Pre- and Postsynaptic Dopaminergic Activities of Indolizidine and Quinolizidine Derivatives of 3-(3-Hydroxyphenyl)-*N*-(*n*-propyl)piperidine (3-PPP). Further Developments of a Dopamine Receptor Model

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Pre- and postsynaptic dopaminergic activities of a series of indolizidine and quinolizidine analogues of 3-(3-hydroxyphenyl)-*N*-(*n*-propyl)piperidine (3-PPP) have been studied. The pharmacological data have been interpreted in terms of a previously reported model for interactions with dopamine pre- and postsynaptic D2-receptors and molecular mechanics (MM2(85)) calculated geometries and conformational energies. The model has been further developed with respect to the receptor topography in the vicinity of the nitrogen binding site. In particular, a novel spatial orientation of the important "propyl cleft" has been proposed. This cleft is suggested to be located mainly above a plane through the receptor-bound substrate. The biologically active agonist and antagonist conformations of the enantiomers of 3-PPP have been reinvestigated.

In an attempt to rationalize the pre- and postsynaptic dopaminergic profiles of the enantiomers of 3-(3-hydroxyphenyl)-*N*-(*n*-propyl)piperidine (3-PPP, (*R*)- and (*S*)-1; Chart I) and related compounds of the phenylpiperidine series, we recently reported a tentative model for central dopamine (DA) D2 receptors.[1] The proposed model was based on conformational energy calculations (molecular mechanics) and molecular superimposition studies. We concluded that a conformation of (*S*)-1 with a calculated energy 2.4 kcal/mol higher than the global energy minimum is responsible for its agonist properties at central DA receptors, while another rotameric form of lower energy is responsible for its antagonist properties at such receptors. The model rationalized presynaptic selectivity in terms of a difference in the effect of *N*-alkyl substituents on the two types of receptors.

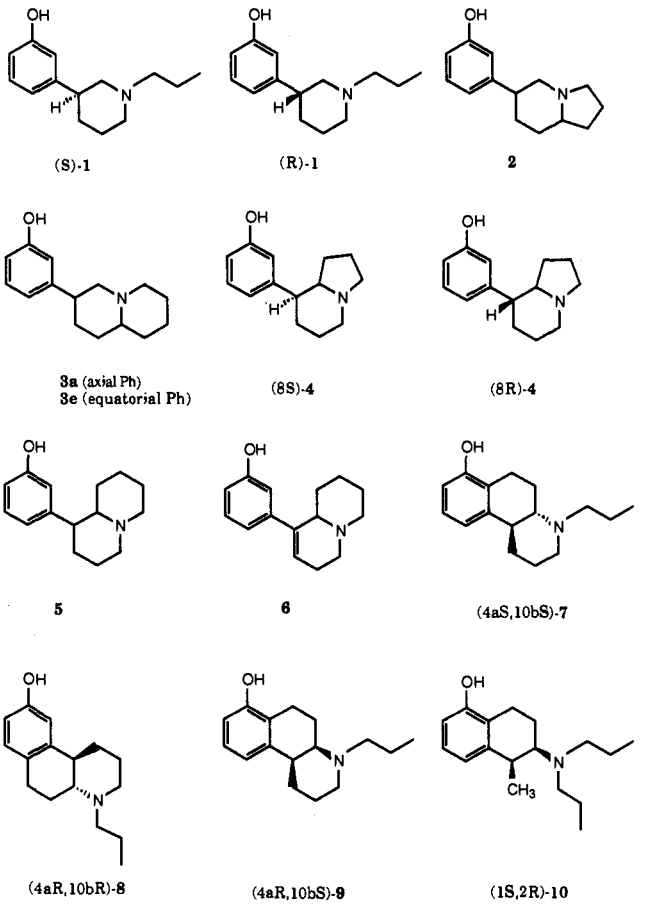
Also recently, Bøgesø et al. reported a series of 3-(3-hydroxyphenyl)indolizidine and -quinolizidine analogues of compound 1 (compounds **2**–**6**, Chart I) together with their pharmacological effects.[2] All isomers of these compounds with an axial phenyl group were found to be inactive as directly acting DA agonists or antagonists, and among the equatorial isomers, (8*R*)-**4** was the only DA agonist in this series. Compounds (8*S*)-**4** and **6** exhibited antagonist properties while compounds **2**, **3e**, and **5** were either very weakly active or inactive. Compound (8*R*)-**4** was found to have a pharmacological profile similar to that of (*R*)-1. Compound (8*S*)-**4**, in contrast to (*S*)-1, did not behave as a full DA agonist in reserpinized rats. In addition, the quinolizidine derivative of **4**, i.e. compound **5**, was found to be inactive while the olefinic derivative **6** showed DA antagonistic properties.

Since the compounds studied by Bøgesø et al. are all structurally very similar to 3-PPP (**1**), these problems are well-suited to be studied in terms of our previously reported DA receptor interaction model. In the present paper we employ the model in a structure–activity analysis of the equatorial isomers of compounds **2**–**5** and compound **6**. It will be shown that this analysis suggests a solution to problems concerning the shape and dimensions of the receptor model which were left open in our previous work.[1]

To be able to compare the biochemical data of the compounds used to construct the receptor model[1] with those of the indolizidine and quinolizidine analogues, we undertook the testing of the equatorial isomers of compounds **4** and **5** and compound **6** in the DOPA and 5-HTP accumulation assays in both naive and reserpinized animals. In addition, in vitro D2 receptor binding using [^{3}H]spiperone and [^{3}H]N-0437 was determined.

Bøgesø et al. observed that axial isomer **3a** unexpectedly induced ipsilateral circling in 6-OHDA-lesioned rats.[2] In order to further investigate the mechanism behind this observation, **3a** was also included in the pharmacological testing.

