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Interactions of ligands with active and inactive conformations of the dopamine D_2 receptor

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Abstract

The affinities of 19 pharmacologically diverse dopamine D_2 receptor ligands were determined for the active and inactive conformations of cloned human dopamine D_2 receptors expressed in Ltk$^-$ cells. The agonist [^{3}H]quinpirole was used to selectively label the guanine nucleotide-binding protein-coupled, active receptor conformation. The antagonist [^{3}H]raclopride, in the presence of the non-hydrolysable GTP-analogue Gpp(NH)p and sodium ions and in the absence of magnesium ions, was used to label the free inactive receptor conformation. The intrinsic activities of the ligands were determined in a forskolin-stimulated cyclic AMP assay using the same cells. An excellent correlation was shown between the affinity ratios (K_R/K_{RG}) of the ligands for the two receptor conformations and their intrinsic activity ($r = 0.96$). The ligands included eight structurally related and enantiopure 2-aminotetralin derivatives; the enantiomers of 5-hydroxy-2-(dipropylamino)tetralin, 5-methoxy-2-(dipropylamino)tetralin, 5-fluoro-2-(dipropylamino)tetralin and 2-(dipropylamino)tetralin. The (*S*)-enantiomers behaved as full agonists in the cyclic AMP assay and displayed a large K_R/K_{RG} ratio. The (*R*)-enantiomers were classified as partial agonists and had lower ratios. The structure–affinity relationships of these compounds at the active and the inactive receptor conformations were analysed separately, and used in conjunction with a homology based receptor model of the dopamine D_2 receptor. This led to proposed binding modes for agonists, antagonists and partial agonists in the 2-aminotetralin series. The concepts used in this study should be of value in the design of ligands with predetermined affinity and intrinsic activity.

Keywords: 2-Aminotetralin derivative; Dopamine D_2 receptor; Dopamine receptor agonist; Dopamine receptor antagonist; Dopamine receptor partial agonist; Intrinsic activity; Ternary complex model

1. Introduction

The theoretical framework dealing with interactions between guanine nucleotide-binding protein-coupled (G protein-coupled) receptors and their ligands defines our understanding of G protein-coupled receptor pharmacology. The ternary complex model describes the interactions between ligand, receptor and G protein (De Lean et al., 1980; Kent et al., 1980). It defines an equilibrium between free receptor (R) and receptor coupled to G protein (RG) which influences the basal activity of a G protein-coupled receptor. An antagonist does not affect this equilibrium because it has the same affinity for R and RG. In contrast, an agonist shifts the conformational equilibrium because it stabilises RG relative to R by binding with higher affinity to RG. Conversely, an inverse agonist stabilises R. The ternary complex model, has now been extended into an allosteric ternary complex model that accommodates recent experimental observations including constitutively active mutant G protein-coupled receptors (Samama et al., 1993; Lefkowitz et al., 1993). This extended, allosteric model assumes a spontaneous G protein independent isomerization step from R to a second receptor conformation (R*) in the absence of ligand. R* interacts with the G protein (R*G) which in turn leads to a biological response.

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In the ternary complex model, K_{high} and K_{low} are binding constants for the high and the low affinity agonist binding sites. The high affinity binding site is guanine nucleotide sensitive and should represent an active receptor conformation coupled to G protein (RG) whereas the low affinity binding site should represent the free receptor in an inactive receptor conformation (R). According to the extended ternary complex model two different conformations of free receptor exists, R and R*. It seems, however, not possible to separate binding to R and R* in receptor binding experiments neither in constitutively nor in non-constitutively active systems (Samama et al., 1993; Gether et al., 1997). In this study the G protein coupled and the free receptor are referred to as the active and inactive receptor, respectively.

The well established relationship between the affinity ratio K_{low}/K_{high} (K_R/K_{RG}) and intrinsic activity (De Lean et al., 1980; Kent et al., 1980; Freedman et al., 1988; Lahti et al., 1992) opens up the possibility to develop separate structure–affinity relationships for the interactions between ligands and the active and inactive receptor conformations, respectively. Such information would be most valuable in the design of ligands with predetermined affinity and intrinsic activity.

In the present study we have verified that the ratio K_R/K_{RG} correlates with the intrinsic activity of a series of 11 structurally and pharmacologically diverse dopamine D_2 receptor ligands. The binding of the ligands to the active and the inactive receptor conformations of the cloned human dopamine D_2 receptor was investigated using [^{3}H]agonist and [^{3}H]antagonist labelling, respectively, and the intrinsic activity of the ligands was determined in a forskolin-stimulated cyclic AMP assay. We have also investigated the interactions of a series of eight structurally related and enantiopure 2-aminotetralin derivatives with the active and the inactive conformations of the dopamine D_2 receptor. The results are encouraging as distinctly different structure–affinity relationships were obtained for the interactions with the two receptor conformations. This information is used to address molecular details of putative interaction modes of agonists, partial agonists and antagonists with active and inactive dopamine D_2 receptor conformations.

