British Journal of Pharmacology (1998) 125, 526–532
http://www.stockton-press.co.uk/bjp

Blockade of 5-Hydroxytryptamine and noradrenaline uptake by venlafaxine: a comparative study with paroxetine and desipramine

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1 Venlafaxine is an antidepressant agent which blocks *in vitro* the reuptake of both 5-HT and NA. The present *in vivo* electrophysiological studies were undertaken, in the rat, to compare the effects of venlafaxine on 5-HT and NA reuptake to those of the selective 5-HT reuptake inhibitor paroxetine and the selective NA reuptake inhibitor desipramine.

2 Administered acutely, venlafaxine dose-dependently prolonged the time required for a 50% recovery (RT_{50}) of the firing activity of dorsal hippocampus CA_3 pyramidal neurons from the suppression induced by microiontophoretic applications of 5-HT and NA. Venlafaxine and paroxetine increased with a similar potency the RT_{50} values for 5-HT, while desipramine was more potent than venlafaxine at increasing the RT_{50} values for NA. Moreover, venlafaxine demonstrated a greater potency at increasing the RT_{50} values for 5-HT compared to that of NA.

3 A two-day treatment with venlafaxine (delivered s.c. by osmotic minipumps) increased the RT_{50} values for both 5-HT and NA applications. The RT_{50} values for 5-HT were significantly increased at a dose of 10 mg kg^{-1} day^{-1}, whereas those for NA were increased at a dose of 20 mg kg^{-1} day^{-1}, consistent with the data obtained following the acute administration of venlafaxine.

4 Taken together, these results indicate that, *in vivo*, venlafaxine blocks both reuptake processes, with a potency greater for the 5-HT than for the NA reuptake process. This dual action, combined with the differential potency of venlafaxine, might constitute the biological *substratum* responsible for its apparent unique clinical efficacy in major depression.

Keywords: Antidepressant; reuptake inhibition; microiontophoresis; major depression

Introduction

Several lines of evidence point towards an involvement of the serotonergic (5-HT) and noradrenergic (NA) systems in the pathophysiology of major depression (see Maes & Meltzer, 1995; Schatzberg & Schildkraut, 1995 for reviews). As for the therapeutic efficacy of currently available antidepressant agents, a considerable corpus of data suggests that it would be mediated *via* an increase of 5-HT neurotransmission (Blier & de Montigny, 1994). The latter increase has been documented with different classes of antidepressant drugs and shown to occur either through a direct action on the 5-HT system (de Montigny & Aghajanian, 1978; de Montigny, 1984; Blier *et al*., 1986; Chaput *et al*., 1986; Blier & de Montigny, 1990) or through indirect mechanisms *via* the noradrenergic system (Mongeau *et al*., 1994a,b; Haddjeri *et al*., 1995). Consequently, it is conceivable that a drug that would act simultaneously on the two systems could increase 5-HT transmission to a greater extent, thus potentially providing a greater clinical efficacy and/or display an earlier onset of action.

Clinical evidence has suggested a potential therapeutic benefit of a dual blockade of the 5-HT and NA reuptake processes. Indeed, it was reported that the combination regimen of the selective 5-HT reuptake inhibitor (SSRI) fluoxetine and the NA reuptake inhibitor desipramine resulted in a more rapid antidepressant action than desipramine alone (Nelson *et al*., 1991), while it was also suggested that a similar combination therapy was effective in refractory depression (Weilburg *et al*., 1989; Seth *et al*., 1992; Zajecka *et al*., 1995). It is noteworthy, however, that these results have yet not been verified in double-blind, placebo controlled trials.

Venlafaxine (1-[2-(dimethylamino)-1-(4-methyoxyphenyl)-ethyl]cyclohexanol) is a phenylethylamine derivative, which was shown to possess antidepressant properties (Schweizer *et al*., 1991; Guelfi *et al*., 1992; Samuelian *et al*., 1992). It was also reported to display an early onset of therapeutic action (Rickels, 1991; Derivan *et al*., 1995; Benkert *et al*., 1996) and to be effective in treatment-resistant depression when used at high doses (Nierenberg *et al*., 1994; de Montigny *et al*., 1998). This drug was shown to inhibit, *in vitro*, the synaptosomal uptake of both [^{3}H]5-HT and [^{3}H]NA (Muth *et al*., 1986; Bolden-Watson & Richelson, 1993), whilst showing no significant affinity for classical receptor subtypes in rat (Muth *et al*., 1986) and human brain (Cusack *et al*., 1994). In this regard, venlafaxine differs from all other antidepressants available and hence shows a unique pharmacological profile that is predictive of a more favorable side-effect profile and, more important, that might confer a greater efficacy. However, a recent radioligand binding study has shown that venlafaxine binds to the 5-HT transporter with only a moderate affinity and to the NA transporter with a very low affinity (Béïque *et al*., 1998), thus raising important considerations with regard to the exact mechanism whereby venlafaxine exerts its reuptake blocking action.

