

Potentialiation by (-)Pindolol of the Activation of Postsynaptic 5-HT_{1A} Receptors Induced by Venlafaxine

Jean-Claude Béïque, Ph.D., Pierre Blier, M.D., Ph.D., Claude de Montigny, M.D., Ph.D., and Guy Debonnel, M.D.

The increase of extracellular 5-HT in brain terminal regions produced by the acute administration of 5-HT reuptake inhibitors (SSRI's) is hampered by the activation of somatodendritic 5-HT_{1A} autoreceptors in the raphe nuclei. The present in vivo electrophysiological studies were undertaken, in the rat, to assess the effects of the coadministration of venlafaxine, a dual 5-HT/NE reuptake inhibitor, and (-)pindolol on pre- and postsynaptic 5-HT_{1A} receptor function. The acute administration of venlafaxine and of the SSRI paroxetine (5 mg/kg, i.v.) induced a suppression of the firing activity of dorsal hippocampus CA₃ pyramidal neurons. This effect of venlafaxine was markedly potentiated by a pretreatment with (-)pindolol (15 mg/kg, i.p.) but not by the selective β -adrenoceptor antagonist metoprolol (15 mg/kg, i.p.). That this effect of venlafaxine was mediated by an activation of postsynaptic 5-HT_{1A} receptors was suggested by its complete reversal by the 5-HT_{1A}

antagonist WAY 100635 (100 μ g/kg, i.v.). A short-term treatment with VLX (20 mg/kg/day \times 2 days) resulted in a ca. 90% suppression of the firing activity of 5-HT neurons in the dorsal raphe nucleus. This was prevented by the coadministration of (-)pindolol (15 mg/kg/day \times 2 days). Taken together, these results indicate that (-)pindolol potentiated the activation of postsynaptic 5-HT_{1A} receptors resulting from 5-HT reuptake inhibition probably by blocking the somatodendritic 5-HT_{1A} autoreceptor, but not its postsynaptic congener. These results support and extend previous findings providing a biological substratum for the efficacy of pindolol as an accelerating strategy in major depression. [Neuropsychopharmacology 23:294-306, 2000] © 2000 American College of Neuropsychopharmacology. Published by Elsevier Science Inc. All rights reserved.

KEY WORDS: Major depression; Antidepressant; Reuptake inhibitors; Dorsal raphe; Hippocampus; In vivo electrophysiology

It is becoming increasingly clear that the therapeutic efficacy of antidepressant treatments such as selective serotonin reuptake inhibitors (SSRI's) finds its substrate in an enhanced serotonergic (5-HT) neurotransmission (Blier and

de Montigny 1994). At least in the rat, this occurs only in the midst of long-term administration of antidepressant treatments, which is necessary for the development of specific adaptive changes. Indeed, acute administration of SSRI's induces a reduction of firing activity of the 5-HT neurons of the raphe nuclei due to an increased activation of the somatodendritic 5-HT_{1A} autoreceptors, resulting from the increased extracellular 5-HT (de Montigny et al. 1981; Blier et al. 1984; Chaput et al. 1986; Hajós et al. 1995; Béïque et al. 1999). However, in the course of a long-term administration of SSRI's, 5-HT neurons recover their normal firing activity as a consequence of the desensitization of these autoreceptors (Blier and de Montigny 1994).

A way by which the action of the somatodendritic 5-HT_{1A} autoreceptors can be circumvented has been

From the Neurobiological Psychiatry Unit, Department of Psychiatry, McGill University, Montréal, Québec, Canada.

Address correspondence to: Dr. Guy Debonnel, Neurobiological Psychiatry Unit, McGill University, 1033 Pine Ave. West, Montréal, Québec, Canada H3A 1A1.

Received September 16, 1999; revised February 3, 2000; accepted March 6, 2000.

delineated by the electrophysiological observation that a 2-day administration of the mixed β -adrenergic/5-HT_{1A} receptors antagonist (-)pindolol prevents the suppressant effects of paroxetine and of the 5-HT autoreceptor agonist LSD on the firing activity of dorsal raphe 5-HT (Romero et al. 1996). Although microdialysis studies cannot assess the postsynaptic impact, several such studies have corroborated the above by showing that the acute coadministration of (-)pindolol and an SSRI produces a greater elevation of extracellular levels of 5-HT in terminal regions than that achieved with the reuptake inhibitor alone (Dreshfield et al. 1996; Hjorth et al. 1996; Romero et al. 1996).

These observations have directly led to the hypothesis that the combination of 5-HT reuptake inhibitors with a blocker of the somatodendritic 5-HT_{1A} autoreceptor, by mean of preventing the initial decrease in firing activity of the 5-HT neurons, would lead to an earlier elevation of 5-HT levels and hence may shorten the delay of the antidepressant response (de Montigny et al. 1992; Artigas 1993). As a direct outcome of this research endeavour, it has been reported, in six of the seven double blind clinical studies assessing this issue to date, that the addition of pindolol to selected antidepressant agents is a successful strategy to shorten the delay of therapeutic action in major depression (Maes et al. 1996; Berman et al. 1997; Tome et al. 1997; Pérez et al. 1997; Zanardi et al. 1997, 1998; Bordet et al. 1998).

Given that the postsynaptic impact of the enhanced concentration of extracellular 5-HT observed in terminals area, especially in limbic structures following the combination of 5-HT reuptake blockers and (-)pindolol is undoubtedly of profound importance, it was deemed of interest to assess this aspect using an *in vivo* electrophysiological approach whereby the degree of activation of the postsynaptic 5-HT_{1A} receptor by endogenous 5-HT can be measured. Thus, it was first determined whether postsynaptic 5-HT_{1A} receptor activation could be induced by the intravenous administration of the dual 5-HT/norepinephrine (NE) reuptake inhibitor venlafaxine and, second, whether this activation could be potentiated by (-)pindolol.

MATERIALS AND METHODS

Animals

Male Sprague Dawley rats (250–300 g; Charles River, St. Constant, Québec, Canada) were received at least one day before the experiments and housed three to four per cage. They were kept on a 12:12 h light/dark cycle, with access to food and water *ad libitum*.

Short-Term Treatments

Rats were anesthetized with halothane for the subcutaneous implantation of osmotic minipumps (Alza; Palo Alto, CA) that delivered for 48 h vehicle or venlafaxine (20 mg/kg/day) with or without (-)pindolol (15 mg/kg/day). Venlafaxine and (-) pindolol were dissolved in a 50% ethanol/water solution, and control rats were implanted with minipumps containing vehicle. All experiments were carried out while the minipumps were still present.

In Vivo Electrophysiological Experiments

Animals were anesthetized with chloral hydrate (400 mg/kg, i.p.) and mounted in a stereotaxic apparatus. Supplemental doses (100 mg/kg, i.p.) of chloral hydrate were administered to maintain anesthesia monitored by the absence of a nociceptive reaction to pinching of the tail or of a hind paw. Body temperature was maintained at 37°C throughout the experiment and a catheter was installed, prior to recording, in a lateral tail vein for intravenous administration of drugs.

Extracellular Unitary Recordings of CA₃ Dorsal Hippocampus Pyramidal Neurons

Five-barrelled glass micropipettes were pulled in a conventional manner and their tips were broken back to 8–12 μ m under microscopic control. The central barrel, used for extracellular recordings, was filled with a 2 M NaCl solution containing fast green FCF. Two side barrels, used for microiontophoresis, were filled with the following solutions: 5-HT (20 mM in NaCl 200 mM, pH 4), and acetylcholine (ACh; 20 mM in 200 mM NaCl, pH 5). A third barrel was used for automatic current balancing, and contained a 2 M NaCl solution. The microelectrode was lowered 4 mm lateral and 4 mm anterior to lambda, according to the stereotaxic coordinates of Paxinos and Watson (1982).

CA₃ pyramidal neurons were recorded at the depth of 3.5 to 3.8 mm from cortical surface and identified by 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). A leak or a small ejecting current of ACh (0 to +5 nA) was used to activate silent or slowly discharging neurons to a physiological firing rate of 8–14 Hz (Ranck 1973).

The uptake activity following microiontophoretic applications of 5-HT was assessed using the recovery time 50 (RT₅₀) values. RT₅₀ is defined as the time in seconds, from the termination of the microiontophoretic application, required by the neuron to recover 50% of its initial firing frequency. The RT₅₀ value has been shown to provide a reliable index of the *in vivo* activity of the 5-HT reuptake process in the rat amygdala and

lateral geniculate body (Wang et al. 1979) and in the hippocampus (Piñeyro et al. 1994). The RT_{50} value following the microiontophoretic application of 5-HT was used to assess the extent of the lesioning of the 5-HT system induced by the pretreatment with the 5-HT toxin 5,7-dihydroxytryptamine (see below).

