Inverse Agonist Actions of Typical and Atypical Antipsychotic Drugs at the Human 5-Hydroxytryptamine$_{2C}$ Receptor

LAURA RAUSER, JASON E. SAVAGE, HERBERT Y. MELTZER, and BRYAN L. ROTH

Departments of Biochemistry, Psychiatry and Neurosciences (B.L.R.), the National Institute of Mental Health Psychoactive Drug Screening Program (L.R., J.E.S., B.L.R.), Case Western Reserve University Medical School, Cleveland, Ohio; and Departments of Psychiatry and Pharmacology, Vanderbilt University Medical School, Nashville, Tennessee (H.Y.M.)

Received April 16, 2001; accepted June 8, 2001

This paper is available online at http://jpet.aspetjournals.org

ABSTRACT

Atypical antipsychotic drugs, which are distinguished from typical antipsychotic drugs by a lower incidence of extra-pyramidal side effects and less propensity to elevate serum prolactin levels (e.g., clozapine, olanzapine, risperidone, quetiapine, ziprasidone), have become the most widely used treatments for schizophrenia, although their precise mechanism of action remains controversial. It has been suggested that this group of atypical antipsychotic drugs is characterized by preferentially high affinities for 5-hydroxytryptamine (5-HT)$_{2A}$ serotonin receptors and relatively low affinities for D$_2$-dopamine receptors. It has recently been proposed that these atypical antipsychotic drugs may also be distinguished from typical antipsychotic drugs (e.g., haloperidol, fluphenazine, chlorpromazine, and ziprasidone) by inverse agonist actions at the 5-HT$_{2C}$ receptor. Inverse agonist actions at the 5-HT$_{2C}$ receptor were measured for a large number of typical and atypical antipsychotic drugs using human embryonic kidney (HEK)-293 cells stably transfected with the h5-HT$_{2C}$ isoform expressed in HEK-293 cells. We have examined the relationship among 5-HT$_{2C}$ inverse agonist potency, efficacy, and atypical antipsychotic drug status in HEK-293 cells of a large number of typical and atypical antipsychotic drugs using human embryonic kidney (HEK)-293 cells stably transfected with the h5-HT$_{2C}$-INI receptor. Inverse agonist actions at the h5-HT$_{2C}$-INI receptor were measured for both typical and atypical antipsychotic drugs. Thus, some typical antipsychotic drugs (chlorpromazine, mesoridazine, fluphenazine, and loxapine) were efficient inverse agonists, whereas several clinically effective atypical antipsychotic drugs (remoxapine, quetiapine, sulpiride, melperone, amperozide) were not. Additionally, several drugs without significant antipsychotic actions (M100907, ketanserin, mianserin, ritanserin, and amitriptyline) were potent inverse agonists at the 5-HT$_{2C}$-INI isoform expressed in HEK-293 cells. Taken together, these results demonstrate that both typical and atypical antipsychotic drugs may exhibit inverse agonist actions at the 5-HT$_{2C}$-INI isoform of the human 5-HT$_{2C}$ receptor and that no relationship exists between inverse agonist actions and atypicality.

Schizophrenia is a life-long illness that affects approximately 1% of the human population (Lewis and Lieberman, 2000; Meltzer, 1999a,b). For several decades, typical antipsychotic drugs, exemplified by chlorpromazine, represented the only effective treatment for schizophrenia. Over the past several years, atypical antipsychotic drugs, exemplified by clozapine, have supplanted typical antipsychotic drugs in the treatment of schizophrenia because of superior efficacy and reduced side effects (Meltzer, 1999a,b; Nash and Meltzer, 1991). In addition to clozapine, several other atypical antipsychotic drugs are currently approved for use, including olanzapine (Bymaster et al., 1996), risperidone (Janssen et al., 1988), ziprasidone (Daniel et al., 1999), and quetiapine (Wetzel et al., 1995; Borison et al., 1996). Clozapine appears to have several unique actions, including superior efficacy in treatment-resistant schizophrenia and lack of extra-pyramidal side effects (Meltzer and Cola, 1994; Meltzer and Okayli, 1995; Meltzer, 1999a,c). Unfortunately, clozapine has a number of serious side effects, including agranulocytosis, which occurs in 0.9% of individuals, seizures, orthostatic hypotension, and sialorrhea. Discovering the molecular mechanisms responsible for the unique actions of clozapine might lead to a new generation of atypical antipsychotic drugs devoid of the side effects of clozapine.

