Effects of Antipsychotic Drugs on Extracellular Dopamine Levels in Rat Medial Prefrontal Cortex and Nucleus Accumbens

TOSHIHIDE KUROKI, HERBERT Y. MELTZER and JUNJI ICHIKAWA

Department of Psychiatry, Saga Medical School, Nabeshima, Saga, Japan (T.K.); and Department of Psychiatry, Psychopharmacology Division, Vanderbilt University School of Medicine, Nashville, Tennessee (H.Y.M., J.I.).

ABSTRACT

The present study was designed to compare the effects of typical and atypical antipsychotic drugs on extracellular dopamine (DA) levels in the medial prefrontal cortex (mPFC) and the nucleus accumbens (NAC), using in vivo microdialysis with dual probe implantation in awake, freely moving rats. Amperozide (2 and 10 mg/kg), clozapine (5 and 20 mg/kg), and olanzapine (10 mg/kg), all of which are atypical antipsychotics, produced greater increases in extracellular DA levels in the mPFC than in the NAC. Olanzapine (1 mg/kg), risperidone (0.1 and 1 mg/kg), also an atypical antipsychotic, and S(−)-sulpiride (25 mg/kg), a typical antipsychotic, produced comparable increases in extracellular DA levels in the mPFC and the NAC. S(−)-sulpiride (10 mg/kg) and haloperidol (0.1 and 1 mg/kg), another typical antipsychotic, significantly increased extracellular DA levels in the NAC but not in the mPFC. The effects of the six antipsychotic drugs to increase extracellular DA levels in the mPFC relative to those in the NAC was positively correlated with the difference between their pKi values for serotonin (5-hydroxytryptamine, 5-HT2A) and DA-D2 receptors and was inversely correlated to their pKi values for D2 or D3 receptors, but was not for 5-HT2A receptors alone. These results are consistent with the hypothesis that the ability of antipsychotic drugs to produce a greater increase in prefrontal compared with NAC extracellular DA levels may be related, in part, to weak D2 and D3 receptor affinity relative to 5-HT2A receptor antagonism.

Antipsychotic drugs such as haloperidol (HAL) and S(−)-sulpiride (SUL) increase dopamine (DA) release in the striatum (STR) and the nucleus accumbens (NAC), most likely due to the blockade of presynaptic DA-D2-like autoreceptors (Westrink and DeVries, 1989). The atypical antipsychotics clozapine (CLOZ) and amperozide (APZ), both of which have greater affinities for serotonin (5-hydroxytryptamine, 5-HT2A) receptors relative to D2 receptors (Meltzer et al., 1989; Leysen et al., 1993), have been reported to produce greater increases in extracellular DA levels in the medial prefrontal cortex (mPFC) compared with the STR and the NAC (Moghaddam and Bunney, 1990; Nomikos et al., 1994; Pehek and Yamamoto, 1994; Volonté et al., 1997). By contrast, HAL has a limited effect on extracellular DA levels in the mPFC. Compounds like CLOZ and APZ that are relatively more potent as 5-HT2A than as D2 receptor antagonists, e.g., melperone, olanzapine (OLAN), risperidone (RISP), quetiapine, sertindole, and ziprasidone (Meltzer et al., 1989; Leysen et al., 1993), have been commonly referred to 5-HT2A/D2 receptor antagonists (Meltzer, 1995). Therefore, it is of interest to test the hypothesis that all antipsychotic drugs of the 5-HT2A/D2 receptor antagonist type produce a greater increase in extracellular DA levels in the mPFC compared with the NAC.

Dopaminergic hypofunction in the prefrontal cortex has been suggested to be related to the etiology of negative symptoms (Davis et al., 1991; Weinberger and Lipska, 1995) and cognitive dysfunction of schizophrenia (Sawaguchi and Goldman-Rakic, 1991). A number of the 5-HT2A/D2 receptor antagonists, e.g., CLOZ, RISP, OLAN, and sertindole, have been reported to have a greater ability to improve negative symptoms than do neuroleptics such as HAL or chlorpromazine (Kane et al., 1988; Chouinard et al., 1993; Meltzer, 1995; Tollefson and Sanger, 1997). CLOZ and RISP also improve some aspects of cognitive function (Hagger et al., 1993; Green

References

Received for publication April 10, 1998.