2. Materials and methods

2.1. Materials

[^{3}H]Raclopride (specific activity, 77 Ci/mmol) and [^{3}H]quinpirole (specific activity, 40 Ci/mmol) were obtained from DuPont New England Nuclear (Boston, MA). The unlabeled compounds were obtained from the following sources: dopamine and haloperidol, Sigma (St. Louis, MO), quinpirole, (−)-(*R*)-apomorphine and (+)-butaclamol, Research Biochemicals (Natick, MA), (−)-(*S*)-3-(3-hydroxyphenyl)-*N*-propylpiperidine ((−)-(*S*)-3-PPP), raclopride and remoxipride were synthesised at the Department of Medicinal Chemistry, Astra Arcus. (+)-(*R*)-2-(dipropylamino)tetralin ((+)-(*R*)-DPAT), (−)-(*S*)-DPAT (Liu et al., 1993), (+)-(*R*)-5-hydroxy-2-(dipropylamino)tetralin ((+)-(*R*)-5-OH-DPAT), (−)-(*S*)-5-OH-DPAT, (+)-(*R*)-5-methoxy-2-(dipropylamino)tetralin ((+)-(*R*)-5-OMe-DPAT), (−)-(*S*)-5-OMe-DPAT, (+)-(*R*)-5-fluoro-2-(dipropylamino)tetralin ((+)-(*R*)-5-F-DPAT), (−)-(*S*)-5-F-DPAT (Backlund Höök et al., 1996), (+)-(*R*)-7-hydroxy-2-(dipropylamino)tetralin ((+)-(*R*)-7-OH-DPAT), (+)-(1*S*,2*R*)-5-methoxy-1-methyl-2-(dipropylamino)tetralin ((+)-(1*S*,2*R*)-UH-232) and (+)-(1*S*,2*R*)-5-methoxy-1-methyl-2-(propylamino)tetralin ((+)-(1*S*,2*R*)-AJ-76) were synthesised at the Department of Organic Pharmaceutical Chemistry, Uppsala University, Uppsala, Sweden. (±)-5-hydroxy-*N*-propyl-*N*-thienylethyl-2-aminotetralin ((±)-N-0437) was a gift from Dr. Cor J. Grol (Department of Medicinal Chemistry, State University of Groningen, the Netherlands). 5′-guanylylimidodiphosphate (Gpp(NH)p) and 3-isobutyl-1-mexanthine (IBMX) were obtained from Sigma. All cell culture reagents were obtained from GIBCO (Paisley, Scotland, UK). All other chemicals were of analytical grade. Mouse fibroblast (Ltk$^-$) cells expressing human dopamine D_2 (long isoform) receptors were obtained from Dr. O. Civelli (Vollum Institute, Portland, OR).

2.2. [^{3}H]Raclopride and [^{3}H]quinpirole competition experiments

The Ltk$^-$ cells expressing human dopamine D_2 receptors were grown and membranes prepared as described previously (Malmberg et al., 1993). [^{3}H]Raclopride (2 nM) and test compound (10–12 concentrations) were incubated with about 25 μg of membrane protein suspended in buffer (50 mM Tris–HCl, 120 mM NaCl, 5 mM KCl and 1 mM EDTA; pH 7.4) containing 0.1 mM Gpp(NH)p to a final volume of 0.5 ml. [^{3}H]Quinpirole (6 nM) and test compound (10–12 concentrations) were incubated with about 200 μg of membrane protein suspended in buffer (50 mM Tris–HCl, 120 mM *N*-Methyl-D-glucamine, 5 mM KCl, 1 mM EDTA and 4 mM $MgCl_2$; pH 7.4) to a final volume of 2 ml. Both assays were performed in duplicate and incubated at 30°C for 60 min. Nonspecific binding was determined 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 Brandel cell harvester. Scintillation cocktail (Packard Ultima Gold, 4 ml) was added and the radioactivity determined in a Packard 2500TR liquid scintillation counter at about 50% efficiency. The binding curves were analysed by nonlinear regression using the LIGAND program (Munson and Rod-

bard, 1980). The K_d values of [^{3}H]raclopride (1.1 nM) and [^{3}H]quinpirole (3.7 nM) have been reported previously (Malmberg and Mohell, 1995). The B_{max} value, as determined with [^{3}H]raclopride, was 1 pmol/mg of protein (Malmberg and Mohell, 1995). [^{3}H]Quinpirole labels about 25% of the [^{3}H]raclopride binding sites (Malmberg and Mohell, 1995).

2.3. Cyclic AMP accumulation

The cells expressing human dopamine D_2 receptors were grown as described previously (Malmberg et al., 1993). Cells were detached with Ca^{2+} and Mg^{2+} free Earle's Balanced Salt Solution and collected by centrifugation ($250 \times g$ for 5 min). Cyclic AMP accumulation was measured in intact Ltk^- cells suspended in DMEM (Dulbecco's modified Eagle's medium) supplemented with 20 mM HEPES. The cell suspension was preincubated with 1 mM IBMX for 20 min at 37°C. The experiment was started by addition of cells (10^6) to test tubes containing 100 μM forskolin and appropriate drugs giving a total volume of 530 μl. The tubes were incubated for 10 min at 37°C. The reaction was terminated by addition of 1 M HCl and subsequent boiling for 3 min. After cooling, the samples were centrifuged at $1500 \times g$ for 10 min at 4°C and the cyclic AMP levels in the supernatant were assayed according to Brown et al. (1972), as modified by Nordstedt and Fredholm (1990). The protein concentration in the cell pellet was determined by the method of Lowry et al. (1951).

