Short Communication

Antiparkinson Therapeutic Potencies Correlate With Their Affinities at Dopamine D2<sup>High</sup> Receptors

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KEY WORDS Parkinson’s disease; dopamine D2 receptor; high-affinity state of D2; [3H]domperidone; N-propylnorapomorphine

ABSTRACT To determine whether antiparkinson dopamine agonists preferentially act on the high-affinity or the low-affinity states of dopamine D1 and D2 receptors, the agonist potencies were obtained by competition against [3H]SCH23390 for D1<sup>High</sup> and D1<sup>Low</sup>, and against [3H]domperidone for D2<sup>High</sup> and D2<sup>Low</sup>. N-propylnorapomorphine and cabergoline were the most potent at D2<sup>High</sup>, with dissociation constants of 0.18 and 0.36 nM, respectively. Other agonists had D2<sup>High</sup> K<sub>v</sub> values of 0.52 nM for quinagolide, 0.6 nM for (+)PHNO, 0.9 for bromocriptine, 1.8 nM for apomorphine, 2.4 nM for pergolide, 3 nM for quinpirole, and 6.2 nM for lergotrile. There was a clear correlation between the K<sub>v</sub> values at D2<sup>High</sup> and their therapeutic concentrations in the plasma water, as derived from the known concentrations after correction for the fraction bound to the human plasma proteins. The data suggest that D2<sup>High</sup> is the primary and common target for the antiparkinson action of dopamine agonists. Bromocriptine, cabergoline, lergotrile, pergolide, and pramipexole had no affinity for D1<sup>High</sup>, consistent with the clinical observations that the D2-selective bromocriptine and pramipexole elicit low levels of dyskinesia. Synapse 61:1013–1018, 2007. © 2007 Wiley-Liss, Inc.

INTRODUCTION

Dopamine receptors can exist either in a form of high affinity for dopamine, D2<sup>High</sup>, or in a form of low affinity for dopamine, D2<sup>Low</sup> (George et al., 1985; McDonald et al., 1984). In order to develop better antiparkinson medications, it is helpful to know which state of the dopamine receptor is related to the therapeautic action of the dopamine agonists.

Because the therapeutic concentrations of dopamine agonists that suppress the release of prolactin are the same as those that act at D2<sup>High</sup>, the high-affinity state of the D2 receptor, D2<sup>High</sup> in the anterior pituitary gland is considered to be the functional state of the receptor (George et al., 1985; McDonald et al., 1984). However, when considering antiparkinson drugs, it is not known whether the therapeutic concentrations of these drugs selectively prefer the high or low-affinity states of the dopamine D1 or D2 receptors (Foley et al., 2004; Guttmann and Jaskolka, 2001; Jenner, 2003; Rascol et al., 2001; Seeman et al., 2005; Vermeulen et al., 1994).

In addition to the therapeutic relevance of the high- and low-affinity states of dopamine receptors, it is known that D2<sup>High</sup> receptors are increased in all known animal models of dopamine supersensitivity (Seeman et al., 2006a, 2007a,b). Although the condition of behavioral dopamine supersensitivity occurs in Parkinson’s disease (Lee et al., 1978), the molecular basis of this supersensitivity is not known (Seeman et al., 2007b). If the basis of supersensitivity can be related to one of the dopamine receptor states, it may...
further assist in improving drug development for Parkinson’s disease.

Despite many reports on the affinities of various dopamine agonists for D1\textsuperscript{High} and D2\textsuperscript{High} receptors (De Keyser et al., 1995; Lahti et al., 1992; Lidow et al., 1989; Mierau et al., 1995; Miyagi et al., 1996; Seeman et al., 2005), there is no single set of affinities from one source that can be used to determine the selectivity of the drugs at the D1\textsuperscript{High} and D2\textsuperscript{High} receptors. One of the technical difficulties in measuring the affinities of dopamine agonists is that [\textsuperscript{3}H]spiperone has been used as a ligand for the D2 receptor (Seeman et al., 1984). This ligand, however, is very fat-soluble and adheres tightly to the D2 receptor, thereby yielding artifactual high dissociation constants, higher than those with the more water-soluble ligand [\textsuperscript{3}H]raclopride (Seeman and Van Tol, 1995). In fact, when competing various dopamine agonists against the [\textsuperscript{3}H]spiperone ligand, the separation between the high- and low-affinity components is not obvious and requires computer-assisted separation with inherent assumptions (Seeman et al., 1985).

