Novel antipsychotics activate recombinant human and native rat serotonin 5-HT1A receptors: affinity, efficacy and potential implications for treatment of schizophrenia

Adrian Newman-Tancredi, Marie-Bernadette Assie, Nathalie Leduc, Anne-Marie Ormier, Nathalie Danty and Cristina Cosi
Division of Neurobiology 2, Centre de Recherche Pierre Fabre, Castres, France

Abstract
Serotonin 5-HT1A receptors are promising targets in the management of schizophrenia but little information exists about affinity and efficacy of novel antipsychotics at these sites. We addressed this issue by comparing binding affinity at 5-HT1A receptors with dopamine D2 receptors, which are important targets for antipsychotic drug action. Agonist efficacy at 5-HT1A receptors was determined for G-protein activation and adenylyl cyclase activity. Whereas haloperidol, thioridazine, risperidone and olanzapine did not interact with 5-HT1A receptors, other antipsychotic agents exhibited agonist properties at these sites. E<sub>max</sub> values (% effect induced by 10 μM of 5-HT) for G-protein activation at rat brain 5-HT1A receptors: sarizotan (66.5), bifeprunox (35.9), SSR181507 (25.8), nemonapride (25.7), ziprasidone (20.6), SLV313 (19), aripiprazole (15), tiospirone (8.9). These data were highly correlated with results obtained at recombinant human 5-HT1A receptors in determinations of G-protein activation and inhibition of forskolin-stimulated adenylyl cyclase. In binding-affinity determinations, the antipsychotics exhibited diverse properties at r5-HT1A receptors: sarizotan (p<sub>K<sub>i</sub> = 8.65), SLV313 (8.64), SSR181507 (8.53), nemonapride (8.35), ziprasidone (8.30), tiospirone (8.22), aripiprazole (7.42), bifeprunox (7.19) and clozapine (6.31). The affinity ratios of the ligands at 5-HT1A vs. D2 receptors also varied widely: ziprasidone, SSRI81507 and SLV313 had similar affinities whereas aripiprazole, nemonapride and bifeprunox were more potent at D2 than 5-HT1A receptors. Taken together, these data indicate that aripiprazole has low efficacy and modest affinity at 5-HT1A receptors, whereas bifeprunox has low affinity but high efficacy. In contrast, SSR181507 has intermediate efficacy but high affinity, and is likely to have more prominent 5-HT1A receptor agonist properties. Thus, the contribution of 5-HT1A receptor activation to the pharmacological profile of action of the antipsychotics will depend on the relative 5-HT1A/D2 affinities and on 5-HT1A agonist efficacy of the drugs.

Keywords: Aripiprazole, atypical antipsychotics, clozapine, G proteins, hippocampus, [35S]GTP<sub>y</sub>S, 5-HT1A receptors.

Introduction
Whilst blockade of dopamine D2 receptors is a common feature of all agents currently employed for the treatment of schizophrenia, antipsychotics such as clozapine, risperidone and ziprasidone also interact at serotonin (5-HT) receptor subtypes. As well as 5-HT2A and 5-HT2C receptors, which have been extensively investigated (Leysen, 2000), multiple aspects of 5-HT1A receptor function have attracted increasing attention to this site as a promising target for antipsychotic therapy (Bantick et al., 2001; Millan, 2000; Sharma and Shapiro, 1996).

First, in the central nervous system, 5-HT1A receptors are expressed in the raphe nuclei, where they exert inhibitory control of serotonergic neurotransmission, and in the cerebral cortex and hippocampus (Barnes and Sharp, 1999; Kroese and Roth, 1998). The latter structures are implicated in the control of...
cognition and memory, functions which are impaired in schizophrenic patients. Indeed, microdialysis experiments have shown that 5-HT1A receptor activation increases dopamine release in frontal cortex (Ichikawa and Meltzer, 2000; Millan et al., 1998a; Rollema et al., 1997, 2000), suggesting alleviation of the proposed deficiency in dopaminergic neurotransmission in this brain region of schizophrenics (Honey et al., 1999). Amelioration of this ‘hypofrontality’ is associated with improvement in negative and cognitive symptoms of schizophrenia (Honey et al., 1999). Further, the influence of 5-HT1A receptor activation on dopamine release is regionally selective, because similar increases in dopamine release are not observed in the nucleus accumbens or striatum (Ichikawa and Meltzer, 2000; Millan et al., 1998a).

Second, 5-HT1A receptors are up-regulated in schizophrenics, as determined by post-mortem investigations of receptor expression in frontal cortex and other brain regions (Burnet et al., 1997; Hashimoto et al., 1991; Sumiyoshi et al., 1996; Tauscher et al., 2002; but see Yasuno et al., 2004). 5-HT1A receptor expression may be increased as a compensation mechanism subsequent to insufficient activation of this site: a deficiency that could be remedied by administration of 5-HT1A receptor agonists. Further, 5-HT1A receptors are expressed on glutamatergic neurons in frontal cortex (Czyrak et al., 2003) and the non-competitive NMDA antagonist, dizocilpine, rapidly up-regulates 5-HT1A receptors (Wedzony et al., 1997). This suggests that 5-HT1A receptor activation may modulate dysfunctional glutamatergic function in schizophrenic patients. Accordingly, glutamate release is decreased by 5-HT1A receptor activation (Mauler et al., 2001) and evidence of 5-HT1A/glutamatergic interaction has also been described at the neuroendocrine level, where blockade of mGluR5 receptors increases corticosterone release partly via activation of 5-HT1A receptors (Bradbury et al., 2003).

Third, numerous laboratories have demonstrated anti-cataleptic properties of 5-HT1A agonists in rodents, indicating that activation of 5-HT1A receptors should reduce extrapyramidal symptoms (EPS) induced by dopamine D2 receptor blockade (Invernizzi et al., 1988; McMillen et al., 1988; Wadenberg et al., 1999). The extent to which 5-HT1A agonists are able to reverse neuroleptic-induced catalepsy is dependent on the agonist efficacy of the ligand: high efficacy activation is necessary to completely abolish haloperidol-induced catalepsy (Kleven et al., 1996; Prinssen et al., 1999, 2002).

Fourth, the atypical antipsychotic, clozapine, which displays improved capacity to treat negative symptoms with minimal EPS liability, exhibits agonist properties at 5-HT1A receptors in various in vitro models of receptor transduction (Assié et al., 1997; Cussac et al., 2002; Elliott and Reynolds, 1999; Newman-Tancredi et al., 1998). The ability of clozapine to occupy 5-HT1A receptors in non-human primates at clinically relevant doses has been demonstrated by PET scans with [3H]WAY100635 (Bantick et al., 2000; Chou et al., 2003) and both the elevation of dopamine release in frontal cortex and the anticonvulsant properties of clozapine are partially mediated by 5-HT1A receptors (Millan et al., 1998b; Rollema et al., 1997).

Finally, some exploratory clinical augmentation trials have shown that buspirone and tandospirone, which act as partial agonists at 5-HT1A receptors, substantially ameliorate negative symptoms scores and reduce the incidence of EPS in haloperidol-treated schizophrenic patients (Goff et al., 1991; Sovner and Parnell-Sovner, 1989; Sumiyoshi et al., 2001,a,b).

