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Characterization of Dopamine Receptor Subtypes by Comparative Structure-Activity Relationships: Dopaminomimetic Activities and Solid State Conformation of Monohydroxy-1,2,3,4,4a,5,10,10a-octahydrobenz[*g*]quinolines and Its Implications for a Rotamer-Based Dopamine Receptor Model

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SUMMARY

A series of phenolic (*cis*)- and (*trans*)-1,2,3,4,4a,5,10,10a-octahydrobenz[*g*]quinolines were investigated in the D_1 and D_2 dopamine (DA) models, DA-sensitive adenylate cyclase and electrically evoked acetylcholine release, respectively, and were compared with the effects of the corresponding aminotetralins. A similar structure-activity pattern was found at both DA receptor subtypes. The change from the bicyclic to the tricyclic DA analogs resulted in a loss of activity of all β-rotameric 8-hydroxy derivatives, suggesting the presence of a steric barrier. Derivatives of the α-rotameric 6-hydroxy *trans* series, in contrast to their inactive *cis* analogs, showed stimulatory effects that increased from *N*-methyl to *N*-*n*-propyl substitution, indicating an interaction with an *N*-alkyl binding site. The inactivity of the corresponding *N*-*n*-butyl derivative ("*N*-butyl phenomenon") suggests that the *N*-alkyl substituents of this series point toward a "small *N*-alkyl binding site," which can be differentiated from a "large *N*-alkyl binding site." An X-ray of the active (−)-enantiomer of (*trans*)-6-hydroxy-*N*-*n*-propyloctahydrobenz[*g*]quinoline-(*R*)-mandelate revealed a 4a*R*,10a*R* absolute configuration, corresponding to that of (−)-5-hydroxy-2-(*N*,*N*-di-*n*-propylamino)tetralin. The hydrogen bonding interactions of the axial N^+-H proton and the hydroxy group to mandelate anions in the crystal provide a model for a possible drug-receptor interaction.

Molecular modeling served to localize the steric barrier and the boundaries of the small *N*-alkyl binding site, which together form an "extended steric barrier." The results led to the proposal of a refined version of a rotamer-based general DA receptor model, which is supplemented by criteria for the orientation of DA agonists. Its application is demonstrated with apomorphine and ergoline.

Pharmacological and biochemical evidence suggests the existence of two distinct DA receptor subtypes, termed D_1 and D_2 in the central nervous system (1, 2), which appear to be similar to the DA_1 and DA_2 receptors of the cardiovascular system (3). The D_1 site has been linked to the activation of adenylate cyclase, whereas the D_2 receptor is either not associated or negatively coupled with this enzyme (4, 5). Although selective agonists and antagonists are known for both receptor subtypes (6), there are relatively few systematic investigations aimed at a comparison of the structural requirements for agonists at the two sites (7, 8).

In order to gain some insight into the topography of the two categories of DA receptors, which could provide a basis for the design of new selective drugs, we recently studied the effects of a series of phenolic aminotetralins, the simplest semirigid DA analogs. The functional *in vitro* models used represent the two central DA receptor subtypes (9, 10). Activities and discrimination at the two receptor sites showed a strong dependence on the so-called rotamer form, as defined by Cannon (11), i.e., on the conformation of DA in these ring systems (cf. Fig. 1). The results could be interpreted in the sense that the two subtypes possess similar major binding sites for the *meta*-hydroxy and the amino group of DA but differ in secondary sites for the *p*-hydroxy group and the *N*-alkyl substituents. The amino group of the aminotetralins, protonated under physiological conditions, is freely rotating. The orientation of the amino proton(s) and the *N*-alkyl substituents are, thus, not determined, preventing an exact localization of the binding sites for the amino group and the *N*-alkyl substituents. This should be possible, however, with aminotetralin-derived structures in which the conformation of the amino group is restricted by an additional annellated ring.

ABBREVIATIONS: DA, dopamine; ACh, acetylcholine; 5-OH-AT, 5-hydroxy-2-aminotetralin; 7-OH-AT, 7-hydroxy-2-aminotetralin; 5-OH-DPAT, 5-hydroxy-2-(*N*,*N*-di-*n*-propylamino)tetralin; 7-OH-DPAT, 7-hydroxy-2-(*N*,*N*-di-*n*-propylamino)tetralin; EtOH, ethanol; MeOH, methanol; Et_2O, diethyl ether; EGTA, ethylene glycol bis(β-aminoethylether)-*N*,*N*,*N*′,*N*′-tetraacetic acid.

We report the effects of such a series of compounds, phenolic (*cis*)- and (*trans*)-1,2,3,4,4a,5,10,10a-octahydrobenz[*g*]quinolines, on DA-sensitive adenylate cyclase and on electrically stimulated ACh release, the functional *in vitro* models for the D_1 and D_2 DA receptor subtypes, respectively, already used in the aminotetralin studies. There, highest activity had been found with derivatives bearing a hydroxy group in position 5 or 7, corresponding to the *meta*-hydroxy group of DA in its α- and β-rotameric conformation, respectively. Therefore, octahydrobenz[*g*]quinolines with hydroxy groups in the corresponding positions 6 or 8 were investigated (Fig. 1). X-ray crystallography revealed the solid state conformation and absolute configuration of the most effective enantiomer. The results will be compared with those of the aminotetralins and discussed with respect to a refined version of a rotamer-based DA receptor model proposed earlier (9, 10).

Materials and Methods

Drugs. Racemic octahydrobenz[*g*]quinolines were synthesized as previously described (12). *Cis*-fused compounds were isolated as by-products of the ring closure reaction, which mainly yielded the *trans*-fused products. Compounds not yet reported were fully characterized and showed the following melting points (crystallizing solvents): (*trans*)-1-*n*-butyl-1,2,3,4,4a,5,10,10a-octahydro-6-hydroxybenz[*g*]quinoline hydrochloride, 309–310° (EtOH); (*cis*)-1,2,3,4,4a,5,10,10a-octahydro-6-hydroxybenz[*g*]quinoline hydrochloride, 291–293° (MeOH/Et_2O);(*cis*)-1,2,3,4,4a,5,10,10a-octahydro-6-hydroxy-1-*n*-propylbenz[*g*]quinoline hydrochloride, 250–253° (MeOH/Et_2O); (*cis*)-1,2,3,4,4a,5,10,10a-Octahydro-8-hydroxybenz[*g*]quinoline hydrochloride, 255–256° (EtOH/CH_2Cl_2); (*cis*)-1-ethyl-1,2,3,4,4a,5,10,10a-octahydro-8-hydroxybenz[*g*]quinoline hydrochloride 232–236° (MeOH/Et_2O); and (*cis*)-1,2,3,4,4a,5,10,10a-octahydro-8-hydroxy-1-*n*-propyl-benz[*g*]quinoline hydrochloride, 270–272° (MeOH/Et_2O).

