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Effect of Pindolol on the Function of Pre- and Postsynaptic 5-HT_{1A} Receptors: In Vivo Microdialysis and Electrophysiological Studies in the Rat Brain

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In microdialysis studies, somatodendritic 5-HT_{1A} receptors in the dorsal raphe nucleus (DRN) were activated by the local infusion of 50 μM citalopram, a selective 5-HT reuptake inhibitor (SSRI). This reduced extracellular 5-HT by about 50% in dorsal striatum, an area receiving 5-HT afferents exclusively from the DRN. (−)Pindolol dose-dependently attenuated this citalopram-induced reduction of striatal extracellular 5-HT. Consistent with its 5-HT reuptake blocking properties, single doses of the SSRI paroxetine (1 and 3 mg/kg IP) and citalopram (1 mg/kg IP) significantly elevated extracellular 5-HT in the dorsal striatum. Pretreatment with (−)pindolol (15 mg/kg IP) potentiated the effect of 3 mg/kg paroxetine and 1 mg/kg citalopram on striatal extracellular 5-HT. A 2-day treatment with 10 mg/kg/day (SC) of paroxetine reduced by 60% the spontaneous activity of 5-HT neurons of the DRN. However, 5-HT neurons displayed normal activity in rats treated with paroxetine and (−)pindolol for 2 days. The inhibitory effect of LSD on 5-HT neuronal firing activity was also markedly attenuated in (−)pindolol-treated rats, indicating that somatodendritic 5-HT_{1A} receptors were blocked by (−)pindolol. To determine whether (−)pindolol also blocked postsynaptic 5-HT_{1A} receptors in hippocampus, 5-HT and the prototypical 5-HT_{1A} agonist 8-OH-DPAT were applied by microiontophoresis onto CA_3 pyramidal neurons following the same treatment. (−)Pindolol did not modify the responsiveness of these neurons to 5-HT and 8-OH-DPAT. Taken together, these results indicate that (−)pindolol can potentiate the effects of an SSRI on extracellular 5-HT concentration by preventing the activation of somatodendritic 5-HT_{1A} autoreceptors resulting from the blockade of the 5-HT transporter in the raphe. This presumably leads to enhanced 5-HT neurotransmission because (−)pindolol would not alter the responsiveness of certain postsynaptic 5-HT_{1A} receptors, such as those located on hippocampal CA_3 pyramidal neurons. These results provide a neurobiological basis for the reported potentiation of certain antidepressant drugs by pindolol in major depression. ***[Neuropsychopharmacology 15:349–360, 1996]***

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Selective serotonin (5-HT) reuptake inhibitors (SSRIs), such as fluoxetine, paroxetine, citalopram, and fluvoxamine, share only the property to block the 5-HT trans-

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porter (Hyttel 1994). Consequently, their antidepressant effect is thought to derive from this property. Yet, blockade of the 5-HT reuptake process takes place within a few hours, whereas the onset of their therapeutic effect ranges from 1 to 2 weeks of treatment. This suggests that adaptive changes account for the antidepressant activity of SSRIs. Somatodendritic 5-HT_{1A} and terminal 5-$HT_{1B/D}$ autoreceptors are desensitized by long-term SSRI administration, leading to an enhancement of 5-HT neurotransmission (Blier and de Montigny 1994).

In recent years, acute changes of extracellular 5-HT release after the administration of antidepressant drugs have been documented using microdialysis. It was first observed that the systemic administration of clomipramine, a tricyclic antidepressant drug that potently inhibits the 5-HT transporter, elicited a preferential increase of extracellular 5-HT in the midbrain raphe nuclei, as compared with frontal cortex (Adell and Artigas 1991). Further reports have confirmed regional differences upon acute systemic administration of SSRI (Bel and Artigas 1992; Invernizzi et al. 1992; Kreiss and Lucki 1995) and of monoamine oxidase inhibitors (MAOIs; Celada and Artigas 1993). Interestingly, low doses of SSRIs, equivalent to those used in humans, exert little or no effect on the extracellular concentration of 5-HT in the frontal cortex, but increase it markedly in the raphe region (Bel and Artigas 1992; Invernizzi et al. 1992). This suggests that these antidepressant drugs may not be clinically effective upon acute administration because of an attenuated terminal release of 5-HT due to a preferential activation of somatodendritic 5-HT_{1A} receptors resulting from the blockade of 5-HT uptake in the raphe nuclei. Indeed, 5-HT cell bodies and dendrites release 5-HT (Héry et al. 1982; Adell et al. 1993), and in the rat and human brain the dorsal raphe nucleus (DRN) is endowed with the highest density of the 5-HT transporter (Cortés et al. 1988; Hrdina et al. 1990), as well as a very high density of 5-HT_{1A} binding sites (Pazos and Palacios 1985). These elements constitute the neurobiological basis for the cessation of firing of 5-HT neurons of the DRN (Sheard et al. 1972; Quinaux et al. 1982; de Montigny et al. 1984) and why no marked enhancement of 5-HT release occurs in terminal regions after a single dose of an antidepressant drug that inhibits 5-HT uptake (Adell and Artigas 1991; Bel and Artigas 1993).

Hence, the concurrent blockade of somatodendritic 5-HT_{1A} autoreceptors and of 5-HT reuptake could accelerate the antidepressant effect by enabling SSRIs to increase synaptic 5-HT levels in terminal regions because of a prevention of an attenuation of the firing activity of 5-HT neurons (Artigas 1993; de Montigny and Blier 1993). Obviously, such a pharmacological approach should not block postsynaptic 5-HT_{1A} receptors in limbic structures that may be involved in mediating the antidepressant response (de Montigny and Blier 1993). We have therefore investigated whether (−)pindolol (which is the enantiomer with 5-HT_{1A} affinity; Hoyer and Schoefter 1991) can antagonize the effect of 5-HT on somatodendritic 5-HT_{1A} autoreceptors and thus potentiate the effect of the SSRI paroxetine and citalopram on extracellular 5-HT in the forebrain. We have also examined the effect of (−)pindolol on the ability of 5-HT and the 5-HT_{1A} agonist 8-OH-DPAT to suppress the firing activity of hippocampus pyramidal neurons, an effect mediated by 5-HT_{1A} receptors (Chaput and de Montigny 1988; Blier et al. 1993).

MATERIAL AND METHODS

Animals

Male Wistar (n = 88) and Sprague-Dawley (n = 26) rats weighing 250 to 310 were used. They were housed four per cage and kept in a controlled environment (12-hour light–dark cycle and 22 ± 2°C room temperature). Food and water were provided ad libitum. Animal care followed the guidelines of the Society for Neuroscience.

