Dopamine and Serotonin Receptors: Amino Acid Sequences, and Clinical Role in Neuroleptic Parkinsonism

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ABSTRACT—This review summarizes the amino acid sequences of the human dopamine and serotonin receptors and their human variants. The review also examines the receptor basis of the atypical antipsychotic drugs that elicit less parkinsonism than the typical antipsychotics. Because the dissociation constant of a drug varies with the radioligand, the dissociation constants of many neuroleptics are here summarized for the dopamine D2-, D4- and serotonin 5HT2A-receptors using different radioligands. Radioligands of low solubility in the membrane (having low tissue/buffer partition) result in lower values for the neuroleptic dissociation constants, compared to radioligands of high membrane solubility. Such studies yield the intrinsic K value for a neuroleptic in the absence of a competing ligand. Clozapine, for example, has an intrinsic K value of 1.6 nM at the D4-receptor, in agreement with the value of 1.6 nM when directly measured with [³H]clozapine at D4. However, because clozapine competes with endogenous dopamine, the in vivo clozapine concentration to occupy 75% of the dopamine D4-receptors is derived to be ~13 nM. This agrees with the value of 12 to 20 nM in the plasma water (or spinal fluid) observed in treated patients. Moreover, in L-DOPA psychosis (in Parkinson’s disease), the clozapine concentration for 75% blockade of D4 is predicted to be ~3 nM. This agrees with the value of ~1.2 nM observed by Meltzer et al. in plasma water (Neuropsychopharmacology, 12, 39–45 (1995)). This analysis supports the concept and practical value of the intrinsic K values. Some atypical neuroleptics (remoxipride, clozapine, perlapine, seroquel and melperone) have high intrinsic K values (ranging from 30 to 88 nM) at the D2-receptor, making them displaceable by high levels of endogenous dopamine in the caudate/putamen. In contrast, however, typical neuroleptics (i.e., those that typically cause parkinsonism) have intrinsic K values of 0.3 to 6 nM, making them less displaceable by endogenous dopamine. A relationship exists between the neuroleptic doses for rat catalepsy and the D2/D4 ratio of the intrinsic K values. Thus, the atypical neuroleptics appear to fall into two groups, those that bind loosely to D2 and those that are selective at D4.

Keywords: Dopamine D4-receptor, Antipsychotic drug, Serotonin 2-receptor, Parkinsonism, Clozapine, Catalepsy

This review summarizes the amino acid sequences of the human dopamine and serotonin receptors and their human variants. In addition, the review examines the receptor basis of the atypical antipsychotic drugs that elicit less parkinsonism than the typical antipsychotics; that is, this review considers whether the affinities of various antipsychotic drugs for dopamine and serotonin receptors may be related to the extent of animal catalepsy or clinical parkinsonism.

Amino acid sequences for human dopamine and serotonin receptors

In addition to binding to various types of dopamine receptors, neuroleptics also bind to serotonin receptors. The binding of antipsychotic drugs to both these two groups of receptors is related, to some degree, to certain identical amino acids found in the same critical position in these two groups of receptors, as illustrated in Fig. 1.
HUMAN DOPAMINE AND BERTERONIN RECEPTORS

Fig. 1

Fig. 1
For example, the key amino acid for the binding of amines (1), such as neuroleptics, is aspartic acid (D) in the transmembrane region 3, as shown in Fig. 1. This same aspartic acid is also important for the binding of the neurotransmitter amines, such as dopamine (1) and serotonin.

Transmembrane region 5 contains a serine that is critical for the binding of agonists (1, 2), such as dopamine (also see Refs. in Ref. 3), and, presumably, serotonin.

Thus, the general three-dimensional structure of these receptors permits dopamine and/or serotonin to bind to transmembrane regions 3 and 5 (Ref. 4), as depicted in Fig. 2.

The aspartic acid in transmembrane region 2 determines the sensitivity of the receptors to sodium ions, influences the existence of the high-affinity for the agonist, and thereby affects the G-protein-coupling of the receptor to adenyl cyclase (5, 6).

Transmembrane region 7 of serotonin receptors S1D, S1D_{beta} and S1E contains threonine (adjacent to tryptophan, W, see Fig. 1). The replacement of this threonine by asparagine enhances the affinity of these receptors for propranolol by 100- to 1000-fold, matching the affinity of S1A for propranolol (7–10).

