Evaluation of the Effects of the Enantiomers of Reduced Haloperidol, Azaperol, and Related 4-Amino-1-arylbutanols on Dopamine and σ Receptors

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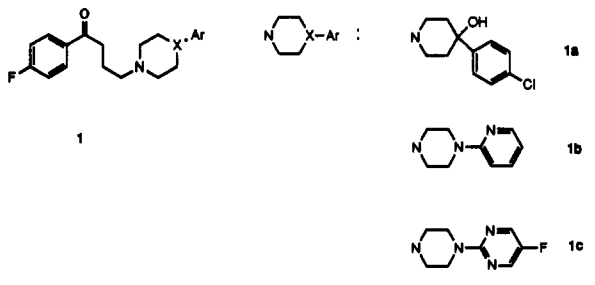
The enantiomers of reduced haloperidol (**3a**), azaperol (**3b**), and the related compound BMY-14802 (**3c**) were prepared in high optical purity. The affinity of these compounds for dopamine D2 and D3 receptors, and σ S1 and S2 sites was determined in vitro. Both enantiomers of **3a** display greatly decreased affinity for D2 and D3 receptors compared to haloperidol, although they still possess affinities in the 100–200-nM range. Both enantiomers of **3a** possess potent and equal affinity for S1 sites (K_i: 1–2 nM), only slightly weaker than haloperidol (K_i: 0.33 nM). At S2 sites, (*R*)-(+)-**3a** displays similar affinity to haloperidol (K_i: 31 and 26 nM, respectively), while (*S*)-(−)-**3a** is slight more potent (K_i: 8.2 nM). The stereoselectivity profile of the enantiomers of **3b** at D2 and D3 receptors is quite similar to that of **3a**, (*S*)-(−)-**3b** being about 4 times more potent than its enantiomer at both receptors. (*R*)-(+)-**3b** binds preferentially to σ S1 over S2 sites, while (*S*)-(−)-**3b** displays the opposite selectivity profile. Both enantiomers of **3c** possess very weak affinity for D2 and D3 receptors. In a manner similar to the enantiomers of **3b**, the affinity of (*R*)-(+)-**3c** is greater for S1 than S2 sites, while (*S*)-(−)-**3c** displays the opposite selectivity profile. Following parenteral administration of both enantiomers of **3a**, dopamine synthesis and turnover in rat striatum, cortex, and mesolimbic areas were increased, in a manner similar to the effects produced by haloperidol itself. Additional studies will be required to assess with certainty whether the effects were due to the compounds themselves or simply were a consequence of the *in vivo* oxidation to haloperidol.

Introduction

Haloperidol (**1a**) is one of the most widely used drugs for the treatment of acute and chronic psychoses.[1] The antipsychotic activity of haloperidol results, at least in part, from its blockade of dopamine (DA) D2 receptors.[2] In addition, like many other antipsychotic drugs, haloperidol also binds with high affinity to DA D3 receptors and to σ binding sites, the significance of which is unknown, since other clinically efficacious antipsychotics display no σ binding.[3] However, since σ receptors have been implicated in the neuronal control of movement, the actions of haloperidol and other classical antipsychotics on σ receptors may contribute to the motor and dystonic side effects produced by these agents.[4–6]



Two major metabolic pathways have been identified for haloperidol in animals and humans. One is an oxidative N-dealkylation pathway that leads to central nervous system (CNS) inactive fragments, 4-(4-chlorophenyl)-4-hydroxypiperidine and 3-(4-fluorobenzoyl)propionic acid.[7] The other one is a reductive pathway that produces alcohol **3a**, generally known as "reduced haloperidol".[8–10] This latter process involves a ketone reductase recently identified in human and guinea pig liver tissues.[11] While **3a** possesses affinity for σ receptors similar to that of haloperidol, its affinity for D2 receptors is greatly reduced.[7,12] The pronounced CNS activity displayed by **3a** following systemic administration[12,13] may reflect the activity of the compound itself or may result from the rapid establishment of an equilibrium between haloperidol and **3a** in vivo.[14,15]

Steady-state concentrations of **3a** are equal to or greater than those of haloperidol in the blood of individuals receiving haloperidol.[13,16–22] Also in humans, **3a** is excreted at a slower rate than haloperidol, leading to the accumulation of **3a** in brain tissue.[10] Given the large interindividual differences observed in haloperidol plasma levels, reductive haloperidol metabolizing activity, and clinical response to haloperidol treatment, monitoring of plasma levels of both haloperidol and **3a** has been suggested as a better clinical indicator than haloperidol levels alone.[13,16–21] Interestingly, some reports suggest that a high blood **3a**/haloperidol ratio correlates with poor clinical responses,[13,18,20] while others have failed to confirm this correlation or found the opposite trend.[21–23] A similar state of confusion exists regarding a possible correlation between the aforementioned ratio and the incidence of extrapyramidal side effects. In any event, it seems clear that a more detailed knowledge of the pharmacological activity of **3a** is required, in order to better understand the clinical profile of haloperidol.

Reduction of the carbonyl group of haloperidol introduces a center of asymmetry, the carbinol carbon of **3a**. However, until recently, little attention has been paid to

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the stereochemical nature of the **3a** produced by animals or humans during the metabolism of haloperidol. A priori, the reduction of haloperidol in vivo could produce either a single enantiomer of **3a**, or mixtures of varying enantiomeric composition, depending on the stereospecificity of the enzymes involved in the reduction. Clinical assays for **3a** levels have consistently failed to address the stereochemical analysis of **3a**. Thus, uncertainty on the clinical significance of **3a** plasma levels has been compounded by the lack of stereochemical information from biological samples. Although chiral assays that are sensitive enough for clinical work do not yet exist, some stereochemical information on the reduction of haloperidol in human tissues was recently published. It was determined in vitro that the haloperidol reductase activity from human brain, blood, and liver produces (*S*)-(−)-**3a** almost exclusively.[25] The relevance of these results to the enantiomeric composition of the **3a** formed in vivo is unknown, but it seems likely that (*S*)-(−)-**3a** is also the predominant enantiomer produced in humans during haloperidol treatment.

