MOLECULAR PHARMACOLOGY, 16, 369-381

The Conformation of Dopamine at Its Receptor: Binding of Monohydroxy-2-aminotetralin Enantiomers and Positional Isomers

J. L. TEDESCO AND P. SEEMAN

Department of Pharmacology, University of Toronto, Toronto, CANADA M5S 1A8

J. D. MCDERMED

The Wellcome Research Laboratories, Burroughs Wellcome Co., 3030 Cornwallis Road, Research Triangle Park, N.C. 27709, U.S.A.

(Received July 5, 1978)
(Accepted March 19, 1979)

SUMMARY

TEDESCO, 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).

In order to test the identity of the [^{3}H]-apomorphine receptor with the [^{3}H]-dopamine receptor in brain, and to determine the conformation of dopamine at that receptor, we tested the binding affinities of some semi-rigid dopamine analogues. The enantiomers of 5-hydroxy-N,N-di-n-propyl-2-aminotetralin, a semi-rigid analogue of dopamine in the α-rotamer conformation, showed stereospecific binding to the [^{3}H]-dopamine, [^{3}H]-apomorphine and [^{3}H]-spiperone receptors in crude homogenate of calf-brain striatum. The affinity of the β-rotamer analogue of dopamine, 7-hydroxy-N,N-di-n-propyl-2-aminotetralin, to these receptors was from 5 to 22 times weaker. Substitution of the hydroxyl into the 6-position, analogous to p-tyramine, resulted in the lowest affinity for these receptors. The results are consistent with the identity of the [^{3}H]-apomorphine with the [^{3}H]-dopamine receptor. The identity of the dopamine agonist receptor with the antagonist receptor, however, remains controversial. Thus, the probable absolute conformation of dopamine at this agonist receptor is defined in terms of the torsion angles of the ethylamine side-chain of the dopamine moiety in R-(−)-apomorphine, with the nitrogen lone electron pair orientation at the agonist receptor also defined.

INTRODUCTION

Various 2-aminotetralin derivatives have dopamine-mimetic properties. McDermed *et al.* (1) demonstrated stereotyped behavior in rats and emesis in dogs following parenteral administration of low doses of 5,6-dihydroxy-2-aminotetralins. The compound (±)-6,7-dihydroxy-2-aminotetralin or ADTN elicits contralateral turning (2) and intense stereotypy (3) in rats. Costall *et al.* have recently concluded (4) that generally the 5,6-dihydroxy-2-aminotetralins were more potent than the corresponding 6,7-dihydroxyl derivatives in evoking both hyperactivity and stereotyped sniffing and biting. Another indication that these 2-aminotetralin derivatives activate the dopamine receptor is that ADTN is equipotent with dopamine in activating striatal adenylate cyclase (5).

This work was supported by the Ontario Mental Health Foundation and the Medical Research Council of Canada (MT-2951).

0026-895X/79/050369-13$02.00/0

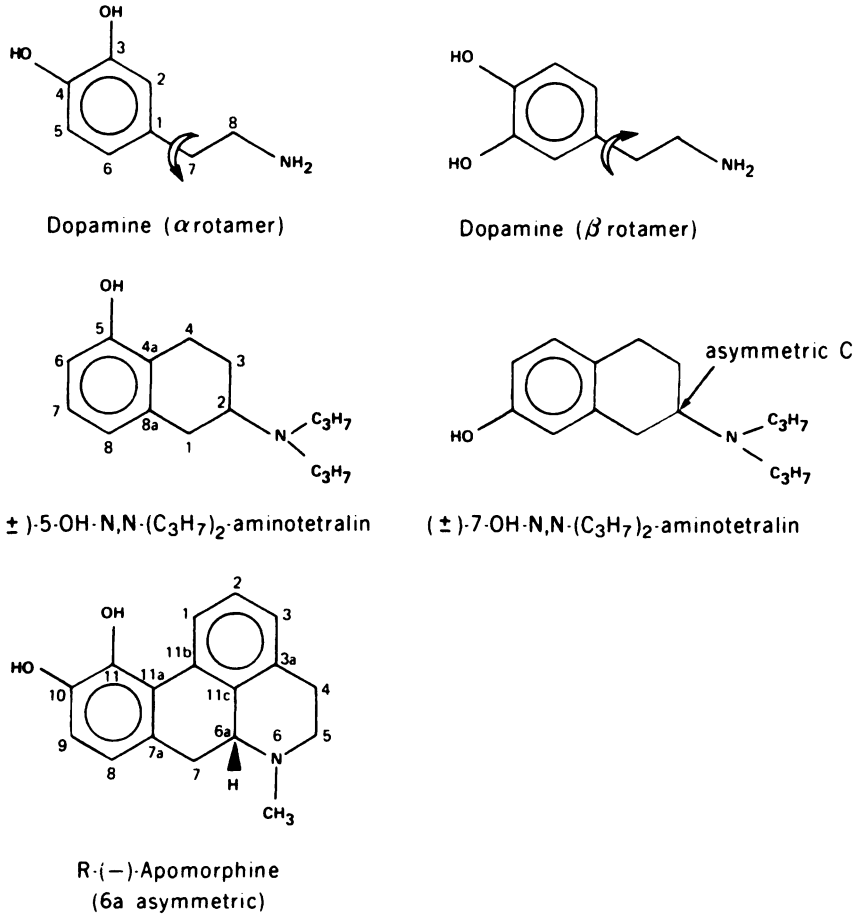

FIG. 1. *Numbering schemes for the carbon skeletons of dopamine, 2-aminotetralins and aporphines.* The two rotameric conformations of dopamine are illustrated in the top row. Below each are representative semi-rigid dopamine analogues in that same conformation. Thus, (±)-5-hydroxy-N,N-di-n-propyl-2-aminotetraline [(±)-5-OH-AT] and R-(−)-apomorphine are α-rotamer dopamine analogues while (±)-7-hydroxy-N,N-di-n-propyl-2-aminotetralin [(±)-7-OH-AT] is a β-rotamer dopamine analogue. The absolute stereoconformation of R-(−)-apomorphine is indicated by the orientation towards the viewer of the hydrogen atom bonded to the 6a asymmetric carbon.

In 1976, one of us reported that monohydroxylated derivatives of N,N-di-n-propyl-2-aminotetralins also produced both stereotyped behavior in rats and emesis in dogs (6). The most potent was (−)-5-hydroxy-N,N-di-n-propyl-2-aminotetralin[1]. The active (−)-enantiomer was about 10 times more potent than R-(−)-apomorphine and was only slightly weaker than the dihydroxyl analogue (±)-5,6-dihydroxy-N,N-di-n-propyl-2-aminotetralin. The (+)-enantiomer was less active than the (−)-enantiomer by a factor of at least 35.