Given the relative paucity of neurobiological studies that have examined the neurobiological actions of venlafaxine, it was deemed necessary to further characterize the purported reuptake inhibition properties of venlafaxine using an *in vivo* electrophysiological paradigm in the rat dorsal hippocampus. To this end, the effects of acute intravenous administration of venlafaxine were assessed on the recovery of firing activity of pyramidal neurons from microiontophoretic application of 5-HT and NA. The effects of a short-term treatment (2 days, s.c.) with venlafaxine, which allows the drug to reach steady state

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concentration, were also assessed. Moreover, in order to fully evaluate the dual 5-HT/NA reuptake inhibition potency of venlafaxine, its acute effect on the reuptake of 5-HT was compared to that of the SSRI paroxetine, and on NA reuptake with that of the NA reuptake inhibitor desipramine.

Methods

Male Sprague Dawley rats (250–300 g: Charles River, St. Constant, Québec, Canada) were received 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 weighing between 250 and 275 g were anaesthetized with halothane for the subcutaneous implantation of osmotic minipumps (Alza, Palo Alto, CA, U.S.A.) that delivered doses of venlafaxine of 10, 20 or 40 mg kg^{-1} day^{-1} or vehicle for 48 h. Venlafaxine was 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 on board.

In vivo *electrophysiological experiments*

Animals were anaesthetized with chloral hydrate (400 mg kg^{-1}, i.p.) and mounted in a stereotaxic apparatus. Supplemental doses (100 mg kg^{-1}, i.p.) of chloral hydrate were given to maintain anaesthesia, 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 in a lateral tail vein, prior to recording, for intravenous administration of drugs.

Extracellular unitary recordings of CA_3 dorsal hippocampus pyramidal neurons

Five-barrelled glass micropipettes were pulled in a conventional manner and their tips were broken back to 8–12 μM diameter under microscopic control. The central barrel, used for extracellular recordings, was filled with a 2 M NaCl solution containing fast green FCF. Three side barrels, used for microiontophoresis, were filled with the following solutions: 5-HT (20 mM in NaCl 200 mM, pH: 4), NA (20 mM in NaCl 200 mM, pH: 4) or acetylcholine (ACh; 20 mM in 200 mM NaCl, pH: 5). The fourth 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 & Watson (1986). CA_3 pyramidal neurons were recorded at a depth of 3.5–3.8 mm from the cortical surface and were 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 & 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). 5-HT and NA were both retained with a current of −12 nA. The uptake activity following microiontophoretic applications of 5-HT and NA was assessed using the recovery time 50 (RT_{50}) values. RT_{50} 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 RT_{50} value has been shown to provide a reliable index of the *in vivo* activity of the NA reuptake process in the rat hippocampus (de Montigny *et al*., 1980; Gravel & de Montigny, 1987) and 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} values were used to assess reuptake activity before and 2–5 min after the acute intravenous administration of venlafaxine and paroxetine on the 5-HT reuptake process and of venlafaxine and desipramine on the NA reuptake process. The effect of a short-term treatment (2 days) with venlafaxine on the reuptake inhibition of both these monoamines was also assessed using the RT_{50} index.

Drugs

Besides venlafaxine (Wyeth-Ayerst Research, NJ, U.S.A.), the following drugs were used: paroxetine (Smith Kline Beecham, West Sussex, U.K.), desipramine (RBI, Natick, MA, U.S.A.), 5-HT, NA and ACh (Sigma Chemical, St-Louis, MO, U.S.A.). Drugs were either prepared in physiological saline for i.v. administration or in a 200 mM NaCl solution for microiontophoretic applications.

Statistical analysis

Results are expressed throughout as means ± s.e.m., unless otherwise specified. When two means are compared, the statistical significance of the difference was assessed using either a paired or a non-paired Student's *t*-test, as indicated in the legend to figures. Probability values of $P<0.05$ were considered to be statistically significant.

