Pindolol Augmentation of Antidepressant Treatment: Recent Contributions from Brain Imaging Studies

Diana Martinez, Allegra Broft, and Marc Laruelle

Preclinical studies suggest that augmentation of selective serotonin (5-HT) reuptake inhibitors by the 5-HT<sub>1A</sub> receptor agent pindolol might reduce the delay between initiation of treatment and antidepressant response, an effect largely mediated by blockade of 5-HT<sub>1A</sub> autoreceptors in the dorsal raphe nuclei. Although some controlled clinical trials suggest that pindolol might reduce latency to selective serotonin reuptake inhibitor response in acute depressive episodes, the effect is moderate and highly variable. Recent positron emission tomography studies investigating the occupancy of 5-HT<sub>1A</sub> receptors in humans by pindolol have shown that at the dose used most often in clinical trials the occupancy is low and variable, which might explain the inconsistent clinical results. Positron emission tomography studies also suggest that pindolol might be more potent at blocking 5-HT<sub>1A</sub> autoreceptors than at blocking postsynaptic receptors, a property that may be useful in this pharmacologic strategy. Thus, the positron emission tomography data support the potential of pindolol to augment the antidepressant response of selective serotonin reuptake inhibitors, but also imply that this potential has not been fully evaluated. Here we review the clinical trials, the positron emission tomography studies, and the possible mechanisms of pindolol augmentation. It is also suggested that positron emission tomography may be used to define therapeutic dosing early on in the process of clinical evaluation of new treatment strategies. Biol Psychiatry 2000;48:844–853 © 2000 Society of Biological Psychiatry

Key Words: Serotonin, pindolol, major depression, PET, [11C]WAY 100635

Introduction

The antidepressant effect of the selective serotonin reuptake inhibitors (SSRIs) is thought to be mediated by enhanced serotonin (5-HT) transmission; however, initial exposure to SSRIs is associated with no change or even a decrease in extracellular 5-HT levels in the terminal fields of the forebrain (Chaput et al 1986; Gartside et al 1999; Invernizzi et al 1992; Romero et al 1996), due to stimulation of somatodendritic 5-HT<sub>1A</sub> autoreceptors in the dorsal raphe nuclei (DRN), the major source of serotonin projection fibers to the corticolimbic brain regions (for a review, see Artigas et al 1996). Electrophysiologic rodent studies have demonstrated that sustained administration of SSRIs or 5-HT<sub>1A</sub> agonists is associated with desensitization of 5-HT<sub>1A</sub> autoreceptors (Blier and De Montigny 1983; Blier and de Montigny 1985, 1987; Chaput et al 1986; Godbout et al 1991; Jolas et al 1994; Schechter et al 1990). Desensitization of 5-HT<sub>1A</sub> autoreceptors has been shown to preferentially affect the somatodendritic autoreceptor over the postsynaptic receptor (Blier and de Montigny 1987; Chaput et al 1986; Godbout et al 1991; Jolas et al 1994). Although desensitization has been demonstrated as early as 3 days following treatment (Le Poul et al 1995), the percentage of desensitized neurons reaches 60–80% at around 21 days, a time frame that approximates the time of onset of the antidepressant effect (for reviews, see Artigas et al 1996; Blier and de Montigny 1994).

The above observations led to the hypothesis that blockade of the somatodendritic 5-HT<sub>1A</sub> autoreceptor might decrease the latency to improvement (Artigas et al 1996; Blier and de Montigny 1990). Pindolol, a β-blocker with activity at the 5-HT<sub>1A</sub> receptor (Hoyer and Schoeffter 1991), was therefore proposed as an augmentation strategy in SSRI treatment, to accelerate the clinical response. This strategy has been evaluated in several clinical trials, with inconsistent results. It is noteworthy that the dose of pindolol used in these clinical trials (7.5 mg/day) was selected based on the expectation that this dose would be too low to induce significant cardiovascular side effects. Yet, the extent to which 5-HT<sub>1A</sub> receptors are blocked in humans following this dose of pindolol has only recently been characterized, using positron emission tomography (PET) and the selective 5-HT<sub>1A</sub> receptor radiotracer [11C]WAY 100635 (Andree et al 1999; Martinez et al, in press; Rabiner et al 2000). These studies indicate that the occupancy of 5-HT<sub>1A</sub> receptors achieved in clinical trials has been low and variable, and that the strategy of...
blocking 5-HT$_{1A}$ autoreceptors as an adjunctive treatment of depression has not yet been fully tested.