The monohydroxyl analogues substituted in the 6 or 7 position (Fig. 1) were much weaker than (±)-5-OH-AT (6). They were 30 or 50 times less potent than (−)-apomorphine in producing stereotypy, but were approximately equipotent in producing emesis.

Thus, there is no apparent absolute need for a dihydroxyl, or catechol, substituent on

[1] 5-OH-AT signifies 5-hydroxy-N,N-di-n-propyl-2-aminotetralin; 6-OH-AT signifies 6-hydroxy-N,N-di-n-propyl-2-aminotetralin; 7-OH-AT signifies 7-hydroxy-N,N-di-n-propyl-2-aminotetralin.

a dopamine-receptor agonist. This has also been confirmed by using the aporphine structure: (±)-11-hydroxy-aporphine is about one-fortieth the potency of (−)-apomorphine (7). The likelihood that meta-tyramine, a flexible monohydroxyl dopamine analogue, can activate dopamine receptors was established some time ago (8, 9). This is confirmed in the present work and demonstrates that the para-hydroxyl group is not needed for high-affinity binding. However, because meta-tyramine has a flexible side-chain, we cannot know what position is assumed at the receptor by its meta-hydroxyl group relative to the alpha-carbon and the nitrogen of that side-chain.

As a dopamine receptor agonist, (−)-apomorphine seems to be specific in at least two ways. First, it is stereospecific since (+)-apomorphine apparently does not activate the receptor (10). Second, it is specific as an α-rotamer analogue of dopamine (Fig. 1), since the β-rotamer form, (−)-9,10-dihydroxy-aporphine (isoapomorphine), is much less active (11–13).

Since apomorphine is a fairly rigid α-rotamer analogue of dopamine, we studied whether [^{3}H]-apomorphine and [^{3}H]-dopamine receptors would both show preferential affinity for α-rotamer analogues.

The enantiomers (−)-5-OH-AT and (+)-5-OH-AT were therefore used as semi-rigid analogues of dopamine in the α-rotamer conformation to test for stereospecific binding of an agonist to both [^{3}H]-apomorphine and [^{3}H]-dopamine receptors. We then tested the compounds (±)-5-OH-AT, (±)-6-OH-AT and (±)-7-OH-AT for relative affinities to both types of receptors to determine whether both receptors would show preferential binding for one rotamer.

METHODS

Preparation of calf caudate crude homogenate was as described previously (14). [^{3}H]-apomorphine (14.1 Ci/mmole) was prepared by New England Nuclear Corporation. General catalytic exchange of tritium for hydrogen in R-(−)-apomorphine HCl was followed by purification. Purity was determined as 96% or higher by thin-layer chromatography on silica gel G plates in an ethanol:acetic acid:water (6:3:1 V/V/V) solvent system. The sample was dissolved at 0.35 mM concentration in ethanol containing 15 mM ascorbic acid, shipped on dry ice and stored at −20°. Purity was checked six months later by duplicate thin-layer chromatography using the above system with parallel nonradioactive apomorphine controls. Development by iodine vapor gave identical Rf values of 0.59. Radioactivity scanning of the plates indicated that radiopurity was still greater than 96%.

[^{3}H]-dopamine (15 Ci/mmole; 68 μM in 0.15N tartaric acid aqueous solution) and [^{3}H]-spiroperidol ([^{3}H]-spiperone; 26.4 Ci/mmole; 38 μM in ethanol) were purchased from New England Nuclear Corporation (Boston, MA.) and used without further chemical characterization. The [^{3}H]-dopamine stock was diluted 20-fold with 0.01% ascorbic acid aqueous solution (previously flushed with nitrogen gas) and stored in aliquots at −20°. [^{3}H]-spiroperidol was stored in undiluted aliquots at −20°. Each aliquot was diluted with buffer just prior to its use in an assay.

Binding assays. All assays were performed using buffer containing 15 mM Tris HCl, 5 mM NA_2EDTA, 1.1 mM ascorbic acid and 12.5 μM nialamide with the final pH adjusted to 7.4 with HCl. Assay tubes were Pyrex glass, 12 × 75 mm. Using Gilson variable-volume automatic pipettes, 200 μl of test ligand or control ligand (at specified concentration) or buffer were dispensed into each tube followed by 200 μl of the [^{3}H]-ligand and 200 μl of calf caudate crude homogenate (immediately after final homogenization on a Polytron P10). When measuring stereospecific binding, each tube contained 100 μl of the (+) or (−)-enantiomer (at the specified concentration), 100 μl of test drug or buffer, 200 μl of the [^{3}H]-ligand and 200 μl of the caudate homogenate. Final tube concentrations were 0.5 nM for [^{3}H]-dopamine, 3.5 nM for [^{3}H]-apomorphine and 0.03 nM for [^{3}H]-spiperone. These final assay concentrations for [^{3}H]-apomorphine and [^{3}H]-dopamine had been determined to be those giving the highest signal-to-noise ratio for specific binding while being adequately low to maintain

specificity of binding (i.e., at or below their respective scatchard K_D values). For ^{3}H-spiperone, priorities were reversed because of concern for maintaining specificity of binding to the dopamine/neuroleptic receptor in relation especially to possible binding to the serotonergic or noradrenergic receptor (15). Thus, an exceptionally low final concentration (0.03 nM) was chosen in order to maximize specificity for the highest affinity site (K_D = 0.10 nM; see ref. 16) while maintaining an adequate signal-to-noise ratio.

Membrane protein content varied between assays from 0.18 to 0.22 mg protein per tube, as determined by the Lowry assay (17). Within all assays, any control or test-drug-concentration group contained 5 or 6 replicate tubes.

Incubation occurred at room temperature for 15 minutes ([^{3}H]-dopamine) or 30 minutes ([^{3}H]-apomorphine or [^{3}H]-spiperone) after addition of the membrane with vortexing. A 0.5 ml aliquot was then removed from each tube and spread over the central one-third of the area of a Whatman GF/B glass-fiber filter (2.4 cm D) atop a Millipore filter support under vacuum. Each filter was then washed with 5 ml ([^{3}H]-dopamine) or 10 ml ([^{3}H]-apomorphine or [^{3}H]-spiperone) of buffer at room temperature in about 5 seconds. The filter was transferred to a glass scintillation vial and 8 ml of Aquasol scintillation cocktail (New England Nuclear Corp.) were added. The tritium radioactivity was measured after 6 or more hours equilibration at 10°.

