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Short communication

Pindolol increases extracellular 5-HT while inhibiting serotonergic neuronal activity

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Abstract

The effects of pindolol, a beta-adrenoceptor blocker/putative 5-hydroxytryptamine (5-HT)$_{1A/1B}$ antagonist, on both the single-unit activity of serotonergic neurons in the dorsal raphe nucleus (DRN) and extracellular 5-HT levels in the caudate nucleus, were examined in freely moving cats. Administration of (±)-pindolol (1 and 10 mg/kg, s.c.) decreased neuronal activity and increased 5-HT levels in a dose- and time-dependent manner. The subsequent administration of WAY-100635 {*N*-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-*N*-(2-pyridinyl)cyclohexanecarboxamide} (0.2 mg/kg, s.c.), a selective 5-HT$_{1A}$ receptor antagonist, blocked pindolol-induced neuronal suppression and potentiated 5-HT output. These results indicate that pindolol may be acting at the level of the nerve terminal to increase 5-HT.

Keywords: Pindolol; WAY-100635; 5-HT$_{1A}$ autoreceptor; Serotonergic neuronal activity; 5-HT (5-hydroxytryptamine), extracellular; Serotonin; Microdialysis

1. Introduction

Recent clinical studies indicate that pindolol can enhance the antidepressant response to selective serotonin (5-hydroxytryptamine, 5-HT) reuptake inhibitors (Blier and Bergeron, 1998). This action is generally attributed to the ability of pindolol to block putative 5-HT$_{1A}$ autoreceptors located on the cell bodies and dendrites of central serotonergic neurons (Romero et al., 1996). These receptors are part of a negative feedback mechanism whereby the local release of 5-HT in the raphe region acts to decrease the discharge activity of serotonergic neurons. The indirect activation of somatodendritic 5-HT$_{1A}$ autoreceptors by selective serotonin reuptake inhibitors leads to an inhibition of neuronal activity and neurotransmitter release, which counteracts the ability of selective serotonin reuptake inhibitors to potentiate the action of endogenous 5-HT at post-synaptic target sites. While the precise mechanism underlying the therapeutic action of pindolol remains to be determined, neurochemical studies have shown that pindolol can potentiate the effects of selective serotonin reuptake inhibitors on extracellular 5-HT, presumably by blocking autoreceptor-mediated feedback inhibition of serotonergic neuronal activity (Dreshfield et al., 1996; Hjorth, 1996; Romero et al., 1996). However, we have recently shown in awake cats that pindolol is not an effective 5-HT$_{1A}$ autoreceptor antagonist (Fornal et al., 1999a,b). In fact, pindolol appears to act as a partial agonist at 5-HT$_{1A}$ autoreceptors. These results led us to investigate the effects of pindolol on the in vivo release of 5-HT in the same animal model.

The aim of the present study was to examine the effects of systemic administration of pindolol on the activity of serotonergic neurons in the dorsal raphe nucleus (DRN) and to compare this with its effects on extracellular levels of 5-HT in the striatum, a brain region selectively innervated by DRN serotonergic neurons, in freely moving cats.

2. Materials and methods

2.1. Animals

Male cats ($n = 12$) weighing 2.5 to 5.0 kg were used. They were housed individually in a temperature-controlled

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(22 ± 1°C) and light-controlled (lights on from 0700 to 2100 h) room and had free access to food and water. All cats were cared for and used in strict accordance to 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.

2.2. Surgical procedure

Under pentobarbital anesthesia, cats were implanted with bundles of moveable, insulated nichrome microwires in the DRN for single-unit recording and with electrodes for monitoring behavioral state, as described previously (Bjorvatn et al., 1998). In addition, a stainless-steel guide cannula (18 gauge) was stereotaxically implanted above the right caudate nucleus (anterior, 15.0 mm; lateral, 5.0 mm; horizontal, +10.0 mm) for in vivo microdialysis. Animals were allowed a minimum of 2 weeks to recover from surgery.

2.3. Electrophysiological recording

Single-unit activity was monitored continuously on an oscilloscope and separated from background noise using a window discriminator. The output of the discriminator was used to obtain an on-line record of cell discharge through a speaker, an electronic counter and a polygraph. Serotonergic neurons were identified by their slow and regular discharge activity (~ 1–4 spikes/s), long duration action potentials (≥ 2 ms), and complete suppression of spontaneous activity during rapid-eye-movement sleep and in response to systemic administration of the 5-HT_{1A} receptor agonist 8-OH-DPAT {8-hydroxy-2-(di-*n*-propylamino)tetralin} (10 μg/kg, i.v. or 100 μg/kg, s.c.).

