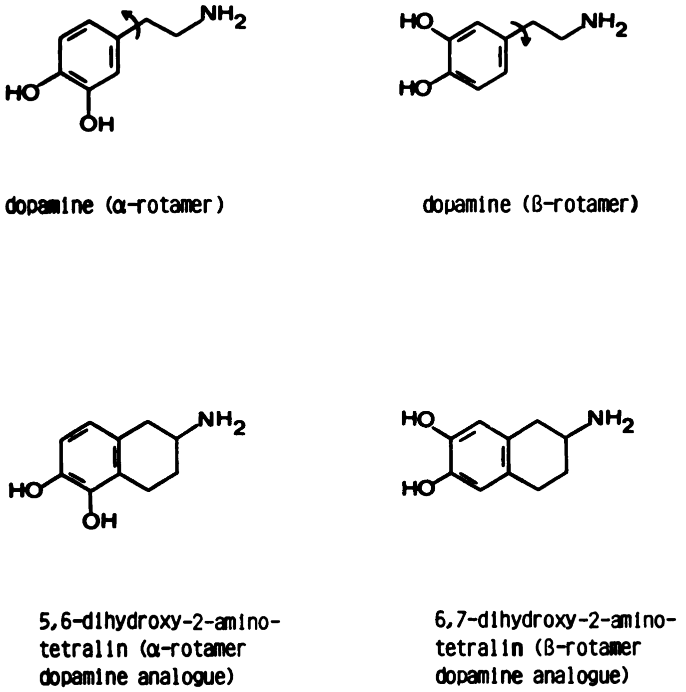


FIG. 1. *Chemical structures of the rotamers of dopamine and its aminotetralin correlates*

Among the different chemical structural types used to study structure-activity relationships of dopamine agonists, dihydroxyaminotetralin derivatives play an important role, since they contain the DA molecule incorporated in a semirigid skeleton (Fig. 1). Thus, Miller *et al.* (8) concluded from a study with 6,7-$(OH)_2$-AT[2] that optimal agonist activity would require an extended transoid conformation of the aminoethyl side chain of DA. The question of whether DA would interact with its receptor in the so-called α-rotamer or β-rotamer conformation (Fig. 1) was studied by comparing the effects of 5,6-$(OH)_2$-AT derivatives, representing the frozen α-rotameric form, with those of 6,7-$(OH)_2$-AT derivatives, representing the frozen β-rotameric form. In studying dopamine D-1 receptor stimulation, Woodruff *et al.* (9) found that β-rotameric 6,7-$(OH)_2$-AT was 40–60 times more potent than the corresponding α-rotameric 5,6-$(OH)_2$-AT, using adenylate cyclase from rat nucleus accumbens and striatum. Cannon *et al.* (10) could confirm these results with the enzyme from rat striatum. However, their data show that the potency order is reversed among the two corresponding tetralin derivatives, with tertiary amino groups, β-rotameric 6,7-$(OH)_2$-DPAT, being less active than α-rotameric 5,6-$(OH)_2$-DPAT.

We present here the results of our investigations with monohydroxyaminotetralins containing either a primary or a tertiary (di-*n*-propyl-substituted) amino group on DA-sensitive adenylate cyclase from rat striatum in order to study the influence of the position of the hydroxy group in combination with the substitution pattern of the amino group on D-1 agonist activity in comparison with the above-mentioned dihydroxyaminotetralins. In addition, we determined the dopaminergic agonist and antagonist binding of the same aminotetralin derivatives in homogenates from calf caudate nucleus in order to compare the binding data with results from the adenylate cyclase assay. Information concerning the stereochemical requirements of the receptor site was obtained by the use of the enantiomers of crucial aminotetralin derivatives with known absolute configurations.

MATERIALS AND METHODS

Synthesis of Aminotetralin Derivatives

The racemates of the aminotetralin derivatives were either known or synthesized by standard methods (11).

The known enantiomers of 5-OH-DPAT were prepared according to the literature (12), starting from resolved *N*-benzylamino-5-methoxytetralin via the intermediate 2-amino-5-methoxytetralin. The same intermediate, upon ether cleavage, gave the hitherto unknown (+)-5-OH-AT [HCl salt: $\alpha_D^{20} = +72.2°$ ($c = 1$, MeOH)] and, respectively, (−)-5-OH-AT [HCl salt: $\alpha_D^{20} = -72.9$ ($c = 1$, MeOH)]. The absolute configuration of (+)-5-OH-DPAT has been elucidated by X-ray analysis as 2*R* (13). Since both (+)-5-OH-DPAT and (+)-5-OH-AT derive from (+)-2-amino-5-methoxytetralin, the absolute configuration of (+)-5-OH-AT is therefore determined as 2*R* (compare Fig. 2).

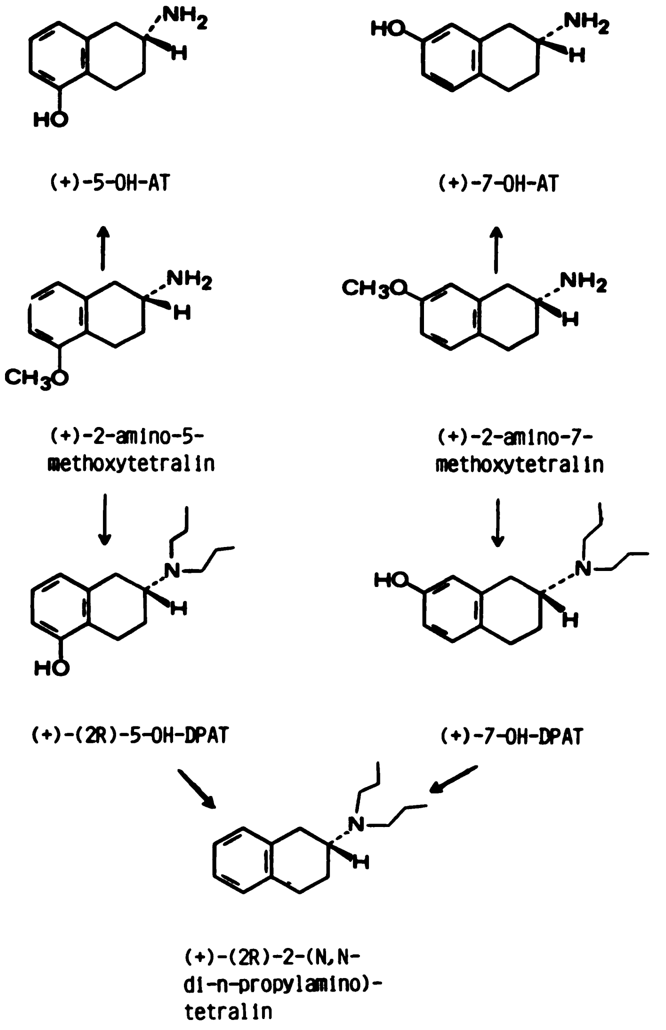


FIG. 2. *Elucidation of absolute configurations of 5- and 7-hydroxyaminotetralin derivatives by correlation with (+)-(2R)-5-OH-DPAT and (+)-2-(N,N-di-n-propylamino)-tetralin*

---

[2] Also referred to as ADTN.