Extracellular Unitary Recordings of 5-HT Neurons of the Dorsal Raphe Nucleus

Single-barrelled glass micropipettes were prepared in a conventional manner (Haigler and Aghajanian 1974), with the tips broken back to 1–3 μ m and filled with a 2 M NaCl solution saturated with Fast Green FCF. A burr hole was drilled on midline, 1 mm anterior to lambda for recording of dorsal raphe neurons. Spontaneously active dorsal raphe 5-HT neurons were encountered on a distance of 1 mm starting at the ventral border of the Sylvius aqueduct, and were identified using the criteria of (Aghajanian 1978): slow, regular firing rate (0.5–2.5 Hz) and positive action potential of long duration (0.8–1.2 ms). The mean firing activity of dorsal raphe 5-HT neurons was determined by carrying out systematic microelectrode descents in the dorsal raphe while recording the firing activity of encountered 5-HT neurons. Usually, five microelectrode descents were carried out in the dorsal raphe of each rat.

5,7-Dihydroxytryptamine Pretreatments

Rats weighing between 175 and 200 g were anesthetized with chloral hydrate (400 mg/kg, i.p.). Lesions of 5-HT neurons were performed by injecting the 5-HT neurotoxin 5,7 dihydroxytryptamine (5,7-DHT; 200 μ g of free base in 20 μ l of 0.9% NaCl and 0.1% ascorbic acid) intracerebroventricularly (1 mm lateral; 0.5 mm posterior to the coronal suture and 2–2.5 mm below the surface of the brain) 1 h after the injection of desipramine (25 mg/kg, i.p.) in order to protect NE neurons. The electrophysiological experiments were carried out 14 to 16 days after the lesions were made.

Drugs

Venlafaxine and WAY 100635 were given by Wyeth-Ayerst Research (NJ, U.S.A.), whereas paroxetine was given by Smith Kline Beecham (West Sussex, U.K.). 5-HT, ACh and metoprolol were purchased from Sigma Chemical (St-Louis, MO). (-) Pindolol was purchased from RBI chemicals (Natick, MA). Drugs were either prepared in physiological saline for i.v. administration or in a 200 mM NaCl solution for microiontophoretic applications. (-)Pindolol was first dissolved in a small volume of ethanol, then diluted to the appropriate concentration with saline for its acute i.p. administration in a volume of *ca.* 1 cc.

Statistical Analysis

Results are expressed throughout as means \pm S.E.M., unless otherwise specified. When means are compared, the statistical significance of the difference was assessed using either a paired, a non-paired Student's t-test or ANOVA, as indicated in the legends to figures. Probability was considered as statistically significant when the *p* was smaller than .05.

RESULTS

Effects of Acute Administration of Venlafaxine and Paroxetine on the Firing Activity of Dorsal Hippocampus CA₃ Pyramidal Neurons

Hippocampal pyramidal neurons were brought to a physiological (8–14 Hz; Ranck 1973) firing frequency by the sustained leak or microiontophoretic application of ACh (0 to +5 nA) and recorded for at least 2 minutes to establish baseline firing activity. The acute intravenous administration of 5 mg/kg of venlafaxine (Figure 1A and 1C) or paroxetine (Figure 1B and 1D) resulted in a 73% and 80% suppression of the firing activity of CA₃ pyramidal neurons, respectively. These effects were completely reversed by the subsequent intravenous administration (100 μ g/kg) of the 5-HT_{1A} antagonist WAY 100635 (Forster et al. 1995; Fletcher et al. 1996; see Figure 1). Moreover, and as previously reported (Haddjeri et al. 1998a), the acute intravenous administration of WAY 100635 (100 μ g/kg) did not significantly alter the firing activity of dorsal hippocampus CA₃ pyramidal neurons (data not show). However, it significantly reduced the suppressant effect of the microiontophoretic application of 5-HT (number of spikes suppressed; *p* < .01 using the paired Student's t-test; Figure 2). These results indicate that the dose administered was sufficient to block postsynaptic 5-HT_{1A} receptors without altering the basal firing activity of the neuron.

Effect of a 5,7-Dihydroxytryptamine Lesion on the Suppressant Effect on the Firing Activity of Dorsal Hippocampus CA₃ Pyramidal Neurons Induced by the Acute Administration of Venlafaxine

The effect of the acute intravenous administration of venlafaxine on the firing activity of CA₃ pyramidal neurons was assessed in rats that had been treated with the serotonergic neurotoxin 5,7-dihydroxytryptamine (5,7-DHT). First, the effectiveness of the lesion of the 5-HT system was determined by assessing the reuptake activity following the microiontophoretic applications of 5-HT. This was achieved using the RT_{50} values method which provides an index on the reuptake activity of microiontophoretically applied 5-HT by serotonergic terminals (Piñeyro et al. 1994). These microiontophoretic applica-

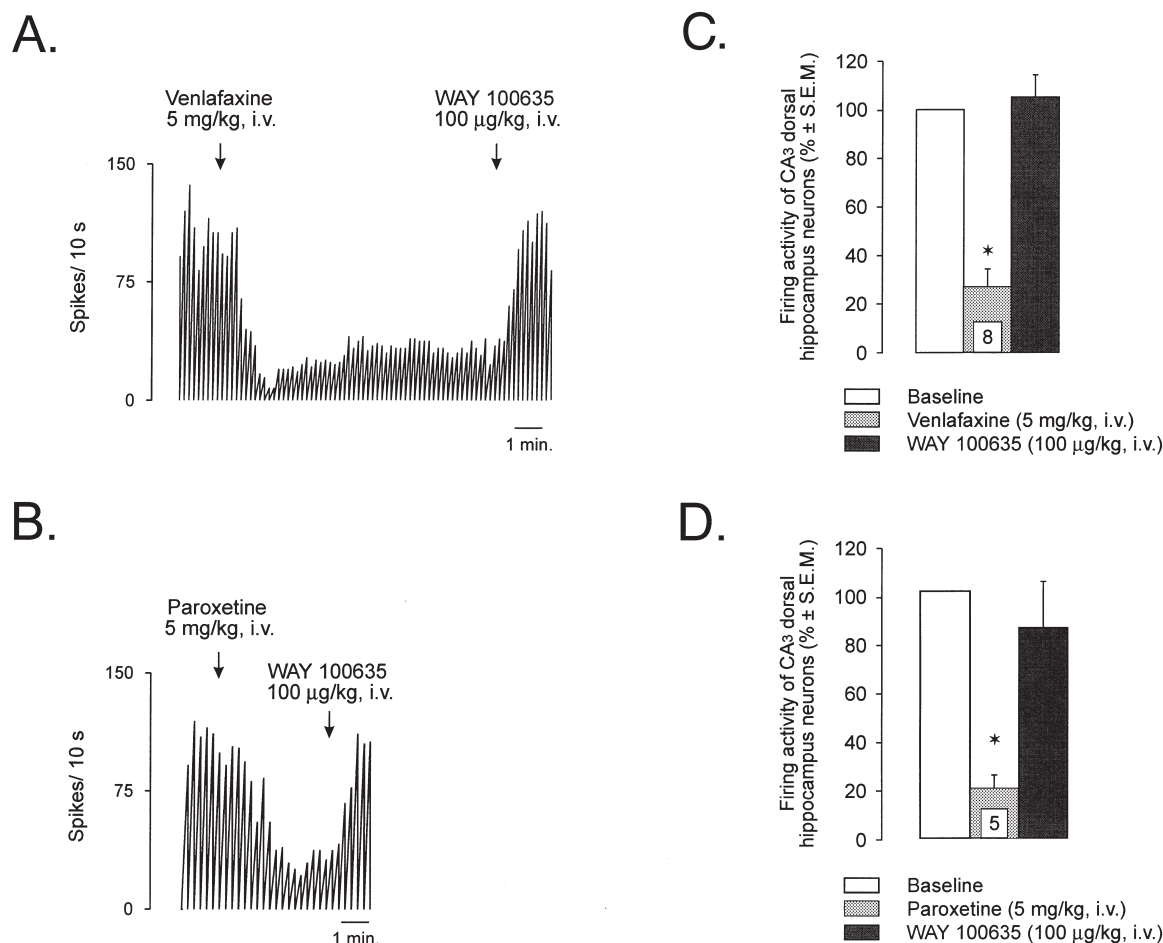


Figure 1. Integrated firing rate histograms showing the effects of acute intravenous administration of venlafaxine (**A**) and of paroxetine (**B**) on the firing activity of ACh-activated dorsal hippocampus CA₃ pyramidal neurons. The effect of a subsequent acute intravenous administration of the selective 5-HT_{1A} antagonist WAY 100635 is also shown. The suppressant effects of venlafaxine (**C**) and of paroxetine (**D**) as well as their reversal by the intravenous administration of WAY 100635 (100 µg/kg) on the firing activity of CA₃ pyramidal neurons are expressed as a percentage (mean ± S.E.M.) of the basal firing activity. **p* < .01 compared to pre-administration values using the paired Student's *t*-test. In this and subsequent figures, the numbers in the columns represent the numbers of neurons tested.

tions of 5-HT were performed in the same control and 5,7-DHT-lesioned rats from which the effects of the intravenous administration of venlafaxine were to be subsequently determined.