Recently, however, it has been found that the separation between the high- and low-affinity components for the D2 receptor is much more reliably and clearly demarcated when using [\textsuperscript{3}H]domperidone as a ligand for the D2 receptor (Seeman et al., 2003). Therefore, using [\textsuperscript{3}H]domperidone to label D2 receptors, the present study was done to provide a single set of affinities for the dopamine agonists at D2\textsuperscript{High} and D2\textsuperscript{Low}. We here report that the therapeutic drug potencies correlate with their affinities for the dopamine D2\textsuperscript{High} receptors.

\section*{MATERIALS AND METHODS
Tissue preparation}

Rat striata were used from either CO\textsubscript{2}-euthanized Sprague–Dawley rats or from purchased frozen rat brains (Pel-Freez Biologicals, Rogers, AR). The brains were stored at −70°C until use. The brain was partly thawed and the striata removed. The striata were homogenized in buffer (4 mg frozen tissue per ml buffer), using a teflon-glass homogenizer (with the piston rotating at 500 rpm) and 10 up and down strokes of the glass container. The buffer contained 50 mM Tris–HCl (pH 7.4 at 20°C), 1 mM EDTA, 5 mM KCl, 1.5 mM CaCl\textsubscript{2}, 4 mM MgCl\textsubscript{2}, and 120 mM NaCl. The homogenate was not washed, centrifuged, or preincubated, because previous work found that 30–50% of the D2 receptors were lost by these procedures (Seeman et al., 1984).

\section*{Agonist/[\textsuperscript{3}H]ligand competitions}

The dopamine D2 receptors in the striatum were measured with [\textsuperscript{3}H]domperidone (2 nM final concentration; custom-synthesized as [phenyl-\textsuperscript{3}H(N)]domperidone; 68 Ci/mmol; PerkinElmer Life Sciences, Boston, MA; Seeman et al., 2003). Each incubation tube (12 × 75 mm\textsuperscript{2}, glass) received, in the following order, 0.5 ml buffer (with or without a final concentration of 10 μM S-sulpiride to define nonspecific binding to the dopamine D2 receptors), 0.25 ml [\textsuperscript{3}H]domperidone, and 0.25 ml of tissue homogenate. The tubes, containing a total volume of 1 ml, were incubated for 2 h at room temperature (20°C), after which the incubates were filtered using a 12-well cell harvester (Titertek, Skatron, Lier, Norway) and buffer-presoaked glass fiber filter mats (Whatman GF/C). After filtering the incubate, the filter mat was rinsed with buffer for 15 s (7.5 ml buffer). The filters were pushed out and placed in scintillation minivials (7 ml, 16 × 54 mm\textsuperscript{2}; Valley Container, Bridgeport, CT). The minivials received 4 ml each of scintillant (Research Products International, Mount Prospect, IL), and were monitored 6 h later for tritium in a Beckman LS5000TA scintillation spectrometer at 55% efficiency. The specific binding of [\textsuperscript{3}H]domperidone was defined as total binding minus that in the presence of 10 μM S-sulpiride. The competition data were analyzed as previously described (Seeman et al., 1985, 2003); the program provided two statistical criteria to judge whether a two-site fit was better than a one-site fit, or whether a three-site fit was better than a two-site fit.