Chart I



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Pharmacological Methods

Determination of Presynaptic Activity. The in vivo biochemical test utilizes the well-established phenomenon of receptor-mediated feedback inhibition of the presynaptic DA neuron.[3] The synthesis rate of DA and norepinephrine (NE) are inhibited by agonists (and activated by antagonists) at dopaminergic and α-adrenergic receptors, respectively. The tyrosine hydroxylase activity is affected via agonist or antagonist activity at the presynaptic DA (auto) receptors.[4] Similarly, the synthesis rate of 5-HT is inhibited by 5-HT receptor agonists.[5] The DOPA accumulation, following decarboxylase inhibition by means of (3-hydroxybenzyl)hydrazine (NSD 1015), was used as a measure of the DA synthesis rate in the DA-rich areas (i.e. the limbic system, corpus striatum) and the NE synthesis rate in NE-rich hemispheres (primarily cortex.[6,7] The levels of NE are approximately 10 times higher than the levels of DA in the remaining cortical (mainly hemispheral) brain portions, thus the formation of DOPA in this brain part is suggested to reflect the synthesis of NE (rather than the synthesis of DA). 5-HTP accumulation was taken as an indicator of the 5-HT synthesis rate in each of the three brain areas.

Determination of Postsynaptic Activity. Postsynaptic dopaminergic effects of the test compounds were assessed by the increase (reserpine pretreated rats) or increase/decrease (nonpretreated rats) in locomotor activity. Motor-activity recordings were carried out as previously described with the use of motility meters.[8]

In Vitro D2 Receptor Binding. The D2 receptor affinity for a number of the compounds were determined by measuring their ability to inhibit [^{3}H]spiperone and [^{3}H]-2-[*N*-propyl-*N*-(2-thienylethyl)amino]-5-hydroxytetralin ([^{3}H]N-0437)[9] binding to rat striatal membranes.

Computational Methods

Calculations of energy-minimized structures and conformational energies were performed with the molecular mechanics program MM2(85) using the full treatment of conjugated systems.[10-13] Potential energy curves were calculated with the MM2 driver option with full energy minimization, except for the dihedral angle employed as the driving angle. A dihedral angle increment of 10° was used in these calculations.

Table I. Effects on DA Synthesis Rates and on Locomotor Activity in Reserpine-Pretreated Rats[a]

	DOPA accumulation: ED_{50}, μmol/kg sc			motor activity	
compd	limb	stri	cortex	dose, μmol/kg	accum counts/30 min mean ± SEM[b]
(*S*)-1[c]	0.8	1.7	I[d]	213	12 ± 2*
(*R*)-1[c]	1.0	1.3	I[d]	13	78 ± 14*
3a	*e*	*e*	*e*	100	262 ± 30*
rac-4	0.44–3.5[f]	*g*	I[h]	50	13 ± 6
(8*S*)-4	*i*	*i*	*i*	50	21 ± 9
(8*R*)-4	1.1–6.5	1.7–4.0	*j*	50	25 ± 11
5	*k*	*k*	*k*	50	14 ± 7
6	I[h]	I[h]	I[h]	50	8 ± 4

[a] Compound **3a** gave a 30–40% decrease in 5-HTP accumulation at a dose of 100 μmol/kg sc. A corresponding decrease of 25% was observed for compound **5**. No other compounds in the table had any effects on the 5-HT synthesis rate. [b] Statistics according to Student's *t* test: * $p < 0.05$. [c] Data taken from ref 15. [d] *I* = inactive; no significant effects at the dose tested (213 μmol/kg). [e] Partial agonist, 30–40% decrease was noted at the highest dose (100 μmol/kg sc) tested. [f] Maximal decrease was 50%. [g] Maximal decrease was 65%. No ED_{50} value could be determined due to too large a spread in the data. [h] Inactive at 50 μmol/kg sc. [i] Partial agonist, a 20–25% decrease was noted at 50 μmol/kg sc. [j] No ED_{50} value could be determined due to too large a spread in the data. [k] Partial agonist, a 45–55% decrease was noted at 100 μmol/kg sc.

Table II. Effects on DA Synthesis Rates and on Locomotor Activity in Normal Rats

	DOPA accumulation				motor activity	
	dose,	% of controls			dose,	% of
compd	μmol/kg	limb	stri	cortex	μmol/kg	controls[a]
(*S*)-1[b]	27	I[c]	147	I	0.8	74*
	213	95		I	213	40*
(*R*)-1[b]	0.8	86	81	I	1.6	75*
	27	40	45	I	213	230*
3a[d]	100	69	56	62	100	220 ± 26*
rac-4	50	I	I	57	50	48 ± 14*
(8*S*)-4	50	153	245	I	3.1	135 ± 22
					50	72 ± 11
(8*R*)-4	50	46	46	37	12.5	42 ± 8*
					50	83 ± 19
5	100	61	54	48	100	142 ± 22
6	12.5	165	200	I	3.1	82 ± 11
					12.5	45 ± 13*
					25	51 ± 11*

[a] Statistics according to Student's *t* test: * $p < 0.05$. [b] Data taken from ref 15. [c] I = inactive; no significant effects at the dose tested. [d] For this compound a 35% and 45% decrease in 5-HTP accumulation was observed at 50 μmol/kg in the limbic and striatal regions, respectively. No other compounds in this table showed any effects on the 5-HT synthesis rate in this assay.

As previously,[1] all calculations were done on the unprotonated amines with the nitrogen lone pair treated as a pseudoatom. The choice of nitrogen atom type, amine or ammonium, does not significantly influence the calculated conformational energies in the present type of molecules (cf. the calculations in ref 1 and 14). Since the polar functional groups are too far apart to affect the calculations through electrostatic interactions, this is to be expected.

The van der Waals surfaces were calculated with radii according to the MM2(85) force field.

Input structures to MM2(85) and least-squares molecular superimpositions were done by using the molecular modeling program system MIMIC.[15,16] Unless otherwise stated, the hydroxyl oxygen, the center of the aromatic ring, the nitrogen atom, and the nitrogen lone pair were used as

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Table III. In Vitro D2 Receptor Binding

	IC_{50}, nm			IC_{50}, nm	
	[^{3}H]N-0437	[^{3}H]SPI		[^{3}H]N-0437	[^{3}H]SPI
(*S*)-1	55	3400	(8*R*)-4	45	4900
(*R*)-1	98	23000	**5**	580	13000
3a	7900	40000	**6**	320	5300
rac-**4**	75	3800	N-0437	0.62	8.8
(8*S*)-4	170	6500			

fitting points in the superimposition studies.

