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Intrinsic Activity of Enantiomers of 8-Hydroxy-2-(di-*n*-propylamino)tetralin and Its Analogs at 5-Hydroxytryptamine$_{1A}$ Receptors That Are Negatively Coupled to Adenylate Cyclase

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SUMMARY

Although many different types of compounds have been tested for 5-hydroxytryptamine$_{1A}$ (5-HT$_{1A}$) binding affinity, much remains to be learned about the structural requirements associated with 5-HT$_{1A}$ agonism, partial agonism, and antagonism. The present study uses the forskolin-stimulated adenylate cyclase (FSC) assay as a functional screen in rat hippocampal membranes to examine structure-activity relationships for a series of enantiomers of novel analogs of the prototypic 5-HT$_{1A}$ agonist 8-hydroxy-2-(di-*n*-propylamino)tetralin (8-OH-DPAT). The findings illustrate that there can be large enantiomeric differences in intrinsic activity at the 5-HT$_{1A}$ receptor, independent of enantiomeric effects on binding affinity. Generally, for each enantiomeric pair exhibiting stereoselective 5-HT$_{1A}$ binding, the enantiomer with the higher affinity also displayed the greater amount of 5-HT$_{1A}$ intrinsic activity in the FSC assay. Interestingly, the enantiomers of 8-OH-DPAT itself displayed stereoselective differences in intrinsic activity but not 5-HT$_{1A}$ affinity. Several of the compounds, namely (*S*)-UH-301, (2*R*,3*R*)-CM-12, and (1*S*,2*R*)-LEA-146, may have potential as prototypes for selective 5-HT$_{1A}$ antagonists, and (*S*)-UH-301 itself may be useful as a selective 5-HT$_{1A}$ antagonist. The FSC data presented here are in good agreement with reported measures of *in vivo* 5-HT$_{1A}$ activity, which were in part the basis of a recently proposed model for the 5-HT$_{1A}$ pharmacophore [*J. Med. Chem.* **34**: 497–510 (1991)].

A variety of different putative serotonin (5-HT) receptor types and subtypes have currently been identified. Of these, the 5-HT$_{1A}$ receptor has been of great interest due to its proposed involvement in a variety of different systems (for reviews see Refs. 1–3). Although development of a selective 5-HT$_{1A}$ antagonist has proven very difficult, the selective 5-HT$_{1A}$ agonist 8-OH-DPAT has been an invaluable aid in defining 5-HT$_{1A}$ receptor sites, and a substantial number of reported analogs of 8-OH-DPAT have been used to investigate structural features required for 5-HT$_{1A}$ agonist binding (4–12). Because of the unique selectivity of 8-OH-DPAT for the 5-HT$_{1A}$ site, a great deal of effort has been expended in using this structure to define the agonist pharmacophore for this receptor subtype. The two *n*-propyl groups of 8-OH-DPAT have previously been shown to be necessary for optimal activity in a biochemical measure of serotonergic activity (specifically, the receptor-mediated feedback inhibition of 5-HTP accumulation). Aminotetralin analogs with alkyl groups smaller than *n*-propyl were less active, whereas those with larger substituents were essentially inactive (5). The di-*n*-propyl portion of 8-OH-DPAT has since been shown to make a significant contribution to its affinity for 5-HT$_{1A}$ sites and to affect enantiomeric specificity of the 8-hydroxy-2-aminotetralins (7). Although the selectivity for 8-OH-DPAT among certain 5-HT receptor subtypes can be related to the di-*n*-propyl substitutions, the lack of the pyrrole ring that is present in the tryptamine agonists may also account for the low affinity for 8-OH-DPAT of the 5-HT$_2$ subtypes (13). Ring-expanded 8-OH-DPAT analogs have also been investigated (14). Despite all this work, the pharmacophore for the 5-HT$_{1A}$ receptor, including its stereochemical requirements, has yet to be fully elucidated.

Recently, Mellin *et al.* (15, 16) have expanded on the use of 8-OH-DPAT to explore the 5-HT$_{1A}$ pharmacophore. In this case, the enantiomers of 8-OH-DPAT and a series of analogs

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ABBREVIATIONS: 5-HT, 5-hydroxytryptamine; 8-OH-DPAT, 8-hydroxy-2-(di-*n*-propylamino)tetralin; FSC, Forskolin-stimulated adenylate cyclase; EGTA, ethylene glycol bis(β-aminoethyl ether)-*N,N,N′,N′*-tetraacetic acid; SAR, structure-activity relationships; 5-HTP, 5-hydroxytryptophan.

and structural homologs were examined for affinity at the 5-HT_{1A} binding site and for their activity *in vivo*. The use of these compounds resulted in the development of a novel model for the agonist pharmacophore for the 5-HT_{1A} receptor (15, 16). In discussing pharmacophores, a variety of definitions have been used (e.g., see Refs. 17 and 18). For the present work, however, this term is used to mean the combination of geometric or structural features of ligands that determine both high affinity and selectivity for the 5-HT_{1A} receptor. Furthermore, in the present case, the discussion of the 5-HT_{1A} pharmacophore is restricted to the aminotetralins and similar structures that are related to 8-OH-DPAT. The combination of this series of enantiomeric pairs of novel 8-OH-DPAT analogs with the *in vitro* FSC assay provides a unique perspective to assess the effect of structure on intrinsic activity for 5-HT_{1A} ligands, and the present study is the first time that the 5-HT_{1A} receptor that is negatively linked to adenylate cyclase in rat hippocampal membranes has been characterized in this manner.