Prior studies have clearly demonstrated that typical antipsychotic drugs are characterized by relatively high D$_2$-dopamine receptor affinities (Seeman and Lee, 1975; Creese et al., 1976). Additionally, it is now clear that atypical antipsychotic drugs are characterized, as a group, by having relatively weak D$_2$-dopamine receptor affinities and relatively high 5-HT$_{2A}$ serotonin receptor affinities (Meltzer et al., 1989). Indeed, several clinically effective atypical antipsychotic drugs have been developed that have in common this

ABBREVIATIONS: 5-HT, 5-hydroxytryptamine; FCS, fetal calf serum; DMEM, Dulbecco’s modified Eagle’s medium; IP, inositol monophosphate; PI, phosphoinositide; HEK, human embryonic kidney.
high 5-HT2A/D2 affinity ratio, including olanzapine, risperidone, sertindole, ziprasidone, melperone, andquetiapine (Meltzer, 1999b).

In addition to 5-HT2A receptors, clozapine has high affinity for a number of other 5-HT receptors (e.g., 5-HT1A, 5-HT2c, 5-HT6, and 5-HT7) (Meltzer et al., 1989; Roth et al., 1992, 1994; Seeger et al., 1995; Meltzer, 1999b), the D4-dopamine receptor (Van Tol et al., 1991; Roth et al., 1995), all five muscarinic receptors (m1–m5) (Peroutka et al., 1980; Zeng et al., 1997), and several adrenergic and histamine receptors (Peroutka et al., 1980). At present, it is not known whether interactions of clozapine and related atypical antipsychotic drugs at these other biogenic amine receptors are essential for their atypical features.

Recently, many groups have proposed that 5-HT2C receptors represent a pharmacologically important site of action of atypical antipsychotic drugs (Canton et al., 1990, 1994; Duinkerke et al., 1993; Herrick-Davis et al., 1998, 2000; Kuoppamaki et al., 1995). However, based on studies with a large number of typical and atypical antipsychotic drugs, we have concluded that 5-HT2C receptors were not central to the unique actions of clozapine and related atypicals which differentiate them from typical antipsychotic drugs (Roth et al., 1992). On the other hand, Herrick-Davis et al. (2000) and others (Niswender et al., 1999) have reported that clozapine and other atypical antipsychotic drugs were inverse agonists at a naturally occurring, constitutively active isoform of the 5-HT2C receptor: the 5-HT2C-INI isoform. In a recent report, atypical antipsychotic drugs with high affinity for 5-HT2C receptors (with the sole exception of loxapine) were devoid of inverse agonist actions at the 5-HT2C-INI isoform (Herrick-Davis et al., 2000). These authors proposed that atypical antipsychotic drugs with high affinities for 5-HT2C receptors are inverse agonists and that inverse agonist actions of atypical antipsychotic drugs play a role in their unique clinical actions. If true, this finding could provide a new paradigm for the design and testing of novel atypical antipsychotics.

In the course of evaluating a large number of typical and atypical antipsychotic drugs for unique actions at a variety of cloned neurotransmitter receptors, we discovered that several typical antipsychotic drugs activated basal phosphoinositide (PI) hydrolysis in untransfected COS-7 but not HEK-293 cells. We also recently reported that receptors expressed in COS-7 cells may not be properly targeted to the plasma membrane, but that faithful targeting occurs in HEK-293 cells (Kristiansen et al., 2000). Because prior studies evaluating the inverse agonist actions of typical and atypical antipsychotic have been mainly performed in COS-7 cells (Niswender et al., 1999; Herrick-Davis et al., 2000), we reevaluated the inverse agonist actions of a large number of typical and atypical antipsychotic drugs, as well as several reference compounds, at two isoforms of the h5-HT2C receptor: h5-HT2C-INI and h5-HT2C-VGI. We now report that 1) many typical and atypical antipsychotic drugs have potent inverse agonist activity at h5-HT2C-INI receptors and 2) inverse agonism does not reliably predict whether or not a drug can be classified as a typical or atypical antipsychotic drug.