This work was supported in part by U.S. Public Health Services Grant MH 41864, Department of Veterans Affairs grant GCRC M01RR00080, and the National Alliance for Research on Schizophrenia and Depression (NARSAD) Young Investigator Award grant, as well as grants from Elisabeth Severance Prentiss Foundation and Mr. Larry Freedman. Preliminary data from this study have been reported in abstract forms of the annual meetings of the Society for Neuroscience, 1995, and the Society of Biological Psychiatry, 1996.

ABBREVIATIONS: HAL, haloperidol; SUL, S(−)-sulpiride; DA, dopamine; STR, striatum; NAC, nucleus accumbens; CLOZ, clozapine; APZ, amperozide; mPFC, medial prefrontal cortex; OLAN, olanzapine; RISP, risperidone; 5-HT, 5-hydroxytryptamine; AUC, area under the curve; VEH, vehicle control.
et al., 1997) which may be related to frontal lobe function. Thus, the greater ability of 5-HT2A/D2 receptor antagonists to increase extracellular DA levels in the cortical regions compared with subcortical regions may contribute to their ability to improve negative symptoms (Deutch et al., 1991). This hypothesis is consistent with preferential in vivo and in vitro binding of 5-HT2A/D2 receptor antagonists to cortical 5-HT2A compared with striatal D2 receptors (Meltzer et al., 1989; Lysen et al., 1993; Stockmeier et al., 1993).

Recently, evidence for the modulation of the activity of mesolimbocortical DA neurons by 5-HT2A receptors has been reported. Systemic administration of the 5-HT2A/D2 receptor antagonist ritanserin has been demonstrated to enhance the activity of midbrain DA neurons (Ugedo et al., 1989). Ritan- serin has also been reported to potentiate the ability of the selective D2 receptor antagonist raclopride to increase DA release in the mPFC but not in the STR, whereas ritanserin alone had no effect on DA release in either region (Anderson et al., 1995). These data suggest that the 5-HT2A receptor antagonist, together with blockade of D2-like receptors (D2, D3, and perhaps D4 subtype), may contribute to the preferential increase in extracellular DA levels in the mPFC compared with the NAC and the STR produced by APZ and CLOZ. However, this hypothesis is not consistent with the reports that RISP and OLAN, which are more potent as 5-HT2A than as D2 receptor antagonists (Bymaster et al., 1995). These data suggest that the 5-HT2A receptor antagonistic activity in vivo and in vitro is greater than that in the mPFC compared with the NAC and the STR (Hertel et al., 1996; Volonté et al., 1997).

The present study was designed to characterize antipsy- chotic drugs of the 5-HT2A/D2 receptor antagonist type in terms of their relative ability to produce a greater increase in extracellular DA levels in the mPFC compared with the NAC. Importantly, antipsychotic drugs differ in their affini- ties for D1, D5, D4, 5-HT2C, 5-HT6, and 5-HT7 receptors (Meltzer et al., 1989; Roth et al., 1992; Schotte et al., 1996). These affinities may also influence their effects on extracel- lular DA levels in the mPFC, the NAC, and the STR. There- fore, this study also investigated the relationships between relative potency to increase extracellular DA levels and in vivo affinities for multiple 5-HT and DA receptors as indicated by pKi values obtained from published sources (Table 1). Six antipsychotic drugs were chosen on the basis of the difference in in vitro and in vivo affinity between rat cortical 5-HT2A and striatal D2 receptors in the following rank order: APZ > CLOZ > RISP > OLAN > HAL > SUL.

Materials and Methods

Animals. Male Sprague-Dawley albino rats (Zivic-Miller, Pitts-burgh, PA) weighing 250 to 300 g were used throughout the study. Rats were housed two or three per cage and were maintained on a 12-h light/dark cycle and under constant temperature at 22°C, with ad libitum access to food and water.

Surgery and Microdialysis. Rats were anesthetized with a combina- tion of xylazine (Rompun, 6 mg/kg, i.p.; Miles, Kansas City, KS) and ketamine hydrochloride (Ketaset, 70 mg/kg, i.p.; Fort Dodge Lab., Fort Dodge, IA) and mounted in a stereotaxic frame (David Kopf Instruments, Tujunga, CA). Two stainless guide cannula (21 gauge) with a dummy probe were placed and fixed by cranioplastic cement onto the cortex dorsal both to the right mPFC and the left NAC (dual probe implantation). Stereotaxic coordinates of each probe when implanted were A:+3.2, L:+9.8 (10° inclination), V:+5.5 mm for the mPFC and A:+2.0, L:+1.7, V:+7.5 mm for the NAC, respectively, relative to bregma; incision bar level: −3.0 mm, according to the atlas of Paxinos and Watson (1986).