Microdialysis Experiments

We performed two different sets of experiments. They aimed at examining (1) the effects of (−)pindolol on the reduction of terminal 5-HT release elicited by the activation of somatodendritic 5-HT_{1A} receptors; and (2) the effects of the combination of the SSRI paroxetine and citalopram with (−)pindolol on extracellular 5-HT. Animals used in the first set of experiments bore a dual implant, consisting of a microdialysis probe in the dorsal striatum and a steel cannula in the DRN. The striatum receives 5-HT fibers exclusively from the DRN, and it is therefore a good model to examine the role of somatodendritic 5-HT_{1A} receptors in the DRN in the control of 5-HT release in a postsynaptic structure (Imai et al. 1986; Romero et al. 1994). Somatodendritic 5-HT_{1A} receptors were activated by the increased extracellular 5-HT that resulted from the application of citalopram in the DRN. Animals of the second set of experiments were implanted with one microdialysis probe and drugs were administered systemically.

Anesthetized rats (using 60 mg/kg IP of pentobarbital) were implanted stereotaxically with 0.25-mm-OD, 4-mm-long concentric dialysis probes (Adell and Artigas 1991) in the dorsal striatum (coordinates in mm with respect to bregma: AP = 0.2, L = 3.0, D = −8.0; Paxinos and Watson 1982). Animals in the first experimental group were also implanted with a 27-gauge stainless steel tube aimed at the DRN, with a lateral angle of 30° and the following coordinates in mm with respect to bregma: AP = −7.8, L = −3.1, D = −6.8. In these animals, the perfusion fluid was infused through the steel tube in the DRN at a rate of 0.05 µl/minute (basal period) and then changed to a perfusion fluid

containing 50 μM of the potent SSRI citalopram (CIT), which was infused at the same rate following an initial 5-minute period at 0.5 μl/minute. To examine the influence of the infusion in the DRN, a group of control animals was also implanted with the steel cannulas and microdialysis probes, and artificial CSF was perfused in the DRN. Microdialysis experiments were conducted 18 to 20 hours after probe implants in conscious, freely moving animals. Probes were perfused with artificial cerebrospinal fluid (125 mM NaCl, 2.5 mM KCl, 1.26 mM $CaCl_2$, and 1.18 mM $MgCl_2$; pH 6.2) at 0.5 μl/minute using a CMA/100 microinjection pump (Carnegie Medicin, Stockholm, Sweden). Sample collection started 60 minutes after the beginning of perfusion. Four to five basal fractions were collected to obtain basal values before either local infusion or systemic administration of drugs. Successive 20-minute dialysate samples were collected. Because of the marked reduction of striatal extracellular 5-HT release elicited by the infusion of citalopram in the DRN observed in pilot experiments, striatal probes of this experimental group only were infused with artificial CSF containing 1 μM citalopram. This enhanced the detectability of 5-HT and reduced variability without affecting the experimental model (i.e., control of terminal release by activation of 5-HT_{1A} receptors in the DRN). 5-HT and 5-hydroxyindoleacetic acid (5-HIAA) were analyzed by high-performance liquid chromatopraphy (HPLC) with amperometric detection, using a modification of previously described methods (Adell and Artigas 1991). The column used was a 3-μm octadecyl silica column (Beckman), coupled to a Hewlett Packard 1049 amperometric detector set at +0.60 V, with a detection limit of 0.5 to 1 fmol for 5-HT. Results are expressed as means ± SEM.

Recordings from 5-HT Neurons of the Dorsal Raphe Nucleus

Dorsal raphe 5-HT neurons were recorded with single-barrelled glass micropipettes, with their tips broken back to 1 to 3 μm under microscopic control. These were filled with a 2-M NaCl solution. The impedance of the electrodes typically ranged from between 3 and 5 MΩ. The rats were anesthetized with chloral hydrate (initial dose: 400 mg/kg IP) with subsequent doses (100 mg/kg IP) to prevent nociception to paw pinching. The pipette was positioned 1 mm anterior to lambda on the midline and lowered on to the dorsal raphe, usually attained at a depth of 4.5 to 5.5 mm from the surface of the brain. The 5-HT neurons were identified according to the following criteria: a slow (0.5 to 2.5-Hz) and regular firing rate and a long-duration (0.8 to 1.2-ms) positive action potential. In order to assess possible changes

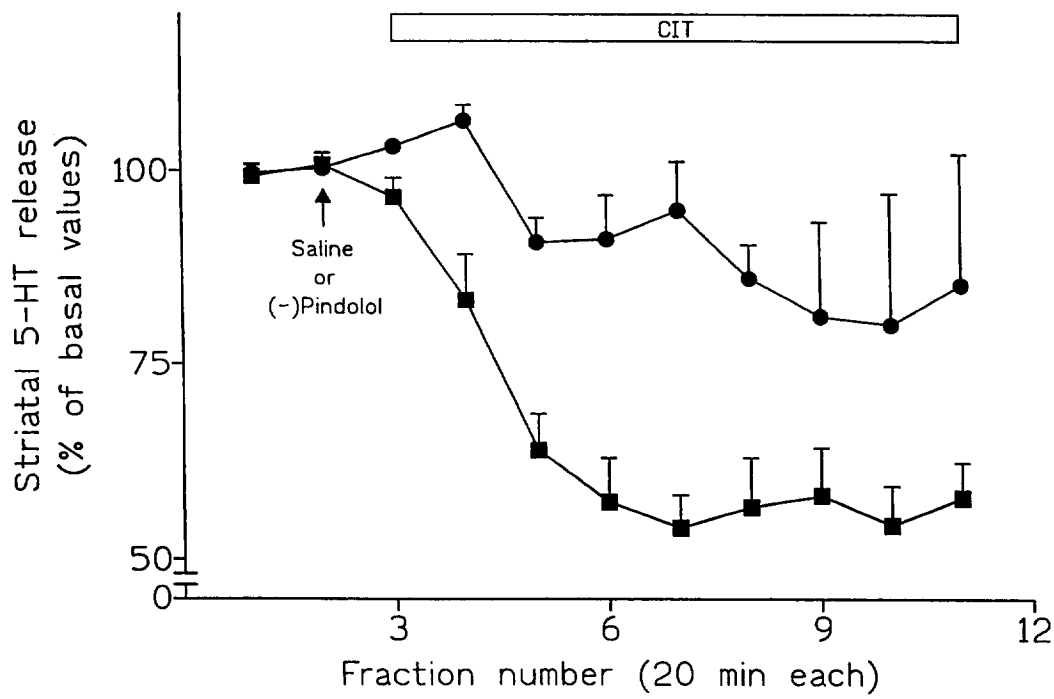

Figure 1. Antagonism by (−)pindolol (15 mg/kg, i.p.) of the reduction of striatal 5-HT output elicited by the local application of 50 μM citalopram in the DRN. Bar indicates the time of application of citalopram in the DRN. Saline (*squares*) or (−)pindolol (*circles*) were injected intraperitoneally 20 min before the application of citalopram was started. The two curves were statisically different ($p < 0.02$ using one-way ANOVA).