Results

Effect of the acute intravenous administration of venlafaxine and paroxetine on the recovery time from microiontophoretic applications of 5-HT

The recovery time, from the suppression of hippocampus pyramidal neuron firing activity following microiontophoretic application of 5-HT, was assessed using the RT_{50} values before and after the acute intravenous administrations of venlafaxine and paroxetine. As the acute injection of venlafaxine and paroxetine (Piñeyro *et al*., 1994) decreased the firing activity of these pyramidal neurons, the current of ACh had therefore to be increased in order to maintain a rate of firing activity similar to that before the injection (Figure 1). Microiontophoretic applications of 5-HT in control rats resulted in a suppression of the firing activity which gradually recovered upon cessation of the application (Figure 1). Following the acute administration of 1 mg kg^{-1} i.v. venlafaxine, the RT_{50} values were significantly increased following application of 5-HT with a current of 5 nA but not with one of 1 nA. At the doses of 5, 10 and 20 mg kg^{-1}, the RT_{50} values for 5-HT with both currents used were significantly increased (Figure 2a and b). Successive intravenous administrations of 5 mg kg^{-1} paroxetine dose-dependently increased the RT_{50} value following 5-HT microiontophoretic application of 1 nA (Figure 2a) and of 5 nA (Figure 2b). With both currents used for 5-HT applications, venlafaxine and paroxetine did not significantly differ in their capacity to prolong the RT_{50} values.

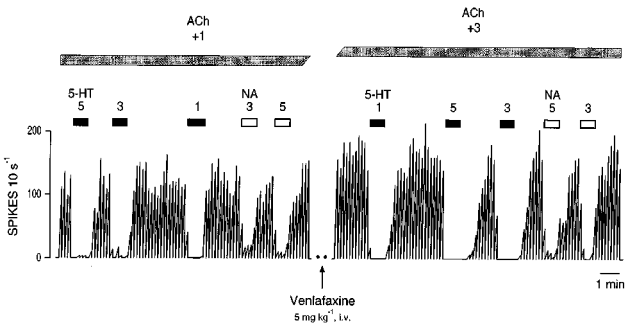

Figure 1 Integrated firing rate histogram of a CA_3 dorsal hippocampus pyramidal neuron showing the effects of microiontophoretic applications of 5-HT and NA, before and following the intravenous administration of 5 mg kg^{-1} of venlafaxine. The solid bars above the trace indicate the duration of microiontophoretic application, for which the ejection current is given in nA. The two dots represent an interval of 2 min.

Effect of the acute intravenous administration of venlafaxine and desipramine on the recovery time from microiontophoretic applications of NA

The effects of the acute administration of venlafaxine and desipramine on the NA reuptake process were assessed as for that of 5-HT. It is important to state that the effects of venlafaxine on the duration of microiontophoretic applications of both 5-HT and NA were assessed on the same neurons. At the dose of 5 mg kg^{-1} i.v., venlafaxine did not modify the RT_{50} values following NA applications (Figure 3a). A significant increase was observed with the intravenous administration of 10 mg kg^{-1}, while with 20 mg kg^{-1}, an approximately two fold increase of the RT_{50} values was obtained.

The effect of venlafaxine on the RT_{50} values following NA applications was compared to that of desipramine (Figure 3b). As previously observed (Lacroix *et al*., 1991), desipramine significantly and dose-dependently prolonged the RT_{50} values following NA applications at doses of 2 and 5 mg kg^{-1} (Figure 3b).

Effect of a short-term treatment with venlafaxine on the recovery from microiontophoretic applications of 5-HT or NA

Rats were implanted with osmotic minipumps delivering doses of venlafaxine of 10, 20 or 40 mg kg^{-1} day^{-1} or vehicle. The electrophysiological experiments were carried out 48 h after the beginning of the treatment with the minipumps still in place. All three dose regimens of venlafaxine administration were individually tested in parallel with their own controls. The control rats and their matching venlafaxine-treated rats were tested with the same microiontophoretic pipettes (see Figures 4, 5 and 6). The 2-day treatment with a dose of 10 mg kg^{-1} day^{-1} of venlafaxine increased significantly the RT_{50} values obtained following microiontophoretic applications of 5-HT (1 and 5 nA), but not those following the applications of NA (3 and 10 nA), when compared to control rats (Figures 5 and 6). Following 2-day treatments with doses of 20 mg kg^{-1} day^{-1} or 40 mg kg^{-1} day^{-1} of venlafaxine, the RT_{50} values obtained following microiontophoretic applications of 5-HT (1 or 5 nA) or NA (3 and 10 nA) were significantly increased (Figures 4, 5 and 6). Interestingly, the increases of the RT_{50} values for both 5-HT and NA did not appear to be dose-dependent, inasmuch as the high dose

