# Enantiomers of 3-(3,4-Dihydroxyphenyl)- and 3-(3-Hydroxyphenyl)-*N*-*n*-propylpiperidine: Central Pre- and Postsynaptic Dopaminergic Effects and Pharmacokinetics

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This study emphasizes the importance of the metabolic conversion of the enantiomers of 3-(3-hydroxyphenyl)-*N*-*n*-propylpiperidine (3-PPP) into their catechol analogues, the enantiomers of 3-(3,4-dihydroxyphenyl)-*N*-*n*-propylpiperidine. These isomers are both shown to be excellent substrates for COMT, with a slight preference for the *S*-(−) entantiomer. Assessment of the dopaminergic activity of these catechols and the results from the determination of brain levels of the enantiomers of 3-PPP and their metabolites indicate that the metabolites probably do not alter the pharmacological profiles established for (*R*)-(+)- and (*S*)-(−)-3-PPP. The conversion of the monophenols into catecholic metabolites is only 1–5%, and the further conversion of these catecholic metabolites into methoxylated analogues is very rapid. However, the very interesting observation was made that, when inhibiting COMT by means of tropolone and subsequently treating the rats with high doses of (*S*)-(−)-3-PPP (ip), postsynaptic dopaminergic activity was elicited. This has never been seen for (*S*)-(−)-3-PPP without tropolone pretreatment and might indicate that, in this special case, the catecholic metabolite affects the in vivo pharmacological profile of (*S*)-(−)-3-PPP.

Several studies have been published on the pharmacological effects of the enantiomers of 3-(3-hydroxyphenyl)-*N*-*n*-propylpiperidine (3-PPP, **1**). In vivo studies showed that *S*(−)-**1** is an agonist at presynaptic dopamine (DA) receptors (DA autoreceptors)[1] but an antagonist at postsynaptic DA receptors. *R*(+)-**1**, on the other hand, was found to have agonistic activity at both receptor sites.[2-4] However, other studies on the enantiomers of **1** failed to demonstrate agonistic effects of *S*(−)-**1**, which acted as an antagonist, while agonistic activity of *R*(+)-**1** was found for postsynaptic and D2 receptors but was difficult to establish for presynaptic and D1 receptors.[5-7] The problems concerning the interpretation of these data have recently been thoroughly discussed in a review.[2]

It has been suggested that metabolic activation of **1** might be responsible for the discrepancies observed between in vivo and in vitro activities.[3-8] One possible metabolite, the catechol analogue of **1**, 3-(3,4-dihydroxyphenyl)-*N*-*n*-propylpiperidine (**2**), has previously been shown to exhibit both pre- and postsynaptic agonistic effects.[9]

Recently, we found that hydroxylation of **1** to **2** does take place in vitro and in vivo with a slight stereoselective preference for the *S* enantiomer.[8] Compound **2** was also shown to be an excellent substrate for catechol-*O*-methyltransferase (COMT), which might explain the low brain levels of **2** after administration of **1**.[8] Compound **2** was only detectable after inhibition of COMT with tropolone in these experiments.

In order to study the enantiomers of **2** and the in vivo relationship between **1** and **2** in more detail, we decided to resolve compound **2** and determine the absolute configuration of the enantiomers of **2** by means of X-ray single-crystal analysis and to study their dopaminergic effects.

The enantiomers of **2** were tested in vivo for their ability to stimulate central pre- and postsynaptic DA receptors in models distinguishing between these two receptor types. In addition, these enantiomers were tested for their susceptibilities to O-methylation by COMT both in vivo and in vitro. Brain levels of the enantiomers and their corresponding O-methyl metabolites were determined by HPLC with electrochemical detection. Since our assays also give data on the ability of the compounds tested to stimulate central 5-HT receptors, we include this information for completeness.

**Table I.** Physical Data

| compd | yield, % | mp, °C | $[\alpha]^{22}_D$, deg (MeOH, *c* 1.0) | formula |
|---|---|---|---|---|
| *S*-**3** | 59 | 175–177 | +7.8 | $C_{13}H_{19}NO_2$·HCl |
| *R*-**3** | 59 | 170–172 | −6.3 | $C_{13}H_{19}NO_2$·HCl |
| *S*-**4** | 69 | oil | −8.3 | $C_{16}H_{25}NO_2$·HCl |
| *R*-**4** | 48 | oil | +7.5 | $C_{16}H_{25}NO_2$·HCl |
| *S*-**2**[a] | 93 | 212–213 | −9.3 | $C_{14}H_{21}NO_2$·HBr |
| *R*-**2**[a] | 94 | 210–211 | +9.2 | $C_{14}H_{21}NO_2$·HBr |

[a] 500-MHz spectrum was recorded for racemic **2**·HBr (MeOH-$d_4$): δ 1.0 (t, 3 H), 1.6–2.1 (m, 6 H), 2.88–3.03 (m, 3 H), 3.08 (t, 2 H), 3.5 (d, 1 H), 3.6 (d, 1 H), 6.61 (dd, 1 H), 6.73 (d, 1 H), 6.74 (d, 1 H).

**Chemistry.** A new coupling reaction between 1-iodo-3,4-dimethoxybenzene and 3-pyridylcopper was used for

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**Table II.** Biochemical Effects of the Enantiomers of 1 and 2

| compd | Dopa accumulation:[a] $ED_{50}$, μmol/kg limb. | Dopa accumulation:[a] $ED_{50}$, μmol/kg stri | DOPAC:[b] % of controls, stri | HVA:[b] % of controls, stri |
|---|---|---|---|---|
| *R*-1 | 1.0[c] | 1.3[c] | 50 ± 3*[d] | 32 ± 3* |
| *S*-1 | 0.8[c] | 1.7[c] | 118 ± 7 ns[d] | 123 ± 11 ns |
| *R*-2 | 6.2 | 7.0 | 65 ± 1* | 49 ± 10* |
| *S*-2 | 8.5 | 8.2 | 83 ± 7 ns | 65 ± 5* |

[a] For details see Experimental Section. No effects on Dopa formation were seen in the hemispheres (indicating that no central α-adrenergic effects were elicited by the test compounds) and no effects on 5-HTP formation were seen in any of the three brain areas for any of the compounds tested (indicating that central 5-HT neurons are not stimulated by the test compounds). [b] Dose was 45 μmol/kg sc. Control values were DOPAC 1.05 ± 0.04 μg/g of tissue and HVA 0.80 ± 0.07 μg/g of tissue. [c] Data taken from ref 11. [d] Statistics according to Student's *t* test. * means $p \leq 0.05$ and ns means not significantly different from controls.

