When this method was applied, it was always verified that the experimental data generated a line whose derived slope was not significantly different from unity ($p > 0.05$).

All the compounds tested and carbachol (carbamoylcholine chloride, Fluka) were dissolved in double-distilled water.

Data are presented as means ± SE of n experiments. Differences between mean values were tested for significance by the Student's t test.

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Registry No. (+)-1, 121757-87-7; (−)-1, 121842-00-0; (+)-**2**, 121842-01-1; (−)-**2**, 121842-02-2; (+)-**3**, 121757-88-8; (−)-**3**, 121842-03-3; (+)-4, 121842-04-4; (−)-4, 121842-05-5; (+)-**5**, 121842-06-6; (−)-**5**, 121842-07-7; (+)-**6**, 121842-08-8; (−)-**6**, 121842-09-9; (+)-**7**, 121842-10-2; (−)-**7**, 121842-11-3; (+)-**8**, 121842-12-4; (−)-**8**, 121842-13-5.

Supplementary Material Available: A table of ^1H NMR and ^{13}C NMR data for racemates 1–8 (Table V) and tables listing atomic coordinates (Table VI), thermal parameters (Table VII), bond angles (Table VIII), and bond lengths (Table IX) for (−)-**2** (6 pages). Ordering information is given on any current masthead page.

Resolved 6,7,8,9-Tetrahydro-*N*,*N*-dimethyl-3*H*-benz[*e*]indol-8-amine: Central Dopamine and Serotonin Receptor Stimulating Properties

Håkan Wikström,*,† Bengt Andersson,† Arne Svensson,† Leslie G. Humber,‡ Andre A. Asselin,‡ Kjell Svensson,§ Agneta Ekman,§ Arvid Carlsson,§ Ingemar Nilsson,∥ and Connie Chidester⊥

Organic Chemistry Unit, Department of Pharmacology, University of Göteborg, S-400 33 Göteborg, Sweden, Wyeth-Ayerst Research, CN8000, Princeton, New Jersey 085403-8000, Department of Pharmacology, University of Göteborg, S-400 33 Göteborg, Sweden, AB Hässle, S-431 83, Mölndal, Sweden, and Department of Physical and Analytical Chemistry, The Upjohn Company, Kalamazoo, Michigan 49001. *Received November 28, 1988*

The enantiomers of 6,7,8,9-tetrahydro-*N*,*N*-dimethyl-3*H*-benz[*e*]indol-8-amine (**1a**) were prepared and tested for their actions on central dopamine and serotonin (5-HT) receptors. The dopaminergic effects were shown to reside in the (+)-*R* enantiomer. It was shown that compound **1a** and its (+)-*R* enantiomer possess potent central 5-HT_{1A} receptor stimulating properties.

Very recently, Wikström et al. presented a study dealing with the question as to which part of the dopaminergic ergolines constitutes the dopaminergic moiety of these structures.[1] In particular, the potential meta hydroxylation in vivo of ergolines and other indole-containing structures was discussed in great detail. The potential dopaminergic stereoselectvity of the 6,7,8,9-tetrahydro-*N*,*N*-dialkyl-3*H*-benz[*e*]indol-8-amines (represented by structures **1a** and **1b**; Scheme I and Table I) was discussed, and it was stated that, if active per se, the dopaminergic effects should reside in the *R* enantiomers. This would be in accordance with the receptor concept of McDermed,[3] on the basis of the stereoselectivities of the potent dopaminergic 2-aminotetralins (*S*)-5-hydroxy-2-(di-*n*-propylamino)tetralin (*S*-5-OH-DPAT, *S*-**4**) and *R*-7-OH-DPAT (*R*-**5**) (Scheme II). The same prediction has previously been made by Asselin et al. from conformational analysis and from fitting **1a** to a dopamine (DA) receptor model, which includes a putative acceptor nucleus, which forms a hydrogen bond with the indolic NH of **1a**.[2] However, if metabolic meta hydroxylation would take place in vivo, the dopaminergic effects of racemic **1a,b** should emanate from the *S* enantiomer of the 5-OH analogue of compounds **1a,b** (structures **2a** and **2b**), again in accordance with McDermed's receptor concept[3] (Scheme II).