The competition between dopamine and [\textsuperscript{3}H]SCH23390 for binding at the dopamine D1 receptors was done by the same method as that for [\textsuperscript{3}H]domperidone, except that the final concentration of [\textsuperscript{3}H]SCH23390 (71 Ci/mmol; PerkinElmer Life Sciences) was 0.6–1 nM. Nonspecific binding was defined by the presence of 1 μM (+)butaclamol. Independently, the Cheng–Prusoff equation (Cheng and Prusoff, 1973) was also used to derive the dissociation constants (K\textsubscript{i} values) of the dopamine agonist from the concentration that inhibited 50% of the high-affinity component (IC\textsubscript{50%}) or 50% of the low-affinity component for [\textsuperscript{3}H]domperidone and [\textsuperscript{3}H]SCH23390, as indicated in the results. The form of the Cheng–Prusoff equation used was K\textsubscript{i} = IC\textsubscript{50%}/(1 + C\textsuperscript{*}/K\textsubscript{d}), where C\textsuperscript{*} was the final concentration of the radioligand and K\textsubscript{d} was the dissociation constant of [\textsuperscript{3}H]SCH23390 (K\textsubscript{d} = 0.5 nM) and of [\textsuperscript{3}H]domperidone (K\textsubscript{d} = 0.43 nM), as determined directly by saturation binding (i.e., Scatchard plot) to the striatal homogenate.

\section*{Drugs}

The following drugs were generously provided by pharmaceutical companies: pramipexole monohydrate by Boehringer Ingelheim Pharma GmbH, KG; (+)PHNO [originally designated and donated as L-647.339-007N011 VII-15(+)] by Merck Sharp and Dohme Research Laboratories, West Point, PA, and
goline (PNU-142,799, for which we thank Dr. Stevin Zorn and Stodola) by Pfizer, Groton, CT.

RESULTS AND DISCUSSION

The dissociation constants of various agonists, including dopamine, as well as the proportion of D1High and D2High receptors in the rat striatal homogenate were measured by competition versus [3H]SCH23390 and [3H]domperidone, respectively. For example, dopamine inhibited the binding of [3H]SCH23390 and [3H]domperidone with dissociation constants averaging 30 ± 10 nM at D1High and 6.1 ± 3.5 nM at D2High, as shown in Figure 1 (top) and Table I. The proportion of D1 receptors in the D1High state was consistently 10–12%, as recognized by dopamine, while the proportion of D2 receptors in the D2High state was generally 15–20% (see Fig. 1, top).

The dissociation constants of apomorphine were 4.6 ± 1.2 nM at D1High and 1.8 ± 0.9 nM at D2High (Fig. 2, middle; Table I). The latter value at D2High compares with 0.5 ± 0.3 nM for D2High for the human cloned D2Long receptor (Seeman et al., 2005). Overall, these values indicated that apomorphine was several fold more potent than dopamine at D1 and D2 receptors.

N-propylnorapomorphine (NPA) was even more potent than apomorphine, with dissociation constants of 1 ± 0.2 nM at D1High and 0.18 ± 0.03 nM at D2High, as shown in Figure 1 (bottom) and Table I. The present Kᵢ value of 0.18 nM at D2High compares with 0.09 nM for the human cloned D2Long receptor (Seeman et al., 2005). The higher potency of NPA at D2High, compared with apomorphine, is consistent with its higher clinical potency.

(+)-PHNO was highly selective for D2High, with a Kᵢ value of 0.6 ± 0.3 nM, compared with its Kᵢ value of 38 ± 7 nM at D1High (Fig. 2, top; Table I).

Bromocriptine, pramipexole, pergolide, and (±)-quinagolide were unusual, however, insofar as they did not recognize a high-affinity state for D1, but did recognize D2High with Kᵢ values of 0.9 ± 0.2, 4.7 ± 1.9, 2.4 ± 0.8, and 0.52 ± 0.16 nM, respectively (Figs. 2 and 3; Table I).