Results and Discussion

Biochemical Results. The compounds were tested in reserpine-pretreated rats and in intact (normal) rats (Tables I and II, respectively). According to the present data, racemic compound **4** can be classified as a partial DA receptor agonist. In the present test system, a partial DA receptor agonist would be expected to behave like the classical DA autoreceptor agonist (*S*)-1, i.e. it would not induce pronounced locomotor stimulation in reserpine-pretreated rats. However, in such rats the biochemical changes should be present, i.e. the DOPA accumulation should be affected (lowered) as compared to controls. Compound **4** decreases DOPA accumulation in reserpine-pretreated animals (Table I) with an ED_{50} slightly higher than that of (*S*)-1 and produces only weak signs of postsynaptic stimulatory effects in reserpinized rats. This compound also appears to possess weak central α-receptor agonistic effects since the cortical DOPA formation was significantly reduced at the highest dose tested in normal rats (Table II). However, since no effects were noted in reserpinized rats (Table I), the α-adrenergic effects may be of indirect nature. In nonpretreated animals compound **4** produced hypomotility at 50 μmol/kg, which suggests a partial agonist effect of this drug.

The 8*S* enantiomer of compound **4** was a weak partial agonist in reserpinized rats (Table I). In normal rats, however, (8*S*)-4 increased limbic and striatal DOPA formation which suggest DA receptor blocking properties. This classifies (8*S*)-4 as a partial D2 agonist with a very low intrinsic efficacy. The antagonism appears to be rather weak since no hypolocomotion was noted at the highest dose (50 μmol/kg sc) tested.

The 8*R* enantiomer of compound **4** shows DA receptor agonist properties (preferentially at presynaptic receptors) in reserpinized rats. Only weak postsynaptic stimulatory effects were noted (weak sniffing). A pronounced decrease in cortical DOPA formation also suggests central α-agonistic effects. Low doses of (8*R*)-4 produced hypomotility in normal rats with a concomitant decrease in limbic and striatal DOPA formation. This indicates a preferential presynaptic DA receptor agonist action of this compound at low doses. The in vitro receptor binding data (Table III) support the conclusion that compound **4** and its *R* enantiomer are the most potent DA receptor ligands in the present study.

Compound **5** was essentially inactive. No behavioral effects were observed in either reserpine-pretreated or normal rats. In addition, only a minor decrease in brain DOPA and 5-HTP formation was monitored.

Compound **6** appears to be a weak DA receptor antagonist in the present experiments. The drug was inactive in reserpine-pretreated rats. In normal animals, however, the compound increased DOPA accumulation in limbic and striatal brain regions, furthermore, the motor activity was reduced. Catalepsy was not observed at the highest dose (25 μmol/kg) tested.

In reserpine-pretreated rats axial isomer **3a** produced motor stimulation coupled with strong tremor and occasional convulsions. However, in the same rats only minor biochemical effects were monitored (partial decreases in brain DOPA and 5-HTP accumulation). In nonpretreated rats this compound produced a pronounced locomotor stimulation, as well as typical stereotyped behavior (gnawing, sniffing, rearing). Biochemically, decreased (partially) DOPA and 5-HTP levels were monitored. These data suggest that **3a** produces behavioral stimulation via *indirect* actions on central monoamine receptors. It is a well-known fact that full, directly acting DA agonists produce strong biochemical effects in doses lower than those required to activate e.g. reserpine-pretreated rats. This result is in complete accordance with the observed ability of **3a** to induce *ipsilateral* circling in 6-OHDA-lesioned rats.[2] In vitro receptor binding data (Table III) support that **3a** has no direct effect on D2 receptors in the central nervous system (CNS).

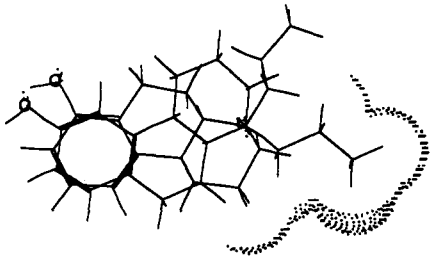

Figure 1. Molecular superimposition of (4a*S*,10b*S*)-**7** and (4a*R*,10b*R*)-**8**. The clipped van der Waals surface in the "downward" direction indicates the size and orientation of the "propyl cleft".

The Receptor-Interaction Model. Our previously described receptor model is defined by a superimposition of the highly potent compounds (4a*S*,10b*S*)-**7** and (4a*R*,10b*R*)-**8** (Chart I).[1] We assume that pre- and postsynaptic receptors have very similar geometrical properties. The superimposition, shown in Figure 1, defines two directions of the *N*-alkyl groups, "upward" and "downward", which were found to be of decisive importance for the understanding of dopaminergic activity and presynaptic selectivity.[1] The combined van der Waals volumes of the molecules in the superimposition were used to define the shape and dimensions of certain parts of the receptor cavity, in particular that of the "propyl cleft", and to indicate areas of possible steric hindrance (Figure 1).[1]

Agonist conformations for (*S*)- and (*R*)-1 were evaluated by least-squares fitting to compounds (4a*S*,10b*S*)-**7** and (4a*R*,10b*R*)-**8**, respectively.[1] Optimal fits were obtained by rotating the global energy-minimum conformer of (*S*)- and (*R*)-1 about the interring bond until the 3-hydroxyphenyl ring and the piperidine ring become almost coplanar. This conformational rearrangement is calculated by MM2(85) to require a conformational energy of 2.1 kcal/mol. (The calculated value for (*S*)-1 in the present work is slightly lower than that given in ref 1, which was calculated by an older version of the MM2/MMP2 program.)