(−)-(*trans*)-1,2,3,4,4a,5,10,10a-Octahydro-6-hydroxy-1-*n* -propylbenz[*g*]quinoline was prepared by resolution of (*trans*)-1,2,3,4,4a,5,10,10a-octahydro-6-methoxybenz[*g*]quinoline with (+)-benzoyl-D-tartaric acid in EtOH, followed by *N*-alkylation with *n*-propylbromide and methyl ether cleavage ($[\alpha]_D^{20} = -142.0°$; $c = 1.0$ in EtOH). The corresponding (+)-enantiomer was obtained analogously, using (−)-benzoyl-D-tartaric acid as resolving agent ($[\alpha]_D^{20} = +143.0°$; $c = 1.0$ in EtOH).

The aminotetralins were prepared as already described (9); CV 205–502 (Fig. 2) was synthesized in our laboratories by R. Nordmann. LY 141865 dihydrochloride was a gift from Eli Lilly and Company (Indianapolis IN).

Crystal structure determination of (−)-(*trans*)-1,2,3,4,4a,5,10,10a-octahydro-6-hydroxy-1-*n*-propylbenz[*g*]quinoline-(*R*)-(−)-mandelate. The compound crystallized from MeOH as thin, colorless, orthorhombic needles. Crystal data: $C_{16}H_{23}NO \cdot C_8H_8O_3$; formula weight, 397.7; space group, $P2_12_12_1$; $a = 7.689(6)$; $b = 7.762(3)$; $c = 35.989(7)$ Å; $V = 2147.9$ Å^3; $d_{calc} = 1.229$ g cm^{-3}; $z = 4$; $\mu = 6.29$ cm^{-1}. Intensities were measured on an Enraf-Nonius CAD-4 diffractometer using monochromated CuK_α radiation to $\theta < 60°$. There was no measurable crystal decay and no absorption corrections were applied. Of the 1892 measured reflections, 1334 had $I > 2.5\ \sigma(I)$ and were considered observed.

The structure was solved by direct methods using SHELX-86 (13). All hydrogen atoms attached to carbon atoms were included in calculated positions; all other hydrogen atoms were located from a difference Fourier map and were included in fixed positions. The final *R* factor was 0.041. Fractional atomic coordinates and anisotropic temperature factors of the non-hydrogen atoms have been deposited and are available on request from the Director of the Cambridge Crystallographic Data Center, University Chemical Laboratory (Lensfield Road, Cambridge CB2 IEW, England).

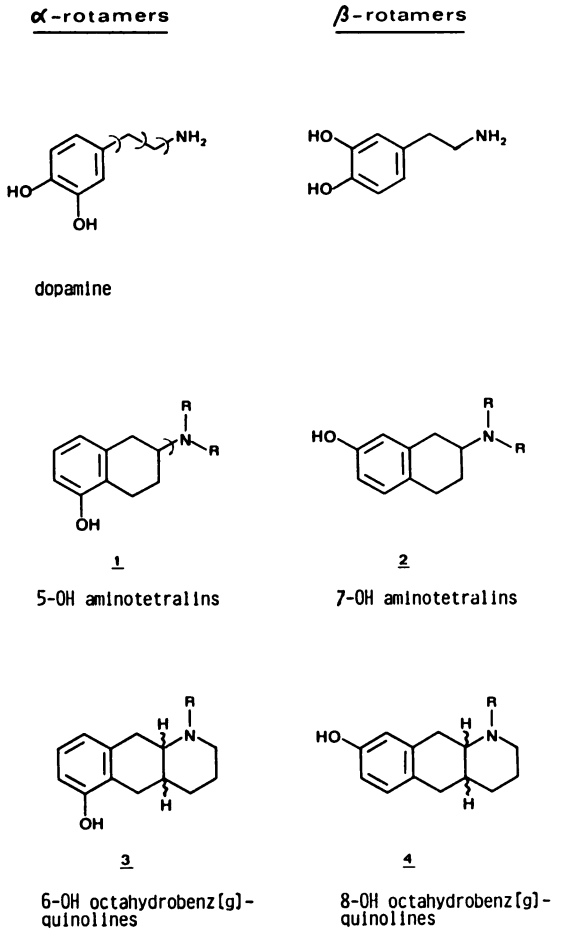


Fig. 1. Rotamers of DA and of conformationally constrained bi- and tricyclic phenolic analogs.

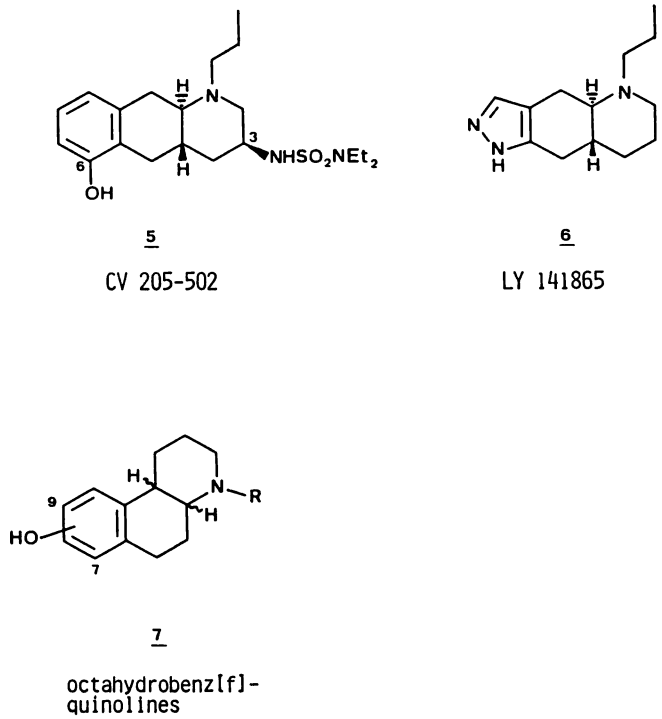


Fig. 2. Compounds structurally related to the octahydrobenz[*g*]quinolines **3** and **4**.