Also surprising was the observation that (−)-quinpirole, a drug frequently used for its apparent selectivity for D2, actually recognized D1High at molarities only fourfold higher than at D2High (Fig. 3, top; Table I).

Clinical correlation

Using the Kᵢ values in Table I, it is possible to relate them to the clinical approximate molarities found in the plasma water of patients being treated with the dopamine agonists. The concentrations of the antiparkinson drugs in the plasma water have

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Rahway, NJ; apomorphine(−)-HCl by Merck Frosst Laboratories, Montreal (Quebec, Canada); N-propylnorapomorphine(−)-HCl by Research Biochemicals, Wayland, MA; pergolide mesylate (LY 127,809), lergotrile mesylate, and quinpirole.HCl (LY 171,555) by Lilly Research Laboratories, Indianapolis, IN; bromocriptine mesylate and (±)-quinagolide.HCl (CV205, 502; for which we thank Dr. Hans Kalkman) by Novartis Pharma AG, Basel, Switzerland; and cabergoline (PNU-142,799, for which we thank Dr. Stevin Zorn and Stodola) by Pfizer, Groton, CT.

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**TABLE I.** Dopamine agonist potencies at D1 and D2 receptors

Dissociation constants (nM) for rat striata

<table>
<thead>
<tr>
<th></th>
<th>(K_{i \text{High}})</th>
<th>(K_{i \text{Low}})</th>
<th>(K_{i \text{High}})</th>
<th>(K_{i \text{Low}})</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
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</tr>
<tr>
<td>Adrenaline</td>
<td>36 ± 4.5</td>
<td>6260 ± 2900</td>
<td>11 ± 2</td>
<td>1500 ± 460</td>
</tr>
<tr>
<td>Apomorphine-R-(-)-HCl</td>
<td>4.6 ± 1.2</td>
<td>652 ± 210</td>
<td>1.8 ± 0.9</td>
<td>98 ± 64</td>
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<tr>
<td>Bromocriptine base</td>
<td>No D1\text{high}</td>
<td>2040 ± 350 ((K_{i 50%}))</td>
<td>0.9 ± 0.2</td>
<td>51 ± 6</td>
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<tr>
<td>Cabergoline</td>
<td>No D1\text{high}</td>
<td>3800 ± 2000 ((K_{i 50%}))</td>
<td>0.36 ± 0.02</td>
<td>560 ± 150</td>
</tr>
<tr>
<td>Dopamine</td>
<td>30 ± 10</td>
<td>5105 ± 2260</td>
<td>6.1 ± 3.5</td>
<td>3650 ± 1600</td>
</tr>
<tr>
<td>Lergotrile</td>
<td>No D1\text{high}</td>
<td>335 ± 44 ((K_{i 50%}))</td>
<td>6.2 ± 4.5</td>
<td>428 ± 48</td>
</tr>
<tr>
<td>Noradrenaline</td>
<td>224 ± 63</td>
<td>6300 ± 3000</td>
<td>9.8 ± 3.5</td>
<td>945 ± 68</td>
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<tr>
<td>N-propyl-norapomorphine-R(—)</td>
<td>1 ± 0.2</td>
<td>492 ± 220</td>
<td>0.18 ± 0.03</td>
<td>54 ± 20</td>
</tr>
<tr>
<td>(+)-PHNO</td>
<td>38 ± 7</td>
<td>4356 ± 625</td>
<td>0.6 ± 0.3</td>
<td>144 ± 80</td>
</tr>
<tr>
<td>Pergolide mesylate</td>
<td>No D1\text{high}</td>
<td>1290 ± 210 ((K_{i 50%}))</td>
<td>2.4 ± 0.8</td>
<td>270 ± 90</td>
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<tr>
<td>Pramipexol monohydrate</td>
<td>No D1\text{high}</td>
<td>8150 ± 150 ((K_{i 50%}))</td>
<td>4.7 ± 1.9</td>
<td>580 ± 190</td>
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<tr>
<td>Quinagolide-(-)-HCl</td>
<td>No D1\text{high}</td>
<td>7350 ± 5600 ((K_{i 50%}))</td>
<td>0.52 ± 0.16</td>
<td>304 ± 160</td>
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<tr>
<td>Quinpirole-(-)-HCl</td>
<td>No D1\text{high}</td>
<td>13 ± 2</td>
<td>10,300 ± 750</td>
<td>3 ± 2</td>
</tr>
</tbody>
</table>

All values are mean ± SE (n = 4–5 experiments).