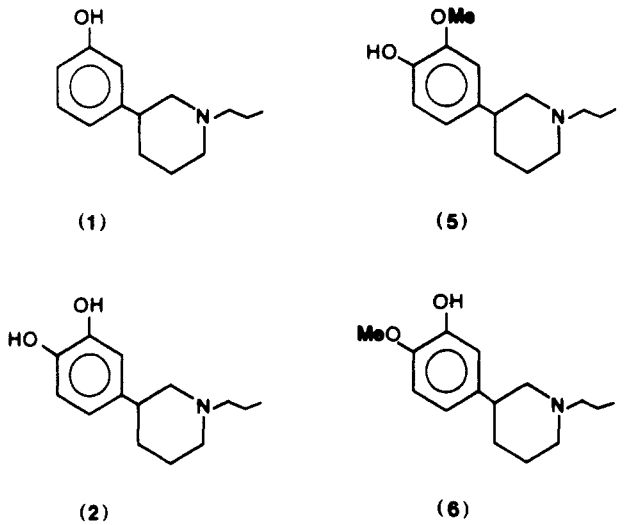


**Figure 1.**

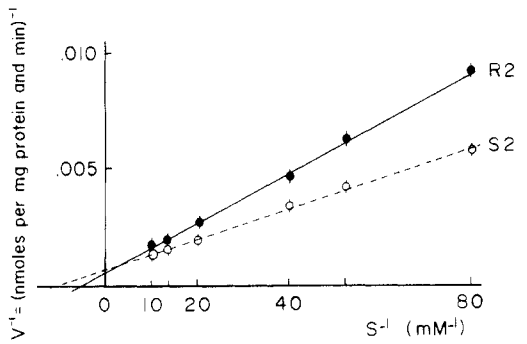


**Figure 2.** Lineweaver–Burk plots for the formation of the enantiomers of 5 from the enantiomers of 2. Data represent means ± SEM of nine experiments.

the formation of 3-(3,4-dimethoxyphenyl)pyridine.[9] This reaction was performed by Hallnemo et al.,[10] who kindly supplied us with this starting material. Catalytic reduction (Pd/C 10%) yielded the piperidine derivative (3), which was resolved by the chromatographic method previously described[11] with use of (*R*)-(−)-α-methoxyphenylacetyl chloride as acylating agent. N-Alkylation was performed by refluxing in benzene a mixture of $NaBH_4$, propionic acid, and either of the enantiomers of 3, yielding the methoxylated enantiomers of 4. These were converted into the enantiomers of 2 by 48% aqueous HBr.

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**Table III.** Motor Activity Effects in Reserpinized Rats of the Enantiomers of 1 and 2, with or without Tropolone Pretreatment

| compd | reserpine pretreatment[a] dose, μmol/kg, sc | reserpine pretreatment[a] acc counts[c] | reserpine plus tropolone pretreatment[b] dose, μmol/kg | reserpine plus tropolone pretreatment[b] acc counts[c] |
|---|---|---|---|---|
| saline | | 4 ± 1 | | 5 ± 2 ns[d] |
| *R*-1 | 13 | 78 ± 14*[e] | | |
| *S*-1 | 213 | 12 ± 2*[e] | 10[f] | 23 ± 12 ns |
| | | | 50[f] | 77 ± 13* |
| *R*-2 | 50 | 24 ± 8* | 5[g] | 20 ± 4* |
| *S*-2 | 50 | 78 ± 8* | 5[g] | 69 ± 20* |

[a] Rats pretreated with reserpine 18 h before drug treatment. [b] Rats pretreated with reserpine (see *a* above) and tropolone 30 min before drug treatment. [c] Accumulated counts 0–30 min after drug treatment. Data are given as means ± SEM ($n$ = 3–10). [d] Statistics according to footnote *d*, Table I. [e] Data taken from ref 11. [f] Administered ip. [g] Administered sc.

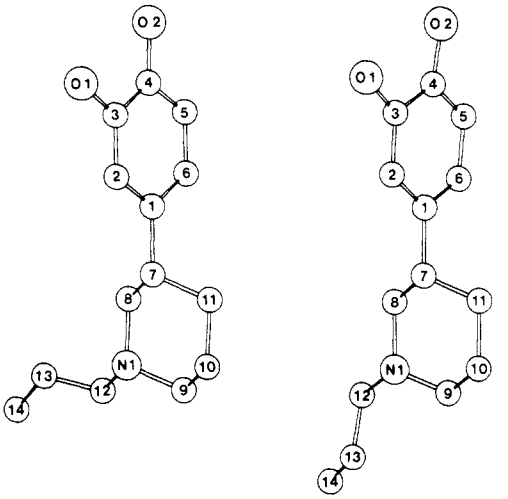


**Figure 3.** Molecular conformation and atom numbering system of the two independent conformations of *S*-2, A (left) and B (right). The plane defined by C(1), C(7), and C(11) is parallel with the plane of the paper.

Single-crystal X-ray analysis was performed on (−)-3-(3,4-dihydroxyphenyl)-*N*-*n*-propylpiperidine hydrobromide ((−)-2·HBr), which was shown to have the *S* absolute configuration.