As exemplified in Figures 3A and 3B, the RT₅₀ values following 5-HT applications in lesioned rats were greater than those obtained in control rats (*p* < .01, using the non-paired Student's *t*-test). *Post hoc* analysis revealed that, in all five lesioned-rats tested, the RT₅₀ values following the microiontophoretic applications of 5-HT (mean value of 103 ± 15 seconds) were greater than the mean value obtained in control rats, which was of 44 ± 8 seconds. This confirmed that the 5-7-DHT lesion had destroyed 5-HT neurons in all lesioned animals. This, as can be seen from Figures 3C and 3D, prevented the suppressant effect of venlafaxine on the firing activity of CA₃ pyramidal neurons, even when

venlafaxine was administered at cumulative doses up to 10 mg/kg, i.v.

Effects of Pre-Treatments with (-)Pindolol and with Metoprolol on the Suppression of the Firing Activity of Dorsal Hippocampus CA₃ Pyramidal Neurons by Venlafaxine

The acute intravenous administration of two successive doses of 500 µg/kg of venlafaxine induced a dose-dependent suppression of the firing activity of dorsal hippocampus CA₃ pyramidal neurons (one-way ANOVA, *p* < .05; Figures 4A and 5) in rats that had received an injection of saline 20 minutes before (1 cc, i.p.). The degree of suppression of firing activity by the same two successive doses of venlafaxine was significantly greater in rats pre-treated with 15 mg/kg i.p. of

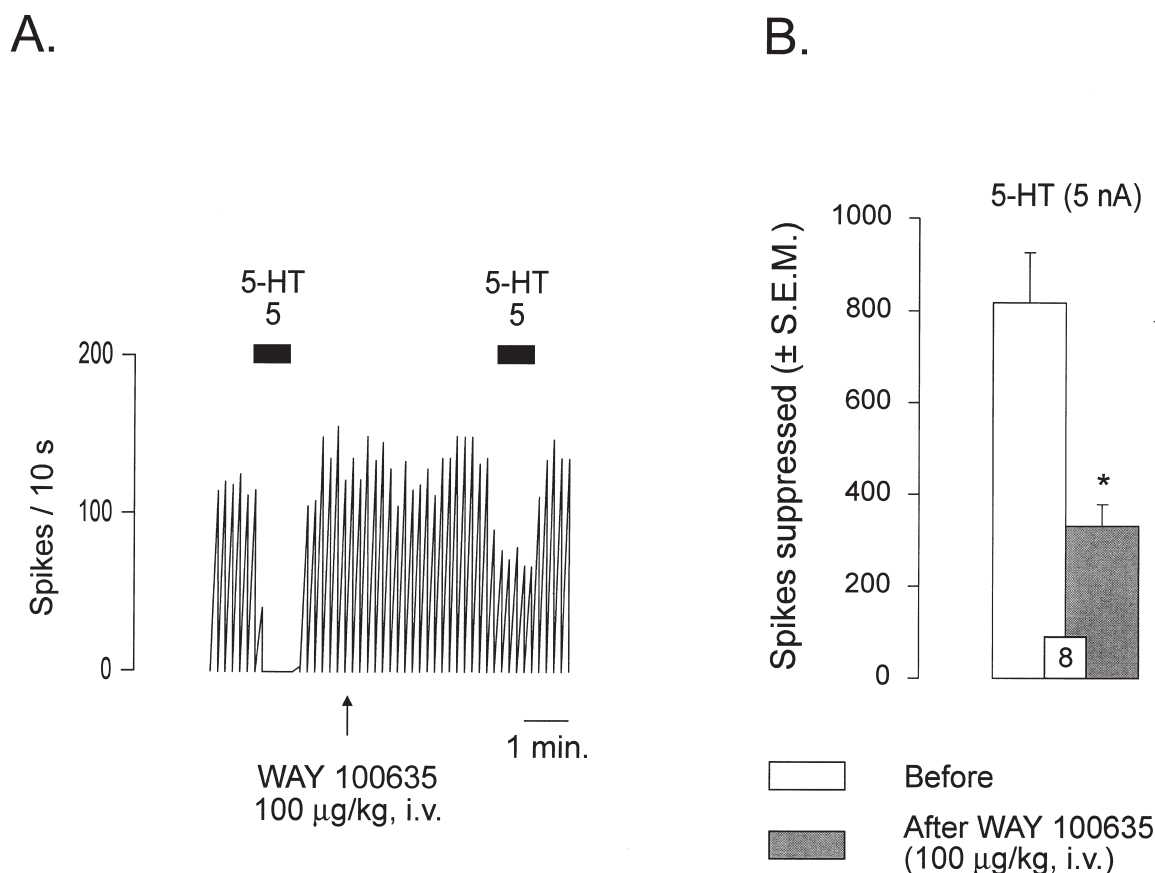


Figure 2. (A) Integrated firing rate histogram of a CA₃ pyramidal neuron showing the effects of microiontophoretic applications of 5-HT (5 nA) before and after the intravenous administration of WAY 100635. The solid bars above the trace indicate the duration of the applications for which the ejection current is given in nA. (B) Effectiveness, expressed as the number of spikes suppressed, of microiontophoretic applications of 5-HT before and after the intravenous administration of WAY 100635. * $p < .01$ using the paired Student's *t*-test.

(-) pindolol (two-way ANOVA, $p < .01$) (Figures 4B and 5). However, a pretreatment with 15 mg/kg i.p. of the selective β -adrenergic antagonist metoprolol (Ablad et al. 1973) failed to alter the degree of suppression induced by the same two doses of venlafaxine (two-way ANOVA, $p = .29$) (Figure 5).

In all three groups, the acute administration of the 5-HT_{1A} antagonist WAY 100635 (100 µg/kg, i.v.) reversed the suppressant effect of venlafaxine to or above baseline value (Figure 5).

Effects of Two-Day Treatments with Venlafaxine Alone and with Venlafaxine and (-)Pindolol on the Firing Activity of Dorsal Raphe 5-HT Neurons

Several studies from our laboratory have shown that, in the course of a sustained administration of a 5-HT reuptake blocking agent, the degree of suppression of firing activity of 5-HT neurons is maximal at two days of

treatment (Blier et al. 1984; Chaput et al. 1986). We have, thus, made use of this paradigm to assess the ability of (-)pindolol to prevent the effect of venlafaxine on somatodendritic 5-HT_{1A} receptors.

A total of 218 serotonergic neurons were recorded during multiple electrode descents (of ca. 750 µm each) in the dorsal raphe of control rats and of rats treated with 20 mg/kg/day of venlafaxine with or without (-)pindolol (15 mg/kg/day, s.c.) for two days while the minipumps were still in place (Figure 6A and 6B). In control rats, 5-HT neurons displayed a mean firing activity of about 1 Hz (Figure 6B). A two-day treatment with venlafaxine (20 mg/kg/day s.c., delivered by an osmotic minipump) reduced by 88% the spontaneous firing activity of dorsal raphe 5-HT neurons ($p < .01$, using the Student's *t*-test) (Figure 6B). In rats treated concomitantly with venlafaxine (20 mg/kg/day, s.c.) and (-)pindolol (15 mg/kg/day, s.c.), the mean firing activity of dorsal raphe neurons was no different than that obtained in control rats (Figure 6B).