The enantiomers of the 7-hydroxyaminotetralin series were obtained as follows: resolution of 2-amino-7-methoxytetralin with (+)-mandelic acid yielded (+)-2-amino-7-methoxytetralin, which on the one hand was cleaved to give (+)-7-OH-AT [HCl salt: α_D^{20} = +71.7° (c = 1, MeOH)], and on the other hand *N*-dialkylated with *n*-propylbromide and subsequently cleaved to yield (+)-7-OH-DPAT [HCl salt: α_D^{20} = +79.6° (c = 1, MeOH)]. The corresponding (−)-enantiomers accordingly were obtained from (−)-2-amino-7-methoxytetralin: (−)-7-OH-AT [HCl salt: α_D^{20} = −70.0° (c = 1, MeOH)] and, respectively, (−)-7-OH-DPAT [HCl salt: α_D^{20} = −79.7° (c = 1, MeOH)].

For elucidation of the absolute configuration, (+)-7-OH-DPAT was converted into its dicyclohexylcarbodiimide adduct, which was hydrogenated to yield (+)-2-(*N*,*N*-di-*n*-propylamino)tetralin (Fig. 2). The latter is known to bear a 2*R* configuration, as was shown independently by stereospecific synthesis from aspartic acid (14) and respective degradation from (+)-(2R)-5-OH-DPAT (15). Therefore, the 2*R* configuration can be assigned to (+)-7-OH-DPAT as well as to (+)-7-OH-AT since the latter compound arises from the common intermediate (+)-2-amino-7-methoxytetralin. The correlation scheme of the enantiomeric monohydroxyaminotetralins is summarized in Fig. 2.

Adenylate Cyclase

Enzyme source. Male rats (Sandoz OFA strain) were killed by decapitation and the brains were rapidly removed. Striatal tissue was obtained from frontal sections approximately between the anterior planes A9200–7200 (16). The tissue was homogenized for 1 min at 200 rpm in 25 volumes (w/v) of cold 2 mM Tris acetate/2mM ethylene glycol bis(β-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid buffer (pH 7.4) in an all-glass homogenizer. The homogenate was then pressed through a nylon net (pore size 35 μm), and 2-ml aliquots of the homogenate were quickly frozen by immersing the tubes into a mixture of dry ice and acetone. The homogenate was stored at −70° until use. The enzyme is stable for several months when stored in this way.

Enzyme assay. Adenylate cyclase activity was routinely assayed by a standard procedure which is based on the method described by Kebabian *et al.* (17). The compounds alone or in combination with agonists were preincubated at 4° together with the tissue homogenate in 400 μl of a mixture containing Tris acetate, 47.1 μmoles (pH 7.4); $MgCl_2$, 2 μmoles; ethylene glycol bis(β-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid, 0.45 μmole; isobutylmethylxanthine, 0.1 μmole; and bovine serum albumin, 0.15%. After 13.5 min, the temperature of the mixture was increased to 30° over 1 min and the enzyme reaction was started by adding 100 μl of 2.5 mM ATP/0.5 mM guanyl-5′-yl imidodiphosphate solution (pH 7.4) and terminated 3 min later by heating the mixture at 95° for 3.5 min. After centrifugation at 10,000 × *g* for 5 min, 50 μl of the supernatant were removed for cyclic AMP determination by radioimmunoassay. All determinations were made in triplicate. For the assay, a multipipetting machine was used which allowed the simultaneous determination of adenylate cyclase activity in 24 tubes.

DA-stimulated cyclic AMP formation occurred in a dose-dependent manner with maximal stimulation at a concentration of 125 μM. The EC_{50} value for DA was 2 μM. Mean basal cyclic AMP production was 113.3 ± 12 pmoles of cyclic AMP/mg of protein per minute (n = 8), rising to 206 ± 6 pmoles of cyclic AMP/mg of protein per minute (n = 8) in the presence of 125 μM DA. The selective DA receptor agonist apomorphine also stimulated cyclic AMP formation at low concentrations but inhibited the enzyme activity at concentrations higher than 10 μM. The maximal stimulation obtained with apomorphine was 76% of that of DA, and the EC_{50} value was 0.6 μM.

[^{3}H]DA and [^{3}H]Spiroperidol Binding

The methods for determining [^{3}H]DA and [^{3}H]spiroperidol binding were adapted from those described in the literature by Burt *et al.* (18) and Creese *et al.* (19). Frozen calf brain (stored at −30° for several weeks) was thawed overnight at 4°. The caudate nucleus was dissected and homogenized in 100 volumes (w/v) of ice-cold 50 mM Tris-HCl buffer (pH 7.7 at 25°) for 30 sec with a Polytron PT-20 ST at a setting of 7 (full scale 10). The homogenate was centrifuged twice at 50,000 × *g* for 10 min at 4° with rehomogenization of the intermediate pellet in the same volume of fresh Tris-HCl buffer. The final pellet was resuspended (Polytron, 30 sec) in 150 volumes of cold 50 mM Tris-HCl buffer containing 0.1% ascorbic acid, 10 μM pargyline, 120 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$, and 1 mM $MgCl_2$. For [^{3}H]DA binding, 0.1 mM pyrocatechol was also added. The tissue suspension was preincubated in a 37° water bath for 5 min in the case of the [^{3}H]DA binding and for 10 min in the case of [^{3}H]spiroperidol binding and kept ice-cold until use.