**Pharmacology.** One in vivo biochemical test utilizes the well-established phenomenon of receptor-mediated feedback inhibition of the presynaptic neuron.[12] Thus, the synthesis rate of the catecholamines DA and norepinephrine (NE) is inhibited by agonists (and activated by antagonists) at dopaminergic and α-adrenergic receptors, respectively. Similarly, the synthesis rate of 5-HT

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**Table IV.** Brain Levels of the Enantiomers of **2**, with or without Tropolone Pretreatment (50 mg/kg ip, 30 min before Administration of **2**), and **5** in Striatum, Cerebellum, and Cortex, 60 min after the Injection (sc) of the Enantiomers of **2**[a]

| | | levels of **2**, pmol/g of tissue | | | | | | levels of **5**, pmol/g of tissue | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | no pretreatment | | | tropolone pretreatment | | | | | |
| compd | dose, μmol/kg | stri | cer | cort | stri | cer | cort | stri | cer | cort |
| *R*-**2** | 8 | ND[b] | 22 ± 3.0 | 18 ± 3.9 | 1380 ± 25 | 1910 ± 109 | 2050 ± 440 | 1620 ± 90 | 1550 ± 170 | 2750 ± 180 |
| | 40 | 112 ± 21 | 150 ± 21 | 227 ± 102 | 5850 ± 330 | 7200 ± 340 | 8470 ± 300 | 6000 ± 750 | 4340 ± 250 | 9540 ± 640 |
| *S*-**2** | 8 | ND | 10 ± 3.9 | 16 ± 2.5 | 2535 ± 450 | 2290 ± 265 | 3250 ± 68 | 2330 ± 250 | 2020 ± 205 | 3410 ± 375 |
| | 40 | 61 ± 8.6 | 63 ± 9.7 | 167 ± 86 | 9720 ± 560 | 9330 ± 720 | 13200 ± 730 | 10330 ± 255 | 9160 ± 275 | 17030 ± 310 |

[a] Values are means ± SEM ($n$ = 3). [b] ND: not determined.

**Table V.** Brain Levels of the Enantiomers of **1** and **5** in Striatum, Cerebellum, and Cortex, 60 min after the Injection (ip and sc) of the Enantiomers of **1**[a]

| | | levels of **1**, pmol/g of tissue | | | levels of **5**, pmol/g of tissue | | |
|---|---|---|---|---|---|---|---|
| compd | dose, μmol/kg | stri | cer | cort | stri | cer | cort |
| *R*-**1** | 45 (ip) | 7040 ± 860 | 4910 ± 570 | 7410 ± 815 | 151 ± 12 | 98 ± 11 | 243 ± 9 |
| *S*-**1** | 45 (ip) | 5380 ± 755 | 3430 ± 360 | 5790 ± 870 | 174 ± 35 | 104 ± 7 | 280 ± 40 |
| *R*-**1** | 45 (sc) | 17550 ± 810 | 15200 ± 245 | 18340 ± 1030 | 93 ± 21 | 114 ± 15 | 211 ± 21 |
| *S*-**1** | 45 (sc) | 20330 ± 3190 | 16130 ± 830 | 21080 ± 1980 | 89 ± 27 | 174 ± 37 | 243 ± 12 |

[a] Values are means ± SEM ($n$ = 3).

**Table VI.** Kinetic Constants of O-Methylation (Probably Meta) of the Enantiomers of **2** by COMT[a]

| compd | $K_m$, mM | $V_{max}/K_m$ |
|---|---|---|
| *R*-**2** | 0.121 ± 0.02 | 346 ± 67 |
| *S*-**2** | 0.077 ± 0.01 | 485 ± 74 |
| ratio *S*/*R* | 0.64 | 1.40 |

[a] Data were obtained with the concentration range 0.1–0.00125 mM substrate. Each concentration was measured in triplicate; results shown are means ± SEM ($n$ = 4).

is inhibited by 5-HT receptor agonists.[13,14] The Dopa accumulation, following decarboxylase inhibition by means of 3-hydroxybenzylhydrazine (NSD 1015), was thus used as an indicator of the DA-synthesis rate in the DA-rich areas (i.e., limbic system, corpus striatum) and the NE-synthesis rate in the NE-dominant hemispheres (mainly cortex). The 5-HTP accumulation was taken as an indicator of the 5-HT-synthesis rate in the three brain areas. A second biochemical test measures the presynaptic activity at the level of DA metabolism by determining the effects of the test compounds on the striatal levels of the DA metabolites DOPAC and HVA, which are decreased after agonists and increased after antagonists. These metabolite levels were measured after administration of the drugs without pretreatment (Table II).

Postsynaptic effects of the compounds were assessed by behavioral tests: an increase in locomotor activity (with or without reserpine pretreatment) and the induction of stereotyped behavior are believed to be due to the stimulation of postsynaptic DA receptors. The occurrence of stereotyped behavior was only qualitatively scored by gross behavioral observations during the various experiments. Motor activity recordings were carried out as previously described with the use of motility meters[15,16] (Table III).

The in vitro affinities of the compounds tested for DA receptors were determined by measuring their abilities to displace [$^3$H]-DP-5,6-ADTN and [$^3$H]-SCH 23390 from striatal dopaminergic binding sites[17,18] and to stimulate cAMP formation in striatal homogenates[19] (Table VII).

**Brain Concentrations and in Vitro O-Methylation.** Concentrations of the enantiomers of **1** and **2** and their O-methylated metabolites were measured by HPLC with electrochemical detection[8] in three brain regions after the administration of the test compounds with and without pretreatment with the COMT inhibitor tropolone. The rate of O-methylation was estimated in vitro by incubation of the enantiomers with COMT and the methyl donor *S*-adenosyl-L-methionine and determination of the formed *O*-methyl derivatives by HPLC and electrochemical detection, as previously described[8] (Tables IV and V).

## Results and Discussion

**Brain Levels and O-Methylation of 2.** As reported earlier,[8] compound **2** is extensively O-methylated by COMT in vitro to two isomeric methoxy hydroxy compounds (**5** and **6**). The first-eluting peak in the chromatograms from the incubation with racemic **2** and its enantiomers is identical with the methoxy hydroxy compound found in the in vivo samples. As in vivo catecholamines are mainly meta-O-methylated, we assume that this peak represents 3-(4-hydroxy-3-methoxy)-*N*-*n*-propylpiperidine (**5**). Figure 2 shows Lineweaver–Burk plots for the formation of the meta-O-methylated compounds (**5**) from the enantiomers of **2**. The kinetic constants calculated from four separate experiments are given in Table VI. Although both enantiomers are obviously excellent substrates for COMT, it is evident that *S*-**2** is the better substrate, its $K_m$ value being about 2-fold lower and its apparent first-order rate constant ($V_{max}/K_m$) about 1.4-fold higher than those of *R*-**2**.