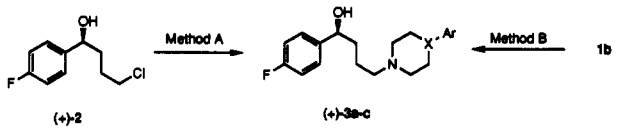
The pharmacological properties of the enantiomers of **3a** have not been described. This information will be essential for properly interpreting plasma levels of **3a** enantiomers, once these results become available through the incorporation of chiral HPLC methods into clinical assays. This report describes the affinities of both enantiomers of **3a** for dopamine D2 and D3 receptors and σ S1 and S2 binding sites in vitro, as well as some preliminary in vivo work exploring the enantiomers' effects on rat brain DA synthesis.

The structural features responsible for the high affinity of haloperidol toward D2 and σ receptors have been the subject of previous investigations.[26-28] While haloperidol binds with high affinity to both types of receptor, structurally related compounds bind selectively to one type only. For this reason, in order to explore further the dopamine and σ pharmacophores of compounds related to haloperidol and **3a**, two additional sets of enantiomerically pure 4-amino-1-arylbutanols were included in this study: the enantiomers of azaperol (**3b**),[29] a metabolite of the animal tranquilizer azaperone (**1b**), and of BMY-14802 (**3c**), a putative σ antagonist with potential antipsychotic activity.[30,31]

Chemistry

Butyrophenones of general formula **1** were prepared in about 50% yield by reaction of 4-chloro-4′-fluorobutyrophenone with the corresponding secondary amine, although higher yields have been recently reported by using ketals of the butyrophenone instead of the butyrophenone itself.[30] The enantiomers of **3a** were prepared by reaction of either enantiomer of **2** with 4-(4-chlorophenyl)-4-hydroxypiperidine. We have previously shown that 4-chloro-4′-fluorobutyrophenone can be reduced stereospecifically with either enantiomer of commercially available *B*-chlorodiisopinocamphenylborane to provide the enantiomers of **2** in ≥97% ee.[32] Similarly, the enantiomers of **3c** were also prepared directly by reaction of (*R*)-(+)-**2** or (*S*)-(−)-**2** with 5-fluoro-2-(1-piperazinyl)-pyrimidine[30] to produce (*R*)-(+)-**3c** and (*S*)-(−)-**3c**, respectively (Scheme I). In terms of conservation of the (fluoropiperazinyl)pyrimidine, which is difficult to prepare, this procedure is a good alternative to the literature method, which obtains the enantiomers of **3c** by formation

Scheme I[a]



[a] For clarity, only the *R* enantiomer of all compounds is shown. Similar reactions were used to produce the *S* enantiomers (see the Experimental Section). Method A: 4-(4-chlorophenyl)-4-hydroxypiperidine or 5-fluoro-2-(1-piperazinyl)pyrimidine, $NaHCO_3$, NaI, DMF, 80 °C. Method B: (−)-*B*-chlorodiisopinocamphenylborane, THF, 20 °C.

of a mixture of diastereomeric carbamates of (±)-**3c**, followed by a series of recrystallizations and the final deprotection of the carbamate.[30]

The enantiomers of **3b** are also accessible by a procedure similar to the one described above for **3a** and **3c**. However, an alternative method involves the stereospecific reduction of **1b** with (+)- and (−)-*B*-chlorodiisopinocamphenylborane to give (*S*)-(−)-**3b** and (*R*)-(+)-**3b**, respectively (Scheme I). Interestingly, the stereospecificity of this reduction was even greater (≥99% ee) than when the reduction was performed on 4-chloro-4′-fluorobutyrophenone.

Pharmacology

Human D2 and D3 Receptor Binding. Until fairly recently, the existence of two DA receptor subtypes, D1 and D2, was widely accepted. However, this situation has dramatically changed following the cloning of cDNAs for D1, D2, D3, D4, and D5 receptor subtypes.[33] Of these, the D3 receptor is of particular interest as it exhibits various properties that suggest that it may be a target of antipsychotic drugs.[34] While most antipsychotics are nearly equipotent at D2 and D3 receptors, the D3 receptor mRNA is expressed in high levels in brain limbic areas (e.g. nucleus accumbens) which are thought to be more associated with psychiatric disorders than the caudate where D2 receptors are highly expressed and appear more related to motor disorders.

In view of the importance of the D3 receptor and the high affinity of haloperidol for it,[33] the present report documents the effects of the enantiomers of **3a**, **3b**, and **3c** on the binding of [^{3}H]spiperone to human D2A (long isoform) and D3 receptors expressed separately in Chinese hamster ovary cells (CHO-K1).[35,36]

σ-1 and σ-2 Binding. σ receptors are unique non-opiate binding sites that are recognized with high affinity by certain antipsychotic drugs, (+)-opiates, and a variety of other agents. Some of these sites appear to play a role in motor function control, and compounds that interact with them have been proposed as potential antipsychotic drugs that will not induce motor side effects, although their exact biological function(s) still remains unclear.[37,38] Recently, two types of σ binding sites have been proposed: σ-1 (S1) and σ-2 (S2).[38] S1 sites appear to predominate in guinea pig brain. On the other hand, S2 sites have been found predominantly in rat liver, rat brain, and several clonal cell lines, including NB41A3 and NIE115 neuroblastomas, C6 glioma, and NG108-15 neuroblastoma–glioma hybrids. S1 sites are recognized with high affinity by (+)-benzomorphans, haloperidol, and 1,3-di(*o*-tolyl)guanidine (DTG), whereas the S2 sites have low affinity for (+)-benzomorphans but relatively high affinity for haloperidol and DTG. In addition, at S1 sites the (+)-benzomorphans are more potent than the (−)-benzomorphans, while at S2 sites the stereoselectivity is reversed. In addition to the suggested