Specific binding was defined as total

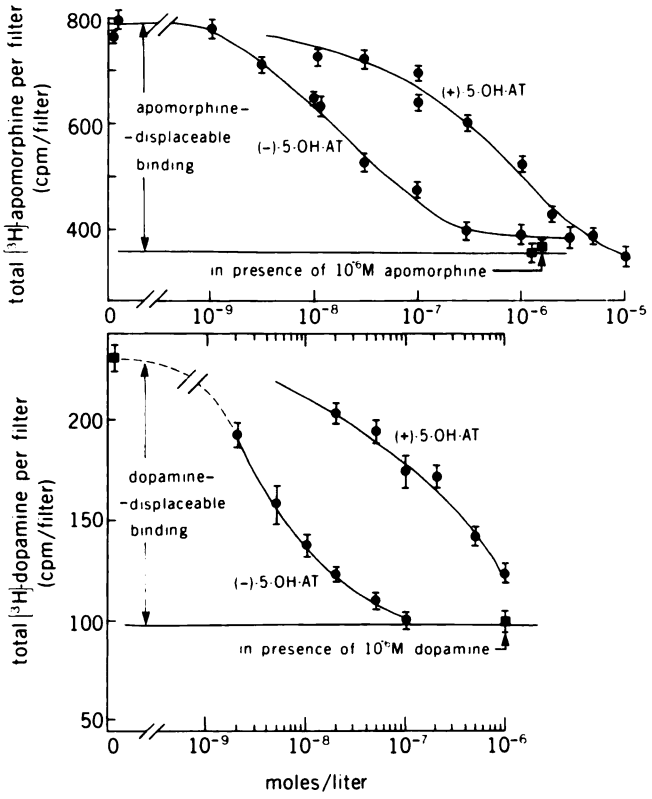

FIG. 2. *The displacement of [^{3}H-apomorphine and [^{3}H]-dopamine by stereoisomers of 5-hydroxy-N,N-di-n-propyl-2-aminotetralin [5-OH-AT]*

Specific binding was defined as the amount of radioligand displaced by an excess (1 μM) of apomorphine or dopamine. Each point is the mean ± standard error of sextuplicate determinations.

binding of [^{3}H]-ligand minus binding in the presence of an excess concentration of non-radioactive control ligand (200 nM or 1 μM apomorphine for [^{3}H]-apomorphine assays; 1 μM dopamine for [^{3}H]-dopamine assays and 50 nM spiperone for [^{3}H]-spiperone assays). Stereospecific binding was defined as binding in the presence of the less active enantiomer (e.g., (+)-5-OH-AT at 70 nM; (−)-butaclamol at 100 nM) minus binding in the presence of the same excess of the more active enantiomer for any given concentration of test-ligand.

RESULTS

Competition by (−)-5-OH-AT and (+)-5-OH-AT for specific [^{3}H]-apomorphine and specific [^{3}H]-dopamine binding is shown in Fig. 2. In competing for [^{3}H]-apomorphine binding, the (−)-enantiomer with an IC_{50} of 18 nM showed 20-fold higher affinity than the (+)-enantiomer. The active (−)-5-OH-AT showed saturable competition above about 200 nM. Almost all [^{3}H]-apomorphine binding which was displaceable by 1 μM apomorphine was also displaceable by (−)-5-OH-AT. Competition for [^{3}H]-dopamine binding followed the same pattern, with (−)-5-OH-AT displaying an IC_{50} of 4 nM, that is, a 50-fold higher affinity than (+)-5-OH-AT. Again, (−)-5-OH-AT at 100 nM displaced almost as much [^{3}H]-dopamine binding as did a 1 μM dopamine excess.

Maximal differences in competition by the 5-OH-AT enantiomers for [^{3}H]-apomorphine sites occurred at concentrations between 50 and 500 nM (Fig. 2). Accordingly a concentration of 70 nM for both the (−) and (+) enantiomers of 5-OH-AT was used in measuring the number of [^{3}H]-apomorphine sites that are stereospecific to this agonist. The binding isotherm for [^{3}H]-apomorphine in the presence of 70 nM (−)- or (+)-5-OH-AT (Fig. 3, top) indicated that these sites were saturated by [^{3}H]-apomorphine above 10 nM. Analysis of the corresponding Scatchard plot of these data (Fig. 3, bottom) gave a K_D for the sites of 5.4 nM with the total number of sites as 110 fmoles/mg protein. These results were similar to previous results (12) from this laboratory for [^{3}H]-apomorphine binding ster-

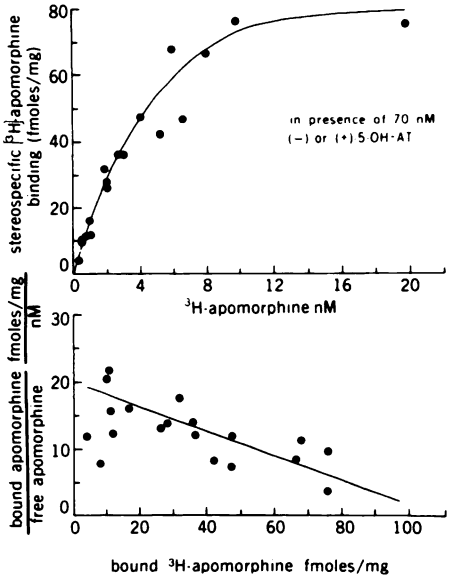

FIG. 3. *Stereospecific adsorption of [^{3}H]-apomorphine in the presence of (+)- or (−)-5-hydroxy-N,N-di-n-propyl-2-aminotetralin [(+)- or (−)-5-OH-AT]*

Stereospecific binding was defined as [^{3}H]-apomorphine bound in the presence of 70 nM (+)-5-OH-AT minus that bound in the presence of 70 nM (−)-5-OH-AT. Scatchard analysis indicated the total number of such sites to be 110 fmoles/mg protein. The dissociation constant for the sites was 5.4 nM. Each point is the result of determinations in quadruplicate of the binding in the presence of each stereoisomer.

eospecific to the dopamine-receptor antagonist (+)-butaclamol (at 10^{-6}M concentration for its enantiomers). A K_D of 3.5 nM and total sites of 124 fmoles/mg protein had been measured in that instance.

In Fig. 4, competition for [^{3}H]-apomorphine sites stereospecific to 10^{-6}M (+)-butaclamol is shown for the enantiomers of 5-OH-AT. The IC_{50} values were 18 nM for (−)-5-OH-AT and 350 nM for (+)-5-OH-AT. Their values were thus virtually identical to the corresponding IC_{50} values obtained when competition for total specific [^{3}H]-apomorphine sites had been measured (cf. Fig. 2).