The differences in extent of O-methylation between the enantiomers of **2** found in vitro are in good agreement with the in vivo results, as reflected by the differences in levels of **2** and **5** after the injection of *R*-**2** and *S*-**2**, respectively. Concentrations of compound **2** in three brain regions 60 min after various doses of subcutaneously administered *R*-**2** and *S*-**2** are given in Table IV, together with estimated concentrations of the O-methylated metabolites (**5**) of these

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**Table VII.** In Vitro Affinities of the Enantiomers of **2** for D1 and D2 Receptors in Rat Striatal Homogenates[a]

| compd | $K_i$, [$^3$H]-DP-5,6-ADTN, $\mu$M | $K_i$, [$^3$H]-SCH 23390, $\mu$M | $K_m$, adenylate cyclase, $\mu$M | maximal cAMP production, pmol (mg of protein)$^{-1}$ min$^{-1}$ |
|---|---|---|---|---|
| *R*-**2** | 0.011 | 57.8 | 65 | 46 |
| *S*-**2** | 0.012 | 13.5 | 21 | 138 |
| DA | 0.015 | 4.4 | 7.8 | 164 |
| *R*-**1** | 0.189 | | | |
| *S*-**1** | 0.086 | | | |

[a] D2: $K_i$ ($\mu$M) for [$^3$H]-DP-5,6-ADTN displacement. D1: $K_i$ ($\mu$M) for [$^3$H]-SCH 23390 displacement; $K_m$ ($\mu$M) for adenylate cyclase stimulation of cAMP formation (pmol (mg of protein)$^{-1}$ min$^{-1}$). For comparison, data for dopamine (DA) are also given.

**Table VIII.** Atomic Fractional Coordinates and Equivalent Isotropic Temperature Factors ($\times 10^2$) for the Non-Hydrogen Atoms[a]

| atom | *x* | *y* | *z* | $U_{eq}$ |
|---|---|---|---|---|
| | | Conformation A | | |
| Br(1) | 0.0316 (2) | −0.7096 (−) | 0.8065 (2) | 7.9 (0.2) |
| N(1) | 0.1910 (14) | −0.4929 (17) | 0.9672 (13) | 7.2 (0.8) |
| O(1) | 0.6993 (12) | −0.7141 (16) | 0.8112 (10) | 7.1 (0.7) |
| O(2) | 0.8740 (13) | −0.5578 (16) | 0.9378 (12) | 8.0 (0.8) |
| C(1) | 0.5236 (17) | −0.5925 (19) | 0.9759 (15) | 6.1 (0.9) |
| C(2) | 0.5502 (17) | −0.6606 (20) | 0.8991 (15) | 6.1 (0.9) |
| C(3) | 0.6676 (18) | −0.6483 (19) | 0.8865 (15) | 5.8 (0.9) |
| C(4) | 0.7565 (17) | −0.5736 (21) | 0.9467 (16) | 6.1 (1.0) |
| C(5) | 0.7333 (19) | −0.5080 (21) | 1.0264 (18) | 6.2 (1.1) |
| C(6) | 0.6164 (21) | −0.5124 (22) | 1.0381 (18) | 6.7 (1.2) |
| C(7) | 0.3945 (18) | −0.6067 (20) | 0.9891 (15) | 6.6 (1.0) |
| C(8) | 0.3213 (19) | −0.4829 (22) | 0.9584 (17) | 7.9 (1.1) |
| C(9) | 0.1943 (23) | −0.5309 (28) | 1.0740 (17) | 9.4 (1.4) |
| C(10) | 0.2666 (26) | −0.6483 (30) | 1.1062 (19) | 10.5 (1.5) |
| C(11) | 0.4011 (23) | −0.6413 (28) | 1.0986 (19) | 8.9 (1.4) |
| C(12) | 0.1166 (20) | −0.3773 (25) | 0.9353 (19) | 8.0 (1.3) |
| C(13) | 0.0944 (35) | −0.3442 (32) | 0.8300 (30) | 11.2 (2.1) |
| C(14) | 0.0015 (37) | −0.2385 (31) | 0.7873 (34) | 12.7 (2.3) |
| | | Conformation B | | |
| Br(1) | 1.4642 (2) | 0.1132 (3) | 0.6728 (2) | 7.4 (0.2) |
| N(1) | 1.2808 (17) | −0.0302 (24) | 0.4562 (14) | 9.7 (1.2) |
| O(1) | 0.8174 (13) | 0.0808 (14) | 0.7058 (10) | 7.3 (0.7) |
| O(2) | 0.6354 (14) | −0.0641 (20) | 0.5761 (14) | 9.2 (1.0) |
| C(1) | 0.9946 (19) | −0.0616 (20) | 0.5515 (16) | 7.1 (1.0) |
| C(2) | 0.9684 (18) | 0.0029 (19) | 0.6246 (15) | 7.0 (0.9) |
| C(3) | 0.8503 (18) | 0.0126 (18) | 0.6322 (15) | 6.5 (0.9) |
| C(4) | 0.7538 (18) | −0.0552 (22) | 0.5649 (19) | 7.8 (1.1) |
| C(5) | 0.7789 (22) | −0.1289 (27) | 0.4890 (22) | 7.2 (1.5) |
| C(6) | 0.8990 (21) | −0.1353 (25) | 0.4807 (19) | 7.6 (1.3) |
| C(7) | 1.1326 (18) | −0.0812 (22) | 0.5551 (17) | 8.1 (1.0) |
| C(8) | 1.1441 (20) | −0.0125 (23) | 0.4558 (17) | 7.6 (1.1) |
| C(9) | 1.3206 (24) | −0.1532 (37) | 0.4602 (25) | 10.2 (1.9) |
| C(10) | 1.3071 (24) | −0.2179 (27) | 0.5477 (25) | 11.5 (1.7) |
| C(11) | 1.1722 (24) | −0.2068 (26) | 0.5541 (24) | 10.5 (1.5) |
| C(12) | 1.2945 (31) | 0.0444 (42) | 0.3689 (20) | 12.7 (2.3) |
| C(13) | 1.4194 (38) | 0.0503 (61) | 0.3658 (25) | 17.4 (3.8) |
| C(14) | 1.4397 (40) | 0.1175 (45) | 0.2853 (27) | 11.9 (2.7) |