Microdialysis probes were constructed in our laboratory according to the method of Robinson and Whishaw (1988). Briefly, the probe was a concentric design consisting of 25-gauge stainless steel tubing (460 μm o.d.) connected to PE20 tubing as an inlet. The outlet consisted of a fused silica capillary (150 μm o.d.; Polymicro Technolo- gies, Phoenix, AZ) passed through the wall of the PE20, threaded down through the stainless steel tubing and into a 2-mm length of sealed dialysis tubing (polyacrylonitrile/sodium methysulfonyl polymer, 310 μm o.d., 220 μm i.d., 40,000 Da cutoff; AN69HF, Hoe- pal; CGH Medical, Lakewood, CO). PE10 tubing was attached to the silica capillary where it exits the probe. All junctions were glued with 2-ton Epoxy (Devon Bearings, Brooklyn Heights, OH). Because the variability in percentage of recovery of DA between probes varied by less than 10%, basal extracellular DA levels in each region were expressed as absolute values and were not corrected by percentage of recovery.

Three to five days after cannulation, the microdialysis probes were implanted into the mPFC and the NAC under slight anesthesia with methoxyflurane (Metofane; Pitman-Moore, Chicago, IL). The probe was perfused with modified Dulbecco’s phosphate-buffered saline including Ca2+ (NaCl, 138 mM; Na2HPO4, 8.1 mM; KCl, 2.7 mM; KH2PO4, 1.5 mM; MgCl2, 0.5 mM; and CaCl2, 1.2 mM, at pH = 7.4) at 0.2 μl/min through the night. After overnight perfusion for approximately 15 h, the perfusion flow rate was increased to 0.4 μl/min, and beginning 1 h later, dialysate samples (12 μl) were collected every 30 min. After obtaining stable baseline values in the dialysate such that a percentage of S.E.M. of the three consecutive DA values in the dialysate differed less than 10% of the mean values, each drug or vehicle was administered s.c. to rats. The effect of the drug was followed for at least another 180 min. After the completion of each experiment, the rats were sacrificed with an overdose of chloral hydrate. The brains were removed and cut coronally along the punc- ture created by the probe. The location of the tip was then verified macroscopically. All experiments were carried out between 10:00 and 16:00 h. The procedures applied in this study were in strict accord- ance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by the Institutional Animal Care and Use Committee of Case Western Reserve Univer- sity in Cleveland, OH, where we completed these experiments.

Dual probe implantation into the mPFC and the NAC used in this study was done to monitor cortico-subcortical interactions and to compare drug effects on the two regions. It has been suggested that the shell, but not the core, of the NAC, projects to the mPFC in the rat brain and may be the target site for the antipsychotic action of antipsychotic drugs (Deutch et al., 1993). In this study, dialysis probes were always implanted above or on the medial side of the anterior limb of the anterior commissure, a location which may indicates preference for the shell of the NAC. However, because of the probe size used in this study, the dialyzed area might also include, in part, the core of the NAC.

Biochemical Assay. DA concentrations in dialysate samples were determined by high-performance liquid chromatography with electrochemical detection as described previously (Kuroki et al., 1996). DA was separated on the reversed phase column (BDS Hy- persil C18 1.0 × 100 mm, 3 μm particle size; Keystone Scientific, Bellefonte, PA). The composition of the mobile phase was 50 mM NaH2PO4 (pH = 6.0), 20% (v/v) methanol, 8% (v/v) acetonitrile, 450 mg/liter SDS, 1 mM Na2EDTA, 10 mM NaCl, and 500 μl/liter triethy- lamine. Electrochemical detection controllers (LC-4C; BAS, West Lafayette, IN) with unijet amperometric detector cells (MF-9080; BAS) set at +550 to +580 mV versus Ag/AgCl reference electrodes were used to detect DA. The column and detector cell were placed within a column oven (831 temperature regulator; Gilson, Middlestone, MA) at 35°C except for the experiment of OLAN administration. Because an OLAN metabolite was coeluted with DA at the same retention
period of the chromatogram under the above-mentioned conditions, the column temperature was lowered to 28°C to obtain clear separation of DA for the experiment of OLAN injection. The data were analyzed with an integrator (HP 3396 Series II; Hewlett-Packard, Avondale, PA). The detection limit was 0.4 fmol for DA at a 3:1 signal-to-noise ratio. All reagents used for high-performance liquid chromatography with electrochemical detection were purchased from Fisher Scientific (Pittsburgh, PA) and Sigma Chemical Co. (St. Louis, MO).