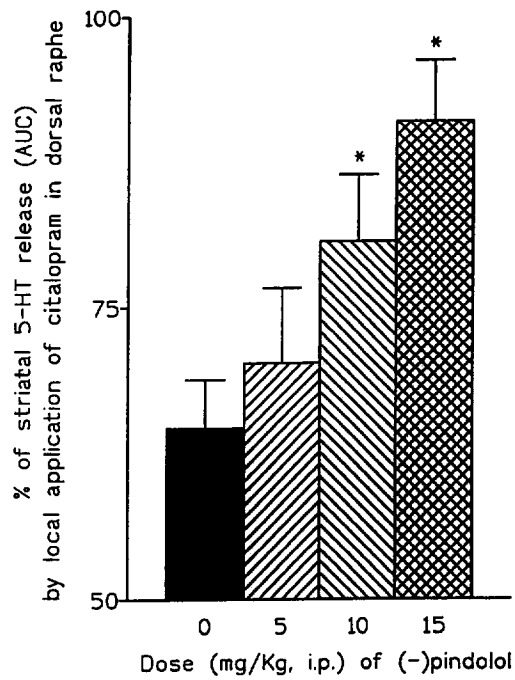

Figure 2. Dose-dependent antagonism of (−)pindolol (5, 10 and 15 mg/kg, i.p.) of the reduction of striatal 5-HT output elicited by the local application of citalopram in the dorsal raphe nucleus. Ordinate represents AUCs of striatal 5-HT dialysates during the period of citalopram infusion, expressed as percentages of their basal (100%) values (see Fig. 1 for data on the 15 mg/kg group). Each group comprised 4 to 7 rats. ANOVA indicated a significant effect of (−)pindolol on the attenuation of 5-HT output in striatum ($p < 0.013$). Asterisks denote significant differences ($p < 0.05$) vs. saline-treated animals.

in the firing activity of 5-HT neurons during the course of paroxetine and/or pindolol administration, five electrode descents through the dorsal raphe were usually carried out in each control and each treated rat: the first one 1 mm anterior to lambda on the midline, the following two 200 μm anterior and posterior to the first descent, and the last two 200 μm anterior and posterior to the first descent. The number of spontaneously active 5-HT neurons encountered, as well as their average firing activity, were assessed for each of the five descents. The sensitivity of the somatodendritic 5-HT_{1A} autoreceptor was assessed using intravenous injections of the 5-HT autoreceptor agonist lysergic acid diethylamide (LSD). In previous studies, the responsiveness of this autoreceptor assessed with intravenous injections of LSD or microiontophoresis of 5-HT was consistently modified to a similar degree by different treatments, thus validating the former approach. (Blier et al. 1984; Blier and de Montigny 1987). The maximum decrease in firing rate induced by a single dose of LSD (2–30 μg/kg) in each rat was taken as the response.

Recordings from CA_3 Pyramidal Neurons of the Dorsal Hippocampus

In order to promote capillary filling, five-barrelled glass micropipettes, preloaded with fiberglass strands, were pulled in the conventional manner and their tips broken back to 8 to 12 μm under microscopic control. The central barrel, used for extracellular unit recording, was filled with a 2-M NaCl solution. The impedance of the central barrel was between 2 and 5 MΩ. Three of the side barrels contained the following solutions: 5-HT creatinine sulphate (0.5 mM in NaCl 200 mM, pH 4; Sigma Chemical, St Louis, Missouri), acetylcholine (ACh; 20 mM in NaCl 200 mM, pH 4; Sigma), and 8-OH-DPAT (1 mM in 100 mM NaCl, pH 4). The fourth barrel was filled with a 2-M NaCl solution used for automatic current balancing. The impedance of the side barrels ranged between 40 and 80 MΩ. All drugs used were ejected as cations and retained with a current of −10 nA.

The rats were anesthetized with chloral hydrate (initial dose: 400 mg/kg IP), with subsequent doses (100 mg/kg IP) to prevent nociception to paw pinching. The micropipette was lowered at 4.2 mm lateral and 4.2 mm anterior to lambda into the CA_3 region of the dorsal hippocampus (3.3 to 3.9 mm from the cortical surface). Pyramidal neurons were identified according to their characteristic large amplitude (0.5–1.2 mV) and long duration (0.8–1.2 ms) single action potentials, alternating with complex spike discharges (Kandel and Spencer 1961). Because most pyramidal neurons of the hippocampus are not spontaneously active under chloral hydrate anaesthesia, a leak or a small ejection current of ACh (0–5 nA) was used in order to activate them within their physiological firing rate (8–12 Hz; Ranck 1975). The depressant effect of the microiontophoretic application of 5-HT on the firing activity or pyramidal neurons is expressed as the number of spikes suppressed/nA of ejection current used. This was performed on-line by a computer equipped with a Tecmar interface. Microiontophoretic ejection periods were kept constant at 50 s for 5-HT and at 40 s for 8-OH-DPAT. An experiment consisted in testing a control and a treated rat with the same micropipette and the same currents of 5-HT and 8-OH-DPAT on the same day so as to minimize the variation due to the efficacy of the pipettes.