Experimental Procedures

Materials. The 5-HT2C-INI and 5-HT2C-VGI isoforms of the h5-HT2C receptor were obtained by amplification of human brain cDNA (Quickclon cDNA; Stratagene, San Diego, CA) via polymerase chain reaction using a proof-reading polymerase (Pfu; Stratagene) using the following primers: 5-HT2C-UP: AAAGCGGCCGCTTAAGACTCTGTGCTAATTCTTCGC. The products were subcloned into the NotI site of pIRESEVector and the complete inserts were verified by automated dSNA sequencing (Cleveland Genomics, Inc, Cleveland, OH). The rat 5-HT2C receptor (VGI isoform) and the PO1C cell line were used as previously described (Roth et al., 1992; Nash et al., 1994). Sources of all drugs have been previously detailed (Roth et al., 1992, 1994, 1995). [3H]Inositol (16 Ci/mmol) was from PerkinElmer Life Science Products (Boston, MA), FUGENE-6 was from Roche Molecular Biochemicals (Indianapolis, IN), cell culture materials were from In Vitrogen (Carlsbad, CA), and molecular biology reagents from New England Biolabs (Boston, MA) or Stratagene.

Transfection and Cell Culture. Transient transfection of COS-7 and HEK-293 cells was as previously detailed, using FUGENE-6 (Kristiansen et al., 2000). At 24 h after transfection in 100-mm plates, cells were split into 24-well plates using Dulbecco’s modified Eagle medium (DMEM) containing 5% dialyzed fetal calf serum (FCS), and 24 h later plates were rinsed with inositol-free, FCS-free DMEM and incubated with 1 μCi/ml [3H]inositol. The next

Fig. 1. Effect of various typical and atypical antipsychotic drugs on the basal PI hydrolysis at r5-HT2C-VGI receptors stably expressed in NIH-3T3 cells. Shown are typical results from a representative experiment which has been replicated three times in which test agents were evaluated for their ability to inhibit basal PI hydrolysis at 1 μM final concentration in NIH-3T3 cells stably expressing the r5-HT2C-VGI receptor (PO1C). Data represent mean cpm ± S.E.M. of triplicate determination; *p < 0.05 versus vehicle control.
day, cells were used for PI hydrolysis measurements. Construction of stable cell lines expressing 5-HT$_{2C}$-INI or 5-HT$_{2C}$-VGV using pIRES-NEO was as previously detailed with selection in DMEM containing 10% FCS and 1 μg/mL G418 (Kristiansen et al., 2000).

PI Hydrolysis Measurements. Prior to use, medium was removed and replaced with a modified Krebs-Bicarbonate buffer (Roth et al., 1987) containing 15 mM LiCl. Cells were then incubated with various concentrations of test agents for 1 h and the reaction terminated as previously detailed (Kristiansen et al., 2000). Following lipid extraction, [3H]IP was measured as previously described (Roth et al., 1987) containing 15 mM LiCl. Cells were then incubated with various concentrations of test agents for 1 h and the reaction terminated as previously detailed (Kristiansen et al., 2000). Following lipid extraction, [3H]IP was measured as previously described (Roth et al., 1987).}

**Results**

A large number of 5-HT$_{2C}$ Receptor Antagonists Display Inverse Agonist Activity at Cloned Rat 5-HT$_{2C}$-VGV Receptors Expressed in NIH-3T3 Cells. In preliminary experiments, we tested 11 atypical antipsychotic drugs (remoxapine, quetiapine, melperone, amperozide, zotepine, ziprasidone, olanzapine, clozapine, risperidone, fluperlapine, and sertindole), 9 typical antipsychotic drugs (sulpiride, setoperone, loxapine, amoxapine, mesoridazine, thioridazine, chlorpromazine, spiperone, and haloperidol), and 4 drugs with equivocal antipsychotic activity (mianserin, ritanserin, M100907, and isofoxapine) for inverse agonist activity at cloned rat 5-HT$_{2C}$ receptors. For these studies, a stable cell line (PO1C) that expresses the 5-HT$_{2C}$-VGV isoform of the rat 5-HT$_{2C}$ receptor at high levels was used. Compounds were screened for activity at 1 μM, since preliminary studies indicated that several compounds induce a non-specific activation of PI hydrolysis at concentrations of 10 μM or greater (not shown). As can be seen in Fig. 1, nearly all of the tested compounds that have been previously shown to be 5-HT$_{2C}$ antagonists depressed basal [3H]IP accumulation, including setoperone, zotepine, ziprasidone, olanzapine, clozapine, risperidone, M100907, fluperlapine, sertindole, loxapine, amoxapine, thioridazine, chlorpromazine, isofoxapine, and ritanserin. All of these drugs have been previously demonstrated to have moderate (K$_i$ < 300 nM) to high (K$_i$ < 50 nM) affinities for this isoform of the rat 5-HT$_{2C}$ receptor (Roth et al., 1992, 1995, 1998).