3. Results

3.1. Determination of affinities for active and inactive receptor conformations

Table 1 presents the affinities of the various ligands to the active (G protein coupled; K_{RG}) and the inactive (free; K_R) conformations of the dopamine D_2 receptor. This series of compounds includes a number of well characterised agonists (dopamine, quinpirole, (−)-apomorphine, (±)-N-0437 and (*R*)-7-OH-DPAT) and antagonists (haloperidol, raclopride, remoxipride, (1*S*,2*R*)-UH-232 and (1*S*,2*R*)-AJ-76)) together with the partial agonist (−)-3-PPP (Fig. 1; Table 1). We have also studied a number of

Table 1
Affinities and intrinsic activities of dopamine receptor agonists and antagonists determined at cloned human dopamine D_2 receptors

Compounds	[^{3}H]Raclopride K_R (nM)	[^{3}H]Quinpirole K_{RG} (nM)	Ratio (K_R/K_{RG})	Inhibition of forskolin-stimulated cyclic AMP production (%)
Dopamine	1794 ± 97	1.89 ± 0.25[a]	949	63 ± 2
Quinpirole	1874 ± 153	3.70 ± 0.41[a]	506	67 ± 3
(−)-Apomorphine	33.0 ± 3.5	0.12 ± 0.03[a]	266	63 ± 3
(*S*)-5-OH-DPAT	33.7 ± 7.2	0.144 ± 0.012[b]	234	66 ± 3
(±)-N-0437	8.36 ± 0.40	0.09 ± 0.01	91	61 ± 2
(*R*)-7-OH-DPAT	54.6 ± 2.9	0.70 ± 0.19[a]	78	64 ± 1
(*S*)-DPAT	374 ± 63	5.50 ± 0.45[b]	68	66 ± 1
(*S*)-5-F-DPAT	294 ± 32	4.97 ± 0.36	59	62 ± 3
(*S*)-5-OMe-DPAT	269 ± 34	10.2 ± 0.8	26	57 ± 2
(*R*)-DPAT	554 ± 32	31.7 ± 4.7[b]	17	47 ± 3[c]
(−)-3-PPP	244 ± 11	18.2 ± 2.6[a]	13	40 ± 2[c]
(*R*)-5-F-DPAT	288 ± 27	26.0 ± 1.7	11	41 ± 2[c]
(*R*)-5-OH-DPAT	146 ± 20	20.2 ± 1.9	7.2	31 ± 2[c]
(*R*)-5-OMe-DPAT	34.3 ± 4.5	6.35 ± 0.15	5.4	37 ± 3[c]
Haloperidol	0.30 ± 0.03	0.16 ± 0.03[a]	1.8	−2 ± 6
(1*S*,2*R*)-UH-232	12.3 ± 1.9	7.25 ± 1.4[a]	1.7	7 ± 4
(1*S*,2*R*)-AJ-76	90.3 ± 4.4	75.9 ± 12[a]	1.2	−12 ± 4
Raclopride	0.95 ± 0.08	1.30 ± 0.19[a]	0.7	7 ± 3
Remoxipride	53.2 ± 2.4	111 ± 12[a]	0.5	−4 ± 6

The experiments were performed as described in Section 2. The K_R and K_{RG} values are means ± S.E.M. of 3–5 experiments. The intrinsic activity of each drug was determined in a concentration of 1000-fold the K_{RG} value. The percent inhibition of forskolin-stimulated cyclic AMP production (100%) are means ± S.E.M. of 4–6 experiments. Basal and forskolin-stimulated (100 μM) cyclic AMP levels were 10 ± 2 and 120 ± 6 pmol/mg of protein, respectively.

[a] From Malmberg and Mohell (1995).

[b] From Yu et al. (1996).

[c] Statistically significant difference for compounds with ratios between 949-5 as compared to dopamine ($P < 0.01$, one-way ANOVA with Dunnet's post test). No statistically significant difference was found for the antagonists (ratios between 1.8–0.5) as compared to forskolin stimulation ($P > 0.05$, one-way ANOVA with Dunnet's post test).