[a] $U_{eq} = {}^1/_3(U_{11} + U_{22} + U_{33} + 2U_{13}\cos\beta)$.

enantiomers. It is evident from these data that the *R* enantiomer consistently reaches higher brain levels than the *S* enantiomer after equimolar doses and that at the same time O-methylated metabolite levels are higher after *S*-**2**. In both cases, however, the high susceptibility of the catecholic enantiomers of **2** to inactivation via O-methylation is clearly shown by the much higher levels of the O-methyl derivatives as compared to the levels of the catechols given. The amount of parent compound (**2**) as compared to its metabolite (**5**) is 0.5–2% and 0.3–1% for *R*-**2** and *S*-**2**, respectively. When the COMT activity is inhibited by pretreatment with tropolone, levels of **2** are indeed increased (Table IV). A comparison between the brain concentrations of compounds **1** and **2** (Tables IV and V) shows that levels of **1** are about $10^2$–$10^3$ times higher than those of **2** after almost equimolar doses, which is in the same range as our preliminary results.[8] The brain levels of the metabolite **5** reached after the administration of **1** (Table V) are in the same range as the levels of **2** found after the adminsitration of **1** to rats pretreated with tropolone (Table IV). These data support our previous conclusion that the conversion of **1** into **2** is 1–5%, as estimated by the brain concentrations of either **5** (no pretreatment) or **2** (tropolone pretreatment) in relation to the brain concentration of the administered compound **1**.

**Dopaminergic Activity.** The results from both in vivo tests measuring presynaptic dopaminergic effects of the enantiomers of **2** (Table II) are in reasonable agreement with each other, and the potency of *R*-**2** appears to be somewhat higher than that of *S*-**2**. However, when the difference in the actual brain concentrations reached after equimolar doses is taken into account, the potencies of *R*-**2** and *S*-**2** appear to be virtually similar. This lack of potency difference is substantiated by the in vitro affinities, measured as their abilities to displace [$^3$H]-DP-5,6-ADTN, a ligand that has been shown to predominanly label D2

binding sites in rat striatal homogenates (Table VII).[17] $K_i$ values of *R*-2 and *S*-2 were not significantly different. It is apparent that the enantiomers of 2 have about 10 (*S*-2) and 20 (*R*-2) times the affinity of the enantiomers of 1, respectively. Recently, racemic 2 was found to have 2 and 7 times higher affinity for [$^3$H]spiperone binding sites as compared to *S*-1 and *R*-1, respectively.[20]

In vivo postsynaptic effects, such as stereotypy, of the enantiomers of 2 were usually absent when naive rats were used. However, in the case of tropolone pretreatment, the animals showed pronounced stereotyped behavior (continuous sniffing and licking). No differences between *R*-2 and *S*-2 were apparent during these biochemical experiments, in which only gross behavioral observations were made. However, from the data in Table III it follows that postsynaptic activity is more pronounced after *S*-2 than after an equimolar dose of *R*-2. This is also valid after tropolone pretreatment. In order to further investigate the pharmacological relevance of the formation of *S*-2 from the administration of *S*-1, reserpinized rats were treated with tropolone (50 mg/kg ip) and *S*-1. There would now be an opportunity for the *S*-2 formed to overcome the blockade of *S*-1, at least in the long run. As seen from the results from these experiments (Table III), there is indeed a postsynaptic effect elicited by *S*-1 when administered to these animals. Roughly, 50 μmol/kg ip of *S*-1 gives the same stimulation as 5 μmol/kg sc of *S*-2, indicating at least a 10% conversion of *S*-1 to *S*-2. This figure seems a little high when compared to the results presented in Table V, where the conversion can be estimated to be 1–5% (cf also ref 8). This might imply that effects other than central dopamine D2 receptor stimulation now come into play. One such obvious effect of the catechol *S*-2 might be D1 receptor stimulation, since we now are dealing with a catechol structure instead of a mono-phenol (*S*-1). Also, postsynaptic potency assessed in the reserpine locomotor test model was reported to be much higher for mixed D1–D2 agonists than for agonists selective for either receptor.[21] To investigate whether the enantiomers of 2 are active upon central D1 receptors, the stimulation of cAMP formation by *R*-2 and *S*-2 in striatal homogenates was measured, by use of an automated cyclase assay,[19] as well as their capacities to displace the D1 antagonist [$^3$H]-SCH 23390 from rat striatal membranes according to Schulz et al.[18] The results of these experiments are given in Table VII and show that *S*-2 is a rather good D1 agonist, having a potency (as reflected by the $K_m$ for adenylate cyclase and the $K_i$ for [$^3$H]-SCH 23390 displacement) only 3 times lower than DA itself. The fact that the maximal stimulation of cAMP formation is similar for *S*-2 and DA indicates that *S*-2 is a full D1 agonist. The (+) enantiomer *R*-2, on the other hand, has low D1 activity, being about 10 times less potent than DA in stimulating adenylate cyclase and displacing [$^3$H]-SCH 23390. At saturating concentrations, its maximal stimulation of cAMP formation was only 27% of the maximal stimulation by DA, which shows that *R*-2 is at best a weak partial D1 agonist but possibly a D1 antagonist (Schulz, personal communication). Interestingly, earlier studies on the enantiomers of 1 reported that *R*-1 and *S*-1 are both inactive as D1 agonists, while *S*-1 was shown to have D1 antagonistic properties.[7,22]

From our in vitro and in vivo results, it can be concluded that both of the enantiomers of 2 are DA agonists. *S*-2 is a mixed D1–D2 agonist, while *R*-2, being virtually equipotent to *S*-2 as a D2 agonist, lacks D1 agonist activity.

