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Molecular Basis for the Binding of 2-Aminotetralins to Human Dopamine D_{2A} and D_3 Receptors

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SUMMARY

The affinities of a series of stereochemically well defined 2-aminotetralin derivatives for cloned human dopamine D_{2A} (443 amino acids) and D_3 receptors expressed in mammalian cell lines have been determined using [^{3}H]raclopride as radioligand. Several of the compounds tested showed high selectivity for the D_3 receptor. Notably, (*R*)-7-hydroxy-2-dipropylaminotetralin displayed 70-fold selectivity for the D_3 receptor and its *cis*-C1-methyl analog, (1*S*,2*R*)-AJ-148, displayed 38-fold selectivity. Large differences in receptor binding affinities between the compounds were obtained, despite the close structural relationship of the compounds. To better understand the receptor interactions of these compounds, we have constructed homology-based receptor models of the human D_{2A} and D_3 receptors by using bacteriorhodopsin as a template. The resulting model was used in conjunction with an indirect model. The indirect model describes a proposed active agonist conformation for dopaminergic 2-aminotetralins and related compounds and consists of a receptor excluded volume that was used to define the agonist binding site. We docked a number of ligands into the D_{2A} and D_3 binding sites by optimizing attractive interactions and minimizing repulsive interactions. In the binding site model of the D_{2A} receptor, the protonated nitrogen of the ligands interacts with Asp-114 in transmembrane region (TM) 3 through a reinforced ionic bond. The aspartic acid is surrounded by aromatic residues that may stabilize the ion pair formed with the protonated ligands. In addition, a hydrogen bond is formed from the phenolic hydrogen of the agonist ligands to Ser-193 (TM 5). Aromatic edge-to-face interactions occur between Phe-390 (TM 6) and the aromatic ring of the agonists. 2-Aminotetralin-based dopaminergic antagonists [e.g., (1*S*,2*R*)-UH-232] structurally related to agonists have a different but partly overlapping mode of binding, with the aromatic ring located more extracellularly, compared with agonists. The structure-activity relationships that are apparent from this and previous studies are qualitatively rationalized by the binding site models.

Five major DA receptor subtypes (D_1–D_5), all belonging to the family of GPC receptors, have been cloned and characterized by the use of molecular biological techniques (1). The D_1 and D_5 receptors are structurally related and stimulate adenylyl cyclase. On the other hand, the D_3 and D_4 subtypes show great similarities with the D_2 receptor, which couples inhibitorily to adenylyl cyclase.

Because the D_2 receptor has been the major target receptor for drugs used in the treatment of many neuropsychiatric diseases, including schizophrenia, the additional "D_2-like" receptors are of great interest. The recently cloned D_3 receptor seems to be expressed preferentially in the limbic regions of the brain (2, 3). This restricted distribution suggests that the D_3 receptor may be of interest in the development of antipsychotic agents devoid of extrapyramidal side effects. Sokoloff *et al.* (2, 4) have described the binding characteristics of a number of DA receptor agonists and antagonists with cloned rat and human D_3 receptors expressed in CHO cells. Interestingly, the only antagonists that displayed any, albeit weak, D_3 versus D_2 selectivity were (1*S*,2*R*)-AJ-76 and (1*S*,2*R*)-UH-232. Furthermore, the isotope-labeled racemic agonist [^{3}H]7-OH-DPAT appears to bind with pronounced selectivity to D_3 receptors in brain tissue (3). Clearly, derivatives of 2-aminotetralin constitute an interesting structural class of dopaminergic ligands with a potential for development into truly D_3-selective compounds.

The recently acquired knowledge about the amino acid sequences of an array of GPC receptors and the availability of a high-resolution three-dimensional structure of bacteriorhodopsin makes it attractive to construct homology-based GPC receptor models. In fact, a number of such models have already been reported (5–7). Previously, information about the receptor binding site was obtained by receptor mapping, using conformationally restricted ligands (indirect modeling) (8). Herein we use an approach in which indirect and homology-based models

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ABBREVIATIONS: DA, dopamine; CHO, Chinese hamster ovary; GPC, G protein-coupled; TM, transmembrane region; (1*S*,2*R*)-AJ76, (1*S*,2*R*)-5-methoxy-1-methyl-2-propylaminotetralin; (2*R*,3*S*)-AJ-166, (2*R*,8*S*)-5-hydroxy-3-methyl-2-dipropylaminotetralin; (1*S*,2*R*)-UH-232, (1*S*,2*R*)-5-methoxy-1-methyl-2-dipropylaminotetralin; 7-OH-DPAT, 7-hydroxy-2-dipropylaminotetralin; 5-OH-DPAT, 5-hydroxy-2-dipropylaminotetralin.

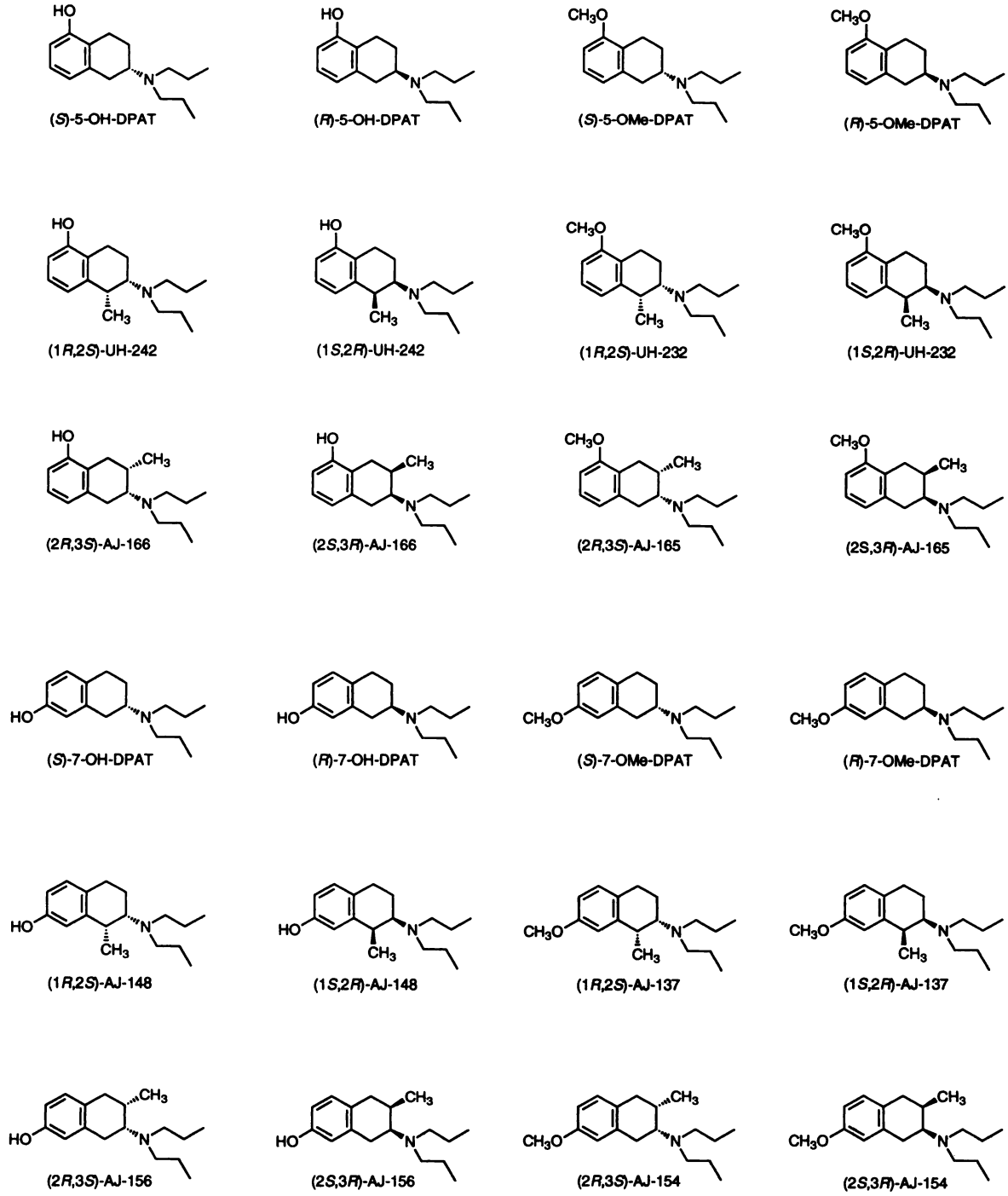

Fig. 1. Structures of the 2-dipropylaminotetralin derivatives included in the receptor binding assays (see also Table 1). *OMe*, methoxy.

are combined (7) to rationalize D_{2A} and D_3 receptor binding data in terms of ligand-receptor interactions. The affinities of a series of stereochemically well defined 2-aminotetralin derivatives for human D_{2A} and D_3 receptors have been determined with *in vitro* receptor binding. Deduced D_{2A} and D_3 receptor binding site models appear to satisfactorily accommodate the experimental data in a qualitative sense. Consequently, these models may be useful in the design of novel drugs.

Experimental Procedures

Materials. Mouse fibroblast (Ltk^-) cells expressing human D_{2A} (long isoform) receptors were obtained from Dr. O. Civelli (Vollum Institute, OR). CHO cells expressing human D_3 receptors were purchased from INSERM Institute (Paris, France). (+)-Butaclamol was purchased from Research Biochemical Inc. (Natick, MA). [^{3}H]Raclopride (batch OA 654/21; specific activity, 46.0 Ci/mmol) was synthesized at Astra Arcus AB. The syntheses of the 2-aminotetralin derivatives have been described earlier (9–12).