Table I. Binding of Test Compounds to Human D2, Human D3, σ-1 and σ-2 Receptors

compound	K_i (nM)[a]			
	D2[b,c]	D3[c]	σ-1[d]	σ-2[e]
1a	0.44 ± 0.07	0.96 ± 0.04	0.33 ± 0.06	26 ± 5.4
(R)-(+)-3a	279 ± 45	1296 ± 277	1.8 ± 0.3	31 ± 9.0
(S)-(−)-3a	73 ± 16	330 ± 85	2.2 ± 0.9	8.2 ± 2.0
1b	52 ± 8.5	53 ± 18	342 ± 46	51 ± 5
(R)-(+)-3b	403 ± 116	374 ± 50	32.5 ± 0.2	280 ± 60
(S)-(−)-3b	101 ± 9	125 ± 25	183 ± 8	38 ± 4
(R)-(+)-3c	>5000[f]	>2000[g]	64 ± 15	1235 ± 58
(S)-(−)-3c	3346[f]	>2000[g]	490 ± 45	96 ± 23

[a] All values are mean ± SEM of three or more experiments. [b] Human D2L receptor. [c] [^{3}H]Spiperone binding to membranes from CHO-K1 cells expressing the cloned human receptors. [d] [^{3}H]-(+)-Pentazocine binding to guinea pig brain membranes. [e] [^{3}H]DTG binding to NG108-15 membranes. [f] Results of only one experiment. [g] Results of two experiments.

involvement in psychosis, σ ligands have also been proposed as potential anxiolytic, antidepressant, antiischemic, or antiaddiction drugs, based mainly on efficacy in rodent models.[39-42] Thus, the development of high-affinity selective S1 and S2 ligands may be important in a number of disease states. Although haloperidol and (±)-**3a** exhibit good affinity for both σ sites,[7] the affinity of the enantiomers is not known. Thus, the ability of the enantiomers of **3a** and the related compounds **3b** and **3c** to inhibit the binding of [^{3}H]-(+)-pentazocine to guinea pig brain membranes (S1) and of [^{3}H]DTG to NG108-15 cell membranes (S2) were assessed using modifications of published procedures.[43,44]

Rat Brain DA Synthesis. DA antagonists, including haloperidol, have a pronounced stimulant effect on brain DA synthesis and turnover.[45] Usually, brain levels of DA metabolites, dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA), are used as an index of DA neuronal activity. DA turnover can be estimated by the ratio of steady-state levels of DOPAC to DA (DOPAC/DA).[46] In this study, we measured the effects of systemic administration of both enantiomers of **3a** on rat brain DA metabolism and turnover and compared them to results obtained with haloperidol.

Results and Discussion

Table I shows the results of in vitro binding experiments with the test compounds. As previously reported,[34] haloperidol binds with high affinity to cloned human D2 and D3 receptors (K_i values of 0.44 and 0.96 nM, respectively). It also binds with subnanomolar affinity (K_i: 0.33 nM) to S1 sites. Its affinity for S2 sites, however, is about 100 times weaker (K_i: 26 nM). The affinity of haloperidol for S2 sites is in agreement with reported values.[38] Our results do show a slightly higher affinity of haloperidol for S1 sites (0.33 nM) than previous reports, which ranged from about 2 to 115 nM.[37] These latter assays employed different tissues, radioligands, and conditions, probably accounting for the variation in the values reported from different laboratories. High levels of σ receptors in motor areas of the brain are in line with their proposed role in the regulation of movement.[38,40] Some evidence has accumulated, indicating that S2 rather than S1 sites may be related to the motor effects of σ ligands. The evidence for the latter is not conclusive, but it suggests that the motor effects of haloperidol and/or reduced metabolites may be related to their affinity for S2 sites.

(±)-**3a** has been shown to bind to D2 receptors, but its affinity is about 2 orders of magnitude weaker than that of haloperidol.[7,12] In general agreement with this earlier report, both enantiomers of **3a** were found to bind to human D2 receptors with affinities much weaker than haloperidol (K_i values of 279 and 73 nM for (*R*)-(+)-**3a** and (*S*)-(−)-**3a**, respectively). The affinity of both enantiomers for human D3 receptors is somewhat lower than their affinity for D2 receptors, but they display an eudismic ratio[47] almost identical to the one observed for D2 receptors (D3 K_i values: 1296 and 330 nM for (*R*)-(+)-**3a** and (*S*)-(−)-**3a**, respectively). Thus, reduction of the carbonyl group of haloperidol results in a dramatic decrease in affinity for D2 and D3 receptors. Except for perhaps remoxipride and clozapine, the affinity of (*S*)-(−)-**3a** for D2 and D3 receptors is less than most clinically useful antipsychotics.[34,48]

Quite unexpectedly, given the stereospecificity reported for the enantiomers of **3c** at σ sites,[30] both enantiomers of **3a** displayed potent and almost identical binding to S1 sites (K_i values: 1.8 and 2.2 nM for (*R*)-(+)-**3a** and (*S*)-(−)-**3a**, respectively). This result suggests that the hydroxyl group on the asymmetric carbon of **3a** is not part of the S1 pharmacophore, in general agreement with the conclusion reached by other authors on the σ pharmacophore for compounds related to haloperidol.[27] In contrast to the S1 binding, the eudismic ratio of the enantiomers of **3a** at S2 sites is similar to that observed at D2 and D3 receptors, the *S* enantiomer being about 4 times more potent than the *R* enantiomer (K_i values: 31 and 8.2 nM for (*R*)-(+)-**3a** and (*S*)-(−)-**3a**, respectively). Both enantiomers display greater affinity for S1 sites than S2 sites, although the selectivity of (*R*)-(+)-**3a** for S1 is greater than that of (*S*)-(−)-**3a**. Both enantiomers of **3a** display a slightly reduced affinity for S1 sites, compared to haloperidol. On the other hand, while (*R*)-(+)-**3a** is equipotent to haloperidol at S2 sites, (*S*)-(−)-**3a** is 3 times more potent than haloperidol or (*R*)-(+)-**3a**.

