Pindolol, a Putative 5-Hydroxytryptamine$_{1A}$ Antagonist, Does Not Reverse the Inhibition of Serotonergic Neuronal Activity Induced by Fluoxetine in Awake Cats: Comparison to WAY-100635$^1$

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ABSTRACT

The ability of pindolol to enhance the clinical antidepressant response to selective serotonin reuptake inhibitors (SSRIs) is generally attributed to a blockade of the feedback inhibition of serotonergic neuronal activity mediated by somatodendritic 5-hydroxytryptamine (5-HT)$_{1A}$ autoreceptors. The current study examined the ability of pindolol to restore the single-unit activity of serotonergic dorsal raphe nucleus neurons in awake cats after acute treatment with the SSRI fluoxetine. The effects of pindolol were compared with those of N-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-N-(2-pyridinyl)cyclohexanecarboxamide (WAY-100635), a selective 5-HT$_{1A}$ receptor antagonist. Systemic administration of fluoxetine (0.5 and 5 mg/kg i.v.) decreased neuronal firing rates to ~50 and 1%, respectively, of baseline levels. The subsequent administration of cumulative doses of (±)-pindolol (0.1–5 mg/kg i.v.) failed to reverse the neuronal inhibition produced by either dose of fluoxetine. In addition to lacking efficacy as an antagonist in these experiments, (±)-pindolol produced an additional decrease in neuronal activity in animals pretreated with the low dose of fluoxetine. The active enantiomer, (−)-pindolol (1 mg/kg i.v.), also was ineffective in restoring neuronal activity after fluoxetine. In contrast, systemic administration of WAY-100635 completely reversed the effect of fluoxetine (5 mg/kg) at low doses (0.025 mg/kg i.v.), and further elevated the firing rate of these neurons above prefluoxetine baseline levels. Overall, these results indicate that pindolol, unlike WAY-100635, lacks appreciable antagonist activity at 5-HT$_{1A}$ autoreceptors. Thus, the clinical efficacy of pindolol in augmenting the antidepressant response to SSRIs, such as fluoxetine, may be unrelated to a restoration of serotonergic neuronal activity.

Major depression is one of the most common mental illnesses, with a lifetime prevalence of ~15% in the general population (Kessler et al., 1994). Despite advances in the pharmacotherapy of this mood disorder, a major problem associated with all current antidepressant drugs is the slow onset of their clinical action, typically from 4 to 6 weeks. In addition, it has been estimated that ~30% of all patients treated with an antidepressant drug fail to demonstrate a significant improvement in mood, despite an adequate treatment trial (Holden, 1991). Consequently, there has been considerable interest in the development of new pharmacological approaches aimed at enhancing the therapeutic action of antidepressant drugs.

Selective serotonin (5-hydroxytryptamine, 5-HT) reuptake inhibitors (SSRIs), such as fluoxetine, are currently the most widely prescribed class of antidepressant drugs and have proven efficacy in the treatment of major depression. The therapeutic action of these agents is generally thought to result from their ability to enhance central 5-HT neurotransmission by increasing the synaptic availability of 5-HT. Although SSRIs rapidly inhibit 5-HT reuptake upon acute administration, several weeks of treatment is required to elicit a clinical response. A widely held hypothesis suggests that the delayed onset of action of these drugs may be due to the indirect activation of 5-HT$_{1A}$ autoreceptors following reuptake inhibition (Blier et al., 1990; Blier and de Montigny, 1994).

Systemic administration of SSRIs increases the synaptic levels of 5-HT in axon terminal sites throughout the brain and in the raphe region, where the cell bodies of serotonergic neurons are located (Invernizzi et al., 1992; Artigas 1993; Gartside et al., 1995). This increase in raphe 5-HT activates somatodendritic 5-HT$_{1A}$ autoreceptors, resulting in a suppression of serotonergic