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**Fig. 2.** Competition between dopamine agonists and \(^{3}H\)SCH23390 at dopamine D1 receptors, and between the agonists and \(^{3}H\)domperidone at dopamine D2 receptors in homogenates of rat striatum. While (+)-PHNO is selective for D2\text{high} receptors, it also recognizes D1\text{high}. Bromocriptine and pramipexole, however, do not recognize D1\text{high}, and are, therefore, highly selective for D2\text{high}. Further details as in Figure 1.

**Fig. 3.** Competition between dopamine agonists and \(^{3}H\)SCH23390 at dopamine D1 receptors, and between the agonists and \(^{3}H\)domperidone at dopamine D2 receptors in homogenates of rat striatum. Although (-)-quinpirole is about fourfold more selective for D2\text{high} receptors, compared with D1\text{high}, pergolide and (±)-quinagolide, however, do not recognize D1\text{high}, and are, therefore, highly selective for D2\text{high}. Further details as in Figure 1.

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been previously calculated from the reported concentrations in the plasma (e.g., Coleman et al., 1989; Durif et al., 1991; Frisén et al., 1980; Guttman and Jaskolka, 2001; Hoover et al., 2004; Seeman et al., 2005), and calculated for the free, unbound concentration in the plasma water (Seeman et al., 2005).

Therefore, using these approximate clinical concentrations, there is a log–log correlation between the D2\textsuperscript{High} Kᵢ values of four clinically used agonists and their drug concentrations in the plasma water, as shown in Figure 4. None of the other Kᵢ values for D1\textsuperscript{High}, D1\textsubscript{Low}, or D2\textsubscript{Low} exhibit such a correlation (data not shown). Not all the dopamine agonists used against Parkinson’s disease could be included, because there was insufficient clinical information for their therapeutic concentrations in the plasma or for the fraction bound to the human plasma proteins.

Although pramipexole and (+)-PHNO also have significant affinities for dopamine D3 receptors (Freedman et al., 1994; Guttman and Jaskolka, 2001; Narendran et al., 2006; Seeman et al., 2006b), these compounds were found to have higher affinity for the human cloned D2\textsuperscript{High} receptor (Seeman et al., 2005), as compared with the human cloned D3\textsuperscript{High} receptor (but see Freedman et al., 1994; Millan et al., 2002; Newman-Tancredi et al., 2002).

Of considerable clinical importance is the observation that bromocriptine, cabergoline, lergotrile, pergolide, and pramipexole did not have any affinity for the D1\textsuperscript{High} receptor. The high selectivity of these compounds for D2\textsuperscript{High} may underlie the clinical observations that bromocriptine and pramipexole mono-therapy elicit significantly less dyskinesia than L-DOPA treatment in animals (Pearce et al., 2004) and Parkinson patients (Holloway et al., 2004; Rascol et al., 2000). In other words, the combined action of dopamine synergistically on D1\textsuperscript{High} and D2\textsuperscript{High}, although highly effective in promoting locomotion, may lead to dyskinesia more readily than the D2\textsuperscript{High}-selective action of bromocriptine or pramipexole.

In conclusion, the present Kᵢ values for the antiparkinson dopamine agonists at the D2\textsuperscript{High} receptor builds on previous work in this field (Foley et al., 2004; Gerlach et al., 2003) and may provide an improved guide to predicting and evaluating treatment outcomes for patients on these medications.

ACKNOWLEDGMENTS

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