In view of the importance of 5-HT1A receptors, it is not surprising that recent antipsychotic agents have increasingly been selected to include varying degrees of agonist properties at 5-HT1A receptors. As well as clozapine – zipsadione and nemonapride exhibit partial agonist properties at 5-HT1A receptors, as do aripiprazole and bifeprunox, which are recently commercialized and in late-stage development respectively (Jordan et al., 2002b, 2004; Van Vliet et al., 2000a,b). Further, a new generation of potential antipsychotic agents in early clinical development, such as SSR181507 and SLV313, is specifically targeted at 5-HT1A receptors, in addition to dopamine receptors (Claustre et al., 2003; Depoortere et al., 2003; Feenstra et al., 2002; Glennon et al., 2002). Sarizotan (EMD128907), another compound with potent 5-HT1A agonist properties (Bartoszyk et al., 2004), was also initially in development as an antipsychotic. However, little or no information is available on the clinical or pre-clinical pharmacology of these new-generation ligands and no reports have been published that allow parallel comparisons of their actions at 5-HT1A receptors. The present study addressed this issue by investigating the actions of these antipsychotics in a series of in vitro signal transduction tests of 5-HT1A receptor activation. Compounds were examined in models of G-protein activation at native rat hippocampal 5-HT1A receptors (Newman-Tancredi et al., 2003) and at recombinant human receptors expressed in HeLa cells (Così and Koek, 2001). Agonist efficacy at HeLa-h5-HT1A receptors was also measured by determining adenylyl cyclase activity (Assié et al., 1997), a classical measure of 5-HT1A receptor activation.
Because all the antipsychotics also interact with dopamine D2 receptors, affinity at rat 5-HT1A receptors was compared with that at rat striatal D2 sites to provide 5-HT1A:D2 affinity ratios. The results indicate that antipsychotics show considerable variation in capacity to bind and activate 5-HT1A receptors and suggest that the contribution of 5-HT1A interactions to their therapeutic profile is likely to be highly diverse.

**Methods**

**HeLa-h5-HT1A cell culture and membrane preparations**

HeLa-h5-HT1A (HA7) cells (Fargin et al., 1989) were grown as described (Cosi and Koek, 2001). Briefly, cells were cultured in Dulbecco’s modified Eagle’s medium (DMEM; Caisio, Cergy-Pontoise, France) supplemented with 10% fetal calf serum, gentamicin (100 μg/ml), and 5-HT1A receptors.

**Table 1. Experimental conditions for binding affinity and G-protein activation determinations**

<table>
<thead>
<tr>
<th>Receptor</th>
<th>Parameters</th>
<th>Tissue</th>
<th>Radioligand (nM)</th>
<th>Kd (μM)</th>
<th>Non-specific (μM)</th>
<th>Incubation buffer (μM)</th>
<th>Inc. time and temp.</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>r5-HT1A</td>
<td>pK&lt;sub&gt;i&lt;/sub&gt;</td>
<td>Cortex</td>
<td>[3H]8-OH-DPAT (0.2)</td>
<td>3.1</td>
<td>5-HT (10)</td>
<td>Tris–HCl 50, pargyline 0.01, CaCl&lt;sub&gt;2&lt;/sub&gt; 4, ascorbic acid 1%</td>
<td>30 min, 23 °C</td>
<td>Assié et al. (1993)</td>
</tr>
<tr>
<td>pEC50, E&lt;sub&gt;max&lt;/sub&gt;</td>
<td>Hippocampus</td>
<td>[35S]GTPγS (0.1)</td>
<td>na</td>
<td>GTPγS (10)</td>
<td>Heps 50, NaCl 150, EDTA 0.2, MgCl&lt;sub&gt;2&lt;/sub&gt; 4, GDP 0.1, pargyline 0.01</td>
<td>60 min, 37 °C</td>
<td>Newman-Tancredi et al. (2003)</td>
<td></td>
</tr>
<tr>
<td>h5-HT1A</td>
<td>pK&lt;sub&gt;i&lt;/sub&gt;</td>
<td>HeLa cells</td>
<td>[3H]8-OH-DPAT (1)</td>
<td>0.71</td>
<td>5-HT (10)</td>
<td>Tris–HCl 50, pargyline 0.01, CaCl&lt;sub&gt;2&lt;/sub&gt; 4</td>
<td>30 min, 23 °C</td>
<td>Cosi and Koek (2001)</td>
</tr>
<tr>
<td>pEC50, E&lt;sub&gt;max&lt;/sub&gt;</td>
<td>HeLa cells</td>
<td>[35S]GTPγS (0.1)</td>
<td>na</td>
<td>GTPγS (10)</td>
<td>Heps 20, MgCl&lt;sub&gt;2&lt;/sub&gt; 10, pargyline 0.01, GDP 30 μM, NaCl 100</td>
<td>60 min, 30 °C</td>
<td>Cosi and Koek (2001)</td>
<td></td>
</tr>
<tr>
<td>rD2</td>
<td>pK&lt;sub&gt;i&lt;/sub&gt;</td>
<td>Striatum</td>
<td>[3H]Nemonapride (0.05)</td>
<td>0.036 (+)Butaclamol (1)</td>
<td>Tris–HCl50, NaCl 120, KCl 5</td>
<td>60 min, 23 °C</td>
<td>Assié et al. (1993)</td>
<td></td>
</tr>
</tbody>
</table>

Affinity was determined in competition-binding experiments. G-protein activation was measured by stimulation of [35S]GTPγS binding. Buffer pH was 7.4 in all cases. Data were analyzed by nonlinear regression and values are expressed as mean ± S.E.M. of at least three determinations performed in triplicate. na, Not applicable.
as described by Cosi and Koek (2001) for 1 h at 30 °C. Membranes were cooled to 0 °C for 15 min before addition of [35S]GTPγS for incubation as described in Table 1. [35S]GTPγS binding to rat hippocampal membranes was carried out essentially as described by Newman-Tancredi et al. (2003) with minor modifications. Briefly, frozen brains were thawed in ice-cold buffer A (50 mM Hepes containing 150 mM NaCl, 0.2 mM EDTA, 1 mM GTP, 10 μM pargyline; pH 7.4, 23 °C). Hippocampi were dissected and homogenized in 20 volumes of buffer A before incubation at 37 °C for 10 min to dissociate endogenous neurotransmitters from receptors. The homogenate was centrifuged at 20 000 g for 15 min, at 4 °C. The pellet was resuspended in buffer A and recentrifuged as before. The pellet was resuspended in buffer B (50 mM Hepes containing 150 mM NaCl, 0.2 mM EDTA, 5 mM MgCl2, 100 μM GDP, 10 μM pargyline), centrifuged as before and resuspended in buffer B. Membranes were incubated with test compounds and [35S]GTPγS as described in Table 1. The reaction was terminated by rapid filtration through Whatman filters using a Brandel harvester and radioactivity was counted by liquid scintillation counting.

For measurement of adenyl cyclase activity at HeLa-h5-HT1A cells, cells were pre-incubated (10 min, 23 °C) with DMEM, 10 mM Hepes. Drugs, at concentrations varying from 0.1 nM to 10 μM, were then added to the cells in DMEM, 10 mM Hepes, 100 mM forskolin, and 100 mM 3-isobutyl-1-methylxanthine (IBMX). At the end of the incubation (10 min, 23 °C), the reaction was stopped by aspirating the medium and adding 0.1 N HCl. Cell extract was diluted 1:500 in radioimmunoassay buffer, and cAMP content was measured using a commercially available radioimmunoassay kit (NEK-033; New England Nuclear Life Science Products, Boston, MA, USA). Typical basal cAMP levels were 10 pmol/well.

All binding experiments terminated by rapid filtration, using a Brandel harvester, through Whatman GF-B fibre filters. Radioactivity retained on the filters was measured by liquid scintillation spectroscopy. Data from all experiments were analysed using nonlinear curve-fitting programs (Prism, Graphpad Software, San Diego, CA, USA), and are expressed as mean value ± S.E.M.