The objective of this study was to resolve compound **1a** and test its enantiomers in our biological screening system, involving methods for monitoring locomotor activity and central biochemical effects (DA and 5-HT synthesis rate).

Scheme I. Structures Discussed

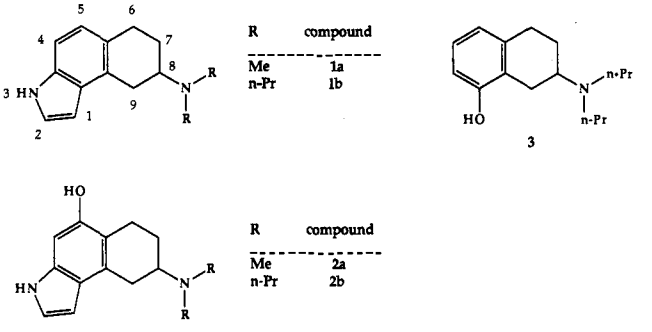

Table I. Physical Data

compd	abs config	$[\alpha]^{20}_D$, deg	% ee	mp, °C
(+)-1a	*R*	+124[a]	94	150–152 dec[b]
(−)-1a	*S*	−109[a]	90	148–150 dec[b]

[a] Determination of optical rotation was performed on the base with (CH_2Cl_2/MeOH 1:1, *c* 0.5). [b] Determination of the melting point was performed on the salt used for resolution, i.e. *R*-1a plus (2*R*,3*R*)-DBTA and *S*-1a plus (2*S*,3*S*)-DBTA, respectively.

In addition, the abilities of the racemate and the enantiomers to displace the radioactively labeled ligands [^{3}H]spiperone (D2 binding), [^{3}H]SCH23390 (D1 binding), and [^{3}H]-8-OH-DPAT (5-HT_{1A} binding) from rat brain homogenate in vitro were tested.

Needless to say, the indirectness of our approach would not allow us to exclude the formation (and possible sub-

† Organic Chemistry Unit, Department of Pharmacology, University of Göteborg.
‡ Wyeth-Ayerst Research.
§ Department of Pharmacology, University of Göteborg.
∥ AB Hässle.
⊥ Department of Physical and Analytical Chemistry, The Upjohn Company.

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0022-2623/89/1832-2273$01.50/0

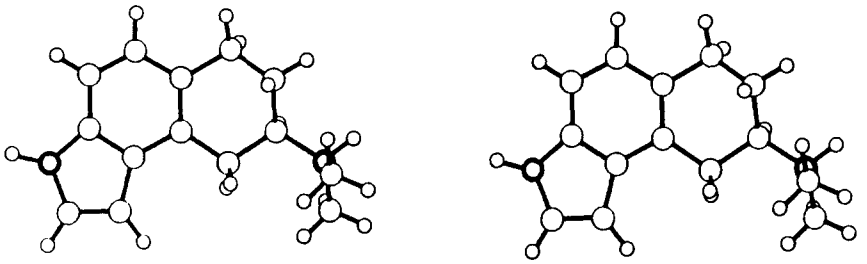
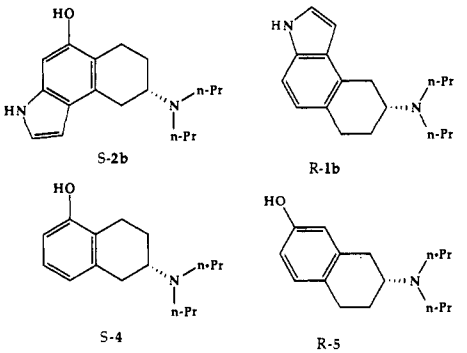


Figure 1. Stereopicture of the X-ray structure of compound (+)-*R*-1a. The dibenzoyltartaric acid part of the structure has been omitted for clarity.