The effect of shifting the position of the hydroxyl group on the aromatic ring was next studied. The compounds (±)-5-OH-AT, (±)-6-OH-AT and (±)-7-OH-AT were each tested for competition for [^{3}H]-apomorphine and [^{3}H]-dopamine sites, using

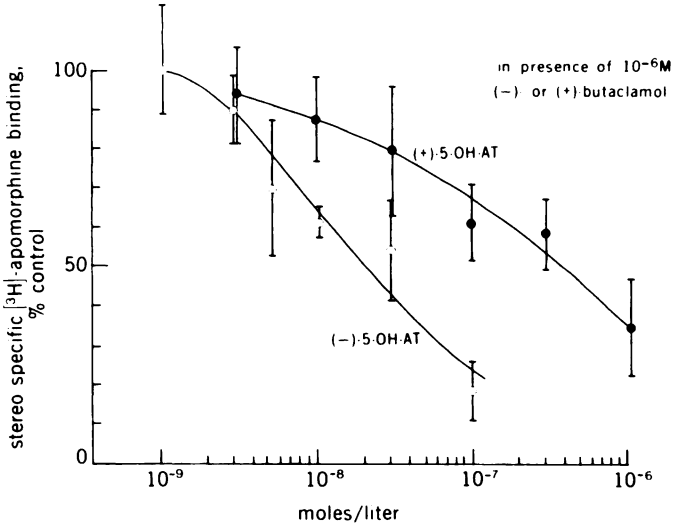

FIG. 4. *The displacement of butaclamol-stereospecific [^{3}H]-apomorphine binding by the (+)- and (−)-enantiomers of 5-hydroxy-N,N-di-n-propyl-2-aminotetralin*

Stereospecific binding of [^{3}H]-apomorphine was here defined as that amount bound in the presence of 1 μM (−)-butaclamol minus that bound in the presence of 1 μM (+)-butaclamol, the active neuroleptic; (−)- or (+)-butaclamol was present in every tube. Points are mean ± SEM (sextuplicate determinations).

the presence of 200 nM apomorphine or 10^{-6}M dopamine as respective controls for non-specific binding. Figure 5 illustrates the relative potencies of the various structural isomers. The IC_{50} values for [^{3}H]-apomorphine specific binding were 24 nM for (±)-5-OH-AT, 520 nM for (±)-7-OH-AT and 900 nM for (±)-6-OH-AT. The corresponding IC_{50} values for specific [^{3}H]-dopamine binding (Table 1) were 12, nM, 88 nM and 290 nM (see also Fig. 6).

In both instances the (±)-5-OH-AT structural isomer, the analogue of dopamine in the α-rotamer conformation, showed higher affinity than did its 6- or 7-hydroxylated congeners. The difference in affinities was more marked for [^{3}H]-apomorphine sites (a 22- to 38-fold difference) than for [^{3}H]-dopamine sites (a 7- to 24-fold difference).

IC_{50} values for inhibition of [^{3}H]-spiperone binding by apomorphine and dopamine (Table 1) were approximately the same as those reported by Leysen *et al.* (18) but were about threefold higher than values reported by Creese *et al.* (19). This difference may simply reflect the fact that at the total concentration of 0.03 nM [^{3}H]-spiperone in our assay system only about half of that concentration is free in the aqueous phase at equilibrium. Most of the remainder is bound to [^{3}H]-spiperone receptors, which exist at a final concentration of about 0.04 nM in our assay system. Because the [^{3}H]-ligand and receptor concentrations are roughly equal and close to the K_D, the effect of increasing concentrations of competing ligands is initially to actually increase the free [^{3}H]-spiperone concentration at equilibrium, thus shifting IC_{50} values upward.

Affinities vary inversely with dissociation constant K_D. The Cheng-Prusoff equation (20) expresses a relation between IC_{50} values and K_D values for a test ligand A competing with another (e.g., radio-labelled) ligand B:

$$A_{K_D} = A_{IC_{50}}/(1 + [B]/B_{K_D}).$$

From scatchard data (to be published) the K_D for [^{3}H]-apomorphine specific binding is 3.2 nM and for [^{3}H]-dopamine 1.6 nM. Thus, to convert any [^{3}H]-apomorphine IC_{50} value in this publication to the corresponding K_D value you must divide the IC_{50} by 2.09. Similarly, with [^{3}H]-dopamine IC_{50} values, division by 1.31 produces the corresponding K_D.

DISCUSSION

These results are the first demonstration of stereoselectivity by a dopamine agonist in binding to the dopamine receptor (excepting enantiomers of norepinephrine and epinephrine in ref. 21).

Both R-(−)-apomorphine and (±)-5-OH-AT can be considered as semi-rigid analogues of dopamine in the α-rotamer (22) or trans-cisoid (23) conformation (Fig. 1). The "trans-cisoid" terminology has sometimes been used inconsistently in the literature (24), and so the α/β-rotamer terminology is to be preferred. The most useful terminology, however, is that in which the mean values of the dihedral or torsion angles (T_1, T_2) for the two degrees of rotational freedom about the ethylamine side-chain are estimated in degrees, as defined by Bergin (25), Pullman (26) and Grol (27). Figure 7 illustrates the conformation of the dopamine moiety in R-(−)-apomorphine as determined by the x-ray crystallographic work of J. Giesecke (28). Giesecke found two almost identical shapes for R-(−)-apomorphine[2] with dihedral angles (T_1, T_2) of (−133°, −178°) and (−146°, −178°). Figure 7 (left) represents the mean conformation (−139.5°, −178°) of the dopamine moiety in these two forms of apomorphine. It is apparent that R-(−)-apomorphine contains the α-rotamer dopamine conformation (Fig. 7, right).

In derivatives of 2-aminotetralins, the 2-amino groups can assume the axial or equatorial position with respect to the saturated ring. However, the presence of bulky N,N-di-n-propyl-substituents on the amine causes severe steric hindrance in the N-axial conformation, as can be readily seen with CPK space-filling models. The equatorial conformation is thus probably preferred. Cannon (22) refers to spectral data that also support this conclusion.