**Drugs.** Six antipsychotic drugs were chosen on the basis of the difference in in vitro and in vivo affinity between rat cortical 5-HT\(_{2A}\) and striatal D\(_2\) receptors (Table 1). The doses of antipsychotic drugs except OLAN were chosen on the basis of the ED\(_{50}\) values for displacing in vivo \[^{3}H\]-ketanserin binding to cortical 5-HT\(_{2A}\)/olfactory tubercle D\(_2\) receptors (Stockmeier et al., 1993). The choice of the doses of OLAN was according to the ED\(_{50}\) values for inhibition of ex vivo \[^{3}H\]-ketanserin binding to cortical 5-HT\(_{2A}\) receptors and inhibition of \(N\)-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline-induced inactivation of striatal D\(_2\) receptors (Bymaster et al., 1996b). APZ, CLOZ, RISP, and OLAN at the doses used in this study may predominantly occupy 5-HT\(_{2A}\) receptors compared with D\(_2\) receptors in rat brain, whereas SUL and HAL predominantly occupy D\(_2\) receptors compared with 5-HT\(_{2A}\) receptors, although discrepancies between in vivo and in vitro data have been reported for some antipsychotic drugs, e.g., racemic SUL (Meltzer et al., 1989; Stockmeier et al., 1993).

APZ-HCl (Pharmacia LEO Therapeutics AB, Malmö, Sweden) was dissolved in deionized water. CLOZ-HCl (Sandoz, East Hanover, NJ), RISP (Janssen, Titusville, NJ), OLAN (Eli Lilly, Indianapolis, IN), HAL (McNeil, Spring House, PA), and SUL (Research Biochemical Inc., Natick, MA) were each dissolved in 0.1 M tartaric acid because of their lesser solubility in water. Drugs or vehicle (0.1 M tartaric acid or deionized water) in a volume of 1.0 ml/kg were administered s.c. to randomly assigned rats.

**Data Analysis.** The mean value of three consecutive samples before drug injection was set at 100% as the predrug basal value. The percentage of net area under the curve (% net AUC) was obtained from the net increase for a 180-min period after drug or vehicle over each predrug basal level, and expressed as percentage of predrug basal levels. All data were statistically evaluated with StatView 4.50 (Abacus Concepts Inc., Berkeley, CA). Basal extracellular DA levels in the mPFC compared with the NAC, as indicated by the difference between the values of log[mPFC] and log[NAC], and the pKi values of antipsychotic drugs for 5-HT and DA receptor subtypes. The values of log[mPFC] and log[NAC] are the logarithms of the % net AUC in the mPFC and the NAC. A probability of less than 0.05 \((p < .05)\) was considered significant in the present study.

**Results**

**Basal Extracellular DA Levels in the mPFC and the NAC.** Basal extracellular DA levels (mean ± S.E.M. fmol/10 \(\mu\)l/30 min, not corrected by % recovery) in the dialysates obtained from all rats used in this study were 1.72 ± 0.09 \((n = 71)\) for the mPFC and 11.04 ± 0.46 \((n = 77)\) for the NAC, respectively. The basal extracellular DA levels in the NAC were about 6-fold higher than in the mPFC (F\(_{1,146}\) = 370.90, \(p < .001\) by one-way ANOVA). There was no significant difference in basal extracellular DA levels in each brain region between the various treatment groups. In a preliminary experiment, the injection of either 0.1 M tartaric acid, deionized water, or physiological saline (0.9% NaCl) had no significant effect on extracellular DA levels in either region. Therefore, the combined data of the effects of both 0.1 M tartaric acid and deionized water on extracellular DA levels were used as the vehicle control (VEH) for the statistical analysis and the graphic presentation (\(n = 6\) for each region).