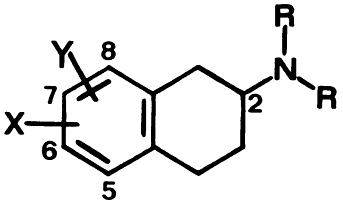
Incubation tubes in triplicate contained 1.8 ml of tissue homogenate, 100 μl of various concentrations of drug in aqueous solution, and 100 μl of a 0.1% ascorbic acid solution of [^{3}H]DA [New England Nuclear Corporation (Boston, Mass.), 15.4 Ci/mmole, prepared to obtain a 2.5 nM final concentration] or [^{3}H]spiroperidol (New England Nuclear Corporation, 25.6 Ci/mmole, prepared to obtain a 0.15 nM final concentration). [^{3}H]DA samples were incubated for 10 min and [^{3}H]spiroperidol samples for 20 min at 37° and rapidly filtered under vacuum through Whatman GF/B filters. After washing twice with 5 ml of cold Tris-HCl (pH 7.7), the filters were transferred to plastic vials containing 10 ml of scintillation liquid. The vials were shaken at room temperature for 4 hr and counted in a liquid scintillation spectrometer. Specific [^{3}H]DA binding was defined as the difference between the binding of [^{3}H]DA in the presence and absence of 1 μM DA; specific [^{3}H]spiroperidol binding was defined as the difference between [^{3}H]spiroperidol binding in the presence and absence of 1 μM (+)-butaclamol. The concentration that reduced binding by 50% (IC_{50}) was estimated on semilog paper. The IC_{50} values in Table 1 represent means and standard errors of the mean of three independent runs.

RESULTS

Adenylate cyclase. All racemic compounds stimulated DA-sensitive adenylate cyclase from rat striatum. Table

TABLE 1

Dopamine-sensitive adenylate cyclase activity and dopamine receptor binding of aminotetralins



Compound			Adenylate cyclase activity[a]		Radioligand binding[b]	
Abbreviation	*X, Y*	*R*	Potency (EC_{50})	Maximal stimulation relative to DA	$[^3H]$Dopamine (IC_{50})	$[^3H]$Spiroperidol (IC_{50})
			μM	%	*nM*	*nM*
Racemates						
5-OH-AT	5-OH	H	107 ± 35	68 ± 14 (4)	2,900 ± 360	25,000 ± 6,600
5-OH-DPAT	5-OH	*n*-Propyl	1.2 ± 0.6	74 ± 9.2 (2)	33 ± 4.5	1,100 ± 60
6-OH-AT	6-OH	H	214 ± 5.5	78 ± 5.2 (3)	2,400 ± 180	>100,000
6-OH-DPAT	6-OH	*n*-Propyl	185 ± 13	54 ± 4.3 (3)	1,200 ± 320	5,900 ± 1,170
7-OH-AT	7-OH	H	4.0 ± 0	73 ± 5.5 (2)	210 ± 27	8,400 ± 1,030
7-OH-DPAT	7-OH	*n*-Propyl	10 ± 0	58 ± 2.8 (2)	170 ± 63	4,300 ± 350
8-OH-AT	8-OH	H	560 ± 279	30 ± 10 (3)	26,000 ± 3,200	>100,000
8-OH-DPAT	8-OH	*n*-Propyl	303 ± 102	46 ± 3.5 (3)	10,000 ± 5,800	17,000 ± 1,500
5,6-$(OH)_2$-AT	5,6-$(OH)_2$	H	5.3 ± 0.3	68 ± 7.1 (2)	230 ± 38	21,000 ± 5,700
5,6-$(OH)_2$-DPAT	5,6-$(OH)_2$	*n*-Propyl	0.8 ± 0	82 ± 6.5 (2)	10 ± 0.5	1,300 ± 570
6,7-$(OH)_2$-AT	6,7-$(OH)_2$	H	1.4 ± 0.2	124 ± 2.5 (2)	8.3 ± 1.3	6,800 ± 1,400
6,7-$(OH)_2$-DPAT	6,7-$(OH)_2$	*n*-Propyl	4.1 ± 0.9	91 ± 2.0 (2)	23 ± 5.4	4,400 ± 350
5,7-$(OH)_2$-AT	5,7-$(OH)_2$	H	10.7 ± 5.1	55 ± 4.2 (2)	560 ± 51	16,000 ± 1,300
5,7-$(OH)_2$-DPAT	5,7-$(OH)_2$	*n*-Propyl	4.5 ± 1.3	30 ± 1.5 (3)	420 ± 30	2,800 ± 620
Enantiomers						
(−)-(2*S*)-5-OH-AT	5-OH	H	79 ± 18	68 ± 15 (6)	1,400 ± 480	27,000 ± 1,700
(+)-(2*R*)-5-OH-AT	5-OH	H	199 ± 21	82 ± 9 (6)	3,300 ± 440	22,000 ± 1,900
(−)-(2*S*)-5-OH-DPAT	5-OH	*n*-Propyl	3.4 ± 2.3	74 ± 11 (3)	19 ± 1.7	340 ± 57
(+)-(2*R*)-5-OH-DPAT	5-OH	*n*-Propyl	35.7 ± 4.1	82 ± 4.0 (2)	1,200 ± 190	3,600 ± 480
(−)-(2*S*)-7-OH-AT	7-OH	H	39.8 ± 0	30 ± 2.0 (2)	13,000 ± 3,300	>100,000
(+)-(2*R*)-7-OH-AT	7-OH	H	2.5 ± 0.7	65 ± 3.5 (3)	130 ± 3	2,500 ± 260
(−)-(2*S*)-7-OH-DPAT	7-OH	*n*-Propyl	0	0 (2)	52,000 ± 8,700	28,000 ± 2,200
(+)-(2*R*)-7-OH-DPAT	7-OH	*n*-Propyl	6.7 ± 4.5	62 ± 5.0 (3)	180 ± 300	2,000 ± 580
dopamine			2.3 ± 0.3	100 (6)	13 ± 2.6	45,000 ± 1,130
Apomorphine			0.6 ± 0.08	76 ± 11 (5)	11 ± 1.4	500 ± 101

[a] Mean basal adenylate cyclase activity was 113.3 ± 12 pmoles of cyclic AMP per milligram of protein per minute ($n = 8$), rising to 206 ± 6 pmoles of cyclic AMP per milligram of protein per minute ($n = 8$) in the presence of 125 μM DA. Values are means of two to six determinations (actual number in parentheses) ± standard error of the means.