Materials and Methods

Chemicals. (±)-8-OH-DPAT HBr was purchased from Research Biochemicals, Inc. (Natick, MA). [α-^{32}P]ATP, [^{3}H]cAMP, and [^{3}H]8-OH-DPAT were obtained from New England Nuclear (Boston, MA). (±)-Pindolol and 5-HT creatinine sulfate were purchased from Sigma Chemical Co. (St. Louis, MO). Dowex (AG 50W-X4, 200–400 mesh) and neutral alumina (AG 7, 100–200 mesh) were purchased from Bio-Rad (Richmond, CA). All other chemicals were purchased from standard commercial sources.

The enantiomers of 8-OH-DPAT and its analogs (Fig. 1) were provided by Dr. Hacksell's laboratory and included the following: (+)-(2*R*)-8-OH-DPAT (5), (−)-(2*S*)-8-OH-DPAT (5), (4a*R*,10b*R*)-JV-26 [(+)-(4a*R*,10b*R*)-1,2,3,4,4a,5,6,10b-octahydro-10-hydroxy-4-propylbenzo[*f*]quinoline·HCl] (16), (4a*S*,10b*S*)-JV-26 [(−)-(4a*S*,10b*S*)-1,2,3,4,4a,5,6,10b-octahydro-10-hydroxy-4-propylbenzo[*f*]quinoline·HCl] (16), (2*S*,3*S*)-CM-12 [(+)-(2*S*,3*S*)-(*trans*)-8-hydroxy-3-methyl-2-(di-*n*-propylamino)tetralin·HBr] (8), (2*R*,3*R*)-CM-12 [(−)-(2*R*,3*R*)-(*trans*)-8-hydroxy-3-methyl-2-(di-*n*-propylamino)tetralin·HBr] (8), (1*S*,2*R*)-ALK-3 [(+)-(1*S*,2*R*)-(*cis*)-8-hydroxy-1-methyl-2-(di-*n*-propylamino)tetralin·HCl] (6), (1*R*,2*S*)-ALK-3 [(−)-(1*R*,2*S*)-(*cis*)-8-hydroxy-1-methyl-2-(di-*n*-propylamino)tetralin·HCl] (6), (1*S*,2*R*)-LEA-146 [(+)-(1*S*,2*R*)-(*trans*)-2-(2-hydroxyphenyl)-*N*,*N*-di-*n*-propylcyclopropylamine·HBr] (19), (1*R*,2*S*)-LEA-146 [(−)-(1*R*,2*S*)-(*trans*)-2-(2-hydroxyphenyl)-*N*,*N*-di-*n*-propylcyclopropylamine·HBr] (19), (*R*)-UH-301 [(+)-(*R*)-5-fluoro-8-hydroxy-2-(di-*n*-propylammino)-tetralin·HBr] (20), and (*S*)-UH-301 [(−)-(*S*)-5-fluoro-8-hydroxy-2-(di-*n*-propylamino)tetralin·HBr] (20).

All drugs were directly soluble in water, with the exception of pindolol, which was dissolved in water after pretreatment with 0.5% glacial acetic acid. Subsequent dilutions were made with water.

Tissue preparation. The conditions used for the tissue preparation were adapted from the method of DeVivo and Maayani (21). Briefly, male Sprague-Dawley rats (150–250 g) were rendered unconscious with carbon dioxide (in a chamber containing dry ice) and then decapitated. Brains were removed and chilled in saline (0.9%), and the hippocampi were rapidly dissected out. The hippocampi were then homogenized in buffer (1:9, original wet weight/volume) containing 300 mM sucrose, 1 mM EGTA, 5 mM EDTA, 20 mM Tris·HCl (pH 7.4), and 5 mM dithiothreitol, using a glass/Teflon homogenizer (20 strokes by hand). The tissue suspension was further diluted 8-fold and centrifuged at 500 × *g* for 5 min at 4°. The resulting supernatant was centrifuged at 39,000 × *g* for 10 min. The pellet was stored on ice for no more than 1-hr before resuspension in buffer for use in the FSC assay.

FSC assay. The conditions used for the FSC assay components were adapted from the method of DeVivo and Maayani (21). The conversion of [α-^{32}P]ATP to [α-^{32}P]cAMP was quantitated by the method of Salomon (22).

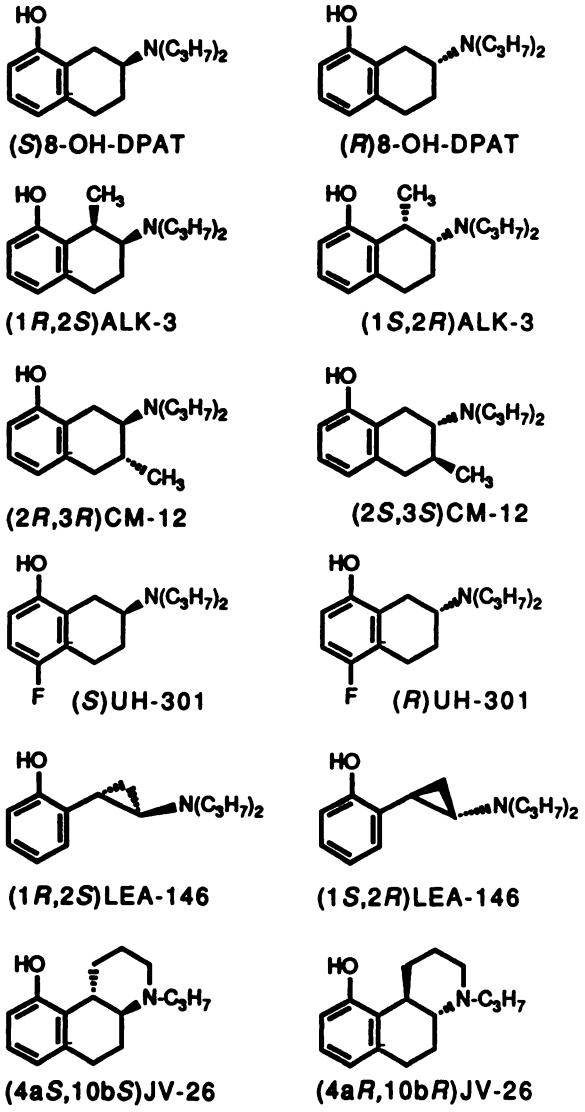

Fig. 1. Structures of the enantiomers of 8-OH-DPAT and its analogs.