It was found that the piperidine ring in the phenylpiperidines is approximately equivalent to a methylamino group with respect to its interactions with the receptor.[1] A single methyl group in the "downward" direction is sufficient for activating presynaptic receptors, while the postsynaptic receptor have a greater demand for lipophilicity around the nitrogen atom.[1]

Analysis of the Equatorial Isomers of Compounds 2 and 3—Implications for the Orientation of the "Propyl" Cleft. These compounds are structurally very similar to 1 and have essentially the same conformational properties as 1 with respect to rotation about the interring

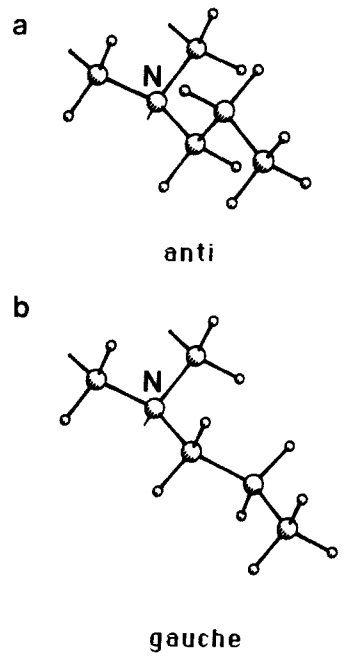

Figure 2. (a) Anti and (b) gauche conformers of the *N*-*n*-propyl group with respect to the nitrogen lone pair.

bond. Thus, they may on this ground be expected to have pharmacological properties similar to those of **1**. Therefore, the observed inactivity of these compounds in vivo[2] is most probably due to repulsive steric interactions between the bicyclic ring systems and the "wall" of the receptor cavity. Our model indicates that the area "in front" of the nitrogen site should be sensitive to steric bulk (Figure 1). However, the precise shape and dimensions of the receptor cavity in this region could not be unambiguously determined in our previous work.[1] The well-defined structures of compounds **2** and **3e** may be employed to further characterize this area of steric hindrance.

When the enantiomers of compound **3e** are fitted to the proposed receptor model in the same way as was previously done for (*S*)- and (*R*)- **1**,[1] the van der Waals volumes of both enantiomers of **3e** significantly exceed that of the receptor cavity, rationalizing the inactivity of this compound. However, this is not the case for compound **2**. The van der Waals volumes of both enantiomers of **2** are compatible with the shape and dimensions of the proposed receptor cavity in Figure 1. Thus, the observed inactivity of compound **2** indicates that our suggested shape of the receptor cavity needs to be refined in the region "in front" of the interaction site for the nitrogen atom.

The size and shape of the cavity in the actual region is largely determined by the choice of the active conformation of the propyl groups in compounds (4a*S*,10b*S*)-**7** and (4a*R*,10b*R*)-**8** (Figure 1). In our previous study we analyzed the propyl group conformations of phenylpiperidine derivatives in an attempt to determine the biologically active conformation of a propyl group in the two directions, "upward" and "downward".[1] We concluded that there were two possible solutions to this problem. The "downward" propyl group could be anti with respect to the nitrogen lone pair as in Figure 2a or gauche as in Figure 2b. It was also concluded that if the "downward" propyl group has a gauche conformation the "upward" one must have an anti conformation and vice versa. In compound **1** the anti conformer (Figure 2a) has a calculated conformational energy of 0.9 kcal/mol above the energy of the gauche conformer. In compounds (4a*S*,10b*S*)-**7** and (4a*R*,10b*R*)-**8** the anti conformer is the global energy minimum and the gauche conformer is calculated to be 0.3 kcal/mol higher in energy.

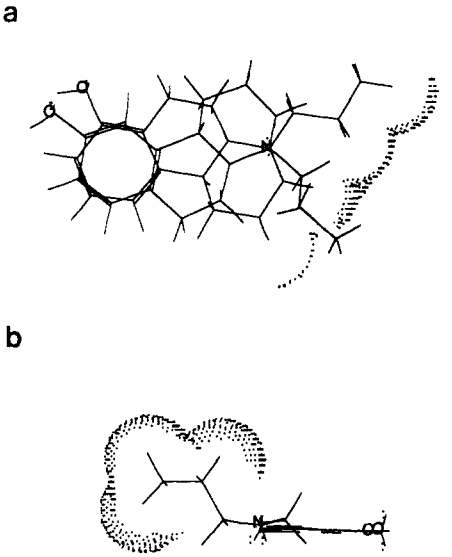

Figure 3. (a) Molecular superimposition of (4a*S*,10b*S*)-**7** and (4a*R*,10b*R*)-**8** with anti and gauche conformations, respectively, of the *N*-*n*-propyl groups. The clipped van der Waals dot surface indicates the shape of the receptor cavity about a plane through the molecular ring systems. The surface of the propyl group has been removed for clarity. (b) The same superimposition as in part a shown along a plane through the ring systems. Parts of the ring systems have been removed for clarity. The clipped van der Waals surface indicates the orientation of the "propyl cleft".

At the time our previous study was done we had no data which could be used to decide which of these two possibilities was the most probable one and we arbitrarily assigned the "downward" propyl group to have a gauche conformation (Figures 1 and 2b). With this choice the propyl group lies approximately in the plane of the substrate molecule and the "propyl cleft" is located "in front" of the nitrogen atom of the substrate and circumscribing a plane through the bound substrate. However, if the alternative choice of the "downward" propyl group conformation is made (anti; Figure 2a), the shape of the receptor cavity becomes drastically different. As shown in Figure 3a and b, a plane through the propyl group in this case becomes approximately orthogonal to a plane through the ring systems of the molecule. Then, the "propyl cleft" becomes located mainly above this plane (Figure 3b) and the "wall" of the receptor cavity moves much closer to the nitrogen interaction site (compare Figure 1 and 3a).

With the receptor topography shown in Figure 3a and b, the van der Waals volumes of both enantiomers of compound **2** (and consequently of the larger compound **3e**) significantly exceeds the dimensions of the receptor cavity "in front" of the nitrogen atom as shown in Figure 4. Thus, the new receptor topography is able to rationalize the inactivity of compounds **2** and **3e** and still make all the conclusions drawn in ref 1 valid. We conclude that the most probable orientation of the "propyl cleft" in the DA receptor cavity is as shown in Figure 3b.