[^{3}H]Raclopride binding to membranes from cells expressing cloned human DA receptors. The cells expressing human D_{2A} (long isoform) (Ltk^- cells) and D_3 (CHO cells) receptors were grown and membranes were prepared as described previously (13). In brief, the cells were detached with 0.05% trypsin and 0.02% EDTA, collected by centrifugation, and homogenized in 10 mM Tris·HCl, 5 mM $MgSO_4$. The homogenate was washed in binding buffer (50 mM Tris·HCl, 120

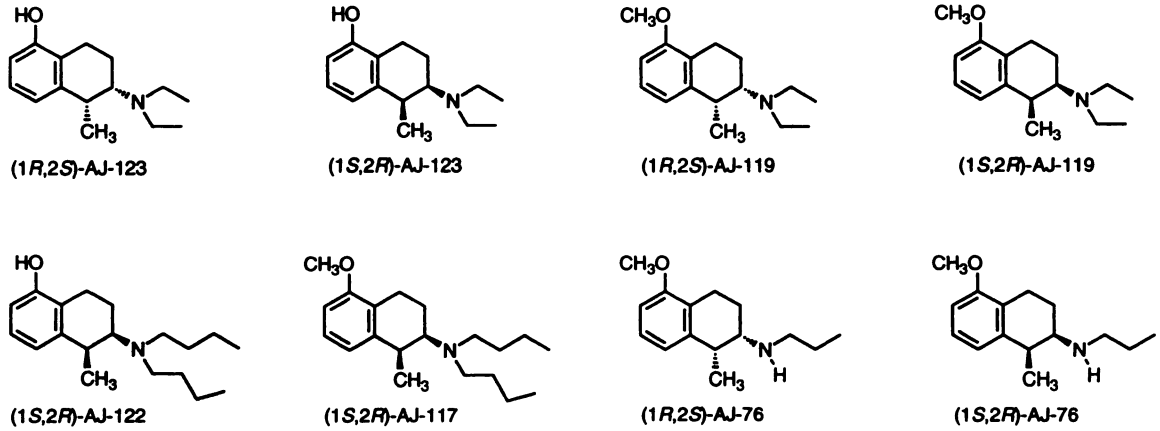

Fig. 2. Structures of the various *N*-alkyl and *N,N*-dialkyl 5-oxygenated *cis*-1-methyl-2-aminotetralin derivatives included in the receptor binding assays (see also Table 2).

TABLE 1

Potencies of various 2-(*N,N*-dipropylamino)tetralin derivatives to inhibit [^{3}H]raclopride binding to cloned human D_{2A} and D_3 receptors

The competition studies were performed and the K_i values were calculated as described in Experimental Procedures. The K_i values and Hill coefficients (n_H) are means ± standard errors of *n* experiments. (*S*)-5-OH-DPAT was best described with two affinity sites, as follows: K_{high} = 9.52 ± 2.3 nM (73%) and K_{low} = 240 ± 61 nM.

Compound	D_{2A} (Ltk$^-$ cells)			D_3 (CHO cells)			D_{2A}/D_3
	K_i	n_H	*n*	K_i	n_H	*n*	
	nM			nM			
(*S*)-5-OH-DPAT	27.0 ± 3.7	0.66 ± 0.001	4	0.893 ± 0.051	0.71 ± 0.046	3	30
(*R*)-5-OH-DPAT	199 ± 52	0.84 ± 0.037	4	7.90 ± 1.8	0.83 ± 0.027	3	25
(*S*)-5-OMe-DPAT[a]	231 ± 28	0.91 ± 0.011	4	10.0 ± 3.2	0.76 ± 0.013	3	23
(*R*)-5-OMe-DPAT	57.7 ± 11	0.89 ± 0.071	4	2.95 ± 0.60	0.67 ± 0.045	3	20
(1*R*,2*S*)-UH-242	211 ± 38	0.71 ± 0.062	3	11.1 ± 1.1	0.80 ± 0.033	4	19
(1*S*,2*R*)-UH-242	68.0 ± 5.6	1.00 ± 0.14	3	14.2 ± 2.4	0.78 ± 0.021	3	4.8
(1*R*,2*S*)-UH-232	1100 ± 100	0.91 ± 0.053	4	102 ± 8.4	0.73 ± 0.019	4	11
(1*S*,2*R*)-UH-232[b]	14.2 ± 2.9	0.87 ± 0.066	5	2.89 ± 0.62	0.77 ± 0.033	4	4.9
(2*R*,3*S*)-AJ-166	15.4 ± 5.3	0.72 ± 0.050	5	3.66 ± 0.36	0.85 ± 0.063	3	4.2
(2*S*,3*R*)-AJ-166	1080 ± 110	0.92 ± 0.086	4	597 ± 140	0.85 ± 0.052	4	1.8
(2*R*,3*S*)-AJ-165	77.3 ± 12	0.76 ± 0.081	3	36.5 ± 11	0.68 ± 0.042	3	2.1
(2*S*,3*R*)-AJ-165	310 ± 79	0.83 ± 0.083	3	108 ± 20	0.77 ± 0.055	3	2.9
(*S*)-7-OH-DPAT	2440 ± 260	1.00 ± 0.12	3	243 ± 43	0.83 ± 0.012	3	10
(*R*)-7-OH-DPAT	43.1 ± 12	0.70 ± 0.081	7	0.614 ± 0.052	0.66 ± 0.094	3	70
(*S*)-7-OMe-DPAT	2120 ± 170	0.78 ± 0.022	4	101 ± 4.4	0.82 ± 0.030	4	22
(*R*)-7-OMe-DPAT	1930 ± 560	0.96 ± 0.014	3	77.8 ± 7.2	0.83 ± 0.070	3	25
(1*R*,2*S*)-AJ-148	3870 ± 440	0.86 ± 0.067	3	463 ± 91	0.86 ± 0.070	4	8.4
(1*S*,2*R*)-AJ-148	22.1 ± 6.5	0.80 ± 0.079	6	0.574 ± 0.14	0.77 ± 0.049	3	38
(1*R*,2*S*)-AJ-137	2720 ± 400	0.92 ± 0.075	3	421 ± 68	0.87 ± 0.020	3	6.5
(1*S*,2*R*)-AJ-137	835 ± 230	0.93 ± 0.072	3	79.9 ± 5.0	0.93 ± 0.072	3	10
(2*R*,3*S*)-AJ-156	508 ± 99	0.83 ± 0.043	4	446 ± 100	0.84 ± 0.018	3	1.1
(2*S*,3*R*)-AJ-156	1710 ± 380	1.01 ± 0.12	3	112 ± 18	0.82 ± 0.040	3	15
(2*R*,3*S*)-AJ-154	472 ± 98	0.89 ± 0.037	3	232 ± 44	0.82 ± 0.018	3	2.0
(2*S*,3*R*)-AJ-154	2070 ± 440	0.95 ± 0.042	3	867 ± 130	0.85 ± 0.040	3	2.4

[a] OMe-DPAT, methoxy-2-dipropylaminotetralin.
[b] From Ref. 12.

mM NaCl, 5 mM KCl, 1.5 mM $CaCl_2$, 4 mM $MgCl_2$, 1 mM EDTA, pH 7.4 at 22°) and stored in aliquots at −70° until use.

The frozen membranes were thawed, homogenized with a Branson 450 sonifier, and suspended in binding buffer to a final concentration of 5–25 μg of protein/0.5 ml (80–100 pM receptors). The binding assays were initiated by the addition of membranes and were carried out, in duplicate, at 22 ± 1° for 60 min. In saturation experiments, eight to 16 concentrations (0.15–12 nM) of [^{3}H]raclopride were used. In competition experiments, 1–2 nM [^{3}H]raclopride was incubated with 10–12 concentrations of the competing ligand. The substances were dissolved in 0.1% ascorbic acid and dilution series were made in binding buffer using a BIOMEK 1000 robot (Beckman). Nonspecific binding was defined with 1 μM (+)-butaclamol. The incubations were terminated by rapid filtration through Whatman GF/B filters and subsequent washing with cold buffer (50 mM Tris·HCl, pH 7.4) using a cell harvester (Brandel). Scintillation cocktail (Packard Ultima Gold, 4 ml) was added and the radioactivity was determined in a Packard 2200CA liquid scintillation analyzer at 50% efficiency. Protein concentration was determined by the method of Markwell *et al.* (14), using bovine serum albumin as a standard.

The binding curves were analyzed individually by nonlinear regression using the LIGAND program (15). One- and two-site curve fittings were tested in all experiments, and the two-site model was accepted when it significantly improved the curve fit ($p < 0.05$; *F* test) and when each site accounted for >20% of the receptors. The Hill coefficients were calculated for each individual experiment.

Receptor modeling. Receptor models were constructed using Sybyl 5.5 (TRIPOS Associates Inc., St. Louis, MO). The coordinates for bacteriorhodopsin were obtained from Henderson *et al.* (16). The amino acid sequences for the human D_{2A} and D_3 receptors were obtained from

TABLE 2

Potencies of various *N*-alkyl and *N,N*-dialkyl 5-oxygenated *cis*-1-methyl-2-aminotetralin derivatives to inhibit [^{3}H]raclopride binding to cloned human D_{2A} and D_3 receptors

The competition studies were performed and the K_i values were calculated as described in Experimental Procedures. The K_i values and Hill coefficients (n_H) are means ± standard errors of *n* experiments.

Compound	D_{2A} (Ltk$^-$ cells)			D_3 (CHO cells)			D_{2A}/D_3
	K_i	n_H	*n*	K_i	n_H	*n*	
	nM			nM			
(1*R*,2*S*)-AJ-123	943 ± 390	0.85 ± 0.055	2	67.4 ± 11	0.95 ± 0.085	2	14
(1*S*,2*R*)-AJ-123	294 ± 32	1.04 ± 0.090	2	173 ± 36	0.96 ± 0.010	2	1.7
(1*R*,2*S*)-AJ-119	6460 ± 1400	1.02 ± 0.070	2	1050 ± 20	0.95 ± 0.095	2	6.2
(1*S*,2*R*)-AJ-119	85.6 ± 10	1.00 ± 0.040	2	44.6 ± 2.2	0.86 ± 0	2	1.9
(1*S*,2*R*)-AJ-122	796 ± 250	1.00 ± 0.11	2	301 ± 120	1.05 ± 0.11	2	2.6
(1*S*,2*R*)-AJ-117	171 ± 49	1.06 ± 0.10	2	60.5 ± 26	0.74 ± 0.17	2	2.8
(1*R*,2*S*)-AJ-76	>10 μM[a]		3	636 ± 23	0.92 ± 0.089	3	
(1*S*,2*R*)-AJ-76[b]	80.2 ± 20	0.95 ± 0.031	3	35.0 ± 4.9	0.89 ± 0.080	4	2.3

[a] IC_{50} value.
[b] From Ref. 12.