Azaperone (1b), a DA antagonist widely used in veterinary medicine as an antiaggression agent, is also a substituted butyrophenone structurally related to haloperidol. As shown in Table I, the affinity of **1b** for D2 and D3 receptors is about 100 times lower than that of haloperidol. In fact, (*S*)-(−)-**3a** and **1b** possess similar affinities for D2 receptors. Interestingly, as a S1 ligand, **1b** is 3 orders of magnitude weaker than haloperidol, while their difference at S2 sites is only about 2-fold. The affinity of **1b** for S1 and S2 sites is within 2–3-fold of the published K_i of 131 nM for σ sites labeled with [^{3}H]-(+)-3-(3-hydroxyphenyl)-1-propylpiperidine ([^{3}H]-(+)-3-PPP) in rat brain membranes.[3]

The stereoselectivity profile of the enantiomers of reduced azaperone (**3b**) at D2 and D3 receptors is quite similar to that of **3a**. (*S*)-(−)-**3b** is about 3–4 times more potent than (*R*)-(+)-**3b** at both receptors. Interestingly, unlike the enantiomers of **3a**, neither enantiomer of **3b** binds to one of the DA receptor subtypes preferentially over the other. Thus, (*R*)-(+)-**3b** binds to D2 and D3 receptors with K_i values of 403 and 374 nM, respectively, while (*S*)-(−)-**3b** displays affinities of 101 and 125 nM, respectively.

An interesting situation occurs with the affinity of the enantiomers of **3b** for σ sites. Thus, while (*R*)-(+)-**3b** displays about 9-fold selectivity for S1 sites (K_i values of 32 and 280 nM, for S1 and S2 sites, respectively), (*S*)-(−)-**3b** displays opposite selectivity (K_i values of 183 and 38 nM for S1 and S2 sites, respectively). The drastic decrease

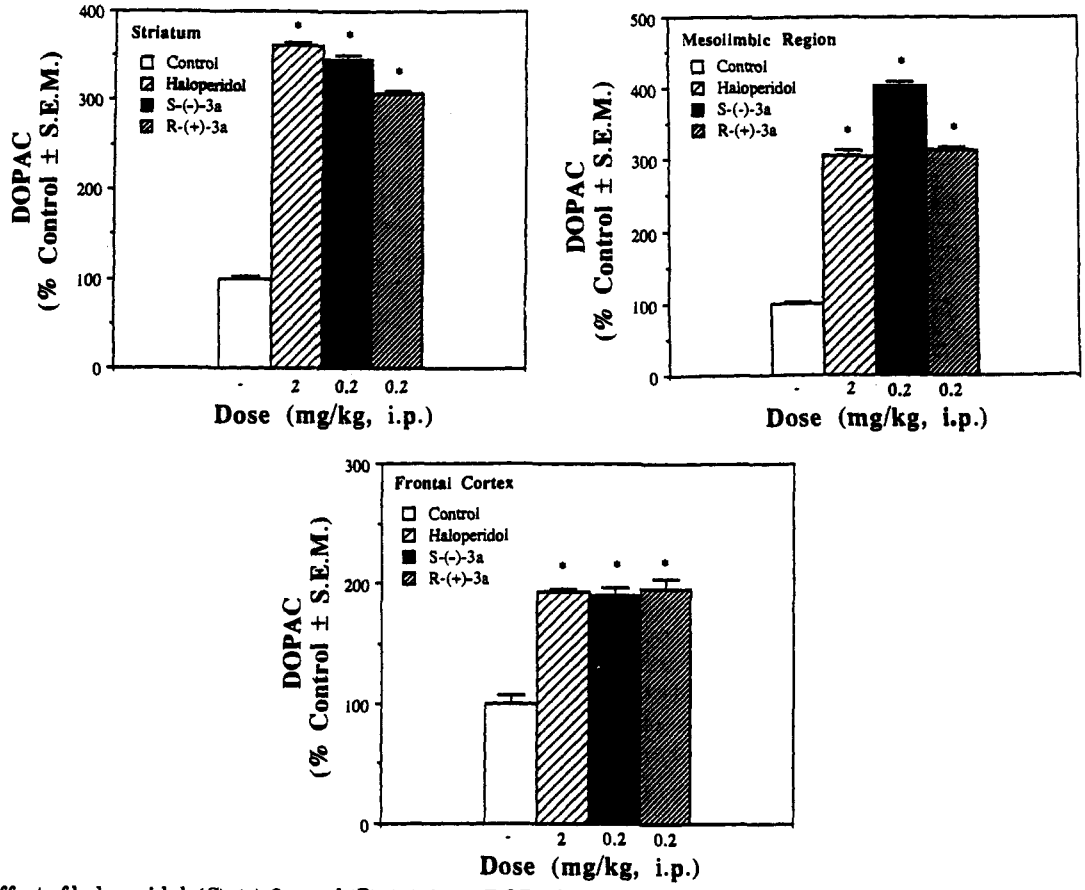


Figure 1. Effect of haloperidol, (*S*)-(−)-**3a**, and (*R*)-(+)-**3a** on DOPAC concentrations in various rat brain regions. Values are expressed as percent of control values ±SEM (see Table I for actual values) and are means of four animals in each group $^*P < 0.05$ versus control group.

