Equivalent Occupancy of Dopamine D\textsubscript{1} and D\textsubscript{2} Receptors With Clozapine: Differentiation From Other Atypical Antipsychotics

Johannes Tauscher, M.D.
Tabasum Hussain, Ph.D.
Ofer Agid, M.D.
N. Paul L.G. Verhoeff, M.D., Ph.D.
Alan A. Wilson, Ph.D.
Sylvain Houle, M.D., Ph.D.
Gary Remington, M.D., Ph.D.
Robert B. Zipursky, M.D.
Shitij Kapur, M.D., Ph.D.

Objective: Clozapine, the prototype of atypical antipsychotics, remains unique in its efficacy in the treatment of refractory schizophrenia. Its affinity for dopamine D\textsubscript{4} receptors, serotonin 5-HT\textsubscript{2A} receptor antagonism, effects on the noradrenergic system, and its relatively moderate occupancy of D\textsubscript{2} receptors are unlikely to be the critical mechanism underlying its efficacy. In an attempt to elucidate the molecular/synaptic mechanism underlying clozapine’s distinctiveness in refractory schizophrenia, the authors studied the in vivo D\textsubscript{1} and D\textsubscript{2} receptor profile of clozapine compared with other atypical antipsychotics.

Method: Positron emission tomography with the radioligands \(^{11}\text{C}\)SCH23390 and \(^{11}\text{C}\)raclopride was used to investigate D\textsubscript{1} and D\textsubscript{2} receptor occupancy in vivo in 25 schizophrenia patients receiving atypical antipsychotic treatment with clozapine, olanzapine, quetiapine, or risperidone.

Results: Mean striatal D\textsubscript{1} occupancies ranged from 55% with clozapine to 12% with quetiapine (rank order: clozapine > olanzapine > risperidone > quetiapine). The striatal D\textsubscript{2} occupancy ranged from 81% with risperidone to 30% with quetiapine (rank order: risperidone > olanzapine > clozapine > quetiapine). The ratio of striatal D\textsubscript{1}/D\textsubscript{2} occupancy was significantly higher for clozapine (0.88) relative to olanzapine (0.54), quetiapine (0.41), or risperidone (0.31).

Conclusions: Among the atypical antipsychotics, clozapine appears to have a simultaneous and equivalent occupancy of dopamine D\textsubscript{1} and D\textsubscript{2} receptors. Whether its effect on D\textsubscript{1} receptors represents agonism or antagonism is not yet clear, as this issue is still unresolved in the preclinical arena. This distinctive effect on D\textsubscript{1}/D\textsubscript{2} receptors may be responsible for clozapine’s unique effectiveness in patients with schizophrenia refractory to other typical and atypical antipsychotics.
receptor antagonist fananserin proved to be an ineffective antipsychotic (18). Further, the moderate D2 occupancy hypothesis was rejected because patients who had been receiving oral neuroleptics and who were switched to clozapine did not differ from patients who had been receiving depot medications (19). In the oral discontinuation group, one could expect to find a faster decline in D2 occupancy, and if moderate D2 blockade were the key to clozapine’s uniqueness, this group should have shown a more rapid response, which was not the case.

Clozapine’s relatively high affinity for the dopamine D1 receptor may be related to its unique clinical efficacy, as D1 receptors mediate the reward function in animal models, a principle thought relevant for the therapeutic action of antipsychotics (20). In vitro, clozapine shows a relatively high affinity for D1 receptors together with a moderate affinity for D2 receptors (21, 22). In man, clozapine showed a distinctively lower D2 occupancy and a higher D1 receptor occupancy compared with the typical neuroleptics (23), but no comparisons with the newer atypical antipsychotics are available.

We used positron emission tomography (PET) with the radioligands [11C]SCH23390 and [11C]raclopride to investigate D1 and D2 receptor occupancy in vivo in 25 schizophrenia patients being treated with the atypical antipsychotics clozapine, olanzapine, quetiapine, or risperidone.