**Drugs**

BMY7378 (8-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-8-azaspiro[4,5]decane-7,9-dione; buspirone; 5-carboxamidotryptamine (5-CT); haloperidol; 5-hydroxytryptamine(5-HT); dihydroergotamine (DHE); (+)-8-hydroxy-dipropylaminotetralin [(+)-8-OH-DPAT]; (+)-8-OH-DPAT; thioridazine and risperidone were purchased from Sigma–RBI (S. Queen Fallavier, France). Aripiprazole; bifeprunox (DU127090); mecamylamine; olanzapine; repinotan (BAY3732); S14506 (1H-[2-(4-fluorobenzoylamino)ethyl]-4-(7-methoxynaphthy)piperazine); sarizotan (EMD128130); SSR181507 ([3-exo]-8-benzoyl-N-[[(2S)7-chloro-2,3-dihydro-1,4-benzodioxin-1-yl][methyl]-8-azabicyclo-[3,2,1] octane-3-methanaminemonohydrochloride); WAY100135 (N-[2-(4-methoxyphenyl)-1-piperazinyl]-N-(2-pyridinyl)cyclohexancarboxamide) and ziprasidone were synthesized by Jean-Louis Maurel (Chemistry Dept, Centre de Recherche Pierre Fabre). Clozapine was purchased from Tocris (Illkirch, France). SLV313 (piperazine, 1-(2,3-dihydro-1,4-benzodioxin-5-yl)-4-[5-(4-fluorophenyl)-3-pyridinyl)methyl) and SLV314 ((2R)-2H-1,

**Table 2. Affinity and efficacy of serotonergic ligands at native rat 5-HT1A receptors**

<table>
<thead>
<tr>
<th>Compound</th>
<th>Cortex 5-HT1A Affinity (pKᵢ)</th>
<th>pEC₅₀</th>
<th>Hippocampus 5-HT1A Eₘₐₓ (%) 5-HT</th>
</tr>
</thead>
<tbody>
<tr>
<td>5-CT</td>
<td>9.82 ± 0.02</td>
<td>7.14 ± 0.07</td>
<td>104.5 ± 4.8</td>
</tr>
<tr>
<td>5-HT</td>
<td>8.66 ± 0.13</td>
<td>6.47 ± 0.13</td>
<td>94.5 ± 6.1</td>
</tr>
<tr>
<td>DHE</td>
<td>9.08 ± 0.04</td>
<td>8.28 ± 0.07</td>
<td>93.9 ± 4.3</td>
</tr>
<tr>
<td>(+)-8-OH-DPAT</td>
<td>8.66 ± 0.07</td>
<td>6.93 ± 0.03</td>
<td>66.7 ± 4.8</td>
</tr>
<tr>
<td>Fleinoxan</td>
<td>8.91 ± 0.03</td>
<td>7.45 ± 0.08</td>
<td>55.0 ± 1.2</td>
</tr>
<tr>
<td>S14506</td>
<td>9.05 ± 0.07</td>
<td>9.00 ± 0.02</td>
<td>52.8 ± 2.9</td>
</tr>
<tr>
<td>Repinotan</td>
<td>10.29 ± 0.05</td>
<td>8.65 ± 0.08</td>
<td>51.4 ± 3.1</td>
</tr>
<tr>
<td>(+)-8-OH-DPAT</td>
<td>8.85 ± 0.07</td>
<td>7.00 ± 0.09</td>
<td>50.2 ± 4.0</td>
</tr>
<tr>
<td>Roxindole</td>
<td>9.10 ± 0.07</td>
<td>7.38 ± 0.08</td>
<td>38.3 ± 2.3</td>
</tr>
<tr>
<td>Buspirone</td>
<td>7.65 ± 0.12</td>
<td>6.47 ± 0.31</td>
<td>19.5 ± 2.5</td>
</tr>
<tr>
<td>BMY7378</td>
<td>8.94 ± 0.04</td>
<td>6.92 ± 0.31</td>
<td>10.6 ± 0.6</td>
</tr>
<tr>
<td>WAY100135</td>
<td>8.35 ± 0.05</td>
<td>nc</td>
<td>−7.2 ± 4.7*</td>
</tr>
<tr>
<td>WAY100635</td>
<td>9.02 ± 0.02</td>
<td>nc</td>
<td>−0.6 ± 2.5*</td>
</tr>
</tbody>
</table>

Affinity (pKᵢ) at r5-HT1A receptors was determined in competition-binding experiments as described in Table 1. Agonist efficacy was determined by stimulation of [35S]GTPγS binding to hippocampal membranes. Eₘₐₓ values are expressed as % of the action of 10 μM 5-HT (= 100%). Data were analysed by nonlinear regression and values are expressed as mean ± S.E.M. of at least three determinations performed in triplicate.

*Where a nonlinear regression could not be fitted, the effect at the highest concentration tested is shown (i.e. 10 μM); nc, not computable. Compounds are listed in the order of their efficacy (Eₘₐₓ).
Table 3. Affinity and efficacy of antipsychotic agents at native rat 5-HT1A receptors

<table>
<thead>
<tr>
<th>Antipsychotics</th>
<th>Cortex 5-HT1A Affinity (pK_i)</th>
<th>Striatum D2 Affinity (pK_i)</th>
<th>Ki ratio 5-HT1A:D2</th>
<th>Hippocampus 5-HT1A</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>pEC_{50}</td>
</tr>
<tr>
<td>Sarizotan</td>
<td>8.65 ± 0.02</td>
<td>8.18 ± 0.08</td>
<td>0.3</td>
<td>7.11 ± 0.05</td>
</tr>
<tr>
<td>Bifeprunox</td>
<td>7.19 ± 0.14</td>
<td>8.83 ± 0.05</td>
<td>44</td>
<td>6.37 ± 0.11</td>
</tr>
<tr>
<td>SSR181507</td>
<td>8.53 ± 0.10</td>
<td>8.41 ± 0.05</td>
<td>0.8</td>
<td>6.99 ± 0.27</td>
</tr>
<tr>
<td>Nemonapride</td>
<td>8.35 ± 0.03</td>
<td>9.92 ± 0.03</td>
<td>37</td>
<td>6.94 ± 0.09</td>
</tr>
<tr>
<td>Ziprasidone</td>
<td>8.30 ± 0.09</td>
<td>7.90 ± 0.14</td>
<td>0.4</td>
<td>6.78 ± 0.28</td>
</tr>
<tr>
<td>SLV313</td>
<td>8.64 ± 0.01</td>
<td>8.58 ± 0.06</td>
<td>0.9</td>
<td>8.16 ± 0.03</td>
</tr>
<tr>
<td>Aripiprazole</td>
<td>7.42 ± 0.13</td>
<td>8.59 ± 0.15</td>
<td>15</td>
<td>6.43 ± 0.09</td>
</tr>
<tr>
<td>Tiosporone</td>
<td>8.22 ± 0.07</td>
<td>8.24 ± 0.04</td>
<td>1.1</td>
<td>nc</td>
</tr>
<tr>
<td>Clozapine</td>
<td>6.31 ± 0.06</td>
<td>6.83 ± 0.05</td>
<td>3.3</td>
<td>nc</td>
</tr>
<tr>
<td>SLV314</td>
<td>7.27 ± 0.07</td>
<td>9.37 ± 0.05</td>
<td>126</td>
<td>nc</td>
</tr>
<tr>
<td>Olanzapine</td>
<td>5.38 ± 0.14</td>
<td>7.82 ± 0.18</td>
<td>275</td>
<td>nc</td>
</tr>
<tr>
<td>Haloperidol</td>
<td>5.77 ± 0.04</td>
<td>9.01 ± 0.01</td>
<td>1738</td>
<td>nc</td>
</tr>
<tr>
<td>Risperidone</td>
<td>6.03 ± 0.09</td>
<td>8.70 ± 0.03</td>
<td>468</td>
<td>nc</td>
</tr>
<tr>
<td>Thioridazine</td>
<td>6.20 ± 0.04</td>
<td>8.29 ± 0.03</td>
<td>123</td>
<td>nc</td>
</tr>
</tbody>
</table>

Affinity (pK_i) at r5-HT1A and rD2 receptors was determined in competition-binding experiments as described in Table 1. Agonist efficacy was determined by stimulation of [35S]GTP\_S binding to hippocampal membranes. E_{max} values are expressed as % of the action of 10 μM 5-HT (= 100%). Data were analysed by nonlinear regression and values are expressed as mean ± S.E.M. of at least three determinations performed in triplicate.