Adenylate cyclase. The retinas were dissected from bovine eyes obtained from the slaughterhouse within 2 hr of death of the cattle. They were homogenized for 1 min at 200 rpm in 25 volumes (w/v) of cold 2 mM Tris acetate/2 mM EGTA buffer (pH 7.4) using an all-glass homogenizer with a glass pestle. After filtration through a Nylon net (pore size, 35 μm), 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 was found to be stable for several months when stored in this way.

Adenylate cyclase activity was routinely assayed by a standard procedure, which is based on the method described by Kebabian *et al.* (14). The compounds were preincubated at 4° together with the tissue homogenate in 400 μl of a mixture containing 47.1 μM Tris acetate, (pH 7.4), 2 μM $MgCl_2$, 0.45 μmM EGTA, 0.1 μM isobutylmethylxanthine, and 0.15% bovine serum albumin. 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 GTP 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 done in triplicate. For the assay, a multipipetting machine was used, which allowed the simultaneous determination of adenylate cyclase activity in 24 tubes.

Superfusion experiments. Male rats (Sandoz OFA strain, weighing approximately 150 g) were used. The animals were pretreated twice with reserpine (2.5 mg/kg subcutaneously) 18 and 12 hr before sacrifice and were killed by decapitation, and the brains were rapidly removed and dissected over a chilled plate.

Tissue cylinders of rat striatum with a diameter of 3 mm were punched out from frontal sections approximately between the frontal planes A 9200–7200 (15) and cut into 0.3-mm thick slices (wet weight, approximately 2 mg/slice), using a McIllwain tissue chopper. About 25 slices were incubated in 6 ml of Krebs medium containing 0.16 μM [^{3}H] choline at room temperature (≈22°) for 30 min. Composition of Krebs medium was (millimolar): NaCl, 118; KCl, 5; $CaCl_2$, 1.2; $MgCl_2$, 2; $NaHCO_3$, 25; KH_2PO_4, 2; Na_2EDTA, 0.02; and glucose, 11.1. It was saturated with 95% O_2/5% CO_2; the pH was 7.4. After incubation, the slices, (two per chamber) were transferred into superfusion chambers (two per chamber) and superfused with Krebs medium at a rate of 1.2 ml/min at 30°.

Collections of 5-min fractions (6 ml) of the superfusate began after 60 min of superfusion. Two-minute periods of electric stimulation (frequency, 2 Hz; rectangular pulses of 2 msec with 12 mA current strength) were applied after 75 (S1) and 150 (S2) min of superfusion. Test substances were added 30 min before S2 and were present in the medium between 120 and 170 min of superfusion. At the end of the experiment, the slices were solubilized in concentrated formic acid and total tritium was measured in superfusates and solubilized slices. Tritium outflow was expressed as the fractional rate per min (i.e., tritium outflow per 5 min/tritium content at the onset of the 5-min collection period).

Stimulation-evoked tritium overflow was calculated by subtracting the extrapolated basal outflow from the total outflow during the 2 min of stimulation and the following 13 min; the stimulation-evoked overflow was then expressed as a percentage of the tritium content of the tissue at the onset of stimulation.

Drug effects on the stimulation-evoked overflow are expressed as the ratios of the overflows evoked by S2 to that by S1 (i.e., S2/S1), as percentage of control. Each value is the mean of two or three independent experiments, performed in duplicate.

Results

Solid state conformation and absolute configuration of (−)-(*trans*)-1,2,3,4,4a,5,10,10a-octahydro-6-hydroxy-1-*n*-propylbenz[*g*]quinoline-(*R*)-(−)-mandelate. Stereoscopic views of the solid state conformation are shown in Fig. 3, A and B, and a drawing of the unit cell contents is given in Fig. 3C. All bond lengths and angles are within the expected ranges. The *N*-*n*-propyl group is equatorial to the heterocyclic ring; the N^+-H proton is axial and forms a hydrogen bond to the carbonyl group of the mandelate anion (($N\cdots O$ = 2.752 Å). The phenolic hydroxy group participates in a strong hydrogen bond to the other mandelate carboxyl oxygen ($O\cdots O$ = 2.592 Å). These hydrogen-bonding interactions in the crystal, as shown in Fig. 3B, provide a model for a possible drug-receptor interaction. In addition, an intramolecular hydrogen bond is present between a carbonyl oxygen and the hydroxy group of the mandelate anion. The absolute configuration of (−)-(*trans*)-1,2,3,4,4a,5,10,10a-octahydro-6-hydroxy-1-*n*-propylbenz[*g*]quinoline in the crystal could be determined as 4a*R*,10a*R*, based on the known *R*-configuration of the mandelate anion.

Adenylate cyclase. The effects of the octahydrobenz[*g*]quinolines together with the effects of some reference compounds are summarized in Table 1. Among the racemic octahydrobenz[*g*]quinolines, only three *N*-alkyl derivatives of the 6-hydroxy *trans* series stimulated the adenylate cyclase. Maximal stimulation increased from the *N*-methyl derivative **3b** to the *N*-ethyl derivative **3c** and the *N*-propyl derivative **3d**,

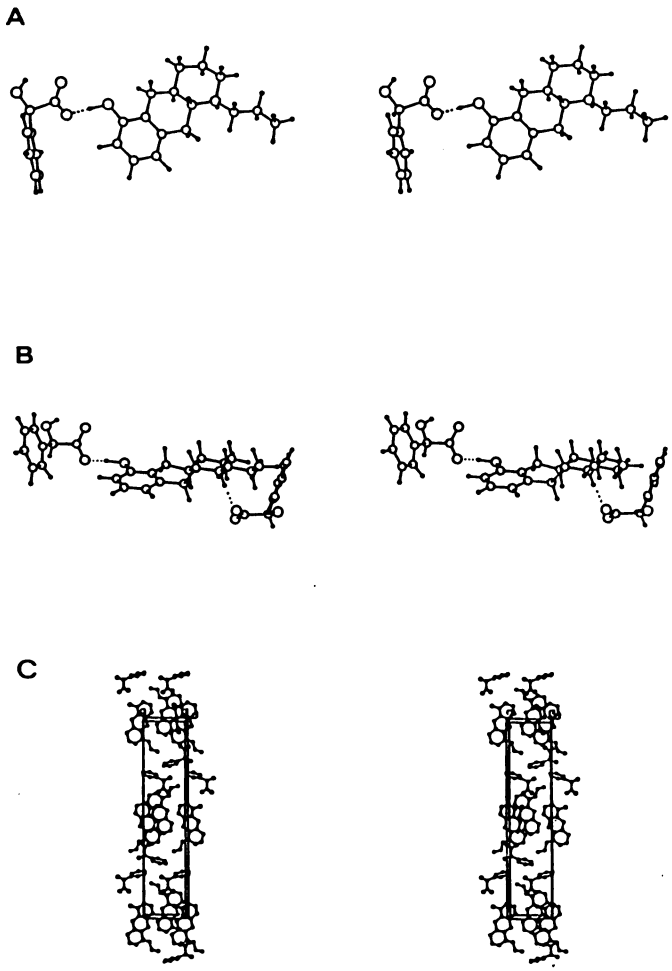
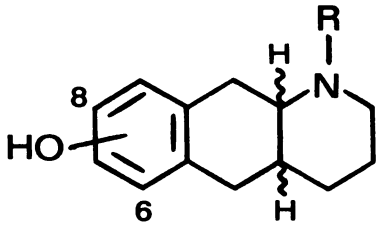