[b] Specific binding was defined as the amount of radioligand displaced by an excess of 1 μM DA ($[^3H]$DA binding) or 1 μM (+)-butaclamol ($[^3H]$spiroperidol binding). Values are means of three independent determinations ± standard error of the mean.

1 shows the list of compounds with their respective activities at the enzyme.

The racemic monohydroxyaminotetralins with primary amino groups revealed smaller values in potency and maximal stimulation than did DA. Among the corresponding tertiary amines, only 5-OH-DPAT showed higher potency than did DA. The change in potency induced by varying the position of the —OH group paralleled the changes in maximal stimulation in the series of tertiary amines but not in the series of primary amines. The following rank orders were established: *primary amines*: potency DA > 7-OH-AT > 5-OH-AT > 6-OH-AT > 8-OH-AT; maximal stimulation DA > 7-OH-AT > 6-OH-AT > 5-OH-AT > 8-OH-AT; *tertiary amines*: potency 5-OH-DPAT > DA > 7-OH-DPAT > 6-OH-DPAT > 8-OH-DPAT; maximal stimulation DA > 5-OH-DPAT > 7-OH-DPAT > 6-OH-DPAT > 8-OH-DPAT.

In the series of primary amines, 7-OH-AT was therefore the most active compound; in the series of tertiary amines as well as over the whole group of monohydroxyaminotetralins, 5-OH-DPAT had the strongest effect. The 8-hydroxyaminotetralins were the least active compounds in the series of primary and tertiary amines. The introduction of *N,N*-di-*n*-propyl substituents had a marked effect in the case of 5-OH-AT: the potency increased about 10-fold, and maximal stimulation was almost doubled. In contrast, in the case of the 7-OH derivative the potency did not change significantly, and maximal stimulation even decreased upon *N,N*-dialkylation. The effects of other derivatives were less influenced by the *N,N*-di-*n*-propyl substituents.

The dihydroxyaminotetralins of the 5,6- and 6,7-dihydroxy series, corresponding to the α- and β-rotamer form of DA, respectively, were generally more active at the adenylate cyclase than the monohydroxy derivatives. Variation of the two adjacent —OH groups induced almost parallel changes in potency and maximal stimulation. However, the rank order in the series of primary amines was different from that in the series of tertiary amines: 6,7-$(OH)_2$-AT > DA > 5,6-$(OH)_2$-AT, respectively, and 5,6-$(OH)_2$-DPAT > DA > 6,7-$(OH)_2$-DPAT. The introduction of *N*,*N*-di-*n*-propyl substituents increased both potency and maximal stimulation of the 5,6-dihydroxy derivative, whereas in the case of the 6,7-dihydroxy compound both values were clearly decreased. 5,6-$(OH)_2$-DPAT was the most potent compound on the whole, and 6,7-$(OH)_2$-AT showed the highest maximal stimulation of all investigated aminotetralins. The 5,7-dihydroxyaminotetralins, which contain the —OH groups in the optimal positions found in the monohydroxy series, showed no stronger effects with regard to the monohydroxy compounds: potency and maximal stimulation of 5,7-$(OH)_2$-AT corresponded to the mean of the values of the respective 5- and 7-hydroxy derivatives, the potency of 5,7-(OH)-DPAT was between that of 5-OH-DPAT and 7-OH-DPAT, and the maximal stimulation was even smaller than that of both monohydroxy compounds.

The activities of the enantiomers of the most interesting monohydroxyaminotetralins with hydroxy groups in positions 5 and 7, respectively, were compared with those of the racemates. In the 5-hydroxy series, the (−)-enantiomers of the primary and tertiary amines had potency and maximal stimulation similar to the racemate, whereas the corresponding (+)-enantiomers were less potent. In contrast, in the 7-OH series the (+)-enantiomers of the primary and tertiary amine had potency and maximal stimulation similar to the racemates, but the (−)-enantiomer of the primary amine was clearly less active and that of the tertiary amine was even inactive. The dose-response curves for the enantiomers investigated are shown in Fig. 3.

[^{3}H]Dopamine and [^{3}H]spiroperidol binding. All aminotetralin derivatives investigated displaced [^{3}H]DA and [^{3}H]spiroperidol binding from specific binding sites in membrane fractions from calf caudate nucleus (Table 1). The compounds tested had a lower affinity for the [^{3}H]spiroperidol binding sites than for the [^{3}H]DA binding sites, as would normally be expected from DA agonists. The [^{3}H]DA binding showed excellent correlation with the potency at adenylate cyclase, whereas the correlation with [^{3}H]spiroperidol binding was rather poor (compare Fig. 4).

DISCUSSION

The activities found for the monohydroxylated aminotetralin derivatives at DA-sensitive adenylate cyclase from rat striatum imply that in these semirigid DA analogues a single phenolic hydroxy group is sufficient to fulfill the requirements for D-1 receptor activation. Our results are in accordance with the finding of Schorderet *et al.* (20), who showed that 5-OH-DPAT stimulates DA-sensitive adenylate cyclase from rabbit retina. In contrast, it has been reported that in order to activate the vascular DA receptor, which is also linked to adenylate cyclase and therefore is of the D-1 type, a catechol group would be essential (21, 22). However, the observed maximal stimulations of the monohydroxyaminotetralins at striatal adenylate cyclase are smaller than that of DA itself.

Our investigations revealed that 5-OH-DPAT was the most active compound in the series of tertiary amines, followed by 7-OH-DPAT; 6-OH-DPAT was considerably less active. These findings parallel the effects of these derivatives in other tests of DA-like activity, including emesis, stereotyped behavior, reduction of striatal DA metabolism, and DA receptor binding (11, 23, 24). In the hitherto rather neglected series of monohydroxyaminotetralins with primary amino groups, however, we found interestingly that the rank order of activity was reversed: 7-OH-AT was the most potent compound with the highest maximal stimulation, followed by 5-OH-AT and again, with considerably weaker activity, by 6-OH-AT. The prominent activities of the 5-OH and 7-OH derivatives of both series stand out clearly against those of the 6-OH derivatives and even more clearly against those of the 8-OH derivatives. The 6-OH group in the aminotetralins corresponds to the *p*-hydroxy group of DA, both the 5-OH and the 7-OH group correspond to the *m*-hydroxy group of DA in its α- and β-rotamer conformations of the aminoethyl chain, respectively. Our results would thus imply that the *m*-hydroxy function in DA represents a major binding site to the D-1 receptor.