**Method**

We included 25 patients (18 men and seven women; mean age=35.4 years, range=18–58) with a DSM-IV diagnosis of schizophrenia or schizoaffective disorder in the study. Their diagnosis of schizophrenia was ascertained with the Structured Clinical Interview for DSM-IV, which was administered by an experienced psychiatrist (J.T., N.P.L.G.V., or O.A.). We recruited the patients from the Schizophrenia and Continuing Care Program of the Centre for Addiction and Mental Health in Toronto, where each patient received ongoing antipsychotic medication either as an inpatient or outpatient. All subjects gave their written consent after the procedure had been fully explained. The study and recruitment procedures were approved by the Research Ethics Board of the Centre for Addiction and Mental Health and the University of Toronto.

Patients had been receiving atypical antipsychotic treatment for at least 14 days before the PET study. A summary of the treatment regimens received by the patients is presented in Table 1. In general, medications were prescribed once daily, twice daily if that was not tolerated. At the day of the PET scans, all patients took their total daily dose of medication at once approximately 2 hours before the first PET scan, and their striatal D1 and D2 occupancy were measured subsequently.

**PET Scanning Procedures**

Striatal dopamine D2 receptor occupancy was determined by using 10.8 mCi (SD=2.7) of high-specific-activity [11C]raclopride (mean=692.9 Ci/mmol, SD=465.0) administered as a bolus plus continuous infusion. Striatal D1 receptor occupancies were determined by using 9.9 mCi (SD=0.6) of high-specific-activity [11C]SCH23390 (mean=875.7 Ci/mmol, SD=528.8) administered as a bolus. Imaging was performed with a Scanditronix/GEMS PC-2048–15B head scanner as described in previous publications (24–27). The PET scans were performed in a fixed order starting with the [11C]SCH23390 scan approximately 2 hours after the last dose and continuing with the [11C]raclopride scan approximately 5–6 hours postdose.

A magnetic resonance imaging (MRI) scan was obtained for each of the patients (GE Signa 1.5-T scanner, proton density maps) and was coregistered to the composite [11C]raclopride and [11C]SCH23390 PET scans by using RView8/mpr software (28). As described in previous publications (24–27), we drew the striatal (caudate plus putamen) and cerebellar regions of interest on two contiguous PET slices with reference to the overlapping coregistered MRI scan. The cerebellar time-activity curve was taken as an estimate of the free and nonspecific [11C]raclopride binding (29), while the striatal time-activity curve provided an estimate of specific binding to the D2 receptors plus free and nonspecific binding. Under these assumptions, it can be shown that the striatal-cerebellar ratio minus one, at the time when the binding is at equilibrium (30–75 minutes in the aforementioned scans), provides an index proportional to the Bmax/Kd ratio of [11C]raclopride for dopamine D2 receptors (referred to as the binding potential). In previous studies (30) we have demonstrated that this ratio correlates very well (r=0.95) with analytically derived estimates of D2 binding potential, is highly reliable with a scan-rescan standard deviation of 6%, and has been standardized in our laboratory with excellent inter- and intrarater reliability (intraclass correlation coefficients >0.95). For estimation of specific binding to D1 receptors (plus free and nonspecific binding) in the striatum and frontal cortex, a ratio was calculated between those regions and the cerebellum as a reference region (27). Data from the left and right hemispheres were pooled for all subsequent calculations, since there was no significant asymmetry in D1 or D2 binding potentials.

Since we did not have baseline measures of D1 or D2 binding potentials for the patients, we used an age-corrected estimate from a comparison group of 29 untreated healthy volunteers who had no current or past axis I DSM-IV psychiatric disorder as determined with the Structured Clinical Interview for DSM-IV, nonpatient version, and had not taken any psychotropic medication in the 3 months preceding this study. Twelve subjects (five men and seven women) with a mean age of 32 years (SD=11, range=20–49) served as the comparison group for D1 binding potential, and 17 subjects (nine men and eight women) with a mean age of 29 years (SD=6, range=20–40) served as baseline D2 binding potential values.