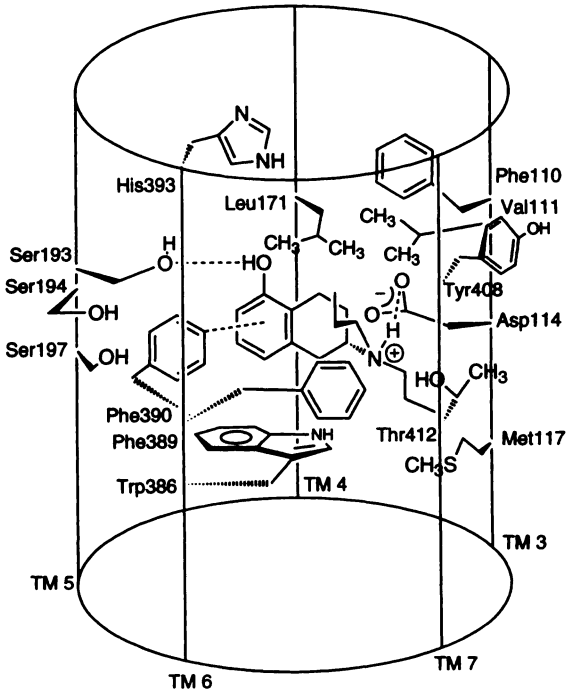

Fig. 3. Schematic representation of the ligand binding site of the D_{2A} receptor model. The agonist (*S*)-5-OH-DPAT is located in the binding site. Interactions occur between the cationic nitrogen of the ligand and Asp-114 and between the phenolic group and Ser-193. In addition, an aromatic edge-to-face interaction can be seen between the ligand and Phe-390.

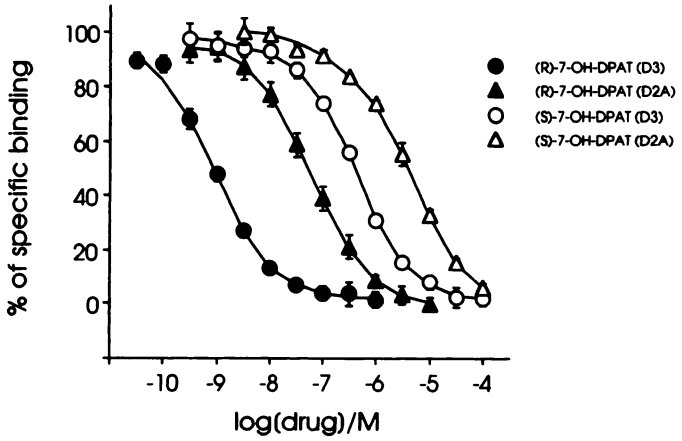

Fig. 4. Dose-response curves for (*S*)- and (*R*)-7-OH-DPAT inhibition of [^{3}H]raclopride binding to D_{2A} and D_3 receptors. The competition experiments were performed as described in Experimental Procedures. The results are means ± standard errors of three to seven experiments performed in duplicate. Where not shown, the standard error is smaller than the size of the symbol.

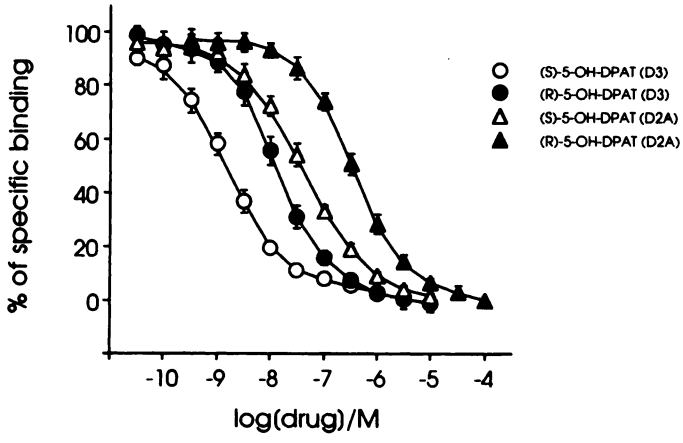

Fig. 5. Dose-response curves for (*S*)- and (*R*)-5-OH-DPAT inhibition of [^{3}H]raclopride binding to D_{2A} and D_3 receptors. The competition experiments were performed as described in Experimental Procedures. The results are means ± standard errors of three or four experiments performed in duplicate. Where not shown, the standard error is smaller than the size of the symbol.

published sequences (17–19). The receptor models of the human D_{2A} and D_3 receptors were constructed in a similar way according to a strategy previously described for the muscarinic m1 receptor (7). In short, the models are based on a presumed homology in three-dimensional structure between bacteriorhodopsin and the GPC receptors. α-Helices were constructed from the primary structure of the D_{2A} and D_3 receptors. TMs were determined by examining hydropathy plots and multiple sequence alignments of a number of GPC receptors. The relative rotations of the helices were estimated by considering conserved amino acids and hydrophobic moment plots. The α-helices ($\phi = -55.02$ and $\psi = -50.43$) were constructed from the amino acid sequences of the TMs, and proline kinks were taken into account. Side-chain conformations from rotamer libraries were used. The helices were energy minimized using the AMBER all-atom force field. Fitting of the backbone of these helices onto the backbone of bacteriorhodopsin

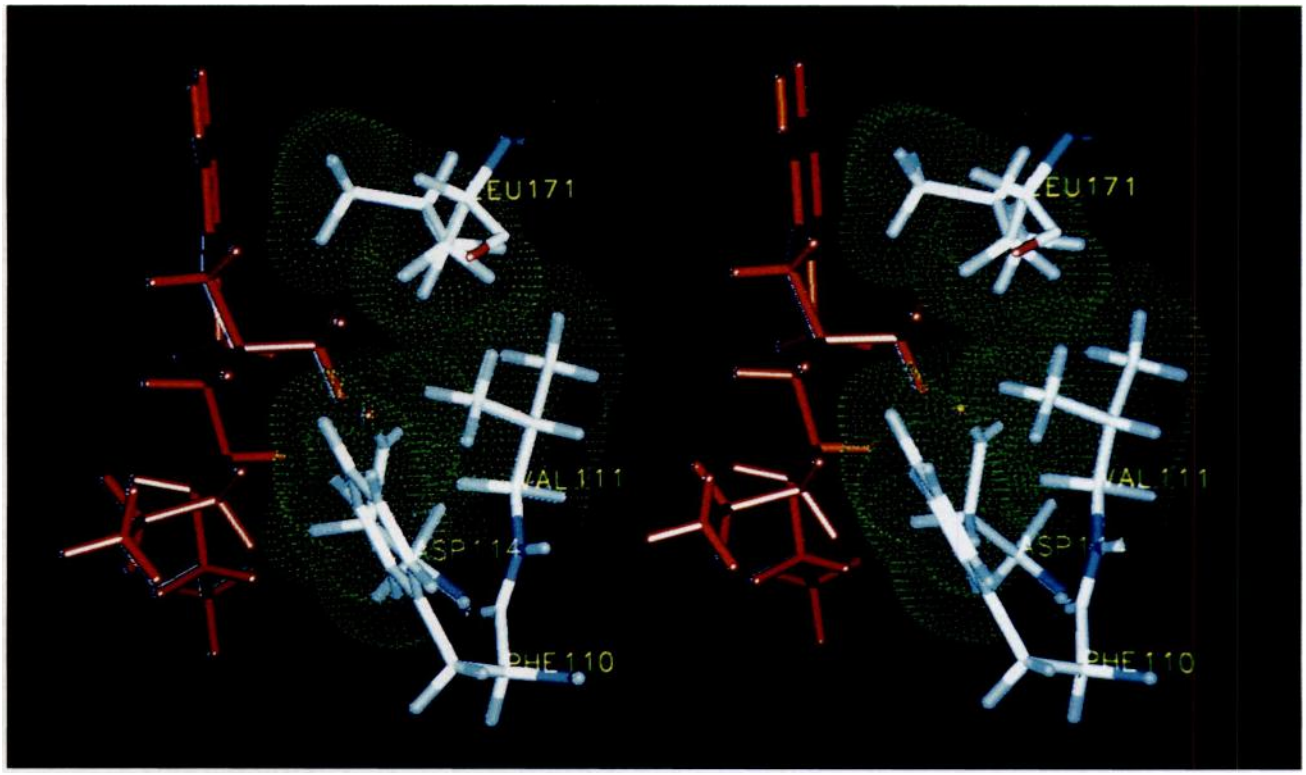

Fig. 6. Stereo representation of the interactions between (2*R*,3*S*)-AJ-166 and the D_{2A} receptor model. The methyl group of the ligand fits into a lipophilic binding pocket of the receptor defined by the residues Val-111 and Leu-171 together with Phe-110 and Asp-114.

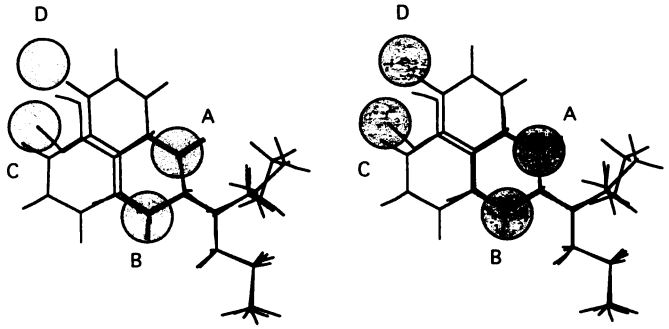

Fig. 7. Stereo representation showing the overlap between the agonist (*S*)-5-OH-DPAT and the antagonist (*R*)-5-OH-DPAT as docked into the D_{2A} receptor model. *Area A*, region where the location of a methyl group increases affinity (the methyl pocket); *area B*, region located close to Asp-114, where a methyl group decreases affinity; *areas C* and *D*, putative interaction points with the *O*-methyl groups of antagonist and agonist ligands, respectively.

produced the TM bundle of the receptors. The loop regions were not included in the modeling. The side chains were adjusted manually to avoid overlap produced by the fitting procedure. The resulting TM bundle was energy minimized using the AMBER united-atom force field (2000 iterations) and the all-atom force field (500 iterations), with no restriction of the backbone, a distance-dependent dielectric constant of 4.0, and a nonbonded cut-off of 9.0 Å.