Additional features on the molecular biology of these receptors may be found in other reviews on dopamine receptors (11) and serotonin receptors (12–14).

**Human variants of dopamine and serotonin receptors**

The known amino acid variations of the human dopamine and serotonin receptors are given in Fig. 2, Tables 1 and 2, and the Refs. therein.

Of all the amino acid variations summarized in Fig. 2 and Tables 1 and 2, the only receptor variant that exhibits a significantly different sensitivity to drugs is the valine-to-glycine variation at position 194 in the human dopamine D4-receptor (3, 15). Although the sensitivity of this receptor to spiperone is the same as that for the common D4-receptor, the sensitivity of this variant to clozapine and olanzapine falls 100- to 1000-fold compared to the common D4-receptor (15). Moreover, this receptor variant apparently has no functional high-affinity state for dopamine, and, therefore, the receptor does not inhibit adenylate cyclase, unlike the common D4-receptor (15).

**Receptor basis for low level of parkinsonism caused by atypical neuroleptics**

Dopamine D2-receptor blockade alleviates psychosis but produces parkinsonism (16–20). Those neuroleptics that cause less parkinsonism are commonly referred to as "atypical antipsychotics" (21). There are several current views on the receptor basis for this atypical action of these particular neuroleptics:

1. Atypical neuroleptics may have low affinity for D2 and are, therefore, readily displaced by high endogenous concentrations of dopamine in the human striatum.
2. Atypical neuroleptics may have both anti-D2 and anticholinergic action.
3. Atypical neuroleptics may block both D2- and serotonin S2A-receptors (22–25).
4. Atypical neuroleptics may produce a selective blockade of dopamine D4-receptors.

These various hypotheses will later be considered. It is first essential to summarize the values for the neuroleptic dissociation constants at the dopamine D2-, D4- and serotonin 2A-receptors.

**Neuroleptic dissociation constant varies with the tissue/buffer partition of the ligand**

To calculate the therapeutic concentrations of neuroleptics and to examine the hypotheses underlying the action of atypical neuroleptics, it is essential to use neuroleptic dissociation constants which have been measured under conditions that reveal the intrinsic dissociation constant.

The dissociation constant of a particular neuroleptic generally varies between laboratories, particularly when different ligands are used. For example, the dissociation constant for clozapine at the dopamine D2-receptor is 150 nM (range: 70 to 400 nM) when [3H]spiperone is used as a ligand (19). However, when using [3H]raclopride as a ligand, the clozapine dissociation constant at D2 is 35 to 60 nM (26).

This dependence of the neuroleptic dissociation constant on the ligand has been examined (27, 28). It was found that the neuroleptic dissociation constant depended on the tissue/buffer partition coefficient of the ligand. A similar finding has recently been made by Durcan et al.
Fig. 2. Dopamine receptors and their human variations. Dopamine is shown attaching to the aspartic acid (D) of transmembrane 3 and to the two serine (S) amino acids in transmembrane 5, as supported by experiments using site-directed mutagenesis. The amino acid sequences are for the human dopamine receptors except for D2 that illustrates the rat sequence (118). There are no variants for D1 and D5, except the two D5 pseudogenes that abruptly stop at 154 amino acids. There are five different forms of D2 and five different forms of D3. The valine at position 194 is found to be glycine in about 10–15% of individuals of African origin. This D4valine194glycine variant is the only dopamine receptor variant that significantly differs in its sensitivity to drugs. (See Table 2 for additional details.)
(29). It was found (27) that clozapine at D2 had a K value (i.e., dissociation constant) of 390 nM using [3H]nemonapride, 186 nM using [3H]spiperone and 83 nM using [3H]raclopride. Haloperidol, as another example, revealed a K at D2 of 9.6 nM using [3H]nemonapride, 2.7 nM using [3H]spiperone and 0.67 nM using [3H]raclopride. These neuroleptic K values are related to the tissue /buffer partition coefficients of the ligands (see Figs. 3 and 4).