^a Where a nonlinear regression could not be fitted, the effect at the highest concentration tested is shown (i.e. 10 μM, except for for clozapine, 100 μM); nc, not computable. Compounds are listed in the order of their efficacy (E_{max}).

4-benzoxazin-3(4H)-one, 8-[4-[3-(5-fluoro-1H-indol-3-yl)propyl]-1-piperazinyl]-2-methyl) was kindly donated by Solvay Pharmaceuticals (Weesp, The Netherlands). Tiosporine was kindly donated by Bristol-Myers (Princeton, NJ, USA). [3H]8-OH-DPAT (reference TRK850; 160–240 Ci/mmoll) and [35S]GTP\_S (1000–1200 Ci/mmoll) were purchased from Amersham Biosciences (Orsay, France). [3H]Nemonapride (reference NET1004; 70–87 Ci/mmoll) was purchased from PerkinElmer Life Sciences (Courtaboeuf, France).

**Results**

**Affinity at rat brain 5-HT1A receptors**

Experiments at native rat cortical 5-HT1A receptors indicated marked affinity for a series of reference serotonergic agents (Table 2). Thus, the agonists 5-HT, 5-CT and (±)-8-OH-DPAT exhibited high affinity, as did the selective antagonist, WAY100635 (pK_i values >8.5). The partial agonist, buspirone exhibited moderate affinity (pK_i = 7.65) whereas repinotan was very potent at rat cortical 5-HT1A receptors (pK_i > 10). Among the antipsychotic agents, haloperidol, thioridazine, risperidone and olanzapine yielded modest affinities at 5-HT1A receptors (pK_i values <6.5, Table 3). In contrast, more recent antipsychotic agents, such as ziprasidone, nemonapride, aripiprazole, bifeprunox, SSR181507 and SLV313, as well as tiosporine, were more potent (pK_i values 7-9, Table 3). When affinity at r5-HT1A receptors was compared with that at rD2 receptors, haloperidol exhibited the highest ratio of selectivity in favour of rD2 receptors (>1700-fold) whereas more recent antipsychotics all exhibited lower ratios. Thus, aripiprazole and bifeprunox exhibited selectivity ratios of 15- and 44-fold respectively. Clozapine and tiosporine exhibited balanced affinities at r5-HT1A vs. rD2 receptors (ratios 3.3 and 1.1 respectively) whereas SSR181507 and SLV313 had slightly higher affinity at r5-HT1A than at rD2 receptors (ratios ≈ 0.8; Table 3).

**Agnost properties at rat brain 5-HT1A receptors**

The non-selective 5-HT1 receptor agonists, 5-HT and DHE, efficaciously stimulated [35S]GTP\_S binding at rat brain hippocampal membranes (E_{max} ≈100% relative to 5-HT), whereas the selective 5-HT1A antagonists, WAY100635 and WAY100135, induced no stimulation (Table 2). Repinotan and S14506 induced intermediate stimulation of [35S]GTP\_S binding (E_{max} ≈50%), consistent with selective activation of 5-HT1A receptors. Buspirone was less efficacious (E_{max} ≈ 20%), consistent with partial agonist properties
at rat hippocampal 5-HT1A receptors, whereas (+)-8-OH-DPAT, the more active enantiomer of (±)-8-OH-DPAT, was more efficacious (67%). WAY100635, inhibited most, but not all, of the stimulation induced by 10 μM 5-HT (pIC₅₀ = 7.90 ± 0.09), leaving a residual stimulation of 14.4 ± 0.6%. Residual stimulation was also observed for 5-CT (11.2 ± 5.1%) and for DHE (7.1 ± 2.1%). In contrast, WAY100635 completely abolished all of the stimulation induced by other serotonergic agonists and antipsychotics, indicating that it was specifically mediated by 5-HT1A receptors (see below). The conventional antipsychotic agents, haloperidol and thioridazine, exhibited no agonist properties but showed a tendency to reduce [³⁵S]GTPγS binding at the highest concentrations tested (10 μM). This was also true of the ‘atypical’ antipsychotics, olanzapine and risperidone (Table 3). In contrast, the more recent antipsychotics, ziprasidone, nemonapride, aripiprazole, bifeprunox, SSR181507 and SLV313, all acted as agonists at rat hippocampal 5-HT1A receptors, although their efficacies showed marked diversity (Figure 1). Ziprasidone, nemonapride, SSR181507 and SLV313 exhibited buspirone-like stimulation of [³⁵S]GTPγS binding (Eₘₐₓ values 19–26%) whereas the efficacy of aripiprazole was lower (15%) and that of bifeprunox was markedly higher (36%). Clozapine (10⁻⁴ M) induced slight stimulation (~8%) under these conditions, whereas sarizotan exhibited efficacy resembling that of (+)-8-OH-DPAT (66%). The older antipsychotic, tiospirone, weakly influenced [³⁵S]GTPγS binding, increasing it just above basal levels.

**Affinity at cloned human 5-HT1A receptors**

Affinity at cloned human 5-HT1A receptors expressed in HeLa cells was high for a range of reference serotonergic agents, such as 5-CT, DHE and S14506 (pKᵢ values > 9; Table 4). High affinity was also observed for WAY100635 and (+)-8-OH-DPAT.

Among the antipsychotic agents, the ‘typical’ neuroleptics, such as haloperidol and thioridazine, exhibited modest or low affinity. Risperidone and olanzapine also exhibited low affinity, but clozapine, ziprasidone, nemonapride and tiospirone as well as ‘new-generation’ antipsychotic agents, aripiprazole, bifeprunox, SSR181507 and SLV313, displayed marked affinity at h5-HT1A receptors (pKᵢ = 7–9).