Binding site modeling. The DA receptors have been studied by site-directed mutagenesis. Several conclusions can be drawn from these studies. (a) The aspartic acid in TM 3 (Asp-114 in D_2) is of importance for the binding of agonists as well as antagonists (20). This is by analogy with other GPC receptors where the endogenous ligand has a protonated/quaternary nitrogen, e.g., the adrenergic and muscarinic receptors. (b) The aspartic acid in TM 2 (Asp-80 in D_2) has been substituted by an alanine or a glutamate (21). These substitutions abolish or decrease, respectively, the inhibition of adenylyl cyclase. In addition, the sodium and pH regulation of the affinities for agonists and substituted benzamide antagonists was impaired (21). Thus, Asp-80 seems to be of importance for agonist efficacy by binding sodium and thereby stabilizing a certain receptor conformation needed for signal transduction. Similar results have been obtained with the α_2-adrenergic receptor (22). (c) There are three serine residues in TM 5, all of which may have some importance for ligand binding and/or receptor activation. These residues have been mutated in the D_{2A} (20) and D_{2B} receptors (23). Results from these partly contradictory studies seem to indicate that primarily Ser-193, but also Ser-197, is important for agonist binding (affinity). Ser-194 seems to be crucial for the ability of DA to inhibit adenylyl cyclase activity, whereas mutation of Ser-193 and Ser-197 decreased the affinity of DA (23). When trying to locate the binding site interacting with the DA receptor agonists examined here, we have considered interactions with the residues discussed above. Furthermore, we have attempted to accommodate these interactions in the binding site model.

The homology-based model described above represents one of an infinite number of models. The relevance of these models can be assessed only by evaluating their ability to accomodate experimental data. To simplify the evaluation procedure and to better define the binding sites of the D_{2A} and D_3 receptors, we have used a DA receptor excluded volume, which is based on the indirect model of Johansson *et al.* (24). The potent DA receptor agonists (*S*)-5-OH-DPAT, (*R*)-apomorphine, (2*R*,3*S*)-AJ-166, and (4a*S*,10b*S*)-*N*-propyl-7-hydroxy-1,2,3,4,4a,5,6,10b-octahydrobenzo[*f*]quinoline (8, 10, 24, 25) were used in their pharmacophore conformations and were aligned by fitting the nitrogen, the oxygen, and the center of the aromatic ring (25).[1] The combined volume of these ligands (the DA receptor excluded volume)

[1] For simplicity, pharmacophore conformations of the dimethylamino analogs of the compounds were used.

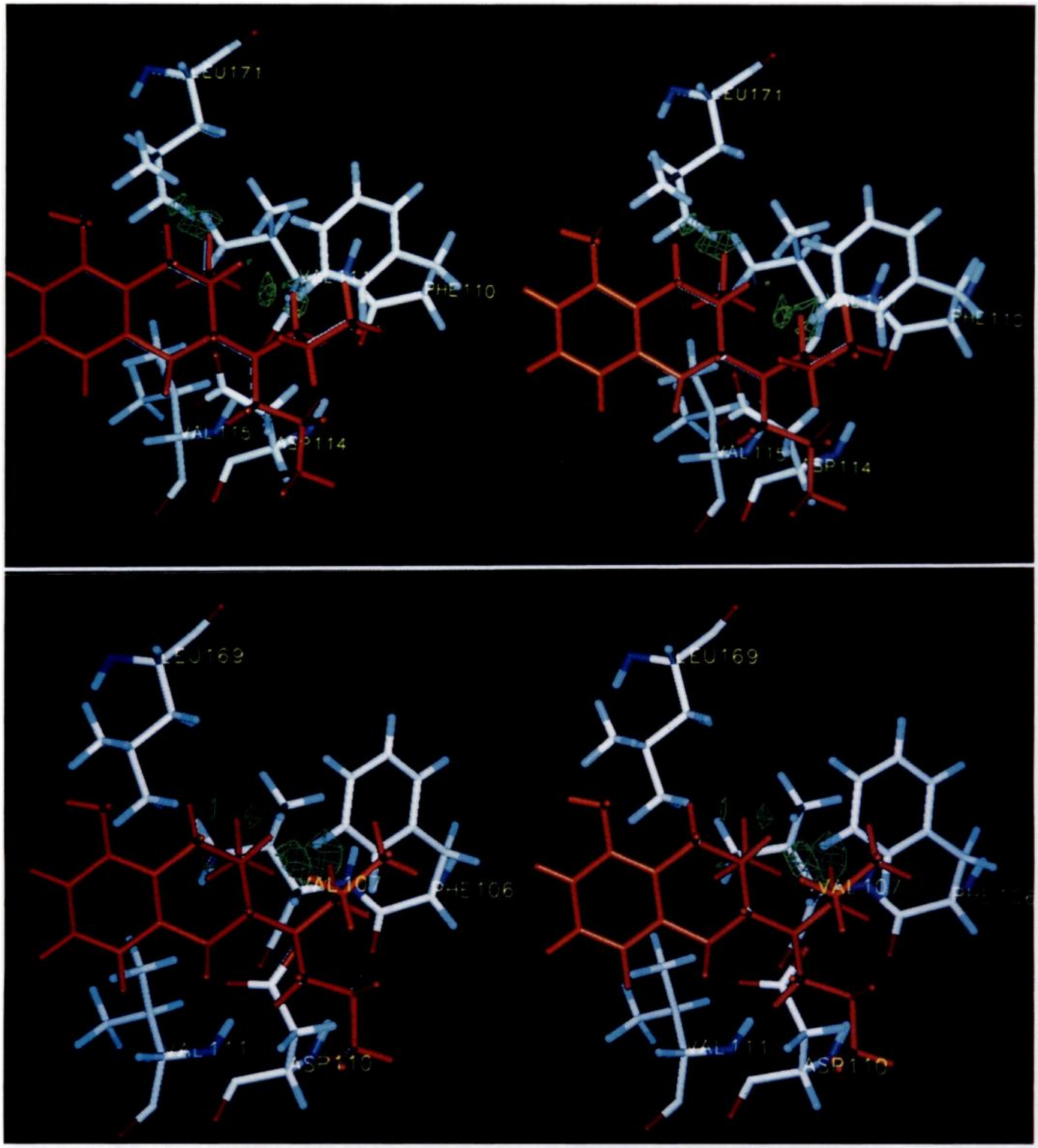

Fig. 8. Stereo representation of the interactions between (2*R*,3*S*)-AJ-166 and the D_{2A} (*top*) and D_3 (*bottom*) receptor models. The ligand has been docked optimally into the D_{2A} receptor model. The docking into the D_3 receptor model has been made by optimizing the Asp-110-nitrogen and Ser-192-hydroxyl distances. *Green areas*, van der Waals interactions between the methyl group of the ligand and the amino acid residues defining the methyl pocket. This interaction is significantly larger for the D_3 receptor, where the methyl group produces van der Waals overlap with Phe-106. Consequently, when interacting with the D_3 receptor, (2*R*,3*S*)-AJ-166 moves away from the aspartate, thus making this interaction less optimal.

was used to probe a common binding site for the agonists. The docking procedure done in Sybyl involved manual docking of the receptor excluded volume into the homology-based receptor model using one of the oxygens in Asp-114 (TM 3) as an anchoring point. Bond lengths and angles of attractive interactions were optimized and repulsive van der Waals interactions were minimized during the docking. In addition, the conformations of the side chains forming the putative binding site were changed manually to minimize overlap between the homology-based receptor models and the receptor excluded volume (using the Sybyl command MVOLUME). After definition of the binding site

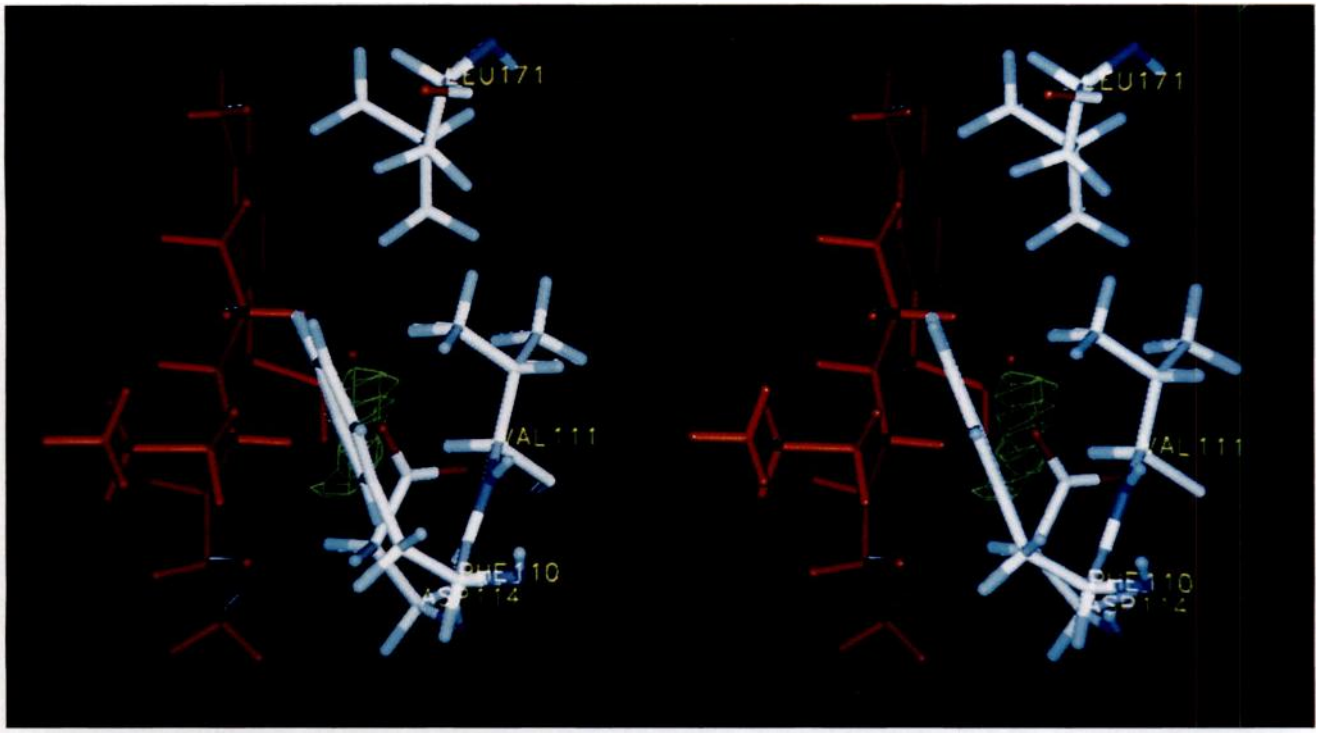

Fig. 9. Stereo representation showing (1*R*,2*S*)-UH-242 in the D_{2A} binding site. The ligand has been fitted in the same way as (*S*)-5-OH-DPAT. *Green area*, van der Waals overlap produced by the methyl group of (1*R*,2*S*)-UH-242 and Asp-114. (1*R*,2*S*)-UH-242 binds optimally when the nitrogen-aspartate distance is increased, which leads to a relief of steric interactions between the methyl group and Asp-114.

models by use of the indirect model, we performed manual docking experiments with individual ligands to achieve optimal interactions with each ligand. The conformational analyses of the 2-aminotetralin derivatives discussed herein have been described previously (26), and conformations corresponding to their proposed pharmacophore conformations (8, 10, 11, 24, 25) were used in the dockings.