The possibility that the D-1 receptor could contain separate binding sites for the α- and β-rotameric *m*-hydroxy group of DA, assuming identical orientation of the phenethylamine moiety, was tested with the help of 5,7-dihydroxyaminotetralins. The finding that the activity of 5,7-$(OH)_2$-AT and that of 5,7-$(OH)_2$-DPAT were between those of the corresponding 5-OH and 7-OH derivatives argues against such a receptor model.

Our results with aminotetralins at DA-sensitive adenylate cyclase discussed so far are most consistent with the assumption that the D-1 receptor contains two major binding sites, one for the *m*-hydroxy group and one for the amino group of DA, which together represent the active site. The *p*-hydroxy group and the aromatic ring of DA would interact with accessory binding sites and thereby contribute to the activity. This is reflected by the greater effects of the dihydroxyaminotetralins with respect to those of the corresponding monohydroxy derivatives.

Examination of the enantiomers of the interesting 5-OH and 7-OH aminotetralins at the adenylate cyclase showed that the preference for the D-1 receptor with respect to the asymmetrical center bearing the amino group does not change upon *N*,*N*-dialkylation. However, if one changes the position of the hydroxy group from position 7 (the β-rotameric *m*-position) to position 5 (the α-rotameric *m*-position), a reversal of the preferred configuration takes place within the series of both primary and tertiary amines. This leaves (−)-(2*S*)-5-OH-AT and (−)-(2*S*)-5-OH-DPAT as preferred enantiomers of the 5-OH series and (+)-(2R)-7-OH-AT and (+)-(2R)-7-OH-DPAT as preferred enantiomers of the 7-OH series. The question then arises whether these results are compatible

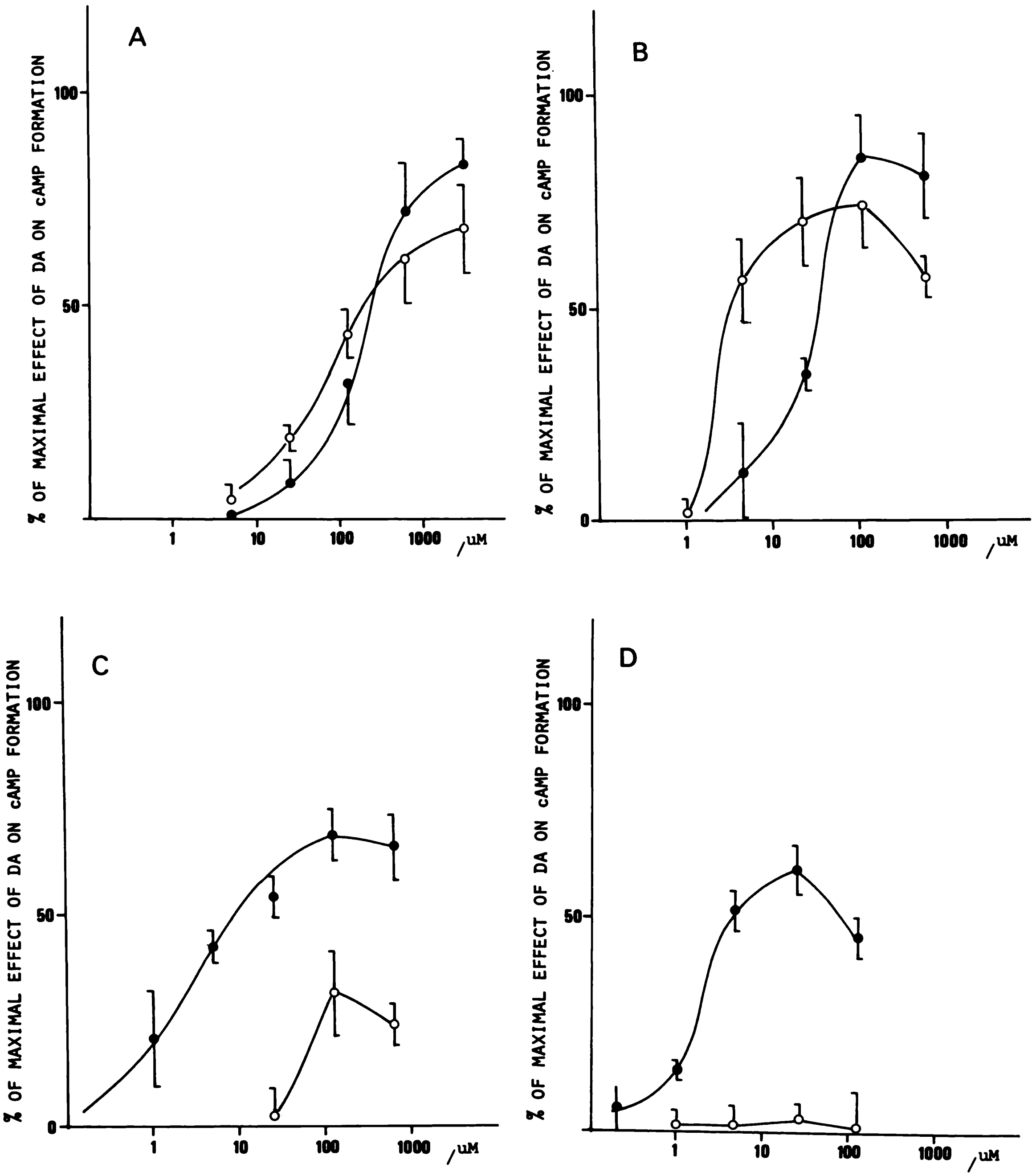


FIG. 3. ***Effect of various concentrations of enantiomers of several monohydroxyaminotetralins on cyclic AMP formation in homogenates from rat striatum***

The effect of the tested substances on cyclic AMP formation is expressed as a percentage of the effect of 125 μM dopamine, which is defined as 100%. Each value represents the mean ± standard error of the mean of two to six experiments. ●, (+)-Form; ○, (−)-form. A, 5-OH-AT; B, 5-OH-DPAT; C, 7-OH-AT; D, 7-OH-DPAT.

with the proposed two-site D-1 receptor model. The aminotetralins contain a quasi-axis of symmetry through the amino group and carbon atom 6 (compare Fig. 5). A rotation around this axis brings the hydroxy group in position 5 into the vicinity of the hydroxy group in position 7 and at the same time reverses the configuration of the amino group and the hydrogen atom at the asymmetrical carbon atom relative to the plane of the aromatic ring. Thus, by such a rotation, (−)-(2*S*)-5-OH-AT achieves an orientation toward the presumed major binding sites similar to that of (+)-(2R)-7-OH-AT (Fig. 6); i.e., both compounds can interact almost identically with the D-1 receptor. Our results with the enantiomers are thus consistent with the proposed D-1 receptor model and would contradict an earlier proposition that the α- and β-rotamer forms of DA affect different subtypes of DA receptors (25). The finding that the configuration of the higher-affinity (−)-enantiomer of 5-OH-DPAT on [^{3}H]DA binding (rat striatum) is opposite to the configuration of the higher-affinity (+)-enantiomer of 6,7-$(OH)_2$-AT has led to a similar proposition by McDermed *et al.* (15) for an unspecified DA receptor.