Data Analysis

Microdialysis data are expressed as fmol/fraction (uncorrected for recovery). To facilitate comparisons between different experimental groups, data were also expressed as percentage of basal (predrug) values. Statistical analysis of single drug effects has been performed using repeated-measures analysis of variance (ANOVA). A two-way ANOVA design has been used to

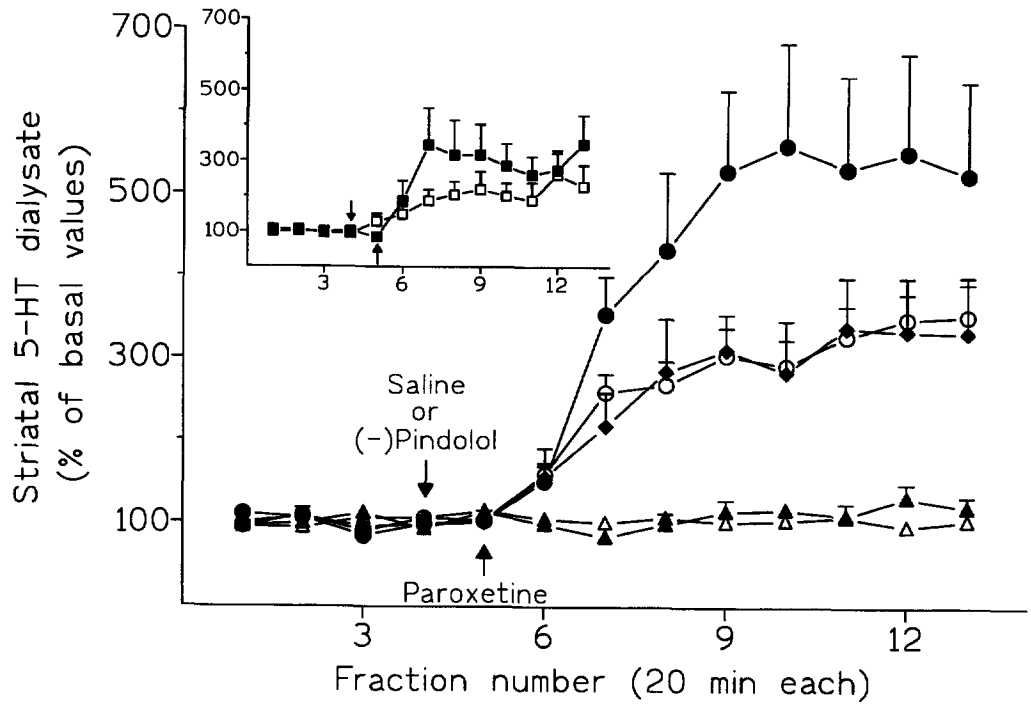

Figure 3. Effects of 3 mg/kg (i.p.) of paroxetine on dialysate 5-HT concentration in the stratum of rats pretreated with i.p. saline (*open circles*, $n = 9$) or (−)pindolol (8 mg/kg, *diamonds*, $n = 5$; 15 mg/kg, *circles*, $n = 7$). The effects of 15 mg/kg of (−)pindolol (*filled triangles*, $n = 5$) and of saline ($n = 6$; *open triangles*) are also depicted. Inset: effects of 1 mg/kg (i.p.) of paroxetine on extracellular 5-HT in striatum of rats pretreated with saline (*open squares*, $n = 6$) or (−)pindolol (*filled squares*, $n = 7$). See text for statistical details.

examine the effects of (−)pindolol on dialysate 5-HT concentrations, with time (repeated measures) and treatment (independent) factors. Data are given as means ± SEM. Statistical significance was set at the 95% level.

Chemicals

8-OH-DPAT and pindolol (racemic and (−)isomer) were purchased from Research Biochemicals (RBI; Natick, MA); 5-HT and ACh from Sigma (St. Louis, MO) and RBI; citalopram and paroxetine were gifts of H. Lundbeck A/S and Smith Kline Beecham, respectively.

RESULTS

Effect of 5-HT Reuptake Blockers and Pindolol on Extracellular 5-HT in the Striatum.

Basal concentrations of 5-HT in dialysates from dorsal striatum were 15.4 ± 1.4 fmol/fraction ($n = 22$) and 2.2 ± 0.2 fmol/fraction ($n = 48$), using probes with and without 1 μM citalopram, respectively. Striatal extracellular 5-HT release was not reduced by the application of artificial CSF in DRN, indicating a lack of effect of the perfusion *per se* (data not shown). However, the concentration of 5-HT in dialysates from the striatum was markedly reduced by the blockade of the transporter in DRN by 50 μM citalopram ($p < .001$, ANOVA for repeated measures). This reduction of the 5-HT output was counteracted in a dose-dependent manner by the prior intraperitoneal administration of (−)pindolol ($p < .02$, one-way ANOVA; Figures 1 and 2). The dose of 15 mg/kg OF(−)pindolol completely prevented the effect of citalopram in DRN and was used in subsequent experiments.

Paroxetine (1 and 3 mg/kg IP) increased extracellular 5-HT in the striatum (Figure 3) ($p < .001$ for both doses, ANOVA for repeated measures). Interestingly, extracellular 5-HT concentration increased gradually so that at the end of the experiment (i.e., 160 minutes after the injection of paroxetine), it was still increasing. This is consistent with the prolonged delay required to reach peak plasma concentrations after a single dose of paroxetine (see Dechant and Clissold 1991 for review). The combination of paroxetine (1 and 3 mg/kg) and (−)pindolol increased striatal 5-HT output more than did paroxetine alone (Figure 3), although statistical significance was reached only with the combination of 3 mg/kg of paroxetine and 15 mg/kg of (−)pindolol. Maximal differences between both experimental groups were present shortly (i.e., between 1 and 2 hours) after paroxetine administration. A two-way ANOVA revealed a significant