Pre- and Postsynaptic Agonist Activities of Compounds 4 and 5. As discussed above, a conformational energy of 2.1 kcal/mol is required to bring (*S*)-**1** into its active agonist conformation, according to our model. This conformational energy nicely accounts for the observed decrease in activity for this compound compared to the activity of the rigid compound (4a*S*,10b*S*)-**7**.[1,17] The same

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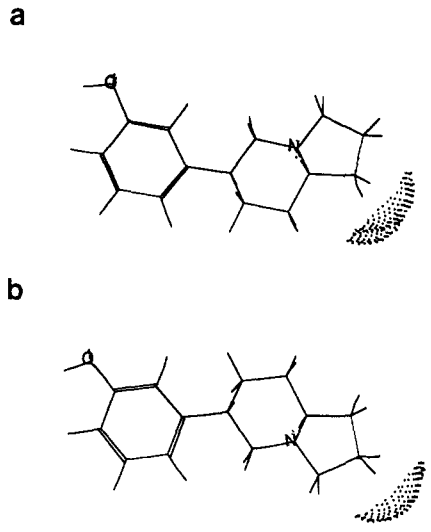

Figure 4. Dot surfaces indicating the parts of van der Waals volumes of (a) (8*S*)-**2** and (b) (8*R*)-**2** exceeding the dimension of the receptor cavity when the molecules are least-squares fitted to the appropriate template compounds in Figure 3a. The orientation of the receptor cavity is identical in this figure and in Figure 3a.

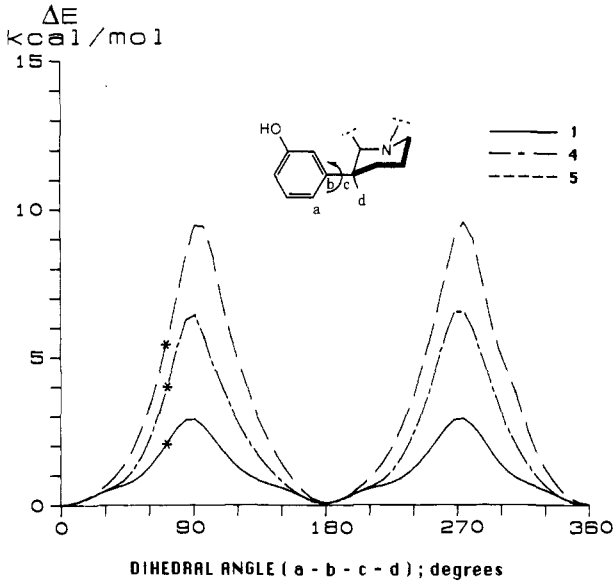

Figure 5. Calculated potential energy curves for rotation about the central bond in compounds **1**, **4**, and **5**. The sign of the dihedral angle refers to the *S* enantiomer. The asterisks denote the proposed agonist conformations (see the text).

is true for (*R*)-**1** in comparison to (4a*R*,10b*R*)-**8**.[1] Thus, in the structure–activity analysis of compounds **4** and **5** it is of interest to calculate the corresponding conformational energies for these compounds. Figure 5 shows the calculated potential energy curves for rotation about the interring bond in **4** and **5**. For comparison, Figure 5 also includes the calculated potential energy curve for **1**. (The sign of the dihedral angles in this plot refer to the *S* enantiomers.) In the calculation of the potential energy curve for **1** the propyl group conformation is gauche as required for the active agonist conformation with a propyl group in the "upward" direction according to our model, as discussed above.

In the calculated lowest energy conformation of **4** and **5** the plane of the phenyl ring is perpendicular to a plane through the bicyclic ring system (dihedral angle a–b–c–d = 0°; Figure 5). This is identical with the conformation found for the hydrochloride of compound (8*S*)-**4** in the crystalline state.[2] The calculated agonist conformations are denoted by asterisks in Figure 5. The energy for the active conformation of (8*S*)-**4**, 4.0 kcal/mol, is significantly higher than the one calculated for (*S*)-**1**, 2.1 kcal/mol. This difference is due to strong van der Waals repulsions of the "forbidden" pentane type between the bicyclic ring system and the aromatic ring in (8*S*)-**4**. These repulsions increase fast as the dihedral angle a–b–c–d approaches 90° (Figure 5).

The lack of presynaptic agonist activity for (8*S*)-**4** may be rationalized by the high conformational energy required to bring this compound into the active agonist conformation. This energy is 1.9 kcal/mol higher than that required for (*S*)-**1**. In addition, (8*S*)-**4** has only a methylene group in the "upward" direction, while (*S*)-**1** has a propyl group in this direction. This suggests[1] that (8*S*)-**4** in this respect should be compared to the *N*-methyl analogue of (*S*)-**1** which is a factor of 6–8 less active at presynaptic receptors than (*S*)-**1**.[18] Thus, these two factors taken together are sufficient to rationalize the lack of presynaptic activity of (8*S*)-**4**.

Previously we explained the lack of postsynaptic agonist activity of (*S*)-**1** by the high conformational energy of its agonist conformation and the presence of only a methylene group in the "downward" direction.[1] Since the corresponding conformational energy for (8*S*)-**4** is 1.9 kcal/mol higher, this explanation is equally valid for the lack of postsynaptic agonist activity of this compound. However, as will be discussed in detail below, both compounds are antagonists at postsynaptic receptors in normal rats.

In contrast to (8*S*)-**4**, the enantiomer (8*R*)-**4** shows DA agonist properties, preferentially at presynaptic receptors. Compound (8*R*)-**4** is found to be a factor of 2–3 less active than (*R*)-**1** (Table I).