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ABBREVIATIONS: 5-HT, 5-hydroxytryptamine; SSRI, selective serotonin reuptake inhibitor; DRN, dorsal raphe nucleus; WAY-100635, N-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-N-(2-pyridinyl)cyclohexanecarboxamide; EEG, electroencephalogram; EOG, electrooculogram; EMG, electromyogram; 8-OH-DPAT, 8-hydroxy-2-(di-n-propylamino)tetralin.
neuronal activity, thereby limiting the ability of SSRIs to increase 5-HT levels at forebrain target sites (e.g., frontal cortex). Consequently, only small increases in extracellular 5-HT are seen in target regions after acute SSRI treatment, which may explain the failure of these drugs to produce an immediate therapeutic effect. The long-term administration of these drugs is suggested to desensitize the 5-HT1A autoreceptor to the inhibitory feedback action of 5-HT, thus allowing serotonergic neurons to resume their normal firing rate in the presence of continued reuptake inhibition (Blier and et al., 1990). The resumption of serotonergic neuronal activity (and hence neurotransmitter release) is thought to lead to an overall enhancement of 5-HT neurotransmission which, in turn, may mediate the therapeutic effect. The time course of these changes is consistent with the delayed onset of action of these drugs in the clinic.

It has been hypothesized that the combined administration of a highly potent and selective 5-HT1A autoreceptor antagonist and an SSRI might lead to a more rapid or more effective antidepressant response by rapidly achieving the same functional consequences seen with autoreceptor desensitization (i.e., decreased autoinhibition) (Blier and de Montigny, 1994; Artigas et al., 1996). Recent clinical studies with pindolol, a β-adrenoceptor blocker with putative 5-HT1A autoreceptor properties, support this idea. For example, pindolol was found to accelerate and/or potentiate the antidepressant effect of SSRIs in most studies (for review, see Blier and Bergeron, 1998). Furthermore, the addition of pindolol to the therapeutic regimen of depressed patients resistant to SSRI treatment was reported to induce a rapid and dramatic antidepressant response in a significant proportion of these patients (Artigas et al., 1994; Blier and Bergeron, 1995), although one study failed to replicate these findings (Moreno et al., 1997). Although the precise mechanism underlying the therapeutic action of pindolol remains to be determined, neurochemical studies have shown that 5-HT1A antagonists in general potentiate the ability of SSRIs to increase extracellular 5-HT levels in forebrain areas, presumably by blocking autoreceptor-mediated feedback inhibition of serotonergic neuronal activity (Hjorth, 1993; Hjorth and Auerbach, 1994; Gartside et al., 1995).

In the current study, we examined the ability of pindolol to restore the discharge of serotonergic neurons in the dorsal raphe nucleus (DRN) of freely moving cats after acute treatment with the SSRI fluoxetine. The effects of pindolol were compared with those of the selective 5-HT1A antagonist N-[2-(4-methoxyphenyl)-1-piperazinyl]ethyl-N-(2-pyridinyl)cyclohexanecarboxamide (WAY-100635). Our results indicate that pindolol, unlike WAY-100635, is not an effective 5-HT1A autoreceptor antagonist because it did not reverse the inhibition of serotonergic neuronal activity produced by fluoxetine. In the companion article (Fornal et al., 1999b), we characterized the effects of pindolol on the spontaneous activity of serotonergic DRN neurons after different routes of administration, and assessed the putative antagonist properties of the drug at presynaptic 5-HT1A receptors.

Materials and Methods

Animals. Male cats (2.5–5.0 kg b.w.t.) were housed individually in a temperature-controlled (22 ± 1°C) and light-controlled (lights on from 7:00 AM to 9:00 PM) room and had free access to food (commercial diet) and tap water. All cats were caged for and used in strict accordance with the Public Health Service Guide for the Care and Use of Laboratory Animals. All procedures were reviewed and approved by the Institutional Animal Care and Use Committee of Princeton University.