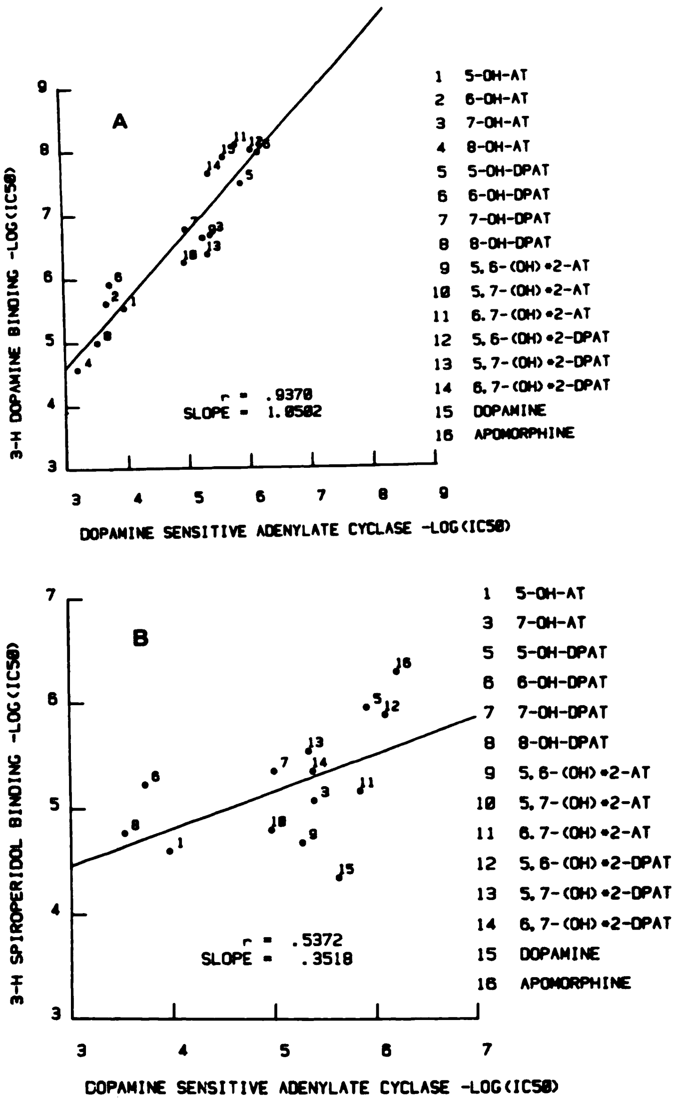


FIG. 4. *Relationship between the EC$_{50}$ values of various racemic aminotetralins at dopamine-sensitive adenylate cyclase and their IC$_{50}$ values at the [^{3}H]dopamine-binding site (A) and [^{3}H]spiroperidol-binding site (B)*

Data are taken from Table 1.

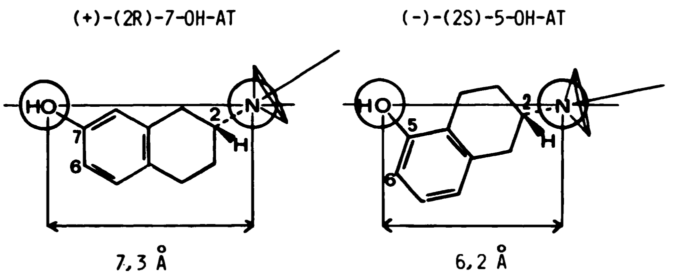


FIG. 6. *Orientation of (+)-(2R)-7-OH-AT and (−)-(2S)-5-OH-AT toward the proposed two major binding sites of the D-1 receptor*

The aminotetralins are displayed above the receptor represented by the two major binding sites (*circles*). The *cones*, centered around the amino group in the prolongation of the N-C$_2$-axis, indicate the possible orientation of the freely rotating *N*-substituents (hydrogen atoms or C$_1$ of the *n*-propyl group) and the electron lone pair, respectively.

The distance between the nitrogen atom of the amino group and the 5-OH and 7-OH groups, respectively, in the aminotetralins differs considerably (5-OH—N, 6.2 Å; 7-OH—N, 7.3 Å), as has already been pointed out by Cannon (25). This difference indicates a certain flexibility in the receptor-agonist interaction. The question arises, therefore, why the position of the OH group for optimal activity changes from 7 in the series of primary amines to 5 in the series of tertiary amines, a structure-activity pattern which was also found in the corresponding dihydroxy series with *β*-rotameric 6,7-(OH)$_2$-AT as the optimal compound among the primary amines and *α*-rotameric 5,6-(OH)$_2$-DPAT as the optimal compound among the tertiary amines. A possible explanation for this change in preferred rotameric form upon *N*,*N*-dipropylation could be seen in changes in the acid strength of the phenolic —OH group depending on its position and the substitution pattern of the amino group. However, this is not the case, as can be seen from the measured pK$_a$ values of the phenolic —OH group of 5-OH-AT, 5-OH-DPAT, 7-OH-AT, and 7-OH-DPAT in Table 2 (all of which are around 12.6). The ionization constants of the protonated amino group (Table 2) show, as expected, higher values for the more basic primary amines than for the tertiary amines but do not vary considerably between positional isomers, therefore ruling out differences in ionization behavior between the 5-OH and 7-OH series.