**Agonist properties at cloned human 5-HT1A receptors**

Agonist efficacy at h5-HT1A receptors was determined by means of two methodologies: activation of

![Figure 1](https://academic.oup.com/ijnp/article-abstract/8/3/341/910003)
coupled G proteins by \(^{35}\)S-GTP\(_{i}\) binding and determination of second-messenger activity (inhibition of adenylyl cyclase activity). Regarding the antipsychotics, aripiprazole, bifeprunox, SSR181507 and SLV313 exhibited marked efficacy in G-protein activation experiments: \(E_{\text{max}}\) > 45% relative to 5-HT (10 \(\mu\)M). Similar values were determined for other antipsychotics, such as clozapine and ziprasidone (Figure 2, Table 5). Sarizotan exhibited very high efficacy, similar to that of 5-CT and DHE, whereas SLV314, also proposed as an antipsychotic agent, displayed efficacy only slightly above background (\(E_{\text{max}} \approx 9.8\%\)). Tiospirone slightly increased \(^{35}\)S-GTP\(_{i}\) binding from basal values. In measures of adenylyl cyclase activity, 5-HT, 5-CT, (+)-8-OH-DPAT and repinotan maximally inhibited adenylyl cyclase activity (\(E_{\text{max}} \approx 100\%\) relative to 5-HT). Buspirone exhibited partial agonist activity (\(E_{\text{max}} = 45\%\)) whereas several antipsychotic agents, including haloperidol, thioridazine and risperidone, tended to increase cAMP accumulation at the highest concentrations. The recent potential antipsychotics, SSR181507 and SLV313, inhibited, but did not abolish, adenylyl cyclase activity (\(E_{\text{max}} = 70-75\%\)), whereas clozapine, aripiprazole, bifeprunox and SLV314, exhibited modest inhibition of adenylyl cyclase, probably reflecting their lower potency observed in \(^{35}\)S-GTP\(_{i}\) binding experiments (Figure 3).

**Correlations between actions at rat brain and recombinant human 5-HT1A receptors**

Values of affinity and maximal stimulation listed in Tables 2–5 were used to determine correlations between rat and human receptors. Using Pearson correlation, affinity (\(pK_i\)) values gathered at rat cortex 5-HT1A receptors correlated closely with \(pK_i\) values at HeLa-h5-HT1A receptors (\(r = 0.9718\), \(p < 0.0001\), slope = 0.88; Figure 4a). Similarly, \(pEC^{50}\) values for G-protein activation at rat hippocampal membranes correlated well with those determined in HeLa-h5-HT1A receptors (\(r = 0.8501\), \(p < 0.0001\), slope = 0.83; Figure 4b). It was not possible to determine \(pEC^{50}\) values for some drugs tested in adenylyl cyclase determinations (Tables 4 and 5), but \(pEC^{50}\) values for the remaining compounds correlated with \(pEC^{50}\) values from \(^{35}\)S-GTP\(_{i}\) binding experiments in HeLa-h5-HT1A cells (\(r = 0.6611\), \(p < 0.01\), slope = 0.80).

**Table 4. Affinity and efficacy of serotonergic ligands at recombinant human 5-HT1A receptors**

<table>
<thead>
<tr>
<th></th>
<th>Affinity (pK_i)</th>
<th>G-protein activation</th>
<th>Adenylyl cyclase</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(pEC^{50})</td>
<td>(E_{\text{max}})</td>
<td>(pEC^{50})</td>
</tr>
<tr>
<td>DHE</td>
<td>9.11 ± 0.03</td>
<td>8.60 ± 0.06</td>
<td>131 ± 14</td>
</tr>
<tr>
<td>(+)-8-OH-DPAT</td>
<td>9.22 ± 0.03</td>
<td>7.70 ± 0.03</td>
<td>129 ± 5(^{b})</td>
</tr>
<tr>
<td>5-CT</td>
<td>9.19 ± 0.20</td>
<td>8.40 ± 0.03</td>
<td>119 ± 1(^{b})</td>
</tr>
<tr>
<td>SI4506</td>
<td>9.44 ± 0.01</td>
<td>9.37 ± 0.05</td>
<td>108 ± 13(^{b})</td>
</tr>
<tr>
<td>5-HT</td>
<td>8.97 ± 0.03</td>
<td>7.31 ± 0.04</td>
<td>100 ± 1(^{b})</td>
</tr>
<tr>
<td>(+)-8-OH DPAT</td>
<td>8.92 ± 0.09</td>
<td>7.59 ± 0.04</td>
<td>82 ± 4</td>
</tr>
<tr>
<td>Repinotan</td>
<td>10.35 ± 0.03</td>
<td>9.11 ± 0.13</td>
<td>77 ± 16</td>
</tr>
<tr>
<td>Roxindole</td>
<td>9.55 ± 0.01</td>
<td>8.02 ± 0.11</td>
<td>65 ± 15</td>
</tr>
<tr>
<td>Flesinoxan</td>
<td>8.77 ± 0.26</td>
<td>7.65 ± 0.08</td>
<td>61 ± 5</td>
</tr>
<tr>
<td>Buspirone</td>
<td>7.87 ± 0.20</td>
<td>7.02 ± 0.08</td>
<td>58 ± 9</td>
</tr>
<tr>
<td>BMY7376</td>
<td>8.69 ± 0.07</td>
<td>7.96 ± 0.15</td>
<td>46 ± 3(^{b})</td>
</tr>
<tr>
<td>WAY100135</td>
<td>8.29 ± 0.02</td>
<td>7.74 ± 0.26</td>
<td>7 ± 2(^{b})</td>
</tr>
<tr>
<td>WAY100635</td>
<td>9.20 ± 0.13</td>
<td>9.54 ± 0.16</td>
<td>– 3 ± 0.1(^{b})</td>
</tr>
</tbody>
</table>

Affinity (\(pK_i\)) at h5-HT1A receptors was determined in competition-binding experiments as described in Table 1. Agonist efficacy was determined by stimulation of \(^{35}\)S-GTP\(_{i}\) binding to HeLa-h5-HT1A cell membranes and by inhibition of forskolin-stimulated cAMP accumulation in HeLa-h5-HT1A whole cells. \(E_{\text{max}}\) values are expressed as % of the action of 10 \(\mu\)M 5-HT (\(100\%\)). Data were analysed by nonlinear regression and values are expressed as mean ± S.E.M. of at least three determinations.

\(^{a}\) Where a nonlinear regression could not be fitted, the effect at the highest concentration tested is shown (i.e. 10 \(\mu\)M).

\(^{b}\) Data previously acquired using the same procedure are shown for comparison (Assié et al., 1997, 1999; Cosi and Koek, 2000; Koek et al., 2001). Compounds are listed in the order of their efficacy (\(E_{\text{max}}\) for G-protein activation; nc, not computable.)
activation at rat hippocampal membranes correlated closely with those determined in HeLa-h5-HT1A cell membranes ($r_S = 0.9553, p < 0.0001$; Figure 4c). Finally, $E_{\text{max}}$ values determined for $[^{35}\text{S}]\text{GTP} \gamma \text{S}$ binding in HeLa-h5-HT1A cell membranes correlated closely with $E_{\text{max}}$ values derived for inhibition of forskolin-stimulated adenylyl cyclase activity in HeLa-h5-HT1A cells ($r_S = 0.9476, p < 0.0001$; Figure 4e).

**Discussion**

Serotonin 5-HT1A receptors sites have been increasingly recognized as key targets for the management of schizophrenia and other psychiatric disorders and recent antipsychotic agents have been selected partly on the basis of activity at this site (see Introduction). The present study indicates that new-generation antipsychotics vary widely in both their affinity and efficacy at 5-HT1A receptors in vitro.

**Affinity at native rat and recombinant human 5-HT1A receptors**

Initial experiments showed that the affinities of serotonergic agents, including 5-HT, 5-CT, and (+)-8-OH-DPAT, yielded affinity ($p_{\text{Ki}}$) values at both recombinant human and native rat 5-HT1A receptors which were consistent with those previously reported in such systems (Table 2; Alper and Nelson, 1998; Barnes and Sharp, 1999; Cosi and Koek, 2001; Newman-Tancredi et al., 1998). Thus, the selective antagonist, WAY100635, exhibited high affinity ($p_{\text{Ki}} > 10$), whereas repinotan (BAY3702) exhibited very high affinity at r5-HT1A receptors ($p_{\text{Ki}} > 10$, Tables 2 and 4). Affinity at native rat and recombinant human receptors was highly correlated (Figure 4a), indicating considerable pharmacological similarity between human and rat 5-HT1A receptors.