The potential curves shown in Figure 5 are of course also valid for the *R* isomers (with opposite sign of the dihedral angle). Since (*R*)-**1** in its active agonist conformation has its propyl group in the "downward" direction, this propyl group should, according to our revised model described above, be in an anti conformation (Figure 2a and 3). The conformational energy required to bring the propyl group in (*R*)-**1** from the gauche conformation used in the calculation of the potential energy curve in Figure 5 to the anti conformation is calculated to be 0.9 kcal/mol. This implies that the total conformational energy for the agonist conformation of (*R*)-**1** is 3.0 kcal/mol. Thus, the agonist conformation of (8*R*)-**4**, with a calculated energy of 4.0 kcal/mol, is only 1.0 kcal/mol higher in energy than that for (*R*)-**1** compared to ca. 2 kcal/mol for the corresponding *S* enantiomers. Furthermore, (8*R*)-**4** has a methylene unit in the "downward" direction and should therefore (see above) be compared to the *N*-methyl analogue of (*R*)-**1**. This analogue is only a factor of 2 less active than (*R*)-**1**.[18] From these comparisons it is to be expected that the *R* enantiomers of compounds **1** and **4** should have much more similar presynaptic activities than the *S* enantiomers, but with compound **4** in both cases as the less active one. This is in complete agreement with the pharmacological data (Tables I and II).

Compound (8*R*)-**4** is a weak agonist at postsynaptic receptors, weaker than (*R*)-**1** (Tables I and II and ref 2). This is compatible with the higher energy for the agonist conformation of (8*R*)-**4** (Figure 5) and the presence of only a

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methylene group in the "propyl cleft".

Compound **5** has experimentally only been studied as its racemate. The calculated energy for its agonist conformation is 5.5 kcal/mol (Figure 5). This value is 1.5 kcal/mol higher than the corresponding value for **4**. This energy increase should make compound **5** significantly less active than **4** on pre- as well as postsynaptic receptors. This is in agreement with the observed inactivity of **5** as a DA receptor agonist and its low affinity in the receptor binding assays (Table III).

Thus, the shape and dimensions of the receptor cavity as derived from an analysis of compounds **2** and **3e** and the calculated conformational energies for the agonist conformation, according to our model, satisfactorily rationalize the observed DA agonistic properties of compounds **2–5**.

Antagonist Models and Conformations. Previously we rationalized the antagonistic properties of (*S*)-**1** on postsynaptic receptors as the inability of the piperidine ring (equivalent to a methylamino group) in the "downward" direction to stabilize the agonist conformation.[1] We proposed that the molecule instead binds in a lower energy conformation which was determined by superimposition of (*S*)-**1** and the postsynaptic antagonist (4a*R*,10b*S*)-**9** (Chart I).

Independently, Froimowitz et al. developed and published a model for dopaminergic agonist and antagonist activity.[14,19] Their proposed stereochemical requirements for agonist activity are very similar to ours. However, the deduced antagonist conformations were different from those which we proposed.[1] Froimowitz et al. suggested that the antagonist conformation of (*S*)-**1** is a mirror image of the agonist conformation of (*R*)-**1**, the main difference between the two binding modes being different (opposite) directions of the N lone pair (N-H) vector.[14,19]

An unsatisfactory consequence of our previously proposed antagonist pharmacophore is that very high energy, ca. 6 kcal/mol, was calculated for the antagonist (1*S*,2*R*)-**10** in its least-squares fit (including nitrogen lone pairs) with the antagonist conformation of (*S*)-**1**.[1] In the antagonist model proposed by Froimowitz et al. (1*S*,2*R*)-**10** and (*S*)-**1** can be nicely superimposed with (1*S*,2*R*)-**10** in a low-energy conformation.[14] The model proposed by these authors also has the attractive feature that the propyl groups in the enantiomeric pairs are pointing in the same general direction, making it possible for agonist as well as antagonist to position their propyl groups in the "propyl cleft".

For these reasons we have adopted the model proposed by Froimowitz et al. and analyzed compounds **4** and **6** within its framework. We have also reinvestigated compound **1**.

Antagonist Conformation of (*S*)-1. We have employed our proposed agonist conformation of (*R*)-**1**[1] and least-squares fitted (*S*)-**1** to this conformation. In this fitting the interring angle and the propyl group conformation of (*S*)-**1** were varied until an optimal fit of the hydroxyl oxygens, the centers of the aromatic rings, the nitrogen atoms, and the carbon atoms of the propyl group was achieved. The result is shown in Figure 6. The interring dihedral angle (a–b–c–d; Figure 5) of (*S*)-**1** in this fit is 237° and the calculated conformational energy is 1.2 kcal/mol. The mean distance between fitted atoms is 0.19 Å.

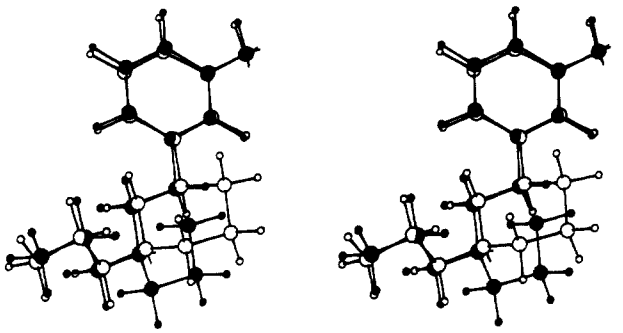

Figure 6. Least-squares superimposition of (*R*)-**1** in its agonist conformation and (*S*)-**1** (filled atoms). The conformation of (*S*)-**1** corresponds to its antagonist conformation.

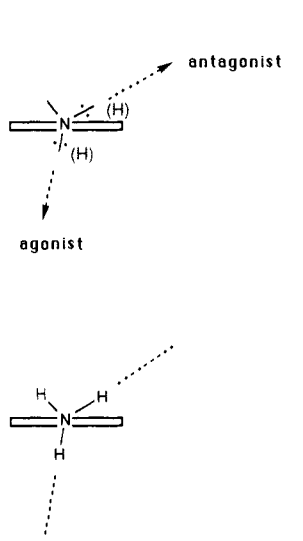

Figure 7. (a) Calculated lone pair (or NH bond) directions for agonist and antagonist binding to the DA receptor and (b) possible NH interactions between protonated dopamine and the DA receptor.