### Table 1. Human dopamine receptors and variants

<table>
<thead>
<tr>
<th>Receptors and variants</th>
<th>Amino acids</th>
<th>Characteristics</th>
<th>%Prevalence</th>
<th>Refs.</th>
</tr>
</thead>
<tbody>
<tr>
<td>D1-like</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>D1</td>
<td>446</td>
<td></td>
<td>~100%</td>
<td>95</td>
</tr>
<tr>
<td>D5</td>
<td>477</td>
<td></td>
<td>~100%</td>
<td>100</td>
</tr>
<tr>
<td>D5 pseudo-1</td>
<td>154</td>
<td>Stops before TM 4</td>
<td>~100%</td>
<td>126</td>
</tr>
<tr>
<td>D5 pseudo-2</td>
<td>154</td>
<td>Stops before TM 4</td>
<td>~100%</td>
<td>126</td>
</tr>
<tr>
<td>D2-like</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>D2 long</td>
<td>443</td>
<td></td>
<td>~97–100%</td>
<td>96</td>
</tr>
<tr>
<td>D2 short</td>
<td>414</td>
<td>Loop 3 is missing 29 AA</td>
<td>~97–100%</td>
<td>121</td>
</tr>
<tr>
<td>D2 V96A</td>
<td>443</td>
<td>Valine replaced by alanine at 96 in TM 2</td>
<td>~0.8%</td>
<td>127</td>
</tr>
<tr>
<td>D2 P310S</td>
<td>443</td>
<td>Proline replaced by serine at 310 (loop 3)</td>
<td>~0.4%</td>
<td>127</td>
</tr>
<tr>
<td>D2 S311C</td>
<td>443</td>
<td>Serine replaced by cysteine at 311 (loop 3)</td>
<td>~3%</td>
<td>127, 128</td>
</tr>
<tr>
<td>D3</td>
<td>400</td>
<td></td>
<td>~72%</td>
<td>97</td>
</tr>
<tr>
<td>D3 S9G</td>
<td>400</td>
<td>Serine replaced by glycine at 9</td>
<td>~28%</td>
<td>129</td>
</tr>
<tr>
<td>D3 nf</td>
<td>342</td>
<td>Stops before TM 6</td>
<td>in SZ &amp; Aff.D.</td>
<td>130</td>
</tr>
<tr>
<td>D3 (TM4del1)</td>
<td>138</td>
<td>Stops after TM 3; no binding</td>
<td></td>
<td>131</td>
</tr>
<tr>
<td>D3 (TM3del1)</td>
<td>109</td>
<td>Stops after TM 2; no binding</td>
<td></td>
<td>132</td>
</tr>
<tr>
<td>D4</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>D4.2</td>
<td>387</td>
<td>Loop 3 has 2 repeats of 16 amino acids each</td>
<td></td>
<td>99, 133</td>
</tr>
<tr>
<td>D4.3</td>
<td>403</td>
<td>Loop 3 has 3 repeats</td>
<td></td>
<td>99, 133</td>
</tr>
<tr>
<td>D4.4</td>
<td>419</td>
<td>Loop 3 has 4 repeats</td>
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<td>99, 133</td>
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<tr>
<td>D4.5</td>
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<td>Loop 3 has 5 repeats</td>
<td></td>
<td>99, 133</td>
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<td>D4.6</td>
<td>451</td>
<td>Loop 3 has 6 repeats</td>
<td></td>
<td>99, 133</td>
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<td>D4.7</td>
<td>467</td>
<td>Loop 3 has 7 repeats</td>
<td></td>
<td>99, 133</td>
</tr>
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<td>D4.8</td>
<td>483</td>
<td>Loop 3 has 8 repeats</td>
<td></td>
<td>99, 133</td>
</tr>
<tr>
<td>D4.9</td>
<td>499</td>
<td>Loop 3 has 9 repeats</td>
<td></td>
<td>99, 133</td>
</tr>
<tr>
<td>D4.10</td>
<td>515</td>
<td>Loop 3 has 10 repeats</td>
<td></td>
<td>99, 133</td>
</tr>
<tr>
<td>D4 (del)</td>
<td></td>
<td>AlaSerAlaGly missing before TM 1</td>
<td>~8% (Italy)</td>
<td>134</td>
</tr>
<tr>
<td>D4 V194G</td>
<td></td>
<td>Valine replaced by glycine at 194</td>
<td>10–15% Africans</td>
<td>3, 15</td>
</tr>
</tbody>
</table>

**del** = Has deletion, AA = amino acid, nf = non-functional, SZ = schizophrenia, Aff.D. = affective disorder, TM = hydrophobic transmembrane region. There are over 20 different types of repeat units, each repeat unit being designated by a different Greek letter (133).