Surgical Procedures. Cats were pretreated with atropine sulfate (0.2 mg s.c. for 30 min) and tranquilized with acepromazine maleate (0.5 mg/kg i.m.) and ketamine hydrochloride (20 mg/kg i.m.) for preoperative preparation. Before placing the animal in a stereoanistic instrument, pentobarbital sodium was administered i.v. (doses individually titrated; range = 6–18 mg/kg) until deep anesthesia was achieved, as determined by loss of corneal and pedal reflexes. Supplemental doses of pentobarbital (~2 mg/kg) were administered as needed to maintain an adequate level of anesthesia. Rectal temperature was maintained between 36 and 38°C with a heating pad. A microdrive, consisting of two inner stainless steel cannulas (23 gauge) separated by 1 mm, which could be lowered through two outer guide cannulas (19 gauge), was stereotaxically implanted toward the DRN at an angle of 40° posterior to the vertical. Microelectrode bundles were then lowered through both cannulas so that their tips were positioned 1 mm above the DRN. Stereotaxic coordinates for the anterior bundle were as follows: posterior, 1.5 mm; lateral, 0 mm; and horizontal, +1.0 mm (Berman, 1968). Each bundle consisted of three 32-μm- and four 64-μm-diameter Formvar-insulated nichrome wires. Additionally, electrodes for recording the cortical electroencephalogram (EEG), the electrooculogram (EOG), and the nuchal electromyogram (EMG) were implanted as described in Fornal et al. (1994). The leads from all electrodes were soldered to a 25-pin connector, and the entire apparatus was anchored to the skull with dental acrylic. Cats were removed from the stereotaxic unit and a Tygon catheter (0.040-inch i.d., 0.070-inch o.d.) containing sterile normal saline was inserted into the right external jugular vein and advanced to the vena cava. The distal end of the catheter, which had previously been attached to a three-way stopcock, was capped and cemented to the implant.

Postoperative Animal Care. After surgery, all animals received an analgesic dose of butorphanol (0.2 mg/kg s.c.), with additional doses given as needed. A topical antibiotic powder (nitrofurazone) was regularly applied to the implant incision. Cats were treated, twice daily, for up to 10 days with ampicillin (20 mg/kg i.m., or 250 mg p.o.). Intravenous catheter patency was maintained by weekly flushing with heparinized sterile saline (500 IU/ml). The headpiece and surrounding tissue were cleaned at regular intervals. Cats were allowed to recover for at least 2 weeks before experiments were initiated. All cats were determined by veterinary examination to be in excellent health when these trials were conducted.

Electrophysiological Recording. Electrical potentials were recorded from each cat with a counterweighted low-noise cable system and 24-channel slip ring assembly. Microelectrode potentials were amplified (Grass 7P511 a.c. preamplifier; Grass Instrument Co., Quincy, MA), filtered (band-pass, 0.1–3.0 kHz), and monitored continuously on a storage oscilloscope. A second microelectrode served as an indifferent electrode. Single-unit activity was separated from background noise by means of a time-amplitude window discriminator (model DIS-1; Bak Electronics, Clarksburg, MD). The acceptance pulse output of the window discriminator was used to produce online records of cell discharge through a speaker and an electronic counter, and on a polygraph (Grass model 7C). Cortical EEG, EOG, and EMG potentials were amplified (Grass 7P5 or 7P511 a.c. preamplifier), band-pass filtered (EEG, 1–35 Hz; EOG, 1–35 Hz; EMG, 30–90 Hz), and recorded continuously on the polygraph.

All experimental trials were conducted in an electrically shielded, sound-attenuating chamber (65 × 65 × 95 cm in height) with a transparent Plexiglas door, which allowed for remote television monitoring of the animals. Each animal was habituated daily to the recording chamber over a 1-week postoperative period. After habituation, the microdrive was slowly advanced through the DRN in discrete steps (~80 μm) until stable single-unit recordings characteristic of serotonergic neurons were encountered. Only recordings...
that displayed a signal-to-noise ratio of at least 3:1 were used for data analysis. Such neurons could be recorded typically for several hours and often for several days.

**Neuronal Identification.** During quiet waking, individual neurons in the DRN were initially identified on-line as serotonergic according to previously established criteria (Fornal and Jacobs, 1988) as follows: 1) slow and highly regular discharge activity (−1−4 spikes/s); 2) biphasic action potentials of relatively long duration (>2 ms); and 3) complete or nearly complete suppression of spontaneous activity during rapid-eye-movement sleep. The neurochemical identity of these neurons was further established by determining the response to i.v. administration of the 5-HT_{1A} agonist 8-hydroxy-2-(di-n-propylamino)tetrain (8-OH-DPAT) (10 µg/kg), which has been shown to potently and selectively inhibit the spontaneous discharge of central serotonergic neurons via a direct action on somatodendritic autoreceptors (Sprouse and Aghajanian, 1986). Finally, localization of recording sites to the DRN was verified histologically.