in affinity for S1 sites in going from haloperidol to **1b** and from **3a** to **3b** suggests that the S1 pharmacophore in haloperidol probably includes the 4-(4-chlorophenyl)-4-hydroxypiperidine moiety. This result is in contrast to the work of Ablordeppey et al., who concluded that the 4-chlorophenyl and hydroxy substituents on the piperidine ring of haloperidol could be removed with only a 4-fold decrease in σ binding affinity.[27]

BMY-14802 (**3c**) has been described as a selective σ ligand with potential antipsychotic activity.[31] The binding profile of its enantiomers has recently been described as well.[30] As shown in Table I, both enantiomers of **3c** possess almost negligible affinity for D2 and D3 receptors. In general, our D2 binding results are in agreement with those previously published by Yevich et al.,[30] who determined the D2 affinity of (±)-**3c** to be about 6430 nM. The pattern of stereoselectivity previously described for **3c** binding to σ sites is reflected in our S1 binding. Yevich et al.[30] performed three different binding assays, utilizing [^{3}H]-3-PPP and [^{3}H]-(+)-*N*-allylnormetazocine, which label predominantly S1 sites, and [^{3}H]-1,3-di(*o*-toluoyl)guanidine ([^{3}H]DTG), which labels both S1 and S2, as the radioligands. They reported (*R*)-(+)-**3c** binding to σ sites to be about 4–11 times more potent than its enantiomer. We found that (*R*)-(+)-**3c** displays affinity for S1 sites about 7.6 times greater than its enantiomer, in close accord with the published values.[30] Their range of IC_{50} values for (*R*)-(+)-**3c** (28–43 nM) is close to our value of 64 nM for S1. Interestingly, in a manner similar to that described above for **3a** and **3b**, the stereoselectivity at S2 is reversed from S1 sites. Thus, (*R*)-(+)-**3c** binds to S1 receptors with moderate affinity (K_i: 64 nM) and selectivity versus S2 receptors (K_i: 1235 nM) and D2 and D3 receptors (K_i >5000 and >2020 nM, respectively), while (*S*)-(−)-**3c** displays greater affinity for S2 receptors (K_i: 96 nM) than for S1 receptors (K_i: 490 nM) or DA receptors (K_i(D2): 3346 nM, K_i(D3): >2020 nM).

Neurochemical Effects of 3a Enantiomers in Vivo. We were interested in exploring how the slight differences in binding affinities exhibited by the enantiomers of **3a** might influence their in vivo effects on rat brain DA neurochemistry. Figure 1 and Table II illustrate the effects of both enantiomers on DA synthesis and metabolism in rat striatum, mesolimbic region, and frontal cortex, three brain areas that receive rich dopaminergic innervations. Also shown, for comparison, are the effects of haloperidol. It should be noticed that the dose of haloperidol used in this study (0.2 mg/kg ip) was 10 times less than the dose of either enantiomer of **3a** (2.0 mg/kg ip). Both enantiomers of **3a** profoundly affected the levels of the DA metabolites DOPAC and HVA, as well as the DOPAC/DA ratio, in the three brain areas under study (Figure 1, Table II). Levels of DOPAC and HVA were greatly increased, to as much as 464% of controls (mesolimbic HVA levels after administration of (*S*)-(−)-**3a**). In general, HVA levels were affected slightly more than DOPAC levels, and (*S*)-(−)-**3a** had a slightly more pronounced effect than its enantiomer, although the differences were usually very modest, relative to the magnitude of the effect. In general, the effects of haloperidol and the enantiomers of **3a** on brain levels of serotonin and its metabolite 5-hydroxy-3-indoleacetic acid (5-HIAA) were minor and did not reach levels of statistical significance in most cases, suggesting a lack of interaction with serotonin receptors.

Table II. Effect of Haloperidol (0.2 mg/kg, ip), (S)-(−)-3a (2 mg/kg, ip), and (R)-(+)-3a (2 mg/kg, ip) on the concentrations of DA, DOPAC, HVA, 5-HT, and 5-HIAA in Rat Striatum, Mesolimbic Region, and Frontal Cortex[a]

	control	haloperidol	(S)-(−)-3a	(R)-(+)-3a
		Striatum		
DA	6370 ± 174	5140 ± 148	5233 ± 173	3753 ± 204*
DOPAC	794 ± 72	2868 ± 26*	2735 ± 153*	2443 ± 197*
HVA	469 ± 12	2189 ± 38*	1977 ± 98*	1782 ± 30*
5-HT	375 ± 11	407 ± 12	367 ± 8	397 ± 25
5-HIAA	376 ± 10	552 ± 21*	437 ± 6	510 ± 230*
DOPAC/DA	0.125	0.558	0.523	0.651
		Mesolimbic		
DA	1494 ± 133	1680 ± 113	2233 ± 51*	1656 ± 64
DOPAC	324 ± 2	986 ± 90*	1313 ± 80*	1020 ± 33*
HVA	170 ± 17	669 ± 54*	788 ± 50*	641 ± 24*
5-HT	553 ± 43	657 ± 27	639 ± 14	627 ± 32
5-HIAA	621 ± 23	648 ± 36	527 ± 20	492 ± 25
DOPAC/DA	0.217	0.587	0.588	0.616
		Frontal Cortex		
DA	25 ± 1.1	27 ± 1.9	19 ± 1.3	18 ± 1.2
DOPAC	17 ± 1.2	32 ± 0.9*	32 ± 1.8*	32 ± 2.6*
HVA	39 ± 3.2	102 ± 7.0*	80 ± 0.7*	98 ± 8.3*
5-HT	193 ± 8.2	160 ± 12	165 ± 5.4	154 ± 6.3
5-HIAA	165 ± 4.4	180 ± 2.0	156 ± 8.1	172 ± 9.9
DOPAC/DA	0.66	1.18	1.70	1.81

[a] Values are ng/g tissue and are mean ± SEM of four animals in each group. *$P < 0.05$ versus control group.