2.4. Microdialysis measurement of extracellular 5-HT

On the day of the experiment, a concentric microdialysis probe, having a 5-mm length of exposed nitrocellulose membrane (0.22 mm OD, 6000 Da cut-off; Spectrum, Houston, USA) was lowered through the guide cannula, so that the tip extended 8 mm beyond the cannula. The probes were perfused with a modified Ringer's solution (147.2 mM NaCl, 4.0 mM KCl, 1.8 mM $CaCl_2$) at a constant flow rate of 1.5 μl/min. Dialysate samples were assayed immediately by high-performance liquid chromatography coupled with electrochemical detection, as described previously (Mendlin et al., 1998). The detection limit for 5-HT was approximately 0.9 pg based on a signal-to-noise ratio of 3:1. Stable 5-HT levels were typically obtained 4 to 5 h after probe implantation. Experiments were conducted between 0900 and 1800 h in a chamber (65 × 65 × 95 cm high) with a transparent Plexiglas front door.

2.5. Data collection

Unit activity (six consecutive 10-s counts) and dialysate (30 μl) samples were obtained at 20-min intervals before and after the systemic administration of drugs, while animals were in a quiet but alert behavioral state, based on polygraphic monitoring and direct visual observation. This was done because both the activity of serotonergic neurons and the release of 5-HT have been shown to vary directly in association with the level of behavioral arousal (cf. Bjorvatn et al., 1998). For each experimental trial, the average of the four samples obtained immediately prior to pindolol administration served as a baseline, and data were calculated as a percentage of this baseline. The single-unit and microdialysis experiments were performed separately under similar experimental conditions.

2.6. Drugs

(±)-8-OH-DPAT hydrobromide (Research Biochemicals International, Natick, USA) and WAY-100635 {*N*-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-*N*-(2-pyridinyl)-cyclohexanecarboxamide} trihydrochloride (courtesy of Wyeth Research, Taplow, England) were dissolved in sterile normal saline, whereas (±)-pindolol (Sigma, St. Louis, USA) was dissolved in sterile water with the addition of a minimal quantity of glacial acetic acid. Drugs were administered subcutaneously. The injection volume was 0.1–0.2 ml/kg body weight. All dosages refer to the chemical form noted.

2.7. Statistical analysis

Data are expressed as means ± SEM. Unit activity and 5-HT levels were analyzed using one-way repeated-measures analysis of variance (ANOVA) and post-hoc Student–Newman–Keuls test, or where appropriate, paired *t*-test. A probability value ≤ 0.05 was taken as statistically significant.

3. Results

3.1. Effects of (±)-pindolol on serotonergic neuronal activity and extracellular 5-HT

Figs. 1 and 2 show the effects of pindolol (1 and 10 mg/kg, s.c.) on the activity of serotonergic DRN neurons in relation to its effects on extracellular 5-HT levels in the striatum (i.e., caudate nucleus). Pindolol produced a marked, dose-related reduction of neuronal activity. This effect was apparent within 20 min and persisted for at least 2 h. The suppression of neuronal activity induced by pindolol was temporally correlated with an increase in striatal extracellular 5-HT, as shown in Fig. 1. The maximal increase in 5-HT output produced by pindolol was

45 ± 4% at 1 mg/kg and 125 ± 30% at 10 mg/kg (Fig. 2).

3.2. Effects of WAY-100635 on pindolol-induced changes in serotonergic neuronal activity and extracellular 5-HT

To examine the role of 5-HT_{1A} autoreceptors in the action of pindolol, the selective 5-HT_{1A} receptor antagonist, WAY-100635 (0.2 mg/kg, s.c.), was administered 2 h after pindolol. As shown in Figs. 1 and 2, WAY-100635 completely reversed the inhibitory effect of pindolol on neuronal activity and, at the same time, potentiated the ability of pindolol to increase 5-HT output. This potentiation was particularly pronounced in animals treated with the high dose of pindolol, even though WAY-100635 increased neuronal activity to a greater extent in animals treated with the low dose of pindolol.