The dopamine moiety contained within (−)-5-OH-AT is then described as an α-rotamer of conformation (−160°, −170°)[3]

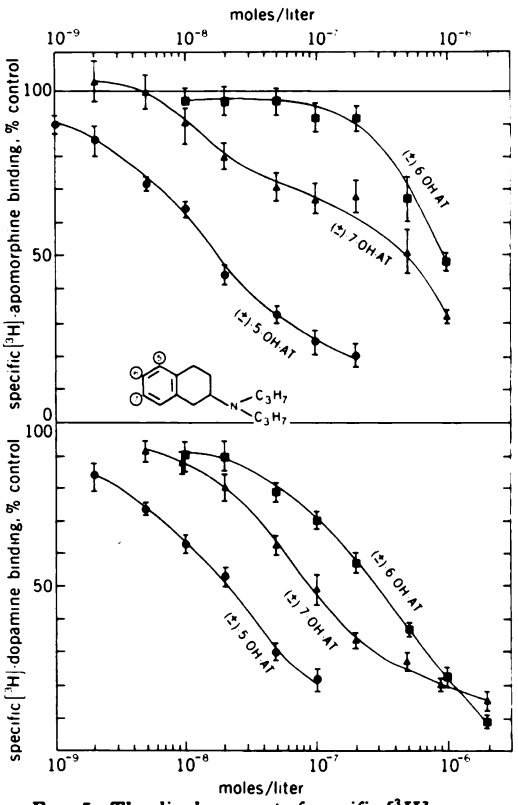

FIG. 5. *The displacement of specific [^{3}H]-apomorphine binding and specific [^{3}H]-dopamine binding by the racemates of 5-OH-AT, 6-OH-AT and 7-OH-AT*

Specific binding was defined as the total binding of the radioligand minus that in the presence of either 200 nM apomorphine or of 1 μM dopamine (for the respective [^{3}H]-ligands). Each point represents a determination in quintuplicate or sextuplicate ± SEM.

or its mirror image (+160°, +170°). We cannot distinguish between these two absolute conformations[4] since no suitable spectroscopic or synthetic data have yet

[2] Actually Giesecke's data inadvertently described S-(+)-apomorphine, but the transformation to R-(−)apomorphine is trivial, involving only a change of sign for T_1 and T_2.

[3] Measured ± 5° using metal Dreiding models (Büchi/Brinkmann Co.).

[4] Confusion persists in the proper use of the terms conformation and configuration. Conformation refers to the shape of an entity as described by signed torsion angles, while configuration describes the order of groups about an asymmetric center. We have used the term absolute conformation to describe a structure with a set of torsion angles of a particular sign, to differentiate between it and one of the opposite conformation, that is, one having equal torsion angles but of opposite sign. Such compounds, if nonsuperimposable and rigid, must be enantiomers, while, if flexible, they may be of the same configuration (or may indeed lack an asymmetric center, as does dopamine).

TABLE 1

Concentrations of ligands producing 50% inhibition of specific binding (i.e., IC_{50} values) of [^{3}H]-dopamine (0.5 nM), [^{3}H]-apomorphine (3.5 nM), or [^{3}H]-spiperone (0.03 nM). Specific binding was defined as in Fig. 5. Assays involved four or six concentrations of a test-ligand, each done in quintuplicate or sextuplicate. Each assay was replicated at least once and standard errors (or range if N = 2) of log IC_{50} values were always less than 0.15.

	IC_{50} (nM)		
	[^{3}H]-Apomorphine	[^{3}H]-Dopamine	[^{3}H]-Spiperone
Dopamine	2.0 ± 0.7	1.5 ± 0.2	17,500 ± 2500
N,N-di-n-propyldopamine	75 ± 16	15	–
R-(−)-apomorphine	3.8 ± 0.5	3.7 ± 0.3	750 ± 130
R-(−)-N-n-propylnorapomorphine	4.0 ± 0.8	3.4 ± 0.2	150
m-tyramine	112 ± 18	37 ± 8	–
p-tyramine	520 ± 100	–	–
(−)-5-OH-N,N-(C_3H_7)$_2$-2-aminotetralin[(−)-5-OH-AT]	18 ± 2[a]	4 ± 0.8	190 ± 60
(+)-5-OH-N,N-(C_3H_7)$_2$-2-aminotetralin[(+)-5-OH-AT]	360 ± 40[a]	200 ± 30	2050 ± 450
(±)-5-OH-N,N-(C_3H_7)$_2$-2-aminotetralin[(±)-5-OH-AT]	24 ± 6	12 ± 4	290 ± 60
(±)-6-OH-N,N-(C_3H_7)$_2$-2-aminotetralin[(±)-6-OH-AT]	900 ± 100	290 ± 38	6000 ± 1000
(±)-7-OH-N,N-(C_3H_7)$_2$-2-aminotetralin[(±-7-OH-AT]	520 ± 70	88 ± 9	1450 ± 150

[a] Concentration of apomorphine for specific binding controls was 10^{-6}M for [^{3}H]-apomorphine assays of these ligands.

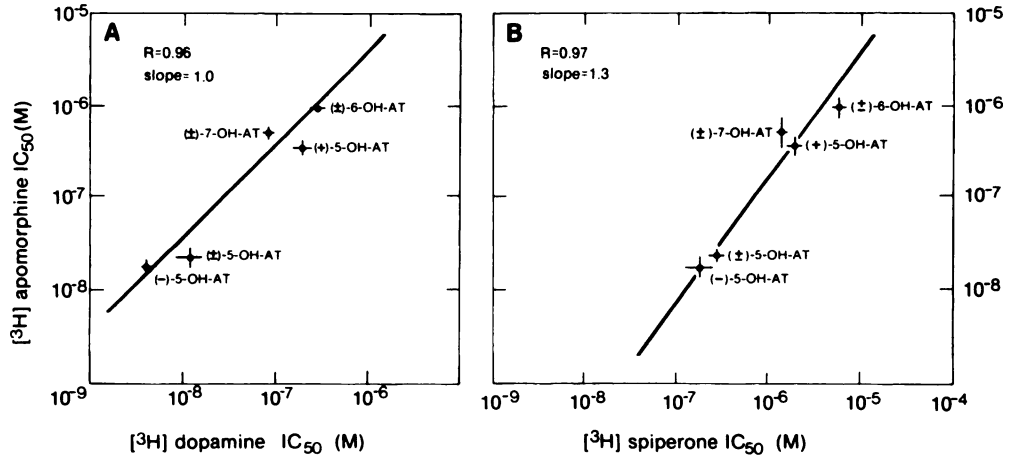

FIG. 6. *Correlation between IC_{50} values for monohydroxylated-2-aminotetralins competing for [^{3}H]-apomorphine and [^{3}H]-dopamine sites (in A), and [^{3}H]-apomorphine and [^{3}H]-spiperone sites (in B). Data are taken from Table 1*

In our assay the dissociation constants (K_D) for [^{3}H]-apomorphine, [^{3}H]-dopamine and [^{3}H]-spiperone specific binding are respectively 3.2 nM, 1.6 nM and 0.10 nM. Thus, the inhibitory dissociation constants (K_D), as determined by the Cheng-Prusoff equation, are essentially identical to the IC_{50} values above for [^{3}H]-dopamine, but not for [^{3}H]-apomorphine assays where the K_D values are 0.48 times the IC_{50} values, nor for [^{3}H]-spiperone assays, where K_D values may be ½ or less of IC_{50} values (see RESULTS).

been published for these stereoisomers. However, we can see that the first conformation, (−160°, −170°) (i.e., the 2S enantiomer) is rather similar to the dopamine conformation (−139.5°, −178°) in R-(−)-apomorphine (Fig. 7). Since the receptor for [^{3}H]-apomorphine shows high stereospecific affinity for both R-(−)-apomorphine and for (−)-5-OH-AT we may predict that (−)-5-OH-AT will prove to be the 2S enantiomer with conformation (−160°, −170°).

