5-HT\textsubscript{1A} Agonist Potential of Pindolol: Electrophysiologic Studies in the Dorsal Raphe Nucleus and Hippocampus

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**Background:** The ability of pindolol to block 5-HT\textsubscript{1A} autoreceptors on serotonin-containing neurons in the raphe nuclei is thought to underlie the clinical reports of enhanced efficacy and rate of improvement in depressed patients treated with pindolol/selective serotonin reuptake inhibitor (SSRI) combinations. Selectivity for somatodendritic 5-HT\textsubscript{1A} autoreceptors is a crucial requirement, as blockade of postsynaptic 5-HT\textsubscript{1A} sites may jeopardize the therapeutic response. Previous investigators have probed the effects of pindolol on serotonergic dorsal raphe cell firing in animal species; here we confirm their findings and extend them to include observations on postsynaptic 5-HT\textsubscript{1A} receptors in the hippocampus.

**Methods:** Extracellular single-unit recordings were made in rats using standard electrophysiologic techniques. Firing rates of serotonin-containing neurons in the dorsal raphe nucleus and CA3 hippocampal pyramidal neurons were monitored and the effects of pindolol given alone or in combination with an SSRI (fluoxetine) or a 5-HT\textsubscript{1A} antagonist (WAY-100,635) were determined.

**Results:** Pindolol inhibited the firing rates of serotonergic dorsal raphe neurons in a dose-dependent manner. Recovery to baseline firing rates was gradual, but this inhibition could be acutely reversed by WAY-100,635. A range of pindolol doses failed to block the inhibitory effects of fluoxetine on dorsal raphe cell firing. In the hippocampus, pindolol also inhibited cell firing as a function of dose, although these effects were insensitive to WAY-100,635 treatment.

**Conclusions:** The ability of pindolol to inhibit serotonergic dorsal raphe cell firing is indicative of its agonist potential and is consistent with previous studies. The lack of observable antagonism of the SSRI-induced slowing of raphe unit activity casts doubt on the suitability of this mechanism of action to account for the positive findings in clinical studies utilizing pindolol/SSRI combinations. The 5-HT\textsubscript{1A}-independent inhibition of hippocampal CA3 cell firing by pindolol suggests that this compound invokes multiple pharmacologic actions, all of which need to be assimilated into any proposed mechanism of action.

**Introduction**

The slow rate of improvement observed clinically with antidepressant treatment has long been attributed to the desensitization of serotonin 1A (5-HT\textsubscript{1A}) autoreceptors in the raphe nuclei (Blier and de Montigny 1994; Briley and Moret 1993). The activity of these neurons, once restored to normal firing rates, allows selective serotonin reuptake inhibitors (SSRIs) to enhance firing-dependent 5-HT release in raphe projection areas. From this scenario it has been predicted that 5-HT\textsubscript{1A} receptor blockade would be useful in accelerating antidepressant activity (Artigas 1993; Blier and de Montigny 1994). As a test of this hypothesis, pindolol has been combined with SSRIs in small clinical studies due to its reported 5-HT\textsubscript{1A} antagonist properties (Artigas et al 1996; McAskill et al 1998). Claims of more rapid onset or improved efficacy in clinical depression with the pindolol/SSRI combination rely on this mechanism, with the important added feature that pindolol is selective for cell body 5-HT\textsubscript{1A} autoreceptors compared with postsynaptic 5-HT\textsubscript{1A} receptors (Romero et al 1996). Such selectivity would appear to be crucial, as significant blockade of postsynaptic sites risks loss of the therapeutic response if activation of postsynaptic 5-HT\textsubscript{1A} receptors is required for clinical activity, as is commonly thought (Blier et al 1987; de Vry 1995).