In the case of antipsychotic agents, data for haloperidol, thioridazine, risperidone and olanzapine confirm previous reports of low affinity at 5-HT1A receptors (Cosi and Koek, 2001; Leysen, 2000; Newman-Tancredi et al., 1998) with a several hundred-fold selectivity for D2 receptors. In contrast, the ‘atypical’ agents, clozapine and ziprasidone, had affinity at 5-HT1A receptors similar to that determined at D2 receptors (3-fold and 0.4-fold ratio of affinity at 5-HT1A vs. D2 receptors). These data confirm those reported previously in a variety of tissues and expression systems (Leysen, 2000; Mason and Reynolds, 1992; Newman-Tancredi et al., 1998). We now show that more recent antipsychotics, aripiprazole and bifeprunox, as well as the novel agents, SSR181507, SLV313 and sarizotan, all display marked affinity at 5-HT1A receptors ($p_{\text{Ki}} > 7$). However, when these data are compared with affinities at D2 receptors, considerable variation becomes apparent. Whereas the r5-HT1A:rD2 affinity ratio for bifeprunox was 44, SSR181507, SLV313 and sarizotan exhibited ratios of $< 1$ and aripiprazole showed a 15-fold higher affinity at D2 than at 5-HT1A receptors. An older neuroleptic,
Efficacy was determined by stimulation of 35clozapine, 100m

The ligands varied in their ability to stimulate G-

Efficacy at native rat and recombinant human

5-HT1A receptors

The ligands varied in their ability to stimulate G-

membranes. Hence, the residual stimulation of 5-HT-induced [35S]GTPyS binding remains even when 5-HT1A receptors are occluded by high concentrations of the selective antagonist, WAY100635 (see Newman-Tancredi et al., 2003 and the Results section). In contrast, all of the stimulation induced by 5-HT1A-selective agonists and by the antipsychotics was abolished by WAY100635, indicating that it was specifically mediated by 5-HT1A receptors (Figure 1). When the antipsychotics were tested in this system, no stimulation of [35S]GTPyS binding was observed for haloperidol, thioridazine, risperidone and olanzapine, as expected (Newman-Tancredi et al., 1998). However, these compounds tended to reduce basal [35S]GTPyS binding at high concentrations, suggesting a possible inverse agonist action. This effect, which remains to be clarified for hippocampal membranes, is corroborated by the increase in forskolin-stimulated cAMP accumulation seen in adenylyl cyclase assays (Table 5) and the WAY100635-reversible decrease in [35S]GTPyS binding observed in HeLa-h5-HT1A cell membranes (Cosi and Koek, 2001). As concerns agonist properties, the present experiments revealed the diverse capacity of recent antipsychotics to stimulate G-protein

<table>
<thead>
<tr>
<th>Affinity</th>
<th>G-protein activation</th>
<th>Adenylyl cyclase</th>
</tr>
</thead>
<tbody>
<tr>
<td>pKᵢ</td>
<td>pEC₅₀</td>
<td>Eₘₐₓ</td>
</tr>
<tr>
<td>Sarizotan</td>
<td>8.86 ± 0.09</td>
<td>7.57 ± 0.01</td>
</tr>
<tr>
<td>Nemonapride</td>
<td>8.42 ± 0.06ᵇ</td>
<td>7.26 ± 0.04</td>
</tr>
<tr>
<td>Bifeprunox</td>
<td>7.69 ± 0.07</td>
<td>6.49 ± 0.20</td>
</tr>
<tr>
<td>SSR181507</td>
<td>9.06 ± 0.01</td>
<td>7.77 ± 0.03</td>
</tr>
<tr>
<td>Ziprasidone</td>
<td>8.52 ± 0.14ᵇ</td>
<td>7.69 ± 0.09</td>
</tr>
<tr>
<td>Clozapine</td>
<td>6.99 ± 0.01ᵇ</td>
<td>5.79 ± 0.22</td>
</tr>
<tr>
<td>Aripiprazole</td>
<td>7.33 ± 0.03</td>
<td>6.61 ± 0.10</td>
</tr>
<tr>
<td>SLV313</td>
<td>8.75 ± 0.07</td>
<td>8.23 ± 0.06</td>
</tr>
<tr>
<td>SLV314</td>
<td>7.84 ± 0.01</td>
<td>6.88 ± 0.13</td>
</tr>
<tr>
<td>Tiospirone</td>
<td>8.73 ± 0.11ᵇ</td>
<td>8.43 ± 0.25</td>
</tr>
<tr>
<td>Olanzapine</td>
<td>5.84 ± 0.04ᵇ</td>
<td>nc</td>
</tr>
<tr>
<td>Risperidone</td>
<td>6.56 ± 0.03ᵇ</td>
<td>6.66 ± 0.32</td>
</tr>
<tr>
<td>Haloperidol</td>
<td>5.97 ± 0.20ᵇ</td>
<td>6.48 ± 0.23</td>
</tr>
<tr>
<td>Thioridazine</td>
<td>6.77 ± 0.05ᵇ</td>
<td>7.02 ± 0.24</td>
</tr>
</tbody>
</table>

Affinity (pKᵢ) at h5-HT1A receptors was determined in competition-binding experiments as described in Table 1. Efficacy was determined by stimulation of [35S]GTP binding to HeLa-h5-HT1A cell membranes and by inhibition of forskolin-stimulated cAMP accumulation in HeLa-h5-HT1A whole cells. Eₘₐₓ values are expressed as % of the action of 10μM 5-HT (= 100%). Data were analysed by nonlinear regression and values are expressed as mean ± S.E.M. of at least three determinations.

ᵇ Where a nonlinear regression could not be fitted, the effect at the highest concentration tested is shown (i.e. 10μM, except for clozapine, 100μM).

ⁿ Where a nonlinear regression could not be fitted, the effect at the highest concentration tested is shown (i.e. 10μM, except for clozapine, 100μM).

Table 5. Affinity and efficacy of antipsychotic agents at recombinant human 5-HT1A receptors stably expressed in HeLa cells.

Data previously acquired using the same procedure are shown for comparison (Assié et al., 1997; Cosi and Koek, 2001).

Compounds are listed in the order of their efficacy (Eₘₐₓ) for G-protein activation; nc, not computable.

Data previously acquired using the same procedure are shown for comparison (Assié et al., 1997; Newman-Tancredi et al., 2003 and the Results section). In agreement with previous studies employing similar conditions (Alper and Nelson, 1998; Newman-Tancredi et al., 2003), 5-HT induced more [35S]GTPyS binding than agonists which are selective for 5-HT1A receptors, such as (+)-8-OH-DPAT, repinotan and S14506 (Table 2). This reflects both the higher efficacy of 5-HT and 5-CT for activation of 5-HT1A receptors and their activation of other receptor subtypes present in hippocampal membranes.
activation. The present data confirm and extend reports of the partial agonist properties of clozapine, ziprasidone and nemonapride at 5-HT1A receptors (Assié et al., 1997; Cosi and Koek, 2001; Newman-Tancredi et al., 1998; Seeger et al., 1995) and provide the first comparative study of novel antipsychotic agents (see below). The following order of efficacy was observed at r5-HT1A receptors and correlated well with that observed at h5-HT1A receptors (Figure 4c): sarizotan > bifeprunox > nemonapride > SSR181507 > ziprasidone > SLV313 > aripiprazole > tiospirone. In fact, a feature of the present study, employing three different measures of efficacy at 5-HT1A receptors is the high degree of correlation between the activities (potency and efficacy) of the drugs in the different assays.