The basic line of argument used above is that high affinity of a ligand for a high-affinity receptor of a rigid dopamine analogue implies a similar dopamine-moiety

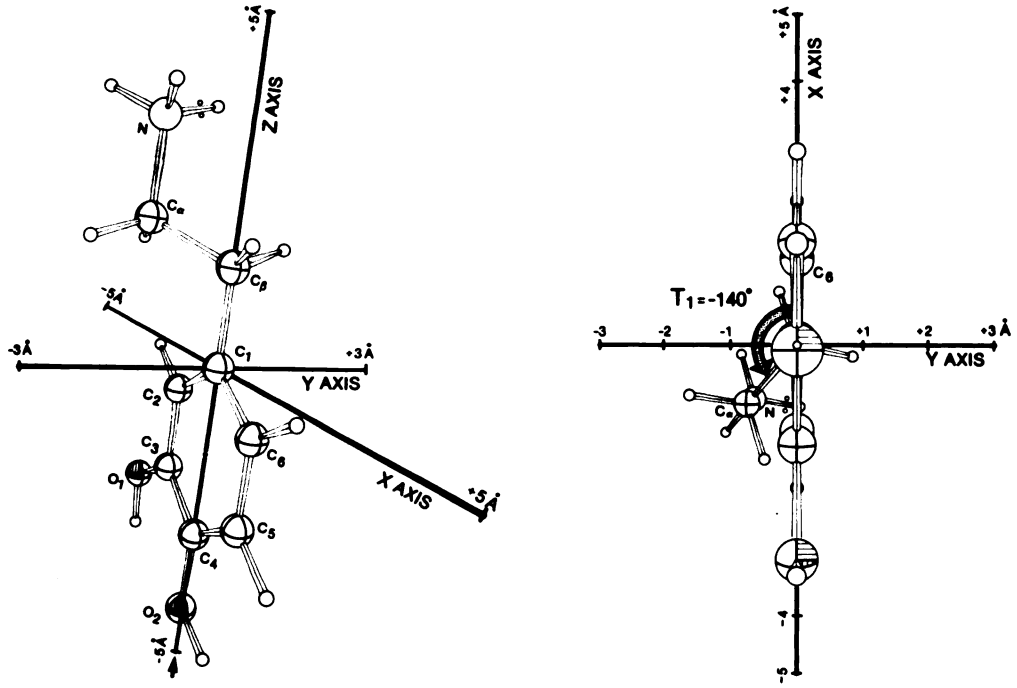

FIG. 7. *The dopamine molecule in the conformation assumed by the dopamine moiety in R-(−)-apomorphine*

This seems the most probable conformation of dopamine at the apomorphine receptor. The dihedral or torsion angle T_1 = T (C_6-C_1-C_β-C_α) is defined as the angle between the planes C_6-C_1-C_β and C_1-C_β-C_α when viewed along their common intercept line C_1-C_β from the negative Z direction (indicated by the small arrow in the left figure). The sign of this torsion angle is negative by convention, since counterclockwise rotation of the second plane (C_1-C_β-C_α) occurred about the line C_1-C_β from an initial position coincident (T_1 = 0) with the first plane. This is illustrated by the figure on the right, which is a view of the same dopamine conformation from the negative Z direction. The dihedral angle T_1 = −140° is indicated by the curved arrow traveling counterclockwise. In the left figure, the two small circles straddling one of the nitrogen bonds signify a lone-pair of electrons. Its probable orientation at the receptor can be inferred from Giesecke's crystallographic data (28) to be as shown above. Since in R-(−)-apomorphine the nitrogen is a tertiary amine with the methyl group in the equatorial position then, assuming sp^3 hybridization about the nitrogen, the dihedral angle T_3 = T(C_β-C_α-N-lone-pair) is T_3 = +55°. If this lone-pair is involved in hydrogen-bonding to the receptor it probably acts as a proton acceptor, as is generally the case with tertiary amines.

conformation in the binding ligand. It is then not surprising that the α-rotamer analogue (±)-5-OH-AT has a much higher affinity for the [^{3}H]-apomorphine receptor than does the β-rotamer analogue (±)-7-OH-AT (or than the symmetric rotamer analogue (±)-6-OH-AT). The fact that the [^{3}H]-dopamine receptor also displays preferential affinity for the α-rotamer (±)-5-OH-AT is good evidence that both [^{3}H]-dopamine and [^{3}H]-apomorphine are in fact binding to the same receptor.

The good correlation between binding of these ligands to [^{3}H]-apomorphine and [^{3}H]-dopamine receptors as shown in Fig. 6A also supports the identity of these two binding sites. It is further probable that, since both radioligands bind to a receptor that shows preferential affinity for (−)-5-OH-AT over (+)-5-OH-AT, the absolute conformation of dopamine preferred at the receptor-site is close to (−160°, −170°).

The Cheng-Prusoff equation implies that if [^{3}H]-apomorphine and [^{3}H]-dopamine sites are identical, then IC_{50} values of [^{3}H]-apomorphine should be greater by a constant factor of 1.6 from corresponding IC_{50} values of [^{3}H]-dopamine. That this in

fact does not hold for some ligands (Table 1), though the general correlation does hold, is consistent with the existence of a minor component of [^{3}H]-apomorphine binding that is saturated under the conditions of this assay. Further evidence for two high-affinity receptor populations comes from a biphasic scatchard analysis of [^{3}H]-apomorphine binding[5] indicating a small number (50 fmoles/mg protein) of very high affinity (0.48 nM) [^{3}H]-apomorphine receptors in calf striatum. The strong suggestion in Fig. 5 of a biphasic pattern to the competition curve for (±)-7-OH-AT is possibly a data-artefact ascribable to different personnel having contributed to different concentration ranges for that particular compound. If in fact the biphasic element can be replicated it would provide further support for the existence of two high-affinity [^{3}H]-apomorphine sites.

The presence of a dipropyl function on the amine nitrogen of dopamine seems to lower its affinity for the [^{3}H]-apomorphine and [^{3}H]-dopamine receptor at least 10-fold (Table 1). However, since R-(−)-N-n-propylnorapomorphine is about equipotent with dopamine and apomorphine, the presence of bulky propyl functions must not per se interfere with binding. Rather their presence on the flexible side-chain of dopamine probably reorients the nitrogen or its lone electron pair to a position unfavorable for binding.