In this review, we will briefly discuss the preclinical evidence supporting the potential usefulness of pindolol in augmenting 5-HT system response to SSRIs. Results of clinical trials are reviewed next, followed by a summary of the recent PET studies evaluating pindolol occupancy of 5-HT$_{1A}$ receptors in humans. The implications of the brain imaging studies for further development and validation of this augmentation strategy are then discussed.

Preclinical Evidence Supporting the Rationale for Pindolol Augmentation

The pindolol augmentation strategy stems from the rationale that pindolol should potentiate the increase in 5-HT transmission induced by SSRIs in cortical and limbic areas. To produce this effect, pindolol should preferentially block the 5-HT$_{1A}$ autoreceptors in the DRN relative to the postsynaptic 5-HT$_{1A}$ receptors in corticolimbic areas. Although the augmentation of the SSRIs effect on 5-HT levels by pindolol is well supported by preclinical studies, the potential selectivity of pindolol for DRN autoreceptors remains controversial.

The coadministration of pindolol with an SSRI has been shown to either prevent the immediate reduction in 5-HT transmission or augment 5-HT levels in the terminal fields of the brain, including the dorsal striatum (Romero et al 1996), hippocampus (Hjorth 1996; Hjorth and Auerbach 1994, 1996), and hypothalamus (Dreshfield et al 1996). In the prefrontal cortex, pindolol was shown to enhance 5-HT transmission in conjunction with fluoxetine, duloxetine, and imipramine (Dawson and Nguyen 2000; Gobert and Millan 1999; Maione et al 1997), although this was not seen in a study with paroxetine (Gartsdie et al 1999). Pindolol has also been shown to attenuate the SSRI-induced reduction in the firing rate of the 5-HT neurons in some studies (Artigas et al 1996; Romero et al 1996) but not in others (Fornal et al 1999b). Pindolol also attenuates the decrease in 5-HT release induced by 5-HT$_{1A}$ agonists (Bosker et al 1994; Gobert and Millan 1999; Sharp et al 1989, 1993; Sharp and Hjorth, 1990).

For pindolol to be maximally effective, it would be important for pindolol to have, relative to the postsynaptic receptor, a preferential effect at the presynaptic receptor. In these conditions, selective blockade of the presynaptic 5-HT$_{1A}$ receptor would result in maximized 5-HT release in the terminal fields, without interfering with 5-HT transmission at the postsynaptic 5-HT$_{1A}$ receptor, as these receptors may also be involved in the antidepressant effect of increased 5-HT transmission (Lopez et al 1998; Martin et al 1990). Some preclinical studies have demonstrated a greater effect of pindolol at the 5-HT$_{1A}$ autoreceptor relative to the postsynaptic receptor. Romero et al (1996) found that pindolol failed to prevent 5-HT$_{1A}$ agonist–induced inhibition in the hippocampus, and Tada et al (1999) demonstrated that pindolol itself did not affect neuronal activity of hippocampal cells, nor did it attenuate the inhibition of neuronal activity induced by the agonist buspirone. Other studies, however, have demonstrated that pindolol does have a pharmacologic effect at the postsynaptic receptor (Hadrava et al 1995; Millan et al 1993), and Corradetti et al (1998) demonstrated that pindolol antagonizes the effects of a 5-HT$_{1A}$ receptor agonist equally in both the DRN and the hippocampus.

In vivo binding studies in rodents have also reported contradictory data. Hirani et al (2000) reported preferential inhibition of binding of the selective 5-HT$_{1A}$ receptor antagonist $[^{14}]$CWAY 106535 by various doses of (-)-pindolol (ranging from 0.001 to 3 mg kg$^{-1}$ intravenously) in the DRN in rats studied with a small animal PET scanner. The median effective dose (ED$_{50}$) of (-)-pindolol was significantly lower in the DRN (ED$_{50}$ = 0.26 ± 0.05 mg kg$^{-1}$) than in the hippocampus (0.48 ± 0.12 mg kg$^{-1}$) and the frontal cortex (0.44 ± 0.13 mg kg$^{-1}$); however, Corradetti et al (1998) reported blocking studies of $[^{1}H]$WAY 106535 binding by (-)-pindolol (15 mg kg$^{-1}$ intraoperitoneally [IP]) and showed an almost complete and equivalent binding inhibition in DRN (−76%), CA1 (−69%), CA3 (−79%), and the parietal cortex (−82%).