The difference in the conformational energies for (*R*)- and (*S*)-**1** in the superimposition shown in Figure 6 is 1.8 kcal/mol in favor of (*S*)-**1**. This nicely accounts for the higher affinity of (*S*)-**1** (Table III and ref 20) provided that the lone pairs (or N–H bonds) in the two compounds have similar interaction energies with (different) sites in the receptor cavity. This is not unreasonable considering that the lone pair (or N–H bond) directions in the agonist and antagonist pharmacophores, according to the superimposition in Figure 6, differ by 120° (Figure 7a). Thus, they may interact with sites which are used by two of the N–H bonds in protonated DA as shown in Figure 7b. Such a pattern of interactions for agonist and antagonists is an alternative to the interaction at the same site and in the case of antagonists via delocalized charge at the back of the nitrogen atom, as proposed by Froimowitz et al.[14]

The agonist and antagonist conformations of (*S*)-**1** derived above are shown in Figure 8. The antagonist conformation should predominate pharmacologically due to its lower energy and its ability to position its propyl group in the "propyl cleft". Froimowitz and Baldessarini suggest that this conformation predominates until the *N*-alkyl group becomes larger than a propyl.[19] As pointed out by these authors, the conformation of (*R*)-**1** with the *N*-propyl group in the "upward" direction, and with the nitrogen lone-pair pointing in the same direction as in the antagonist conformation of (*S*)-**1**, does not seem to be connected with antagonistic activity. A superimposition of this conformation of (*R*)-**1** and the antagonist conformation of

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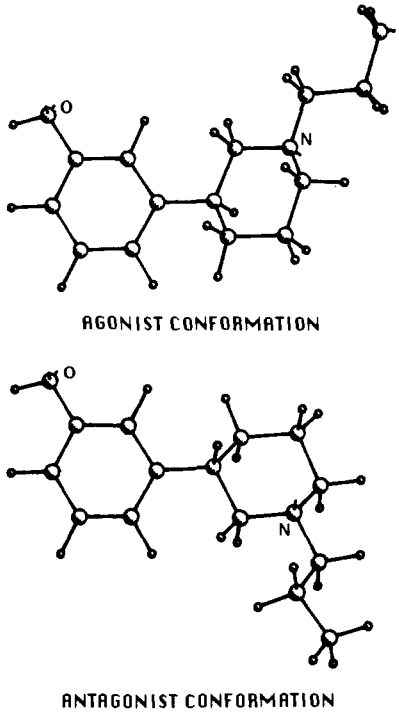
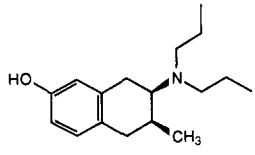


Figure 8. Agonist and antagonist conformations of (*S*)-1.

(*S*)-1 shows a bad fit. The nitrogen atom in (*S*)-1 is in this conformation located 0.2 Å *above* the plane of the phenyl ring, while the nitrogen atom in the corresponding conformation of (*R*)-1 is located 0.8 Å *below* this plane. Thus, the *R* enantiomer is not compatible with the antagonist pharmacophore as defined above. Furthermore, for (*R*)-1 the antagonist conformation has its propyl group in the "upward" direction. This further lowers the probability of this conformation in its interactions with the receptor in comparison with the agonist conformation, which has its propyl group in the "downward" direction interacting with the "propyl cleft".

Johansson et al.[21] observed that the racemate of **11** increases DOPA accumulation in nonpretreated rats, indi-



(2R,3S)-11

cating an antagonistic action at DA receptors. These authors speculate that the DA antagonistic properties reside in the 2*R*,3*S* enantiomer. This is supported by our antagonist model. Compound (2*R*,3*S*)-**11** gives an excellent fit to the antagonist conformation (*S*)-1 as derived above.

Antagonistic Properties of Compounds 6 and (8*S*)-4. The antagonist conformation of (*S*)-1 derived above is supported by the conformational properties of antagonist **6**. The olefinic bond in the bicyclic system of this compound gives the molecule different conformational properties compared to the phenylpiperidines. The calculated potential energy curve for rotation about the partial double bond in **6** is shown in Figure 9. The conformation with a dihedral angle a–b–c–d = 155°, which is

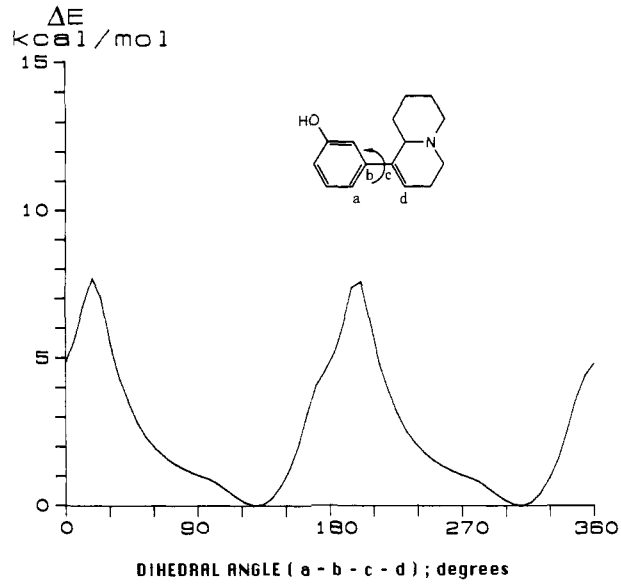


Figure 9. Calculated potential energy curve for rotation about the central bond in compound **6**.

close to the global energy minimum, gives a very good fit between compound **6** and our proposed antagonist conformation of (*S*)-1. The mean distance between fitted points (see Computational Methods) is 0.07 Å and the conformational energy for **6** in this conformation is 1.6 kcal/mol above the global energy minimum. As the energy for a conformation of **6** which fits the agonist conformations of (*S*)-1 is larger than 5 kcal/mol (a–b–c–d = 4°; Figure 9) the antagonist conformation of **6** should predominate pharmacologically, in agreement with experimental data.

According to our model, compound (8*S*)-**4** has a calculated conformational energy for its antagonist conformation of 1.9 kcal/mol, similar to the value calculated for **6**. Accordingly, compound (8*S*)-**4** should have antagonist properties similar to **6**. This is in agreement with in vitro receptor binding data (Table III).