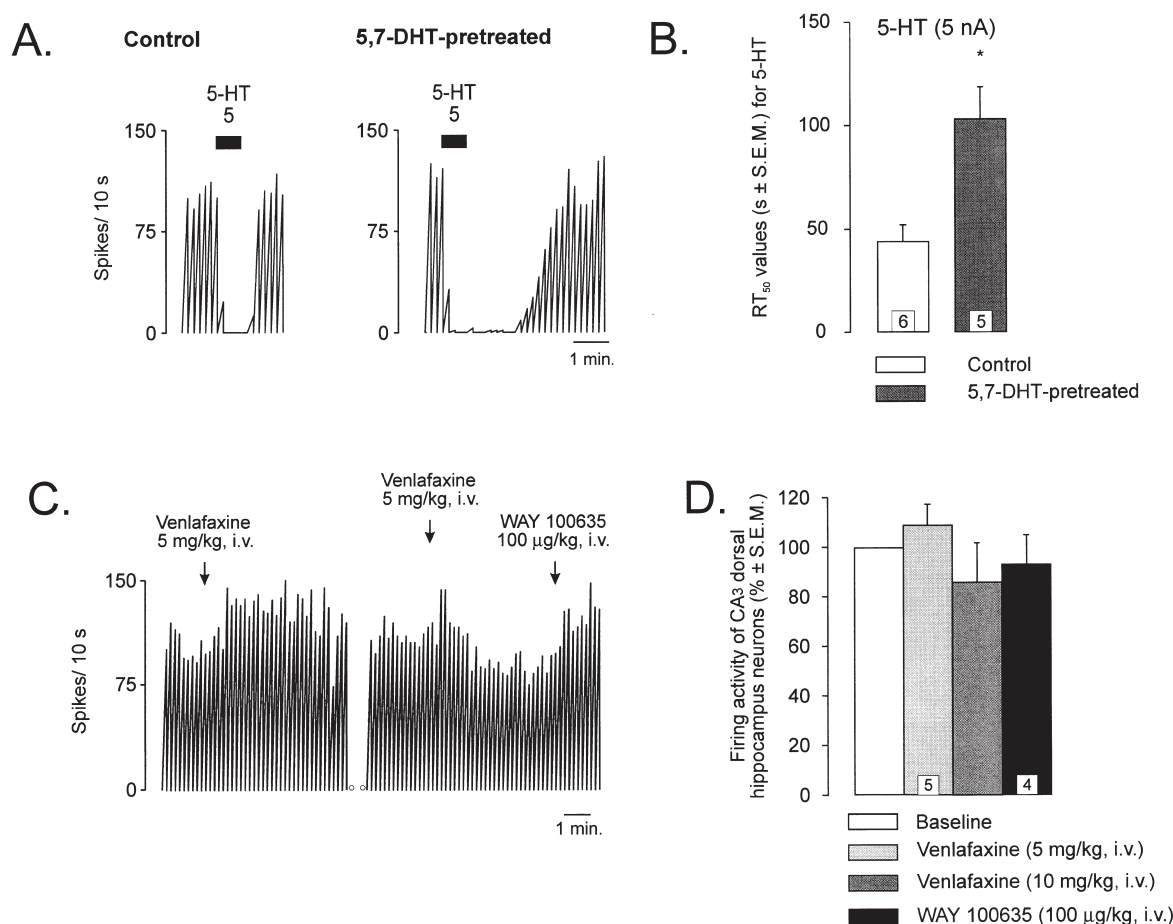


Figure 3. Integrated firing rate histograms of CA₃ dorsal hippocampus pyramidal neurons showing the effects of microiontophoretic applications of 5-HT in control and in 5,7-DHT-lesioned rats (**A**). The solid bars above each trace indicate the duration of the applications for which the ejection current is given in nA. The recovery time, expressed as RT₅₀ values (means ± S.E.M.), of CA₃ dorsal hippocampus neurons obtained with microiontophoretic applications of 5 nA of 5-HT on ACh-induced firing activity in control and in 5,7-DHT-lesioned rats is shown in **B**. * $p < .01$, compared to control values using the non-paired Student's *t*-test. The integrated firing rate histogram in **C** shows the effect of acute intravenous administration of venlafaxine on the firing activity of an ACh-activated dorsal hippocampus CA₃ pyramidal neuron of a 5,7-DHT-lesioned rat. The effect of a subsequent acute intravenous administration of the selective 5-HT_{1A} antagonist WAY 100635 is also shown. The two dots represent an interval of 2 min. The effect of venlafaxine (**D**), as well as that of the intravenous administration of WAY 100635 (100 µg/kg), on the firing activity of CA₃ pyramidal neurons of 5,7-DHT-lesioned rats are expressed as a percentage (mean ± S.E.M.) of the basal firing activity.

DISCUSSION

The data presented herein show that the acute intravenous administration of venlafaxine and that of paroxetine, two 5-HT reuptake inhibitors, induced a suppression of firing activity of CA₃ dorsal hippocampus pyramidal neurons, which effect was reversed by the 5-HT_{1A} antagonist WAY 100635. Moreover, a pretreatment with the mixed β -adrenoceptor/5-HT_{1A} antagonist (-)pindolol, but not with the selective β -adrenoceptor antagonist metoprolol, potentiated the suppression of firing activity of CA₃ pyramidal neurons induced by venlafaxine. Finally, the observation that a two-day treatment with the coadministration of (-)pindolol and

venlafaxine prevented the suppression of firing activity of dorsal raphe 5-HT neurons induced by a two-day treatment with venlafaxine alone, confirmed the antagonistic activity of (-)pindolol at the level of the somatodendritic 5-HT_{1A} autoreceptor.

The acute intravenous administration of 5-HT reuptake inhibitors suppresses the firing activity of the 5-HT neurons of the dorsal and median raphe nuclei (de Montigny et al. 1981; Blier et al. 1984; Chaput et al. 1986; Hajós et al. 1995) due to an enhanced activation of the somatodendritic 5-HT_{1A} autoreceptor by an increased level of extracellular 5-HT in the raphe (Adell and Artigas 1991; Bel and Artigas 1993; Hajós et al. 1995; Gartside et al. 1995; Rutter et al. 1995; Béïque et al. 1999).

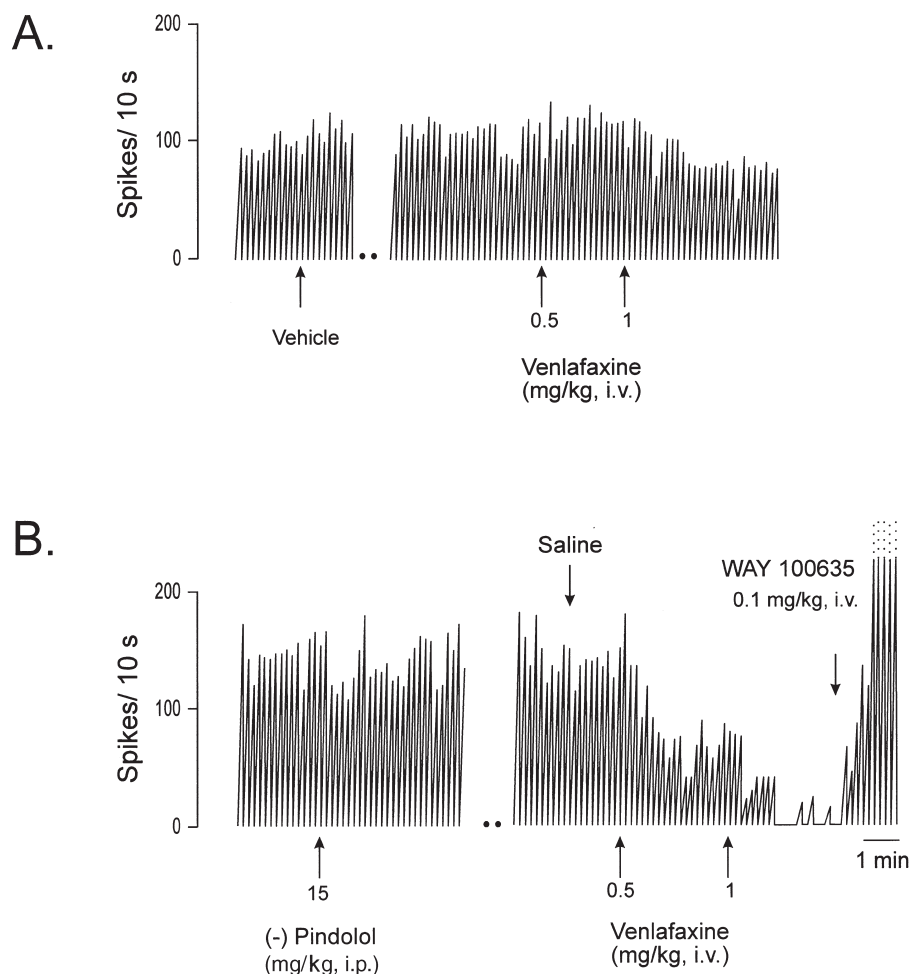


Figure 4. Integrated firing rate histograms showing the response of dorsal hippocampus CA₃ pyramidal neurons to two successive acute intravenous administration of venlafaxine (of 500 μ g/kg each) in rats that had received 20 min prior either the vehicle (1 cc, i.p.; **A**) or (-)pindolol (15 mg/kg, i.p.; **B**). The effect of a subsequent administration of WAY 100635 is also shown. The dots at the bottom of the histograms represent a period of *ca.* 20 min.

This is consistent with the fact that the potencies with which reuptake inhibitors suppress the firing activity 5-HT neurons are correlated with their affinities for the 5-HT transporter (Béïque et al. 1999).

The functional outcome and relative importance of the suppression of firing activity of 5-HT neurons by the acute administration of 5-HT reuptake inhibitors were put into perspective by *in vivo* microdialysis studies which showed that the acute systemic administration of 5-HT reuptake inhibitors results in an enhanced level of 5-HT in terminal regions and this, despite the presence of presumably silent 5-HT neurons (Invernizzi et al. 1995; Kreiss and Lucki 1995; Malagie et al. 1995). In light of these data, it can be assumed that the suppression of the firing activity of pyramidal neurons of the CA₃ region of the dorsal hippocampus produced by the acute intravenous administration of venlafaxine and paroxetine, is mediated by the activation of the inhibitory postsynaptic 5-HT_{1A} receptors by the increased

level of endogenous 5-HT. This is supported by the observation that this suppression was reversed by the selective 5-HT_{1A} antagonist WAY 100635 (Figure 1).