**Time-Dependent Effects on Extracellular DA Levels in the mPFC and the NAC during the 180-min Period after Drug Injections Compared With VEH.** Repeated-measure ANOVA for each treatment group in both regions with post hoc comparison, as compared with VEH, is indicated as follows: APZ (10 mg/kg) produced time-dependent effects on extracellular DA levels in the mPFC and the NAC, whereas APZ (2 mg/kg) had no time-dependent effect in either region. Both doses of CLOZ (5 and 20 mg/kg), OLAN (1 and 10 mg/kg), and RISP (0.1 and 1 mg/kg) had significant time-dependent effects in both regions. SUL (25 mg/kg) significantly increased extracellular DA levels in both regions, whereas SUL (10 mg/kg) had a significant effect in the NAC but not in the mPFC. Both doses of HAL (0.1 and 1 mg/kg) increased extracellular DA levels in the NAC but not in the mPFC (Figs. 1 and 2).

The time course of extracellular DA levels in the mPFC or the NAC was comparable with peak DA levels in the mPFC at 60 min for all drugs studied except OLAN and SUL, which peaked at 90 to 120 min. Elevated extracellular DA levels were still present in the mPFC 180 min after drug administration. The same was true for the increase in extracellular DA levels in the NAC.

**TABLE 1**

<table>
<thead>
<tr>
<th>Drug</th>
<th>D(_1)</th>
<th>D(_2)</th>
<th>D(_3)</th>
<th>D(_4)</th>
<th>5-HT(_{1A})</th>
<th>5-HT(_{2A})</th>
<th>5-HT(_{3\alpha})</th>
<th>5-HT(_{2A}/D_2)</th>
<th>ED(_{50}) D(_2)</th>
<th>5-HT(_{1A})</th>
<th>5-HT(_{2A})</th>
<th>5-HT(_{3\alpha}/D_2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>APZ</td>
<td>6.2</td>
<td>6.3</td>
<td>6.3(^a)</td>
<td>5.9(^a)</td>
<td>8.0</td>
<td>5.9</td>
<td>1.7</td>
<td>&gt;100</td>
<td>1.1</td>
<td>&lt;0.01</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CLOZ</td>
<td>6.8</td>
<td>7.0</td>
<td>6.4(b)</td>
<td>8.0</td>
<td>6.7(b)</td>
<td>8.3</td>
<td>1.3</td>
<td>5.2</td>
<td>0.9</td>
<td>0.2</td>
<td>0.3</td>
<td>0.1</td>
</tr>
<tr>
<td>RISP</td>
<td>6.2(b)</td>
<td>8.9</td>
<td>7.9(b)</td>
<td>7.8</td>
<td>6.9(b)</td>
<td>10.1</td>
<td>1.2</td>
<td>15</td>
<td>6</td>
<td>0.4</td>
<td>15</td>
<td>10</td>
</tr>
<tr>
<td>OLAN</td>
<td>6.6(b)</td>
<td>7.8</td>
<td>7.3(b)</td>
<td>7.6(b)</td>
<td>&lt;6.0(b)</td>
<td>8.7(b)</td>
<td>8.1(b)</td>
<td>0.9</td>
<td>15</td>
<td>10</td>
<td>86</td>
<td>&gt;340</td>
</tr>
<tr>
<td>HAL</td>
<td>7.0</td>
<td>9.0</td>
<td>7.7(b)</td>
<td>8.0</td>
<td>5.5(b)</td>
<td>7.7</td>
<td>5.6</td>
<td>&lt;1.3</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SUL</td>
<td>&lt;5.0(c)</td>
<td>7.9(c)</td>
<td>7.6(c)</td>
<td>6.0(c)</td>
<td>&lt;5.0(c)</td>
<td>&lt;5.0(c)</td>
<td>&lt;1.3</td>
<td>0.2</td>
<td>15</td>
<td>10</td>
<td>86</td>
<td>&gt;340</td>
</tr>
</tbody>
</table>

The pKi values for D\(_1\), D\(_2\), 5-HT\(_{1A}\), and 5-HT\(_{3\alpha}\) receptors were obtained from Meltzer et al. (1989), Stockmeier et al. (1993), Roth et al. (1992, 1995), except where noted. The ED\(_{50}\) values (mg/kg) for in vivo 5-HT\(_{2A}\) and D\(_2\) receptor occupancy were obtained from Stockmeier et al. (1993) except for OLAN. Data for OLAN refers to Bymaster et al. (1996b). The ED\(_{50}\) values of sulpiride are data of a racemic compound.

\(^a\) Data refers to Meltzer et al. (1993).