The final composition of the assay medium was 100 mM NaCl, 2 mM magnesium acetate, 10 μM forskolin, 80 mM Tris·HCl (pH 7.4), 2 mM magnesium acetate, 100 mM sodium chloride, 0.2 mM ATP, 1 mM cAMP, 10 μM GTP, 60 mM sucrose, 4 mM theophylline, 0.2 mM EGTA, 1 mM EDTA, 1 mM dithiothreitol, 0.1 mg/ml creatine phosphokinase, 5 mM creatine phosphate, and 1–2 μCi of [α-^{32}P]ATP (specific activity, 20–40 Ci/mmol) per sample. Each drug concentration was tested in triplicate.

Tubes containing 150 μl of the cyclase assay medium and 50 μl of drug dilution (or water) were preincubated at 30° for 5 min, staggering samples by 15-sec intervals. The assay was then started with the addition of 50 μl of the tissue suspension (100–125 μg of protein/tube). After a 5.25-min incubation at 30°, the reaction was terminated with the addition of 100 μl of a solution containing 2% sodium lauryl sulfate, 45 mM ATP, and 1.3 mM cAMP. The internal standard, [^{3}H]cAMP (diluted in water to give approximately 30,000 dpm/50 μl), was then added to the samples, which were subsequently put in a boiling water bath for 3 min. After cooling of the samples to room temperature, the conversion of [α-^{32}P]ATP to [α-^{32}P]cAMP was quantitated using sequential chromatography. Samples were loaded onto Dowex columns and then eluted with water onto neutral alumina columns. The final fraction of interest (as determined previously from column elution

profiles) was eluted from the alumina columns with 0.1 mM imidazole (pH 7.3), directly into scintillation vials containing 10 ml of scintillation fluid (Safety Solve; RPI Corp., Mount Prospect, IL). These samples were then counted in a Packard scintillation counter (B460C), using a program for $^{3}H/^{32}P$ dual labeling.

5-HT$_{1A}$ receptor binding assay. The [^{3}H]8-OH-DPAT binding assay was performed using cortex from male Sprague-Dawley rats, as previously described (23).

Data analysis. Adenylate cyclase activity was corrected for relative recovery and expressed as fmol of cAMP/min/μg of protein or as a fraction of 5-HT-sensitive adenylate cyclase, as defined by the maximal inhibition of FSC achieved with 10 μM 5-HT. 5-HT routinely inhibited 25–30% of FSC activity in the rat hippocampal membrane preparation. Protein concentration was measured by the method of Lowry *et al.* (24), using bovine serum albumin as the standard.

The IC_{50} value is the concentration of inhibitor causing 50% inhibition of [^{3}H]8-OH-DPAT binding. The EC_{50} value refers to the agonist concentration that yielded 50% of maximal 5-HT-sensitive inhibition of FSC. IC_{50} and EC_{50} values were calculated using the nonlinear regression analysis software PC-NONLIN (Statistical Consultants Inc., KY). K_i values were calculated from binding data using the Cheng-Prusoff equation (25),

$$K_i = \frac{IC_{50}}{1 + (L/K_d)}$$

such that L is the concentration of [^{3}H]8-OH-DPAT (1 nM) and K_d is the dissociation constant for [^{3}H]8-OH-DPAT (4 nM).

The screening of these compounds for 5-HT$_{1A}$ activity was carried out as follows. Any compound that inhibited FSC in a dose-dependent manner and whose inhibition curve was shifted to the right in the presence of 10 μM pindolol [an antagonist of 5-HT$_{1A}$ receptors (26)] was considered to possess 5-HT$_{1A}$ agonistic activity. A compound was considered a full agonist if it produced an inhibition of adenylate cyclase activity that was equivalent to the maximal inhibition produced by 5-HT (defined with 10 μM 5-HT in this assay). Anything less would indicate partial agonism. If a compound did not appear to significantly inhibit FSC in a dose-dependent fashion, it was then tested in the presence of a fixed concentration (i.e., 1 μM) of 5-HT, to determine the extent to which the compound was able to reverse the 5-HT-induced inhibition of FSC. Full reversal of the inhibition of FSC produced by 5-HT would suggest antagonistic activity. Anything less would suggest partial agonistic activity.

Results

All 5-HT$_{1A}$ binding affinity data for the enantiomers of 8-OH-DPAT and its analogs (see structures in Fig. 1) are summarized in Table 1, along with the corresponding estimates of EC_{50} for the compounds demonstrating agonistic activity in the FSC assay. For compounds for which an accurate EC_{50} value could be determined, the majority of the calculated EC_{50}/K_i ratios ranged between 12 and 22 [compared with a range of 5 to 14 for reference 5-HT$_{1A}$ agonists 5-HT, 8-OH-DPAT, and buspirone (27)]. All cyclase EC_{50} values were greater than the corresponding binding K_i values. This observation is consistent with other reports comparing 5-HT$_{1A}$ binding affinity with FSC assay data (3, 21, 28). Only a qualitative assessment of intrinsic activity was assigned to compounds that inhibited less than 45% of 5-HT-sensitive FSC, due to the difficulty of determining reliable quantitative estimates from nonlinear regression analysis. None of the compounds inhibited FSC to a greater extent than 5-HT itself. The effect of each enantiomeric pair of compounds on the inhibition of FSC is discussed individually below.