Fig. 3. X-ray structure of (−)-(*trans*)-1,2,3,4,4a,5,10,10a-octahydro-6-hydroxy-1-*n*-propylbenz[*g*]quinoline-(*R*)-(−)-mandelate. Stereoscopic representation of the molecule. A, Top view; B, frontal view displaying the two intermolecular hydrogen bonds to the mandelate anions. C, Stereoscopic view of a unit cell along *a*.

TABLE 1

Effects of octahydrobenz[g]quinolines on DA-sensitive adenylate cyclase and on electrically evoked ACh release

Me, methyl; Et, ethyl; Pr, propyl; Bu, butyl.



Compounds				Adenylate Cyclase[a]		ACh release[b]	
No.	Position of OH	Ring Junction	R	Potency	Maximal Stimulation	Potency	Maximal Inhibition
				pD_2	% relative to DA	pD_2	% of control
3a	6-OH	*trans*	H		0		0
3b	6-OH	*trans*	Me	6.15	14	7.30	−62
3c	6-OH	*trans*	Et	5.95	40	8.38	−90.5
3d	6-OH	*trans*	*n*-Pr	5.68	43	8.55	−91
(−)-3d	6-OH	*trans*	*n*-Pr	5.90	50	9.15	−86
(+)-3d	6-OH	*trans*	*n*-Pr	4.75	24.5	7.63	−82.5
3e	6-OH	*trans*	*n*-Bu	0	0	0	0
3f	6-OH	*cis*	H	0	0	0	0
3g	6-OH	*cis*	Me	0	0	0	0
3h	6-OH	*cis*	Et	0	0	0	0
3i	6-OH	*cis*	*n*-Pr	0	0	0	0
4a	8-OH	*trans*	H	0	0	0	0
4d	8-OH	*trans*	*n*-Pr	0	0	0	0
4f	8-OH	*cis*	H	0	0	0	0
4i	8-OH	*cis*	*n*-Pr	0	0	0	0
References[c]							
5-OH-AT (**1a**, *R* = H)				4.45	23	0	0
5-OH-DPAT (**1d**, *R* = *n*-Pr)				5.90	34.5	8.40	−90
7-OH-AT (**2a**, *R* = H)				5.05	48.5	6.45	−66
7-OH-DPAT (**2d**, *R* = *n*-Pr)				5.35	29.5	8.31	−83
CV 205-502 (**5**)				5.80	34.5	8.65	−90
LY 141865 (**6**)				0	0	7.33	−81

[a] Mean basal adenylate cyclase activity was 70.9 ± 1.9 pmol of cyclic AMP/mg of protein/min (n = 22), rising to 179.5 ± 3.9 pmol of cyclic AMP/mg of protein/min (n = 22) in the presence of 125 μM DA. The values are based on the means of two or three independent experiments performed in triplicate (SE < 10%).

[b] The maximal drug effect is expressed as the observed maximal change of S_2/S_1 ratio, as the percentage of the control ratio. Control ratio was 0.78 ± 0.013 (n = 90). The EC_{50} values were estimated graphically using four or five drug concentrations separated by 1 log unit. The values are based on the means of two or three independent experiments performed in duplicate (SE <10%).

[c] All data concern the racemic compounds.

whereas the potency showed the reverse rank order. Change from *N*-propyl to *N*-butyl substitution in this series (**3e**) resulted in a complete loss of activity. Among the enantiomers of the *N*-propyl derivative **3d**, the (−)-form showed higher potency and maximal stimulation. The 6-hydroxy derivatives of the *cis* series were all found to be inactive, irrespective of their *N*-alkyl substituent. Similarly, none of the 8-hydroxy derivatives showed an activity, regardless of whether the ring fusion was *cis* or *trans* or whether the amine group was unsubstituted or *N*-propylated.

The 3-substituted octahydrobenz[*g*]quinoline CV 205-502, **5** (16) (Fig. 2), tested as a reference compound, revealed effects in the range of the corresponding 3-unsubstituted derivative **3d**, as did 5-OH-DPAT, **1d**, the bicyclic aminotetralin analog of **3d**. LY 141865, **6** (17), a heterocyclic analog of **3d** with reported selectivity for the D_2 receptor subtype (18), as expected showed no activity in the D_1 receptor model. The bicyclic analogs of the inactive 8-hydroxy octahydrobenz[*g*]quinolines, the 7-hydroxy aminotetralins **2a** and **2d**, stimulated the adenylate cyclase from bovine retina, as already shown earlier with an enzyme from rat striatum (9).

ACh release. The effects of the octahydrobenz[*g*]quinolines in the superfusion experiments (Table 1) showed extensive parallelism with the effects in the cyclase assay, except that the potency of the active derivatives ranked, as expected in the nanomolar range, whereas in the D_1 model they were in the micromolar range. Among the racemic octahydrobenz[*g*]quinolines, only the three *N*-alkyl derivatives of the 6-hydroxy *trans* series, which were already found to stimulate the adenylate cyclase, also showed agonist activities at the striatal D_2 receptor modulating ACh release. Potency increased with increasing size of the *N*-alkyl substituent from *N*-methyl to *N*-propyl, whereas a further extension by one carbon atom also resulted in an inactive compound in this model (**3e**). The superior activity of the (−)-enantiomer of the *N*-propyl derivative **3d** over its (+)-form was also found in the release assay. Similarly, neither the *cis*-derivatives of the 6-hydroxy series nor the 8-hydroxy derivatives of the *cis* and *trans* series revealed any effects, irrespective of their *N*-substitution.