Results and Discussion

***In vitro* receptor binding studies using [^{3}H]raclopride.** The present series of compounds includes resolved C5- and C7-oxygenated 2-aminotetralins, non-methyl substituted or methyl substituted at C1 or C3 (Figs. 1 and 2). Only *cis*-methyl-substituted compounds have been investigated, because the corresponding *trans*-stereoisomers are of low potency or inactive as D_2 receptor ligands (25). The affinities of these closely related 2-aminotetralin derivatives for cloned human D_{2A} and D_3 receptors were determined using the *in vitro* receptor binding method (Tables 1 and 2). The substituted benzamide [^{3}H] raclopride was used as radioligand. It has been shown to label cloned human D_{2A} and D_3 receptors with high affinity and low nonspecific binding (13). In the present experiments the K_d values of [^{3}H]raclopride for D_{2A} and D_3 receptors were 1.43 ± 0.09 nM (eight experiments) and 1.58 ± 0.06 nM (five experiments), respectively. The B_{max} values were 2.70 ± 0.2 and 3.57 ± 0.1 pmol/mg of protein, respectively.

Most D_2 receptor agonists display high and low affinity binding sites in *in vitro* receptor binding assays (27). These sites are believed to represent different conformations of the receptor protein. Various factors, such as sodium ions, GTP, and incubation temperature, seem to affect the receptor conformation (27). In the present series of compounds, high and low affinity binding sites were not easily detected. (*S*)-5-OH-DPAT was the only compound with a clear biphasic binding profile at the D_{2A} receptor, with 73% in the high affinity state (K_{high} = 9.5 nM). In addition, DA displayed two binding sites for the D_{2A} and D_3 receptors under the same experimental conditions. The high affinity site of the D_{2A} receptor is sensitive to GTP (data not shown). It is noteworthy, however, that the D_2 receptor agonists (*S*)-5-OH-DPAT, (1*R*,2*S*)-UH-242, (2*R*,3*S*)-AJ-166, and (*R*)-7-OH-DPAT all have Hill coefficients significantly different from 1 (Student's *t* test, $p < 0.05$) (Table 1).

To confirm that the lack of two binding sites for most of the agonists was not due to the use of an artificial test system (cloned receptors expressed in cell lines), we investigated the binding of (*R*)-7-OH-DPAT to rat striatal D_2 receptors. Using [^{3}H]raclopride and the same experimental conditions as described above, (*R*)-7-OH-DPAT displayed a monophasic displacement curve with a Hill coefficient of 0.92 ± 0.033 and a K_i value of 36.6 ± 9.5 nM (three experiments). This is in agreement with its affinity for cloned human D_{2A} receptors (Table 1), as well as the high affinity value previously reported by Seeman *et al.* (28), where (*R*)-7-OH-DPAT displaced [^{3}H] spiperone in a biphasic manner, with a K_{high} of 36.1 nM and a K_{low} of 2305 nM (in the absence of sodium). The fact that we use sodium in the incubation buffer and that some of the 2-aminotetralins tested probably are partial agonists may influence our ability to resolve high and low affinity binding sites.

Binding site models for the D_{2A} and D_3 receptors. Interactions of the ligands with the binding site models for the D_{2A} and D_3 receptors are qualitatively similar. In the binding site model for the D_{2A} receptor, the protonated nitrogen of the ligands interacts with Asp-114 (Asp-110 in D_3) in TM 3 through a reinforced ionic bond (29). The aspartic acid is surrounded

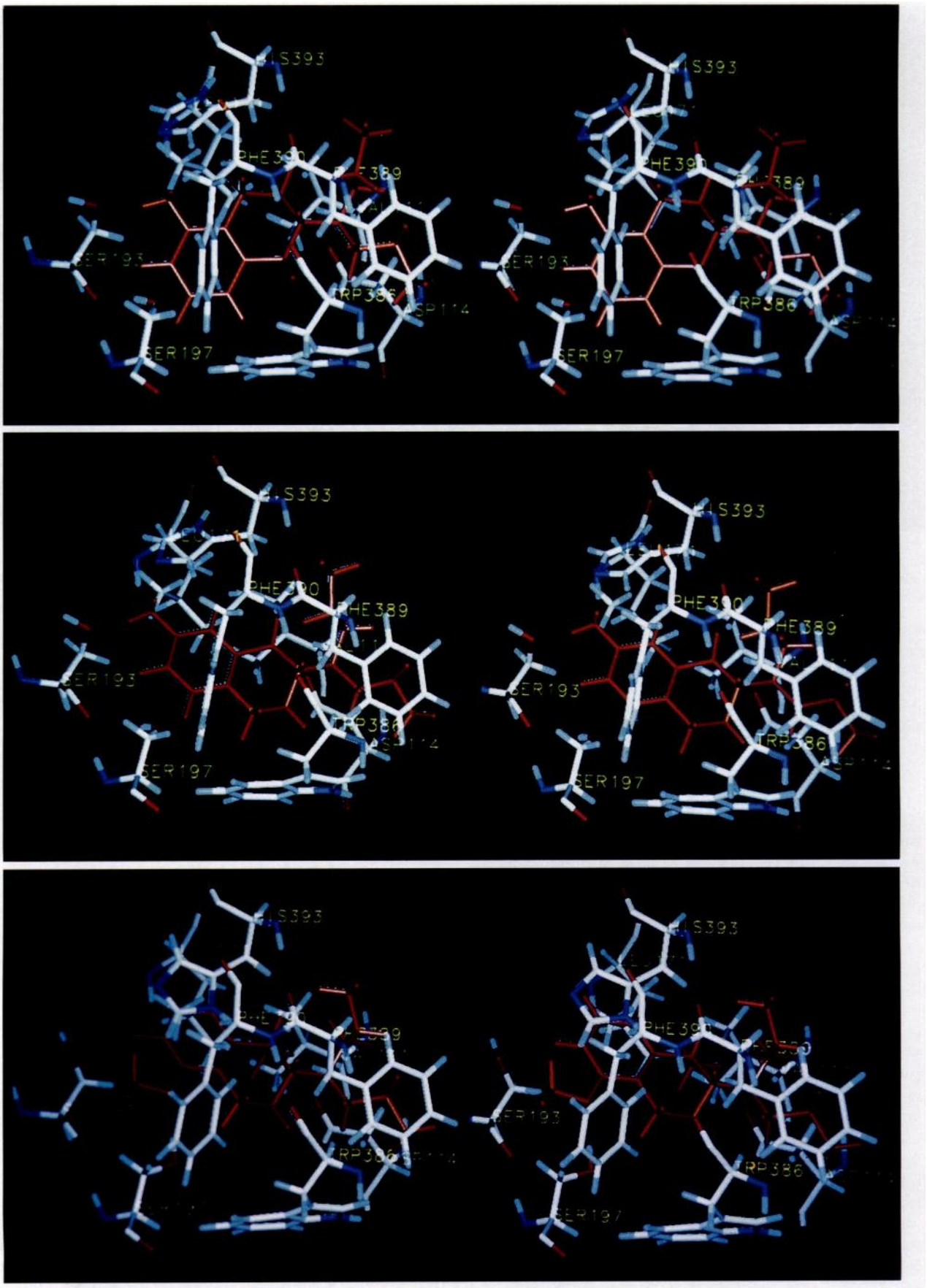

Fig. 10. Dockings of the agonists (*S*)-5-OH-DPAT (*top*) and (*R*)-7-OH-DPAT (*middle*) and the antagonist (1*S*,2*R*)-UH-232 (*bottom*) into the D_{2A} receptor model.