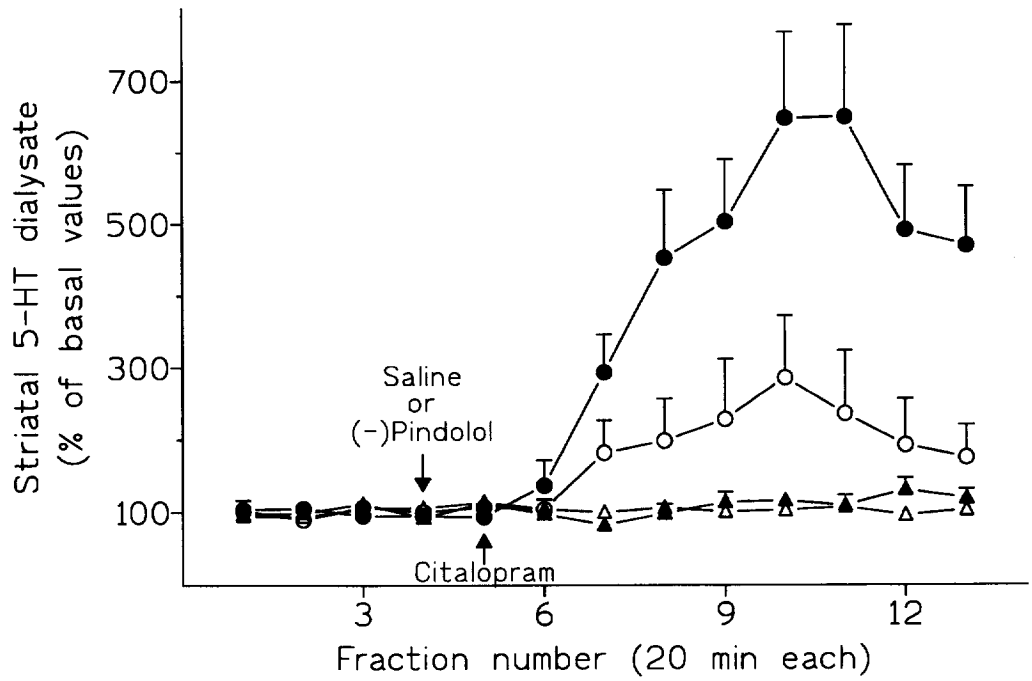

Figure 4. Effects of 1 mg/kg (i.p.) of citalopram on dialysate 5-HT concentrations in rats pretreated with saline (open circles) or 15 mg/kg of (−)pindolol (*filled circles*) ($n = 5$ in both cases). The effects of 15 mg/kg of (−)pindolol (*filled triangles*, $n = 5$) and saline ($n = 6$; *open triangles*) are also depicted.

effect of 15 mg/kg of (−)pindolol on dialysate 5-HT concentrations ($p \leq .05$, treatment factor; $p < .001$ treatment × time interaction). Maximal differences were noted for fractions 8 to 11 ($p < .04$, treatment factor) and tended to diminish with time. The effect of 3 mg/kg IP of paroxetine was not potentiated by 8 mg/kg (−)pindolol (Figure 3).

The pretreatment with 15 mg/kg IP of (−)pindolol also potentiated the effects of 1 mg/kg IP of citalopram on dialysate 5-HT concentration in striatum (significant

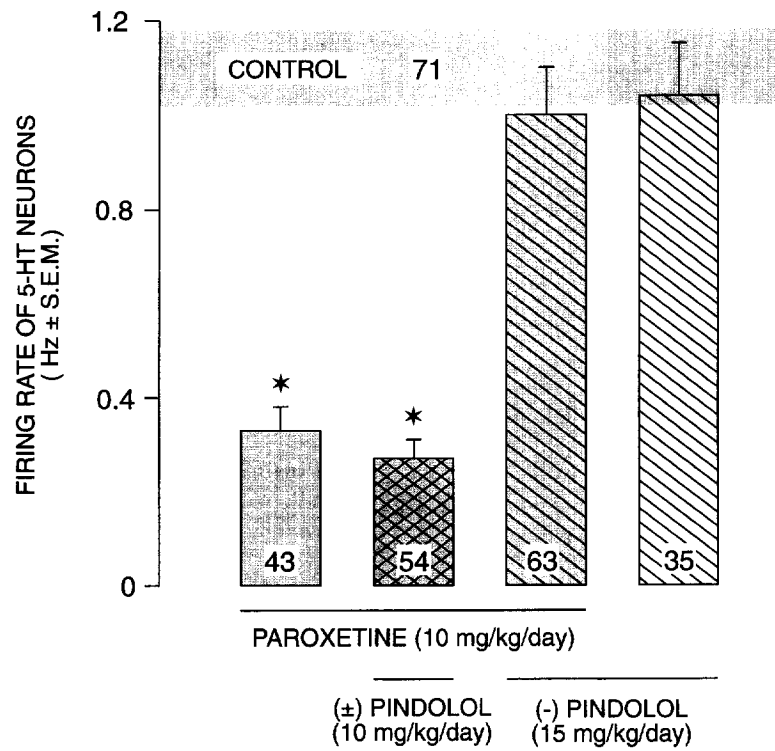

Figure 5. Effects of paroxetine and pindolol on the spontaneous firing activity of dorsal raphe 5-HT neurons recorded in chloral hydrate-anesthetized rats. The drugs were administered for 2 days with osmotic minipumps implanted subcutaneously. The numbers at the bottom of each column and in the shaded area represent the number of 5-HT neurons recorded in each group. $p < 0.05$ with respect to the control group which is depicted by the horizontal shaded area.

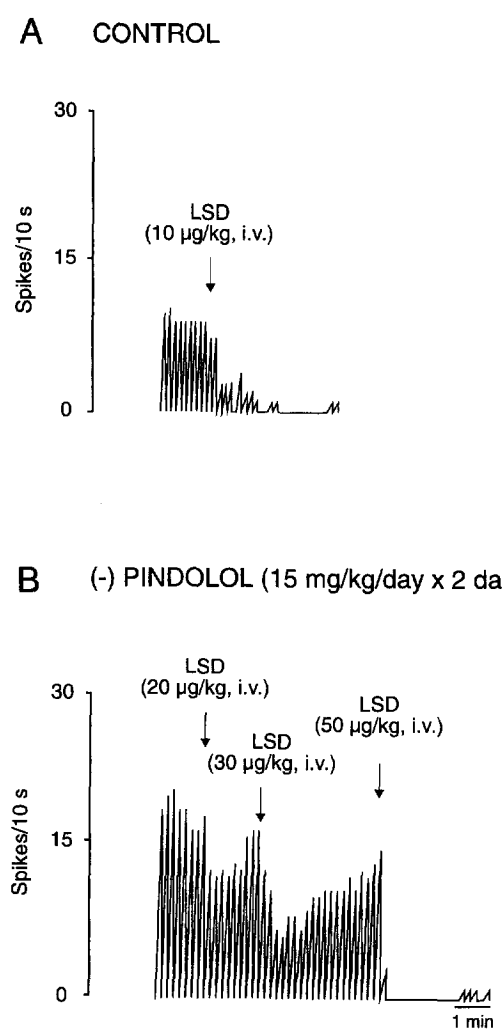

Figure 6. Integrated firing rate histograms of DRN 5-HT neurons recorded in a saline-treated rat **(A)** and in a rat treated for 2 days with (−)pindolol **(B)**, showing their response to intravenous injections of LSD. Time base applies to both traces.

effects of the time factor in both groups, $p < .001$; significant effect of the treatment, $p < .05$; significant treatment × time interaction, $p < .005$; (Figure 4).