8-OH-DPAT. Although the enantiomers of 8-OH-DPAT had virtually identical affinities for the 5-HT$_{1A}$ receptor, there was a dramatic difference between the activities of these two enantiomers at the 5-HT$_{1A}$ receptor that is negatively coupled to adenylate cyclase. Whereas (*S*)-8-OH-DPAT was only a partial agonist, inhibiting FSC by about 50% of the maximal 5-HT effect (Fig. 2A), (*R*)-8-OH-DPAT was a full 5-HT$_{1A}$ agonist (Fig. 2B). Both curves were sensitive to pindolol, as would be expected if the inhibition of FSC was due to activation of 5-HT$_{1A}$ receptors.

ALK-3. The only difference between the structures of ALK-3 and 8-OH-DPAT is that the former compound has a *cis*-methyl substituent in the C1 position. Such a simple modification resulted in tremendous (1000-fold) stereoselectivity in 5-HT$_{1A}$ binding, compared with the parent compound 8-OH-DPAT (Table 1). Although the magnitude of inhibition of FSC did not appear to be stereoselective, the activities of (1*R*,2*S*)-ALK-3 and (1*S*,2*R*)-ALK-3 were dramatically reduced, compared with those of the 8-OH-DPAT enantiomers, such that both ALK-3 enantiomers were rendered weak partial agonists at the 5-HT$_{1A}$ receptor. Pindolol produced a significant shift ($p < 0.05$) in the (1*R*,2*S*)-ALK-3-induced inhibition curve (Fig. 2C), suggesting that the observed inhibition was consistent with an interaction at 5-HT$_{1A}$ receptors. (1*S*,2*R*)-ALK-3 dose-dependently reversed about 80% of 5-HT-induced inhibition (Fig. 2D), consistent with it acting as a partial agonist at 5-HT$_{1A}$ receptors.

CM-12. The structure of CM-12 differs from that of 8-OH-DPAT by only a *trans*-methyl group in the C3 position. Although this minor change in the prototypic structure evoked stereoselectivity with respect to 5-HT$_{1A}$ binding affinity, the overall affinities of both CM-12 enantiomers were decreased, compared with those of the 8-OH-DPAT enantiomers. The CM-12 enantiomeric pair also displayed stereochemical differences in functional activity at the 5-HT$_{1A}$ receptor that is negatively coupled to adenylate cyclase. (2*R*,3*R*)-CM-12 produced no measurable agonist activity and appeared to fully reverse 5-HT-induced inhibition (Fig. 2E). In contrast, (2*S*,3*S*)-CM-12 had high intrinsic activity, inhibiting almost 78% of the 5-HT-sensitive adenylate cyclase activity, and this inhibition was pindolol sensitive (Fig. 2F).

UH-301. UH-301 is identical in structure to 8-OH-DPAT, with the exception of the presence of a fluorine in the C5 position. This enantiomeric pair demonstrated stereoselectivity in both 5-HT$_{1A}$ affinity and activity. (*S*)-UH-301 appeared to be a putative 5-HT$_{1A}$ antagonist, because it did not significantly inhibit FSC and it produced full reversal of 5-HT-induced inhibition of FSC (Fig. 2G). (*R*)-UH-301 was a weak partial agonist, because its reversal of 5-HT-induced inhibition converged to its level of inhibition of FSC (Fig. 2H).

LEA-146. LEA-146 is more flexible than 8-OH-DPAT, but like 8-OH-DPAT it does contain a phenethylamine moiety. The phenylcyclopropylamine LEA-146 showed stereoselectivity with respect to both 5-HT$_{1A}$ binding affinity and intrinsic activity. (1*R*,2*S*)-LEA-146 was an efficacious 5-HT$_{1A}$ agonist, inhibiting 90% of maximal 5-HT-sensitive adenylate cyclase (Fig. 2I). This inhibition curve was shifted to the right in the presence of pindolol. In contrast, (1*S*,2*R*)-LEA-146 was only a very weak partial agonist, as demonstrated by its ability to almost fully reverse the inhibitory effect of 10 μM 5-HT (Fig. 2J). Because the intrinsic activity of (1*S*,2*R*)-LEA-146 was so low, no quantitative measure of cyclase potency was attempted.

TABLE 1

Comparison of 5-HT$_{1A}$ binding affinities with FSC assay potencies for enantiomers of 8-OH-DPAT and its analogs

Values are the mean ± standard error for at least three separate experiments [except for the K_i values for the UH-301 enantiomers (two experiments)].

Compound	Affinity, K_i[a]	Potency, EC_{50}[b]	Ratio, EC_{50}/K_i	Maximal inhibition[c]
	nM	nM		%
(*S*)-8-OH-DPAT	6.1 ± 0.6	135 ± 51	22	47 ± 3
(*R*)-8-OH-DPAT	4.1 ± 0.3	57.4 ± 11	14	101 ± 8
(1*R*,2*S*)-ALK-3	2920 ± 219	—[d]		35 ± 9
(1*S*,2*R*)-ALK-3	2.9 ± 0.3	—		22 ± 5
(2*R*,3*R*)-CM-12	1389 ± 31	—		NS[e]
(2*S*,3*S*)-CM-12	49.6 ± 4.2	643 ± 63	13	78 ± 2
(*S*)-UH-301	126 (136, 116)	—		NS
(*R*)-UH-301	32.7 (16, 49)	356 ± 198	10.9	47 ± 3
(4a*S*,10b*S*)-JV-26	3.9 ± 0.2	47.1 ± 8.3	12	82 ± 3
(4a*R*,10b*R*)-JV-26	32.3 ± 3.2	—		28 ± 9
(1*R*,2*S*)-LEA-146	8.0 ± 1.1	176 ± 79	22	90 ± 5
(1*S*,2*R*)-LEA-146	923 ± 66	—		NS

[a] 5-HT$_{1A}$ binding affinity against [^{3}H]8-OH-DPAT. Apparent K_i values were determined using the Cheng-Prusoff equation (25).
[b] EC_{50} determined in the FSC assay, as calculated by nonlinear regression analysis.
[c] Percentage of inhibition of the 5-HT-sensitive component of FSC.
[d] —, an accurate EC_{50} could not be calculated due to low intrinsic activity.
[e] NS, no statistically significant inhibition of cyclase detected.