These 5-HT_{1A} receptors, which also mediate the suppressant effect of microiontophoretically-applied 5-HT (Figure 2), are indeed known to have an inhibitory action on firing activity mediated by the hyperpolarization of the cells through opening of a potassium channel (Andrade et al. 1986). Moreover, the fact that the intravenous administration of the 5-HT_{1A} agonists flesinoxan, 8-OH-DPAT and flibanserin suppresses the firing activity of dorsal hippocampus CA₃ pyramidal neurons (Hadrava et al. 1995; Rueter et al. 1998) corroborates this conclusion. The observation that a lesion of the 5-HT system with 5,7-DHT prevented the suppressant effect of paroxetine (Piñeyro et al. 1994) as well as that of venlafaxine (Figure 3C and 3D) on the firing activity of CA₃ pyramidal neurons, also provides strong credence to the idea that their suppressant effects result from in-

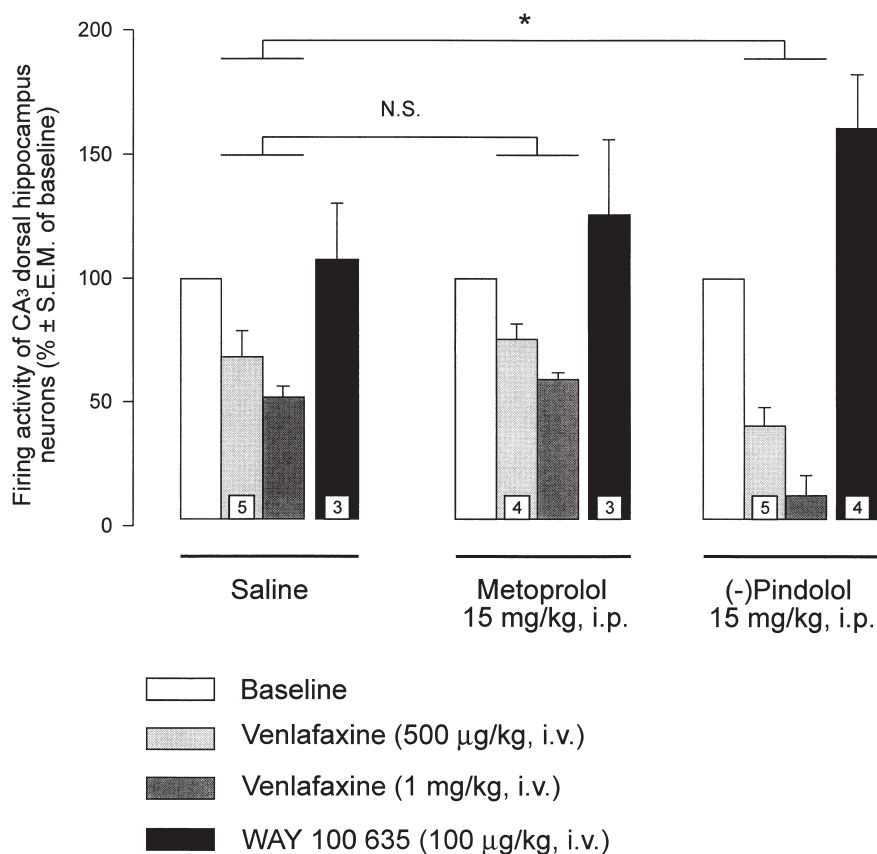


Figure 5. Firing activity of dorsal hippocampus CA₃ pyramidal neurons following the acute intravenous administration of two successive doses of 0.5 mg/kg of venlafaxine (cumulative doses of 0.5 mg/kg and of 1 mg/kg) expressed as the percentage of pre-injection (baseline) value in rats that were pre-treated 20 min prior with either the vehicle (1 cc, i.p.), metoprolol (15 mg/kg, i.p.), or (-) pindolol (15 mg/kg, i.p.). * $p < .01$, compared to the suppressant effect obtained in rats pre-treated with saline, using a two-way ANOVA.

creased levels of extracellular 5-HT subsequent to re-uptake blockade.

Given that venlafaxine blocks also the reuptake of NE, albeit with a weaker potency than that of 5-HT (Muth et al. 1986; Béique et al. 1998a,b, 1999), the possibility that this property could, at least in part, mediate the suppressant effect of venlafaxine on the firing activity of CA₃ pyramidal neurons has to be considered. The abolishment of this suppressant effect of venlafaxine by 5,7-DHT, combined with the observation that WAY 100635 completely reversed the effect of venlafaxine, strongly infers that the suppressant effect of venlafaxine was mainly the result of its effect on the 5-HT rather than on the NE system. This contention is further supported, first, by the lack of suppressant effect of intravenous administration of the NE reuptake inhibitor desipramine of the firing activity of hippocampal pyramidal neurons (Lacroix et al. 1991; Curet et al. 1992; Béique et al. 1998a) and second, by the fact at the dose used in the present study (5 mg/kg, i.v.), venlafaxine does not block, *in vivo*, the uptake of NE in the hippocampus (Béique et al. 1998a).

The suppression of firing activity of CA₃ pyramidal neurons induced by venlafaxine was potentiated by a pretreatment with (-)pindolol (Figure 5). Since this suppression was also totally reversed by the subsequent intravenous administration of the 5-HT_{1A} antagonist WAY 100635, these results suggest that (-)pindolol potentiates the degree of activation of postsynaptic 5-HT_{1A} receptors induced by venlafaxine. Several possibilities must be envisaged in the attempt to identify the pharmacological properties of (-)pindolol that underlie this potentiation. Some relevant issues will hence be discussed below.

The primary clinical indication of pindolol is for the treatment of mild to moderate hypertension which is ought to its β -adrenergic receptor blockade. That the effects observed herein with (-)pindolol are not consequent of the latter property is shown by the observation that a pretreatment with metoprolol, a mixed $\beta_{1/2}$ -adrenergic antagonist devoid of significant affinity for 5-HT_{1A} receptors, failed to potentiate the effect of venlafaxine in the present paradigm (Figure 5). Importantly, this is fully consistent with the clinical observa-

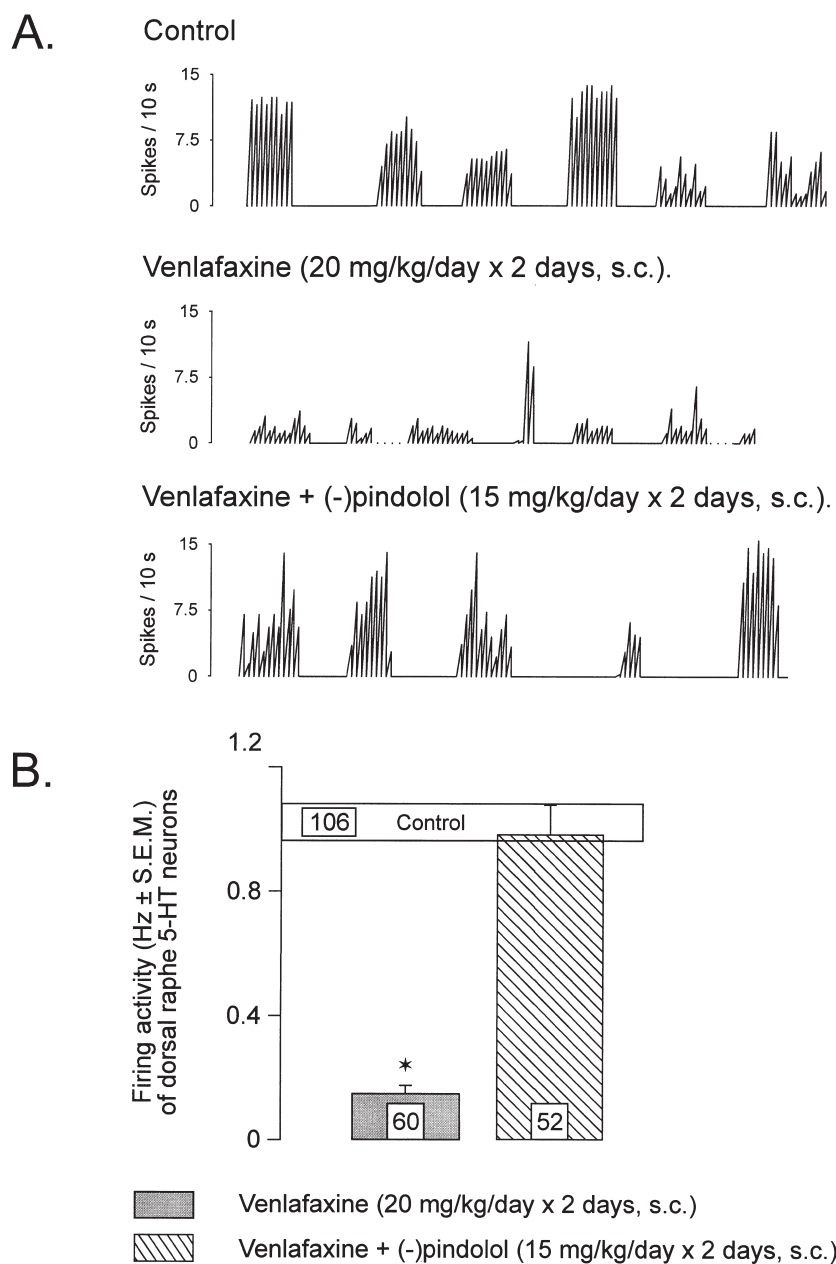


Figure 6. In **A**, integrated firing rate histograms of dorsal raphe 5-HT neurons recorded in the dorsal raphe showing their spontaneous firing activity in rats treated for two days with the vehicle, venlafaxine alone, or venlafaxine plus (-)pindolol. In **B** are shown the effects of a 2-day treatment with venlafaxine alone and of a cotreatment with venlafaxine and (-) pindolol on the spontaneous firing activity of dorsal raphe 5-HT neurons. The open horizontal bar represents the range (S.E.M. \times 2) of the firing activity of 5-HT neurons in rats treated with vehicle. * $p < .01$ using the non-paired Student's *t*-test.

tion of Zanardi et al. (1997), that metoprolol failed to accelerate the antidepressant response of paroxetine.