The reference compounds CV 205-502, **5**, and 5-OH-DPAT, **1d**, showed potencies and maximal effects similar to their closest analog, the *N*-propyloctahydrobenz[*g*]quinoline **3d**,

again a parallelism with the situation at the D_1 receptor. Compared with these most active compounds, the D_2-selective DA agonist LY 141865, **6**, proved to be an order of magnitude less potent. The 7-hydroxy aminotetralins **2d** and **2a**, in contrast to their tricyclic analogs of the 8-hydroxybenz[*g*]quinoline series, are able to stimulate the D_2 receptor as previously shown (10).

Discussion

Structure-activity relationships. A transition from the bicylic aminotetralins to the tricyclic octahydrobenz[*g*]quinolines represents an increase in the size of the molecule combined with an added conformational constraint on the amino group. Both factors could influence activity as well as subtype selectivity. The nearly identical structure-activity pattern found with the monohydroxyoctahydrobenz[*g*]quinolines in the D_1 and D_2 model, however, implies that, like the aminotetralins, none of these tricyclic compounds are able to discriminate between the two DA receptor subtypes. In contrast, angularly annellated 8,9-dihydroxyoctahydrobenz[*f*]quinolines **7** appear to be selective DA_2 agonists (19). The 3-substituent of CV 205-502, **5**, has no influence on subtype preference, as revealed by a comparison with the "naked analog" **3d**. D_2 selectivity is obtained, however, by replacement of the phenol ring in **3d** by a pyrazole, ring as demonstrated with LY 141865, **6**.

Although incorporation of the amino group into a linearly annellated third ring had no influence on subtype selectivity, it led to a dramatic differentiation between the activity of α- and β-rotameric compounds. In the aminotetralin series, both conformers are effective DA agonists; however, in the octahydrobenz[*g*]quinoline series, the activity is only preserved in α-rotameric 6-hydroxy *trans*-derivatives and is lost in all β-rotameric 8-hydroxy compounds. Cannon *et al.* (19, 20) found a similar discrimination between the corresponding 6,7- and 7,8-dihydroxy analogs. How can we explain the inactivity of the β-rotameric linear tricycles? A steric hindrance by the *N*-alkyl group can be ruled out, because the effects are independent of *N*-substitution. An unfavorable orientation of the nitrogen lone pair (or N^+-H), as suggested for Cannon's dihydroxy analogs by Nichols (21), seems rather unlikely, because such an axial N^+-H proton appears to be very effective in the case of the corresponding α-rotameric compounds **3b–3d**. An unfavorably directed N^+-H proton, however, might explain the inactivity of the *cis*-derivatives **3f–3i**. The biologically active β-rotameric aminotetralins differ structurally from their inactive tricyclic analogs by the fixed peripheral carbon atoms of the *N*-containing ring. We favor the idea that they prevent a receptor docking by interacting with a "steric barrier." McDermed (22) proposed another "steric barrier," which is supposed to repulse the unsubstituted phenyl ring of isoapomorphine. Our results suggest, however, that the inactivity of isoapomorphine is due to its inactive partial structure, 8-hydroxyoctahydrobenz[*g*]quinoline **4a**.

In both receptor models, the (−)-enantiomer of the octahydrobenz[*g*]quinoline **3d** proved to be the more active form. Its determined 4a*R*,10a*R* absolute configuration corresponds to the 2*S* absolute configuration of the active (−)-enantiomer of 5-OH-AT and 5-OH-DPAT at both DA receptor subtypes (9,10). Because (-)-5-OH-DPAT is a partial structure of **(−)-3d**, a similar receptor orientation of the two compounds may be assumed.

From the structure-activity relationships concerning *N*-substitution, we expect information about one of the *N*-alkyl binding sites postulated by us on the basis of the aminotetralin studies. The inactivity of the *N*-unsubstituted (*trans*)-6-hydroxy derivative **3a** parallels the low activity of the corresponding aminotetralin **1a**. *N*-Methyl substitution renders the tricyclic compound active (**3b**), but maximal effects are reached with *N*-ethyl or *N*-*n*-propyl substitution (**3c, 3d**) emphasizing the crucial of *N*-alkyl substituents in the α-rotameric series. Because in the (*trans*)-octahydrobenz[*g*]quinolines the crucial direction of the N^+-H is fixed, a conformational change due to a steric interaction of the *N*-alkyl group can be excluded. This strongly supports the idea that the *N*-alkyl substituents bind to an accessory site, which leads to an increase in affinity[1] and activity. The optimal tricyclic compounds **3c** and **3d** are as active as 5-OH-DPAT, suggesting that in the α-rotameric (*trans*)-octahydrobenz[*g*]quinolines the *N*-alkyl substituent is favorably oriented in order to reach this binding site. The reverse rank order of potency of these derivatives in the D_1 as compared with the D_2 model confirms our previous observations (10) that secondary binding sites differ slightly at the two DA receptor subtypes.

In important studies with monohydroxyoctahydrobenz[*f*]quinolines **7**, Wikstroem *et al.* (23, 24) recently showed that replacing a *N*-propyl substituent with *N*-butyl has different effects depending on the rotamer form; in the β-rotameric 9-hydroxy *trans* series the replacement caused a loss of dopaminergic activity, whereas in the α-rotameric 7-hydroxy *trans* series the same variation resulted in an increase in potency. These findings led to the suggestion that, at the receptor, the *N*-substituent of the two conformer series point in different directions and that the space available in one direction does not allow substituents larger than *n*-propyl. Because these results are based on DA turnover rates and behavioral data measuring presynaptic D_2 and unspecified postsynaptic effects, no conclusion can be drawn with respect to the situation at the D_1 receptor. In our active (*trans*)-6-hydroxy-octahydrobenz[*g*]quinoline series, a change from *N*-*n*-propyl to *N*-*n*-butyl substitution also resulted in a complete loss of activity, an effect we like to call "*N*-butyl phenomenon." Together with the increase in activity following *N*-ethyl or *N*-propyl substitution, it suggests that, in these α-rotameric derivatives as well as in Wikstroem's β-rotameric (*trans*)-octahydrobenz[*f*]quinolines, the *N*-alkyl substituent is directed toward an accessory site with limited space, a "small *N*-alkyl binding site." It contrasts with a "large *N*-alkyl binding site" capable of accommodating the *N*-*n*-butyl group of the (*trans*)-7-hydroxyoctahydrobenz[*f*]quinolines as well as the *N*-*n*-pentyl or functionalized *N*-alkyl substituent of 5-hydroxyaminotetralins (25, 26). The two different *N*-alkyl binding sites explain the high dopaminergic activity of *N*-*n*-butyl-*N*-*n*-propyl-5-hydroxyaminotetralin, as compared with the corresponding inactive *N*,*N*-di-*n*-butyl derivative (25). The observation of a *N*-butyl phenomenon in both our models indicates that a small *N*-alkyl binding site exists at both DA receptor subtypes.

