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Venlafaxine: Discrepancy Between In Vivo 5-HT and NE Reuptake Blockade and Affinity for Reuptake Sites

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KEY WORDS antidepressant; dorsal raphe; locus coeruleus; paroxetine; desipramine; fenfluramine; tyramine; releaser

ABSTRACT Using an in vivo electrophysiological paradigm, venlafaxine and paroxetine displayed similar potency for suppressing the firing activity of dorsal raphe 5-HT neurons (ED_{50}: 233 and 211 μg/kg i.v., respectively), while venlafaxine was three times less potent than desipramine (ED_{50}: 727 and 241 μg/kg i.v., respectively) to suppress the firing activity of locus coeruleus NE neurons. The selective 5-HT_{1A} receptor antagonist WAY 100635 (100 μg/kg, i.v.) reversed the suppressant effect of venlafaxine and paroxetine on the firing activity of 5-HT neurons and the α_2-adrenoceptor antagonist piperoxane (1 mg/kg, i.v.) reversed those of venlafaxine and desipramine on the firing activity of NE neurons. The ED_{50} of venlafaxine on the firing activity of 5-HT neurons was not altered (ED_{50}: 264 μg/kg) in noradrenergic-lesioned rats, while the suppressant effect of venlafaxine on the firing activity of NE neurons was greater in serotonergic-lesioned rats (ED_{50}: 285 μg/kg). Taken together, these results suggest that, in vivo, venlafaxine blocks both reuptake processes, its potency to block the 5-HT reuptake process being greater than that for NE. Since the affinities of venlafaxine for the 5-HT and NE reuptake carriers are not in keeping with its potencies for suppressing the firing activity of 5-HT and NE neurons, the suppressant effect of venlafaxine on the firing activity of 5-HT and NE neurons observed in vivo may not be mediated solely by its action on the [^{3}H]cyanoimipramine and [^{3}H]nisoxetine binding sites. In an attempt to unravel the mechanism responsible for this peculiarity, in vitro superfusion experiments were carried out in rat brain slices to assess a putative monoamine releasing property for venlafaxine. (±)Fenfluramine and tyramine substantially increased the spontaneous outflow of [^{3}H]5-HT and [^{3}H]NE, respectively, while venlafaxine was devoid of such releasing properties. **Synapse 32:198–211, 1999.**

INTRODUCTION

Venlafaxine (1-[2-(dimethylamino)-1-(4-methyoxyphenyl)-ethyl]cyclohexanol), a phenylethylamine derivative, has been shown to be effective in the treatment of major depression (Schweizer et al., 1991; Guelfi et al., 1992). Moreover, an increasing number of studies are suggesting that venlafaxine would display a unique clinical profile in that: 1) it would display an earlier onset of therapeutic action (Schweizer et al., 1991; Benkert et al., 1996); 2) it would display a positive dose–response relationship with regard to clinical efficacy (Kelsey, 1996); 3) it would be more efficacious than fluoxetine (Clerc et al., 1994; Dierick et al., 1996; Rudolph et al., 1997) and imipramine (Schweizer et al., 1994; Lecrubier et al., 1997) in treating major depression; and, 4) it would show a significant efficacy in treatment-resistant depression (Nierenberg et al., 1994; de Montigny et al., 1999). Since in vitro studies carried out in rat brain synaptosomes have demonstrated that venlafaxine inhibits the uptake of both serotonin (5-hydroxytryptamine; 5-HT) and norepinephrine (NE) (Muth et al., 1986a; Bolden-Watson and Richelson, 1993), these clinical properties have been suggested to be attributable to a dual 5-HT/NE reuptake inhibition. However, in a recent radioligand binding study we have shown that venlafaxine displayed only a moderate affinity for the 5-HT transporter and low affinity for the

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NE transporter, as assessed with [^{3}H]cyanoimipramine and [^{3}H]nisoxetine competitive binding, respectively (Béïque et al., 1998b). Accordingly, venlafaxine displayed a much lower affinity for both reuptake sites than prototypical reuptake inhibitors such as paroxetine and desipramine. These results thus put into evidence a clear discrepancy with regard to the mechanism of action of venlafaxine in major depression. In order to clarify this issue, it was deemed necessary to further assess the purported reuptake blocking properties of venlafaxine using a functional in vivo paradigm. In this regard, the suppression of the firing activity of dorsal raphe nucleus 5-HT and locus coeruleus NE neurons achieved by reuptake blockers is believed to provide a reliable and precise index of their potencies to inhibit 5-HT and NE reuptake as these two functions were shown to be correlated (Quinaux et al., 1982). In the present study, we have thus assessed: 1) the potencies of venlafaxine and paroxetine for suppressing the firing activity of dorsal raphe 5-HT neurons; 2) the potency of venlafaxine, desipramine, paroxetine, and fluoxetine for suppressing the firing activity of locus coeruleus NE neurons; 3) the effect of a 5-HT lesion and that of a NE lesion on the potencies of venlafaxine to suppress the firing activity of locus coeruleus NE and dorsal raphe 5-HT neurons, respectively; 4) to compare the potencies of reuptake inhibitors with those already published with other reuptake inhibitors and to correlate these potencies with their respective affinities, taken from previous studies, for the 5-HT and NE transporters; and 5) the effects of fenfluramine, tyramine, and venlafaxine on the spontaneous outflow of radioactivity from rat brain slices preloaded with [^{3}H]5-HT or [^{3}H]NE in order to determine putative monoamine-releasing properties for venlafaxine.