\(^b\) Data refers to Schotte et al. (1996).

\(^c\) Data refers to Leysen et al. (1993).

\(^d\) Data refers to Bymaster et al. (1996a).

\(^e\) Data refers to Seeman and Van Tol (1995).
Effects on the Percentage of Net AUC of Extracellular DA Levels in the mPFC and the NAC. Both doses of antipsychotic drugs studied except HAL produced significant increases in the % net AUC in the mPFC compared with VEH. APZ (2 mg/kg) showed a significant increase in the % net AUC in the mPFC when determined by one-way ANOVA despite nonsignificant time-dependent effects determined by repeated-measure ANOVA with post hoc comparison. HAL (1 but not 0.1 mg/kg) had a significant effect in the mPFC. APZ (10 but not 2 mg/kg) produced a significant increase in the % net AUC in the NAC. Both doses of all of the other antipsychotic drugs had significant effects in the NAC (Table 2).

Because of the great difference in absolute values of extracellular DA levels and variance of % converted values after drug injection between the mPFC and the NAC, statistical analysis by ANOVA is not applicable to the regional comparison of the effects of antipsychotic drugs on extracellular DA levels. However, as can be seen in Table 2, APZ (2 and 10 mg/kg) and CLOZ (5 and 20 mg/kg) clearly showed greater effects on extracellular DA levels in the mPFC than in the NAC. OLAN (10 mg/kg) had a slightly greater effect on extracellular DA levels in the mPFC.
tracellular DA levels in the mPFC than in the NAC, whereas OLAN (1 mg/kg) produced a comparable increase in the mPFC compared to the NAC. RISP (0.1 and 1 mg/kg) produced comparable effects on extracellular DA levels in the two regions. SUL (25 mg/kg) also showed a comparable increase in the mPFC compared to the NAC, whereas SUL (10 mg/kg) as well as HAL (0.1 and 1 mg/kg) increased extracellular DA levels in the NAC to a greater extent than in the mPFC.

Thus, the six antipsychotic drugs may be grouped on the basis of their effects on extracellular DA levels in the mPFC and the NAC. Three of the drugs (CLOZ, APZ, and OLAN) produced greater increases in extracellular DA levels in the mPFC compared with the NAC at at least one dose studied, whereas two drugs (SUL and HAL) had greater effects in the NAC compared with the mPFC at at least one dose studied.

**Relationship Between the Affinities (pKi Values) for Receptor Subtypes and the Ability to Increase Extracellular DA Levels in the mPFC Compared With the NAC.** To determine the relationship between pKi values and regional effects of antipsychotic drugs on extracellular DA levels, the difference in the values of log[net AUC of DA] between the mPFC and the NAC (log[mPFC] – log[NAC]) was determined in this study (Table 2). This measure is more appropriate for this purpose than the comparison of absolute extracellular DA levels in each region between the mPFC and the NAC because basal extracellular DA levels are significantly different (6-fold) between these regions, and the % net AUC values are less affected by the difference in basal levels.

<table>
<thead>
<tr>
<th>Drug</th>
<th>mg/kg</th>
<th>mPFC</th>
<th>NAC</th>
<th>log[mPFC]–log[NAC]</th>
</tr>
</thead>
<tbody>
<tr>
<td>VEH</td>
<td>0</td>
<td>9±376</td>
<td>−13±14</td>
<td>1.35</td>
</tr>
<tr>
<td>APZ</td>
<td>2</td>
<td>351±82**</td>
<td>16±23</td>
<td>0.99</td>
</tr>
<tr>
<td>CLOZ</td>
<td>5</td>
<td>1310±287***</td>
<td>133±21***</td>
<td>0.05</td>
</tr>
<tr>
<td>OLAN</td>
<td>1</td>
<td>221±70**</td>
<td>78±347</td>
<td>0.63</td>
</tr>
<tr>
<td>RISP</td>
<td>0.1</td>
<td>556±142**</td>
<td>133±386</td>
<td>0.60</td>
</tr>
<tr>
<td>SUL</td>
<td>0.1</td>
<td>202±66*</td>
<td>181±70*</td>
<td>0.30</td>
</tr>
<tr>
<td>HAL</td>
<td>0.1</td>
<td>560±82***</td>
<td>280±39*</td>
<td>0.10</td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>208±26*</td>
<td>265±76***</td>
<td>0.11</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>358±79***</td>
<td>454±32***</td>
<td>0.00</td>
</tr>
<tr>
<td></td>
<td>66</td>
<td>153±36*</td>
<td>384±49***</td>
<td>0.40</td>
</tr>
<tr>
<td></td>
<td>266</td>
<td>88±294</td>
<td>300±100**</td>
<td>0.54</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>137±456</td>
<td>371±52***</td>
<td>0.43</td>
</tr>
</tbody>
</table>