**Drug Administration.** Single-unit activity was recorded before and after i.v. bolus injections of various test compounds. Drugs were administered remotely from an adjacent room via an infusion line connected to the venous catheter. Injections were made by loading the infusion line with drug (0.1−0.5 ml/kg) and flushing the line with 2 to 4 ml of sterile normal saline. All injections were made while cats were in a quiet but alert behavioral state, based on direct visual observations and polygraphic monitoring. Pulse injections of an equivalent volume of sterile saline served as a control.

To assess the effects of (-)-pindolol and WAY-100635 on the neuronal suppression produced by fluoxetine, each putative 5-HT_{1A} antagonist was administered in increasing doses at 6-min intervals, starting 15 min after fluoxetine administration (0.5 mg/kg or 5 mg/kg). Drug effects were measured after each injection and dose-response curves were constructed for both drugs. At the end of each pindolol trial, WAY-100635 (0.1 mg/kg) was administered to verify that the cells tested with pindolol were, indeed, responsive to 5-HT_{1A} autoreceptor blockade. In addition, we examined the effects of sequential i.v. administration of (−)-pindolol (1 mg/kg) and WAY-100635 (0.1 mg/kg) on the neuronal suppression produced by fluoxetine. These drugs were administered 3 h apart, beginning −24 to 30 h after fluoxetine administration (5 mg/kg).

**Data Collection.** Firing rate data for the drug conditions were obtained in comparable behavioral states, based on polygraphic and behavioral criteria, because the activity of serotonergic neurons varies directly with the level of behavioral arousal (Fornal and Jacobs, 1988). For the cumulative dose antagonism trials, firing rates were calculated from consecutive 10-s samples beginning 1 min before the drug injection and continuing for 5 min after the injection. The percent change in firing rate was determined by comparing the discharge rate taken over a 1-min period (i.e., six consecutive 10-s samples) during the time of maximal drug effect to the baseline rate obtained during the 1-min period immediately preceding the first drug injection. For the single-dose antagonism trials, firing rates were calculated from six consecutive 10-s samples at predefined time intervals.

**Drugs.** The following drugs were used: (−)-fluoxetine hydrochloride (courtesy of Eli Lilly, Indianapolis, IN), (−)-pindolol (Sigma Chemical Co., St. Louis, MO), (−)-pindolol (courtesy of Wyeth-Ayerst, Princeton, NJ), WAY-100635 trihydrochloride (courtesy of Wyeth Research, Taplow, England), and (−)-8-OH-DPAT hydrobromide (Research Biochemicals Inc., Natick, MA). All drug solutions were prepared immediately before each trial. 8-OH-DPAT, fluoxetine, and WAY-100635 were dissolved in sterile normal saline, whereas (−)-pindolol and (−)-pindolol were dissolved in sterile water. Pindolol required mild heating, sonication, and the addition of glacial acetic acid (1 µM/kg of drug) to dissolve. All dosages refer to the chemical form noted.

**Histology.** Animals were anesthetized with ketamine hydrochloride (20 mg/kg i.m.), and direct anodal current was passed through the recording electrode (20 µA for 40 s) at sites from which acceptable units were recorded. Cats were overdosed with pentobarbital sodium (100 mg/kg i.v.) and then perfused intracardially with physiological saline, followed by 10% formaldehyde (Formalin), and 5% potassium ferrocyanide in formaldehyde to produce a Prussian blue reaction. Frozen sections (50 µm) were cut through the midbrain region, mounted on slides, and stained with neutral red.

**Statistical Analysis.** Data are expressed as means ± S.E. Unit activity was analyzed by either a one-way or two-way repeated measures ANOVA and post hoc Student-Newman-Keuls test, or where appropriate, an unpaired t test. In all cases, a probability value ≤0.05 was considered to be statistically significant.

**Results**

**General Characteristics.** Data were obtained from 30 serotonergic neurons recorded in 21 cats. All neurons exhibited the characteristic changes in firing rate across the sleep/wake cycle, as described in Fornal et al. (1996). The mean discharge rate of these neurons was 3.14 ± 0.11 spikes/s during quiet waking and declined to 0.08 ± 0.05 spikes/s during rapid-eye-movement sleep, when most (27 of 30) of the recorded cells completely ceased firing. All cells tested with systemic administration of 8-OH-DPAT (n = 24) also displayed the characteristic inhibitory response to this 5-HT_{1A} agonist. The average decrease in neuronal activity produced by a 10-µg/kg dose of 8-OH-DPAT was 97 ± 2%. There were no significant differences with respect to either mean baseline firing rates or neuronal responsiveness to 8-OH-DPAT in the various drug-treatment groups.