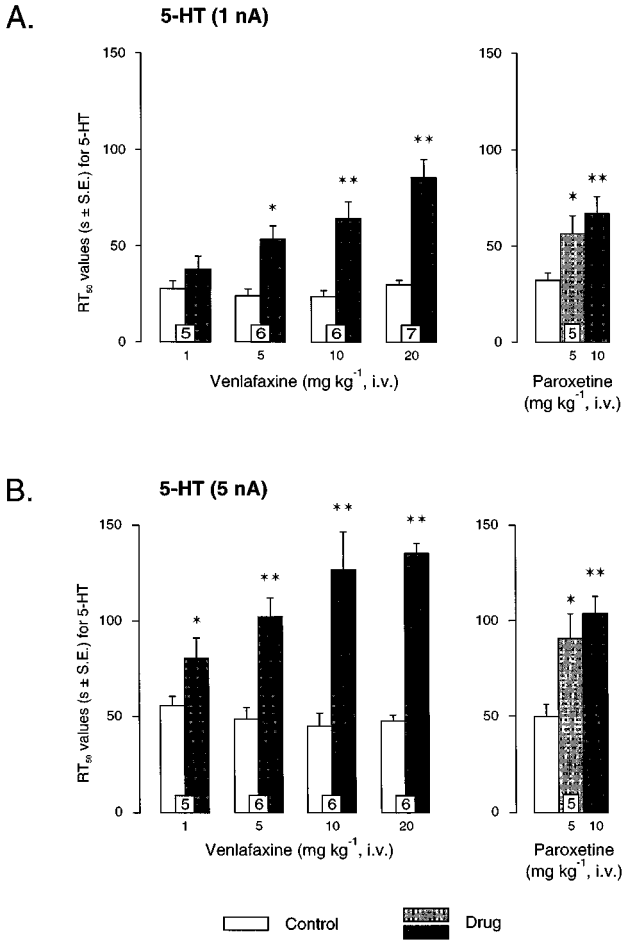

Figure 2 Recovery time, expressed as RT_{50} values (means ± s.e.m.), of CA_3 dorsal hippocampus neurons obtained with microiontophoretic applications of 1 (a) and 5 nA (b) of 5-HT on ACh-induced firing activity, before and after the acute intravenous administration of venlafaxine or of two successive doses of 5 mg kg^{-1} (cumulative doses of 5 and 10 mg kg^{-1}, respectively) of paroxetine. $^{*}P<0.05$, $^{**}P<0.01$ compared to control values using the paired Student's *t*-test. In this and subsequent figures, the numbers in squares represent the numbers of neurons tested.

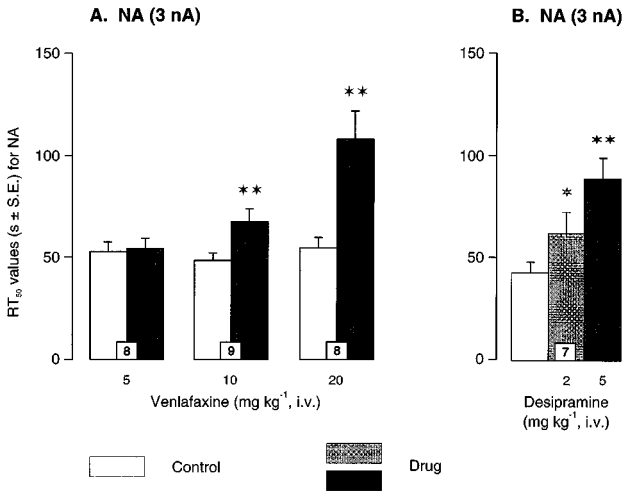

Figure 3 Recovery time, expressed as RT_{50} values (means ± s.e.m.), of CA_3 dorsal hippocampus neurons obtained with microiontophoretic applications of 3 nA of NA on ACh-induced firing activity, before and after the acute intravenous administration of venlafaxine (a) or of 2 and 3 mg kg^{-1} (cumulative doses of 2 and 5 mg kg^{-1}, respectively) of desipramine (b). $^{*}P<0.05$, $^{**}P<0.005$ compared to control values using the paired Student's *t*-test.