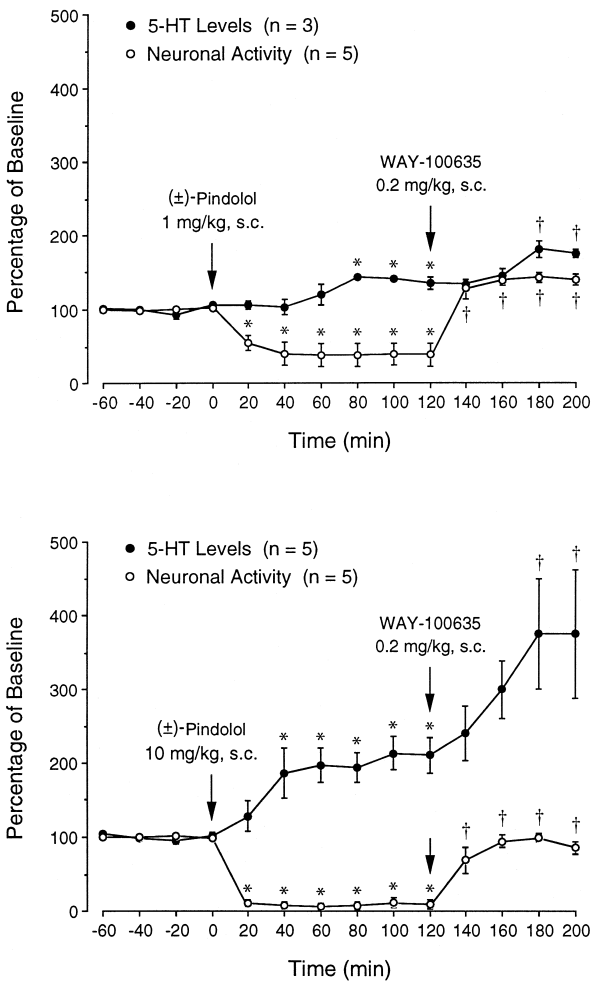

Fig. 1. Effect of sequential administration of (±)-pindolol (1 and 10 mg/kg, s.c.) and WAY-100635 (0.2 mg/kg, s.c.) on serotonergic DRN neuronal activity and striatal extracellular 5-HT levels in awake cats. Top panel: pindolol 1 mg/kg; bottom panel: pindolol 10 mg/kg. Values are means ± SEM. Arrows indicate time of drug injections. Mean baseline firing rates were 3.35 ± 0.56 and 3.25 ± 0.29 spikes/s for the 1 and 10 mg/kg doses of pindolol, respectively. Mean baseline dialysate 5-HT levels were 0.064 ± 0.015 and 0.056 ± 0.007 pg/μl (values not corrected for probe recovery) for the 1 and 10 mg/kg doses of pindolol, respectively. $^{*}p < 0.05$ vs. respective baseline (−60 to 0 min); $^{\dagger}p < 0.05$ vs. respective pindolol levels (60 to 120 min post-injection).

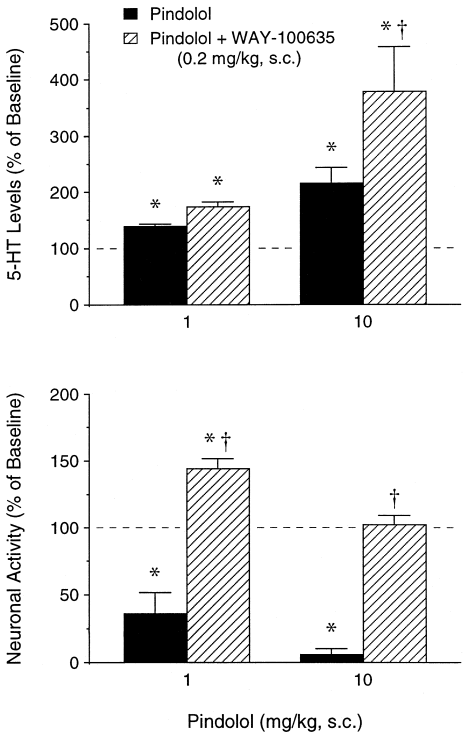

Fig. 2. Summary of the maximal effects induced by (±)-pindolol (1 and 10 mg/kg, s.c.) and WAY-100635 (0.2 mg/kg, s.c.) on striatal extracellular 5-HT levels (top panel) and serotonergic DRN neuronal activity (bottom panel). Values are means ± SEM. $^{*}p < 0.05$ vs. respective baseline; $^{\dagger}p < 0.05$ vs. respective pindolol level.

4. Discussion

These results show that pindolol increases extracellular 5-HT levels in the awake cat, despite the fact that the drug strongly inhibits the activity of serotonergic neurons. Furthermore, WAY-100635 potentiated the effect of pindolol on 5-HT output, presumably by blocking the inhibitory action of pindolol on neuronal activity. Thus, the pharmacological profile of pindolol in these experiments resembles that of an indirect 5-HT agonist (e.g., a selective serotonin reuptake inhibitor).

Previous microdialysis studies conducted in rats have reported inconsistent effects of pindolol on extracellular 5-HT levels. For example, in the frontal cortex, systemic administration of pindolol resulted in either an elevation (Maione et al., 1997), a reduction (Clifford et al., 1998; Hjorth and Bengtsson, 1998), or no change (Gur et al., 1997; Lejeune et al., 1998) in 5-HT levels. Similarly, in the dorsal hippocampus, a median raphe-innervated area, pindolol either produced an increase in 5-HT (Bosker et al., 1994) or had no effect (Hjorth and Bengtsson, 1998).