**Effects of Fluoxetine on Serotonergic Neuronal Activity.** Administration of fluoxetine (0.5 and 5 mg/kg i.v.) depressed the spontaneous activity of serotonergic DRN neurons in a dose-dependent manner. Figure 1 shows the inhibitory response of serotonergic neurons to i.v. injections of 0.5 and 5 mg/kg fluoxetine during the period of maximal drug effect. Significant reduction of neuronal activity occurred within 60 s of the injection and the peak drug effect was reached within 5 min. Fluoxetine at 0.5 mg/kg decreased the firing rate from a mean baseline level of 3.52 ± 0.17 spikes/s to a minimum of 1.67 ± 0.26 spikes/s (n = 5), whereas the 5-mg/kg dose decreased the firing rate from a mean baseline level of 2.97 ± 0.16 spikes/s to a minimum of 0.02 ± 0.01 spikes/s (n = 14). The mean percentage decrease of neuronal activity produced by fluoxetine was 53 ± 5% (n = 5) at 0.5 mg/kg and 99 ± 1% (n = 14) at 5 mg/kg. The suppression of neuronal activity produced by 5 mg/kg of fluoxetine was significantly greater than that produced by the lower dose (p < .05; t test). The inhibitory action of fluoxetine at 5 mg/kg persisted for at least 24 h, with little or no recovery of spontaneous neuronal activity noted during this time. Thus, the firing rate of individual DRN neurons (n = 9) was still suppressed by 83 to 100% when examined 24 to 48 h after fluoxetine administration (5 mg/kg).

Control saline injections had no effect on neuronal activity. The maximal decrease in firing rate observed after saline administration was 8 ± 2% (n = 10). The decrease in neuronal activity produced by both doses of fluoxetine was significantly different from saline control (p < .05, one-way ANOVA and Student-Newman-Keuls test).

**Effects of WAY-100635 on the Neuronal Suppression Produced by Fluoxetine.** To determine whether the suppression of serotonergic DRN neuronal activity produced by fluoxetine was sensitive to autoreceptor blockade, the effect
of WAY-100635, a well established presynaptic 5-HT\textsubscript{1A} antagonist (Fletcher et al., 1996; Fornal et al., 1996), was examined in animals pretreated with fluoxetine. Figure 2 shows the response of a typical serotonergic DRN neuron to i.v. administration of cumulative doses (0.005–1 mg/kg) of WAY-100635 after acute treatment with fluoxetine (5 mg/kg for 15 min). WAY-100635 potently antagonized the inhibitory action of fluoxetine in a dose-dependent manner. As shown in Fig. 3, the two lowest doses of WAY-100635 tested, 0.005 and 0.01 mg/kg, produced no significant reversal, whereas total cumulative doses $\geq$0.025 mg/kg completely restored cell firing after fluoxetine and significantly elevated neuronal activity above prefluoxetine baseline levels. The predrug baseline firing rate of these neurons was 2.92 ± 0.29 spikes/s. The horizontal dashed line represents baseline unit activity. *$p$ < .05 versus 0 mg/kg (saline) by one-way ANOVA and Student-Newman-Keuls test.

Effects of (±)-Pindolol on the Neuronal Suppression Produced by Fluoxetine. The ability of (±)-pindolol to restore the discharge of serotonergic DRN neurons after acute treatment with fluoxetine (5 mg/kg i.v.) is shown in Figs. 4 and 5. In contrast to the effects of WAY-100635,
The combined administration of pindolol with an SSRI represents a new treatment strategy aimed at accelerating the clinical response to antidepressant drugs and increasing their therapeutic effectiveness. This approach is based on the capacity of pindolol to block 5-HT1A autoreceptors, thereby attenuating the feedback inhibition of serotonergic neuronal activity produced by SSRIs. This hypothesis was tested by examining the ability of pindolol to restore serotonergic DRN neuronal activity after acute treatment with fluoxetine, a popular SSRI antidepressant drug. Surprisingly, racemic pindolol (the chemical form used in clinical studies) failed to antagonize the effects of either a low or high dose of fluoxetine on serotonergic neurons. Pindolol produced an additional decrease in neuronal activity in animals pretreated with the low dose of fluoxetine. These results do not support the hypothesis that pindolol acts by blocking 5-HT1A autoreceptors.