There are several possible explanations for the in vivo effects produced by the enantiomers of **3a**. One possibility would be that both enantiomers are being oxidized in vivo, generating an undetermined amount of haloperidol, which is directly responsible for the observed increases in DA synthesis. The second possibility is that some of the effects observed are produced by the enantiomers themselves, without prior oxidation to haloperidol. We will come back to these two explanations after briefly reviewing key information previously obtained for (±)-**3a**.

In a behavioral paradigm, systemic administration of (±)-**3a** was found to block apomorphine-induced stereotypy in rats, an effect typical of DA antagonists, but with only one-fourth the potency of haloperidol.[49] (±)-**3a** has been shown to increase serum prolactin levels in rats[50] and in humans,[51] another effect typical of DA antagonists, but once again its potency was much lower than that of haloperidol itself.

Intraperitoneal administration of (±)-**3a** to rats and guinea pigs produces a pronounced increase in DA turnover in striatal and cortical areas.[12,52,53] Here again the potency of (±)-**3a** is about 20–50% (depending on the species) that of haloperidol. The authors unanimously explained the activity of (±)-**3a** on the basis of its in vivo oxidation to haloperidol. In fact, rat brain concentrations of haloperidol following parenteral administration of (±)-**3a** have been found to be as high as the concentrations of (±)-**3a** itself.[12,52] In addition to the in vivo data, several reports document the in vitro oxidation of (±)-**3a** to haloperidol using human[54] or rat microsomal enzymes.[14] That the central activity observed with (±)-**3a** is due primarily to its in vivo oxidation to haloperidol is further supported by the lack of direct antidopaminergic activity of (±)-**3a** when injected directly into rat striatum.[55]

Turning our attention back to the enantiomers of (±)-**3a**, and their observed effects on rat brain DA metabolism, how much of these effects results from oxidation to haloperidol and what is the activity of the enantiomers themselves? (S)-(−)-**3a** is slightly more active in vivo, and it also displays somewhat higher affinity for D2, D3, and S2 receptors than its enantiomer. Reports that in rats (S)-(−)-**3c** is oxidized to the corresponding ketone at a faster rate than the corresponding (R)-(+)-enantiomer[48] are consistent with the hypothesis that the in vivo effects seen with (S)-(−)-**3a** are the result of a faster oxidation to haloperidol. However, the effects of (R)-(+)-**3a** on brain DA neurochemistry are almost as potent as those of (S)-(−)-**3a**. If the stereoselectivity observed in the oxidation of **3c** by rats should apply to the closely related **3a**, then we would not expect as much haloperidol to have formed following administration of (R)-(+)-**3a** as following (S)-(−)-**3a**. It is intriguing to speculate that some of the effects we observed with (R)-(+)-**3a** in vivo were due to the actions of the compound itself. Even more intriguing is the question of how humans might respond to either enantiomer of **3a**. For one thing, handling of the haloperidol–**3a** metabolic equilibrium in humans might be different than in rats. For example, it has been shown that, unlike humans, rats reduce haloperidol to **3a** only to a very small extent.[12] If similar interspecies differences exist in the oxidative pathway, the results we obtained with the enantiomers of **3a** in rats might not be predictive of humans. Since humans appear to only produce (S)-(−)-**3a**,[25] it is unknown whether they possess the ability to efficiently oxidize (R)-(+)-**3a** to haloperidol. On the basis of our in vitro binding data, (R)-(+)-**3a** is reasonably selective for S1 sites versus D2, D3, and S2 receptors, more selective in fact that either haloperidol or (S)-(−)-**3a**. It is intriguing to speculate that (R)-(+)-**3a** might possess some utility in one of the therapeutic areas that have been associated with σ activity.[39–42]

Conclusions

As previously shown for (±)-**3a**, both enantiomers of **3a** display high affinity for S1 and S2 σ sites, while their affinity for D2 and D3 receptors is greatly decreased compared to haloperidol. (S)-(−)-**3a** is somewhat more potent than its enantiomer at dopamine receptors, but (R)-(+)-**3a** is the more selective of the enantiomers at S1 sites. Following parenteral administration of both isomers, rat brain DA synthesis was affected in a manner similar to haloperidol itself. Additional studies will be required to determine with certainty whether these effects were due to the compounds themselves or the consequence of in vivo oxidation to haloperidol.

Our results provide a framework for the eventual interpretation of clinical information on the enantiomeric composition of **3a** from human biological samples. It also suggests a series of experiments, such as exploring the ability of human microsomal enzymes to oxidize the individual **3a** enantiomers, that might help define the potential therapeutic utility of one of the enantiomers in humans.

Experimental Section

Melting points were determined on a Thomas-Hoover capillary melting point apparatus and are uncorrected. Proton NMR spectra were recorded on a Varian XL200 NMR spectrometer (200 MHz) or a Bruker AM250 (250 MHz) and were consistent with the proposed structures. The peaks are described in ppm downfield from TMS (internal standard). The mass spectra were obtained on a Finnigan 4500 mass spectrometer or a VG Analytical 7070E/HF mass spectrometer; the spectra are described by the molecular peak (M) and its relative intensity as well as the base peak (100%). Elemental analyses were performed by the Analytical Research Section at Parke-Davis, Ann Arbor, MI. Where analyses are indicated by the symbols of the elements,

the results are within 0.4% of the theoretical values. TLC was performed on 0.25-mm silica gel F254 (E. Merck) glass plates. Medium-pressure liquid chromatography (MPLC) was performed on silica gel, E. Merck, grade 60, 230–400 mesh, 60 A, with a RB-SY pump (FMI). Chiral HPLC was performed according to the method previously described.[32] Haloperidol (**1a**) was obtained from Aldrich Chemical Co.