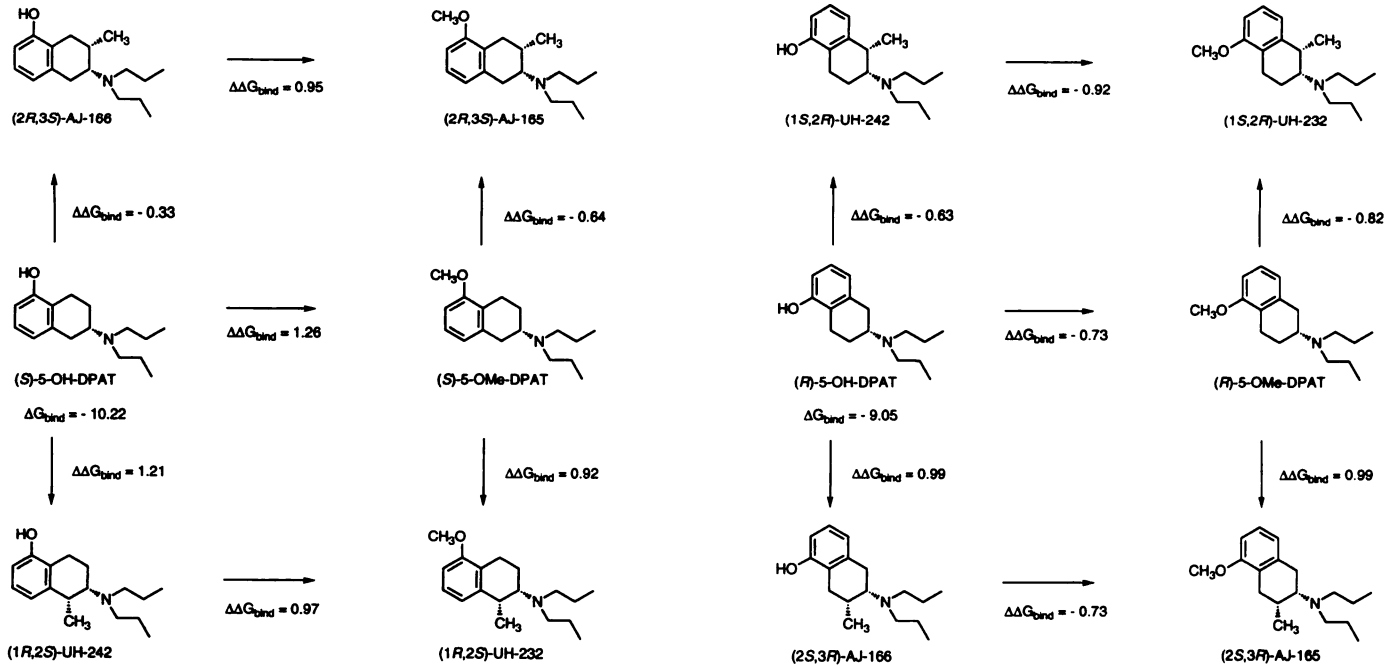

Fig. 11. Thermodynamic cycle describing the experimentally determined shifts in free energies of D_{2A} receptor binding ($\Delta\Delta G_{bind}$), obtained by perturbing (*S*)- and (*R*)-5-OH-DPAT into methyl-substituted analogs by O, C1, or C3 methylation. The contribution of each methyl group to the binding affinity can be estimated by assuming that the relative mode of binding of these compounds is that indicated (see also Fig. 7). The free energy of binding (ΔG_{bind}) was calculated for each drug-receptor interaction from the D_{2A} receptor binding affinities given in Table 1, using the formula $\Delta G_{bind} = RT \ln K_i$. The change in ΔG_{bind} ($\Delta\Delta G_{bind}$) associated with a particular perturbation (of a hydrogen to a methyl group) was determined from the difference in ΔG_{bind} values between the perturbed and nonperturbed compounds. A negative $\Delta\Delta G_{bind}$ corresponds to an increase in affinity due to the perturbation, whereas a positive value corresponds to a decrease in affinity. $\Delta\Delta G_{bind}$ values are given in kcal/mol. A perturbation of a hydrogen into a methyl group in *area A* (Fig. 7) favors binding and a methyl group introduced in *area B* (Fig. 7) disfavors binding. Perturbing hydroxy to methoxy (*OMe*) in compounds homochiral with (*R*)-5-OH-DPAT favors binding (Fig. 7, *area C*), whereas it disfavors binding in compounds homochiral with (*S*)-5-OH-DPAT (Fig. 7, *area D*). Derived values (average ± standard error) of $\Delta\Delta G_{bind}$ for methyl groups in *areas A* (−0.61 ± 0.10 kcal/mol), *B* (1.0 ± 0.06 kcal/mol), *C* (−0.79 ± 0.06 kcal/mol), and *D* (1.1 ± 0.10 kcal/mol) may be used to rationalize the almost identical binding affinities of the following pairs of compounds: (2*R*,3*S*)-AJ-166 and (1*S*,2*R*)-UH-232 (15.4 nM and 14.2 nM, respectively); (2*R*,3*S*)-AJ-165 and (1*S*,2*R*)-UH-242 (77 nM and 68 nM); (1*R*,2*S*)-UH-242 and (2*S*,3*R*)-AJ-165 (211 nM and 310 nM); and (1*R*,2*S*)-UH-232 and (2*S*,3*R*)-AJ-166 (1100 nM and 1080 nM).

by aromatic residues, which may stabilize the ion pair formed with the protonated ligands. In addition, a hydrogen bond is formed from the phenolic hydrogen of the ligands to Ser-193 (Ser-192 in D_3, TM 5). Aromatic edge-to-face interactions occur between Phe-390 (Phe-346 in D_3, TM 6) and the aromatic ring of the ligands. Similar interactions have also been suggested by Hibert and co-workers (5, 6). A schematic representation of the interaction of (*S*)-5-OH-DPAT with the binding site of the D_{2A} receptor is presented in Fig. 3. Below is a detailed analysis of the observed structure-activity relationships and their rationalization, in qualitative terms, based on molecular modeling studies of the interactions between the various ligands and the D_{2A} and D_3 binding models.

Mode of agonist binding of (*S*)-5-OH-DPAT and (*R*)-7-OH-DPAT. On the basis of various biochemical and behavioral studies, several 2-aminotetralin derivatives have been classified as selective D_2 receptor agonists or antagonists; the regioisomers 5-OH-DPAT and 7-OH-DPAT are potent agonists (30) and display high stereoselectivity and affinity for the D_2 receptor. As shown in Table 1, the D_{2A} and D_3 receptor affinities reside entirely or predominately in (*S*)-5-OH-DPAT and (*R*)-7-OH-DPAT. McDermed and Freeman (30) rationalized the heterochirality[2] of these potent agonists by suggesting a model in which different faces of the compounds interact with a putative three-point pharmacophore.[3] An attractive feature of this model is that it allows for superposition of the nitrogens, the nitrogen lone pairs, the oxygens, and the aromatic rings, the pharmacophore elements of the two compounds (24).

In an attempt to elucidate the molecular basis for the binding characteristics described above, we performed a flexible docking of the potent agonists (*S*)-5-OH-DPAT and (*R*)-7-OH-DPAT into the binding site model of the D_{2A} receptor (Fig. 3). This docking orients the two regioisomeric 2-aminotetralin derivatives in relative spatial positions similar to those in the indirect D_2 receptor agonist model proposed by McDermed and Freeman (30). An almost identical docking of (*S*)-5-OH-DPAT and (*R*)-7-OH-DPAT could also be performed with the binding site model of the D_3 receptor (to Asp-110 and Ser-192).

D_3 selectivity of 2-aminotetralin derivatives. The D_{2A}/D_3 affinity ratios of the compounds vary between 1.1 and 70 (Table 1). The most D_3-selective ligands are (*R*)-7-OH-DPAT (70-fold) (Fig. 4), (1*S*,2*R*)-AJ-148 (38-fold), and (*S*)-5-OH-DPAT (30-fold) (Fig. 5), which also have high D_3 receptor affinities (0.61 nM, 0.57 nM, and 0.89 nM, respectively). The binding affinities of (*R*)-7-OH-DPAT for cloned human D_2 and D_3 receptors are similar to those reported previously for racemic 7-OH-DPAT with cloned rat D_2 and D_3 receptors (3). In addi-

[2] Two compounds are denoted heterochiral when they have opposite senses of chirality but not necessarily opposite absolute configurations.

[3] A different but interesting mode of superposition has been proposed by Grol *et al.* (39).

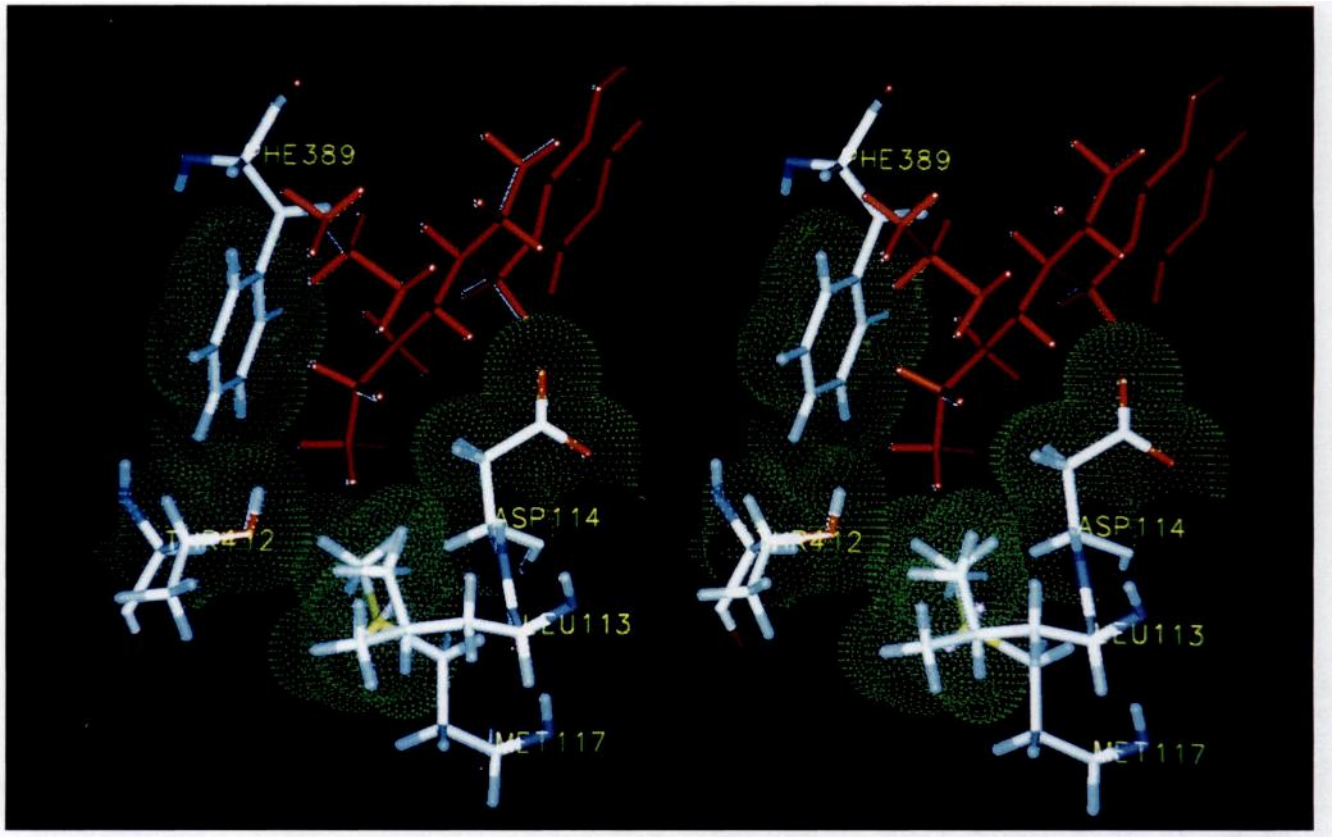

Fig. 12. Stereo representation of the interaction between the propyl group of (*S*)-5-OH-DPAT and the D_{2A} receptor model. The propyl cleft is defined by the following amino acids: Leu-113, Asp-114, Met-117, Phe-389, Tyr-408, and Thr-412.