The failure of pindolol to antagonize fluoxetine’s effects on neuronal activity cannot be attributed to the use of inadequate doses. A wide range of doses was used in this study, and increasing the dose of pindolol only served to further depress neuronal activity. Furthermore, (±)-pindolol has a relatively high affinity (Kᵢ = 34 nM) for 5-HT1A-binding sites (Winter and Rabin, 1993), and the drug readily penetrates into the brain after systemic administration. For example,
with [carbonyl-¹¹C]-WAY-100635 binding, a positron emission tomography study in monkeys reported a high (70%) in vivo occupancy of 5-HT₁A receptors in the raphe region following an i.v. injection of 1 mg/kg (±)-pindolol (Farde et al., 1997). In a microdialysis study, (−)-pindolol at doses as low as 0.1 mg/kg s.c. was reported to potentiate the effect of fluoxetine (10 mg/kg i.p.) on 5-HT output in the hypothalamus of conscious rats (Dreshfield et al., 1997). In general, the doses of pindolol used in the current study are considerably higher than those reported to facilitate the clinical effect of SSRIs. Thus, the doses of pindolol used in our study were more than sufficient to block 5-HT₁A autoreceptors, even though no antagonism was demonstrated.

The inability of pindolol to antagonize fluoxetine in our study contrasts with the results of a study in anesthetized rats, which reported that a 2-day treatment with (−)-pindolol (15 mg/kg/day) but not (±)-pindolol (10 mg/kg/day) prevented the reduction of serotonergic DRN neuronal activity produced by the SSRI paroxetine (Romero et al., 1996). In the latter study, both drugs were administered s.c. via osmotic minipumps, and drug effects were assessed by comparing the firing rates of spontaneously active cells encountered during electrode descents through the DRN. However, such neuronal sampling procedures measure the activity of different cells, rather than the activity of the same cells before and after a particular drug treatment. In addition, it is possible that the continuous infusion of pindolol or paroxetine could have altered the action of either drug by promoting changes in receptor sensitivity. Thus, the variance between our study and that of Romero et al. (1996) may be related to the experimental paradigm.

In contrast to pindolol, WAY-100635 proved to be a highly effective antagonist of fluoxetine-induced neuronal inhibition. WAY-100635, at doses as low as 0.025 mg/kg, completely reversed the effect of fluoxetine, presumably by blocking the negative feedback action of increased synaptic levels of 5-HT at 5-HT₁A autoreceptors. WAY-100635 also reversed the effect of fluoxetine in all cells tested with, and shown to be either unaffected or inhibited further by, systemic pindolol. Because 5-HT₁A autoreceptors are tonically activated by endogenous 5-HT release in the awake animal (Fornal et al., 1994, 1996; Bjorvatn et al., 1998), the blockade of these receptors results in an increase in baseline firing, as ob-

![Fig. 6. Firing rate histogram of a single serotonergic DRN neuron showing the effect of cumulative i.v. administration of (±)-pindolol (0.1–5 mg/kg) on the neuronal suppression produced by fluoxetine (0.5 mg/kg i.v.). Pindolol failed to reverse the effect of this low dose of fluoxetine and produced an additional decrease in neuronal activity. The subsequent i.v. administration of WAY-100635 (0.1 mg/kg) rapidly restored unit activity back to prefluoxetine baseline levels.](image)

![Fig. 7. Effects of cumulative i.v. doses of (±)-pindolol (0.1–5 mg/kg) and subsequent administration of WAY-100635 (WAY, 0.1 mg/kg i.v.) on the suppression of serotonergic DRN neuronal activity produced by fluoxetine (0.5 mg/kg i.v.). Pindolol was administered in increasing doses at 6-min intervals starting 15 min after fluoxetine administration. The maximal increase in firing rate produced by each dose of pindolol is shown. Pindolol failed to reverse the effect of this low dose of fluoxetine and produced a further decrease in neuronal activity. The i.v. administration of WAY-100635 after pindolol completely restored neuronal activity to prefluoxetine baseline levels. The horizontal dashed line represents baseline unit activity. *p < .05 versus respective preinjection level (time zero) by two-way ANOVA and Student-Newman-Keuls test.](image)