Finally, data from human postmortem autoradiographic binding studies are also contradictory. One study showed similar potencies of (-)-pindolol to displace $[^{1}H]$WAY 106535 in DRN and the hippocampus ($K_{i}$ (-)pindolol was 17.0 ± 5.9 nmol/L in the DRN and 17.3 ± 3.7 nmol/L in the hippocampus; Raurich et al 1999), whereas, Castro et al (1999) reported a significantly higher affinity of pindolol for the 5-HT$_{1A}$ receptors in the DRN ($K_{i}$ = 8.9 ± 2.2 nmol/L) than for the receptors in the hippocampus ($K_{i}$ = 14.4 ± 2.9 nmol/L in CA1, $p < .05$), using the same ligand ($[^{1}H]$WAY 106535) and same number of human subjects ($n = 4$). Thus, some, but not all, preclinical pharmacologic and human postmortem data support the hypothesis that pindolol might act preferentially at the autoreceptor.

Clinical Trials

Open Clinical Trials

The initial open-label clinical study by Artigas et al (1994) reported that the coadministration of pindolol with the SSRI paroxetine resulted in a decreased latency to improvement and augmentation of the antidepressant effect in refractory depression. Following this report, subsequent open-label studies continued to report both a decreased latency and augmentation in treatment-refractory patients.
Blier and Bergeron 1995; Blier et al 1997; Vinar et al 1996). Pindolol was also shown to function as an augmentation strategy with buspirone (Blier et al 1997) and nefazodone (Bakish et al 1997). On the other hand, the combination of pindolol with tricyclic antidepressant drugs devoid of effect on the 5-HT reuptake process (desipramine or trimipramine) did not appear to have an effect (Blier et al 1997). Of the open-label studies, only one reported no improvement in treatment-refractory patients (Dinan and Scott 1996).

**Double-Blind Controlled Trials**

While initial open-label clinical trials suggested that this regimen of pindolol might accelerate and/or enhance clinical response, subsequent double-blind placebo-controlled studies provided mixed results. Table 1 lists published double-blind placebo-controlled studies of pindolol augmentation of antidepressant treatment with SSRIs. When results from the same data sets were included in more than one article, only the latest article with the largest number of subjects is listed. Studies are sorted by type of SSRI and publication year; published studies include studies of pindolol versus placebo augmentation of fluoxetine \((n = 4, \text{ Berman et al 1999; Maes et al 1999; Moreno et al 1997; Perez et al, in press})\), paroxetine \((n = 3, \text{ Bordet et al 1998; Tome et al 1997; Zanardi et al 1997})\), fluvoxamine \((n = 1, \text{ Zanardi et al 1998})\), and various antidepressant drugs (Perez et al 1999). The pindolol regimen was typically 7.5 mg/day, with one study using higher doses \((15 \text{ mg/day, Bordet et al 1998})\). Duration of the trials varied from 10 days to 6 weeks. The number of subjects per study varied from 8 to 111; a total of 621 subjects were included in these studies. All studies included patients who met criteria for a major depressive episode, and inclusion criteria generally included a score greater than 18 on the Hamilton Depression Scale \((\text{HAM-D; Hamilton 1960})\). Other than these criteria, a considerable heterogeneity in clinical presentation is noted. Some studies excluded comorbid axis I diagnoses, whereas others did not. The degree to which each study included patients whose current episode showed resistance to previous treatment also varied (two studies were specifically targeted at treatment-resistant patients: Moreno et al 1997; Perez et al 1999).

Two types of clinical outcomes were evaluated. First, increased efficacy of pindolol versus placebo augmentation was tested by comparing the reduction in HAM-D or the response rate at the end point. Increased efficacy was demonstrated in three studies and was not demonstrated in six studies. Second, decreased latency was evaluated as a difference in the time to reach clinical response between groups. Decreased latency was demonstrated in five studies but not in four studies. Studies that reported decreased latency mostly included patients with recent onset of the current episode (Bordet et al 1998; Perez et al, in press; Tome et al 1997; Zanardi et al 1997, 1998). The two studies restricted to treatment-resistant patients (Moreno et al 1997; Perez et al 1999) failed to report superiority of pindolol augmentation versus placebo augmentation.

Finally, a common finding in these clinical studies is a high degree of variability in response. For example, even in the largest of the positive studies with fluoxetine the proportion of patients demonstrating a sustained response (greater than 19 days) was 38/55 in the pindolol group and 27/56 in the placebo group (Perez et al, in press). It is of note that the one study conducted with 15 mg/day of fluoxetine (Blier and Bergeron 1995; Blier et al 1997; Vinar et al 1996). Pindolol was also shown to function as an augmentation strategy with buspirone (Blier et al 1997) and nefazodone (Bakish et al 1997). On the other hand, the combination of pindolol with tricyclic antidepressant drugs devoid of effect on the 5-HT reuptake process (desipramine or trimipramine) did not appear to have an effect (Blier et al 1997). Of the open-label studies, only one reported no improvement in treatment-refractory patients (Dinan and Scott 1996).