**Relevance of Catechol Formation from 3-PPP.** We have hypothesized earlier[8] that the postsynaptic actions of *R*-1 might be partly dependent on the formation of small amounts of *R*-2 in vivo but also that the formation of catecholic metabolites might have some significance for the in vivo presynaptic effects exhibited by the enantiomers of 1. However, when considering the two metabolic steps discussed in this paper, that is, hydroxylation (1–5%) and methylation (98–99.7%), it is obvious that only a very small fraction ($0.3 \times 10^{-4}$–$10 \times 10^{-4}$) of 2, as compared to 1, is present after the administration of 1. Therefore it seems highly unlikely that 2 gives any significant contribution to the pharmacological effects seen after the administration of 1, either at pre- or at postsynaptic DA receptors.

Interestingly, ip administration of *S*-1 to animals pretreated with reserpine and tropolone gives rise to pronounced postsynaptic activation in terms of stereotypies and locomotor activity. This finding suggests that malfunction of the O-methylation (e.g., due to low levels of the methyl donor *S*-adenosylmethionine) may lead to accumulation of the catechol metabolite and thus affect the pharmacological profile of *S*-1.

## Experimental Section

**Chemistry.** Melting points (uncorrected) were determined in open glass capillaries on a Thomas-Hoover apparatus. $^1$H NMR spectra were recorded with Brucker 500-MHz and Varian EM-360 instruments ($Me_4Si$). GC was performed with a Hewlett-Packard 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 He, 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 5700 gas chromatograph. One MS spectrum (compound *S*-4) was recorded on a ZAB high-resolution mass spectrometer (VG-analytical) working in the EI mode (60 eV) with direct inlet. All spectra were in accordance with assigned structures.

HPLC was performed on a Waters 5 Si 10 column using hexane/EtOAc/EtOH (different compositions) as the mobile phase, working in the pressure range 1000–3000 psi and with a flow rate of 2 mL/min. Detection was made by a Waters Model 440 UV monitor. Optical purity was estimated by comparing peak areas (height times width at half-height). Optical rotation was measured with a Perkin-Elmer 141 polarimeter equipped with a thermostat.

The elemental analyses (C, H, N) for the new substances were within 0.4% of the theoretical values. For purity tests, TLC was performed on 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.

**3-(3,4-Dimethoxyphenyl)piperidine (3).** 3-(3,4-Dimethoxyphenyl)pyridine[10] (7.3 g, 34 mmol) was converted to its hydrochloride by means of HCl-saturated ethanol and evaporation of the solvent and excess HCl. The residue was dissolved in methanol (200 mL) and HCl-saturated ethanol (50 mL) and hydrogenated for 18 h in a Parr apparatus in the presence of $PtO_2$ (1.0 g). The catalyst was removed by filtration (Celite) and the filtrate was evaporated, yielding a residue, which was crystallized from ethanol–ether, giving 4.4 g (51%) in two crops, with mp 220–222 °C (lit.[9] mp 220–221 °C).

**(+)-3(*S*)-(3,4-Dimethoxyphenyl)piperidine (*S*-3).** (*R*)-(−)-α-Methoxyphenylacetic acid (2.8 g, 17 mmol) was dissolved in $CH_2Cl_2$ (50 mL), $SOCl_2$ (15 mL) was added, and the mixture was refluxed for 30 min. Excess $SOCl_2$ was evaporated, and the residual acid chloride oil was dissolved in $CH_2Cl_2$ (40 mL). The

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solution was added at 20 °C to a vigorously stirred mixture of 3·HCl from above (2.9 g, 11 mmol) and 5% aqueous NaOH (40 mL). After the mixture was stirred for 45 min, the phases were separated and the organic phase was washed once with water and dried ($Na_2SO_4$). Filtration and evaporation of the solvent gave 1(*R*)-(α-methoxy-α-phenylacetyl)-3-(3,4-dimethoxyphenyl)-piperidine as a crude oil (5.0 g), which was chromatographed on a $SiO_2$ column (600 g) together with another batch of the same diastereomeric mixture (1.9 g) with ether as eluant.

The fractions containing the diastereomer that eluted first in nearly pure form were combined, and the solvent was evaporated to give the desired diastereomeric amide as an oil (2.6 g, 6.9 mmol) (containing 3.9% of the other diastereomer according to GLC (260 °C, isothermal)). This was dissolved in dry THF (100 mL), and potassium *tert*-butoxide (9.0 g, 81 mmol) and water (0.73 g, 40 mmol) were added with stirring at room temperature. The mixture was stirred at this temperature overnight and was then partitioned between ether and water. After the organic phase was dried ($Na_2SO_4$), excess HCl-saturated ethanol was added, and the solvent was evaporated. The residue was redissolved twice in absolute ethanol, and the solvent was evaporated, giving a crystalline residue. Recrystallization from ethanol–ether gave 1.1 g (59%) of the desired *S*-**3**·HCl: mp 175–177 °C; $[\alpha]^{20}_D$ +7.8° (*c* 1.0, MeOH). The optical impurity in this product was determined to be less than 1% as shown by recoupling it to optically pure (*R*)-(−)-α-methoxyphenylacetyl chloride and performing GLC analysis, as described above.[11]

**(−)-3(*R*)-(3,4-Dimethoxyphenyl)piperidine (*R*-3).** The other diastereomer, which eluted last (from the preparation of *S*-3 above), was collected and the solvent evaporated to give the desired diastereomeric amide as an oil (2.8 g, 7.7 mmol) (containing 2% of the other diastereomer according to GLC (260 °C, isothermal)). This was cleaved as described above to yield 1.1 g (59%) of the desired *R*-**3**·HCl: mp 170–172 °C; $[\alpha]^{20}_D$ −6.3° (*c* 1.0, MeOH). The optical impurity in this product was determined to be less than 2% according to the method described above.