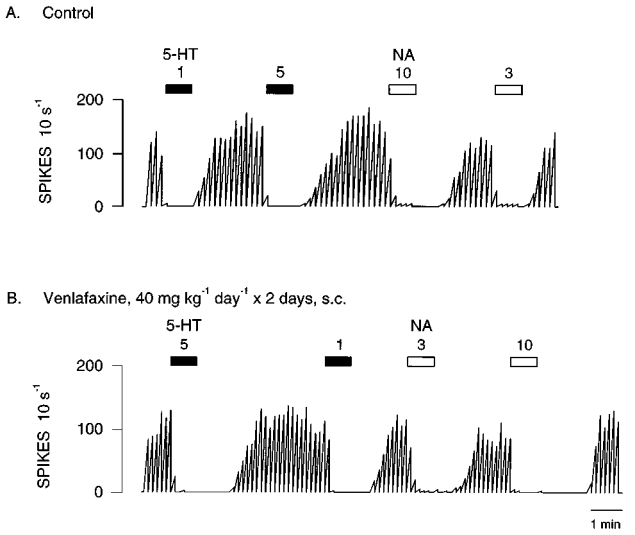

Figure 4 Integrated firing rate histograms of CA_3 dorsal hippocampus pyramidal neuron showing the effects of microiontophoretic applications of 5-HT and NA in rats following a 2 day treatment with vehicle (a) or with 40 mg kg^{-1} day^{-1} s.c. of venlafaxine (b).

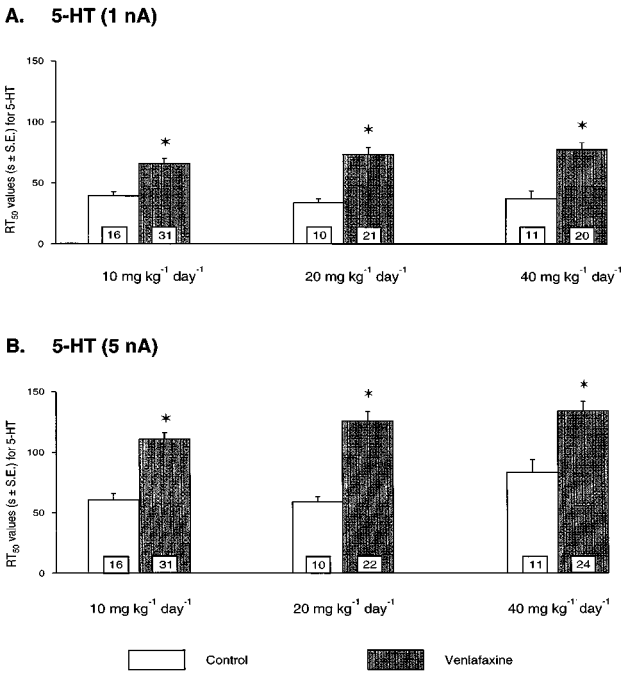

Figure 5 Recovery time, expressed as RT_{50} values (means ± s.e.m.), of CA_3 dorsal hippocampus neurons obtained with microiontophoretic applications of 1 nA (a) and 5 nA (b) of 5-HT on ACh-induced firing activity, in control rats, and following 2 day treatments with 10, 20 or 40 mg kg^{-1} day^{-1} of venlafaxine. $^{*}P<0.005$ compared to control values using the two-tailed Student's *t*-test.

regimen did not further increase significantly the RT_{50} values for both 5-HT and NA applications compared to those obtained with the minimal effective dose.

Effect of the acute intravenous administration of desipramine following acute or short-term administrations of venlafaxine

The effect of acute intravenous administration of desipramine on the NA reuptake process was assessed in rats that had previously received an intravenous dose of 5 mg kg^{-1} venlafaxine. As before, the intravenous dose of 5 mg kg^{-1} of venlafaxine did not modify the RT_{50} values obtained following microiontophoretic applications of NA with a 3 nA current (Figure 7a). The subsequent administration of a dose of 5 mg kg^{-1} of desipramine induced an increase of the RT_{50} values (Figure 7a) which was identical to that produced by this dose of desipramine in naive rats (compare with Figure 3).

In a last series of experiments, the RT_{50} values obtained for NA prior to and following the acute administration of 5 mg kg^{-1} of desipramine were assessed in rats that had been treated for 2 days with doses of 10 or 40 mg kg^{-1} day^{-1} of venlafaxine. After both the 10 and 40 mg kg^{-1} day^{-1} regimens with venlafaxine, the acute administration of desipramine still significantly increased the RT_{50} values following microiontophoretic applications of NA (Figure 7b).