Scheme II. Compounds *S*-**2b**, *S*-**4** and *R*-**1b**, *R*-**5** Arranged Pairwise according to McDermed's Receptor Concept



sequent conjugation) of **2a**. Rather, the approach would indicate the likelihood of **2a** to play a significant role in the biological effects seen after **1a** in vivo. In vitro D2 binding data, excluding metablic events, would indicate if *R*- and/or *S*-**1a** have affinity for this site per se.

Chemistry

6,7,8,9-Tetrahydro-*N*,*N*-dimethyl-3*H*-benz[*e*]indol-8-amine[2] (**1a**) was purified by chromatography (SiO_2 and eluting with MeOH) before any attempts to resolve it were made. Resolution was performed with the enantiomers of di-*O*-benzoyltartaric acid (DBTA) for diastereomeric salt formation and recrystallization from refluxing MeOH/H_2O (6:1). Two crystallizations in each case gave the two enantiomers with high enantiomeric excess (ee). Low-temperature single-crystal X-ray analysis was performed on the (+)-enantiomer (optical rotation is referring to the base form of **1a**) as its (−)-(2*R*,3*R*)-DBTA salt. The analysis showed that this (+)-enantiomer has the *R* absolute configuration (Figure 1).[4]

The determination of ee was performed by NMR analysis of each enantiomer as the base in $CDCl_3$ with the addition of "Pirkle's alcohol" ((+)-1-anthryl-2,2,2-trifluoroethanol).[5]

Pharmacology

Biochemistry. The in vivo biochemical test utilizes the well-established phenomenon of receptor mediated feedback inhibition of the presynaptic neuron.[6] DA and norepinephrine (NE) have the same general biosynthetic pathway, and the synthesis rate of the catecholamines DA and NE is decreased by agonists (and increased by antagonists) at dopaminergic and α-adrenergic receptors, respectively. Similarly, the synthesis rate of 5-HT is inhibited by 5-HT receptor agonists.[7,8] The Dopa accumulation, following decarboxylase inhibition by means of (3-hydroxybenzyl)hydrazine (NSD 1015), was used as an indicator of the DA synthesis rate in the DA-rich areas (i.e. limbic system and corpus striatum) and of the NE synthesis rate in the NE-rich hemispheres (mainly cortex). The 5-HTP accumulation was taken as an indicator of the 5-HT synthesis rate in the three brain areas (Table II).

Table II. Effects on Brain DA and 5-HT Synthesis Rates in Vivo (Subcutaneous Administration)

	ED_{50},[e] μmol/kg					
	Dopa accumltn			5-HTP accumltn		
compd	limb	stri	hem	limb	stri	hem
(±)-**1a**	0.30	0.40	P[a] 4.0	0.30	0.20	0.40a
(+)-*R*-**1a**	0.30	0.40	P[a] 2.0	0.40	0.10	0.40
(−)-*S*-**1a**	4.2	8.0	10.0	3.7	3.7	3.7
(+)-*R*-**3**[b]	I[c]	I	I	0.036	0.047	0.050
(−)-*S*-**3**[b]	I	I	I	0.061	0.065	0.077
(+)-*R*-**5**[d]	0.0095	0.011	I	I	I	I

[a] P means a partial response. Dopa and 5-HT accumulation were 60–75% of those of the controls in the brain areas indicated. [b] Data taken from ref 11. [c] I means inactive at the highest dose given (50 μmol/kg). [d] Data taken from ref 10. [e] Abbreviations: limb = limbic system, stri = corpus striatum, and hem = hemispheres.

Table III. In Vivo Behavioral Data

		motor activity		
compd	abs config	dose, μmol/kg	accumulated counts/30 min[b]	behavioral observation[a]
(±)-**1a**		4.0	90 ± 19*	mixed DA and 5-HT
(+)-**1a**	R	2.0	130 ± 23*	mixed DA and 5-HT
(−)-**1a**	S	12.5	22 ± 7	no change

[a] The gross behavior of the animals was observed during the motility recordings. The DA-mediated behavior was characterized by hyperactivity, sniffing, and licking, whereas the 5-HT mediated behavior consisted of a flat body posture, abducted hind- and forelegs, and forepaw treading, the so-called "5-HT syndrome". [b] Statistics according to Student's *t* test. An asterisk indicates values that are statistically different from controls: $p < 0.05$.