The differences in the activities of the two enantiomers of LEA-146, as measured in the FSC assay, were consistent with the previous findings of Arvidsson *et al.* (19), which were obtained using an *in vivo* measurement of 5-HT$_{1A}$ receptor activation.

JV-26. JV-26 is a conformationally constrained analog of 8-OH-DPAT. Although the enantiomers of JV-26 had only modest stereoselectivity for 5-HT$_{1A}$ binding sites (Table 1), there was greater difference between enantiomers with respect to intrinsic activity. (4a*S*,10b*S*)-JV-26 was an efficacious agonist, with almost 85% of the intrinsic activity of 5-HT in this system (Fig. 2K). This inhibition curve had a large 5-HT$_{1A}$ component, based on the ability of pindolol to shift the curve. The other enantiomer, (4a*R*,10b*R*)-JV-26, produced a much weaker inhibition of FSC (Fig. 2L), which was completely blocked by 10 μM pindolol.

Discussion

The FSC assay has provided interesting results concerning SAR associated with this series of enantiomeric pairs of 8-OH-DPAT analogs. The marked stereoselective differences in intrinsic activity of some of the tested enantiomers emphasize the importance of studying individual enantiomers when evaluating the pharmacology of compounds, because erroneous conclusions may be drawn from results obtained with the racemates (7). Generally, it appeared that, for each pair of enantiomers tested, the enantiomer with the higher 5-HT$_{1A}$ binding affinity also had the greater level of 5-HT$_{1A}$ agonistic activity in the FSC assay. The compounds excluded from this trend are 8-OH-DPAT, whose enantiomers did not show a statistically significant stereoselectivity for affinity at 5-HT$_{1A}$ binding sites, and ALK-3, for which both enantiomers displayed low intrinsic activity. Although the number of compounds examined in this study was too small for development of comprehensive rules regarding the SAR of aminotetralins and related compounds as agonists, partial agonists, and antagonists at the 5-HT$_{1A}$ receptor, the data present some interesting features that provide new information regarding both the recognition of compounds by the 5-HT$_{1A}$ receptor and their ability to activate the receptor once bound to it.

The stereoselective difference in activity, but not 5-HT$_{1A}$ affinity, for the enantiomers of 8-OH-DPAT itself is of particular interest. 8-OH-DPAT, which is commercially available only as the racemic mixture, is widely regarded as a full agonist at the 5-HT$_{1A}$ receptor. Yet, it is clear from the present study that the *S*-enantiomer is a partial agonist, whereas the *R*-enantiomer likely contains full agonist activity. Our previous data with (±)-8-OH-DPAT suggested that, at best, it was a partial agonist, with about 85% of the intrinsic activity of 5-HT (28). This is in contrast to other reports that 8-OH-DPAT was a full agonist at the 5-HT$_{1A}$ receptor linked to adenylate cyclase (26). It was considered that the partial agonistic activity of racemic 8-OH-DPAT might be consistent with the *S*-enantiomer antagonizing the *R*-enantiomer at the higher concentrations (up to 100 μM), because the *S*-enantiomer (as a partial agonist) can also act as a partial antagonist. This possibility was tested by use of an equation that allows the calculation of a response in the presence of two drugs of differing intrinsic activity acting simultaneously on the same receptor subtype (29):

$$\frac{E_{DD'}}{E_{max}} = \frac{(\alpha_D[D]/K_D) + (\alpha_{D'}[D']/K_{D'})}{([D]/K_D) + ([D']/K_{D'}) + 1}$$

where D and D' are two drugs acting on the same receptors, having intrinsic activities of α_D and $\alpha_{D'}$, respectively, and affinities of K_D and $K_{D'}$. $E_{DD'}$ is the response when both compounds act simultaneously.

Fig. 3A shows the actual dose-response curves in the FSC assay for the enantiomers of 8-OH-DPAT, as well as the commercially available racemic mixture. Taking the values for EC_{50} and percentage of maximal inhibition for (*R*)- and (*S*)-8-OH-DPAT from Table 1 and substituting them for K_D and α_D in the equation given above resulted in a theoretical relationship for the racemic mixture relative to the resolved enantiomers (Fig. 3B) that corresponded well to the experimentally derived results (Fig. 3A). It is clear from the equation given above that, if two enantiomers have similar affinities for a receptor and if one is a full agonist and the other an antagonist (or a very weak partial agonist), use of the racemic mixture in functional assays would lead to the erroneous conclusion that