A 5-HT\textsubscript{1A} antagonist selective for cell body autoreceptors would seem to be an unlikely entity given the large degree of receptor reserve reported in the dorsal raphe (Cox et al 1993; Meller et al 1990). Full agonists occupy only a small fraction of available receptors to yield their maximal effect; partial agonists achieve the same net
result by simply occupying more sites (Sprouse 1991; Sprouse and Wilkinson 1995). It is therefore likely that some dose of pindolol will manifest agonist properties as its degree of intrinsic activity, however small, is summed over many receptors. Recent reports attest to this agonist potential in electrophysiologic recordings of dorsal raphe neurons (Clifford et al 1998; Fornal et al 1999a, 1999b, 1999c), although antagonist effects have also been claimed (Haddjeri et al 1999). The goal of our experiments was to independently characterize the agonist potential of acutely administered pindolol in the dorsal raphe and further to gauge its efficacy at postsynaptic 5-HT1A receptors. For these latter studies, pyramidal neurons of the hippocampal CA3 region were chosen for testing, as these cells are known to contain a dense concentration of 5-HT1A sites (Vergé et al 1986) and to receive a moderate input from the dorsal raphe nucleus (Vertes 1991).

**Methods and Materials**

**Electrophysiology**

Extracellular single-unit recordings were made in choral hydrate–anesthetized male Sprague–Dawley rats (250–350 g) using standard electrophysiologic techniques (Sprouse and Aghajanian 1987, 1988). Serotonin-containing neurons in the dorsal raphe nucleus were identified online by their long-duration action potentials (1–2 msec) and slow rhythmic firing rate (0.5–3.0 Hz). Cells with these characteristics have been confirmed to be 5-HT neurons by intracellular double-labeling (Aghajanian and Vandermaelen 1982). Unit activity was computed and stored in 10-sec bins triggered by individual neuronal spikes from an electronic discriminator and continuously plotted as a firing rate histogram (Spike 2, Cambridge Electronic Design, Cambridge, UK). Compounds were administered intravenously and the effects on firing rate noted.

Hippocampal CA3 pyramidal neurons were recorded and identified from their characteristic large amplitude (0.5–1.2 mV) and long duration (0.8–1.2 msec) with single action potentials alternating with complex spike discharges. Because most of these units discharge infrequently in choral hydrate–anesthetized rats, a small iontophoretic current of acetylcholine (1–6 nA) was used to activate silent or slowly discharging neurons to a physiologic firing rate (8–12 Hz). Five-barreled micropipettes, used for this purpose, were arrayed as follows: the central barrel, for recording, was filled with a 2-mol/L NaCl solution and two side barrels, for microiontophoresis, contained 5-HT creatinine sulfate (0.5 mmol/L in 200 mmol/L NaCl, pH 4) or acetylcholine (20 mmol/L in 200 mmol/L NaCl, pH 4). A third side barrel containing a 2-mol/L NaCl solution was used for automatic current balancing. Pipettes were pulled and the tips broken back under microscopic control to a final width of 7–10 μm. All drugs were retained with a −8 nA current between ejections. A slowing of cell firing following brief ejections of 5-HT (10 nA, 1 min) confirmed the presence of serotoninergic receptors on these neurons. As with the dorsal raphe recordings, unit activity was computed and stored in 10-sec bins with changes in firing rate noted after intravenous administration.

**Drugs**

Drugs were synthesized by the Medicinal Chemistry Department of Pfizer Central Research (WAY-100,635, fluoxetine) or obtained from commercial sources ((±)-pindolol, (±)-8-OH-DPAT, RBI, Natick, MA). Solutions for intravenous administration were prepared daily using a saline vehicle (WAY-100,635, (±)-pindolol, (±)-8-OH-DPAT) or an acid/saline mixture (fluoxetine). Doses were scaled to a final volume of 1 mL/kg and administered via lateral tail vein. Pilot studies indicated that the vehicles employed had no effect on cell firing.

**Results**

**Inhibition of Serotonergic Dorsal Raphe Cell Firing by Pindolol**

Following intravenous administration, pindolol inhibited the spontaneous firing rate of serotonergic dorsal raphe neurons in a dose-dependent manner. In general, the onset of the suppressant effect occurred within the first or second 10-sec sampling interval after drug injection (Figure 1A); recovery to baseline rates was typically partial, proceeding gradually over the course of 20–30 min, and occasionally absent. The mean inhibitory dose (ID50) value, calculated as the dose required to reduce firing rate to 50% of baseline, was 1.7 mg/kg (Figure 2). Complete inhibition of cell firing was consistently observed at the highest dose tested, approximately five to 10 times its ID50. The 5-HT1A antagonist WAY-100,635 (Fornal et al 1996), given at doses that do not significantly affect baseline firing rate (10 and 30 μg/kg IV, n = 6), abruptly reversed the inhibition produced by pindolol (Figure 1A). In pilot studies, a similar dose (17 μg/kg IV) reduced the suppression of cell firing induced by 8-OH-DPAT (1 μg/kg IV) from 53% ± 11% to 9% ± 3% of the predrug baseline (n = 5–7).