Locomotor Activity. Postsynaptic effects of the test compounds were assessed by the increase in locomotor activity (reserpine pretreatment). Motor-activity recordings were carried out as previously described with the use of motility meters (Table III).[9]

In Vitro Binding. The abilities of the test compounds to displace the radioactively labeled ligands [^{3}H]spiperone, [^{3}H]SCH23390, and [^{3}H]-8-OH-DPAT from D2, D1, and

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Table IV. In Vitro Binding Data

compd	abs config	IC_{50},[a] nM		
		D2[b]	D1[b]	5-HT_{1A}
(±)-1a		1840 ± 51	19700 ± 4400	82.0 ± 18
(+)-1a	*R*	1050 ± 250	20400 ± 2200	31.0 ± 7.0
(−)-1a	*S*	9510 ± 3500	29200 ± 3800	244 ± 89
(±)-3		5760 ± 810	>100000[c]	4.46 ± 1.1
TDHL[d]		4	69	25

[a] Based on three determinations. [b] The displacement curves were not analyzed according to Seeman and Niznik (D1 and D2 high and low, respectively).[16] Instead, the IC_{50} values were estimated by taking the whole displacement curves into consideration. [c] Only a single determination was made. [d] TDHL means *trans*-dihydrolisuride and was included as a reference compound in this study. The IC_{50} estimations of TDHL come from our binding screening, in which we make three measurements in each concentration with tissue from one single rat (n = 1). Therefore, no statistical evaluation was possible in this case.

5-HT_{1A} sites, respectively, in homogenized rat brain were assessed in vitro.

Results and Discussion

As seen from Table II, the more active enantiomer of compound **1a**, with regard to the dopaminergic activity in vivo, is the (+)-*R* enantiomer ((+)-*R*-**1a**). The (−)-*S* enantiomer ((−)-*S*-**1a**) is about 10 times less potent in this assay. This result supports the prediction initially made by Asselin et al.[2] that the indole NH functionality is isosteric with a phenolic group. Accordingly, compound (+)-*R*-**2b** should be compared to the potent aminotetralin (+)-*R*-7-OH-DPAT ((+)-*R*-**5**; Scheme II).[10] The pharmacological significance of the potential metabolic activation of **1a**, as proposed by Wikström et a.,[1] seems therefore less important. This is further indicated by the stereoselectivity exhibited by the *R*-**1a** enantiomer in the D2 affinity binding assay (Table IV). These results cannot exclude the formation (and potential conjugation) of the enantiomers of **2a** after the in vivo administration of **1a**.

Very interestingly, Table II shows that racemic **1a** and its (+)-*R* enantiomer are very potent 5-HT receptor agonists in the central nervous system. They are 2–8 times less potent than the reference compound (+)-*R*-8-OH-DPAT ((+)-*R*-**3**), one of the most potent and selective 5-HT_{1A} agonists known.[11] The biochemically measured DA and 5-HT receptor agonist potencies (ED_{50} values) of **1a** and its *R* enantiomer in vivo are about equal. The 5-HT receptor agonist effects of compound **1a** have not been reported previously. The stereoselectivity of the (+)-*R* enantiomer, as measured by the ratio of biochemical potency of *R* versus *S* enantiomers, is about 10 with regard to both dopaminergic and serotonergic effects. One might speculate that the serotonergic effects monitored for compound **1a** come from the interaction between the 5-HT receptor surface and the ammonium nitrogen, the aromatic system, and the high density of electronegativity in the indole 3-position (corresponds to position 1 in compounds **1a** and **1b**; Scheme I). That position is equivalent to the oxygen position (8-position) in compound **3** (Scheme I).