Conclusions

The model for the interaction with central DA pre- and postsynaptic D2-receptors previously described by us[1] and the refinements and extensions to this model reported in the present paper satisfactorily rationalize the dopaminergic profiles of compounds **2**–**6**. The structure–activity analysis of compounds **2** and **3e** indicates that the important "propyl cleft" is located above the plane of the receptor-bound substrate. A new antagonist pharmacophore based on a suggestion made by Froimowitz et al.[14,19] successfully rationalizes the antagonistic properties of the investigated compounds.

Experimental Section

Biochemistry. Normal (Intact) Animals. Animals were injected with test drug (sc) and NSD 1015 (100 mg/kg ip) 65 and 30 min before death, respectively. Controls received corresponding saline injections. Shown in Table II are the doses giving a half-maximal increase (DOPA formation) or decrease of the formation of DOPA or 5-HTP in three different brain regions. Maximal increase or decrease of DOPA formation are shown as percent of controls. Control levels were as follows: limbic region, 426 ± 31 ng/g DOPA, 157 ± 32 ng/g 5-HTP; striatum, 885 ± 90 ng/g DOPA, 99 ± 22 ng/g 5-HTP; cortex, 89 ± 7 ng/g DOPA, 109 ± 20 ng/g 5-HTP (mean ± SEM, n = 4).

Biochemistry. Reserpine-Pretreated Animals. Animals were injected with reserpine (5 mg/kg sc) 18 h, test drug 60 min, and NSD 1015 (100 mg/kg ip) 30 min before death. Controls received corresponding saline injections. ED_{50} values were de-

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termined from dose-response curves comprising four to five dose levels (number of determinations = 4) using the statistical program RSI from BBN Research Systems.[22] Maximal decrease in DOPA was as follows: limbic 65%, striatum 80%, and cortex 50%. Maximal decrease in 5-HTP was as follows: limbic, striatum, and cortex 50%. Some of the compounds displayed partial agonist effects.

Motor Activity. The rats were injected with reserpine (5 mg/kg sc) 18 h before and test drug immediately before the activity session. Shown in Table I are the accumulated activity counts/30 min (mean ± SEM, n = 2-4). Reserpine controls were as follows: 3 ± 1 counts/30 min; n = 13. After the motility experiments the rats were injected with NSD 1015 (see above).

Inhibition of [^{3}H]Spiperone Binding. Inhibition of [^{3}H]-spiperone binding to D2 receptors in rat striatal membranes was determined as described by Hyttel.[23]

Inhibition of [^{3}H]N-0437 Binding. Male Wistar (Mol:Wist) rats (125-250 g) were sacrificed and their corpora striata were dissected and weighed. The tissue was homogenized (Ultra Turrax, 10-15 s) in 10 mL of ice-cold 50 nM Tris buffer, pH 7.5 (at 25 °C), containing 1 mM Na_2 EDTA, 5 mM KCl, and 2 mM $CaCl_2$. The homogenate was incubated for 15 min at 37 °C and then centrifuged twice at 20000g for 10 min at 4 °C, with rehomogenization of the pellet in 10 mL of ice-cold buffer. The final pellet was homogenized in 1600 vol (w/v) of ice-cold buffer. Incubation tubes kept on ice in triplicate received 100 μL of drug solution in water (or water for total binding) and 2000 μL of tissue suspension (final tissue content corresponds to 1.25 mg of original tissue). The binding experiment was initiated by addition of 100 μL of [^{3}H]N-0437 (from Amersham International plc., England, specific activity approximately 80 Ci/mmol, final concentration 0.2 nM) and by placing the tubes in a 25 °C water bath. After incubation for 90 min, the samples were filtered under vacuum (100-200 mbar) through Whatman GF/F filters (25 mm). The tubes were rinsed with 5 mL of ice-cold buffer which was then poured on the filters. Thereafter, the filters were washed with 5 mL of buffer. The filters were placed in counting vials, and 4 mL of appropriate scintillation fluid (e.g. Picofluor 15) was added. After shaking for 1 h and storage for 2 h in the dark, the content of radioactivity was determined by liquid-scintillation counting. Specific binding was obtained by substracting the nonspecific binding in the presence of 1 μM of 6,7-ADTN. For determination of the inhibition of binding, five concentrations of drugs covering 3 decades were used. The measured cpm's were plotted against drug concentration on semilogarithmic paper, and the best fitting s-shaped curve was drawn. The IC_{50} value was determined as the concentration at which the binding is 50% of total binding in control samples minus the nonspecific binding in the presence of 1 μM 6,7-ADTN.

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Synthesis of *N*-[*N*-(4-Deoxy-4-amino-10-methylpteroyl)-4-fluoroglutamyl]-γ-glutamate, an Unusual Substrate for Folylpoly-γ-glutamate Synthetase and γ-Glutamyl Hydrolase

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N-[*N*-(4-Deoxy-4-amino-10-methylpteroyl)-4-fluoroglutamyl]-γ-glutamate has been synthesized and its ability to serve as a substrate for folylpolyglutamate synthetase and γ-glutamyl hydrolase has been investigated. It was anticipated that this compound would be a substrate for both of these enzymes. Although the title compound proved to be a good substrate for folylpolyglutamate synthetase, hydrolysis catalyzed by γ-glutamyl hydrolase was unexpectedly slow. These results suggest the use of fluoroglutamate-containing peptides as hydrolase-resistant folates or antifols in a variety of chemotherapeutic regimens.

Poly-γ-glutamate derivatives of folates have been known for many years.[1] Recently, the role of the naturally occurring folylpoly-γ-glutamates in folate-dependent one-carbon biochemistry[2] and the role of analogous derivatives of antifolates such as methotrexate (MTX) in the cytotoxic action of these drugs[3] have been the subject of extensive investigation. The enzyme which catalyzes the formation of poly-γ-glutamate derivatives of both the naturally occurring folates and the antifolates is folylpoly-γ-glutamate synthetase (FPGS, EC 6.3.2.17), an ATP-dependent enzyme which occurs in all species examined.[4] This enzyme specifically activates the γ-carboxyl group of the C-terminal glutamate in the growing polypeptide via formation of a γ-glutamyl phosphate intermediate.[5] Prior to the determination of the chemical structure and unambiguous chemical synthesis of the folylpoly-γ-glutamates,[6] they were referred to collectively as the folate "conjugates".

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