The “intrinsic” dissociation constant

It is possible to remove the dependence of the neuroleptic K on the ligand and thus obtain the intrinsic dissociation constant of the neuroleptic in the absence of any competing ligand. This is done by extrapolating the relation shown (Fig. 4) down to the intercept on the vertical axis (i.e., at low partition coefficient). This intercept represents the intrinsic dissociation constant of the neuroleptic in the absence of any competing ligand. Thus, a low partition or a partition of “zero” indicates that the neuroleptic would be competing against a water-soluble ligand with low or negligible partition and which would be readily displaced by the neuroleptic.

Several examples of this are in Fig. 4. The extrapolated or intrinsic K value for clozapine at D4 is 1.6 nM, in agreement with the Kd value of 1.5 nM as directly measured with [3H]clozapine at D4 (see Fig. 4). This value also agrees with that found (30, 31) for a D4-like site labelled by [3H]clozapine in rat frontal cortex. The present clozapine intrinsic K value of 1.5–1.6 nM is significantly lower than the value of 6 nM reported for a [3H]clozapine binding site in rat brain membranes (32).

The identical values of the extrapolated intrinsic K and
the dissociation constant, $K_d$, as determined directly using the $[^3H]$neuroleptic, holds for $[^3H]$chlorpromazine, $[^3H]$clozapine, $[^3H]$haloperidol and $[^3H]$sertindole (Fig. 4). The $K_d$ values of these $[^3H]$neuroleptics are shown at a low partition (zero). This is because the $[^3H]$ligand does not compete with any other compound for binding to the receptor.

A summary of the intrinsic $K$ values for 17 neuroleptics at the dopamine D2 (cloned)-, D4 (cloned) and serotonin 2A (rat cortex)-receptors is given in Table 3.

Table 3 indicates that the neuroleptics fall into two groups, those that bind “loosely” and have high intrinsic $K$ values at D2 (between 30 and 88 nM) and those that bind tightly to D2 with low intrinsic $K$ values of 0.3 to 6 nM.

**Clozapine therapeutic concentration can be derived from the intrinsic $K$ at D4**

An example of the usefulness of the intrinsic $K$ is that it may be used to derive the therapeutic concentration of a neuroleptic. For example, although Fig. 4 shows that clozapine has an intrinsic $K$ of 1.6 nM at the dopamine D4-receptor, clozapine in vivo must compete with endogenous dopamine in the synapse (of the order of 10 nM, Ref. 33). Thus, the in vivo concentration of clozapine for 50% occupation of dopamine D4-receptors is approximately equal to $iK \cdot (1 + D/[6.2 \text{nM}])$ or 4.2 nM, where $iK$ is the intrinsic $K$ of 1.6 nM for clozapine (Fig. 2), $D$ is the synaptic concentration in the order of 10 nM, and 6.2 nM is the dissociation constant of dopamine at the high-affinity state of the dopamine D4-receptor (Table 2 in Ref. 34).

This approximate synaptic concentration of 4.2 nM clozapine, however, only applies for the blockade of 50% of the D4-receptors. Hence, the synaptic concentration of clozapine required to block 75% of the D4-receptors will be three times higher, or about 13 nM. [Actually, the clinical requirement to block 75% of the dopamine receptors to achieve antipsychotic action applies to the occupation of D2-receptors (35). The percent occupancy of D4-receptors required for the clinical control of psychotic symptoms is not yet known.]

This predicted in vivo concentration of 13 nM clozapine, however, only applies for the blockade of 50% of the D4-receptors. Hence, the synaptic concentration of clozapine required to block 75% of the D4-receptors will be three times higher, or about 13 nM. [Actually, the clinical requirement to block 75% of the dopamine receptors to achieve antipsychotic action applies to the occupation of D2-receptors (35). The percent occupancy of D4-receptors required for the clinical control of psychotic symptoms is not yet known.]