**Determination of Drug and Prolactin Plasma Levels**

At the time of the PET scans, blood was drawn for a plasma drug level and prolactin level analysis. We determined clozapine, olanzapine, quetiapine, and risperidone levels in heparinized plasma using a liquid chromatography/mass spectroscopy method (31, 32). Prolactin levels were determined by using a two-site chemiluminescent immunoassay with a minimum detectable limit of 0.3 ng/ml and a coefficient of variance of 3.6% to 4.5% (ACS, Ciba-Corning Diagnostics, Corning, N.Y.).

**Statistical Analysis**

Statistical analyses were performed with SPSS for Windows 11.0.1 (SPSS, Inc., Chicago, 2001). Dopamine D1 and D2 receptor binding indices of different antipsychotics were compared by using

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**Table 1. Atypical Antipsychotic Treatment Received by 25 Patients With Refractory Schizophrenia**

<table>
<thead>
<tr>
<th>Atypical Antipsychotic</th>
<th>Mean (SD)</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clozapine (N=7)</td>
<td>510.7 (88.8)</td>
<td>400–600</td>
</tr>
<tr>
<td>Olanzapine (N=6)</td>
<td>18.8 (3.1)</td>
<td>15–22.5</td>
</tr>
<tr>
<td>Quetiapine (N=5)</td>
<td>490.0 (159.7)</td>
<td>300–700</td>
</tr>
<tr>
<td>Risperidone (N=7)</td>
<td>4.3 (1.3)</td>
<td>3–6</td>
</tr>
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TABLE 2. Dopamine Receptor Occupancy Rates in 25 Patients With Refractory Schizophrenia Receiving Atypical Antipsychotic Treatment

<table>
<thead>
<tr>
<th>Atypical Antipsychotic</th>
<th>Striatal D1 (%)</th>
<th>Striatal D2 (%)</th>
<th>Striatal D1/D2 Quotient</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clozapine</td>
<td>55 + 15</td>
<td>61 + 10</td>
<td>0.88</td>
</tr>
<tr>
<td>Olanzapine</td>
<td>43 + 10</td>
<td>79 + 6</td>
<td>0.54</td>
</tr>
<tr>
<td>Quetiapine</td>
<td>12 + 10</td>
<td>30 + 18</td>
<td>0.41</td>
</tr>
<tr>
<td>Risperidone</td>
<td>25 + 7</td>
<td>81 + 4</td>
<td>0.31</td>
</tr>
</tbody>
</table>

Discussion

Clozapine occupies dopamine D1 and D2 receptors in vivo, which is in line with previous studies that used PET and $[11C]SCH23390 (23) or $[11C]raclopride (11). Our study represents the first attempt to compare clozapine’s action on D1 and D2 receptors to that of other novel antipsychotics—and we found that it is unique in this regard.

Our receptor occupancy results correspond with the D1 and D2 receptor affinity values found in preclinical studies: the Kᵢ values for clozapine were 290–540 nM for D1 and 130–150 nM for D2, suggesting relatively similar affinity for both receptors (22, 33). Corresponding to the comparatively higher D2 than D1 occupancy with quetiapine and risperidone in our in vivo PET study, preclinical data suggest a 10- to 100-fold higher affinity for D2 receptors than for D1 receptors for both quetiapine and risperidone (22, 33). We found that olanzapine came closest to clozapine with regard to the “balanced” or equivalent occupancy of D1 and D2 receptors, still showing a D1/D2 occupancy ratio of 0.54. In line with that, preclinical data showed olanzapine’s Kᵢ values at D1 of 52 nM and at D2 of 20 nM (33).

While we observed occupancy of D1 receptors, there is no broad consensus on clozapine’s intrinsic efficacy at these receptors. In a number of in vivo assays, clozapine has some preferential, although not selective, action to antagonize D1 receptor-mediated function (34). However, D1 antagonism by itself has not been an effective antipsychotic principle: studies with the selective D1 antagonists SCH23390 (35), SCH39166 (36–38), and NNC 01–0687 (39) by themselves were ineffective as antipsychotics. In addition, relatively brief treatment with SCH39166 in doses inducing a more than 70% occupancy of striatal D1 receptors failed to induce antipsychotic action (40).