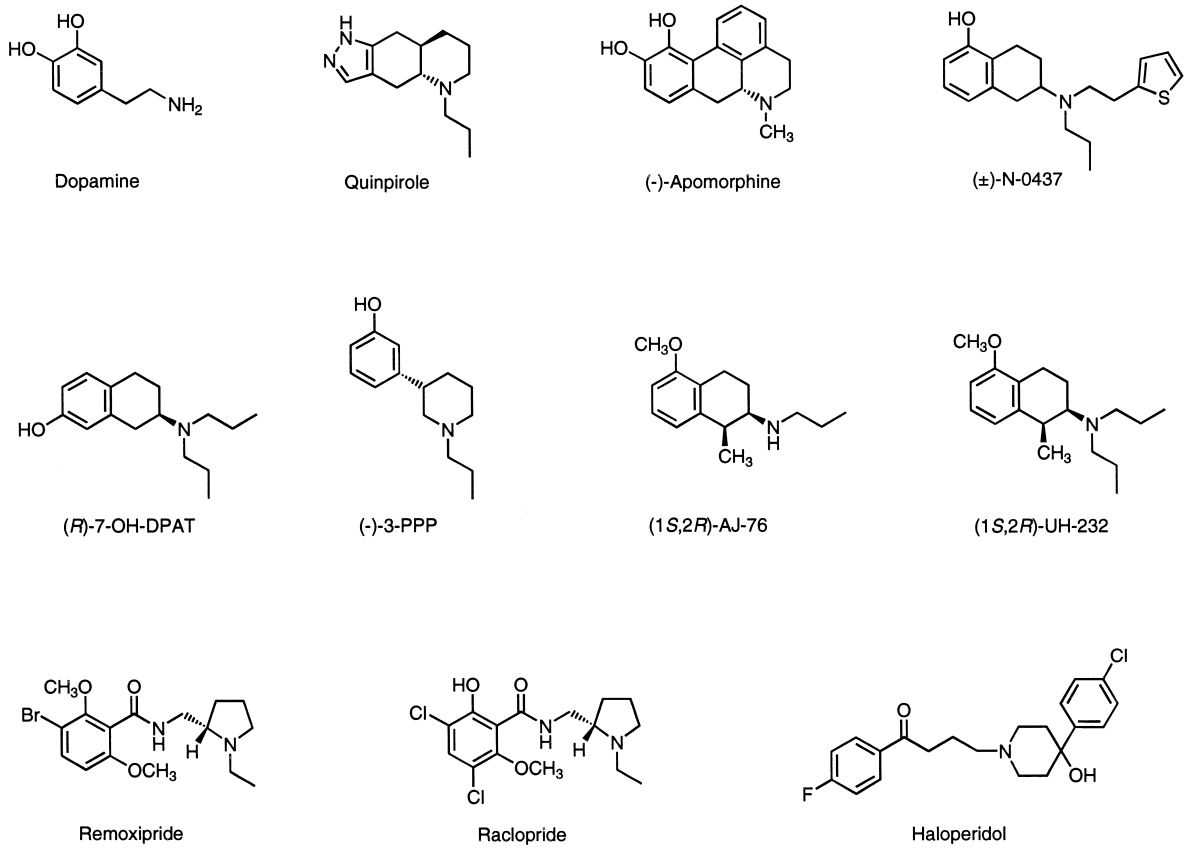

Fig. 1. Structures of the dopaminergic reference compounds included in the study.

closely related 2-dipropylaminotetralin derivatives; the enantiomers of 5-OH-DPAT, 5-OMe-DPAT, 5-F-DPAT and DPAT (Fig. 2; Table 1).

3.2. Determination of intrinsic activity

Stimulation of the dopamine D_2 receptor inhibits adenylyl cyclase and thereby reduces the cyclic AMP production (Vallar and Meldolesi, 1989). The intrinsic activity of the compounds was measured using a forskolin-stimulated cyclic AMP assay (Table 1). In order to compensate for the different affinities of the various compounds, they were tested at a concentration 1000-fold the K_{RG} value, as determined with [^{3}H]quinpirole, in the functional assay. The full agonists dopamine, quinpirole, (−)-apomorphine, (*R*)-7-OH-DPAT and (±)-N-0437 inhibited forskolin-stimulated cyclic AMP production by 61–67% whereas (−)-3-PPP behaved as a partial agonist by inhibiting the cyclic AMP levels by 40%. The antagonists haloperidol, raclopride, remoxipride, (1*S*,2*R*)-UH-232 and (1*S*,2*R*)-AJ-76 did not significantly affect the forskolin-stimulated cyclic AMP levels (Table 1). The (*S*)-enantiomers of 5-OH-DPAT, 5-F-DPAT, 5-OMe-DPAT and DPAT behaved as full agonists whereas the corresponding (*R*)-enantiomers behaved as partial agonists (Table 1).

3.3. Correlation of K_R/K_{RG} ratio with intrinsic activity

The ratio between the affinities for the inactive and the active receptor conformations (K_R/K_{RG}) was calculated and plotted against the experimentally determined intrinsic activity of the reference compounds as well as of the closely related 2-dipropylaminotetralins (Fig. 3). Four of the full agonists (dopamine, quinpirole, (−)-apomorphine

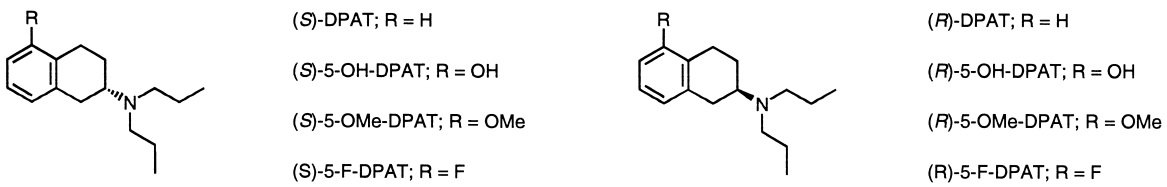

Fig. 2. Structures of the closely related 2-aminotetralins included in the study.