This predicted in vivo concentration of 13 nM clozapine for 75% occupation of dopamine D4-receptors compares to an observed value in the plasma water or spinal fluid of treated patients of between 12 and 20 nM (36, see Refs. and analysis in Ref. 19), using 1.85% as the proportion of free (unbound) clozapine in the plasma (Table 1 in Ref. 19).

These considerations (as well as those below in Parkinson’s disease) provide further support for the idea that clozapine is clinically operative at the dopamine D4-receptor, despite the fact that clozapine is known to bind to many receptors.

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**Table 2. Human serotonin receptors and variants**

<table>
<thead>
<tr>
<th>Receptors and variants</th>
<th>Amino acids</th>
<th>Characteristics</th>
<th>% Prevalence</th>
<th>Refs.</th>
</tr>
</thead>
<tbody>
<tr>
<td>S1A</td>
<td>422</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S1A I(28)V</td>
<td>422</td>
<td>Isoleucine replaced by valine at 28</td>
<td>2.7%</td>
<td>101, 102</td>
</tr>
<tr>
<td>S1A R219L</td>
<td>422</td>
<td>Arginine replaced by leucine at 219</td>
<td>~3%</td>
<td>135, 136</td>
</tr>
<tr>
<td>S1D</td>
<td>377</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
| S1D
| S1D<br> F124C           | 390         | Phenylalanine replaced by cysteine at 124 in TM 3 | 2% | 104–107 |
| S1E (=S31)             | 365         |                |             | 137   |
| S1F                    | 366         |                |             |       |
| S2A                    | 471         |                |             |       |
| S2B                    | 481         |                |             |       |
| S2C (=S1C)             | 458         | Cysteine replaced by serine at 23 in TM 1 | 13% | 111 |
| S2C C23S               | 458         |                |             |       |
| S4 rat long            | 406         |                |             |       |
| S4 rat short           | 387         |                |             |       |
| S5A                    | 357         |                |             |       |
| S5B mouse              | 370         |                |             |       |
| S6                     | 440         |                |             | 114   |
| S7                     | 445         |                |             | 116   |

$TM =$ hydrophobic transmembrane region.
Deriving the clozapine therapeutic concentration in L-DOPA psychosis in Parkinson's disease

Another example of the usefulness of the intrinsic $K$ is in Parkinson's disease. In excellent agreement with the findings of Meltzer et al. (37), who measured plasma clozapine in Parkinson's patients who had become psychotic on L-DOPA, the clozapine concentration (in the plasma water or spinal fluid) for 75% blockade of dopamine D4-receptors can be derived to be approximately 1.7 nM (using the above equation, where the endogenous dopamine concentration, $D$, is known to be less than 5% of normal). This value is in agreement with the value of approximately 1.2 nM found by Meltzer et al. (37), after allowance is made for clozapine binding to plasma proteins (see above).

Clozapine occupation of dopamine receptors, as seen by positron tomography

A third important example of the principle shown in Fig. 4 is the resolution of different positron tomography
Fig. 4. Using the type of data shown in Fig. 3, the dissociation constant (K value) for a neuroleptic at a given receptor depends on the tissue/buffer partition of the [3H]ligand. Extrapolating down to the intercept yields the intrinsic dissociation constant (iK value). This iK value represents the K value for the neuroleptic in the absence of any competing [3H]ligand, and is, therefore, referred to as the intrinsic dissociation constant. The [3H]ligands for the serotonin 5HT2A receptor (rat cerebral cortex) were [3H]ketanserin (*K) and [3H]spiperone (*S). The [3H]ligands for the human cloned D2-receptor were [3H]nemonapride (*N), [3H]spiperone (*S) or [3H]raclopride (*R). The [3H]ligands for the human cloned D4-receptor were [3H]nemonapride (*N), [3H]spiperone or [3H]Sandoz GLC756 (*G). The data using either D2short (118; in GH4C1 cells, Ref. 120) or D2long (Ref. 121; in COS-7 cells) yielded identical K values for each neuroleptic. The [3H]ligands for the cloned human D4-receptor (98, 99) were [3H]nemonapride, [3H]spiperone or [3H]Sandoz GLC756 (*G) (122). The data using either D4.2 or D4.7 (99) yielded identical K values for each neuroleptic. The number of independent measurements is given beside each point. The tissue/buffer partition coefficients for the [3H]ligands were for the postmortem human caudate nucleus (see Fig. 3), except that for *K and *S which were based on the rat cerebral cortex. Using partition coefficients based on the [3H]ligands partitioning between tissue culture cells and buffer yielded essentially similar results for the extrapolated intrinsic dissociation constants (27). Note that the dissociation constants, Kd, using [3H]clozapine, [3H]haloperidol and [3H]sertindole are identical to the extrapolated iK values for clozapine, haloperidol and sertindole, respectively. The Kd values of these [3H]-neuroleptics are shown at a low partition (zero). This is because the [3H]ligand does not compete with any other compound for binding to the receptor.
findings in the proportion of dopamine D2-receptors occupied by clozapine in humans.