As shown in Table III, racemic **1a** readily counteracted the reserpine-induced akinesia. As judged from gross behavioral observations, **1a** produced effects mediated both via central DA and 5-HT receptors: the rats displayed hyperlocomotion, sniffing, flat body posture, and forepaw treading. The same "mixed" behavioral effects were noted for compound *R*-**1a**. These behavioral observations support the lack of D2/5-HT_{1A} selectivity of these two drugs in the in vivo and in vitro tests. Compound *S*-**1a** was essentially inactive regarding the behavioral stimulatory effects in the reserpine pretreated animals. This is also in line with its low potency in the other tests.

It might have been expected that compound **1a**, like some other compounds containing the indole moiety (e.g. some erglines like the reference compound TDHL in Table IV), would display some D1 properties. However, as seen from Table IV, neither racemic **1a** nor either of its enantiomers has affinity for D1 receptor sites.

In summary, the resolution of compound **1a**, the X-ray analysis of (+)-**1a**, and the pharmacological testing in vivo and in vitro of **1a** and its enantiomers showed that the dopaminergic, as well as the serotonergic, effects reside mainly in the (+)-*R* enantiomer. On the basis of the in vitro binding data, it might be argued that these effects are likely to emanate from (+)-*R*-**1a** itself and not from any potential metabolite. Preliminary plasma-concentration data from this laboratory[12] indicate that **1a** has a good oral availability, excluding an extensive first pass elimination.

Experimental Section

Chemistry. Melting points (uncorrected) were determined with a melting point microscope (Reichert Thermovar). ^{1}H NMR spectra for determination of ee were recorded with a Bruker 500-MHz instrument (Me_4Si).

GC was performed with a HP 5830A instrument with a flame-ionization detector. A fused silica column (11 m, 0.22-mm i.d.) coated with cross-linked SE-54 (film thickness 0.3 μm, gas H_2, gas velocity 40 cm/s) was used throughout.

GC/MS spectra were recorded on a HP 5970A Mass Selective Detector working at 70 eV and interfaced with a HP 5700A gas chromatograph. All spectra were in accordance with the assigned structures.

Optical rotations were measured on a Perkin-Elmer 241 polarimeter at room temperature.

The elemental analyses for new crystalline substances (C, H, N) were within 0.4% of the theoretical values. For purity tests, TLC was performed with fluorescent silica gel plates developed in at least two different systems. For all the compounds, only one spot (visualized by UV light and I_2 vapor) was obtained.

(+)-(8*R*)-6,7,8,9-Tetrahydro-*N*,*N*-dimethyl-3*H*-benz[*e*]indol-8-amine ((+)-*R*-1a). Racemic **1a**[2] (3.0 g, 14.0 mmol) was purified by column chromatography (SiO_2, eluting with MeOH) before the resolution procedure was initiated. A total of 2.82 g of light brown crystals of the base was obtained. Part of these crystals (1.29 g, 6.0 mmol) was dissolved in warm 95% EtOH and mixed with a solution of (−)-(2*R*,3*R*)-di-*O*-benzoyltartaric acid [(−)-(2*R*,3*R*)-DBTA, MW 358, 2.15 g, 6.0 mmol] dissolved in warm 95% EtOH. The solvent was evaporated and the residue was dissolved in a refluxing mixture of MeOH (60 mL) and water (10 mL). Large crystals had formed after a night at room temperature. These crystals were filtered, dried, weighed (1.20 g), and recrystallized again from MeOH/H_2O (6:1), affording large crystals (0.66 g, 19%, mp 150–152 °C) after 3 days. Part of these crystals (80 mg) were converted to the base with 10% Na_2CO_3 and extracted with CH_2Cl_2, leaving 24.1 mg of the base as crystals, which were dissolved in CH_2Cl_2/MeOH (1:1, 4.82 mL, *c* 0.5), and the optical rotation was monitored ($[\alpha]^{20}_D$ = +124°).