The agonist activity of pindolol on dorsal raphe 5-HT1A autoreceptors, were it to be observed in these experiments, should be maximal at the 0.3-mg/kg dose, the highest dose tested without significant effects on baseline rate. The preferred agonist in these experiments would be 5-HT itself, generated by blockade of reuptake by an SSRI (Gartside et al 1995; Sheard et al 1972). Accordingly, fluoxetine was dosed to achieve an inhibition of cell firing in the 50–80% range (1–2 mg/kg IV), and pindolol was tested for its ability to reverse at a dose of 0.3 mg/kg. In the five cells examined, pindolol failed to reverse the effects of fluoxetine and, in two cells, appeared to add to the suppressant effect of the SSRI (Figure 1B). A higher dose of pindolol (3 mg/kg), near the ID50 for dorsal raphe...
inhibition, also failed to show antagonist activity in the four cells tested.

**Inhibition of CA3 Hippocampal Cell Firing by Pindolol**

Intravenous administration of pindolol also inhibited hippocampal CA3 pyramidal neurons. As with the dorsal raphe recordings, onset was rapid and recovery was gradual and typically incomplete (Figure 1C). The ID$_{50}$ values for pindolol in the hippocampus and dorsal raphe were similar (1.2 vs. 1.7 mg/kg IV), as were the slopes of the dose–response curves (Figure 2). Unlike the dorsal raphe recordings, however, WAY-100,635 (30 and 100 μg/kg IV) could not reverse pindolol-induced inhibition in the hippocampus, even though lower doses antagonized the inhibitory effects of pindolol in the dorsal raphe (Figure 1A).

**Discussion**

The results of the present study suggest that at high doses pindolol behaves as a 5-HT$_{1A}$ agonist to completely inhibit serotonergic dorsal raphe cell firing, confirming very similar findings published previously (Clifford et al 1998; Fornal et al 1999a, 1999b, 1999c). No evidence of 5-HT$_{1A}$ receptor antagonism was observed employing an SSRI to reduce unit firing rate, again consistent with earlier reports (Clifford et al 1998; Fornal et al 1999a) but inconsistent with the mechanism of action claimed in clinical studies with pindolol/SSRI combinations (McAskill et al 1998). An entirely new finding is the ability of pindolol to suppress CA3 hippocampal cell firing in a dose-dependent manner and, more importantly, in a WAY-100,635–insensitive manner. WAY-100,635 (30 and 100 μg/kg IV) could not reverse pindolol-induced inhibition in the hippocampus, even though lower doses antagonized the inhibitory effects of pindolol in the dorsal raphe. These data also discount the possibility that pindolol may alter hippocampal unit activity indirectly through terminal 5-HT$_{1B}$ antagonism, another reported activity of pindolol (Assie and Koek 1996), as this mechanism would also be sensitive to 5-HT$_{1A}$ receptor blockade. Blockade of cholinergic input provided in the form of the acetylcholine iontophoretic currents would seem unlikely, given the
receptor selectivity of pindolol analogs (van Koppen et al 1984), but blockade of $\beta$-adrenoceptor excitation (Mueller et al 1981) is an obvious possibility. The observation that pindolol does not alter membrane potential in CA3 cells in hippocampal slices (Corradetti et al 1998) indeed suggests an effect on the neuronal input lost in tissue preparation. Regardless of the mechanism at work, these data demonstrate the potential for pindolol’s actions at receptor sites in addition to those scrupulously examined in recent studies and advocate a broader view of its net pharmacologic activities, particularly when one interprets clinical data.