(-)Pindolol bears significant affinity for the 5-HT_{1B} receptor (Hoyer et al. 1985) which is known to negatively regulate the release of 5-HT in terminal areas of the rat brain (Engel et al. 1986). Given that in functional assays (-)pindolol acts as an antagonist at the latter receptor (Schoeffter and Hoyer 1989), it is plausible that the latter property could account, at least in part, for the potenti-

ating effect of (-)pindolol on the suppressant effect of venlafaxine on the firing activity of hippocampal neurons that was observed in the present study. Nonetheless, while *in vivo* microdialysis studies suggest that a 5-HT_{1B} antagonism may increase the output of 5-HT elicited by 5-HT reuptake inhibitors, there is evidence that this would only be so in the presence of normal firing of 5-HT neurons, i.e., in the presence of an adequate 5-HT_{1A} autoreceptor blockade (Hjorth 1993; Sharp et al. 1997).

The 5-HT_{1A} antagonism of (-)pindolol, thus, appears to be a *sine qua non* condition for its 5-HT_{1B} antagonistic property to be of functional relevance. However, the involvement of the antagonism of the 5-HT_{1B} by (-)pindolol in mediating its potentiating effect cannot be ruled out in light of the data presented herein. Nevertheless, one must underscore the fact that the 5-HT_{1B} antagonism of (-)pindolol does not account, most probably, for the efficacy of (-)pindolol as an adjunct in major depression and this, for the following reason. In the human brain, it has been demonstrated that terminal 5-HT release is controlled by the human 5-HT_{1B} receptor [h5-HT_{1B} (Fink et al. 1995), using the nomenclature put forward by Hartig et al. (1996)]. Even if the rat 5-HT_{1B} (r5-HT_{1B}) and h5-HT_{1B} receptor share high sequence homology (Voigt et al. 1991; Weinshank et al. 1992), they have important pharmacological differences one of which being that the h5-HT_{1B} does not bind (-)pindolol with high affinity (Hartig et al. 1992; Parker et al. 1993). Thus, these results indicate that the usefulness of (-)pindolol in major depression is unlikely to be due to an antagonistic activity at the r5-HT_{1B} receptor since the latter receptor does not have a pharmacological equivalent in humans (for review see Hartig et al. 1992, 1996).

Previous studies have shown that the blockade of the somatodendritic 5-HT_{1A} autoreceptors potentiates the elevation of extracellular levels of 5-HT in terminal areas induced by 5-HT reuptake inhibitors (Hjorth 1993; Hjorth et al. 1996; Gartside et al. 1995; Dreshfield et al. 1996; Romero and Artigas 1997). This contention has also been verified with venlafaxine in a recent report published during the preparation of this manuscript (Gur et al. 1999). These *in vivo* microdialysis studies do not, however, ascertain the overall 5-HT neurotransmission as they only can assess the level of extracellular 5-HT and not its effect on the postsynaptic target neurons. The importance of this is exemplified by WAY 100635 which possesses antagonistic properties both at the level of somatodendritic and postsynaptic 5-HT_{1A} receptors (Hajós et al. 1995; Béïque et al. 1999; present study). Indeed, while this drug potentiates the rise in extracellular level of 5-HT induced by SSRI's, it concurrently blocks postsynaptic 5-HT_{1A} receptors, thereby partly cancelling out the effect of the enhanced 5-HT concentration on overall 5-HT neurotransmission (Figures 1 and 2).

Given that activation of postsynaptic 5-HT_{1A} receptors in limbic areas may be involved in the antidepressant response (de Montigny et al. 1992), these results suggest that WAY 100635 most likely would not be an effective adjunct in the treatment of major depression, as previously suggested (Gartside et al. 1995). In this respect, (-)pindolol differs from WAY 100635. Indeed, while at the level of the dorsal raphe it prevented the suppression of the firing activity of 5-HT neurons by two-day treatment of venlafaxine (Figure 6), it did not

block the effect of the venlafaxine-induced rise of endogenous on postsynaptic 5-HT_{1A} receptors of CA₃ pyramidal neurons of the dorsal hippocampus (Figures 4 and 5).

WAY 100635, on the other hand, has been shown to antagonize, contrarily to (-)pindolol, the effects of endogenous 5-HT on 5-HT_{1A} receptors both in the hippocampus (Figure 1) and in the dorsal raphe (Béïque et al. 1999). These observations, thus, demonstrate functionally that (-)pindolol, but not WAY 100635, shows a discriminative antagonism between somatodendritic and hippocampal postsynaptic 5-HT_{1A} receptors, as it blocked the former and not the latter. Taken together, these results thus suggest that the antagonism of somatodendritic 5-HT_{1A} receptors in the absence of a blockade of their postsynaptic congeners by (-)pindolol is the most likely mechanism by which it potentiates the activation of postsynaptic 5-HT_{1A} receptors resulting from 5-HT reuptake inhibition. Moreover, the complementary use of the different paradigms in the present study should prove useful to assess the properties of molecules with a putative differential antagonistic activities on somatodendritic vs. postsynaptic 5-HT_{1A} receptors.

The discriminative pre- and postsynaptic 5-HT_{1A} receptor antagonism of (-)pindolol may appear surprising since there is not yet any definite evidence provided by molecular biology that these two populations of receptors are distinct entities. However, several lines of evidence suggest that the somatodendritic 5-HT_{1A} receptor and its postsynaptic congener have distinct physiological and pharmacological properties (reviewed in de Montigny and Blier 1992). Nonetheless, it needs to be mentioned that in the wake of the increasing interest aroused by the usefulness of pindolol in major depression, a considerable amount of apparent conflicting results has emerged. For instance, it has been shown that when administered acutely, (-) or (±)pindolol in the awake cat (Fornal et al. 1997) as well as (±)pindolol in the anesthetized rat (Haddjeri et al. 1998b), suppresses the firing activity of 5-HT neurons in the dorsal raphe. This effect appears to be mediated by an agonistic action of pindolol on somatodendritic 5-HT_{1A} receptors as, in both studies, it was reversed by the subsequent acute intravenous administration of WAY 100635. Moreover, the intravenous administration of pindolol, as well as its microiontophoretic application directly onto dorsal raphe 5-HT neurons, have been reported to induce a suppression of firing activity (Clifford et al. 1998). Overall, these results suggesting an agonistic activity of pindolol at somatodendritic 5-HT_{1A} receptors are in apparent discrepancy with the present ones showing that a two-day treatment of (-)pindolol produces an efficient antagonism on this population of receptors (Figure 6). In addition, there is other evidence suggesting an antagonism of (-)pindolol on somatodendritic 5-HT_{1A} receptors. In particular, Romero et al. (1996) observed that a

two-day treatment with (-)pindolol prevents the suppressant effect on the firing activity of 5-HT neurons of: 1) paroxetine (administered for two days); 2) microiontophoretically applied 5-HT and 8-OH-DPAT; and 3) the systemic administration of the 5-HT autoreceptor agonist LSD. In keeping with these observations, when administered intravenously at low doses to rats, pindolol prevents the suppressant effect of intravenous administration of the 5-HT receptor agonist LSD on the firing activity of dorsal raphe 5-HT neurons (Haddjeri et al. 1998b).

Although this antagonism was not observed in the awake cats and in the anaesthetized rats with the use of systemic administration of the 5-HT_{1A} agonist 8-OH-DPAT (Fornal et al. 1997; Haddjeri et al. 1998b), *in vitro* electrophysiological studies in the rat have demonstrated that (-)pindolol antagonizes the inhibitory action of 5-CT and ipsapirone on the firing activity of dorsal raphe 5-HT neurons in mesencephalic slices (Corradetti et al. 1998), thus giving further credence to the notion that pindolol possesses somatodendritic 5-HT_{1A} receptor antagonistic activity. Part of the answer to these inconsistencies may lie in the observation that a low dose of (-)pindolol is more effective than a higher dose in antagonizing the decrease of extracellular levels of hippocampal 5-HT induced by 8-OH-DPAT (Assié and Koek 1996). Although definite evidence remains to be provided, the most likely explanation for these apparent discrepancies is that pindolol would display a predominant agonistic activity at high concentrations and an antagonistic one at low concentrations. The latter contention is however questionable in light of the apparent discordant observation observed at the post-synaptic level also.

Using the *in vitro* hippocampal slice preparation, it was shown that pindolol readily blocks the effect of 5-CT (Corradetti et al. 1998), whereas *in vivo* electrophysiological studies suggest that pindolol does not antagonize in the dorsal hippocampus microiontophoretically applied 5-HT (Romero et al. 1996) as well as endogenous 5-HT (present study) where the concentration of the drug is likely to be lower. Interestingly, using a biochemical assay (stimulation of [³⁵S]-GTPγS binding in Chinese Hamster Ovary cells), a recent study has shown that whilst (-)pindolol displayed intrinsic activity (although modest) on human-5-HT_{1A} receptors mediated function, it also blocked the action of 5-HT itself, thus showing a clear partial agonistic activity (Newman-Tancredi et al. 1998). Thus, the notion of a partial agonistic activity of pindolol may reconcile some of the apparently discrepant effects observed using different experimental conditions and approaches.