Effects of Paroxetine and Pindolol on the Firing Activity of Dorsal Raphe 5-HT Neurons

The 2-day treatment with the SSRI paroxetine (10 mg/kg/day SC, with 2ML1 Alza minipumps implanted under halothane anesthesia under aseptic conditions) reduced by 60% the spontaneous firing activity of 5-HT neurons (Figure 5). In rats treated with (±)pindolol (10 mg/kg SC) or (−)pindolol (15 mg/kg SC), also delivered using minipumps for 2 days, the firing activity of 5-HT neurons was similar to that in the controls. The concomitant administration of paroxetine and (±)pindolol (10 mg/kg SC) still produced a marked reduction of the firing activity of 5-HT neurons that was not different from that obtained in the paroxetine-treated rats. However, the 2-day treatment with 15 mg/kg/day of (−)pindolol completely prevented the reduction of firing activity shown to occur with paroxetine (Figure 5). In order to determine if this effect of (−)pindolol was due to a blockade of somatodendritic 5-HT_{1A} autoreceptors, the responsiveness of 5-HT neurons to 5-HT autoreceptor agonist LSD was examined in these (−)pindolol-treated rats after completing the systematic electrode descents. There was a marked attenuation of the suppressant effect of intravenous LSD on 5-HT neuronal firing activity in (−)pindolol-treated rats (ED_{50}: 26 ± 6 µg/kg, IV, $n = 5$) as compared to controls (ED_{50}: 5 ± 1 µg/kg, IV, $n = 5$; see Figure 6).

Lack of Effect of (−)Pindolol on the Responsiveness of CA_3 Dorsal Hippocampus Pyramidal Neurons

The results on DRN 5-HT neurons indicate that a high dose of (−)pindolol effectively blocked somatodendritic 5-HT_{1A} autoreceptors. In order to determine whether this (−)pindolol regimen would also block postsynaptic 5-HT_{1A} receptors in the dorsal hippocampus, 5-HT and the 5-HT_{1A} agonist 8-OH-DPAT were applied by microiontophoresis onto CA3 pyramidal neurons in four pairs of control and treated rats using the same micropipette (Figure 7). The responsiveness of these neurons was similar in (−)pindolol- and saline-treated rats after a 2-day treatment (Figure 8). It is noteworthy that in two (−)pindolol-treated rats in which these experiments on postsynaptic neurons were carried out, the responsiveness of DRN 5-HT neurons to intraveneous LSD also was found to be attenuated.

DISCUSSION

The present results suggest that (−)pindolol attenuates the activation of somatodendritic 5-HT_{1A} autoreceptors by endogenous 5-HT induced by the SSRI paroxetine, as indicated by its prevention of the reduction of 5-HT neuronal firing, thus enhancing extracellular 5-HT in postsynaptic regions. Furthermore, (−)pindolol does not antagonize the actions of either 5-HT itself or of 8-OH-DPAT in the dorsal hippocampus. This provides a neurobiological basis for the reported potentiation by pindolol of the antidepressant effect of SSRI and MAOI in depressed patients (Artigas et al. 1994; Blier and Bergeron 1995).

The local infusion of the SSRI citalopram in the DRN produced a marked reduction of extracellular 5-HT release in the dorsal striatum (Figure 1), an area receiving 5-HT afferents exclusively from the DRN. This confirms earlier data obtained with push-pull cannulae in the cat

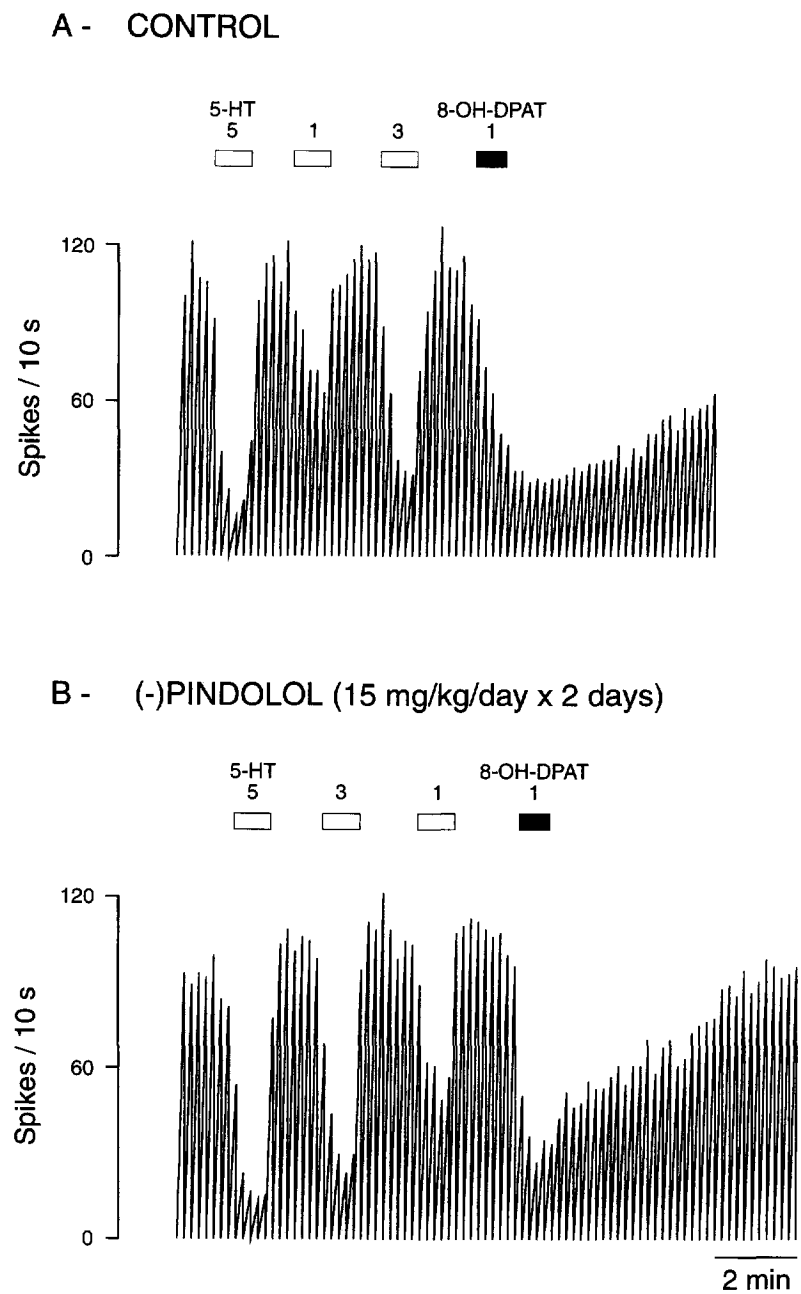

Figure 7. Integrated firing rate histograms of pyramidal neurons of the CA_3 dorsal hippocampus, recorded in a saline-treated rat **(A)** and in a rat treated for 2 days with (–)pindolol **(B)**, showing their responses to microiontophoretic applications of 5-HT and 8-OH-DPAT with the same micropipette. The length of the bars indicates the duration of the microiontophoretic application for which currents are given in nA. Time base applies to both traces.