The change in preferred rotameric form within the aminotetralins upon *N*,*N*-dipropylation is due to an increase in potency and maximal stimulation (adenylate cyclase) and affinity (DA receptor binding) in the 5-OH series, whereas the corresponding values in the 7-OH series remain constant or even decrease. A plausible explanation would therefore be that the *n*-propyl substituents at the amino group of the 5-OH series (but not

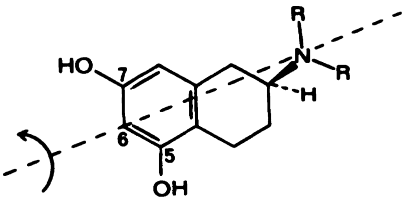


FIG. 5. *Position of the 5-OH and 7-OH groups in the aminotetralins, corresponding to the m-hydroxy group in DA, relative to a quasi-axis of symmetry*

Rotation around the quasi-axis of symmetry through N and C$_6$ brings the hydroxy group in position 5 in the vicinity of the hydroxy group in position 7 and at the same time reverses the configuration of the amino group and the hydrogen atom at the asymmetrical carbon atom relative to the plane of the aromatic ring.

TABLE 2

Ionization constants of monohydroxyaminotetralins

Compound	pK$_a$ (OH)^a	pK$_a$ (NH$^+$)^b
5-OH-AT	12.6	9.0
7-OH-AT	12.6	9.1
5-OH-DPAT	12.7	8.1
7-OH-DPAT	12.6	8.1

[a] Apparent pK$_a$ values were determined by potentiometric titration with tetramethylammonium hydroxide in methyl-Cellosolve/water = 80/20 according to the method of Simon (26).

[b] Apparent pK$_a$ values were determined by UV spectrometry according to the method of Albert and Serjeant (27).

those of the 7-OH series) have access to an accessory binding site which increases the affinity for the receptor and possibly facilitates a proper orientation of the electron lone-pair of the amino group. The discrimination between the two series could be due to differences in the respective distance between the amino group and the hydroxy group, and in the orientation of the substituted amino group as depicted in Fig. 6. The major loss of activity upon *N*,*N*-di-*n*-butyl substitution in both series[3] indicates that limited space is available for the *N*,*N*-dialkyl substituents at the receptor. This would favor the 5-OH tertiary amines with its shorter OH—N distance. The inactivity of the DA derivative, which has its *N*,*N*-di-*n*-propyl groups constrained into a piperidine ring (28), on the other hand, underlines the importance of the stereochemical orientation of the *N*,*N*-dialkyl substituents. The relative inactivity of *N*,*N*-dimethyl dopamine (3) and *N*,*N*-dimethyl-substituted aminotetralins (10) at the adenylate cyclase could be interpreted in the sense that the *N*,*N*-dimethyl substituents do not reach this accessory binding site, which could help to direct the electron lone pair at the nitrogen atom and the *N*-substituents. They could thus be prone to steric repulsion. DA likewise cannot reach this presumed accessory binding site. Therefore, with respect to the superior activities of 7-OH-AT and 6,7-$(OH)_2$-AT, one would assume that DA interacts with the D-1 receptor in the β-rotamer conformation. Upon *N*,*N*-di-*n*-propylation however, DA is supposed to change its mode of receptor interaction, adopting an α-rotamer conformation to meet the new space-filling requirements.

Specific binding with properties similar to a receptor-linked adenylate cyclase would suggest identical recognition sites. Such a correlation has been shown to exist between *cis*-[^{3}H(Z)]flupenthixol binding and DA-sensitive adenylate cyclase activity in rat striatum based on the activities of a series of DA-antagonistic neuroleptics (29). Our results with a series of aminotetralins demonstrate for the first time such a correlation with DA agonists between [^{3}H]DA binding and potency at DA-sensitive adenylate cyclase in striatal tissue. The correlation between affinity (IC_{50} value) for the [^{3}H]DA binding site and the potency (EC_{50} value) at adenylate cyclase showed a high correlation coefficient ($r = 0.94$). However, no relationship could be detected between [^{3}H]DA binding and the maximal stimulation of the enzyme. The series of 14 aminotetralins covers a wide range of activity. Distances between functional groups, and the position of the aromatic ring and the lipophilic centers with respect to these functional groups, vary considerably. The series including DA and apomorphine can therefore be regarded as sufficiently heterogeneous to allow a correlation. This could suggest that [^{3}H]DA binding in the striatum involves binding to D-1 receptors which mediate activation of DA receptors in this region.

In contrast, a poor correlation ($r = 0.54$) was observed between [^{3}H]spiroperidol binding and potency at DA-sensitive adenylate cyclase. This parallels observations concerning a lack of correlation between the binding with another radiolabeled butyrophenone-type neuroleptic, [^{3}H]haloperidol, and activity at the striatal DA-sensitive adenylate cyclase (29). It could mean that [^{3}H]spiroperidol binding does not involve substantial binding to D-1 receptors. However, another interpretation could be that [^{3}H]spiroperidol binds preferentially to DA-antagonistic sites and therefore allows only insufficient discrimination of DA agonists. Studies with the same series of compounds on a model representing interaction with a dopamine D-2 receptor should help to clarify this question. In addition, they should tell us whether the observed structure-activity relationships are also valuable for other subtypes of DA receptors.

ACKNOWLEDGMENTS

The authors wish to thank Miss K.-M. Oksman for skillful technical assistance, Dr. M. Kohler for the pK_a measurements, and Dr. H.-J. Tobler for the computer graphics.