**(−)-3(*S*)-(3,4-Dimethoxyphenyl)-*N*-*n*-propylpiperidine (*S*-4).** $NaBH_4$ (0.90 g, 24 mmol) was added portionwise with stirring to a solution of propionic acid (5.1 g, 66 mmol) in dry benzene (110 mL). The temperature was kept below 15 °C for 3 h, and then 3(*S*)-(3,4-dimethoxyphenyl)piperidine hydrochloride (0.50 g, 1.9 mmol) (*S*-**3**·HCl) was added and the mixture was refluxed for 6 h. The reaction mixture was allowed to reach room temperature, and excess 2 N NaOH was added. After being stirred for 30 min, the reaction mixture was extracted with ether, all the organic phases were mixed and dried ($Na_2SO_4$), the solvent was evaporated, and the residue (0.57 g, 112%) was chromatographed ($SiO_2$) with use of methanol as eluant. The pure fractions were collected, and the solvent was evaporated. The residue was converted to its hydrochloride salt with HCl-saturated ethanol and evaporation of the solvent, but the attempted crystallization from ethanol–ether yielded an oil (0.40 g, 69%) having $[\alpha]^{22}_D$ −8.3° (*c* 1.0, MeOH). High-resolution mass spectrum shows $M^+$ at *m/e* 263 (14.65%) ($C_{16}H_{25}NO_2$: calcd 263.1885, found 263.1887) and base peak at *m/e* 234. No impurities were detected by GC or TLC.

**(+)-3(*R*)-(3,4-Dimethoxyphenyl)-*N*-*n*-propylpiperidine (*R*-4).** Compound *R*-**3** (0.41 g, 1.6 mmol) was converted to its *n*-propyl analogue as described for *S*-**4**·HCl above, yielding 0.23 g (48%) of an oil having $[\alpha]^{22}_D$ +7.5° (*c* 1.0, MeOH). This compound was analyzed only on the HP 5970A system and showed $M^+$ at *m/e* 263 (12%) and base peak at *m/e* 234. This compound performs exactly as *S*-4 in GC/MS, GC, and TLC analyses. No impurities were detected by GC or TLC.

**Demethylation of Methoxy Compounds.** The catechols were obtained by heating of the appropriate methoxy compounds in 48% aqueous HBr for 2 h at 125 °C under nitrogen. The hydrobromic acid was evaporated and the residue was crystallized from methanol–ether.

**Determination of Absolute Configuration by Single-Crystal X-ray Analysis.** Crystals were grown from an acetonitrile solution, and a crystal with the dimensions 0.48 × 0.30 × 0.22 mm was used for data collection with an Enraf-Nonius CAD4F-11 diffractometer. The angular settings of 25 reflections ($7° < \theta < 48°$) were measured to calculate the lattice parameters. Intensity data for reflections within one hemisphere and with $\theta < 60°$ were collected by the $\theta/2\theta$ scan method with use of monochromated Cu Kα radiation. Three intensity control reflections, which were measured every 2 h, indicated no crystal decay. A total of 4712 reflections were recorded, and of these, 4414 reflections with $I > 3\sigma(I)$ were considered observed. All intensities were corrected for Lorenz and polarization effects but not for absorption or extinction.

**Crystal Data.** Molecular formula $C_{14}H_{21}NO_2$·HBr, space group $p2_1$, unit cell $a$ = 11.182 (2) Å, $b$ = 10.657 (1) Å, $c$ = 13.713 (3) Å, $\beta$ = 109.34 (2)°, $V$ = 1542 Å$^3$, $Z$ = 4, $M_r$ = 316.24, $D_{calcd}$ = 1.362 g cm$^{-3}$, $\mu$(Cu Kα) = 39.7 cm$^{-1}$.

The bromine positions, obtained from a Patterson synthesis, were used as input to the direct methods program DIRDIF,[23] which provided the non-hydrogen atom positions. All hydrogen atom positions were obtained from Fourier difference synthesis maps. Refinement was carried out by the full-matrix least-squares method with use of anisotropic temperature factors for the non-hydrogen atoms. The hydrogen atoms were assigned a common temperature factor ($B$ = 5.0 Å$^2$). The hydrogen atom parameters were not refined. In order to determine the absolute configuration of *S*-**2**, anomalous dispersion factors[24] were introduced for the non-hydrogen atoms. The atomic parameters of the non-hydrogen atoms for both enantiomers were then refined. Two sets of unique reflections ($h,k,l$, $h,-k,l$) were used in the refinement, and nonobserved reflections were allowed to contribute when $F_c > F_o$. When the refinement was finished, the residuals for the *S* and *R* enantiomers were calculated as $R$ = 0.049 and $R$ = 0.058 ($R_w$ = 0.059 and $R_w$ = 0.072, respectively). With use of Hamilton's test,[25] the ratio $R_w(R)/R_w(S)$ = 1.22 is sufficiently great to reject the *R* enantiomer at the 0.005 significance level. Furthermore, for the *S* enantiomer, among the 42 Bijvoet pairs for which $|F_c(h,k,l) - F_c(h,-k,l)| > 1.5$, 37 of the $F_c$ differences had the same sign as the corresponding $F_o$ differences. The weighting scheme used in the later part of the refinement was $w = 1/(1 + ((|F_o| - 15)/12)^2)$.[26] The form factors used were those given by Cromer and Mann.[27] All calculations have been performed on a DEC-system-10 computer with use of mainly the X-ray 72 program system.[28] The molecular conformation and the atom numbering scheme of the two independent conformations A and B are shown in Figure 3. The atomic coordinates and the $U_{eq}$ values for the non-hydrogen atoms are listed in Table VIII. Lists of observed and calculated structure factors, anisotropic temperature factors, and corrdinates for the hydrogen atoms can be obtained from the Department of Structural Chemistry, Göteborg, Sweden.

**Pharmacology.** Animals used in the biochemical and motor activity experiments were male rats of a Sprague–Dawley strain (ALAB, Södertälje, Sweden), weighing 200-300 g. 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. Injection volumes were 5 or 10 mL/kg, and all solutions had neutral pH (except for the solutions of reserpine).

**Biochemistry.** The biochemical experiments and the determinations of Dopa and 5-HTP by means by HPLC with electrochemical detection were performed as previously described.[11] Separate dose–response curves based on four to six dose levels

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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 level was estimated (Table II).