**Table 1. Double-Blind Placebo-Controlled Clinical Trials of Pindolol Augmentation of Antidepressant Treatment**

<table>
<thead>
<tr>
<th>Reference</th>
<th>SSRI</th>
<th>SSRI dose</th>
<th>Pindolol dose</th>
<th>Duration (weeks)</th>
<th>n</th>
<th>Chronic/treatment resistant</th>
<th>Other axis I excluded</th>
<th>Increased efficacy</th>
<th>Decreased latency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moreno et al 1997</td>
<td>Fluoxetine</td>
<td>20–40 mg/day</td>
<td>7.5 mg/day</td>
<td>2</td>
<td>8</td>
<td>100%</td>
<td>No</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Maes et al 1999</td>
<td>Fluoxetine</td>
<td>20 mg/day</td>
<td>7.5 mg/day</td>
<td>5</td>
<td>21</td>
<td>70%</td>
<td>Yes</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>Berman et al 1999a</td>
<td>Fluoxetine</td>
<td>20 mg/day</td>
<td>7.5 mg/day</td>
<td>6</td>
<td>86</td>
<td>50%</td>
<td>No</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Perez et al, in pressb</td>
<td>Fluoxetine</td>
<td>20 mg/day</td>
<td>7.5 mg/day</td>
<td>6</td>
<td>111</td>
<td>—</td>
<td>Yes</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Tome et al 1997</td>
<td>Paroxetine</td>
<td>20 mg/day</td>
<td>7.5 mg/day</td>
<td>6</td>
<td>80</td>
<td>—</td>
<td>Yes</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Zanardi et al 1997</td>
<td>Paroxetine</td>
<td>20 mg/day</td>
<td>7.5 mg/day</td>
<td>4</td>
<td>63</td>
<td>—</td>
<td>Yes</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Bordet et al 1998</td>
<td>Paroxetine</td>
<td>20 mg/day</td>
<td>15.0 mg/day</td>
<td>4</td>
<td>100</td>
<td>—</td>
<td>Yes</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Zanardi et al 1998</td>
<td>Fluvoxamine</td>
<td>300 mg/day</td>
<td>7.5 mg/day</td>
<td>6</td>
<td>72</td>
<td>—</td>
<td>Yes</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Perez et al 1999</td>
<td>Fluoxetine</td>
<td>40 mg/day</td>
<td>7.5 mg/day</td>
<td>1.5</td>
<td>80</td>
<td>100%</td>
<td>Yes</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>Paroxetine</td>
<td>40 mg/day</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>Fluvoxamine</td>
<td>200 mg/day</td>
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<tr>
<td></td>
<td>Clomipramine</td>
<td>150 mg/day</td>
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</tr>
</tbody>
</table>

**Notes:**

SSRI, selective serotonin reuptake inhibitor.

Table 2. Occupancy of Corticolimbic and DRN 5-HT1A Receptors by Pindolol in Humans: PET Studies

<table>
<thead>
<tr>
<th>Reference</th>
<th>Pindolol regimen</th>
<th>Scan timing relative to dose</th>
<th>Pindolol plasma level at scan time (ng mL⁻¹)</th>
<th>Occupancy</th>
<th>Corticolimbic regions</th>
<th>DRN</th>
<th>DRN vs. cortical regions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Andree et al 1999</td>
<td>10 mg pindolol p.o. single dose</td>
<td>2 hours</td>
<td>3</td>
<td>32 ± 5</td>
<td>24% ± 5</td>
<td>16% ± 9</td>
<td>ns</td>
</tr>
<tr>
<td>Rabiner et al 2000</td>
<td>5 mg pindolol p.o. single dose</td>
<td>2 hours</td>
<td>3</td>
<td>10 ± 5</td>
<td>9% ± 5</td>
<td>10% ± 20</td>
<td>ns</td>
</tr>
<tr>
<td>Rabiner et al 2000</td>
<td>10 mg pindolol p.o. single dose</td>
<td>2 hours</td>
<td>4</td>
<td>15 ± 3</td>
<td>13% ± 8</td>
<td>37% ± 9</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Rabiner et al 2000</td>
<td>20 mg pindolol p.o. single dose</td>
<td>2 hours</td>
<td>3</td>
<td>55 ± 17</td>
<td>46% ± 10</td>
<td>39% ± 3</td>
<td>ns</td>
</tr>
<tr>
<td>Martinez et al, in press</td>
<td>7.5 mg pindolol CR QD for 7 days</td>
<td>4 hours</td>
<td>8</td>
<td>18 ± 8</td>
<td>18% ± 5</td>
<td>40% ± 29</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Martinez et al, in press</td>
<td>7.5 mg pindolol CR QD for 7 days</td>
<td>10 hours</td>
<td>8</td>
<td>7 ± 3</td>
<td>12% ± 3</td>
<td>38% ± 26</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Martinez et al, in press</td>
<td>30 mg pindolol CR single dose</td>
<td>4 hours</td>
<td>8</td>
<td>58 ± 24</td>
<td>42% ± 4</td>
<td>64% ± 15</td>
<td>&lt;.001</td>
</tr>
</tbody>
</table>

DRN, dorsal raphe nuclei; 5-HT, serotonin; PET, positron emission tomography; p.o., per os; CR, controlled release; QD, quaque die.