(−)-(*S*)-6,7,8,9-Tetrahydro-*N*,*N*-dimethyl-3*H*-benz[*e*]indol-8-amine ((−)-*S*-1a). The solvent of the mother liquor from the last crystallization in the preparation of (+)-*R*-**1a** was evaporated and the residue was basified and the base showed $[\alpha]^{20}_D$ = −90°. This base (410 mg, 1.9 mmol) in warm 95% EtOH was mixed with (+)-(2*S*,3*S*)-DBTA·H_2O (MW 376, 720 mg, 1.9 mmol) dissolved in warm 95% EtOH. The solvent of this mixture was

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evaporated and the residue was recrystallized from refluxing $MeOH/H_2O$ (6:1). After 3 days at room temperature, crystals (610 mg, 18%) were filtered and dried. Part of these crystals (55.5 mg) were converted to the base with 10% Na_2CO_3 and extracted with CH_2Cl_2, leaving 17.2 mg of the base as crystals, which were dissolved in CH_2Cl_2/MeOH (1:1, 3.44 mL, c 0.5) and the optical rotation was monitored ($[\alpha]^{20}_D = -109°$).

Chemical Shift Assignment for the Enantiomers of 1a. The spectra of (+)-*R*-**1a** and (−)-*S*-**1a** were recorded on a Bruker AM 500-MHz spectrometer. NOE difference measurements, 2D ^1H-^1H, and ^1H-^{13}C correlation experiments were used to aid in the chemical shift assignments. Data are given for (+)-*R*-**1a**: ^1H NMR (500 MHz, $CDCl_3$, 30 °C) δ 1.70, 2.20 (2 H, 2 m, 7-CH_2), 2.45 (6 H, s, 8-$N(CH_3)_2$), 2.78 (1 H, m, 8-CH), 2.93, 3.24 (2 H, 2 m, 9-CH_2), 2.97 (2 H, m, 6-CH_2), 6.52 (1 H, d of d, J = 2.1, 3.3 Hz, 1-CH), 6.94 (1 H, d, J = 8.2 Hz, 5-CH), 7.17 (2 H, m, 2-CH, 4-CH), 8.48 (1 H, br s, 3-NH).

Determination of Enantiomeric Excess (ee). The ee of (+)-*R*-**1a** and (−)-*S*-**1a** were determined by the use of a chiral shift reagent, "Pirkle's alcohol" ((+)-1-anthryl-2,2,2-trifluoroethanol).[5] With 3 molar equiv of this alcohol, the ^1H NMR spectrum of **1a** exhibited two well-separated signals at δ 6.40 and 6.44, respectively for the 1-CH resonance. The ee of (+)-*R*-**1a** and (−)-*S*-**1a** were determined to be 94% and 90%, respectively. The accuracy of the determinations was estimated to be better than 3%.

Pharmacology. Animals. Animals used in the biochemical and motor-activity experiments were male rats of the Sprague-Dawley strain (ALAB, Sollentuna, Sweden), weighing 200–300 g.

Materials. All substances to be tested were dissolved in saline immediately before use, occasionally with the addition of a few drops of glacial acetic acid and/or moderate heating in order to obtain complete dissolution. Reserpine was dissolved in a few drops of glacial acetic acid and made up to volume with 5.5% glucose solution. Injection volumes were 5 mL/kg, and all solutions had neutral pH at the time of injection (except for the solutions of reserpine).

Biochemistry. The biochemical experiments and the determinations of Dopa and 5-HTP by means of HPLC with electrochemical detection were performed as previously described.[13] Separate dose-response curves based on four to six dose levels (n = 4) for each substance (sc administration) and each brain area were constructed. From these curves, the dose of the drug yielding a half-maximal decrease (ED_{50} value) of the Dopa (maximal effects, expressed as percent of controls, were limbic system = 35, striatum = 20, and the hemispheres = 50) and the 5-HTP (maximal effects, expressed as percent of controls, were limbic system = 50, striatum = 50, and the hemispheres = 50) levels were estimated separately (Table II).