General rotamer-based DA receptor model. Many DA models make use of artificial receptor boundaries to explain structure-activity relationships (e.g., 22, 27–30). We have used

[1] Using [^{3}H]DA binding, an increase in affinity with increasing size of the *N*-alkyl group was noted which paralleled the increase in activity. The difference in affinity between **3a** and **3d** was found to be more than two orders of magnitude.

molecular modeling techniques (31) to localize anticipated receptor boundaries, namely the small *N*-alkyl binding site and the suggested steric barrier. The superimposition of the α- and β-rotameric aminotetralins (2*S*)-5-OH-AT and (2*R*)-7-OH-AT, proposed earlier (9, 10), served as a basic framework for orienting the tricyclic structures at the receptor. The four enantiomeric active structures chosen (Fig. 4A) were superimposed to form the "receptor-excluded volume" depicted in Fig. 4B. A frontal view of the superimposition shows that all N^+-H protons are pointing down (Fig. 4C, *arrow*), localizing the amino binding site below the plane of the aromatic ring. A similar orientation of the N^+-H proton of **(−)-3d** has been found in its crystal structure (Fig. 3B). The *n*-butyl-6-hydroxyoctahydrobenz[*g*]quinoline **3e** and the 8-hydroxyoctahydrobenz[*g*]quinoline **4a** served as relevant inactive structures[2] (Fig. 5A) for the construction of the "receptor-essential volume" shown in Fig. 5B. It marks the boundary of the small *N*-alkyl binding site (volume a) and the steric barrier (volume b), which prevents a receptor interaction of the β-rotameric octahydro-benz[*g*]quinolines **4**. The two partial receptor boundaries can be regarded as comprising part of an "extended steric barrier."

These results, integrated into our earlier rotamer-based DA receptor model (10), yield the refined version shown in Fig. 6. The small *N*-alkyl binding site (*S*) is localized relative to the two major binding sites of the DA receptor, which are defined

[2] The relevant inactive structures are anticipated to be inactive due to steric interactions.

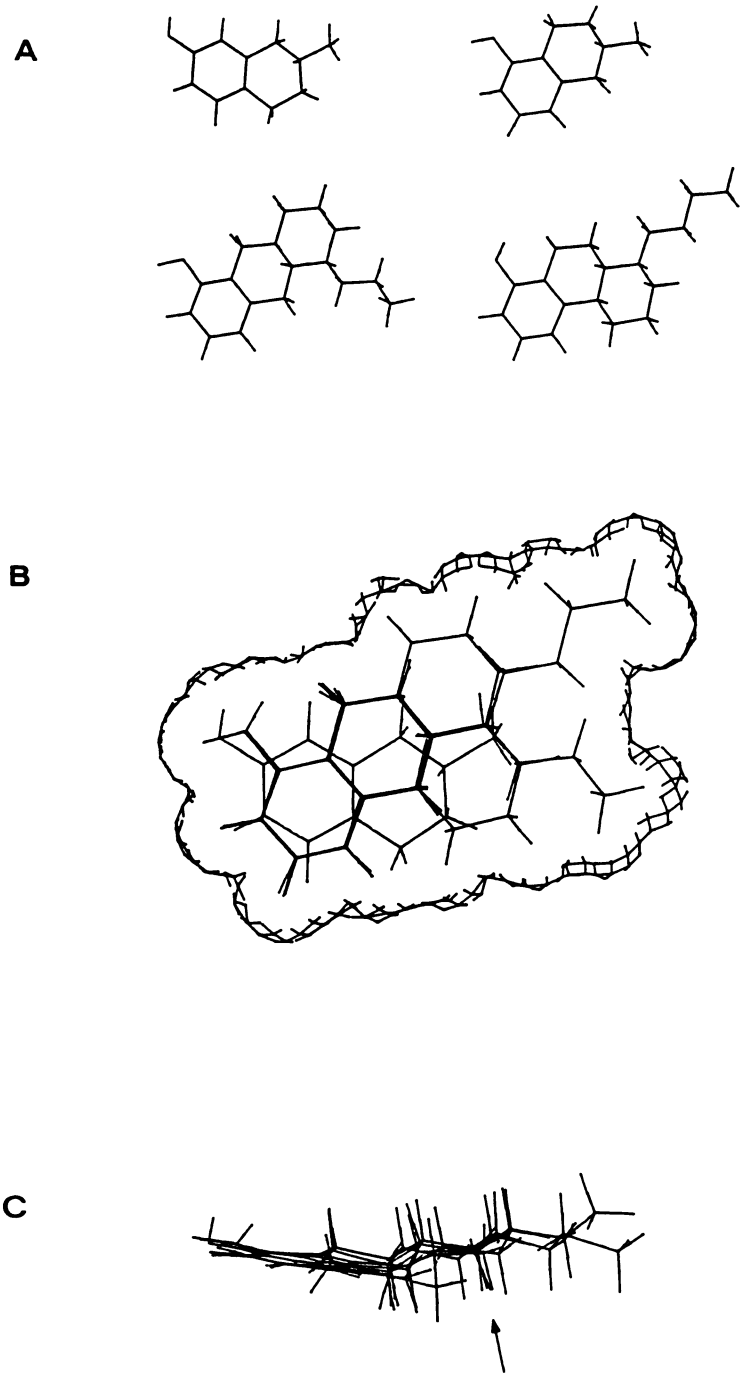


Fig. 4. Superimposition of active structures generating a receptor excluded volume. A shows the active structures used to construct a receptor excluded Van der Waals volume; (*R*)-7-OH-AT, (*S*)-5-OH-AT, (4a*R*,10a*R*)-(*trans*)-*n*-propyl-6-hydroxyoctahydrobenz[*g*]quinoline **(−)-3d**, and (4a*S*,10b*S*)-(*trans*)-*N*-*n*-butyl-1,2,3,4,4a,5,6,10b-octahydro-7-hydroxybenz[*f*]quinoline. The structures were computer modeled starting from presently described **((−)-3d)** or published (32, 33) X-ray coordinates. The aminotetralins were superimposed by a fit of the oxygen, nitrogen, N^+-H proton, and one aromatic carbon. The tricyclic compounds were fitted to the aminotetralin with the corresponding rotamer form and the corresponding absolute configuration. B shows the resulting superimposition and the receptor excluded volume generated therefrom. C represents a frontal view of the superimposition. The *arrow* marks the N^+-H protons pointing down. All modeling was performed using Sybyl software (Tripos) on a Evans and Sutherland PS 350.