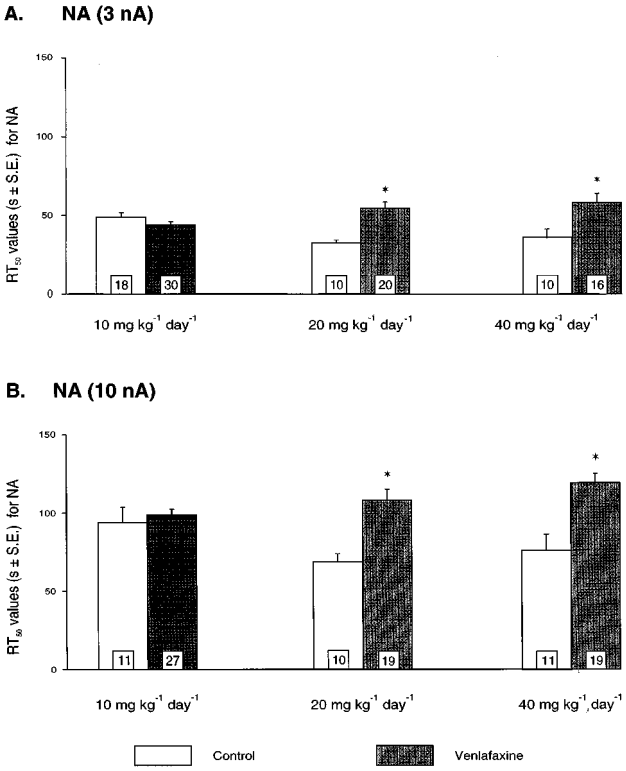

Figure 6 Recovery time, expressed as RT_{50} values (means ± s.e.m.), of CA_3 dorsal hippocampus neurons obtained with microiontophoretic applications of NA (3 and 10 nA) on ACh-induced firing activity, in control rats, and following 2-day treatments with 10, 20 and 40 mg kg^{-1} day^{-1} of venlafaxine. $^{*}P<0.005$ compared to control values using the unpaired Student's *t*-test.

Discussion

The results obtained in the present study indicate that venlafaxine displays both 5-HT and NA reuptake inhibition properties when assessed using an *in vivo* electrophysiological paradigm. Yet, venlafaxine demonstrated a greater potency to inhibit the 5-HT reuptake process over that for NA in these conditions. On the other hand, paroxetine and venlafaxine were equipotent to inhibit 5-HT reuptake, while desipramine was more potent than venlafaxine to inhibit NA reuptake when using this *in vivo* approach.

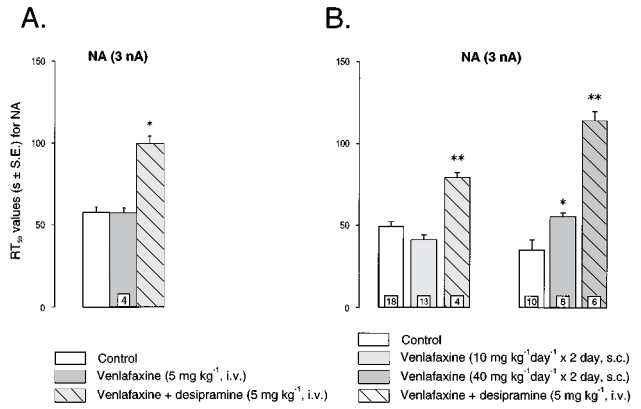

Figure 7 (a) Recovery time, expressed as RT_{50} values (means $\pm$s.e.m.), of CA_3 dorsal hippocampus neurons, obtained with microiontophoretic applications of 3 nA of NA on ACh-induced firing activity, before, and after the acute intravenous administration of venlafaxine (5 mg kg^{-1}) and following the subsequent administration of desipramine (5 mg kg^{-1}) to the same rats. $^{*}P<0.005$ compared to control values using the paired Student's *t*-test. (b) Recovery time, expressed as RT_{50} values (means $\pm$s.e.m.), of CA_3 dorsal hippocampus neurons obtained with microiontophoretic applications of 3 nA of NA on ACh-induced firing activity, before and after a 2-day treatment with 10 or 40 mg kg^{-1} day^{-1} of venlafaxine and following the acute administration of desipramine (5 mg kg^{-1}, i.v.) to the same rats. $^{*}P<0.05$; $^{**}P<0.001$, using the unpaired Student's *t*-test.