Pindolol showed both increased SSRI efficacy and decreased latency period (Bordet et al 1998). With two exceptions (Perez et al 1997, in press), studies did not report pindolol plasma levels.

PET Studies

WAY-100635 [N-(2-(4-(2-methoxyphenyl)-1-piperazinyl)ethyl)-N-(2-pyridinyl) cyclohexane carboxamide] is a potent and selective 5-HT1A antagonist (Kd = 0.1–0.4 nmol/L) (Fletcher et al 1993; Forster et al 1995; Gozlan et al 1995). Labeled with carbon 11 in the carbonyl position, this ligand allows reliable quantification of 5-HT1A receptor availability in the human brain using PET (Farde et al 1998; Gunn et al 1998; Parsey et al, in press; Pike et al 1995). Three studies have measured the occupancy of the 5-HT1A Receptor achieved in humans by pindolol (Andree et al 1999; Martinez et al, in press; Rabiner et al 2000). Findings from these PET studies are summarized in Table 2.

Andree et al (1999) studied inhibition of [¹¹C]WAY 100635 binding in three male volunteers, 2 hours following the oral administration of 10 mg of pindolol. Pindolol occupancy of 5-HT1A receptors in the DRN (16% ± 9%) was not significantly different from the occupancy in temporal and frontal cortices (24% ± 5%), at a mean plasma pindolol level of 32 ng mL⁻¹.

Rabiner et al (in press) reported occupancy of 5-HT1A receptors by pindolol following oral administration of three different doses of pindolol (5 mg, 10 mg, and 20 mg), given as a single dose 2 hours before scanning. At the 5-mg dose (plasma level 10 ± 5 ng mL⁻¹), no significant occupancy was detected in either the DRN or temporal regions, whereas occupancy was seen in all regions examined (DRN, insula, and medial temporal lobe) at 10-mg and 20-mg doses. Following the 10-mg dose (plasma level 15 ± 3 ng mL⁻¹), a significantly larger occupancy was measured in the DRN (37% ± 9%) than in the cortical regions (13% ± 8%, p < .001). Following the 20-mg dose (plasma level 55.1 ± 16.8 ng mL⁻¹), the DRN occupancy (39% ± 3%) was similar to the cortical occupancy (46% ± 10%, p = .24). Rabiner et al (2000) also evaluated the 5-HT1A receptor occupancy of two other beta blockers, penbutolol and tertatolol, which have also been shown to exhibit 5-HT1A activity and to enhance the effect of paroxetine on 5-HT in the frontal cortex (Gartside et al 1999). Penbutolol achieved significant occupancy of 5-HT1A receptors at both doses tested (40 mg and 80 mg), with no differences between DRN and corticolimbic 5-HT1A receptors. Tertatolol did not significantly affect [¹¹C]WAY 100635 binding at the doses tested (5 mg and 10 mg).

Martinez et al (in press) studied eight male subjects under four conditions: at baseline (scan 1), then following 5 days of 7.5-mg controlled release pindolol (scan 2 at 4 hours after dose and scan 3 at 10 hours after dose), and 4 hours following an increase of the oral dose to 30 mg (scan 4). Pindolol occupancy of all brain regions examined (dorsolateral, medial, orbitofrontal, and subgenual prefrontal cortices; anterior cingulate; parietal, occipital, insular, and medial temporal cortices; and DRN) was significant (20% ± 8% at scan 2, 14% ± 8% at scan 3, and 44% ± 8% at scan 4 at plasma pindolol concentrations of 18 ± 8 ng mL⁻¹, 7 ± 3 ng mL⁻¹, and 58 ± 24 ng mL⁻¹, respectively). Occupancy in the DRN was significantly higher than occupancy in all other regions at each dose. Dorsal raphe nuclei occupancy was 40% ± 29% on scan 2, 38% ± 26% on scan 3, and 64% ± 15% on scan 4 compared to an average occupancy in all other regions of 18% ± 5% on scan 2, 12% ± 3% on scan 3, and 42% ± 4% on scan 4. Analysis of the relationship between pindolol plasma level and 5-HT1A occupancy in scans 2 and 4 revealed maximal effect values (± SE) of 95% ± 23% in the DRN and 53% ± 17% in postsynaptic regions and concentration at the half maximal response values of 24 ± 15 ng mL⁻¹ for the DRN and 21 ± 18 ng mL⁻¹ in postsynaptic regions. This result suggests that the sites that display high affinity for pindolol corresponded to the