Not diminished by the present findings are the earlier reports of mixed $\beta$-receptor/5-HT$_{1A}$ receptor antagonists such as pindolol and propranolol as useful tools in dorsal raphe recordings. These studies were only demonstrations to confirm the 5-HT receptor subtype responsible for autoinhibition, as pindolol and propranolol were administered either by iontophoresis (Sprouse and Aghajanian 1986) or by bath application (Gelbach and Vandermaelen, 1987), and considerable effort in terms of experimental design was focused on utilizing iontophoretic currents or bath concentrations that did not suppress unit activity on their own. The use of these agents in vivo, prompted by the publication of the clinical studies, has been hampered by the inexact nature of systemic dosing relative to direct application in achieving extracellular levels that only block and do not activate 5-HT$_{1A}$ autoreceptors. As an example, low doses of pindolol (10–200 $\mu$g/kg IV) have been shown to block the inhibition produced by lysergic acid diethylamide (LSD), whereas higher doses (500 $\mu$g/kg IV) inhibit dorsal raphe by themselves (Haddjeri et al 1999).

Only compounds with extremely low levels of intrinsic activity will not produce a slowing of unit activity at some dose, this being a rather select group (e.g., UH-301, WAY-100,635) with the majority of agents falling into the partial agonist category (e.g., 8-OH-DPAT, buspirone, ipsapirone, gepirone). As noted in earlier work, compounds with partial agonist activity can block other partial agonists—for example, propranolol blocking the effects of 8-OH-DPAT or ipsapirone (Sprouse and Aghajanian 1986) or pindolol blocking the effects of gepirone (Gelbach and Vandermaelen 1987) or LSD (Haddjeri et al 1999). Antagonizing a full agonist such as 5-HT itself, however, appears to be an entirely different matter, with only limited blockade achieved by propranolol (Sprouse and Aghajanian 1986) or the lack of blockade with pindolol reported here and elsewhere. Within the class of mixed $\beta$/5-HT$_{1A}$ antagonists, only tertatolol has been shown to block the effects of endogenous 5-HT, and this conclusion is based only on the ability of this compound to modestly increase baseline firing rate in a subpopulation of dorsal raphe neurons (Jolas et al 1993; Prisco et al 1993).

Growing evidence of a long-loop feedback mechanism, in which activation of postsynaptic 5-HT$_{1A}$ receptors in turn suppresses cell firing in the raphe nuclei (Ceci et al 1994; Hajós et al 1999), suggests that this mechanism too should be considered in the action of pindolol. Overlapping such feedback on somatodendritic autoreceptor function, the effects on raphe firing rate become a function of the direct actions of agents on the autoreceptors and their indirect actions at some 5-HT$_{1A}$ site distant from the raphe nuclei but nevertheless connected through a feedback loop. The inability of propranolol to block the inhibitory effects of 8-OH-DPAT on raphe cell firing when given systemically (Blier et al 1988), paired with its clear ability to do so when administered iontophoretically (Sprouse and Aghajanian 1986), may be evidence of the importance of the long-loop feedback mechanism relative to somatodendritic autoreceptor control (Blier et al 1988). In the case of pindolol, in vitro doses block the hyperpolarizing effects of 5-CT on dorsal raphe neurons (Corradetti et al 1998); its lack of antagonism in vivo again points to the potential importance of the feedback loop.

The profile of pindolol that emerges from the electrophysiologic data is one of 5-HT$_{1A}$ partial agonism. Behavioral studies (Moore et al 1993; Sánchez et al 1996; Zhang and Barrett 1991) also show a mixed profile, as do clinical studies employing endocrine markers or temperature regulation as surrogate endpoints (e.g., Meltzer and Maes...
1996). Still, the positive results of the pindolol/SSRI depression trials are not diminished by classifying pindolol as a 5-HT₁A partial agonist rather than a selective 5-HT₁A autoreceptor antagonist. Some receptor mechanism, likely involving 5-HT neurotransmission itself or regional targets of 5-HT neurotransmission, must be operative, although our results, particularly those in the hippocampus, suggest that pindolol has many pharmacologic actions, all of which need to be assimilated in any proposed mechanism of action.

References


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