1-(4-Fluorophenyl)-4-[4-(2-pyridinyl)-1-piperazinyl]butanone (Azaperone, 1b). A mixture of 4-chloro-4'-fluorobutyrophenone (28.0 g, 0.140 mol), 1-(2-pyridinyl)piperazine (20.5 g, 0.126 mol), sodium bicarbonate (52.8 g, 0.628 mol), and sodium iodide (5.25 g, 35 mmol) in 500 mL of acetone was refluxed under nitrogen for 48 h. The mixture was filtered, concentrated, dissolved in 500 mL of 10% HCl, and washed with ethyl acetate (2 × 500 mL). The aqueous extract was made basic with concentrated ammonium hydroxide and extracted with ethyl acetate. The organic extract was dried over $MgSO_4$ and concentrated to give an oily solid, which was washed with ether and filtered to give 21.49 g (52% yield) of **1b** as an off-white solid, mp 106–109 °C. Anal. ($C_{19}H_{22}FN_3O$): C, H, N.

(*R*)-(+)- and (*S*)-(−)-4-(4-chlorophenyl)-1-[4-(4-fluorophenyl)-4-hydroxybutyl]-4-piperidinol [(*R*)-(+)-3a and (*S*)-(−)-3a] were prepared by the method described previously.[32] The enantiomeric purity of these samples was determined by chiral HPLC (97.0% ee for (*R*)-(+)-**3a** and 98.0% ee for (*S*)-(−)-**3a**).[32]

(*S*)-(−)-α-(4-Fluorophenyl)-4-(2-pyridinyl)-1-piperazinebutanol [(*S*)-(−)-3b]. A solution of **1b** (5.0 g, 15.3 mmol) in 75 mL of THF was added dropwise, under nitrogen, to a solution of (+)-*B*-chlorodiisopinocamphenylborane (6.48 g, 20.2 mmol) in 75 mL of THF at room temperature. After stirring for 16 h, the reaction was carefully quenched by dropwise addition of 100 mL of methanol. The mixture was concentrated and partitioned between CH_2Cl_2 and 1 N NaOH. The organic extract was washed with brine, dried with $MgSO_4$, and concentrated. The residue was purified by MPLC (2.5% methanol in $CHCl_3$) to give 1.53 g (30%) of (*S*)-(−)-**3b** as a white solid, mp 100–102 °C, $[\alpha]_D$ −57° (*c* = 1.05, $CHCl_3$). The enantiomeric purity was determined to be greater than 99.9% by chiral HPLC (>99.8% ee). ^1H NMR ($CDCl_3$): 1.69–1.98 (4H, m), 2.45–2.60 (4H, m), 2.65–2.74 (2H, m), 3.59–3.63 (4H, m), 4.64–4.67 (1H, m), 6.60–6.65 (2H, m), 6.96–7.03 (2H, m), 7.10 (1H, broad s), 7.31–7.36 (2H, m), 7.44–7.51 (1H, m), 8.18–8.19 (1H, m). MS: 329 (M, 5), 107 (100). Anal. ($C_{19}H_{24}FN_3O$): C, H, N.

(*R*)-(+)-α-(4-Fluorophenyl)-4-(2-pyridinyl)-1-piperazinebutanol [(*R*)-(+)-3b]. This compound was prepared in a manner similar to (*S*)-(−)-**3b**, using (−)-*B*-chlorodiisopinocamphenylborane as the reducing agent, to give the product as a white solid, mp 99–102 °C. The ^1H-NMR and MS spectra were identical to those described above for (*S*)-(−)-**3b**, $[\alpha]_D$ +54.0° (*c* = 0.99, $CHCl_3$).

(*R*)-(+)-α-(4-Fluorophenyl)-4-(5-fluoro-2-pyrimidinyl)-1-piperazinebutanol [(*R*)-(+)-3c]. This compound was prepared in 42% yield by reaction of 5-fluoro-2-(1-piperazinyl)pyrimidine[30] with (*R*)-(+)-**2**, in a manner similar to the synthesis of (*R*)-(+)-**3a**.[32] The free base was obtained as an off-white solid: mp 111–121 °C; $[\alpha]_D$ +47.9° (c = 1.19, $CHCl_3$) [lit.[30] mp 114.5–115.5 °C; $[\alpha]_D$ +14.3° (*c* = 0.53, MeOH)]. Anal. ($C_{19}H_{24}F_2N_4O{\cdot}0.33H_2O$): C, H, N.

(*S*)-(−)-α-(4-Fluorophenyl)-4-(5-fluoro-2-pyrimidinyl)-1-piperazinebutanol [(*S*)-(−)-3c]. This compound was prepared by reaction of 5-fluoro-2-(1-piperazinyl)pyrimidine[30] with (*S*)-(−)-**2**, in a manner similar to the synthesis of (*S*)-(−)-**3a**.[32] The free base was obtained as an off-white solid, mp 111–121 °C; $[\alpha]_D$ −50.4° (c = 1.27, $CHCl_3$). Anal. ($C_{19}H_{24}F_2N_4O{\cdot}0.2H_2O$): C, H, N.

Pharmacological Methods. Tissue Preparation. Frozen male Hartley guinea pig whole brains were purchased from ABS (Wilmington, DE). The brains were homogenized in 25 volumes of 50 mM ice-cold Tris-HCl buffer, pH 8.0 (25 °C), with a Polytron (PT-10, Brinkmann Instruments, setting 6, for 20 s). The homogenate was centrifuged at 45000*g* for 10 min at 4 °C. The supernatant fluid was discarded, and the resulting pellets were resuspended in a minimal volume of buffer (0.2 mL) and stored at −80 °C for no more than 1 month.