On the other hand, there is some evidence that clozapine behaves as a D2 agonist: hypothermia produced by clozapine in rats was fully antagonized by either of the selective D2 receptor antagonists SCH23390 or NNC 01–0687 (41). This aspect could be interesting given the clinical and laboratory observations implicating D1 receptor agonism in the prefrontal cortex in cognitive functions (41, 42). Finally, regardless of its agonist/antagonist action, a recent $[18F]fluorodeoxyglucose PET study in patients suffering from treatment-resistant schizophrenia showed that brain metabolic and clinical responses to clozapine were related to D1 receptor genotype (43). After 5 weeks of treatment with clozapine, brain metabolic decreases were found in patients with the 2,2 but not the 1,2 D1 receptor genotype. Moreover, patients with the 2,2 D1 genotype significantly improved with clozapine, whereas those with a 1,2 D1 genotype did not (43).

We did not observe a simple relationship between prolactin plasma levels and D1 or D2 occupancy rates. This can be explained by the differential blood-brain disposition of the atypical antipsychotics under investigation. In line with our findings, risperidone has been shown to lead...
to higher prolactin levels than clozapine, olanzapine, or quetiapine (44). This fact is not directly related to dopamine receptor occupancy in the brain but is mainly due to differential blood-brain barrier penetration of atypical antipsychotics. It has been shown that risperidone has a comparably higher central to peripheral potency for prolactin elevation than olanzapine (44). Compounds with a higher peripheral potency bring about higher prolactin levels for a given level of functional central antagonism, and thus one cannot expect a simple linear relationship between plasma prolactin and dopamine receptor occupancy with different antipsychotics.

There are several limitations of the current study that suggest caution in how these results are interpreted. We compared D1 and D2 receptor binding potential values of patients treated with clozapine and other atypical antipsychotics to that of healthy subjects, since the patients were already receiving treatment and it is very difficult to find neuroleptic-naive patients with similar demographic characteristics. However, this is unlikely to induce a systematic bias in our results, since there is no clear evidence for alterations of striatal D1 or D2 receptor number in schizophrenia (45–50). Moreover, the main intent of the study was to compare antipsychotics. Since the same baseline was used for all of the agents, it is unlikely to have given rise to differences among antipsychotics.

Plasma drug levels were positively correlated with striatal D2 receptor occupancies in clozapine- and olanzapine-treated patients but not with quetiapine or risperidone, nor was there such a correlation between plasma drug levels and D1 occupancy. This is surprising but may be due to the fact that subjects were not randomly assigned to different doses. The apparent difference in the relationships between drug plasma levels and receptor occupancies on D1 and D2 receptors can partly be explained by differences in the central and plasma kinetics of the four antipsychotics. The $[^{11}\text{C}]{\text{SCH23390}}$ PET scans to determine D1 occupancy were performed at around peak plasma levels for all antipsychotics, while the PET scan with $[^{11}\text{C}]$raclopride was performed 3–4 hours later. Different time points of PET scans with regard to intake of the last dose of the medication do not make a difference with antipsychotics that show a sustained high blockade of dopamine receptors, such as olanzapine and risperidone (51). However, it is conceivable that with clozapine and quetiapine, given their more rapid decline from peak plasma concentration (26, 52), the second scan may have underestimated the peak occupancy values for D2 receptors.

In summary, this PET study in schizophrenia patients is consistent with the idea that clozapine has a unique interaction with the D1/D2 system as suggested by animal models. The relatively equivalent D1/D2 occupancy may explain the clinical uniqueness of clozapine in patients with refractory symptoms. These cross-sectional data provide a strong impetus for prospective clinical studies focusing on the role of dopamine D1 receptors, with the caveat that it is still unclear whether agonistic or antagonistic properties are desirable, along with moderate D2 antagonism as a means for enhanced therapeutic efficacy against psychosis.

FIGURE 1. Summation PET Scan Images of D1 and D2 Receptor Occupancy in Patients With Refractory Schizophrenia Receiving Atypical Antipsychotic Treatment
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