An alternative explanation is provided by Ginos *et al.* (29, 30). They have observed that N,N-n-butyl-n-propyl-dopamine has considerable potency in various dopaminergic bio-systems. In contrast, the N,N-di-n-butyl derivatives of both dopamine (31)[5] and 5,6-dihydroxy-2-aminotetralin (1) are essentially without activity. Since corresponding N,N-di-n-propyl homologues are quite potent in these systems, Ginos *et al.* suggest that the N-n-propyl group specifically interacts with the receptor to facilitate binding. This is not incompatible with our above suggestion, since such an interaction might be the mechanism facilitating proper nitrogen lone-pair orientation. This laboratory has recently reported evidence for the necessary involvement of the lone-pair in binding to the dopamine receptor (32).

As Figs. 2 and 4 illustrate, the 20-fold preferential affinity of the [^{3}H]-apomorphine receptor for (−)-5-OH-AT over (+)-5-OH-AT is also found when the [^{3}H]-apomorphine binding is to receptors that are stereospecific to the neuroleptic butaclamol (10^{-6} M). This is consistent with the idea that the anti-psychotic receptor is physically identical to the [^{3}H]-apomorphine/[^{3}H]-dopamine receptor. One laboratory (33) has reported that *in vivo* binding assays of mouse whole brain show that apomorphine may compete effectively at very low doses (ID_{50} = 0.3 mg/kg, i.v.) for [^{3}H]-spiperone labeled receptors. This also would be consistent with identical agonist and antagonist receptors, with perhaps two conformations existing that are readily reversible *in vivo* but not *in vitro*.

However, there exists other evidence that in fact the [^{3}H]-dopamine receptor and the [^{3}H]-neuroleptic receptor are two distinct and separate species. High affinity binding of the dopamine agonists [^{3}H]-dihydroergocryptine and bromocryptine to neuroleptic receptors (after blockade of alpha-noradrenergic receptors with phentolamine) (34) does not seem compatible with the two-conformation hypothesis. Recent animal lesion (35) and human postmortem (36) studies show that the number of [^{3}H]-apomorphine sites in brain can decrease by 40 to 70% while [^{3}H]-haloperidol sites increase or remain constant.

In the present study a correlation was found between affinities for [^{3}H]-apomorphine and [^{3}H]-spiperone receptors for the enantiomers and positional isomers of monohydroxy-2-aminotetralins. This is an interesting phenomenon, but we do not consider it strong evidence for the identity of the neuroleptic and dopamine-agonist receptors for two reasons. First, the slope in Fig. 6B is significantly greater than 1.0. A plot of the dissociation constants K_D instead of the IC_{50} values would simply further increase this slope slightly. Secondly, the affinities of these ligands are 14- to 20-fold less for [^{3}H]-spiperone sites than for [^{3}H]-apomorphine sites. Both of these facts are difficult to reconcile with the idea of

[5] Tedesco, J. L., unpublished data.

identical receptors for both neuroleptics and dopamine agonists.

Rigid monohydroxyl analogues of dopamine are useful in assessing the relative contributions of the hydroxyl positions to receptor-binding. A limitation of this approach is that it cannot control for any interactions between the adjacent ring-hydroxyls that may occur in catechol compounds. Dihydroxy-2-aminotetralins have therefore been of more interest generally since they are closer analogues of dopamine. However, binding affinities of these compounds[5] (37) do not correlate well with their published biological activities in producing stereotyped behavior (4, 38) or in producing peripheral vasodilation (39). Lack of correlation can, of course, be attributed to a number of possible causes. The authors believe an important reason to be that, in biological systems, primary and secondary amines are more susceptible both to compartmentation by lipid barriers and uptake mechanisms as well as to possible metabolization by monoamine oxidases. These factors should not play any major role in assays of binding affinity *in vitro*. Another possibility, though, is that some of these ligands may have some antagonist properties which may contribute to binding but not to bio-activity.

The β-rotamer analogue 6,7-dihydroxy-2-aminotetralin (ADTN) is potent in most of such dopamine-receptor model systems, including the binding to [^{3}H]-apomorphine receptors, despite the fact that apomorphine is itself an α-rotamer analogue. In contrast, the β-rotamer analogue isoapomorphine (9,10-dihydroxy-aporphine) is almost inactive in binding to either [^{3}H]-apomorphine, [^{3}H]-dopamine or [^{3}H]-spiperone.[5] Thus, it is necessary to examine this question of optimal conformation from new perspectives. One aspect to study more closely concerns the relative contribution that electrostatic and electron donor-acceptor complexes (such as charge-transfer and hydrogen-bond complexes) can make in binding to a given receptor active-site when the amine varies from a primary to a secondary to a tertiary state. Another important factor to examine is the absolute configuration of these dihydroxyl compounds that actually binds to the receptor. All such dihydroxyl compounds tested so far have been racemic mixtures.

ACKNOWLEDGMENTS

We thank Mrs. Joan Dumas and Mrs. Anna Banaschuk for excellent assistance. We also thank Drs. J. L. Neumeyer, J. P. Long and B. Costall for the kind gifts, respectively, of R-(−)-N-n-propylnorapomorphine, N,N-di-n-propyl-dopamine and m-tyramine.

REFERENCES

1. 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.
2. Woodruff, G. N., A. O. Elkhawad, A. R. Crossman and R. J. Walker. Further evidence for the stimulation of rat brain dopamine receptors by a cyclic analogue of dopamine. *J. Pharm. Pharmac.* **26:** 740–741, 1974.
3. Elkhawad, A. O. and G. N. Woodruff. Studies on the behavioural pharmacology of a cyclic analogue of dopamine following its injection into the brains of conscious rats. *Br. J. Pharmac.* **54:** 107–114, 1975.
4. Costall, B., R. J. Naylor, J. G. Cannon and T. Lee. Differential activation by some 2-aminotetralin derivatives of the receptor mechanisms in the nucleus accumbens of rat which mediate hyperactivity and stereotyped biting. *Eur. J. Pharmacol.* **41:** 307–319, 1977.
5. Miller, R., A. Horn and L. Iversen. Effects of dopamine-like drugs on rat striatal adenyl cyclase have implications for CNS dopamine receptor topography. *Nature* **250:** 238–241, 1974.
6. 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.
7. Neumeyer, J. L., F. E. Granchelli, K. Fuxe, U. Ungerstedt and H. Corrodi. Aporphines. 11. Synthesis and dopaminergic activity of monohydroxyaporphines. Total synthesis of (±)-11-hydroxyaporphine, (±)-11-hydroxynoraporphine, and (±)-11-hydroxy-N-n-propylnoraporphine. *J. Med. Chem.* **17:** 1090–1095, 1974.
8. Andén, N.-E., S. G. Butcher and J. Engel. Central dopamine and noradrenaline receptor activity of the amines formed from m-tyrosine, α-methyl-m-tyrosine and α-methyldopa. *J. Pharm. Pharmac.* **22:** 548–550, 1970.
9. Ungerstedt, U., K. Fuxe, M. Goldstein, A. Battista, M. Ogawa and B. Anagnoste. Action of m-tyrosine in experimental models: Evidence for possible antiparkinsonian activity. *Eur. J. Pharmacol.* **21:** 230–237, 1973.