**Actions of aripiprazole, bifeprunox and SSR181507 at 5-HT1A receptors**

Several important elements emerge from these results. First, aripiprazole exhibited very modest efficacy in three in-vitro models (G-protein activation at rat and human 5-HT1A receptors and adenylyl cyclase inhibition at h5-HT1A receptors). At r5-HT1A receptors, the efficacy of aripiprazole was substantially less than that of (+)-8-OH-DPAT and was intermediate between that of the weak partial agonist, BMY7378, and that of buspirone (Tables 2 and 3). This observation contrasts with a preliminary report indicating that aripiprazole had marked efficacy at rat hippocampal membranes, exceeding that of buspirone and resembling that of ziprasidone (Jordan et al., 2002a). In comparison, at HeLa-h5-HT1A receptors, aripiprazole displayed an efficacy resembling that of buspirone, consistent with a study of [35S]GTPγS binding at h5-HT1A receptors expressed in CHO cells (Jordan et al., 2002b). However, in that study, all the compounds tested, including buspirone and risperidone, exhibited marked efficacy relative to 5-HT, suggesting that experimental conditions amplified the detection of weak partial agonist activity. In contrast, the present models, which discriminate well between the partial agonist properties of the ligands, show that the efficacy of aripiprazole is low in comparison with a range of other antipsychotic agents possessing 5-HT1A agonist properties. These data underline the importance of ‘calibrating’ the responsiveness of in-vitro transduction systems with a range of reference ligands and suggest that the activation of 5-HT1A receptors by aripiprazole in vivo is likely to be modest. This may account for the absence of marked influence by aripiprazole on frontocortical dopamine or hippocampal 5-HT levels measured by microdialysis (Assié et al., 2004; Jordan et al., 2004; Li et al., 2004), although its weak agonist properties may be sufficient to induce a reduction of 5-hydroxyindole acetic acid levels and generalization to a 5-HT1A agonist cue in drug discrimination experiments (Jordan et al., 2004; Marona-Lewicka and Nichols, 2004).

**Figure 3.** Inhibition by novel antipsychotics of h5-HT1A receptor-mediated adenylyl cyclase activity in HeLa-h5-HT1A cells. Adenylyl cyclase activity was determined by measuring inhibition of forskolin-stimulated cAMP accumulation. HeLa-h5-HT1A cells were incubated with forskolin (10 µM) and agonists for 10 min. cAMP contained in cellular extract was dosed by radioimmunoassay. Isotherms were analysed by nonlinear regression. Points shown are average values from at least three independent experiments performed in triplicate and are expressed as percentage inhibition relative to that of the endogenous agonist, 5-HT (10 µM). Note different y-axis scale for sarizotan.
Second, the recently reported novel agent, SSR181507 (Claustre et al., 2003; Depoortere et al., 2003), consistently displayed marked efficacy at 5-HT1A receptors, greater than that of buspirone but substantially less than (+)-8-OH-DPAT or repinotan.

In contrast, Claustre et al. (2003) reported that SSR181507 exhibited an efficacy of 85% relative to 5-HT, similar to that of (+)-8-OH-DPAT. However, in the experimental conditions they employed, the weak partial agonist, NAN-190 also exhibited marked agonist efficacy \(E_{\text{max}} = 56\%\) and even the antagonist, WAY100635, stimulated \([35\text{S}]\text{GTP}\gamma\text{S}\) binding \(E_{\text{max}} = 10\%\) suggesting that the system was very sensitive to weak partial agonists. In the present study, SSR181507 did exhibit greater efficacy than clozapine, ziprasidone or aripiprazole and had balanced affinity at 5-HT1A relative to D2 receptors (affinity ratio = 0.76). SSR181507 may, therefore, be expected to display marked 5-HT1A agonist actions and, accordingly, suppresses serotonergic neuron firing in the dorsal raphe and decreases 5-HT synthesis in striatum (Claustre et al., 2003). Further, the 5-HT1A agonist properties of SSR181507 are sufficiently pronounced to exert anxiolytic actions in rodents (Depoortere et al., 2003) and reverse social interaction deficits induced by PCP (Boulay et al., 2004). Thus, the 5-HT1A receptor agonist properties of SSR181507, described here in vitro, are likely to markedly affect the therapeutic profile of this ligand.

Third, bifeprunox, an antipsychotic agent in advanced clinical development, displays efficacious but low-potency agonist properties at r5-HT1A receptors, with an \(E_{\text{max}}\) value similar to that of roxindole, more than double that of aripiprazole and greater than that of SSR181507. This suggests that substantial activation of 5-HT1A receptors would occur with bifeprunox, although its 44-fold lower affinity at r5-HT1A than at D2 receptors means that actions at the latter are likely to be predominant at low doses in vivo. This conclusion is reinforced by the observation that bifeprunox only influenced adenyl cyclase activity at the highest dose tested (10 \(\mu\)M), suggesting that its potency and/or efficacy in whole cells may be lower than in membrane preparations. The only available literature data on bifeprunox indicates that it has a \(pK_i\) of 8 at h5-HT1A receptors, with an \(E_{\text{max}}\) of 70% (Van Vliet et al., 2000b), data similar to those reported here for \([35\text{S}]\text{GTP}\gamma\text{S}\) binding. However no experimental details are available and the present study therefore provides the first comparative information enabling the properties of bifeprunox at 5-HT1A receptors to be interpreted in the context of the actions of other antipsychotics. Indeed, the diversity of in-vitro expression systems and experimental conditions employed in different laboratories underlines the necessity for systematic comparisons of drug actions. This is particularly true concerning cellular response measurements, where receptor expression levels and G-protein

**Figure 4.** Correlation of affinity and efficacy values obtained from rat brain and recombinant human 5-HT1A receptors. Values used in correlations are those shown in Tables 2–5. (a) Correlation of affinity (\(pK_i\)) values determined by competition binding in membranes prepared from rat cortex and from HeLa-h5-HT1A cells. (b, c) Correlation of potency (\(p_{\text{EC}_{50}}\)) and efficacy (\(E_{\text{max}}\)) values respectively, determined by stimulation of \([35\text{S}]\text{GTP}\gamma\text{S}\) binding in rat hippocampal and HeLa-h5-HT1A cell membranes. (d, e) Correlation of potency (\(p_{\text{EC}_{50}}\)) and efficacy (\(E_{\text{max}}\)) values respectively, determined by stimulation of \([35\text{S}]\text{GTP}\gamma\text{S}\) binding to HeLa-h5-HT1A cell membranes and by inhibition of forskolin-stimulated adenyl cyclase activity in HeLa-h5-HT1A cells. In panel (d), \(p_{\text{EC}_{50}}\) values could not be calculated for all drugs so the number of points is lower (see Table 5). For illustrative purposes, Pearson correlations are shown in all cases but correlations of \(E_{\text{max}}\) values were calculated using non-parametric Spearman rank correlation.

![Image of Figure 4](https://academic.oup.com/ijnp/article-abstract/8/3/341/910003)
subtypes, as well as ionic concentrations and temperature can dramatically influence agonist/antagonist properties (cf. biphasic activation of G\textsubscript{\alpha} subunits by clozapine and ziprasidone; Newman-Tancredi et al., 2002).