As is clear from Fig. 1, several atypical antipsychotic drugs, including remoxapine, quetiapine, melperone, and amperozide, were devoid of inverse agonist actions at the tested concentrations, as were the typical antipsychotic drugs mesoridazine, spiperone, and haloperidol. Four of these drugs, remoxapine, quetiapine, melperone, and amperozide, have negligible affinities (K$_i$ > 500 nM) for the tested isoform of the rat 5-HT$_{2C}$ receptor (Roth et al., 1995, 1988).

It is also clear from Fig. 1 that the basal activity of the VGV isoform of the 5-HT$_{2C}$ receptor is low (1000–1200 dpm) and that most of the tested drugs had modest inverse agonist activity. This is despite the very high level of expression of the 5-HT$_{2C}$ receptor in this cell line (7 pmol/mg). The low level of constitutive activity precluded detailed dose response studies with the VGV isoform of the 5-HT$_{2C}$ receptor expressed in NIH-3T3 cells. Accordingly, further studies were performed with the INI isoform of the human 5-HT$_{2C}$ receptor, which has previously been demonstrated to have a high degree of constitutive activity (Niswender et al., 1999).

Selected Typical and Atypical Antipsychotic Drugs Increase PI Hydrolysis in Untransfected COS-7 and HEK-293 Cells. In initial experiments, we evaluated the suitability of two cell lines for the evaluation of the inverse agonist activity of typical and atypical antipsychotic drugs. For these studies, we used untransfected COS-7 and HEK-293 cells to determine whether alterations of basal [3H]IP production occur due to nonspecific effects of high concentrations of antipsychotic drugs. As is shown in Fig. 2A, several typical antipsychotic drugs, including thioridazine, fluphenazine, and amoxapine, induced significant elevations of IP accumulation in untransfected COS-7 cells. By contrast, using untransfected HEK-293 cells (Fig. 2B), only risperidone and amperozide, two atypical antipsychotic drugs, caused significant elevations in IP accumulation. Because minimal elevations of basal PI hydrolysis were seen with untransfected HEK-293 cells, they were used for further studies.

As shown in Fig. 3, HEK-293 cells stably transfected with the h5-HT$_{2C}$-INI receptor had a high level of basal activity (5000–9000 dpm), which was inhibited in a dose-dependent fashion by the typical antipsychotic drug fluphenazine. Fig. 4 shows representative dose-response studies for the inhibition of basal [3H]IP accumulation for the reference

![Fig. 2](image-url)  
*Fig. 2. Effect of selected typical and atypical antipsychotic drugs on basal PI hydrolysis in untransfected COS-7 and HEK-293 cells. Shown are typical results from a representative experiment that has been replicated twice in which test agents were evaluated for their ability to inhibit PI hydrolysis in untransfected COS-7 (A) and HEK-293 (B) cells. Data represent mean dpm ± S.E.M. of triplicate determinations; *p < 0.05 versus vehicle control.
inverse agonists mianserin and ritanserin at stably trans- 
fected HEK-293 cells.

Figures 5 and 6 show representative dose-response curves 
for selected typical and atypical antipsychotic drugs as well 
as drugs devoid of antipsychotic actions. Table 1 shows 
\( pEC_{50} \), \( EC_{50} \), and \( E_{\text{max}} \) values for all tested drugs at the 
h5-HT\( _{2C-INI} \) isoform. Two atypical antipsychotic drugs (melper- 
erone and remoxapride) and two typical antipsychotic drugs 
(sulpiride and haloperidol) were devoid of measurable in- 
verse agonist activity. All other tested 5-HT\( _{2C} \) antagonists 
displayed inverse agonist activity.