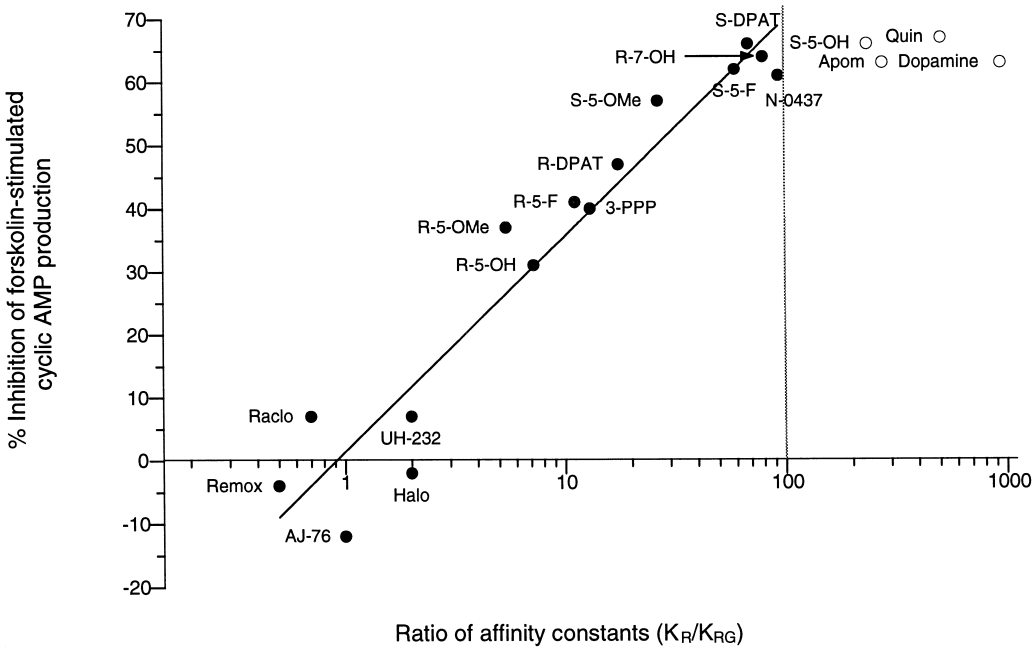

Fig. 3. Correlation plot between affinity ratio (K_R/K_{RG}) and intrinsic activity. The results are presented in Table 1. Quin: quinpirole, Apom: (−)-apomorphine, S- or R-5-OH: (*S*)- or (*R*)-5-OH-DPAT, R-7-OH: (*R*)-7-OH-DPAT, S- or R-5-F: (*S*)- or (*R*)-5-F-DPAT, S- or R-5-OMe: (*S*)- or (*R*)-5-OMe-DPAT, 3-PPP: (−)-3-PPP, Halo: haloperidol, UH-232: (1*S*,2*R*)-UH-232, AJ-76: (1*S*,2*R*)-AJ-76, N-0437: (±)-N-0437 Raclo: raclopride, Remox: remoxipride. Unfilled symbols are not included in the linear regression analysis ($r = 0.96$).

and (*S*)-5-OH-DPAT) displayed considerably higher ratios (949-234) than the other full agonists ((±)-N-0437, (*R*)-7-OH-DPAT, (*S*)-DPAT, (*S*)-5-F-DPAT, and (*S*)-5-OMe-DPAT) which had ratios between 91 and 26. Therefore, compounds with K_R/K_{RG} ratios over 100 were not included in the correlation (indicated with open circles in Fig. 3). The antagonists displayed ratios close to 1 (2–0.5) whereas the partial agonists showed intermediate K_R/K_{RG} ratios (17–5).

4. Discussion

The affinity for the high affinity agonist binding site (K_{RG}) should represent the affinity of a compound for the active G protein coupled receptor conformation (RG or R∗G) and similarly, the affinity for the low affinity agonist binding site (K_R) should represent the binding to the inactive free form of the receptor (R). In the extended ternary complex model, R∗ is also a free receptor to which an agonist binds with high affinity (Samama et al., 1993; Lefkowitz et al., 1993). However, in the absence of G proteins, agonist binding is monophasic and the affinity is lower than to the G protein coupled receptor. Thus, binding to free receptor might be a mixture of R and R∗ but with an apparent affinity to a single low affinity binding site (Samama et al., 1993; Gether et al., 1997). Therefore, in this study the G protein-coupled receptor is referred to as the active receptor conformation and the free receptor as the inactive receptor conformation. The dopamine D_2 receptor agonist [^{3}H]quinpirole has been shown to label a single high affinity site at cloned human dopamine D_2 receptors and was used to selectively label the active dopamine D_2 receptor conformation (Malmberg and Mohell, 1995). The antagonist [^{3}H]raclopride labels both receptor conformations. However, in the presence of the non-hydrolysable GTP-analogue Gpp(NH)p and sodium ions and in the absence of magnesium ions the high affinity component is converted into low (Malmberg and Mohell, 1995).