10. Saari, W. S. and S. W. King. Synthesis and biological activity of (6aS)-10,11-dihydroxyaporphine, the optical antipode of apomorphine. *J. Med. Chem.* **16:** 171–172, 1973.
11. 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 (+)-1,2,9,10-Tetrahydroxyaporphine. *J. Med. Chem.* **16:** 1228–1233, 1973.
12. Pinder, R. M., D. A. Buxton and G. N. Woodruff. On apomorphine and dopamine receptors. *J. Pharm. Pharmac.* **24:** 903–904, 1972.
13. Saari, W. S., S. W. King, V. J. Lotti and A. Scriabine. Synthesis and biological activity of some aporphine derivatives related to apomorphine. *J. Med. Chem.* **17:** 1086–1090, 1974.
14. Seeman, P., T. Lee, M. Chau-Wong, J. Tedesco and K. Wong. Dopamine receptors in human and calf brains, using [^{3}H]apomorphine and an antipsychotic drug. *Proc. Natl. Acad. Sci. USA* **73:** 4354–4358, 1976.
15. Leysen, J. E., W. Gommeren and P. M. Laduron. Spiperone: A ligand of choice for neuroleptic receptors. 1. Kinetics and characteristics of in vitro binding. *Biochem. Pharmacol.* **27:** 307–316, 1978.
16. Hartley, E. J. and P. Seeman. The effect of varying ^{3}H-spiperone concentration on its binding parameters. *Life Sci.* **23:** 513–518, 1978.
17. Lowry, O. H., N. J. Rosebrough, A. L. Farr and R. J. Randall. Protein measurement with the folin phenol reagent. *J. Biol. Chem.* **193:** 265–275, 1951.
18. Leysen, J. E., C. J. E. Niemegeers, J. P. Tollenaere and P. M. Laduron. Serotonergic component of neuroleptic receptors. *Nature* **272:** 168–171, 1978.
19. Creese, I., R. Schneider and S. H. Synder. [^{3}H]-spiroperidol labels dopamine receptors in pituitary and brain. *Eur. J. Pharmacol.* **46:** 377–381, 1977.
20. Cheng, Y.-C. and W. H. Prusoff. Relationship between the inhibition constant (K_i) and the concentration of inhibitor which causes 50 per cent inhibition (I_{50}) of an enzymatic reaction. *Biochem. Pharmacol.* **22:** 3099–3108, 1973.
21. 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.
22. Cannon, J. G. Chemistry of dopaminergic agonists, in *Advances in Neurology*, Vol. 9, (Eds, D. B. Calne, T. N. Chase and A. Barbeau), Raven Press, New York, 1975, 177–183.
23. Dandiya, P. C., H. L. Sharma, S. K. Patni and R. S. Gambhir. An evaluation of apomorphine action on dopamine receptors. *Experientia* **31:** 1441–1442, 1975.
24. Smissman, E. E., S. El-Antably, L. W. Hedrich, E. J. Walaszek and L.-F. Tseng. Synthesis of cisoid and transoid analogs of phenethylamine. *J. Med. Chem.* **16:** 109–113, 1973.
25. Bergin, R. and D. Carlström. The structure of the catecholamines. II. The crystal structure of dopamine hydrochloride. *Acta Cryst.* **B24:** 1506–1510, 1968.
26. Pullman, A. and B. Pullman. New paths in the molecular orbital approach to solvation of biological molecules. *Q. Rev. Biophys.* **7:** 505–566, 1975.
27. Grol, C. J. and H. Rollema. Conformational analysis of dopamine by the INDO molecular orbital method. *J. Pharm. Pharmac.* **29:** 153–156, 1977.
28. Giesecke, J. The crystal and molecular structure of apomorphine hydrochloride hydrate. *Acta Cryst.* **B29:** 1785–1791, 1973.
29. 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.
30. Ginos, J. Z. and F. C. Brown. A potential antiparkinson drug, N-n-propyl-N-n-butyl-β-(3,4-dihydroxyphenyl)ethylamine hydrochloride, and its effects in intact and nigra-lesioned rats. *J. Med. Chem.* **21:** 155–160, 1978.
31. Cannon, J. G., F.-L. Hsu, J. P. Long, J. R. Flynn, B. Costall and R. J. Naylor. Preparation and biological actions of some symmetrically N,N-disubstituted dopamines. *J. Med. Chem.* **21:** 248–253, 1978.
32. Tedesco, J. L. and P. Seeman. Pre-synaptic dopamine receptor binding: Involvement of the nitrogen lone electron pair. *Soc. Neurosci.* **4,** Abst. **1679:** 522, 1978.
33. Höllt, V., A. Czlonkowski and A. Herz. The demonstration in vivo of specific binding sites for neuroleptic drugs in mouse brain. *Br. Res.* **130:** 176–183, 1977.
34. Tittler, M., P. Weinreich and P. Seeman. New detection of brain dopamine receptors with [^{3}H]dihydroergocryptine. *Proc. Natl. Acad. Sci. USA* **74:** 3750–3753, 1977.
35. Nagy, J., T. Lee, P. Seeman and H. C. Fibiger. Direct evidence for presynaptic and postsynaptic dopamine receptors in brain. *Nature* **274:** 278–281, 1978.
36. Lee, T., P. Seeman, A. Rajput, I. J. Farley and O. Hornykiewicz. Receptor basis for dopaminergic supersensitivity in Parkinson's disease. *Nature* **273:** 59–61, 1978.
37. Tedesco, J. L., J. Bowles, J. D. McDermed and P. Seeman. Conformational requirements for the

^{3}H-apomorphine receptor: Stereospecific binding of 2-aminotetralin derivatives. *Soc. Neurosci.* 3, Abst. **1476**: 461, 1977.

38. Cannon, J. G., T. Lee, H. D. Goldman, B. Costal and R. J. Naylor. Cerebral dopamine agonist properties of some 2-aminotetralin derivatives after peripheral and intracerebral administration. *J. Med. Chem.* **21**: 1111–1116, 1977.
39. Volkman, P. H., J. D. Kohli, L. I. Goldberg, J. G. Cannon and T. Lee. Conformational requirements for dopamine-induced vasodilation. *Proc. Natl. Acad. Sci. USA* **74**: 3602–3606, 1977.