REFERENCES

1. Costall, B., and R. J. Naylor. Behavioural aspects of dopamine agonists and antagonists, in *The Neurobiology of Dopamine* (A. S. Horn, ed.). Academic Press, New York, 555–576 (1979).
2. Goldberg, L. I., P. H. Volkman, and J. D. Kohli. A comparison of the vascular dopamine receptor with other dopamine receptors. *Annu. Rev. Pharmacol. Toxicol.* **18:**57–79 (1978).
3. Miller, R. J., and J. McDermed. Dopamine-sensitive adenylate cyclase, in *The Neurobiology of Dopamine* (A. S. Horn, ed.). Academic Press, New York, 159–177 (1979).
4. Brown, E. M., R. J. Carroll, and G. D. Aurbach. Dopaminergic stimulation of cyclic AMP accumulation and parathyroid hormone release from dispersed bovine parathyroid cells. *Proc. Natl. Acad. Sci. U. S. A.* **74:**4210–4213 (1977).
5. Murthy, V. V., J. C. Gilbert, L. I. Goldberg, and J. F. Kuo. Dopamine-sensitive adenylate cyclase in canine renal artery. *J. Pharm. Pharmacol.* **28:**567–571 (1976).
6. Libet, B., H. Kobayashi, and T. Tanaka. Synaptic coupling into production and storage of a neuronal memory trace. *Nature (Lond.)* **258:**155–157 (1975).
7. Kebabian, J. W., and D. B. Calne. Multiple receptors for dopamine. *Nature (Lond.)* **277:**93–96 (1979).
8. Miller, R., A. Horn, L. Iverson, and R. Pinder. Effects of dopamine-like drugs on rat striatal adenylate cyclase have implications for CNS dopamine receptor topography. *Nature (Lond.)* **250:**238–241 (1974).
9. Woodruff, G. N., K. J. Watling, C. D. Adrews, J. A. Poat, and J. D. McDermed. Dopamine receptors in rat striatum and nucleus accumbens: conformational studies using rigid analogues of dopamine. *J. Pharm. Pharmacol.* **29:**422–427 (1977).
10. Cannon, J. G., B. Costall, P. M. Laduron, J. E. Leysen, and R. J. Naylor. Effects of some derivatives of 2-aminotetralin on dopamine-sensitive adenylate cyclase and on the binding of [^{3}H]-haloperidol to neuroleptic receptors in the rat striatum. *Biochem. Pharmacol.* **27:**1417–1420 (1978).
11. McDermed, J. D., G. M. McKenzie, and A. P. Phillips. Synthesis and pharmacology of some 2-aminotetralins: dopamine receptor agonists. *J. Med. Chem.* **18:**362–367 (1975).
12. McDermed, J. D., G. M. McKenzie, and H. S. Freeman. Synthesis and dopaminergic activity of (±)-, (+)- and (−)-2-dipropylamino-5-hydroxy-1,2,-3,4-tetrahydronaphthalene. *J. Med. Chem.* **19:**547–549 (1976).
13. Giesecke, J. The crystal structure of (+)-2-dipropylamino-5-hydroxytetralin hydrochloride. *Acta Crystallogr. Sect. B Struct. Crystallogr. Cryst. Chem.* **36:**110–114 (1980).
14. Zymalkowski, F., and E. Dornhege. Die absolute Konfiguration von (+)- und (−)-2-Aminotetralin sowie der vier isomeren 2-Amino-tetralole-(1). *Liebigs Ann. Chem.* **728:**144–151 (1969).
15. McDermed, J. D., H. S. Freeman, and R. M. 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* (E. Usdin, ed.). Pergamon Press, New York, 568–570 (1979).
16. König, J. F. R., and R. A. Klippel (eds.). *The Rat Brain: A Stereotaxic Atlas of the Forebrain and Lower Parts of the Brain Stem.* Williams & Wilkins, Baltimore (1970).
17. Kebabian, J. W., G. L. Petzold, and P. Greengard. Dopamine-sensitive adenylate cyclase in caudate nucleus of the rat brain and its similarity of the dopamine receptor. *Proc. Natl. Acad. Sci. U. S. A.* **69:**2045–49 (1972).
18. Burt, D. R., I. Creese, and S. H. Snyder. Properties of [^{3}H]haloperidol and [^{3}H]dopamine binding associated with dopamine receptors in calf brain membranes. *Mol. Pharmacol.* **12:**800–812 (1976).
19. Creese, I., R. Schneider, and S. H. Snyder. ^{3}H-Spiroperidol labels dopamine receptors in pituitary and brain. *Eur. J. Pharmacol.* **46:**377–381 (1977).
20. Schorderet, M., J. McDermed, and P. Magistretti. Dopamine receptors and

---

[3] R. Markstein, unpublished results.

cyclic AMP in rabbit retina: a pharmacological and stereochemical analysis using semirigid analogs of dopamine (aminotetralins) and thioxanthene isomers. *J. Physiol. (Paris)* **74**:509–513 (1978).

21. Goldberg, L. I., and J. D. Kohli. Peripheral pre- and post-synaptic dopamine receptors: are they different from dopamine receptors in the central nervous system? *Commun. Psychopharmacol.* **3**:447–456 (1979).
22. Brodde, O. E., F.-J. Meyer, W. Schemuth, and J. Freistühler. Demonstration of specific vascular dopamine receptors mediating vasodilation on the isolated rabbit mesenteric artery. *Nauyn-Schmiedebergs Arch. Pharmacol.* **316**:24–30 (1981).
23. Feenstra, M. G. P., H. Rollema, D. Dijkstra, C. J. Grol, A. S. Horn, and B. H. C. Westerink. Effect of non catecholic 2-amino-tetralin derivatives on dopamine metabolism in the rat striatum. *Nauyn-Schmiedebergs Arch. Pharmacol.* **313**:213–219 (1980).
24. Tedeso, J. L., P. Seeman, and J. D. McDermed. The conformation of dopamine at its receptor: binding of monohydroxy-2-aminotetralin enantiomers and positional isomers. *Mol. Pharmacol.* **16**:369–381 (1979).
25. Cannon, J. G. Chemistry of dopaminergic agonists. *Adv. Neurol.* **9**:177–183 (1975).
26. Simon, W. Zur Mikrotitration organischer Verbindungen: Teil IX, Standardmethodik für die Mikrobestimmung von scheinbaren Dissoziationskonstanten im System 80 Gew.-proz. Methylcellosolve/20 Gew.-proz. Wasser. *Helv. Chim. Acta* **41**:1835–1851 (1958).
27. Albert, A., and E. P. Serjeant (eds.). *The Determination of Ionization Constants.* Chapman and Hall, London, 44–64 (1971).
28. Ginos, J. Z., G. C. Cotzias, E. Tolosa, L. C. Tang, and A. LoMonte. Cholinergic effects of molecular segments of apomorphine and dopaminergic effects of *N,N*-dialkylated dopamines. *J. Med. Chem.* **18**:1194–1200 (1975).
29. Hyttel, J. Effects of neuroleptics on ^{3}H-haloperidol and ^{3}H-cis(Z)-flupenthixol binding and on adenylate cyclase activity in vitro. *Life Sci.* **23**:551–556 (1978).

Send reprint requests to: Dr. M. P. Seiler, Preclinical Research, Sandoz Ltd., Lichtstrasse, CH-4002 Basel, Switzerland.