The affinities and intrinsic activities of 11 reference compounds and 8 closely related 2-dipropylaminotetralins have been determined (Table 1). We found a good correlation between the K_R/K_{RG} ratio and the intrinsic activity ($r = 0.96$) (Fig. 3). This type of correlation is well established (De Lean et al., 1980; Kent et al., 1980; Freedman et al., 1988; Lahti et al., 1992; Lahti et al., 1996) and may be used to rapidly estimate the intrinsic activity of a compound using receptor binding studies (Saunders et al., 1990). It is noteworthy that the intrinsic activity of a compound may vary depending on the test system. A full agonist in one system may be classified as a partial agonist in another, due to, for example, the number of spare receptors and the G proteins present (Kenakin et al., 1995). The system dependent differences in efficacies may also be explained at the receptor level by a different equilibrium between R and R∗ in various tissues (Leff, 1995). In the present system an affinity ratio of about 30 between

the receptor conformations appears to give a sufficient shift in the equilibrium in order to obtain full agonism. An increased K_R/K_{RG} ratio does not lead to higher intrinsic activity (cf., e.g., dopamine and quinpirole).

The ternary complex model also predicts a correlation between the amount of high affinity sites labelled by an agonist and its intrinsic activity (De Lean et al., 1980; Kent et al., 1980). In a recent study by Roth et al. (1997) no correlation between the ability of a number of agonists to label the high affinity agonist state at the 5-HT_{2A} receptor and to augment phosphoinositide hydrolysis was found. However, the results appear to be consistent with the extend ternary complex model (Roth et al., 1997).

4.1. Structure–affinity relationships of the enantiomers of DPAT, 5-OH-DPAT, 5-OMe-DPAT and 5-F-DPAT

The series of closely related 2-dipropylaminotetralin derivatives (Fig. 2) was included in this study to provide information about the ligand binding site in the two receptor conformations. The compounds differ in the nature of the 5-substituent. The hydroxy group (5-OH-DPAT) is a hydrophilic, hydrogen bond donating and accepting group that donates electrons into the aromatic ring. The methoxy group (5-OMe-DPAT) is also an electron donating group but with higher lipophilicity; it is a hydrogen bond acceptor but not a donor and it is slightly larger than the hydroxy group. The fluorine (5-F-DPAT) is a small, slightly lipophilic and electron withdrawing substituent. Thus, these substituents differ in terms of hydrogen bonding ability, electronic influence on the aromatic ring, lipophilicity, and to some extent also in steric bulk. In addition, the enantiomers of DPAT, 5-OH-DPAT, 5-OMe-DPAT and 5-F-DPAT display a spectrum of pharmacological profiles: 5-OH-DPAT is a well studied dopamine D_2 receptor agonist. The agonist activity resides in (*S*)-5-OH-DPAT whereas the (*R*)-enantiomer is reported to behave as a dopamine D_2 receptor antagonist (Karlsson et al., 1990). [1] The 5-deoxy antipodes (*S*)- and (*R*)-DPAT exhibit interesting pharmacological profiles. The (*S*)-enantiomer induces a typical dopaminergic behaviour in animals whereas the (*R*)-enantiomer induces a behaviour indicative of 5-HT_{1A} receptor agonist properties (Yu et al., 1996). The functional effects of the enantiomers of 5-OMe-DPAT and 5-F-DPAT at dopamine D_2 receptors have not been reported previously (for serotonergic effect of 5-F-DPAT, see Backlund Höök et al., 1996).

Below follows a detailed examination of the affinities of the 2-aminotetralin derivatives for the active and the inactive receptor conformations. The (*S*)- and the (*R*)-enantiomers are considered separately because they may not necessarily interact in the same way with the receptor.

4.1.1. (S)-2-aminotetralins

The unsubstituted 2-dipropylaminotetralin (*S*)-DPAT displayed high affinity for the active receptor conformation (5.5 nM) whereas the affinity for the inactive receptor conformation was considerably lower (374 nM). An introduction of a 5-methoxy or a 5-fluoro group in (*S*)-DPAT produced only minor changes in affinity at the active and inactive receptor conformations. However, a 5-hydroxy group increased the affinity 40-fold at the active receptor conformation whereas only a 10-fold increase was seen at the inactive receptor conformation. This indicates that (*S*)-5-OH-DPAT donates a hydrogen bond to the active receptor conformation. The smaller increase in affinity at the inactive receptor conformation may indicate that in this conformation the putative hydrogen bond interaction is weaker than in the active receptor conformation. The ability of a ligand to accept a hydrogen bond ((*S*)-5-OMe-DPAT) does not affect the affinity at either the active or the inactive receptor conformation. The similar affinities of the (*S*)-enantiomers of 5-DPAT, 5-F-DPAT and 5-OMe-DPAT, respectively, suggest that the electronic effects on the aromatic ring induced by the 5-substituents do not affect the affinities to the active or the inactive receptor conformation.