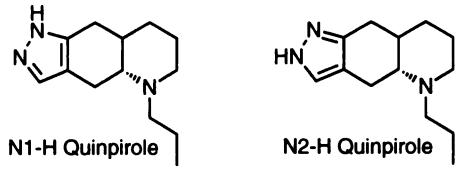

Fig. 13. Structures of both tautomeric forms of the DA receptor agonist quinpirole.

tion, the D_2 receptor antagonist (*R*)-5-OH-DPAT (31) displays relatively high selectivity and affinity for the D_3 receptor (25-fold and 7.9 nM, respectively) (Fig. 5).

The binding site models appear to provide insight into the molecular basis for the observed differences in subtype selectivity. In (*R*)-7-OH-DPAT the distance between the protonated nitrogen, which binds to Asp-114, and the phenolic oxygen, which donates a hydrogen bond to Ser-193, is slightly longer than in the less D_3-selective (*S*)-5-OH-DPAT (7.4 Å versus 6.6 Å, respectively; a difference of 0.8 Å). The binding site models of the D_{2A} and D_3 receptors exhibit a similar difference in distances between the binding aspartic acid and serine residues (Asp-114 and Ser-193 in D_{2A} and Asp-110 and Ser-192 in D_3) (this difference in distances between the D_{2A} and D_3 receptor models is about 1.0 Å when the same χ angles for these residues are used in both models). The more pronounced D_3 selectivity of (*R*)-7-OH-DPAT, compared with (*S*)-5-OH-DPAT, may be related to a slightly less optimal fit to the D_{2A} model [due to the longer N-O distance of (*R*)-7-OH-DPAT] than that of (*S*)-5-OH-DPAT. The location of Asp-110 and Ser-192 in the D_3 binding site model appears to allow for the formation of more optimal bonds between the oxygenated 2-aminotetralin ligands and these two crucial interaction points, in terms of bond lengths and angles. In addition, a closer edge-to-face contact is possible between Phe-346 and the aromatic ring of the ligands in the D_3 receptor model than between the corresponding amino acid in the D_{2A} receptor (Phe-390) and the ligands. These observations appear to rationalize the fact that almost all agonist ligands described herein exhibit selectivity for the D_3 receptor.

It is noteworthy, however, that the D_{2A} and D_3 receptors have identical amino acids in their binding sites in our models. These receptors show about 48% overall sequence identity and about 80% identity in the TMs. The differences are mainly located on the outside of TM 1, TM 4, TM 5, TM 6, and TM 7.

Effects of the introduction of methyl substituents in the 2-aminotetralin derivatives. The introduction of a methyl group in the nonaromatic ring (C1 or C3) of the 2-aminotetralin moiety influences affinity, subtype selectivity, and stereoselectivity (Table 1). The observation that DA receptor subtype selectivity and affinity of the 2-aminotetralin derivatives are modulated by introduction of methyl groups in the nonaromatic ring may be rationalized in molecular terms. Inspection of the interaction between the D_{2A} binding site model and (2*R*,3*S*)-AJ-166 and (1*S*,2*R*)-AJ-148, the two most potent methyl-substituted agonists, reveals that the respective methyl groups occupy approximately the same volume in space. The methyl groups fit smoothly into a lipophilic cavity that is large enough to accommodate a methyl group but not larger

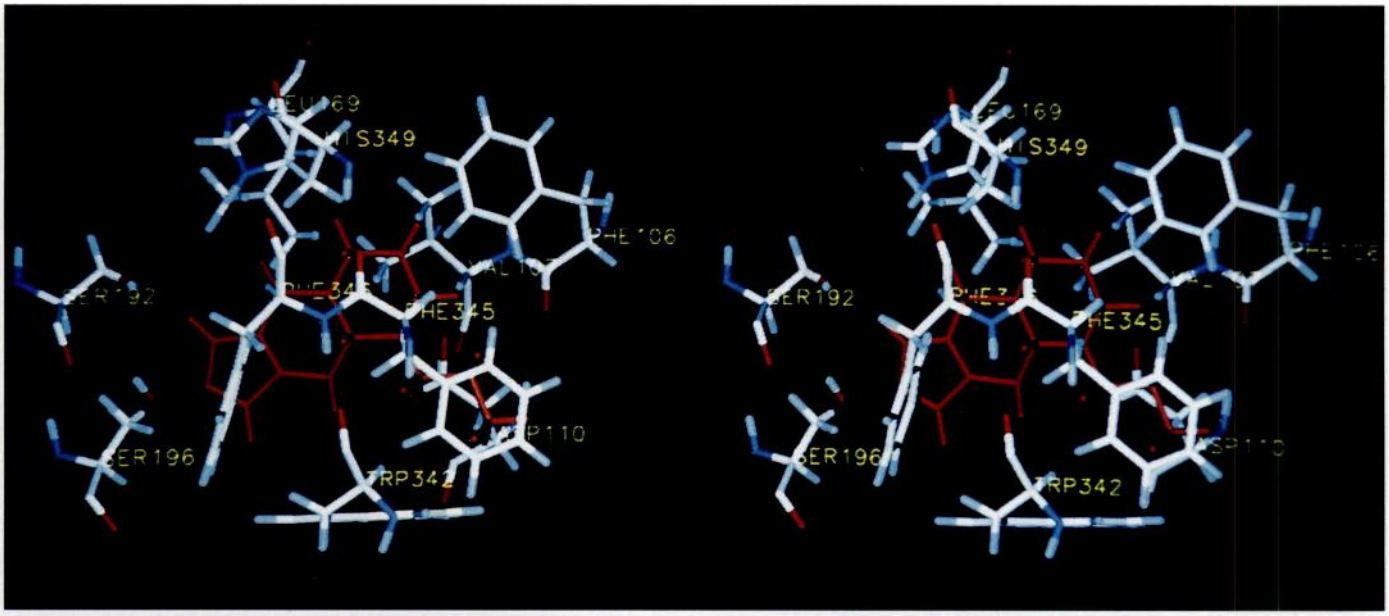

Fig. 14. Docking of quinpirole into the D_3 receptor model. The N1-H tautomer forms a good hydrogen bond to Ser-192 and an aromatic interaction with Phe-346 in the binding site.

substituents.[4] As indicated in Fig. 6, the interaction with this cavity (the "methyl pocket") (Fig. 7, *area A*) increases the total van der Waals contribution to the binding between these ligands and the D_{2A} receptor. A number of other 2-aminotetralin derivatives [(2*R*,3*S*)-AJ-165, (1*S*,2*R*)-UH-242, (1*S*,2*R*)-UH-232, and (1*S*,2*R*)-AJ-137] also show increased affinity (2–4-fold) with the introduction of a methyl group. This trend may be rationalized by the observation that all of these compounds have a methyl group in the same position of the receptor, i.e., in the methyl pocket.

None of the methyl-substituted 2-aminotetralin derivatives tested show any significant increase in D_3 receptor affinity, compared with the unsubstituted compounds. This may be related to slightly different locations of the methyl pocket in the D_{2A} and D_3 binding sites, relative to the binding aspartate and serine residues. The center of the methyl pocket is located farther away from the aspartate in the D_3 binding site than in the D_{2A} binding site. These observations seem to rationalize the binding data, because the methyl pocket in the D_3 receptor is less accessible to the derivatives studied than is that in the D_{2A} receptor (Fig. 8).

Some methyl-substituted 2-aminotetralins [(2*S*,3*R*)-AJ-166, (2*S*,3*R*)-AJ-165, (1*R*,2*S*)-UH-242, and (1*R*,2*S*)-UH-232] exhibit a decrease in affinity for D_{2A} (about 5-fold) and D_3 (10–75-fold) receptors, compared with the non-methyl-substituted analogs. When the methyl-substituted compounds interact with the D_{2A} and D_3 receptor binding sites, the methyl group interferes with one oxygen of the carboxylate group of Asp-114 (in D_{2A}; Asp-110 in D_3) (Fig. 9). This steric repulsion rationalizes the observed decrease in affinity.

Different modes of binding of agonists and antagonists. It has been previously shown that C5-oxygenated 2-aminotetralins should be homochiral[5] to (*S*)-5-OH-DPAT to be able to stimulate D_2 receptors and that antagonists should be homochiral to (*R*)-5-OH-DPAT (32). For example, (1*R*,2*S*)-UH-242 is an agonist, whereas (1*S*,2*R*)-UH-242 displays a D_2 receptor antagonist profile (33).

Replacement of the hydroxyl group with a methoxy group in the C5-oxygenated 2-aminotetralins homochiral with (*S*)-5-OH-DPAT decreases the affinity at both the D_{2A} and D_3 receptors. Conversely, in the C5-oxygenated compounds that are homochiral with (*R*)-5-OH-DPAT a replacement of the hydroxyl group with a methoxy group produces an increase in affinity. As noted previously (32), this may suggest that the agonists prefer to donate a hydrogen bond to the receptor, whereas the antagonists preferably accept a hydrogen bond.

Most likely, antagonists with large structural differences bind to distinct areas of the receptor. Similarly, it is likely that most antagonists bind to different regions of the receptor than do the agonists. However, the present series of structurally related 2-aminotetralins show similar trends in D_{2A} receptor affinity when a C1-methyl or C3-methyl group is introduced in the agonists (*R*)-7-OH-DPAT and (*S*)-5-OH-DPAT and in the antagonist (*R*)-5-OH-DPAT. This suggests that the agonists and the antagonists have similar modes of binding and that they may interact with the same binding site (32).