The values of log[mPFC] – log[NAC] are the differences in the logarithm of % net AUC between the mPFC and the NAC. Numbers of rats in each treatment group are in parentheses. * p < .05, ** p < .01, *** p < .001 by one-way ANOVA compared to the vehicle control.

**Discussion**

The major findings of the present study are: 1) at certain doses, some but not all atypical antipsychotic drugs have preferential effects to increase extracellular DA levels in the mPFC compared with the NAC; and 2) the ability of antipsychotic drugs to increase extracellular DA levels in the mPFC compared with the NAC is positively correlated with the difference in the affinities of antipsychotic drugs for 5-HT2A and D2 receptors. It is also inversely correlated with the affinities for D3 or D4 receptors. There is no correlation between the affinities for 5-HT2A receptors alone and the preferential effects in the mPFC.

This study was undertaken to clarify the importance of the affinities of antipsychotic drugs for 5-HT2A and D2 receptors for the differential clinical and biochemical effects of typical and atypical antipsychotic drugs. It was previously postulated that the difference in pKi values for these two receptors was relevant to their ability to produce minimal extrapyramidal symptoms and might also contribute to their possible advantages as antipsychotic drugs (Meltzer et al., 1989). These drugs may also differ with regard to their ability to treat negative symptoms and to improve cognitive function. Negative symptoms and cognitive impairment have been suggested to be related to frontal lobe function and, in particular, to the alteration of cortical dopaminergic activity (Sawaguchi and Goldman-Rakic, 1991; Murphy et al., 1996). 5-HT2A receptors located on pyramidal cells in the cerebral cortex may also be the site of action of antipsychotic drugs (Jakab and Goldman-Rakic, 1998). Therefore, it is of importance to determine whether typical and atypical antipsychotic drugs differ with regard to their ability to increase extracellular DA levels in the mPFC relative to the NAC. If so, this difference may be related to their affinities for 5-HT2A and D2 receptors.

The six antipsychotic drugs studied included four atypical antipsychotic drugs (CLOZ, APZ, RISP, and OLAN) and two typical antipsychotic drugs (HAL and SUL) (Meltzer, 1995). Despite the fact that only six drugs were studied, the range of
the differences in their relative affinities for 5-HT$_{2A}$ and D$_2$ receptors was 1000-fold. Because the D$_2$ and D$_3$ receptor affinities of the six drugs are highly correlated, it was not possible to separate out the importance of D$_2$ versus D$_3$ receptor blockade with regard to the effect on extracellular DA levels in the mPFC and NAC. Further study is needed to determine the relative importance of D$_2$ and D$_3$ receptors to the ability to increase extracellular DA levels in the two regions.

Both doses of APZ, CLOZ, OLAN, and RISP had greater or comparable effects on extracellular DA levels in the mPFC compared with the NAC. By contrast, SUL (10 mg/kg) and both doses of HAL had much greater effects on extracellular DA levels in the NAC than in the mPFC, leaving open the possibility that an equal or greater effect on extracellular DA levels in the mPFC compared with the NAC may be characteristic of an atypical antipsychotic drug. These results are generally consistent with previous reports by others (Moghaddam and Bunney, 1990; Nomikos et al., 1994; Hertel et al., 1996). Volonté et al. (1997) reported a greater effects of OLAN (0.3, 1 and less than 3 mg/kg) in the STR than in the mPFC, whereas we found a greater effect of OLAN (10 but not 1 mg/kg) in the mPFC than in the NAC. It should be noted that SUL (25 mg/kg) produced a comparable increase in extracellular DA levels in the mPFC relative to the NAC. This effect may be related to the reported beneficial effects of SUL to decrease negative symptoms (Mauri et al., 1996). HAL, which has minimal beneficial effect on negative symptoms, produced the smallest increase in extracellular DA levels in the mPFC at both doses studied. These data support the possibility that there could be a relationship between extracellular DA levels in the mPFC and improvement in negative symptoms.