Animals used for the determination of brain concentrations and DOPAC/HVA levels were female Wistar rats (170–200 g, CDL Groningen, The Netherlands). Drugs were dissolved in saline immediately before use. The enantiomers of **2** were given sc in volumes of 1 mL/kg 60 min before death.

Tropolone pretreatment was performed by injection of tropolone (Aldrich) (50 mg/kg, ip, 2 mL/kg) 15 min before administration of the test compound.

Rats were killed by decapitation, brains were taken out, and cerebellum, cortex, and striatum were dissected, immediately frozen on dry ice, and kept at −80 °C until assayed.

Striatal DOPAC and HVA concentrations were determined by HPLC with electrochemical detection.[29]

**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.[15,16] Eighteen hours prior to the motility testing (carried out between 9 a.m. and 1 p.m.), the rats were intraperitoneally injected with reserpine (5 mg/kg). The different compounds under investigation were 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 shows the results of administration of *R*-**2** and *S*-**2** to rats pretreated either with reserpine (5 mg/kg, 18 h before) or with reserpine plus tropolone (50 mg/kg, 30 min before).

**Brain Concentrations.** Compound **2** was determined by essentially the same method as described previously.[8] Briefly, tissue is homogenized in 0.1 M perchloric acid, and the supernatant is purified over Sephadex G10 columns. The compounds are eluted with formic acid, and this fraction (2.5 mL) is injected onto a HPLC system (500-μL loop, Nucleosil 5 C18 column of 150 × 4.6 mm) using phosphate/citrate pH 4.0 with 15% methanol as the mobile phase, delivered at 1.0 mL/min, at 600 mV, and a rotating carbon-paste amperometric detector for detection. Tissue concentrations were calculated with the aid of standards of compound **2** and are corrected for recovery (86.8 ± 5.6%). For the detection of the O-methylated compounds (**5** and **6**), the same procedure could be used when the methanol concentration of the mobile phase was increased to 27% and the detector potential was set at 750 mV. As the pure *O*-methyl derivatives of **2** (**5** and **6**) were not available, concentrations of O-methylated compounds were "quantified" by comparison with standards of **2**: assuming a 5-fold lesser sensitivity of *O*-methyl vs. parent catechol, the calculated concentrations were multiplied by 5 in order to obtain an estimate of O-methylated metabolite concentrations, which could be used for comparison between *R*-**2** and *S*-**2**.

**In Vitro O-Methylation.** Catechol-*O*-methyltransferase (COMT) was prepared by homogenizing rat liver in 4 vol of cold 0.15 M KCl, centrifugation during 20 min at 10000*g*, and centrifugation of the supernatant for 60 min at 105000*g*. The supernatant obtained was used as such as a crude COMT preparation and contained 15 mg/mL protein. Incubations were performed in 10-mL glass tubes in a shaking water bath at 37 °C. Incubation constituents were as follows (in final concentrations): *R*-**2** or *S*-**2**, 0.1–0.00125 mM; *S*-adenosyl-L-methionine (Boehringer), 150 mM; $MgCl_2$ (Merck), 10 mM; phosphate buffer pH 7.4 (Merck), 50 mM; COMT, 100 μL (1.5 mg of protein/mL); and water to a final volume of 1.0 mL. After 15 min, 200-μL samples were taken to which 50 μL of perchloric acid was added; after centrifugation for 15 min at 5000 rpm, the supernatants were injected onto the HPLC system (see above) using a loop volume of 20 μL. $K_m$ and "$V_{max}$" values were calculated from Lineweaver–Burk plots constructed from the results of four separate experiments.

**In Vitro Binding to Striatal Homogenates.** Displacement of [$^3$H]di-*n*-propyl-5,6-dihydroxy-2-aminotetralin ([$^3$H]-DP-5,6-ADTN, Amersham, UK, sp act. 229 Ci/mmol) from rat striatal membranes by the enantiomers of compounds **2** and **1** was studied according to Mulder et al.:[17] 50-μL (2.5-mg) membrane suspensions (rat striata in 50 mM Tris·HCl pH 7.5, 1 mM EDTA, 0.01% ascorbic acid (TEA buffer)) were added to incubation tubes containing 0.5 mM [$^3$H]-DP-5,6-ADTN, $10^{-5}$–$10^{-11}$ M **1** or **2** enantiomers, and TEA buffer to a final volume of 1 mL. After incubation (20 min at 25 °C), samples were filtrated through Whatman GF/B filters and washed twice with cold TEA buffer, and the filters were transferred to glass scintillation vials. After addition of 7 mL of Emulsifier Scintillation 299 (Packard) and standing for one night, $^3$H was counted (Beckman LS 1800) at an efficiency of 45%.

$K_i$ values are the means of two independent experiments, in which each concentration was measured in triplicate, and were calculated by use of the Cheng–Prusoff equation with $K_D$ = 0.57 nM and ligand concentration = 0.5 nM.

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**Registry No.** (±)-**1**, 83228-38-0; (*R*)-(+)-**1**, 85976-54-1; (*S*)-(−)-**1**, 85966-89-8; (*R*)-(+)-**2**, 103150-17-0; (*S*)-(−)-**2**, 103150-18-1; (*S*)-(−)-**2**·HBr, 103150-16-9; (±)-**3**·HCl, 103301-77-5; (*R*)-(−)-**3**·HCl, 103150-13-6; (*S*)-(+)-**3**·HCl, 103150-10-3; (*R*)-(+)-**4**, 103150-15-8; (*S*)-(−)-**4**, 103150-14-7; COMT, 9012-25-3; DOPAC, 102-32-9; HVA, 306-08-1; (*R*)-(−)-PhCH(OMe)$CO_2$H, 3966-32-3; dopamine, 51-61-6; 3-(3,4-dimethoxyphenyl)pyridine hydrochloride, 79412-30-9; 1-(*R*)-(α-methoxy-α-phenylacetyl)-3-(3,4-dimethoxyphenyl)-piperidine (isomer 1), 103150-11-4; 1(*R*)-(α-methoxy-α-phenylacetyl)-3-(3,4-dimethoxyphenyl)piperidine (isomer 2), 103150-12-5.

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