The table includes data from four studies: Andree et al 1999, Rabiner et al 2000, Martinez et al, in press, and Rabiner et al (in press). The table summarizes the occupancy of 5-HT1A receptors in the DRN and cortical regions at different pindolol doses and plasma levels. The results show a significant increase in occupancy with higher doses, particularly in the DRN.
whole population of 5-HT\textsubscript{1A} receptors in the DRN and only about half of the 5-HT\textsubscript{1A} receptors in the postsynaptic areas.

In summary, PET studies demonstrate a significant occupancy of 5-HT\textsubscript{1A} receptors in humans by pindolol but also suggest that the occupancy resulting from the regimens used in clinical trials (2.5 mg thrice daily) is low and highly variable between subjects. Higher occupancy and lower variability are observed at large doses.

Differences in DRN versus corticolimbic occupancy have not been consistently reported by PET studies (Table 2). These differences might be related to differences in experimental design and imaging protocol. Martinez et al (in press) measured occupancy following sustained administration of a controlled release formulation of pindolol, rather than following a single dose of pindolol (Andree et al 1999; Rabiner et al 2000). The duration of the scans varied and data were analyzed using different methods; however, none of these differences are suspected a priori to bias the comparison of the DRN versus cortical occupancy. More likely, the failure to observe DRN selectivity in the study of Andree et al (1999) and in the high dose used by Rabiner et al (2000) might be due to the noise associated with the measurement of DRN \([^{11}C]\)WAY 100635 binding potential (BP) and the small number of subjects (three to four) included in these two studies.

Lastly, it is important to note that the partial volume effect will influence \([^{11}C]\)WAY 100635 BP measurements more in the DRN than in corticolimbic regions, given the difference in the respective sizes of these regions. Yet, the magnitude of the partial volume effect is dependent only on the volume of the structure and not on the level of activity (Kessler et al 1984). Therefore, the partial volume effect would be expected to affect both the baseline and pindolol occupancy scans equally, and would not explain the greater pindolol-induced decrease in DRN binding relative to corticolimbic regions.

**Potential Mechanisms Underlying Pindolol DRN Selectivity**

The apparent relative selectivity of pindolol for DRN 5-HT\textsubscript{1A} autoreceptors might result from the fact that pindolol is a weak agonist rather than a pure antagonist. Pindolol was initially characterized as an antagonist at the 5-HT\textsubscript{1A} receptor, given that it was shown to have no effect on 5-HT levels (Hjorth and Sharp 1990, 1993; Romero et al 1996; Sharp et al 1989) or was reported to increase extracellular 5-HT in the prefrontal cortex (Maione et al 1997), hippocampus (Assie and Koek 1996; Bosker et al 1994; Gur et al 1997; Matos et al 1996), and caudate (Fornal et al 1999a), as well as the DRN itself (Maione et al 1997; Matos et al 1996), via blockade of the 5-HT\textsubscript{1A} autoreceptor. More recent studies, however, have indicated that pindolol acts as a partial agonist at the 5-HT\textsubscript{1A} autoreceptor. Pindolol alone has been shown to inhibit the cells of the DRN (Clifford et al 1998; Fornal et al 1999c; Haddjeri et al 1999), decrease 5-HT in the frontal cortex (Gobert and Millan 1999), and decrease 5-HT synthesis and metabolism (Hjorth and Carlsson 1986). Newman-Tancredi et al (1998) demonstrated that pindolol produces an increase in guanosine-5'-O-(3-[^35]S]thio)-triphosphate binding in cells transfected with the human 5-HT\textsubscript{1A} receptor, but that the increase is only 20% that of induced by 5-HT, consistent with weak partial agonist activity.

Taking all of these findings together, it appears that the effect of pindolol as an antagonist or weak partial agonist at the 5-HT\textsubscript{1A} receptor may ultimately depend upon the local concentration of endogenous 5-HT (Gobert and Millan 1999).