(Bourgoin et al. 1981; Becquet et al. 1990) and microdialysis in the rat brain (Adell and Artigas 1991). The presence of at least three different subtypes of 5-HT_1 receptors has been documented in the rat DRN (5-HT_{1A}, 5-HT_{1B}, 5-$HT_{1D\alpha}$; Pazos and Palacios 1985; Bruinvels et al. 1993). Current evidence indicates that the reduction of striatal release is due to 5-HT_{1A} autoreceptor activation.

First, these 5-HT_{1A} receptors are located on the soma and dendrites of 5-HT neurons (Sotelo et al. 1990) and activate a hyperpolarizing potassium channel (Aghajanian and Lakoski 1984). Thus, the local application of 5-HT or 5-HT_{1A} agonists reduces firing of DRN 5-HT neurons (Blier and de Montigny 1987; Sprouse and Aghajanian 1987, 1988), 5-HT synthesis (Hjorth et al. 1987; Hutson et al. 1989; Invernizzi et al. 1991), and the terminal release of 5-HT (Hutson et al. 1989; Sharp et al. 1989; Adell et al. 1993). In contrast, 5-$HT_{1B/D}$ agonists do not alter DRN firing activity in anesthetized rats (Sprouse and Aghajanian 1987).

Second, 5-HT_{1A} receptors of the DRN are coupled to a G_i/G_o protein since a pretreatment with pertussis toxin prevents the hyperpolarizing actions of 5-HT and 5-HT_{1A} agonists (Innis and Aghajanian 1987). In agreement with these data, the infusion of the SSRI citalopram in rats treated with pertussis toxin in the DRN does not reduce striatal 5-HT release, indicating the involvement of a G protein–coupled receptor (Romero et

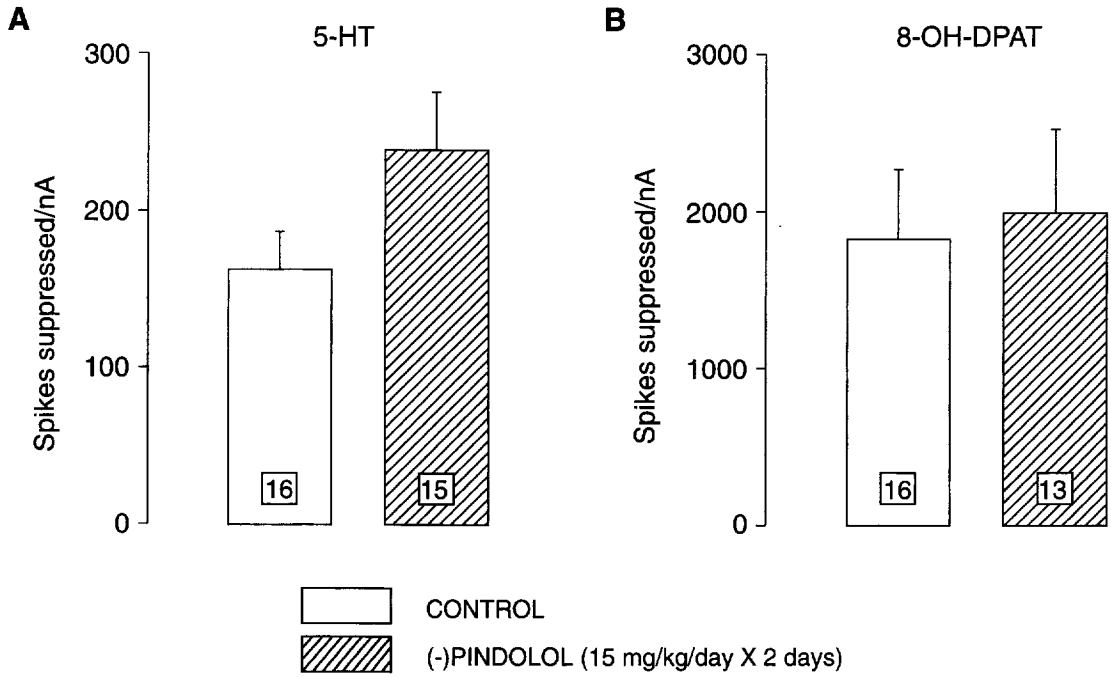

Figure 8. Effectiveness of microiontophoretic applications of 5-HT and 8-OH-DPAT onto pyramidal neurons of the CA_3 dorsal hippocampus in saline-treated rats and in rats treated with (−)pindolol for 2 days. Values are expressed as the number of spikes suppressed/nA of ejection current (± S.E.M.). The number within each column indicates the number of neurons tested. There were no statistical difference between the treated and the control group ($p > 0.05$ using the two-tailed Student's t test).

al. 1994). Finally, pindolol has moderate affinity for 5-$HT_{1A/1B}$ receptors (Pazos et al. 1985; Hoyer and Schoefter 1991) and blocks certain neuroendocrine, physiological, and behavioral responses that are mediated by either pre- or postsynaptic 5-HT_{1A} receptors (Middlemiss et al. 1985, Gehlbach and VanderMaelen 1987; Aulakh et al. 1988, Chaouloff et al. 1990; Lesch et al. 1990). The affinity of (−)pindolol for 5-$HT_{1D\alpha}$ receptors is almost two orders of magnitude lower (Bruinvels 1993). Furthermore, the citalopram-induced reduction of striatal extracellular 5-HT also is blocked by (−)tertatolol, a 5-HT_{1A}/β-adrenoceptor antagonist (Romero et al. 1994). Taken together, these data indicate that the reduction of striatal 5-HT release elicited by citalopram is mediated exclusively by 5-HT_{1A} receptors.