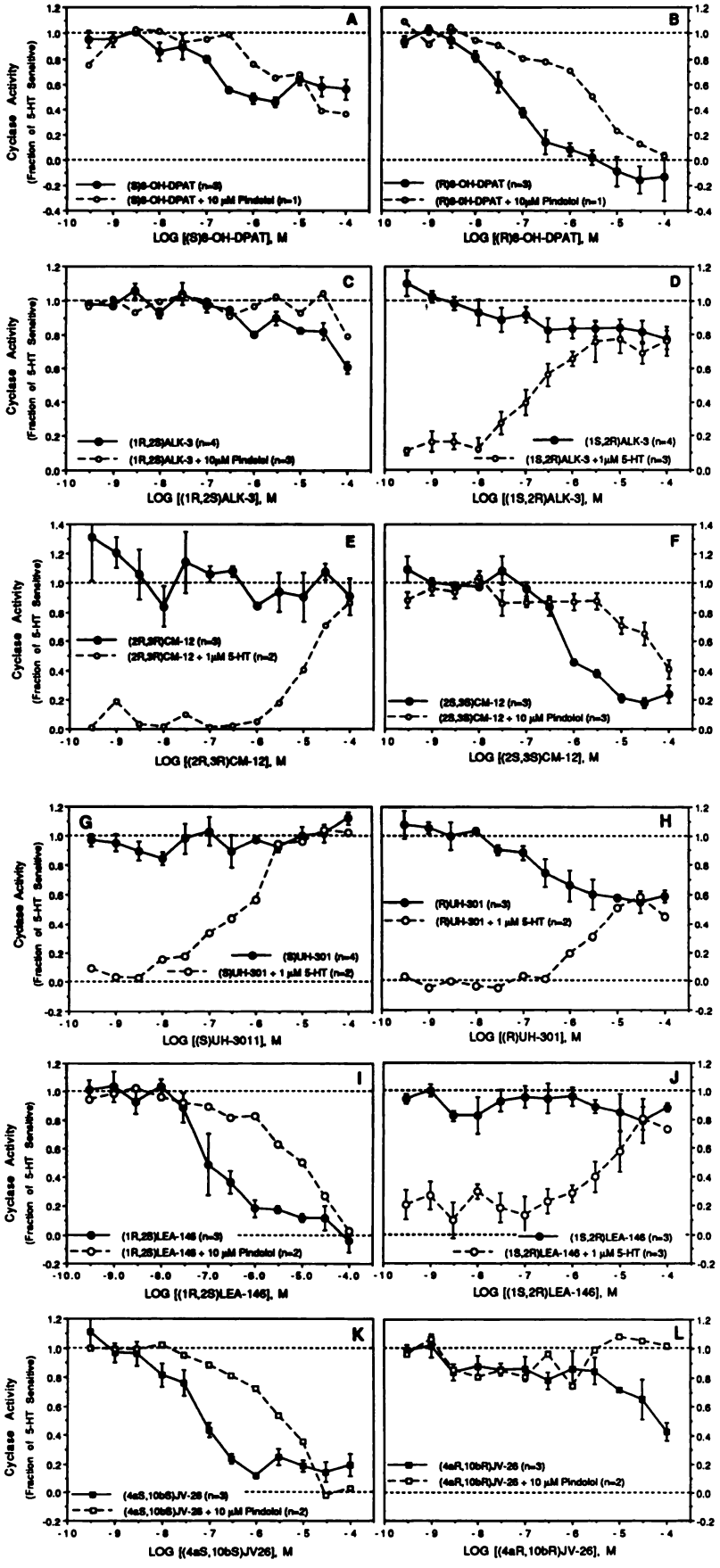

Fig. 2. Inhibition of FSC by the enantiomers of 8-OH-DPAT and its analogs. A, (*S*)-8-OH-DPAT alone (three experiments) (●) and in the presence of 10 μM pindolol (one experiment) (○). B, (*R*)-8-OH-DPAT alone (three experiments) (●) and in the presence of 10 μM pindolol (one experiment) (○). C, (1*R*,2*S*)-ALK-3 alone (four experiments) (●) and in the presence of 10 μM pindolol (three experiments) (○). D, (1*S*,2*R*)-ALK-3 alone (four experiments) (●) and in the presence of 1 μM 5-HT (three experiments) (○). E, (2*R*,3*R*)-CM-12 alone (three experiments) (●) and in the presence of 1 μM 5-HT (two experiments) (○). F, (2*S*,3*S*)-CM-12 alone (three experiments) (●) and in the presence of 10 μM pindolol (three experiments) (○). G, (*S*)-UH-301 alone (four experiments) (●) and in the presence of 1 μM 5-HT (two experiments) (○). H, (*R*)-UH-301 alone (three experiments) (●) and in the presence of 1 μM 5-HT (two experiments) (○). I, (1*R*,2*S*)-LEA-146 alone (three experiments) (●) and in the presence of 10 μM pindolol (two experiments) (○). J, (1*S*,2*R*)-LEA-146 alone (three experiments) (●) and in the presence of 1 μM 5-HT (three experiments) (○). K, (4a*S*,10b*S*)-JV-26 alone (three experiments) (■) and in the presence of 10 μM pindolol (two experiments) (□). L, (4a*R*,10b*R*)-JV-26 alone (three experiments) (■) and in the presence of 10 μM pindolol (two experiments) (□). All *points* are the mean ± standard error.