It has been well established that the RT_{50} value provides a reliable, quantitative, index to assess, *in vivo*, the uptake activity following microiontophoretic applications onto CA_3 dorsal hippocampus pyramidal neurons of both 5-HT (Piñeyro *et al*., 1994) and NA (de Montigny *et al*., 1980; Gravel & de Montigny, 1987). In the present study, the ability of venlafaxine to increase the RT_{50} values following 5-HT and NA microiontophoretic applications was assessed both acutely and following a 2-day treatment with venlafaxine. Following its acute intravenous administration, venlafaxine dose-dependently increased the RT_{50} values for both 5-HT and NA, thus confirming its purported dual reuptake blocking properties. However, whereas a dose of 1 mg kg^{-1} was sufficient to induce a significant increase of the RT_{50} value for 5-HT, a dose of 10 mg kg^{-1} was necessary to produce a significant increase of the RT_{50} values for NA (Figure 3). This suggests that venlafaxine is more potent in blocking the 5-HT transporter than the NA transporter.

The direct comparison of the RT_{50} values following acute intravenous administration of venlafaxine and paroxetine reveals that these two drugs displayed an equipotent inhibitory effect on the 5-HT reuptake process (Figure 2a and b). However, venlafaxine was less potent (about five times) than desipramine in inhibiting NA reuptake, as shown by the higher dose of venlafaxine required to significantly increase the RT_{50} values following NA applications (Figure 3). Most interestingly, and perhaps even surprisingly, these relative potencies (i.e. venlafaxine *vs* paroxetine and desipramine for 5-HT and NA reuptake blockade, respectively) are incongruent with those previously reported from an *in vitro* uptake study done in rat brain synaptosomes (Bolden-Watson & Richelson, 1993), as well as with those generated from a radioligand binding study (Béïque *et al*., 1998). Indeed, Bolden-Watson & Richelson (1993) have reported that paroxetine is 53 times more potent than venlafaxine in inhibiting 5-HT reuptake whereas we have reported, in a previous radioligand binding study, that paroxetine displayed an affinity 2000 times greater than that of venlafaxine for the [^{3}H]-cyanoimipramine binding site on the 5-HT transporter (Béïque *et al*., 1998). As for the NA reuptake process, desipramine has been reported to be 344 times (Bolden-Watson & Richelson, 1993) and four times (Muth *et al*., 1986) more potent than venlafaxine to block the uptake of [^{3}H]-NA from rat brain synaptosomes. The reasons for the discrepancy between these later studies is unclear considering that both studies were caried out with essentially similar protocols except that; (1) while total brain was used in the study of Muth *et al*., (1986) only hippocampal tissue was used in that of Bolden-Watson & Richelson (1993) and (2) the uptake assay in the study of Muth *et al*., (1986) was carried out with 100 nM [^{3}H]-NA, while Bolden-Watson & Richelson (1993) used a concentration of 8 nM [^{3}H]-NA. How such methodological factors may alter the relative potencies of venlafaxine and desipramine to inhibit [^{3}H]-NA uptake is however obscure. Nonetheless, we have reported that desipramine displays an affinity for the [^{3}H]-nisoxetine binding site on the NA transporter that is 2000 times greater than that of venlafaxine for this same site (Béïque *et al*., 1998). Altogether, the relative reuptake blocking potencies of paroxetine, desipramine and venlafaxine derived from the present study, when compared to those previously published, clearly expose a discrepancy between the functional 5-HT and NA reuptake blocking potencies of venlafaxine with its binding characteristics to both the 5-HT and NA transporters. Consequently, it can be argued that the sole existence of this discrepancy challenges the otherwise valid assumption that venlafaxine, as the other reuptake blockers do, would block the reuptake of both 5-HT and NA by its sole binding to the [^{3}H]-cyanoimipramine and [^{3}H]-nisoxetine binding site, respectively. The reasons underlying this very important and crucial peculiarity with regard to venlafaxine's mechanism of action is at present unresolved and thus remains to be elucidated.

The short-term treatments with venlafaxine (10, 20 and 40 mg kg^{-1} $day^{-1} \times 2$ days), which permitted the drug to reach a steady state concentration given its short half-life in rodents (1 h; Howell *et al*., 1994), further corroborated the dual 5-HT/NA reuptake blocking properties of venlafaxine, because the RT_{50} values for both 5-HT and NA were increased. Yet, while a dose of 10 mg kg^{-1} day^{-1} resulted in a significant increase of the RT_{50} following 5-HT applications, 20 mg kg^{-1} day^{-1} were necessary to observe a similar effect following NA applications. Interestingly, with increasing doses, the effect of venlafaxine administered for 2 days displayed a plateau with regard to the increase of the RT_{50} values and therefore was not dose-dependent. This observation is different from that reported with duloxetine, another 5-HT/NA reuptake inhibitor (SNRI), which, under the same experimental conditions, showed a clear dose-dependent relationship with respect to the RT_{50} values for 5-HT and NA (Kasamo *et al*., 1996). The reason for this apparent discrepancy between these two purportedly similar drugs (i.e. both are SNRI's) remains to be elucidated. Notwithstanding this latter observation, the data obtained with the short-term treatments with venlafaxine are congruent with those generated by intravenous administration of venlafaxine with regard to the preferential potency of venlafaxine to inhibit the 5-HT reuptake process over that of NA.