To date, only one other study has examined the effects of pindolol on extracellular 5-HT in the striatum (Romero et al., 1996). That study reported no effect of (−)-pindolol (15 mg/kg, i.p.) on basal 5-HT levels, in contrast to the present results in cats.

Several studies have suggested that the increase in 5-HT output produced by pindolol may be due to a blockade of somatodendritic 5-HT_{1A} autoreceptors, and subsequent increase in the firing rate of serotonergic neurons (Bosker et al., 1994; Matos et al., 1996; Maione et al., 1997). Previous electrophysiological studies have shown that serotonergic neurons are under tonic autoreceptor-mediated feedback inhibition, at least in the unanesthetized animal (Fornal et al., 1996; Bjorvatn et al., 1998). However, in contrast to both selective (WAY-100635 and *p*-MPPI) and non-selective (spiperone) 5-HT_{1A} autoreceptor antagonists, pindolol does not increase, but rather inhibits, the activity of serotonergic DRN neurons in awake animals, as shown previously and in the present study. Therefore, an increase in firing rate cannot account for the elevation in 5-HT levels seen after systemic pindolol administration.

Instead, pindolol may facilitate 5-HT output by acting directly at the level of the nerve terminal. In support of this, pindolol has been shown to increase 5-HT output when locally perfused in the brain (Assie and Koek, 1996; Matos et al., 1996), presumably by blocking terminal 5-HT_{1B} autoreceptors, which inhibit the presynaptic release of 5-HT. In addition, recent evidence suggests that 5-HT_{1B} autoreceptors may also modulate the activity of the 5-HT transporter, since 5-HT_{1B} antagonists reduce the rate of clearance of exogenously applied 5-HT from the extracellular space (Frazer and Daws, 1998). Interestingly, almost all of the studies which reported an increase in 5-HT after systemic pindolol administration employed a selective serotonin reuptake inhibitor in the perfusion medium, which may have artificially elevated endogenous 5-HT tone at inhibitory nerve terminal autoreceptors. Likewise, the ability of pindolol to potentiate the effects of systemically administered selective serotonin reuptake inhibitors on 5-HT output may involve an interaction with 5-HT_{1B} and not 5-HT_{1A} autoreceptors, as suggested by the results of a recent microdialysis study (Nguyen and Dawson, 1998). Furthermore, the facilitatory action of pindolol on 5-HT output may have obscured the effects of the drug in other functional models of presynaptic 5-HT_{1A} receptor activity.

A number of compounds, notably selective serotonin reuptake inhibitors and monoamine oxidase inhibitors, increase the extracellular concentration of 5-HT in the brain at doses which cause a near total inhibition of serotonergic neuronal activity, as observed in the present study with pindolol. Although suppression of neuronal firing would be expected to counteract the ability of such drugs to elevate extracellular 5-HT, it does not preclude such an effect. These results suggest that significant quantities of 5-HT continue to be released during little or no impulse flow. The finding that WAY-100635 completely reversed the neuronal suppressant action of pindolol and also potentiated the increase in extracellular 5-HT induced by pindolol suggests that impulse-dependent transmitter release plays a major role in the 5-HT-enhancing action of pindolol. Another possibility to consider is that pindolol may have a direct releasing action on 5-HT. In vitro studies have shown that pindolol, at relatively high concentrations, can release 5-HT from rat cortical slices and human platelets (Nathan et al., 1977; Middlemiss, 1986). Interestingly, this action may be specific to the (+)-isomer of pindolol. Finally, it is important to emphasize that the in vivo microdialysis technique does not measure neurotransmitter release per se, but rather the net effect of two opposing processes, i.e., release and reuptake (for a review, see Fuller, 1994). Moreover, pindolol may act on both of these processes to enhance 5-HT output, as discussed above. Additional in vitro studies are needed to more fully characterize the pharmacological action of pindolol at the nerve terminal level.

The finding that pindolol inhibits the activity of serotonergic neurons via a mechanism sensitive to WAY-100635 is consistent with the idea that pindolol acts as an agonist at 5-HT_{1A} autoreceptors. Pindolol is known to have a relatively high affinity for the 5-HT_{1A} receptor. However, since pindolol also has been shown to increase 5-HT levels in the DRN (Matos et al., 1996; Maione et al., 1997), an indirect mechanism of action of pindolol on serotonergic neuronal activity cannot be excluded at this time.

In summary, although pindolol may be a useful pharmacotherapeutic agent, its primary mechanism of action remains unknown. This is an important issue because it has an impact on the discovery of future therapeutic agents.

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