We also tested representative typical and atypical antipsy- 
chotic drugs at the h5-HT\( _{2C-VGI} \) isoform, which displays low 
levels of constitutive activity. As can be seen in Fig. 7, none 
of the four tested drugs (chlorpromazine, clozapine, olanzap- 
line, and fluphenazine) was an inverse agonist using the 

inhibition of PI hydrolysis as a measure of inverse agonism in 
stably transfected HEK-293 cells. These results imply that 
RNA editing may alter the ability of selected typical and 
atypical antipsychotic drugs to function as inverse agonists 
at the h5-HT\( _{2C} \) receptor.

**Discussion**

The major finding of this study is that inverse agonism at 
the h5-HT\( _{2C-INI} \) receptor is not a reliable predictor of atypical 
antipsychotic activity. Additionally, several potent 5-HT\( _{\text{ar}} \) 
family antagonists with equivocal (e.g., M100907, ritanserin) 
or no (isoclozapine, mianserin, amitriptyline) antipsychotic 
activity were found to be potent and effective inverse ago- 
nists at the h5-HT\( _{2C-INI} \) receptor. These results indicate that 
inverse agonist activity at the h5-HT\( _{2C-INI} \) receptor does not,
TABLE 1

EC_{50} and E_{max} values for selected drugs at the h5-HT_{2C-INI} isoform

Data represent mean ± S.E.M. of EC_{50} and E_{max} values from two to three separate experiments.

<table>
<thead>
<tr>
<th>Drug</th>
<th>EC_{50}</th>
<th>pEC_{50} ± S.E.M.</th>
<th>E_{max} ± S.E.M.</th>
<th>Typical (T) or Atypical (A)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>nM</td>
<td></td>
<td>%</td>
<td></td>
</tr>
<tr>
<td>Fluphenazine</td>
<td>1412</td>
<td>3.15 ± 0.08</td>
<td>85 ± 2</td>
<td>T</td>
</tr>
<tr>
<td>Sertindole</td>
<td>91.6</td>
<td>1.96 ± 0.1</td>
<td>77 ± 3</td>
<td>A</td>
</tr>
<tr>
<td>Ritanserin</td>
<td>18.8</td>
<td>1.27 ± 0.27</td>
<td>73 ± 8</td>
<td>N</td>
</tr>
<tr>
<td>Ziprasidone</td>
<td>34.5</td>
<td>1.53 ± 0.18</td>
<td>77 ± 2</td>
<td>A</td>
</tr>
<tr>
<td>Clozapine</td>
<td>80.1</td>
<td>1.90 ± 0.23</td>
<td>74 ± 6</td>
<td>A</td>
</tr>
<tr>
<td>Thiothixene</td>
<td>27352</td>
<td>4.43</td>
<td>54 ± 13</td>
<td>T</td>
</tr>
<tr>
<td>Quetiapine</td>
<td>2208</td>
<td>3.34 ± 0.3</td>
<td>45 ± 5</td>
<td>A</td>
</tr>
<tr>
<td>Loxapine</td>
<td>59.2</td>
<td>1.77 ± 0.15</td>
<td>72 ± 8</td>
<td>T</td>
</tr>
<tr>
<td>Amperozide</td>
<td>495.5</td>
<td>2.69 ± 0.48</td>
<td>23 ± 11</td>
<td>A</td>
</tr>
<tr>
<td>Mesoridazine</td>
<td>473</td>
<td>2.67 ± 0.08</td>
<td>46 ± 16</td>
<td>T</td>
</tr>
<tr>
<td>Zetepine</td>
<td>161</td>
<td>2.20 ± 0.09</td>
<td>71 ± 6</td>
<td>T</td>
</tr>
<tr>
<td>Thoridazine</td>
<td>&gt;10,000</td>
<td>&gt;5</td>
<td>N.D.</td>
<td>T</td>
</tr>
<tr>
<td>Remoxapride</td>
<td>&gt;10,000</td>
<td>&gt;5</td>
<td>N.D.</td>
<td>A</td>
</tr>
<tr>
<td>Sulpiride</td>
<td>&gt;10,000</td>
<td>&gt;5</td>
<td>N.D.</td>
<td>A</td>
</tr>
<tr>
<td>Melperone</td>
<td>&gt;10,000</td>
<td>&gt;5</td>
<td>N.D.</td>
<td>A</td>
</tr>
<tr>
<td>Risperidone</td>
<td>95.3</td>
<td>1.97 ± 0.19</td>
<td>76 ± 3</td>
<td>A</td>
</tr>
<tr>
<td>Haloperidol</td>
<td>&gt;10,000</td>
<td>&gt;5</td>
<td>N.D.</td>
<td>T</td>
</tr>
<tr>
<td>Setoperone</td>
<td>195.6</td>
<td>2.29 ± 0.27</td>
<td>64 ± 11</td>
<td>T</td>
</tr>
<tr>
<td>Fluplerapine</td>
<td>1276</td>
<td>3.10 ± 0.5</td>
<td>60 ± 14</td>
<td>A</td>
</tr>
<tr>
<td>M100907</td>
<td>85.1</td>
<td>1.97 ± 0.12</td>
<td>60 ± 17</td>
<td>N</td>
</tr>
<tr>
<td>Chlorpromazine</td>
<td>174</td>
<td>2.24 ± 0.3</td>
<td>57 ± 12</td>
<td>T</td>
</tr>
<tr>
<td>Olanzipine</td>
<td>16</td>
<td>0.38 ± 0.38</td>
<td>68 ± 12</td>
<td>A</td>
</tr>
<tr>
<td>Mianserin</td>
<td>8.6</td>
<td>0.32 ± 0.32</td>
<td>69 ± 10</td>
<td>N</td>
</tr>
<tr>
<td>Isofluprine</td>
<td>4.3</td>
<td>0.64 ± 0.6</td>
<td>68 ± 13</td>
<td>N</td>
</tr>
<tr>
<td>Perphenazine</td>
<td>685</td>
<td>2.18 ± 0.42</td>
<td>32 ± 14</td>
<td>T</td>
</tr>
<tr>
<td>Trifluoperazine</td>
<td>&gt;10,000</td>
<td>&gt;5</td>
<td>N.D.</td>
<td>T</td>
</tr>
<tr>
<td>Amoxapine</td>
<td>122</td>
<td>2.07 ± 0.45</td>
<td>55 ± 7</td>
<td>T</td>
</tr>
<tr>
<td>Amitriptyline</td>
<td>235</td>
<td>2.37 ± 0.31</td>
<td>55 ± 1</td>
<td>N</td>
</tr>
</tbody>
</table>