The increase of extracellular 5-HT release elicited by low doses of paroxetine was gradual (Figure 3), a time course possibly related to its slow absorption and distribution (Dechant and Clissold 1991). This slow increase contrasts with the rapid changes seen after a single dose of other SSRIs, such as citalopram (Invernizzi et al. 1992) and fluvoxamine (Bel and Artigas 1992). The pretreatment with 15 mg/kg of (−)pindolol potentiated the effect of paroxetine in the dorsal striatum at the dose of 3 mg/kg. This potentiation is not due to the effect of (−)pindolol by itself on extracellular 5-HT because the injection of 15 mg/kg of this agent did not alter extracellular 5-HT in the striatum (Figure 3). Interestingly, differences between the paroxetine and the paroxetine plus (−)pindolol groups tended to diminish with time. This may be related to the fact that the elimination half-life of (−)pindolol is shorter than that of paroxetine (Somogyi et al. 1992). Indeed, a lower dose of (−)pindolol (8 mg/kg IP) did not potentiate the effect of the 3-mg/kg dose of paroxetine. Moreover, there was also a potentiation of the effect of citalopram on the striatal 5-HT output with the concurrent treatment with (−)pindolol. In this case, the differences between the citalopram and the citalopram plus (−)pindolol groups were more pronounced, probably because maximal concentrations of (−)pindolol and citalopram are achieved concurrently (Milne and Goa 1991), thus maximizing the effect of the blockade of somatodendritic 5-HT_{1A} autoreceptors by (−)pindolol. Indeed, the potentiation of the effects of SSRIs on striatal 5-HT output is likely to derive from the antagonism of (−)pindolol at 5-HT_{1A} autoreceptors, as discussed above. It is unlikely that a mere increase of the dose of the SSRI could enhance further intracellular 5-HT and the antidepressant response in humans because increasing the IP dose of paroxetine from 3 to 10 mg/kg does not further enhance 5-HT levels in cortical dialysates (Romero and Artigas, unpublished observations).

Local infusion of methiothepin potentiates the in-

crease of 5-HT output in the frontal cortex produced by low doses of citalopram administered systemically (Invernizzi et al. 1992). Similarly, a potentiation of the 5-HT output in the ventral hippocampus has been observed with the systemic co-administration of citalopram and (−)penbutolol, a β-adrenergic blocker also endowed with 5-HT_{1A} antagonistic properties (Hjorth 1993). By analogy to the present results, it can be assumed that (−)penbutolol also potentiates the ability of citalopram to increase 5-HT output by blocking somatodendritic 5-HT_{1A} autoreceptors.

The hippocampus receives a preferential innervation from the median raphe 5-HT neurons (Azmitia and Segal 1978; Imai et al. 1986), which may be less sensitive than DRN 5-HT neurons to the hyperpolarizing actions of 5-HT_{1A} agonists (Sinton and Fallon 1988; Blier et al. 1990). Therefore, it remains to be established whether the degree of potentiation of the effects of SSRIs by (−)pindolol or other 5-HT_{1A} antagonists is the same in areas innervated by the DRN or the median raphe.

Interestingly, there was a parallelism between the doses of pindolol needed to block the effects of paroxetine on 5-HT neuronal firing and those required to increase striatal 5-HT release. A dose of 5 mg/kg of the (−)isomer and 10 mg/kg of the racemic compound were inactive, whereas 15 mg/kg of (−)pindolol produced a complete antagonism in both experimental approaches. The large dose of (−)pindolol needed to antagonize somatodendritic 5-HT_{1A} autoreceptors, as compared to the much smaller one required to block other 5-HT_{1A}–mediated responses, such as the 8-OH-DPAT–induced hypothermia (Aulakh et al. 1988), and the inability of (−)pindolol to antagonize the suppressant effect of 5-HT and 8-OH-DPAT in the hippocampus represent further evidence supporting the heterogeneity of 5-HT_{1A} receptors in the brain.

Pindolol displays a profile similar to that of spiperone (Blier et al. 1993), that is, it blocks pre- but not postsynaptic 5-HT_{1A} receptors, as assessed by us on both populations of neurons using microiontophoresis of 5-HT and 8-OH-DPAT. This differential effectiveness of pindolol cannot be attributed to DRN having a greater receptor reserve than hippocampus neurons as the opposite results should have been obtained. These results, together with the reverse effects of BMY 7378 with the same microiontophoretic approach on pre- versus postsynaptic neurons (Chaput and de Montigny 1988), as opposed to (−)pindolol and spiperone, constitute convincing evidence for the different pharmacological properties of 5-HT_{1A} receptors in different brain regions.

Although (−)pindolol could antagonize the somatodendritic 5-HT_{1A} autoreceptors, a higher dose was necessary than that of paroxetine required to suppress the firing activity of 5-HT neurons (Figure 5). This could possibly be explained, at least in part, by the fact that a supramaximal dose of paroxetine was used to attenuate the firing activity of 5-HT neurons, all the more so as a dose–response study with this SSRI on this parameter has not been carried out. However, this regimen of paroxetine produced a 55% inhibition of [^{3}H]5-HT uptake assessed *in vitro* in hippocampus slices (Piñeyro et al. 1994). Consequently, it appears that an even larger dose of (±)pindolol, on a mg/kg basis, would be necessary to overcome the inhibitory effect of paroxetine on the firing activity of DRN 5-HT neurons. These data would not support our hypothesis for the neurobiological substratum of the more rapid antidepressant effect of the combination of pindolol and paroxetine in major depression (Artigas et al. 1994; Blier and Bergeron 1995). Indeed, in these clinical studies, a dose of 20 mg/day of paroxetine was administered concomitantly with 2.5 mg of racemic pindolol three times a day. Despite extensive studies with other ligands, the affinity of pindolol for 5-HT_{1A} receptors in human brain is not known at present. It is, however, important to mention that human 5-HT_{1A} receptors display high affinity for [^{125}I]cyanopindolol, a ligand commonly used to label β-adrenoceptors in human brain. Using autoradiography, this ligand labels a subpopulation of 5-HT_{1A} receptors in the human DRN and hippocampus (40–55% of total binding), which was displaced by 5-HT with high affinity (pK_i = 8.9; Pazos et al. 1994). [^{125}I]cyanopindolol labels 5-HT_{1B} receptors, but not 5-HT_{1A} receptors in rat brain (Pazos et al. 1985). These results suggest a species difference of the affinity of pindolol derivatives for 5-HT_{1A} receptors. Such a species difference in the affinity of ligands by no means represents a first for receptors with the same amino acid sequence. For instance, 5-HT_3 receptors have a much greater affinity (at least one order of magnitude) for antagonists in rats than in guinea pigs (Hoyer 1990). Consequently, the apparent faster onset of the therapeutic effect of paroxetine in major depression in the presence of pindolol is likely attributable to the prevention of the decreased firing activity of 5-HT neurons, probably produced by an SSRI at the beginning of the treatment. These clinical results are presently undergoing verification in double-blind placebo-controlled trials.

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