The results presented herein must also be considered in a clinical perspective. There is now a large body of evidence suggesting that the activation of the postsynaptic 5-HT_{1A} receptor plays a prominent role in the

therapeutic action of antidepressants, thus validating the clinical significance and relevance of attaining its earlier activation (Kurtz 1992; Haddjeri et al. 1998a). Therefore, that (-)pindolol acts by circumventing the homeostatic activity of the inhibitory somatodendritic 5-HT_{1A} autoreceptor in a way to potentiate the activation of postsynaptic 5-HT_{1A} receptors, provides a tenable and attractive hypothesis for its clinical effectiveness as an accelerating strategy in the treatment of major depression.

It has been suggested that venlafaxine is endowed with a faster onset of action in major depression and that it is effective for treatment-resistant depression (Nierenberg et al. 1994; Derivan et al. 1995; de Montigny et al. 1999). Since the latter phenomena supervene only when high doses are administered, it has been suggested that they would be attributable to the recruitment of the noradrenergic system through NE reuptake blockade as this occurs only when high doses are administered to rats (Béique et al. 1998a). Thus, in light of the results presented herein, one can contemplate the possibility that the combination of pindolol and venlafaxine might prove a powerful strategy to hasten the antidepressant response as well to treat refractory depression.

ACKNOWLEDGMENTS

This work was supported by the Medical Research Council of Canada (MRC; grants MA 6444 and MT 11014) and by Wyeth-Ayerst Research. J.C.B. is in receipt of a fellowship from the Fonds de la Recherche en Santé du Québec (FRSQ); P.B. is recipient of a MRC Scientist award; and G.D. is recipient of a Scholarship from the FRSQ.

REFERENCES

- Ablad B, Carlsson E, Ek L (1973): Pharmacological studies of two new cardioselective adrenergic beta-receptor antagonists. *Life Sci* 12:107–119
- Adell A, Artigas F (1991): Differential effects of clomipramine given locally or systemically on extracellular 5-hydroxytryptamine in raphe nuclei and frontal cortex. An *in vivo* brain microdialysis study. *Naunyn-Schmiedeberg's Arch Pharmacol* 343:237–244
- Aghajanian GK (1978): Feedback regulation of central monoaminergic neurons: Evidence from single cell recording studies. In Youdim MBH, Lovenberg W, Sharman DF, Lagnado JR (eds), *Essays in Neurochemistry and Neuropharmacology*. New-York, John Wiley and Sons, pp 1–32
- Andrade R, Malenka RC, Nicoll RA (1986): A G protein couples serotonin and GABA_B receptors to the same channels in hippocampus. *Science* 234:1261–1265
- Artigas F (1993): 5-HT and antidepressants: New views from microdialysis studies. *Trends Pharmacol Sci* 14:262

- Assié MB, Koek W (1996): (-)-Pindolol and (±)-tertanolol affect rat hippocampal 5-HT levels through mechanisms involving not only 5-HT_{1A}, but also 5-HT_{1B} receptors. *Neuropharmacology* 35:213–222
- Bel N, Artigas F (1993): Chronic treatment with fluvoxamine increases extracellular serotonin in frontal cortex but not in raphe nuclei. *Synapse* 15:243–245
- Berman RM, Darnell AM, Miller HL, Anand A, Charney DS (1997): Effect of pindolol in hastening response to fluoxetine in the treatment of major depression: A double-blind, placebo-controlled trial. *Am J Psychiatry* 154:37–43
- Béique JC, de Montigny C, Blier P, Debonnel G (1998a): Blockade of 5-Hydroxytryptamine and noradrenaline uptake by venlafaxine: A comparative study with paroxetine and desipramine. *Br J Pharmacol* 125:526–532
- Béique JC, de Montigny C, Blier P, Debonnel G (1999): Venlafaxine: Discrepancy between in vivo 5-HT and NE reuptake blockade and affinity for reuptake sites. *Synapse* 32:198–211
- Béique JC, Lavoie N, de Montigny C, Debonnel G (1998b): Affinities of venlafaxine and various reuptake inhibitors for the serotonin and norepinephrine transporters. *Eur J Pharmacol* 349:129–132
- Blier P, de Montigny C (1994): Current advances and trends in the treatment of depression. *Trends Pharmacol Sci* 15:220–226
- Blier P, de Montigny C, Tardif D (1984): Effects of the two antidepressant drugs mianserin and indalpine on the serotonergic system: Single cell studies in the rat. *Psychopharmacology* 84:242–249
- Bordet R, Thomas P, Dupuis B (1998): Effect of pindolol on onset of action of paroxetine in the treatment of major depression: Intermediate analysis of a double-blind, placebo-controlled trial. *Am J Psychiatry* 155:1346–1351
- Chaput Y, de Montigny C, Blier P (1986): Effects of a selective 5-HT reuptake blocker, citalopram, on the sensitivity of 5-HT autoreceptors: Electrophysiological studies in the rat. *Naunyn-Schmiedeberg's Arch Pharmacol* 33:342–349
- Clifford EM, Gartside SE, Umbers V, Cowen PJ, Hajós M, Sharp T (1998): Electrophysiological and neurochemical evidence that pindolol has agonist properties at the 5-HT_{1A} autoreceptor *in vivo*. *Br J Pharmacol* 124:206–212
- Corradetti R, Laaris N, Hanoun N, Laporte AM, Le Poul E, Hamon M, Lanfumey L (1998): Antagonist properties of (-)-pindolol and WAY 100635 at somatodendritic and postsynaptic 5-HT_{1A} receptors in the rat brain. *Br J Pharmacol* 123:449–462
- Curet O, de Montigny C, Blier P (1992): Effect of desipramine and amphetamine on noradrenergic neurotransmission: Electrophysiological studies in the rat brain. *Eur J Pharmacol* 221:59–70
- de Montigny C, Blier P (1992): Electrophysiological properties of 5-HT_{1A} receptors and of 5-HT_{1A} agonists. In Stahl SM, Gastpar M, Keppel Hesselink JM, Traber J (eds), *Serotonin 1A receptors in Depression and Anxiety*. New York, Raven Press, pp 83–98
- de Montigny C, Blier P, Caillé G, Kouassi E (1981): Pre- and postsynaptic effect of zimelidine and norzimelidine on the serotonergic system: single cell studies in the rat. *Acta Psychiatr Scand* 63:S79–S80
- de Montigny C, Chaput Y, Blier P (1992): Classical and novel targets for antidepressant drugs. In Mendlewicz J, Brunello N, Langer SZ, Racagni G (eds), *New pharmacological approach to the therapy of depressive disorders*. Basel, S. Karger, pp 8–17
- de Montigny C, Silverstone P.H, Bakish D, Blier P, Debonnel G (1999): Venlafaxine in treatment-resistant major depression: A Canadian multicentre open-label trial. *J Clin Psychopharmacol* 19:401–406
- Derivan A, Entsuah AR, Kikta D (1995): Venlafaxine: Measuring the onset of antidepressant action. *Psychopharmacol Bull* 31:439–447
- Dreshfield LJ, Wong DT, Perry KW, Engleman EA (1996): Enhancement of fluoxetine-dependent increase of extracellular serotonin (5-HT) levels by (-)-pindolol, an antagonist at 5-HT_{1A} receptors. *Neurochem Res* 21:557–562
- Engel G, Göthert M, Hoyer D, Schlicker E, Hillenbrand K (1986): Identity of inhibitory presynaptic 5-hydroxytryptamine (5-HT) autoreceptors in the rat brain cortex with 5-HT_{1B} binding sites. *Naunyn-Schmiedeberg's Arch Pharmacol* 332:1–7
- Fink K, Zentner J, Göthert M (1995): Subclassification of presynaptic 5-HT autoreceptors in the human cerebral cortex as 5-HT_{1D} receptors. *Naunyn-Schmiedeberg's Arch Pharmacol* 352:451–454
- Fletcher A, Forster EA, Brown G, Cliffe IA, Hartley JE, Jones DE, McLenachan A, Stanhope KJ, Critchley DJP, Childs KJ, Middlefell VC, Lanfumey L, Corradetti R, Laporte A-M, Gozlan H, Hamon M, Dourish CT (1996): Electrophysiological, biochemical, neurohormonal and behavioral studies with WAY 100635, a potent, selective and silent 5-HT_{1A} receptor antagonist. *Behav Brain Res* 73:337–353
- Fornal CA, Martin FJ, Metzler CW, Jacobs BL (1997): Pindolol suppresses serotonergic neuronal activity (by a WAY 100635-sensitive mechanism) and does not block the neuronal inhibition produced by 8-OH-DPAT in awake cats. *Soc Neurosci Abstr* 23:973
- Forster EA, Cliffe IA, Bill DJ, Dover GM, Jones D, Reilly Y, Fletcher A (1995): A pharmacological profile of the selective silent 5-HT_{1A} receptor antagonist, WAY-100635. *Eur J Pharmacol* 281:81–88
- Gartside SE, Umbers V, Hajos M, Sharp T (1995): Interaction between a selective 5-HT_{1A} receptor antagonist and an SSRI *in vivo*: Effects on 5-HT cell firing and extracellular 5-HT. *Br J Pharmacol* 115:1064–1070
- Gur E, Dremencov E, Lerer B, Newman ME (1999): Venlafaxine: acute and chronic effects on 5-hydroxytryptamine levels in rat brain *in vivo*. *Eur J Pharmacol* 372:17–24
- Haddjeri N, Blier P, de Montigny C (1998a): Long-term antidepressant treatments result in a tonic activation of forebrain 5-HT_{1A} receptors. *J Neurosci* 18:10150–10156
- Haddjeri N, de Montigny C, Blier P (1998b): Modulation of the firing activity of rat serotonin and noradrenaline neurons by (±)pindolol. *Biol Psychiatry* 45:1163–1169
- Hadrava V, Blier P, Dennis T, Ortemann C, de Montigny C (1995): Characterization of 5-hydroxytryptamine_{1A} properties of flesinoxan: In vivo electrophysiology and hypothermia study. *Neuropharmacology* 34:1311–1326
- Haigler HJ, Aghajanian GK (1974): Lysergic acid diethylamide and serotonin: A comparison of effects on serotonergic