Fourth, it should be emphasized that both aripiprazole and bifeprunox act as partial agonists at D2 receptors, a property which exerts a major influence on the actions of these ligands, including attenuation of prolactin secretion by pituitary-located D2 receptors and reduction in EPS liability (Inoue et al., 1996; Long et al., 2000; Nakai et al., 2003). Further, aripiprazole has multiple interactions at numerous other receptor subtypes (Shapiro et al., 2003) including 5-HT2A and 5-HT2C, targets which are known to profoundly influence antipsychotic drug action (Leysen, 2000). Prudence is therefore necessary when assessing the influence of 5-HT1A receptor activation on the clinical pharmacology of these agents, but it may be supposed that the pharmacological profile of these ligands in vivo will be influenced by the balance of affinity and efficacy at both D2 receptors and 5-HT1A receptors. If the agonist properties of antipsychotics at D2 receptors are too accentuated, the antipsychotic potential of a compound is likely to be negated. Thus sarizotan, which exhibits very high efficacy at 5-HT1A receptors and partial agonist properties at D2 receptors (Tables 3, 5; Bartoszyk et al., 2004) was initially in clinical trials as an antipsychotic but is now in development as an anti-dyskinetic agent in L-dopa-treated Parkinson’s disease patients (Bartoszyk et al., 2004). Indeed, whilst sarizotan does not induce catalepsy in rodents, it poorly blocks methylphenidate-induced behaviours in rats and apomorphine-induced climbing in mice – two measures predictive of antipsychotic activity (Kleven et al., 2004). Thus, the affinity and efficacy of sarizotan at 5-HT1A receptors and/or its efficacy at D2 receptors may be too high for antipsychotic properties to be satisfactorily expressed.

‘Selectively non-selective’ antipsychotics

Whereas clozapine, olanzapine, ziprasidone and aripiprazole interact with a wide number of target sites, SSR181507 and bifeprunox act more selectively at 5-HT1A and D2-like receptors. They therefore represent a move away from multidopamine receptor antipsychotics and towards the concept of increasingly ‘selectively non-selective’ agents (cf. Roth et al., 2004). Such agents may avoid some of the side-effects (e.g. sedation, weight gain, metabolic problems) associated with previous antipsychotics. Other novel ‘selectively non-selective’ 5-HT1A agonist/D2 antagonists are SLV313 and SLV314. These compounds, like SSR181507 and bifeprunox, exhibit little or no interaction with D1, 5-HT2A or a\textsubscript{1} and a\textsubscript{2} adrenergic receptors (M. B. Assié, unpublished observations). SLV313 exhibits balanced 5-HT1A/D2 affinity and has efficacy at 5-HT1A receptors similar to that of ziprasidone and buspirone. SLV313 blocks psychostimulant-induced behaviours in rodents in the absence of catalepsy (Glennon et al., 2002; Kleven et al., 2004; McCreary et al., 2002) suggesting that its balance of 5-HT1A/D2 properties produces a favourable antipsychotic profile. In contrast, SLV314 exhibits affinity at 5-HT1A receptors which is two orders of magnitude lower than at D2 receptors and its efficacy at 5-HT1A receptors is very low. Accordingly, its absence of prominent induction of catalepsy in rodents (M. Kleven, unpublished observations) is probably due to its potent serotonin reuptake inhibition properties (Kruse et al., 2002; Tuintstra et al., 2002) rather than to direct 5-HT1A receptor activation. In fact, although antagonism of post-synaptic 5-HT1A receptors is reported to benefit performance in models of cognition (Harder et al., 1996; Harder and Ridley, 2000; Meneses, 1999) it is unlikely to reduce EPS liability or increase dopaminergic neurotransmission in frontocortical regions (Ichikawa and Meltzer, 2000; Prinssen et al., 2002). Nevertheless, it is interesting that an older neuroleptic, tiotriprone, which also exhibits very low efficacy at 5-HT1A receptors (Table 3; Newman-Tancredi et al., 1998), exhibited antipsychotic properties in humans comparable with haloperidol but with a lower incidence of EPS (Jain et al., 1987; Moore et al., 1987). These data suggest that even low levels of 5-HT1A receptor activation may be beneficial in schizophrenic patients.

Conclusions

Whereas conventional neuroleptics, such as haloperidol and thioridazine, as well as ‘atypical’ agents including olanzapine and risperidone, do not interact with 5-HT1A receptors, a new generation of potential antipsychotics is emerging, including bifeprunox, SSR181507 and SLV313, which selectively targets 5-HT1A receptors as well as dopamine D2 receptors. The present data indicate that marked diversity exists between these 5-HT1A/D2 agents and raises the question of the desirable balance of affinity at 5-HT1A and D2 receptors, as well as the optimal efficacy at 5-HT1A receptors. These parameters probably need to fall within a target ‘window’ that permits the beneficial properties of 5-HT1A receptor activation, including EPS reduction and actions against negative...
and cognitive symptoms, to be expressed (see Introduction) whilst allowing the blockade of D2 receptors to exert desired antipsychotic actions. The present data provide a framework within which these issues can be addressed.

Acknowledgements

The study was funded by Pierre Fabre Médicament. We thank Dr D. Cussac and Dr F. Colpaert for helpful discussions.

Statement of Interest

All the authors are employees of the Pierre Fabre Research Centre.

References


the cloned 5-HT\textsubscript{1A} receptor. *Journal of Biological Chemistry* 264, 14848–14852.

Feenstra RW, Long SK, Kuipers W, Van der Heyden JAM, Tulp MTM, Kruse CG (2002). New approaches for psychosis treatment: design, synthesis and SAR of ligands binding to dopamine D\textsubscript{2} and serotonin 5-HT\textsubscript{1A} receptors. *Drugs Fut* 27 (Suppl. A). XVIIIth International Symposium on Medicinal Chemistry.


Harder JA, Ridley RM (2000). The 5-HT\textsubscript{1A} antagonist, WAY 106635, alleviates cognitive impairments induced by dizocilpine (MK-801) in monkeys. *Neuropharmacology* 14, 547–552.

Hashimoto T, Nishino N, Nakai H, Tanaka C (1991). Increase in serotonin 5-HT\textsubscript{1A} receptors in prefrontal cortex and temporal cortices of brains from patients with chronic schizophrenia. *Life Sciences* 48, 355–363.


Jordan S, Chen R, Johnson J, Regardie K, Tadoni Y, Kikuchi T (2002a). Aripiprazole is a potent, partial agonist at cloned human D\textsubscript{2} and native rat 5-HT\textsubscript{1A} receptors. *European of Neuropsychopharmacology* 12, S293.

Jordan S, Koprivica V, Chen R, Tottori K, Kikuchi T, Altar CA (2002b). The antipsychotic aripiprazole is a potent, partial agonist at the human 5-HT\textsubscript{1A} receptor. *European Journal of Pharmacology* 441, 137–140.


Kleven M, Prinssen EPM, Koek W (1996). Role of 5-HT\textsubscript{1A} receptors in the ability of mixed 5-HT\textsubscript{1A} receptor agonist/dopamine D\textsubscript{2} receptor antagonists to inhibit methylphenidate-induced behaviors in rats. *European Journal of Pharmacology* 313, 25–34.


Mauler F, Fahrig T, Horvath E, Jork R (2001). Inhibition of evoked glutamate release by the neuroprotective 5-HT\textsubscript{1A} receptor antagonist WAY-100,635. In: Kroeze WK, Roth BL (Eds.), *Antipsychotics* (pp. 149–160). Basel, Switzerland: Birkhäuser Verlag.


Wadenberg MLG, Young KA, Richter JT, Hicks PB (1999). Effects of local applications of 5-hydroxytryptamine into the dorsal or median raphe nuclei on haloperidol-induced catalepsy in the rat. Neuropsychopharmacology 38, 151–156.