4.1.2. (R)-2-aminotetralins

(*R*)-DPAT behaved as a partial agonist with a K_R/K_{RG} ratio of 17. It displayed about 6-fold lower affinity for the active receptor conformation (32 nM) than (*S*)-DPAT (5.5 nM), whereas the affinities for the inactive receptor conformation were similar (554 nM and 374 nM, respectively). The introduction of a 5-fluoro substituent in (*R*)-DPAT did not significantly change the affinity for the active or the inactive receptor conformation. In contrast, a 5-hydroxy substituent increases the affinity more for the inactive (4-fold) than for the active (1.5-fold) receptor conformation. An even larger increase is seen when a 5-methoxy group is introduced in (*R*)-DPAT (16- and 5-fold, respectively). This increase in affinity for the inactive receptor conformation may be rationalised as follows; (i) (*R*)-5-OMe-DPAT accepts an hydrogen bond from the receptor in the inactive conformation or (ii) an electron donating substituent is preferable and increases the affinity for the inactive receptor conformation. However, a hydrogen bond interaction appears to be less likely since (*R*)-5-OH-DPAT, which also should be able to accept a hydrogen bond, displays a modest 5-fold increase in affinity at the inactive receptor conformation as compared to (*R*)-DPAT, which is unable to form hydrogen bonds. (*R*)-5-OMe-DPAT has 3–4 fold higher affinity than (*R*)-5-OH-DPAT at both receptor conformations. This is due to the *O*-methyl group which probably increases the affinity by either interacting

[1] A similar behaviour is reported for the enantiomers of the *N*-(2-thienyl)ethyl derivative of 5-OH-DPAT (N-0437). The (−)-enantiomer is an agonist whereas the (+)-enantiomer is reported to behave as an partial agonist or an antagonist (Timmerman et al., 1989; Lahti et al., 1996).

with a hydrophobic region of the receptor or by decreasing the hydrophilic properties of the ligand and thereby facilitating desolvation. Therefore, it is likely that in the (*R*)-series an electron rich aromatic ring is of importance for binding to the inactive receptor conformation. This idea is supported by the observation that a number of analogues of (1*S*,2*R*)-UH-232 with electron withdrawing groups in the 5-position have low affinity for the dopamine D_2 receptor (Haadsma-Svensson et al., 1994).

All (*R*)-enantiomers have lower intrinsic activity than the (*S*)-enantiomers. Thus, the stereochemistry is an important factor for the ability of the compounds to reduce the forskolin-stimulated cyclic AMP production via the dopamine D_2 receptor. However, the regioisomers (*S*)-5-OH-DPAT and (*R*)-7-OH-DPAT are potent dopamine D_2 receptor agonists but the stereoselectivity of 7-OH-DPAT is reversed as compared to that of 5-OH-DPAT (Table 1, Figs. 1 and 2). It is interesting to note, that (*R*)-DPAT is a partial agonist whereas (*R*)-7-OH-DPAT is a full agonist. In contrast, both (*S*)-DPAT and (*S*)-5-OH-DPAT are full agonists. The addition of a hydroxy group in the 7-position in (*R*)-DPAT increases the affinity 10-fold for the inactive receptor conformation and 45-fold for the active receptor conformation. Thus, in (*R*)-7-OH-DPAT a hydrogen bond interaction appears to be important for high intrinsic activity. A comparable increase in affinities at the two receptor conformations is also apparent when comparing (*S*)-DPAT and (*S*)-5-OH-DPAT. However, this does not lead to any change in intrinsic activity since an increase in ratio above 30 is not reflected in the corresponding increase in intrinsic activity (see above).

4.2. Hypothesis of ligand binding and receptor activation at a molecular level

On the basis of indirect modelling as well as a homology based receptor model of the dopamine D_2 receptor, we have previously proposed that 2-aminotetralin derivatives have two different modes of interaction with the dopamine D_2 receptor; an agonist- and an antagonist binding mode (Grol et al., 1991; Malmberg et al., 1994). We concluded that compounds homochiral [2] to (*S*)-5-OH-DPAT bind in a different orientation in the dopamine D_2 receptor compared to compounds homochiral to (1*S*,2*R*)-UH-232. According to our model the agonist (*S*)-5-OH-DPAT interacts with Asp^{114} (TM3) via a reinforced ionic interaction and displays a hydrogen bond to Ser^{193} in TM5. An aromatic edge-to-face interaction was identified between the Phe^{390} and the aromatic ring of the ligand. A 2-aminotetralin based antagonist has the protonated nitrogen and the nonaromatic ring overlapping with the corresponding structural elements of the agonists, whereas the location of the aromatic ring is more extracellular. These two different ways for ligands to interact with the receptor are referred to as the agonist binding mode and antagonist binding mode, respectively. The ability of a compound to bind in the agonist binding mode and to interact with Phe^{390} might be related to the intrinsic activity [3].