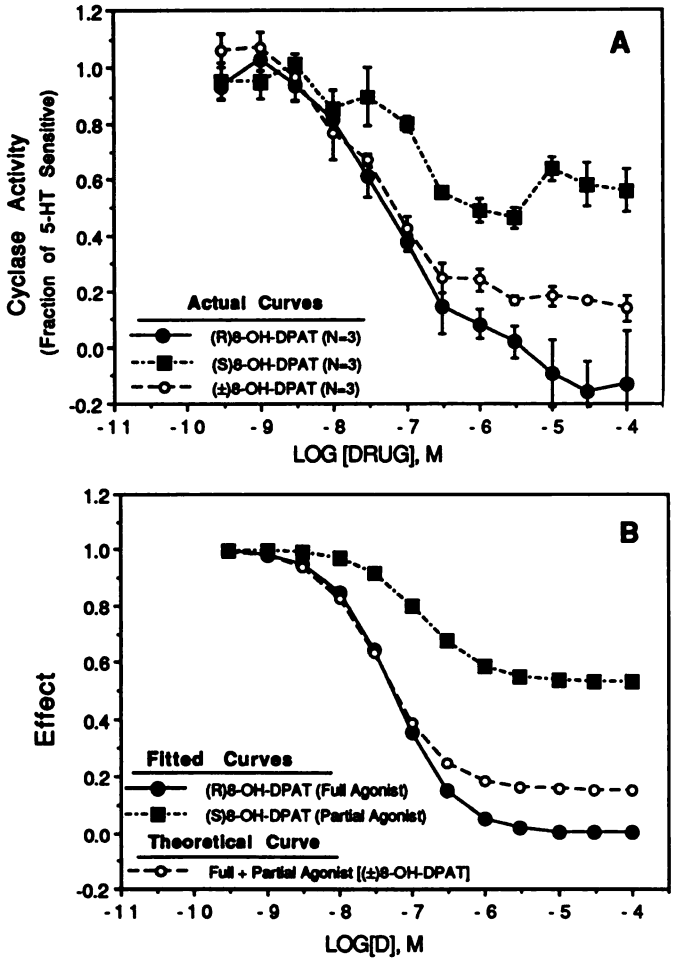

Fig. 3. Comparison of the effects of the enantiomers of 8-OH-DPAT and racemic 8-OH-DPAT on FSC. A, Actual dose-response curves for the inhibition of FSC by (*R*)-8-OH-DPAT (three experiments) (●), (*S*)-8-OH-DPAT (three experiments) (■), and (±)-8-OH-DPAT (three experiments) (○). B, *Curves with solid symbols*, fitted curves for (*R*)- and (*S*)-8-OH-DPAT from the data for the corresponding curves in A. The EC_{50} and intrinsic activity (percentage of maximal inhibition) values derived from these fits are given in Table 1. *Curve with open circles*, theoretical curve (calculated using the equation given in the Discussion) expected from the simultaneous addition of a full and partial agonist having the EC_{50} and intrinsic activity values calculated for (*R*)- and (*S*)-8-OH-DPAT.

one was dealing with a partial agonist of moderate intrinsic activity.

Mellin *et al.* (15, 16) have recently performed a comprehensive study in which the stereochemical and conformational characteristics of the enantiomers listed in Table 1, along with other aminotetralin analogs, were studied using X-ray crystallography and molecular mechanics calculations. They have proposed a somewhat flexible 5-HT_{1A} agonist pharmacophore consisting of an aromatic site and a vector between the nitrogen of the aminotetralin and an oxygen atom (or dummy atom) on the acceptor (or 5-HT_{1A} receptor). In trying to explain the features that determine the differences in intrinsic activity observed between the two enantiomers of 8-OH-DPAT, it is interesting to note that, in the model proposed by Mellin *et al.* (15, 16), (*S*)-8-OH-DPAT must be "flipped" over, relative to the *R*-enantiomer, to satisfy the requirements of the proposed pharmacophore (Fig. 4). Such an orientation would also be consistent with the enantiomeric differences in intrinsic activity seen with certain of the other analogs, such as CM-12 and

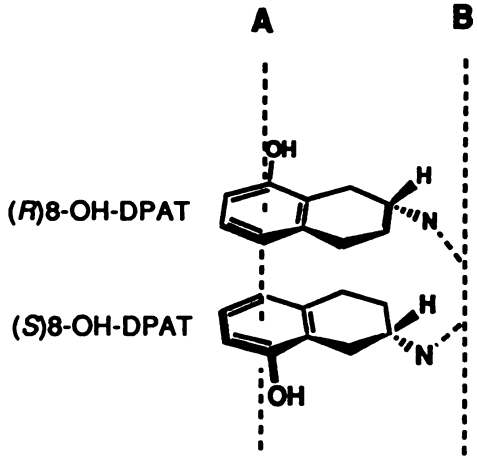

Fig. 4. Diagram of the theoretical fits of (*R*)- and (*S*)-8-OH-DPAT to the 5-HT_{1A} receptor, as adapted from the work of Mellin and co-workers (15, 16). *Line A*, a vector passing through the center of the aromatic ring and perpendicular to it; *line B*, the plane of a dummy atom (oxygen) on the receptor.

UH-301. However, a much larger series of analogs will ultimately have to be examined to establish the importance of the orientation of the aminotetralin nucleus relative to the substitutions (i.e., the 8-hydroxy group) in determining intrinsic activity.

Overall, the 5-HT_{1A} activity of the enantiomers of 8-OH-DPAT and its analogs in the FSC assay corresponded well to their respective *in vivo* actions (15, 16) and, hence, to the proposed model of the 5-HT_{1A} pharmacophore. One difference between the *in vivo* and *in vitro* data was that (*R*)-8-OH-DPAT was considered to be only slightly more active than its antipode *in vivo* (ED_{50} = 0.1 and 0.2 μmol/kg, respectively), whereas there was a large difference in intrinsic activities as measured *in vitro* by the FSC assay. This discrepancy could simply be a reflection of differing receptor densities in the *in vivo* (receptor-mediated feedback inhibition of 5-HTP accumulation) and *in vitro* (FSC) systems. Because this *in vivo* system, which is primarily a measure of 5-HT_{1A} autoreceptors, contains a large receptor reserve (30, 31), even partial agonists can appear as full agonists. In contrast, the FSC assay measures activity at the postsynaptic 5-HT_{1A} receptor, where there is likely little or no receptor reserve (30, 31). Consequently, the FSC assay provides a means of observing partial agonistic activity that would otherwise be seen as full agonistic activity in the presence of a large receptor reserve. In addition, although the *in vivo* tests demonstrated (1*S*,2*R*)-ALK-3 to be a fairly potent 5-HT_{1A} agonist and (1*R*,2*S*)-ALK-3 to be inactive, both of these enantiomers appeared as weak partial agonists in the FSC assay. However, because (1*R*,2*S*)-ALK-3 has very low affinity for the 5-HT_{1A} receptor, relative to its enantiomer, it is possible that no significant agonistic activity could occur *in vivo* with the concentrations tested.