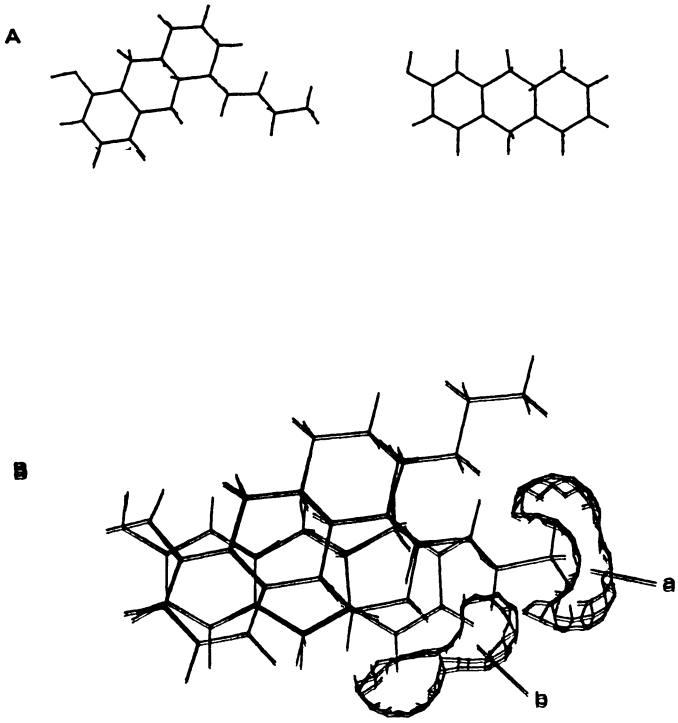


Fig. 5. Inactive structures and the derived steric barriers. The (*trans*)-*N*-*n*-butyl-8-hydroxyoctahydrobenz[*g*]quinoline 3e and the 8-hydroxyoctahydrobenz[*g*]quinoline 4a were used to localize the boundaries of the small *N*-alkyl binding site and the anticipated steric barrier, respectively. A shows their computer-modeled structures, based on X-ray coordinates. Both molecules were fitted to the relevant aminotetralin. They form a Van der Waals volume of inactive structures (not shown). B shows the superimposition of the active and inactive structures in the relevant receptor orientation, together with the receptor essential volume (difference of volumes of active and inactive structures). It represents the receptor boundary around the small *N*-alkyl binding site (*a*), and the steric barrier (*b*), which prevents a receptor interaction of the β-rotameric octahydrobenz[*g*]quinolines.

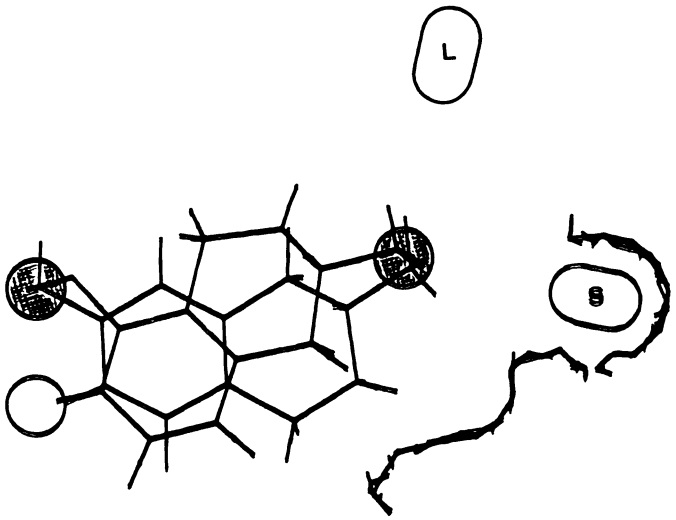


Fig. 6. Rotamer-based general DA receptor model. The two major binding sites for the *meta*-hydroxy and amino group of DA are displayed as *hatched circles*, the amino binding site being situated below the focal plane. The two aminotetralins, α-rotameric (2*S*)-5-OH-AT and β-rotameric (2*R*)-7-OH-AT, superimposed (10), serve as a basic framework for orienting new structures at the model receptor. One of their N^+-H protons is pointing down toward the amino binding site. *Open symbols* indicate accessory binding sites, the *open circle* the binding site for the *p*-hydroxy group of DA, the *ovals* the large (*L*) and small (*S*) *N*-alkyl binding site. The *line* around the small *N*-alkyl binding site and *its continuation* represent the disclosed extended steric barrier. An additional secondary binding site (not shown) exists for the aromatic ring. All features of the model are common to D_1 and D_2 receptors.

by the superimposition of the two aminotetralins. It is surrounded by part of the extended steric barrier. The boundaries of the large *N*-alkyl binding site (*L*) cannot be determined due to lack of relevant analogs. Nevertheless, the *n*-butyl group of Wikstroem's α-rotameric octahydrobens[*f*]quinoline derivative (Fig. 5B) can be used to identify its approximate position. Potential agonists can be oriented at our model DA receptor by applying the following criteria.

(I) Rotamer form. Molecules containing a 5-OH-AT or a 7-OH-AT partial structure are fitted in the model, according to their rotamer form, to α-rotameric (2*S*)-5-OH-AT or β-rotameric (2*R*)-7-OH-AT.

(II) Absolute configuration of asymmetric center α to basic nitrogen (β to aromatic ring). In a correct orientation it should correspond to the absolute configuration of the relevant aminotetralin of the basic framework (*trans*-fused ring systems).

(III) Influence of *N*-*n*-propyl substitution. Increase in activity (compared with the corresponding NH-derivative) indicates an α-rotameric orientation; no change or a decrease in activity indicates a β-rotameric orientation.