![Fig. 8. Effects of sequential i.v. administration of (−)-pindolol (1 mg/kg) and WAY-100635 (0.1 mg/kg) on the suppression of serotonergic DRN neuronal activity produced by fluoxetine (5 mg/kg i.v.). Values are means ± S.E. (n = 5 cells). Time zero indicates time of injection. (−)-Pindolol and WAY-100635 were administered −26 ± 2 and 29 ± 2 h, respectively, after fluoxetine. (−)-Pindolol had no significant effect on neuronal activity following prolonged 5-HT reuptake inhibition produced by fluoxetine, whereas WAY-100635 markedly elevated activity. The predrug baseline firing rate of these neurons was 3.09 ± 0.32 spikes/s. The horizontal dashed line represents baseline unit activity. *p < .05 versus respective preinjection level (time zero) by two-way ANOVA and Student-Newman-Keuls test.](image)
served in the current study when WAY-100635 was administered after fluoxetine. This overshoot, however, was significantly attenuated or abolished in the presence of pindolol, suggesting that pindolol interferes with WAY-100635's ability to increase neuronal activity.

WAY-100635 also blocks the action of other 5-HT reuptake inhibitors (e.g., paroxetine) on DRN serotonergic neuronal activity (Gartside et al., 1995, 1997), although WAY-100635 appears to be substantially less effective in the anesthetized animal. The reason for this finding is not clear, but it may be related to a diminished excitatory input and/or lack of appreciable tone at 5-HT$_{1A}$ autoreceptors under anesthesia.

The antagonistic action of WAY-100635 on neuronal activity appears to be pharmaco logically specific. Thus, although WAY-100635 increases serotonergic neuronal firing rates when administered alone in awake cats, it does not prevent the decrease in neuronal activity that occurs naturally during sleep (Fornal et al., 1996), which is largely mediated by the inhibitory neurotransmitter γ-aminobutyric acid (Levine and Jacobs, 1992). Furthermore, WAY-100635 does not reverse or prevent the inhibition of serotonergic DRN neuronal activity produced by prazosin, a selective α$_1$-adrenoceptor antagonist, which blocks the tonic facilitatory influence of norepinephrine on these cells under anesthesia (Gartside et al., 1997; Hertel et al., 1997).

Both pindolol and WAY-100635 can potentiate the increase in forebrain extracellular 5-HT produced by SSRIs, including fluoxetine, as measured by in vivo microdialysis (Gartside et al., 1995; Dreshfield et al., 1996, 1997; Hjorth, 1996; Romero et al., 1996; Dawson and Nguyen, 1998). Although not fully understood, this potentiation of 5-HT output following reuptake inhibition is attributed to a blockade of 5-HT$_{1A}$-auto receptor-mediated feedback inhibition. The results obtained with WAY-100635 in the current study are consistent with this hypothesis because WAY-100635 completely restored the activity of serotonergic neurons after acute fluoxetine. However, the results obtained with pindolol suggest that its effects on extracellular 5-HT are mediated through a different mechanism, unrelated to 5-HT$_{1A}$ autoreceptor blockade.