It is interesting that even slight modifications to the structure of the reference 5-HT_{1A} agonist, 8-OH-DPAT, affected 5-HT_{1A} affinity and intrinsic activity. For example, the introduction of a single *cis*-methyl substituent in the C1 position (i.e., ALK-3) dramatically improved the stereoselectivity of 5-HT_{1A} binding, compared with that of the parent compound. The presence of this pseudoaxial 1-methyl group decreases mobility of the di-*n*-propylamino moiety and increases steric bulk on one face of the tetralin ring system (32), and it clearly caused a large

decrease in intrinsic activity of the 1*S*,2*R*-enantiomer, relative to that of 8-OH-DPAT. However, no distinction could be made concerning the relative activity of (1*S*,2*R*)- and (1*R*,2*S*)-ALK-3 in the FSC assay. Because (1*S*,2*R*)-ALK-3 has approximately a 1000-fold greater affinity for the 5-HT_{1A} receptor than does (1*R*,2*S*)-ALK-3, an impurity of even 0.1% (1*S*,2*R*)-ALK-3 in the sample of (1*R*,2*S*)-ALK-3 could account for the activity seen with the less potent enantiomer. Such a possibility, thus, limits the interpretation of the FSC data for (1*R*,2*S*)-ALK-3. Compared with the C1 position, a *trans*-methyl substituent in the C3 position (i.e., CM-12) resulted in greater stereoselectivity with respect to functional activity. Thus, (2*S*,3*S*)-CM-12 had high intrinsic activity, whereas the 2*R*,3*R*-enantiomer produced no significant effect on FSC.

Not only do the data emphasize the potential for enantiomeric differences in intrinsic activity, they provide possible structural prototypes for selective 5-HT_{1A} antagonists. In general, the development of agonists for neurotransmitter receptors has been based on structures related to the transmitter they mimicked, whereas antagonists have been large molecules that usually belonged to many different chemical classes and were generally nonspecific in action (33–35). The design of selective antagonists for the 5-HT_{1A} receptor has proven to be particularly difficult. Compounds developed to date either have had significant interactions with other receptor systems or have proven to be partial agonists rather than antagonists. The present work shows that small, agonist-like molecules may have potential as selective 5-HT_{1A} receptor antagonists. Specifically, (2*R*,3*R*)-CM-12, (*S*)-UH-301, and (1*R*,2*R*)-LEA-146 all caused no significant inhibition of FSC but instead antagonized the effect of 5-HT on FSC.

Among the aminotetralins examined, (*S*)-UH-301 shows the greatest potential as a 5-HT_{1A} antagonist. It has relatively good affinity for the 5-HT_{1A} site; average K_i values calculated from binding studies ranged from 52 to 127 nM (20) (Table 1), and the apparent K_i calculated using the Cheng-Prusoff equation (25) and the data in Fig. 2G was 56 nM. In addition to showing no agonist activity in the FSC assay, (*S*)-UH-301 has recently been shown to possess only antagonist activity in the *in vivo* 5-HTP accumulation assay (20). It is interesting that the 5-fluoro substitution from 8-OH-DPAT abolished efficacy in only the *S*-enantiomer. Fluorine differs only slightly, in size, from hydrogen, and its effect on the acidity of the phenol group of UH-301 has been proposed to be minimal (20). Because preliminary NMR experiments and molecular mechanics calculations did not indicate that the fluorine substituent induced any conformational changes, major differences between 8-OH-DPAT and UH-301 in 5-HT_{1A} affinity and activity may, therefore, be related to electronic distribution (20). Ultimately, the efficacy of 8-OH-DPAT may be altered by changes in electronic properties of the aromatic ring. Further extensive screening will be required to determine whether (*S*)-UH-301 is truly selective for 5-HT_{1A} receptors, although the initial report shows that it has very good selectivity between 5-HT_{1A} and D_2-dopaminergic receptors (20).

(*S*)-UH-301 and related structures such as (2*R*,3*R*)-CM-12 will likely serve as prototypes for even more potent 5-HT_{1A} antagonists, and further alterations of the substitution pattern of this series of 8-OH-DPAT-based analogs could possibly lead to further development of selective and potent 5-HT_{1A} antagonists, which would possess high and stereospecific 5-HT_{1A} affinity. Conformational preferences and electrostatic potentials of these compounds will have to be studied in order to establish how these factors may be of importance in determining 5-HT_{1A} receptor affinity and selectivity (36).

The new model for the 5-HT_{1A} pharmacophore proposed by Mellin *et al.* (15, 16) will undoubtedly have predictive value. It accommodates a relatively large set of compounds with different stereoselectivities and agonist potencies and, therefore, appears to have some generality. The 5-HT_{1A} intrinsic activities of this series of enantiomers in the FSC assay were generally in good agreement with the *in vivo* functional data and support the newly proposed 5-HT_{1A} pharmacophore model. Consequently, the FSC assay is a useful *in vitro* biochemical screen to assess 5-HT_{1A} activity of the enantiomers of 8-OH-DPAT analogs and can ultimately be used to provide insights into the SAR that differentiate full agonists from partial agonists from antagonists.

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