N.D., no detectable inverse agonist activity.

Fig. 7. RNA editing alters the inverse agonist actions of selected typical and atypical antipsychotic drugs. Shown are results from an experiment that has been replicated three times in which the ability of chlorpromazine, clozapine, olanzapine and fluphenazine to inhibit constitutive PI hydrolytic activity of the h5-HT_{2C-VGI} expressed in HEK-293 cells was measured. Data represent the mean dpm ± S.E.M. of triplicate determinations.

by itself, reliably distinguish between typical and atypical antipsychotic drugs.

Several prior studies have described inverse agonist actions of typical and atypical antipsychotic drugs at 5-HT_{2C} and 5-HT_{2A} receptors (Barker et al., 1994; Westphal and Sanders-Bush, 1994; Labrecque et al., 1995; Egan et al., 1998; Herrick-Davis et al., 2000). Thus, Egan et al. (1998) were the first to systematically evaluate a large series of typical and atypical antipsychotic drugs for their inverse agonist actions at h5-HT_{2A} receptors. In the Egan et al. (1998) study, variable inverse agonist actions were seen for selected typical and atypical antipsychotic drugs.

Others (Barker et al., 1994; Westphal and Sanders-Bush, 1994; Labrecque et al., 1995) have examined the inverse agonist actions of various 5-HT_{2C} antagonists. Thus, Labrecque et al. (1995) found that chlorpromazine, a typical antipsychotic drug, was a potent inverse agonist at r5-HT_{2C} receptors expressed in Sf9 cells, while Westphal and Sanders-Bush (1994) reported that clozapine was a potent inverse agonist at the same isoform. By contrast, Herrick-Davis and colleagues (2000) have recently reported that, with one exception, all the tested typical antipsychotic drugs were devoid of inverse agonist actions when h5-HT_{2C-INI} receptors were transiently expressed in COS-7 cells. Additionally in the Herrick-Davis et al. (2000) study, all of the tested atypical antipsychotic drugs were effective inverse agonists.