(IV) Influence of *N*-*n*-butyl substitution. Preservation or increase in activity (compared with the corresponding *N*-propyl compound) indicates an orientation of the *N*-alkyl substituent toward the large *N*-alkyl binding site and loss of activity an orientation toward the small *N*-alkyl binding site.

(V) Extended steric barrier. It should not be touched or penetrated.

The criteria are valid for D_1 as well as for D_2 agonists because the underlying structure-activity relationships are relevant at both receptor subtypes. Its practical application is demonstrated with the two DA agonists apomorphine and ergoline. α-Rotameric apomorphine is fitted to (2*S*)-5-OH-AT its partial structure with the corresponding absolute configuration (Fig. 7A). *N*-*n*-butyl substitution leads to a decrease in activity (34), indicating that the *N*-alkyl substituent points toward the small *N*-alkyl binding site. The orientation of the ergoline molecule is more complex, because criterion I cannot be applied. An α-rotameric orientation is suggested by the increase in activity following *N*-propyl substitution (7). It is also compatible with the sterochemistry criterion II, the loss of activity following *N*-butylation[3] and the extended steric barrier. However, in such an orientation, the acidic indole proton cannot form a hydrogen bond to the DA *meta*-hydroxy binding site. That this hydrogen bonding is a prerequisite for a receptor activation is suggested by the inactivity of *O*-methylated phenolic DA agonists and N_1-methylated ergolines. Yet, a slightly turned "quasi-α-rotameric orientation," as shown in Fig. 7B, should also satisfy this requirement. In such an orientation, the 8-substituent of the ergolines, like the 3-substituent of CV 205-502, 5, is situated in the area of the large *N*-alkyl binding site. Ergolines tend to be metabolically hydroxylated in position 13, which results in an increase in dopaminergic activity (37). It can be explained by a change from the "quasi-α-rotameric" to the more efficient β-rotameric orientation, because by the 13-hydroxylation the

[3] R. Markstein, unpublished results.

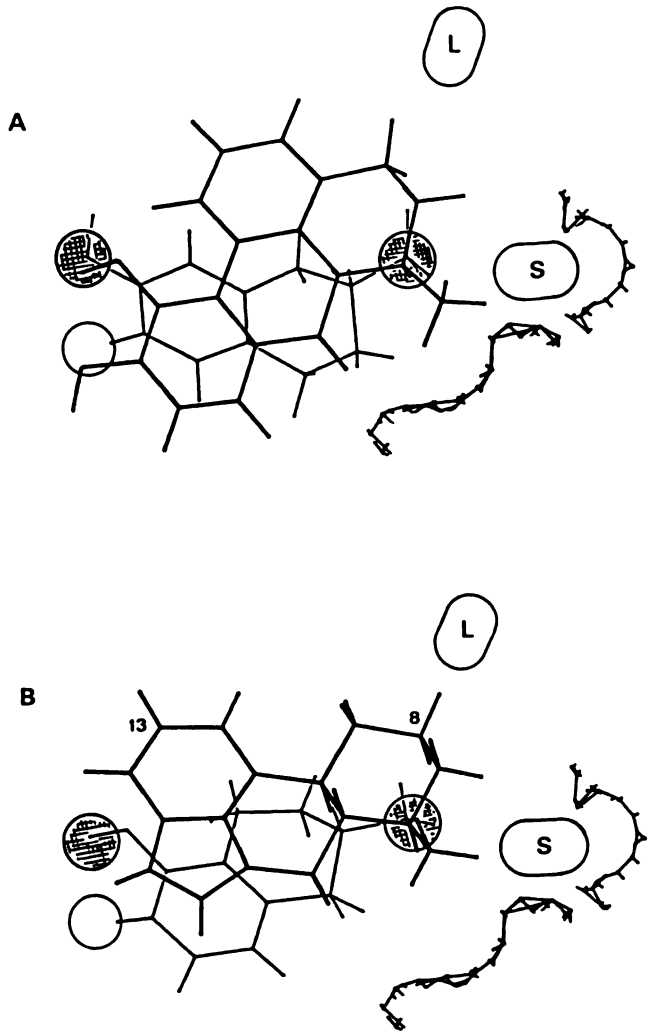


Fig. 7. Orientation of apomorphine and ergoline in the DA receptor model. A, Apomorphine. The molecule contains 5-OH-AT as partial structure and is, thus, fitted to the α-rotameric (2*S*)-5-OH-AT with corresponding absolute configuration. The α-rotameric orientation is confirmed by the increase in activity following *N*-propyl substitution. The NN^{+}-H proton is pointing down toward the amino binding site, the *N*-alkyl group is directed toward the small *N*-alkyl binding site (*S*), explaining the inactivity of its *N*-butyl derivative (34). The extended steric barrier is not touched. For comparison, (2*R*)-7-OH-AT is displayed marking the receptor orientation of DA. B, Ergoline. The molecule does not contain a 5- or 7-OH-AT partial structure to facilitate a receptor orientation. An α-rotameric fitting to (2*S*)-5-OH-AT is indicated by the described increase in activity following *N*-propyl substitution (7). The 5*R* absolute configuration of the ergoline corresponds thereby to that of the aminotetralin. In order to bring the indole proton of the ergoline within hydrogen bonding range of the major binding site for the *meta*-hydroxy group of DA, the molecule is turned clockwise by about 15° around an axis through N_6 perpendicular to the plane of the aromatic ring. It, thus, achieves the quasi-α-rotameric orientation shown. The *N*-alkyl group is oriented towards the small *N*-alkyl binding site, which is consistent with the loss of activity following *N*-butyl substitution. The 8-substituent is directed toward the large *N*-alkyl binding site; the orientation is compatible with the extended steric barrier. For comparison, (2*S*)-5-OH-AT is displayed, indicating the original α-rotameric orientation. Both structures were computer modeled starting from published X-ray coordinates (35, 36); superimpositions were performed as described in the legend to Fig. 4.

ergoline molecule has acquired a β-rotameric (2*R*)-7-OH-AT partial structure.

Our DA receptor model, like the one originally proposed by McDermed (22) and most others subsequently described (e.g., Refs. 24, 27, 28, and 30), postulates two major binding sites for the *meta*-hydroxy and amino group of DA. An exception is the model of Grol *et al.* (29) with different amino binding sites for α- and β-rotameric compounds. We believe that the evidence for a small *N*-alkyl binding site shown with α-rotameric octahydrobenz[*g*]quinolines and β-rotameric octahydrobenz[*f*]quinolines (23) speaks against the suggestion of Grol *et al.*

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