- neurons receiving a serotonergic input. *J Pharmacol Exp Ther* 168:688–699
- Hajós M, Gartside SE, Sharp T (1995): Inhibition of median and dorsal raphe neurones following administration of the selective serotonin reuptake inhibitor paroxetine. *Naunyn-Schmiedeberg's Arch Pharmacol* 351:624–629
- Hartig PR, Branchek TA, Weinshank RL (1992): A subfamily of 5-HT_{1D} receptor genes. *Trends Pharmacol Sci* 13:152–159
- Hartig PR, Hoyer D, Humphrey PPA, Martin GR (1996): Alignment of receptor nomenclature with the human genome: Classification of 5-HT_{1B} and 5-HT_{1D} receptor subtypes. *Trends Pharmacol Sci* 17:103–105
- Hjorth S (1993): Serotonin 5-HT_{1A} autoreceptor blockade potentiates the ability of the 5-HT reuptake inhibitor citalopram to increase nerve terminal output of 5-HT *in vivo*: A microdialysis study. *J Neurochem* 60:776–779
- Hjorth S, Bengtsson HJ, Milano S (1996): Raphe 5-HT_{1A} autoreceptors, but not postsynaptic 5-HT_{1A} receptors or β -adrenoceptors, restrain the citalopram-induced increase in extracellular 5-hydroxytryptamine *in vivo*. *Eur J Pharmacol* 316:43–47
- Hoyer D, Engel G, Kalkman HO (1985): Characterization of the 5-HT_{1B} recognition site in rat brain: Binding studies with (-)[¹²⁵I]iodocyanopindolol. *Eur J Pharmacol* 118:1–12
- Invernizzi R, Bramante M, Samanin R (1995): Extracellular concentrations of serotonin in the dorsal hippocampus after acute and chronic treatment with citalopram. *Brain Res* 696:62–66
- Kandel ER, Spencer WA (1961): Electrophysiology of hippocampal neurons. II. After potentials and repetitive firing. *J Neurophysiol* 24:243–259
- Kreiss DS, Lucki I (1995): Effects of acute and repeated administration of antidepressant drugs on extracellular levels of 5-hydroxytryptamine measured *in vivo*. *J Pharmacol Exp Ther* 274:866–876
- Kurtz N (1992): Efficacy of azapirones in depression. In Stahl SM, Gastpar M, Keppel Hesselink JM, Traber J (eds), *Serotonin 1A receptors in Depression and Anxiety*. New York, Raven Press, pp 163–170
- Lacroix D, Blier P, Curet O, de Montigny C (1991): Effects of long-term desipramine administration on noradrenergic neurotransmission: Electrophysiological studies in the rat brain. *J Pharmacol Exp Ther* 257:1081–1090
- Maes M, Vandoolaeghe E, Desnyder R (1996): Efficacy of treatment with trazodone in combination with pindolol or fluoxetine in major depression. *J Aff Dis* 41:201–210
- Malagie I, Trillat AC, Jacquot C, Gardier AM (1995): Effects of acute fluoxetine on extracellular serotonin levels in the raphe: An *in vivo* microdialysis study. *Eur J Pharmacol* 286:213–217
- Muth EA, Haskins JT, Moyer JA, Husbands GE, Nielsen ST, Sigg EB (1986): Antidepressant biochemical profile of the novel bicyclic compound Wy-45,030, an ethyl cyclohexanol derivative. *Biochem Pharmacol* 35:4493–4497
- Newman-Tancredi A, Chaput C, Gavaudan S, Verrière L, Millan MJ (1998): Agonist and antagonist actions of (-)-pindolol at recombinant, human serotonin_{1A} (5-HT_{1A}) receptors. *Neuropsychopharmacology* 18:395–398
- Nierenberg AA, Feighner JP, Rudolph R, Cole JO, Sullivan J (1994): Venlafaxine for treatment-resistant unipolar depression. *J Clin Psychopharmacol* 14:419–423
- Parker EM, Grisel DA, Iben LG, Shapiro RA (1993): A single amino acid difference accounts for the pharmacological distinctions between the rat and human 5-hydroxytryptamine_{1B} receptors. *J Neurochem* 60:380–383
- Pérez V, Gilaberte I, Faries D, Alvarez E, Artigas F (1997): Randomised, double-blind, placebo-controlled trial of pindolol in combination with fluoxetine antidepressant treatment. *Lancet* 349:1594–1597
- Piñeyro G, Blier P, Dennis T, de Montigny C (1994): Desensitization of the neuronal 5-HT carrier following its long-term blockade. *J Neurosci* 14:3036–3047
- Ranck JB (1973): Studies of single neurons in dorsal hippocampal formation and septum in unrestrained rats. I. Behavioral and firing repertoires. *Exp Neurol* 41:461–531
- Romero L, Artigas F (1997): Preferential potentiation of the effects of serotonin uptake inhibitors by 5-HT_{1A} receptor antagonist in the dorsal raphe pathway: Role of somatodendritic autoreceptors. *J Neurochem* 68:2593–2603
- Romero L, Bel N, Artigas F, de Montigny C, Blier P (1996): Effect of pindolol on the function of pre- and postsynaptic 5-HT_{1A} receptors: *In vivo* microdialysis and electrophysiological studies in the rat brain. *Neuropsychopharmacology* 15:349–360
- Rueter LE, de Montigny C, Blier P (1998): *In vivo* electrophysiological assessment of the agonistic properties of flibanserin at pre- and postsynaptic 5-HT_{1A} receptors in the rat brain. *Synapse* 29:392–405
- Rutter JJ, Gundlach C, Auerbach SB (1995): Systemic uptake inhibition decreases serotonin release via somatodendritic autoreceptor activation. *Synapse* 20:225–233
- Schoeffter P, Hoyer D (1989): 5-Hydroxytryptamine 5-HT_{1B} and 5-HT_{1D} receptors mediating inhibition of adenylate cyclase activity. *Naunyn-Schmiedeberg's Arch Pharmacol* 340:285–292
- Sharp T, Umbers V, Gartside SE (1997): Effect of a selective 5-HT reuptake inhibitor in combination with 5-HT_{1A} and 5-HT_{1B} receptor antagonists on extracellular 5-HT in rat frontal cortex *in vivo*. *Br J Pharmacol* 121:941–946
- Tome MB, Isaac MT, Harte R, Holland C (1997): Paroxetine and pindolol: A randomized trial of serotonergic autoreceptor blockade in the reduction of antidepressant latency. *Int Clin Psychopharmacol* 12:81–89
- Voigt JP, Laurie DJ, Seeburg PH, Bach A (1991): Molecular cloning and characterization of a rat brain cDNA encoding a 5-hydroxytryptamine_{1B} receptor. *EMBO J* 10:4017–4023
- Wang RY, de Montigny C, Gold BI, Roth RH, Aghajanian GK (1979): Denervation supersensitivity to serotonin in rat forebrain: Single cell studies. *Brain Res* 178:479–497
- Weinshank RL, Zgombick JM, Macchi MJ, Branchek TA, Hartig PR (1992): Human serotonin 1D receptor is encoded by a subfamily of two distinct genes: 5-HT_{1Da} and 5-HT_{1Db}. *Proc Natl Acad Sci USA* 89:3630–3634
- Zanardi R, Artigas F, Franchini L, Sforzini L, Gasperini M, Smeraldi E, Perez J (1997): How long should pindolol be associated with paroxetine to improve the antidepressant response? *J Clin Psychopharmacol* 17:446–450
- Zanardi R, Franchini L, Gasperini M, Lucca A, Smeraldi E, Perez J (1998): Faster onset of action of fluvoxamine in combination with pindolol in the treatment of delusional depression: A controlled study. *J Clin Psychopharmacol* 18:441–446