The agonist nature of pindolol at the 5-HT\textsubscript{1A} receptor might account for the regional differences in binding observed in the PET studies. The binding of agonists to the 5-HT\textsubscript{1A} receptor is modulated by G protein coupling (Harrington and Peroutka 1990); the addition of GTP or its analogue guanyllyl imidodiphosphate results in a significant reduction in the percentage of 5-HT\textsubscript{1A} receptors configured in a state of high affinity for agonists (Monticelli et al 1992; Nenonene et al 1994). Thus, addition of GTP results in reduced binding of 5-HT\textsubscript{1A} agonists (Emerit et al 1990; Hall et al 1986; Monticelli et al 1992), whereas the binding of pure antagonists such as \([^3H]\)WAY 100635 is unaffected (Emerit et al 1990; Gozlan et al 1995; Khawaja et al 1995). As a partial agonist, the binding of pindolol is expected to be affected by G protein coupling and affinity state. Therefore, the preferential occupancy by pindolol of the DRN 5-HT\textsubscript{1A} receptors relative to corticolimbic 5-HT\textsubscript{1A} receptors might stem from the presence of a larger proportion of DRN 5-HT\textsubscript{1A} receptors being configured in the high-affinity state. This hypothesis is supported by preclinical data. The number of receptor sites labeled by \([^3H]\)WAY 100635 in corticolimbic areas is 36–60% higher than those labeled with agonist \([^3H]8-OH-DPAT\) (Fletcher et al 1993; Gozlan et al 1995; Khawaja 1995; Khawaja et al 1995), suggesting that only a subset of 5-HT\textsubscript{1A} receptors is in the high-affinity state in these regions. In contrast, Khawaja et al (1995) reported similar \(B_{\text{max}}\) for \([^3H]\)WAY 100635 and \([^3H]8-OH-DPAT\) in the DRN, suggesting that most of the DRN 5-HT\textsubscript{1A} receptors are configured in the high agonist affinity state.

Although differences in G protein coupling may contribute to the regional differences in occupancy, there is also evidence to support a role for cellular processes further downstream. A study by Radja et al (1992) demonstrated that receptor/G protein coupling was similar.
in the hippocampus and DRN, a finding also reported in a more recent study by Meller et al (2000). Although both receptors have been shown to be linked to pertussis toxin–sensitive Gi/Go, regulatory proteins, activation of the somatodendritic autoreceptor results in membrane hyperpolarization secondary to G protein–coupled K\(^+\) conductance, which inhibits firing and results in a reduction of 5-HT synthesis and release in the forebrain (Blier et al 1993; Hjorth and Magnusson 1988; Meller et al 2000); however, unlike the postsynaptic receptor, the presynaptic receptor does not appear to affect adenylyl cyclase activity (Clarke et al 1996; Johnson et al 1997). Furthermore, the postsynaptic receptor appears to have two populations of receptors, one that produces hyperpolarization via increased K\(^+\) conductance and another that inhibits forskolin-mediated stimulation of adenylyl cyclase activity (Meller et al 2000). The relevance of these differences between pre- and postsynaptic 5-HT\(_{1A}\) for the binding of pindolol remains to be elucidated.

**Clinical Implications of PET Data**

**Estimation of Occupancy Achieved in Clinical Trials**

To our knowledge, only two clinical studies reported plasma levels of pindolol. Perez et al (1999) measured pindolol plasma levels of 9.9 ± 5.1 (SD) ng mL\(^{-1}\) in a sample of 40 subjects treated with a combination of pindolol (2.5 mg thrice daily) and SSRIs (fluoxetine, paroxetine, fluvoxamine, or clomipramine). Slightly lower pindolol plasma values (6–7 ng mL\(^{-1}\)) were reported in a group of 55 patients under a pindolol–fluoxetine combination (Perez et al, in press). These data indicate that the pindolol plasma levels reached in clinical studies are in the 6–10 ng mL\(^{-1}\) range. Using pharmacokinetic parameters derived from Martinez et al (in press), a plasma level of 10 ng mL\(^{-1}\) is predicted to be associated with low 5-HT\(_{1A}\) occupancy (28% and 17% in the DRN and corticolimbic regions, respectively). Plasma levels of 6 ng mL\(^{-1}\) are predicted to be associated with only 19% and 12% occupancy in the DRN and corticolimbic regions, respectively. The average DRN 5-HT\(_{1A}\) receptor occupancy is thus in the 25% range in these clinical studies.

It is unclear if 25% occupancy of DRN 5-HT\(_{1A}\) receptors is enough to achieve the desired clinical effect. The minimal occupancy of DRN 5-HT\(_{1A}\) receptors needed to achieve blockade of the SSRI-induced activation of these receptors has not been well documented. In the classic study of Romero et al (1996), a (−)-pindolol dose of at least 10 mg/kg IP was required to significantly block the decrease in striatal 5-HT induced by direct application of citalopram (50 μM) in the DRN. To our knowledge, the occupancy of DRN 5-HT\(_{1A}\) receptors achieved by 10 mg/kg IP pindolol in the rat has not been reported. Yet, a dose of 15 mg/kg IP (−)-pindolol has been reported to occupy 76% of DRN 5-HT\(_{1A}\) receptors (Corradetti et al 1998), and it is likely that the occupancy following 10 mg/kg IP is in the range of 50% (i.e., higher than the 25% achieved in clinical studies). Studies combining occupancy and electrophysiologic or microdialysis measurements are needed to clarify this question.