The potent antagonist (1*S*,2*R*)-UH-232 has to adopt a different orientation in the D_{2A} binding site, compared with the agonists, to be able to have the proper orientation of the nitrogen lone pair towards Asp-114 and to be able to participate in hydrogen bonding with a serine residue (Fig. 7 shows the overlap between the agonist and the antagonist binding modes; Fig. 10 shows agonist and antagonist dockings into the D_{2A} binding site model). In such an interaction mode the antagonist accepts a hydrogen bond from Ser-197, whereas the agonists interact with Ser-193 by donating a hydrogen bond. Another difference between the interaction of (1*S*,2*R*)-UH-232 with the D_{2A} receptor and that of the agonists is the absence of a stabilizing interaction with Phe-390; the aromatic ring of the antagonist is situated more extracellularly in the binding site

[4] The *cis*-3-cyclopentyl derivative of 5-OH-DPAT has been shown to be inactive (C. Mellin, C. J. Grol, and U. Hacksell, unpublished observations).

[5] Two compounds are denoted homochiral when they have the same sense of chirality but not necessarily the same absolute configuration.

than is that of the agonists. Consequently, the interaction with Phe-390 is no longer possible. Instead, an interaction may exist between His-393 (TM 6) and the aromatic ring of the antagonist. The methyl group of the antagonist (1*S*,2*R*)-UH-232 may interact with the methyl pocket of the D_{2A} receptor in a similar way as the methyl group of the agonists (2*R*,3*S*)-AJ-166 and (1*S*,2*R*)-AJ-148, thus rationalizing the favorable contribution to binding of this substituent. It is likely that the other 2-aminotetralin-based D_{2A} receptor antagonists bind to the receptors in the same orientation as (1*S*,2*R*)-UH-232.

Thus, the binding mode of the 2-aminotetralin derivatives is determined by their stereochemistry. Compounds that are homochiral with (*S*)- and (*R*)-5-OH-DPAT have different modes of binding. The dipropylammonium groups and the nonaromatic ring of all potent compounds interact similarly with the receptor, but the positions of the aromatic rings of agonists and antagonists differ considerably, being located more extracellularly in (*R*)-5-OH-DPAT and related derivatives (Figs. 7 and 10).

Experimental evidence for these different modes of binding is provided by the *cis*-methyl-substituted compounds. The methyl group can be situated in two different locations, (a) in the methyl pocket (Fig. 7, *area A*) or (b) in an area close to the carboxylate (Fig. 7, *area B*). The effect of having a methyl group in either of these two areas of the receptor can be generalized in terms of free energy of binding (ΔG_{bind}). The shift in free energy of binding ($\Delta\Delta G_{bind}$) to the D_{2A} receptor caused by introduction of a methyl group (i.e., O, C1, or C3 methylations) in (*S*)-5-OH-DPAT and in (*R*)-5-OH-DPAT is shown in Fig. 11. Methyl substituents in the C5-oxygenated 2-aminotetralins that occupy *area A* (Fig. 7) of the D_3 receptor give positive $\Delta\Delta G_{bind}$ values or no change in ΔG_{bind}, whereas other substitutions produce changes in free energy of binding ($\Delta\Delta G_{bind}$) to the D_3 receptor that are similar to those seen at the D_{2A} receptor but slightly larger.

In the present series, the agonists seem to prefer a hydroxyl group and the antagonists a methoxy group. The lower affinity of methoxylated compounds binding in the "agonist binding mode" may be related to their inability to donate a hydrogen bond to Ser-193. In the agonist binding mode the *O*-methyl group accommodates *area D* (Fig. 7). In the "antagonist binding mode" the *O*-methyl group accommodates *area C* (defined by Ser-193 and Ser-197). This latter interaction increases affinity. Two additional factors that may contribute to the observed affinities are as follows. (a) The antagonists seem to bind to a serine at the receptor by accepting a hydrogen bond. Better hydrogen bond-accepting ability (due to increased basicity) of a methoxy group, compared with at hydroxy group, could give increased affinity. However, this would give increased binding of only about 0.27 kcal/mol, according to measurements in water (34). In the gas phase, however, which more closely resembles the interior of a protein, ethers are much more basic than alcohols and $\Delta\Delta G_{bind}$ may be larger. (b) Hydroxy groups are more solvated than ethers and therefore require more desolvation energy to enter into the hydrophobic environment of the binding site. This may be of particular importance in the antagonist binding mode, because the hydroxy/methoxy group is directed intracellularly (Fig. 7). Although it is impossible to distinguish agonists from antagonists on the basis of the receptor binding data (Table 1 and 2), the consistency of the modeling results strongly supports the hypothesis that all C5-oxygenated 2-aminotetralin derivatives that are homochiral with (*R*)-5-OH-DPAT could be D_2 receptor antagonists, provided that conformational or steric constraints that disfavor binding are absent.[6]

In contrast to the consistent effects of methyl substitution in the C5-oxygenated 2-aminotetralins (Fig. 11), no clear trend was apparent when hydrogens were perturbed into methyl groups in the C7-oxygenated series. This indicates that the mode of binding of these methyl-substituted analogs to the D_{2A} and D_3 receptors is less well defined. Most likely, $\Delta\Delta G_{bind}$ is influenced not only by the perturbation of a hydrogen into a methyl group but also by changes in the global mode of binding of the compounds.

In the C7-oxygenated series, D_2 receptor agonist activities have been unambiguously shown for derivatives that are homochiral with (*R*)-7-OH-DPAT (11), but the possibility that derivatives that are homochiral with (*S*)-7-OH-DPAT would be D_2 receptor antagonists has not been verified. In fact, (*S*)-7-OH-DPAT has been claimed to behave as a weak D_2 receptor agonist (35). It should be noted, however, that this effect could be due to small contaminating amounts of the more potent enantiomer (*R*)-7-OH-DPAT. Among the C7-oxygenated compounds (see Fig. 1) that have been investigated with respect to antagonist properties, i.e., the enantiomers of AJ-148, AJ-137, and AJ-156, as well as racemic AJ-154, only (2*R*,3*S*)-AJ-156 showed D_2 receptor antagonist activity (increased accumulation of dihydroxyphenylalanine) (11, 12).

Because no effector coupling has been unambiguously established for the D_3 receptor, the intrinsic activity of D_2 receptor agonists and antagonists for the D_3 receptor is unknown. Consequently, we refrain from speculating about the mode of interaction of potential agonists and antagonists with the D_3 receptor.

The propyl group phenomenon. The structural requirements for the *N*-substituents of dopaminergic C5-hydroxylated 2-aminotetralins have been described previously (36). On the basis of *in vivo* biochemical and behavioral data it was demonstrated that the nitrogen should be substituted with at least one propyl group for optimal dopaminergic potency. The corresponding *N*-ethyl-substituted compounds are slightly less potent, whereas the absence of an *N*-ethyl or *N*-propyl substituent gives dramatic reductions in potency. When one *N*-propyl group is present the structural requirements for the second *N*-substituent are less stringent (29, 37).

The K_i values of some C5-hydroxy- and C5-methoxy-substituted *cis*-1-methyl-2-aminotetralins with *N*-substituents other than *N*,*N*-dipropyl are shown in Table 2 (see also Fig. 2). The data demonstrate that the *N*,*N*-dipropyl substitution pattern is important for high D_{2A} and D_3 receptor affinity. The D_3 selectivity of the *N*,*N*-diethyl and *N*,*N*-dibutyl derivatives is attenuated (2–3-fold), compared with the *N*,*N*-dipropyl analogs, indicating that the D_3 receptor may be more sensitive to varying lengths of the *N*-substituents than is the D_{2A} receptor.

In the binding site models of the D_{2A} and D_3 receptors, there is a cleft that is large enough to accommodate a propyl group but neither longer nor branched *N*-substituents. This "propyl cleft" extends from Asp-114 (TM 3) towards TM 2 and TM 7

[6] As suggested by one of the reviewers, the different interaction modes of agonists and antagonists might be verified by studies of the binding affinities of selected compounds for previously reported D_2 receptor mutants (Ser-193 to Ala-193, Ser-194 to Ala-194, or Ser-197 to Ala-197) (20, 23).

(in D_{2A}; see Fig. 12). The presence of this cleft rationalizes the present and previous structure-activity relationship studies of the *N*-substituents of dopaminergic 2-aminotetralins. The conformations of the *N*-propyl groups used in the docking procedure were close to minimum energy values described earlier (26). The *N*-propyl group interacting with the propyl cleft in the D_{2A} binding site model has to adopt an extended conformation in relation to the C2-N bond to produce an optimal fit. The other *N*-propyl group, which points in the extracellular direction, adopts a *gauche* conformation.

2-Aminotetralins substituted with one *N*-propyl group and an *N*-phenylethyl (N-0434) or *N*-thienylethyl group (N-0437) are potent D_2 agonists (38). The aromatic moieties of the *N*-substituents may increase the affinities of these compounds by interacting favorably with the aromatic residues located above Asp-114 (Phe-110 and Tyr-408 in D_{2A}).

Further evaluation of the receptor models. The DA receptor agonist quinpirole (Fig. 13) is also accomodated by the binding site models (Fig. 14). The pyrazole ring in quinpirole exists in two tautomeric forms, the N1-H and N2-H tautomers. The N1-H tautomer seems to interact more optimally with the receptors, by forming a hydrogen bond with Ser-193 (in D_2; Ser-192 in D_3) and an edge-to-face aromatic interaction with Phe-390 (in D_2; Phe-346 in D_3). The aromatic receptor interaction of the N2-H tautomer seems less optimal. In addition, the previously reported (2, 4) D_3 selectivity of quinpirole may be explained by the closer edge-to-face aromatic interaction between Phe-346 (D_3) and the pyrazole ring, compared with the corresponding interaction in the D_{2A} receptor binding site.

Conclusions. The ability of the D_{2A} and D_3 receptor binding site models to accommodate experimental data is remarkable, because they each represent one of an infinite number of possible receptor conformations. In fact, the structure-activity relationships described herein can be rationalized by the models. The presence of a methyl pocket and a propyl cleft in the binding site models is particularly interesting, because these structural features of the D_{2A} receptor have been predicted on the basis of indirect modeling and, thus, are supported by a wealth of experimental evidence. The obvious possibility of using the D_{2A} and D_3 binding site models in the design of non-2-aminotetralin-based, potent, and subtype-selective D_2 and D_3 receptor ligands is intriguing.

Acknowledgments

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