Motor Activity. The motor activity was measured by means of photocell recordings (M/P 40 Fc Electronic Motility Meter, Motron Products, Stockholm) as previously described.[9] Eighteen hours prior to the motility testing (carried out between 9 a.m. and 1 p.m.), the rats were subcutaneously injected in the neck region with reserpine (5 mg/kg). The different test compounds were also administered subcutaneously in the neck region (n = 4). Immediately after drug administration, the rats were placed in the test cages (one rat/cage) and put into the motility meters. Motor activity was then followed and recorded for the subsequent 30 min (Table III).

5-HT_{1A} Radioligand Binding.[14] Male Sprague-Dawley rats (160–225 g) were killed by decapitation and the whole brain with the exception of the brainstem and cerebellum was rapidly removed, weighed, and chilled in ice-cold 0.9% NaCl. Each brain was homogenized (Ultra-Turrax, 20 s) in 10 mL of ice-cold 50 mM Tris buffer (pH 8.0 at 25 °C) containing 120 mM NaCl, 4 mM $CaCl_2$, and 4 mM $MgCl_2$ and centrifuged at 20000g at 4 °C for 10 min. Pellets were resuspended in 10 mL of fresh buffer and preincubated for 10 min in a 37 °C water bath and then recentrifuged. Final pellets were homogenized in 100 volumes (w/v) of Tris buffer (as described above) containing 10 μM pargyline. The incubation tubes were kept on ice in triplicates and received 100 μL of drug solution in water (or water for total binding) and 1000 μL of membrane suspension (corresponds to 10 mg of original tissue). The binding experiment was initiated by addition of 100 μL of [^3H]-8-OH-DPAT (specific activity 143–158 Ci/mmol) in ascorbic acid (the final incubation concentration was 1 nM [^3H]-8-OH-DPAT in 0.1% ascorbic acid). After incubation for 15 min at 37 °C, the reaction was terminated by separation of the free radioligand from bound by rapid vacuum filtration using cell harvester equipment (O.M. Teknik, Denmark). The tubes were rinsed with 4 mL of ice-cold 0.9% NaCl and the filters (Whatman GF/F 25 mm) were washed twice with 4 mL of ice-cold 0.9% NaCl.

The radioactivity of the filters was measured in a liquid scintillation counter (efficiency 41%) in 5 mL of Picofluor TM15. Specific binding (70–75% of total binding) was defined as the radioactivity displaced by 10 μM 5-HT. IC_{50} values were calculated by semilog plot and linear-regression analysis.

D1 Radioligand Binding.[15] Binding of [^3H]SCH 23390 (66 Ci/mmol) to rat striatal membranes was, with a few exceptions, determined as described by Hyttel and Arnt.[15] Briefly, membranes were isolated in 50 mM potassium phosphate buffer (pH 7.4 at 25 °C) by homogenization (final pellets were homogenized in 2000 volumes) and centrifugation at 25000g. The membrane suspension was incubated with 0.2 nM [^3H]SCH 23390 in a final volume of 2.2 mL (1 mg of original tissue) for 60 min at 30 °C. The incubation was terminated by filtration as described above. Specific binding (>90% of total binding) was defined as the radioactivity displaced in the presence of 1 μM *cis*-(*Z*)-flupentixol.

D2 Radioligand Binding. Preparation of rat striatal membranes for [^3H]spiperone (specific activity 21–24 Ci/mml) binding was carried out in a similar manner as described for D1 binding.[15] The final pellets were homogenized in 1300 volumes of 50 mM potassium phosphate buffer and the membrane suspension was incubated with 0.5 nM [^3H]spiperone in a final volume of 4.2 mL (3 mg of original tissue) for 10 min at 37 °C. Specific binding was 70–80% of total binding and was obtained by adding 10 μM 6,7-ADTN to the membrane suspension.

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Registry No. (±)-**1a**, 121784-56-3; (+)-(*R*)-**1a**, 121675-44-3; (−)-(*S*)-**1a**, 121675-45-4.

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