The data in Fig. 4, using [3H]raclopride and [3H]spiperone, may explain why clozapine occupies 48% of the D2-receptors in patients when measured with [11C]raclopride (35, 38, 39), but between 0% and 22% when measured with [18F]methylspiperone (40) or [18F]fluoroethylspiperone (41). The in vitro data (Fig. 4), therefore, are qualitatively in agreement with the positron emission tomography findings.

Receptor bases for atypical neuroleptic action

The different receptor hypotheses, mentioned above, for the clinically atypical action of the atypical neuroleptics may be examined using the intrinsic K values.

1. “Loose” neuroleptics displaceable by endogenous dopamine

The first group are those atypical neuroleptics that have low affinity at D2 and thus may be readily displaced by high endogenous concentrations of dopamine in the caudate/putamen. This group includes remoxipride, clozapine, perlapine, seroquel and melperone, all of which have intrinsic K values in the higher range of 30 to 88 nM (Table 3). This is in contrast to most typical neuroleptics that have intrinsic K values in the lower range of 0.3 to 6 nM (Table 3). Molindone is borderline, having an intrinsic K value of 6 nM (Table 3). Of the twelve atypical neuroleptics listed by Roth et al. (42), nine have dissociation constants (using [3H]spiperone) that are between 45 and 1584 nM, suggesting that these compounds would be readily displaced at the D2-receptor by high local concentrations of endogenous dopamine in the striatum. Of the eleven typical neuroleptics tested by Roth et al. (42), ten have dissociation constants (using [3H]spiperone) that are between 0.06 and 8 nM, suggesting that these compounds would be less readily displaced at the D2-receptor by high local concentrations of endogenous dopamine in the striatum.

It should first be noted that the therapeutic concentrations (in spinal fluid or plasma water) of remoxipride and molindone are identical to the concentrations that block 50% of the dopamine D2-receptors (19, 28, 43). Thus, remoxipride and molindone are not exceptions to the general rule that therapeutic levels of neuroleptics occupy D2-receptors (with the exception of clozapine which occupies D4; Refs. 19, 28).

However, the higher range of intrinsic K values of 30 to 88 nM for these atypical drugs indicates that they are loosely attached to the D2-receptors and may, therefore, be readily displaced by endogenous dopamine. The principle of displacement of a neuroleptic by endogenous dopamine has been shown for [3H]raclopride (44, 45), [11C]raclopride (46–50), [3H]spiperone and [3H]methylspiperone (44, 45, 51), [18F]N-methylspiperone (52, 53) and [123I]iodobenzamide (49, 54, 55).

Neuroleptics with high dissociation constants have low tissue/buffer partition values and are more extensively displaced by endogenous dopamine compared to neuroleptics with low dissociation constants that have high tissue/buffer partition values (44). The above atypical neuroleptics (remoxipride, clozapine, perlapine, molindone, seroquel and melperone) would be expected to be readily displaced by endogenous dopamine. For example, 100 nM dopamine displaces about 5% of [3H]nemonapride (K -20 pM), 10% of [3H]spiperone (K -60 pM), 19% of [3H]methylspiperone (K -80 pM) and 50% of [3H]raclopride (K ~1-2 nM) (44). Hence, neuroleptics with K values above 6 nM will be markedly displaced by endogenous dopamine.