Cell Cultures. CHO-K1 (donated by Dr. J. Granneman, Wayne State University, Detroit, MI) and NG 108-15 cells (from American Type Culture Collection, Rockville, MD) were maintained under an atmosphere of 5% CO_2 and 95% air in T-150 flasks. CHO-K1 cells expressing either human D-3[36] or human D-2L[35] receptors were grown in F-12 medium (GIBCO Laboratories, Grand Island, NY) supplemented with 10% fetal bovine serum (Hyclone Laboratories, Logan, UT) in T-150 culture flasks in a humidified atmosphere of 5% CO_2 and 95% air. NG 108-15 cells (passage 4-18) were grown and subcultured in Dulbecco's modified Eagle's medium (GIBCO Laboratories) with 10% fetal bovine serum at 37 °C in a humidified atmosphere of 95% air and 5% CO_2. For subculturing, growth medium was replaced and the cells were rinsed with 0.02% EDTA in phosphate buffer saline solution (Sigma Chemical Co., St. Louis, MO) for 10 min; the cells were centrifuged and resuspended (1–10 seeding concentration) in serum-containing medium for seeding into T-150 flasks.

Dopamine Receptor Binding Assays. Experimental compounds were made up as stock solutions in DMSO. The final concentration of 0.1% DMSO used in the incubation mixture had no effect on specific binding. The cells were obtained by replacing the medium with 0.02% EDTA in phosphate-buffered saline, and the cells were scraped from the flasks and centrifuged at about 1000*g* for 3 min. The pelleted cells were then resuspended in ice-cold TEM buffer (25 mM Tris-HCl, 1 mM EDTA, and 6 mM $MgCl_2$, pH 7.4 at 37 °C) and homogenized with a Brinkman Polytron homogenizer at setting 5 for 10 s). The membranes were pelleted by centrifugation at 40000*g* at 4 °C for 20 min, resuspended in TEM buffer at 1 mL/mg membrane protein, and stored at −80 °C until used in the receptor binding assay. Aliquots (100 μL) of the thawed membrane suspension were added to triplicate assay tubes (1-mL minitubes) containing (final concentrations) 100 μL of [^3H]spiperone (0.6 nM, 107 Ci/mmol, Amersham, Arlington Heights, IL), 100 μL of TEM buffer or test compound, and 300 μL of buffer to a final volume of 0.5 mL. Haloperidol (1 μM) was used to define nonspecific binding which was typically less than 10–15% of total binding. After incubation at 25 °C for 1 h, the assay was terminated by rapid vacuum filtration through Whatmann GF/B filtermats (previously soaked for 1 h in 0.5% polyethyleneamine) using a Brandel MB-48R cell harvester, followed by a rapid washing with 4 × 5 mL of ice-cold buffer. Individual filter disks containing the bound ligand were placed in counting vials with 5 mL of scintillation fluid (Ready-Gel, Beckman Instrument Inc., Fullerton, CA) and counted in a Beckman LS-6800 liquid scintillation counter at a efficiency of 45%. Protein was determined by the Bio-Rad method[57] using the microplate reader for analysis.

σ Binding Assays. Experimental compounds were made up as stock solutions in DMSO. The final concentration of 0.1% DMSO used in the incubation mixture had no effect on specific binding. S1 binding was assayed using [^3H]-(+)-pentazocine (42 Ci/mmol) and guinea pig brain membranes; S2 binding was determined using [^3H]DTG (54.5 Ci/mmol) and NG108-15 cell membranes, in the presence of 200 nM dextramethorphan to mask S1 sites. Briefly, 25 μL of tritiated ligand (final concentration 1 nM for [^3H]-(+)-pentazocine and 2 nM for [^3H]DTG) and 25 μL of drug (for competition studies only) in 50 mM Tris-HCl (pH 8.0) and 200 μL of the appropriate membrane suspension (0.5–0.8 mL of 10 mM Tris-HCl/mg membrane protein, pH 8.0) were added to 1-mL polypropylene test tubes. Tubes were incubated for 120 min at 25 °C. Nonspecific binding was determined in the presence of 10 μM (final concentration) haloperidol. Specific binding was typically about 88% for [^3H]-DTG binding and 95% for [^3H]-(+)-pentazocine. Assays were terminated by the addition of 0.5 mL of ice-cold 10 mM Tris-HCl buffer (pH 7.7 at 25 °C), and rapid filtration through Whatman GF/B filters (previously soaked for at least 2 h in 0.5% polyethyleneamine) and washed twice with 5 mL of ice-cold buffer. Individual filter disks were counted as described above.

Binding Data Analysis. Saturation and competition binding data were analyzed using the iterative nonlinear least-squares curve-fitting program from Lundon Software, Inc. (Chagrin Falls, OH). In some competition experiments, apparent K_i values were calculated from experimentally determined IC_{50} values by the method of Cheng and Prusoff.[58]

Biogenic Amine Metabolism. Male Long-Evans rats (150–200 g) from Harlan Laboratories (Indianapolis, IN) were used in

these studies. Animals had free access to laboratory chow and water and were housed five per cage in a temperature-controlled room with a 12-h light/dark cycle (lights on at 0600 h). Test compounds were administered as suspensions in 0.2% Tween 80 [Sigma Chemical Co., St. Louis, MO]. Test compounds were administered 1 h before the animals were sacrificed by decapitation. The brain was rapidly removed, the striatum and mesolimbic region (nucleus accumbens and olfactory tubercle) and frontal cortex were dissected out over ice. Tissue samples were assayed at once or frozen on dry ice and stored at −80 °C until analyzed. Tissue samples were homogenized in 0.5 mL of 0.1 M phosphate/citrate buffer (pH 2.5) containing 15% methanol and centrifuged. Concentrations of DA and its metabolites in the supernatant fluids were analyzed using an HPLC method with electrochemical detection, as previously described.[59] Statistical comparisons were made with the use of one-way analysis of variance followed with Newman Keuls' test to determine which group means were significantly different. Probability levels of less than 5% were considered significant.

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