Pindolol, unlike WAY-100635, has strong antagonistic properties at terminal 5-HT$_{1B}$ autoreceptors (Middelmiss, 1986), which control the amount of 5-HT released per nerve impulse. The concomitant blockade of these receptors in rats augments the effects of SSRIs on 5-HT release in the forebrain, while producing little or no change in basal extracellular 5-HT in the absence of 5-HT reuptake inhibition (Gobert et al., 1997, Sharp et al., 1997). Thus, in addition to somatodendritic 5-HT$_{1A}$ autoreceptors, nerve terminal autoreceptors appear to restrain the ability of SSRIs to increase central 5-HT levels. By comparing the effects of systemic pindolol alone and in combination with selective 5-HT$_{1A}$ or 5-HT$_{1B}$ receptor antagonists after acute SSRI treatment, Nguyen and Dawson (1998) concluded that the ability of pindolol to potentiate the effect of fluoxetine on 5-HT output was largely mediated through a blockade of 5-HT$_{1B}$ autoreceptors and not 5-HT$_{1A}$ autoreceptors. Similarly, we found that systemic administration of (±)-pindolol (1 and 10 mg/kg s.c.) increases striatal extracellular 5-HT levels in cats, even though the drug strongly inhibits the activity of serotonergic DRN neurons (Fornal et al., 1999a), which selectively innervate this region. This finding demonstrates that pindolol can enhance 5-HT output in the presence of reduced impulse flow, most likely by acting directly at the level of the nerve terminal. Although blockade of 5-HT$_{1B}$ autoreceptors might account for the ability of pindolol to enhance 5-HT output in rats after treatment with an SSRI, the clinical significance of this effect is unclear because pindolol apparently has a much lower affinity for the terminal autoreceptor found in humans, which is of the 5-HT$_{1B}$ subtype (Oksenberg et al., 1992).

The finding that pindolol further decreased serotonergic neuronal activity in animals pretreated with a clinically relevant dose of fluoxetine suggests that pindolol may have intrinsic agonist properties at 5-HT$_{1A}$ autoreceptors. Indeed, both pindolol and its active (−)-isomer were found to inhibit the spontaneous activity of serotonergic DRN neurons in awake cats (Fornal et al., 1999b). This inhibition was completely reversed by WAY-100635, suggesting that the effect is mediated through an activation of 5-HT$_{1A}$ autoreceptors. Additional experiments showed that pindolol had no effect on the suppression of serotonergic DRN neuronal activity produced by 8-OH-DPAT, a selective 5-HT$_{1A}$ agonist. These results indicate that pindolol exerts predominantly an agonist rather than an antagonist action at 5-HT$_{1A}$ autoreceptors in the awake cat. However, it is possible that the pharmacological properties of pindolol (e.g., intrinsic activity) at somatodendritic 5-HT$_{1A}$ autoreceptors in the cat may differ from those seen in humans.

In light of the above results, the ability of pindolol to enhance the clinical antidepressant response to SSRIs may not be explained by antagonism of 5-HT$_{1A}$ autoreceptors, as currently believed. Instead, pindolol's therapeutic action may be related to its agonist activity at 5-HT$_{1A}$ receptors. For example, the coadministration of pindolol with an SSRI, by producing sustained activation, may facilitate the desensitization of 5-HT$_{1A}$ autoreceptors, a process thought to play a crucial role in the action of this class of antidepressant drugs. Furthermore, the efficacy of partial agonists, such as pindolol, at 5-HT$_{1A}$ autoreceptors may be enhanced in depressed patients, as suggested by the finding that 5-HT$_{1A}$ receptors are increased in the DRN of suicide victims with major depression (Stockmeier et al., 1998). Alternatively, pindolol might enhance 5-HT neurotransmission by stimulating certain postsynaptic 5-HT$_{1A}$ receptors, as suggested by functional studies in animals (Aulakh et al., 1988) and humans (Meltzer and Maes, 1996). Interestingly, buspirone, a partial 5-HT$_{1A}$ agonist that also inhibits serotonergic cell firing in the awake animal (Fornal et al., 1994), has been reported to enhance the therapeutic efficacy of SSRIs in preliminary clinical trials (Jacobsen, 1991; Joffe and Schuller, 1993).
Among reserve in the raphe region. Furthermore, pindolol is unique expected if 5-HT1A autoreceptors were blocked by pindolol. Administration of pindolol and SSRIs has not resulted in a dramatic explored. Finally, in controlled clinical studies, the coadministration of pindolol and SSRIs has not resulted in a dramatic acceleration of the antidepressant response, as would be expected if 5-HT1A autoreceptors were blocked by pindolol. Thus, confirmation of the hypothesis that pharmacological blockade of 5-HT1A autoreceptors potentiates the antidepressant response to SSRIs must await further clinical studies with compounds genuinely acting as autoreceptor antagonists.

In summary, the present results show that pindolol does not block the suppression of serotonergic DRN neuronal activity produced by fluoxetine, a widely prescribed SSRI antidepressant. Activity of 5-HT1A antagonist drug administration in behaving cats. J Pharmacol Exp Ther 270: 1343–1358.

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References


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