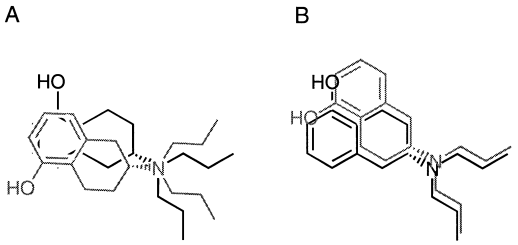

Fig. 4. Proposed binding modes of the agonist (*S*)-5-OH-DPAT (black) and the partial agonist (*R*)-5-OH-DPAT (grey) to active (A) and inactive (B) receptor conformations. (*S*)-5-OH-DPAT interacts in the agonist binding mode with both receptor conformations. (*R*)-5-OH-DPAT interacts in the agonist binding mode with the receptor in the active conformation (A), with its aromatic ring overlapping that of (*S*)-5-OH-DPAT. In the inactive receptor conformation (B), (*R*)-5-OH-DPAT binds in the antagonist binding mode with the aromatic ring differently located from the aromatic ring of (*S*)-5-OH-DPAT whereas the nonaromatic rings are overlapping. The antagonist (1*S*,2*R*)-UH-232 interacts in the antagonist binding mode with both receptor conformations.

The results in this study can be used to extend the previous model by providing insight into the molecular detail of the binding modes of agonists, partial agonists and antagonists in the 2-aminotetralin series to active and inactive receptor conformations, respectively (Fig. 4). A hydrogen bond interaction appears to be important for the affinity of (*S*)-series (agonists) to both receptor conformations. The weaker hydrogen bond interaction at the inactive conformation could be the result of different locations of the TM domains in the two receptor conformations and therefore an altered distance between the ligand and a hydrogen bonding residue. However, (*S*)-DPAT, which has no hydrogen bonding ability, displays a 68-fold difference in affinity at the two receptor conformations. Therefore, the reduced affinity at the inactive receptor conformation would probably include the loss of an aromatic interaction and general bulk changes in the binding site. In summary, these observations suggest that 2-aminotetralin based agonists bind to the same region of the dopamine D_2 receptor. Further, this region appears to undergo a structural rearrangement as the result of the conformational

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

[3] Recently, site-directed mutagenesis of Phe^{390} produced a mutant receptor with no binding of labelled agonist or antagonist (Cho et al., 1995). This, indicates that Phe^{390} is important for the overall receptor conformation. The His^{394} in the rat dopamine D_2 receptor (His^{393} in human), which was suggested to be an interaction point in the antagonist binding mode, has also been mutated (Woodward et al., 1994). However, this mutation had both positive and negative effects on the binding of the antagonists studied, which included mostly substituted benzamides and no 2-aminotetralines.

equilibrium between active and inactive conformations of the dopamine D_2 receptor.

The (*S*)- and the (*R*)-enantiomers appear to interact differently with the inactive receptor conformation. A more electron rich aromatic nucleus in the (*R*)-2-aminotetralins increases the affinity for the inactive receptor conformation whereas the corresponding effect is not seen in the (*S*)-series. This supports the notion that agonists in the (*S*)-series do not bind to the inactive receptor conformation in a similar way as the (*R*)-series (partial agonists) or as an antagonist (e.g., (1*S*,2*R*)-UH-232).

Antagonists have similar affinity for both receptor conformations. Therefore, they should interact with a region of the receptor that is not affected by the conformational change. Thus, the antagonist (1*S*,2*R*)-UH-232 should bind in the antagonist binding mode at both active and inactive receptor conformations (Fig. 4B). It is probable that antagonists from other chemical classes may adopt quite different binding modes to the receptor but have at least one interaction in common, the interaction with Asp^{114} (TM 3).

Our modelling studies suggest the following binding modes of partial agonists: A partial agonist such as (*R*)-5-OH-DPAT may interact with the active receptor conformation by binding in the agonist binding mode, similarly to the full agonist (*S*)-5-OH-DPAT (Fig. 4A). In contrast, (*R*)-5-OH-DPAT interacts with the inactive receptor conformation in the same way as the antagonist (1*S*,2*R*)-UH-232, i.e., in the antagonist binding mode (Fig. 4B). This mode of interaction should be energetically favourable for (*R*)-2-aminotetralin based partial agonists since they are homochiral with the antagonist (1*S*,2*R*)-UH-232.

The agonist binding site in the active receptor conformation appears to be unable to accommodate the C1-methyl group in (1*S*,2*R*)-UH-232. To extrapolate, the introduction of a group that prevents a ligand from binding in the agonist binding mode to the active receptor conformation should produce an antagonist.

In conclusion, in this study we have determined the affinity of a number of dopaminergic ligands for the active and inactive dopamine D_2 receptor conformations using agonist and antagonist radioligands. The ratio of the affinities for the two receptor conformations was correlated with the intrinsic activity as determined by cyclic AMP measurements. Using a selective labelling of the two receptor conformations we have found an excellent correlation between affinity ratio and intrinsic activity. These data suggest that this method, which uses receptor binding, is well suited for the rapid estimation of intrinsic activity.

Furthermore, interpretation of the binding data based on a receptor model has provided hypotheses regarding the molecular interactions of agonists, antagonists and partial agonists with active and inactive receptor conformations of the dopamine D_2 receptor. The approach to examine structure–affinity relationships at the active and inactive conformations separately should make it possible to design ligands with predetermined affinity and intrinsic activity. In addition, mapping of the binding site of the two receptor conformations with appropriate ligands may provide hints about the molecular details of the conformational changes in the receptor protein.

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