As shown in Figure 7a and b, when desipramine was acutely administered to rats that had received either an acute intravenous administration or a 2-day treatment of a maximal non-effective dose of venlafaxine on NA reuptake (5 mg kg^{-1}, i.v. and 10 mg kg^{-1} day^{-1}, s.c. respectively), it was still able to exert its full effect (compare Figures 3 and 7a). These results indicate that venlafaxine did not prevent the effect of

desipramine on the NA reuptake and that these two drugs do not exert an additive effect. Assuming that these maximal non-effective doses of venlafaxine result in a significant transporter occupancy, this lack of an additive effect of these two drugs on the reuptake of NA is compatible with the previous suggestion that they would block NA reuptake by acting on different sites (Lavoie *et al*., 1997; Béïque *et al*., 1998). On the other hand, the fact that desipramine (5 mg kg^{-1}, i.v.) was able to prolong the RT_{50} values for NA after a 2-day treatment with 40 mg kg^{-1} day^{-1} of venlafaxine and that these latter RT_{50} values are well below those obtained following acute administration of either venlafaxine (20 mg kg^{-1}, i.v.) or desipramine (5 mg kg^{-1}, i.v.), indicates that a 2-day treatment with 40 mg kg^{-1} day^{-1} of venlafaxine does not induce a maximal NA reuptake blockade. This latter finding is somewhat surprising since the 40 mg kg^{-1} day^{-1} regimen of venlafaxine appeared to induce a maximal effect on the NA reuptake as the RT_{50} values for NA were not further increased when the dose of 20 mg kg^{-1} day^{-1} was increased to 40 mg kg^{-1} day^{-1} (Figure 6), thus suggestive of a plateau. Whether this apparent incomplete NA reuptake blockade by the 40 mg kg^{-1} day^{-1} regimen is the result of adaptative changes that might have developed during the short-term treatment period or from a pharmacokinetic parameter that would limit the effective brain concentration of venlafaxine remains to be determined.

Taken together, the results presented herein provide functional evidence that venlafaxine exerts a more powerful action on the 5-HT reuptake process than that for NA, a finding that is consistent throughout the different studies that have directly assessed this matter. Indeed, the *in vitro* uptake study in rat brain synaptosomes of Bolden-Watson & Richelson (1993) shows a preferential inhibitory effect of venlafaxine on the reuptake of 5-HT over that of NA with a 3 : 1 ratio, while that of Muth *et al*., (1986) reported a 5 : 1 ratio. Given the differential potencies of venlafaxine for these reuptake processes, it is conceivable that when a low dose of venlafaxine is administered to patients, it would act preferentially on the 5-HT reuptake process whereas when the dose is increased, a progressive recruitment of the noradrenergic system, through NA reuptake blockade, would ensue. This latter property may well represent the biological substratum responsible not only for the positive dose-response relationship observed clinically with venlafaxine which, most interestingly, does not occur with SSRI's (Kelsey, 1996), but also for the apparent remarkable clinical efficacy of high doses of venlafaxine. Indeed, clinical studies are now suggesting that venlafaxine, when used at high doses, demonstrates an early onset of action (Rickels, 1991; Derivan *et al*., 1995; Benkert *et al*., 1996) as well as therapeutic efficacy in treatment-resistant depression (Nierenberg *et al*., 1994; de Montigny *et al*., 1998).

In summary, the differential potencies of venlafaxine to inhibit *in vivo* the reuptake of 5-HT and NA demonstrated herein, provide a tenable and attractive hypothesis for the unique clinical properties of venlafaxine. Furthermore, the present data, by revealing a discrepancy between the functional *in vivo* potencies of venlafaxine to block 5-HT and NA uptake and its *in vitro* binding profile to the two transporters, suggest that venlafaxine might interact with the 5-HT and the NA transporters in a more complex manner than the classical 5-HT and NA reuptake blockers. Further studies are thus warranted in order to clarify this crucial aspect, given the seemingly unique clinical properties of venlafaxine.

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 Royal Victoria Hospital Research Institute Fellowship, P.B. of a MRC Scientist award and G.D. of a Scholarship from the FRSQ.

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(Received March 16, 1998
Revised June 15, 1998
Accepted June 22, 1998)