Quetiapine, another 5-HT$_{2A}$/D$_2$ receptor antagonist, has a low extrapyramidal symptoms profile similar to that of OLAN, yet there is no evidence that it has beneficial effects on negative symptoms or cognition in schizophrenia (Arvanitis et al., 1997). Recently, quetiapine was reported not to increase extracellular DA levels in the mPFC (Volonté et al., 1997). These findings may be consistent with the hypothesis that increased prefrontal extracellular DA levels may be important to the ability of 5-HT$_{2A}$/D$_2$ receptor antagonists to improve negative symptoms. The lesser ability of quetiapine to increase DA levels in the mPFC may be due to much weaker affinity of quetiapine for 5-HT$_{2A}$ and D$_2$ receptors than any other atypical antipsychotic drug studied here (Schotte et al., 1996). More typical and atypical antipsychotic drugs need to be studied to further clarify this relationship between prefrontal extracellular DA levels and negative symptoms. It will also be important to determine the effect of chronic administration of these agents on extracellular DA levels because the effect of chronic treatment would be expected to have greater clinical relevancy.

Although there was a strong inverse correlation between D$_2$ (or D$_3$) receptor affinities and the ability to increase extracellular DA levels in the mPFC compared with the NAC, it is most likely that the relationship determining the preferential effect on extracellular DA levels in the mPFC is 5-HT$_{2A}$ relative to D$_2$ receptor affinity. The Spearman rank correlation coefficient of the difference in pKi values for the two receptors and the logarithm of the % net AUC for the mPFC and the NAC was 0.79, accounting for 62% of the variance in the dependent measure. This correlation is unlikely due to the correlation with pKi values for D$_2$ or D$_3$ receptors because there was no significant correlation between these values and the difference in pKi values for 5-HT$_{2A}$ and D$_2$ receptors. 5-HT$_{2A}$ receptor affinities alone did not correlate with the greater ability to increase extracellular DA levels in the mPFC relative to the NAC, although the selective 5-HT$_{2A}$ receptor antagonists such as M-100,907 were reported to increase extracellular DA levels in the mPFC (Schmidt and Fadayel, 1995). It would appear that 5-HT$_{2A}$ receptor blockade, in the presence of D$_2$ receptor blockade that is relatively less effective than that of 5-HT$_{2A}$ receptor blockade, is able to increase extracellular DA levels in the mPFC more so than in the NAC. This may be due to...
local effects of 5-HT$_{2A}$ receptor blockade in the mPFC and NAC because the density of 5-HT$_{2A}$ receptors is much higher than in the mPFC compared with the NAC. The occupancy of 5-HT$_{2A}$ receptors in the NAC may be more effective than in the mPFC because the density of 5-HT$_{2A}$ receptors is much higher than in the mPFC.

This hypothesis does not explain why OLAN (1 mg/kg) was more effective than OLAN (10 mg/kg) to increase extracellular DA levels in the mPFC compared with the NAC. APZ to increase extracellular DA levels in the mPFC compared with the NAC. APZ, a weak D$_{2}$ receptor antagonist, is reported to inhibit the reuptake of DA (Pettersson, 1995).

This effect might be expected to increase extracellular DA levels in the NAC even more so than the mPFC because Cass and Gerhardt (1995) reported that DA uptake is less effective in the mPFC than in the NAC and STR. Moreover, local perfusion of APZ into the NAC has been reported to produce a greater increase in DA release than in the mPFC (Nomikos et al., 1994). Thus, it may not be excluded that the combination of 5-HT$_{2A}$ receptor blockade and DA reuptake inhibition produced by APZ contribute to the striking preferential effect of APZ on extracellular DA levels in the mPFC. Other factors not yet apparent may also be involved.

The present results suggest that antipsychotic drugs may be grouped on the basis of their ability to increase extracellular DA levels in the mPFC versus the NAC. Although the clinical relevance of such a classification is not clear at present, the assumption that the preferential activation of prefrontal dopaminergic neurotransmission may contribute, at least in part, to the efficacy in treating negative symptoms and cognitive impairment leads to testable hypotheses for developing new antipsychotic drugs.

References


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Send reprint requests to: Toshihide Kuroki, M.D., Ph.D., Department of Psychiatry, Saga Medical School, Nabeshima 5-1-1, Saga 849-8501, Japan. E-mail: kuroki@post.saga-med.ac.jp