As we now demonstrate, however, transiently transfected COS-7 cells are not suitable for the study of the inverse agonist activities of typical and atypical antipsychotic drugs for several reasons. First, as is clear from the present findings, several of the tested drugs actually elevated basal[^3H]IP accumulation in untransfected COS-7 cells. For compounds with potent inverse agonist actions (e.g., risperidone, clozapine, and amoxapine), the modest elevation of basal activity is unlikely to greatly affect the observed inverse agonist activity. On the other hand, drugs with modest potencies (e.g., fluphenazine, thioridazine) are likely to display artifically low efficacies because the weak inverse agonist actions are attenuated by nonspecific elevations of[^3H]IP.
accumulation. Second, as we have previously shown, COS-7 cells do not appropriately target 5-HT receptors to plasma membranes while HEK-293 cells do (Kristiansen et al., 2000). Third, we have found variable levels of constitutive activity in transiently transfected COS-7 cells, which makes detailed analysis of dose-response curves difficult (not shown).

Finally, the present studies indicate that stably transfected HEK-293 cells produce reliable and robust constitutive activity of the h5-HT_2C-INH receptor, which is suitable for detailed pharmacological analysis.

At present, five atypical antipsychotic drugs are approved for use in the United States, including clozapine, olanzapine, risperidone, ziprasidone, and quetiapine. Additionally, four other atypical antipsychotic drugs include fluperoxpin (Fischer-Cornelssen, 1984; Fleischhacker et al., 1986), remoxapride (Chounard, 1990), sertindole (Brown and Levin, 1998), and zotepine (Fenton et al., 2000) have been demonstrated to be clinically effective in large-scale, double-blind, placebo-controlled human trials. Finally, two other drugs, melperone (Christensson, 1989; Harnryd et al., 1989) and amperozide (Christensson and Bjork, 1990), have demonstrated atypical antipsychotic actions in limited human testing. As our findings clearly indicate, a variety of inverse agonist actions at the h5-HT_2C-INH receptor was seen with these atypical antipsychotics, which was not correlated with the atypical nature of the tested compounds.

Of particular interest are two of the tested drugs—clozapine and quetiapine—which have been shown to be virtually devoid of extra-pyramidal side effects in humans, not to elevate serum prolactin levels and to be effective in treating L-dihydroxyphenylacetic acid-induced psychosis without exacerbating Parkinson’s disease. Despite the nearly identical lack of adverse motor side effects, these two atypical antipsychotic drugs differ greatly in inverse agonist potencies. It is also important to note that three drugs with demonstrated atypical antipsychotic actions in humans (remoxapride, melperone, and amperozide) were devoid of inverse agonist activity using [3H]IP accumulation as a measure.

Finally, several typical antipsychotic drugs were potent inverse agonists at the tested isoforms of the h5-HT_2C receptor. Thus, chlorpromazine (a reference typical antipsychotic drug), fluphenazine, thioridazine, and loxapine were potent inverse agonists at the h5-HT_2C-INH receptor. Additionally, several drugs devoid of antipsychotic actions (ritanserin, mianserin, amitriptyline) were potent inverse agonists. Taken together, these results clearly indicate that the inverse agonist actions of drugs at the h5-HT_2C-INH receptor do serve as a reliable indicator of potential atypical antipsychotic action.

In 1989, Meltzer et al. predicted that atypical antipsychotic drugs may be characterized by one of two features: 1) a relatively high 5-HT_2A/D2 affinity ratio or 2) a relatively low affinity for D2-dopamine receptors. Other groups have also predicted that a relatively high 5-HT_2A/D2 affinity ratio is a characteristic of atypical antipsychotic drugs (Altar et al., 1986; Janssen et al., 1988; Rasmussen and Aghajanian, 1988). In support of this concept, several atypical antipsychotic drugs including olanzapine, risperidone, quetiapine, and ziprasidone have been approved for use in the past 12 years that have the characteristic of a high 5-HT_2A/D2 affinity ratio (Janssen et al., 1988; Seeger et al., 1995; Wetzel et al., 1995; Bymaster et al., 1996; Daniel et al., 1999). The present findings support the Meltzer et al. (1989) hypothesis and predict that 5-HT_2C inverse agonist activity in HEK-293 cells does not by itself, reliably predict whether or not a drug may be classified as an atypical antipsychotic.