**Between-Subject Variability**

The second practical implication of the PET studies is the large between-subject variability in both pindolol plasma levels and occupancy, particularly at the lower dose. For example, the occupancy of DRN 5-HT\(_{1A}\) receptors following 1 week of pindolol 7.5 mg/day varied from no detectable occupancy to 48% occupancy in the group studied by Martinez et al (in press). Rabiner et al (2000) also reported a greater degree of variability between subjects at the lower dose. This variability might account for some of the variability in response observed in the clinical studies. In the study by Martinez et al (in press) the variability in DRN occupancy was partially accounted for by between-subject differences in pindolol plasma levels, indicating that plasma level monitoring would be useful in clinical studies to adjust the dose to a level compatible with appropriate occupancy.

**Therapeutic Window**

The selective DRN occupancy of pindolol observed in PET studies implies the existence of a therapeutic window of pindolol plasma concentrations. The therapeutic window includes the range of pindolol concentrations at which a marked difference is reached between DRN occupancy (which should be high to potentiate SSRI effects on 5-HT transmission) and cortical occupancy (which should be low to maintain activation of postsynaptic 5-HT\(_{1A}\) receptors). Positron emission tomography studies predict that a pindolol regimen of 6.25 mg thrice daily (18.75 mg/day) would be necessary to achieve 50% occupancy of DRN 5-HT\(_{1A}\) receptors. At this dose, occupancy in corticolimbic regions would be only 32%, and the difference in occupancy between the two regions would be 18%. The difference in occupancy achieved at this dose is close to the maximal difference in occupancy, which is predicted to occur at a plasma pindolol level of 63 ng/mL (DRN occupancy of 73% and corticolimbic occupancy of 52%, difference of 21%).

**Profile of the Ideal Compound for Augmentation of SSRI Antidepressant Effects**

Assuming that selective blockade of 5-HT\(_{1A}\) autoreceptors is useful for hastening the clinical response to
SSRI, the PET data presented here provide some clues about the desired properties of compounds selected for this application. The data suggest that pindolol might not be the ideal compound, since the side effects associated with its β-adrenergic blocking properties might preclude its use in routine clinical practice at doses (15–25 mg/day) required to block 50% of the DRN 5-HT1A receptors. It is currently assumed that silent 5-HT1A antagonists would be the optimal pharmacologic agents for this application: WAY 100635 or other silent antagonists provide superior potentiation of SSRIs’ acute effects on 5-HT transmission compared with pindolol, a difference attributed to the fact that pindolol is a partial agonist (Clifford et al 1998; Gartside et al 1999; Sharp et al 1993); however, if DRN selectivity is a unique feature of partial agonists and if it is important to minimize blockade of postsynaptic 5-HT1A receptors, a weak and DRN-selective partial agonist might still be the drug of choice for this application. Positron emission tomography studies of other 5-HT1A partial agonists and silent antagonists are warranted to evaluate the relationship between pharmacologic profile and DRN selectivity. The higher the DRN selectivity of the candidate drug, the larger the therapeutic window within which DRN autoreceptors would be inhibited without interfering with 5-HT transmission at the postsynaptic receptors. Finally, given that the goal of this pharmacologic strategy is to hasten therapeutic response, appropriate plasma levels of the candidate drug should be reached rapidly, maybe using an initial loading dose. Again, PET imaging provides a unique tool to define the relationship between pharmacokinetic and therapeutic windows.

Conclusion

Recent PET imaging studies revealed that the occupancy of DRN 5-HT1A receptors achieved during clinical trials aimed at hastening or augmenting the antidepressant effects of SSRIs might have been suboptimal for achieving the desired potentiation of 5-HT transmission. This factor, as well as the important between-subject variability in occupancy, might account for the mixed results reported in double-blind, placebo-controlled studies. On the other hand, pindolol appeared to be associated with significant in vivo selectivity for DRN 5-HT1A autoreceptors, relative to corticolimbic postsynaptic receptors. This DRN selectivity is presumably desirable for potentiation of 5-HT transmission and represents an important proof of concept for the development of new 5-HT1A agents for this application. Early evaluation of new drugs with PET imaging will enable rapid screening of compounds based on DRN selectivity, and more rigorous determination of doses to be tested in clinical trials.

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