It is reasonable to expect, moreover, variations in the synaptic dopamine concentration in different brain regions, based on the different concentrations of homovanillic acid (HVA) found in these various regions. For example, the basal concentration of HVA in the rat striatum is 4 times higher than that in the limbic region (56) and 20 times higher than that in the pre-frontal cortex (57).

Hence, loosely bound neuroleptics (with high dissociation constants) would occupy more dopamine receptors in brain regions having low dopamine output (limbic regions, hypothalamus and pre-frontal cortex), but would occupy fewer dopamine receptors in regions hav-
ing high dopamine output (caudate/putamen) as a result of the neuroleptic competition with endogenous dopamine (see Fig. 5, top). Loose neuroleptics, therefore, would be expected to occupy a lower fraction of dopamine receptors in the caudate/putamen but a higher fraction in the nonstriatal regions, with corresponding fewer extrapyramidal signs compared to the typical neuroleptics with low intrinsic K values. These considerations may explain why seroquel (450 mg) occupies only 27% to 44% of the D2-receptors in patients (using \[^{11}\text{C}]\text{raclopride, Ref. 58} and why clozapine occupies only 48% of the D2-receptors in patients (using \[^{11}\text{C}]\text{raclopride; 35, 38, 39}), compared to the typical neuroleptics that occupy 70% to 80% of the D2-receptors (35, 38, 39).

Although this low D2 occupancy with seroquel or clozapine matches their loose attachment to D2, two other loose atypical neuroleptics (melperone and remoxipride) occupy 70% of the D2 sites (35).

It would appear, therefore, that some loose neuroleptics (seroquel and clozapine) reveal low D2 occupancy, while other loose neuroleptics (melperone and remoxipride) reveal high D2 occupancy. This apparent inconsistency may be resolved by considering Fig. 5 (bottom). This figure proposes two components within the 70% population of D2 sites that are not accessible to \[^{11}\text{C}]\text{raclopride}. One component consists of D2 sites occupied by the nonradioactive neuroleptic (N). The second component of D2 sites is that which is occupied by endogenous dopamine (D). It is proposed that both loose and tight neuroleptics may prevent 70% of the D2 sites from being occupied by \[^{11}\text{C}]\text{raclopride}, except that the proportion of the two components may vary.
2. Combined block of D2- and muscarinic receptors

A second small group of two atypical neuroleptics, clozapine and thioridazine, strongly block both D2- and muscarinic receptors. Clozapine, for example, is of the order of 20- to 50-fold more potent in blocking muscarinic acetylcholine receptors than blocking dopamine D2-receptors (see Refs. in Ref. 60). Clozapine blocks muscarinic receptors at about 15 nM. Because anticholinergic drugs have an anti-Parkinson action, it might appear that the low value of 15 nM may readily account for the atypical action of clozapine. However, isoclozapine is equally anticholinergic (K of 11 nM, Ref. 61), yet elicits catalepsy in animals at low doses, in contrast to clozapine. Moreover, it has been argued that the combination of antagonists for dopamine (i.e., neuroleptic) and acetylcholine (i.e., benztpine) is not as effective in minimizing parkinsonism as clozapine itself (62).

Thioridazine also blocks muscarinic receptors at about the identical concentrations that it blocks D2-receptors (see Refs. in Ref. 60). Thus, the relatively low level of parkinsonism caused by thioridazine may stem from its anticholinergic action.

3. Combined block of D2- and serotonin S2A-receptors

A third mechanism that may account for the clinically atypical action of atypical neuroleptics is that these drugs may simultaneously block both D2- and serotonin S2A-receptors (22–25).

The blockade of serotonin receptors increases the release of dopamine, as measured indirectly by the fall in [3H]raclopride binding to D2-receptors (63–65; see additional Refs. on dopamine-serotonin interactions in Ref. 21). In turn, therefore, the increased release of endogenous dopamine displaces some of the neuroleptic from the D2-receptors, thereby partly alleviating the parkinsonism caused by the D2 blockade.

This mechanism (of enhancing dopamine release) may explain the modest alleviation of neuroleptic-induced catalepsy (in rats) by ritanserin, a serotonin antagonist (66). This alleviation only occurs, however, if the catalepsy is submaximum (66), but not if the catalepsy is maximum as produced by a relatively high dose of haloperidol (67, 68).

Ritanserin has been reported to alleviate neuroleptic-induced parkinsonism and akathisia in patients (69, 70). However, ritanserin does not alleviate haloperidol-induced dystonia in monkeys, unlike clozapine, which is very effective in reversing this extrapyramidal syndrome (71–74).

A more effective alleviation of neuroleptic-induced catalepsy is produced by 8-hydroxy-2-dipropylamino-tetralin (8-OH-DPAT), a serotonin S1A agonist (67, 75–80). There is mixed evidence, therefore, supporting the concept of a balanced block of D2- and serotonin S2A-receptors to account for the low level or absence of parkinsonism by clozapine and other atypical neuroleptics.

In order to investigate this important D2/S2A block hypothesis further, the above intrinsic K values (Table 3) may be used for these two receptors. The intrinsic K values at the S2A-receptor are approximately the same as those reported by others for the neuroleptic dissociation constants at this receptor, using [3H]ketanserin (81, 82).

In principle, the ratio of the neuroleptic intrinsic K values for these two receptors should be related to the dose that elicits either parkinsonism in patients or catalepsy in rats. Such doses vary considerably, depending on how the parkinsonism or the catalepsy is measured.

For purposes of testing the D2/S2A hypothesis, therefore, the catalepsy doses as obtained in a single laboratory are more useful. Figure 6 (top), therefore, attempts to relate the intrinsic K values for the D2/S2A ratio with the rat catalepsy doses for various neuroleptics using catalepsy doses from a single laboratory (83) wherein the same criteria were used to measure catalepsy for all the neuroleptics tested.

In examining Fig. 6 (top), it is important to note that clozapine and isoclozapine have almost identical selectivity for the S2A-receptor (compared to D2). Nevertheless, isoclozapine elicits catalepsy at about 3 mg/kg, while clozapine does not produce catalepsy at 100 mg/kg.

There is a trend toward a correlation between the rat catalepsy doses and the D2/S2A ratios of the intrinsic K values, as indicated by the dashed line in Fig. 6. This trend would agree with other studies which have found a difference between typical and atypical neuroleptics in their relative occupancy of D2- and S2-receptors (84–87).

Drug selectivity for receptors is ligand-dependent: The data for olanzapine (Fig. 4) show that olanzapine is selective for the D4-receptor (compared to D2 or S2A), when using the intrinsic K values. However, if only the data for olanzapine using [3H]spiperone were considered, then olanzapine would be viewed as selective for the serotonin S2A-receptor (see Fig. 4).

The receptor selectivity of haloperidol and isoclozapine (Fig. 4) are also dependent on the ligand considered. Clozapine, the most atypical neuroleptic, is also potent at many other receptors, including serotonin 5HT1C (88, 89), S5- and S6-receptors (32, 42, 90), and a1-adrenoceptors (91), but not potent at serotonin S3-receptors (92). It is possible, therefore, that such other receptor sites contribute to the atypical action of clozapine.

4. Selective block of dopamine D4-receptors

A fourth possible mechanism for atypical neuroleptic
action may be the selective blockade of dopamine D4-receptors. As shown in Fig. 6, there is a relation between the neuroleptic doses for rat catalepsy and the D2/D4 ratio of the intrinsic K values.

A feature of the data in Fig. 6 is that clozapine and isoclozapine are considerably different in their values for the D2/D4 ratio of intrinsic K values, in relation to their different cataleptic potencies. This is in contrast to their identical values for the D2/S2A ratios of intrinsic K values, as noted above.
Roth et al. (93) have also found that perlapine, olanzapine and clozapine are selective for the dopamine D4-receptor, compared to the D2-receptor.

Although sertindole is only now beginning to be tested in large numbers of patients, it has been reported that sertindole does not elicit parkinsonism (94). The low level or absence of extrapyramidal signs with sertindole appears related to its ability to block D4-receptors more selectively than D2-receptors (Fig. 4 and Table 3).

In summary, neuroleptics that elicit relatively low levels of parkinsonism are either loose at D2 or selective for D4.

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