MATERIALS AND METHODS

Male Sprague-Dawley rats (250–275 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.

In vivo electrophysiological experiments

Electrophysiological experiments were performed on animals anesthetized with chloral hydrate (400 mg/kg, i.p.) and mounted in a stereotaxic apparatus. Supplemental doses (100 mg/kg, i.p.) were given to prevent any nociceptive reaction to pinching 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 i.v. administration of drugs.

Extracellular unitary recordings of 5-HT neurons of the dorsal raphe nucleus and of NE neurons of the locus coeruleus

Single-barrelled glass micropipettes were prepared in a conventional manner (Haigler and Aghajanian, 1974), with the tips broken back to 1–3 µm 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 dorsal raphe neurons recordings or 1 mm posterior to lambda and 1 mm lateral to midline for locus coeruleus neurons recordings. Spontaneously active dorsal raphe 5-HT neurons were encountered at 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). Spontaneously active NE neurons of the locus coeruleus were identified using the criteria of Aghajanian (1978): regular firing rate (1–5 Hz) and positive action potential of long duration (0.8–1.2 ms) exhibiting a characteristic burst discharge in response to nociceptive pinch of the contralateral hind paw. 5-HT and NE neurons were recorded for at least 1 min to establish basal firing rate before a single dose of the drug to be tested was administered i.v. in a volume of about 0.1 cc via a lateral vein of the tail. By giving a single dose to one rat, dose–response curves for the effects of venlafaxine, paroxetine, desipramine, or fluoxetine were generated, from which the dose required for a 50% suppression of the firing activity of the neuron (effective dose-50; ED_{50} value) were determined.

6-Hydroxydopamine and 5,7-dihydroxytryptamine pretreatments

Rats weighing between 175 and 200 g were anesthetized with chloral hydrate (400 mg/kg, i.p.). Lesions of NE neurons were performed by injecting the NE neurotoxin 6-hydroxydopamine (6-OHDA; 120 µg free base in 20 µl of 0.9% NaCl and 0.1% ascorbic acid) intracerebroventricularly (i.c.v.; 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 fluoxetine (10 mg/kg, i.p.) in order to protect 5-HT neurons. The same protocol was applied for the injection of the 5-HT neurotoxin 5,7 dihydroxytryptamine (5,7-DHT; 200 µg of free base in 20 µl of 0.9% NaCl, and 0.1% ascorbic acid, i.c.v.), except that the rats received desipramine (25 mg/kg, i.p.) instead of fluoxetine 1 h before the lesion to protect NE neurons. In both cases, the electrophysiological experiments were carried out at least 14 days after the lesions were made.

Superfusion experiments

Rats were sacrificed by decapitation and the brain immediately removed and rapidly dissected on an ice-cold glass plate. Hippocampal or hypothalamic slices

of 400 μm of thickness were prepared using a McIlwain tissue chopper. The slices were then incubated for 30 min at 37°C in Krebs' buffer containing either 20 nM of [^{3}H]5-HT creatinine sulfate (specific activity 1,017 GBq/mmol) or 20 nM of [^{3}H]NE (specific activity of 538.7 GBq/mmol) both purchased from NEN Life Science, (Boston, MA) and bubbled with a mixture of 95% O_2 / 5% CO_2. The composition of the Krebs solution was the following (in mM): NaCl 118, KCl 4.7, $CaCl_2$ 1.3, $MgCl_2$ 1.2, NaH_2PO_4 1, $NaHCO_3$ 25, glucose 11.1, Na_2 EDTA 0.004, and ascorbic acid 0.11. At the end of the incubation period, the slices were transferred to glass superfusion chambers (two slices of hippocampus or one of hypothalamus) and superfused at a rate of 0.5 ml/min with oxygenated Krebs' solution maintained at 37°C. Nineteen consecutive 4-min fractions were collected starting 90 min after the beginning of superfusion. One period of electrical stimulation, S_1, was carried out at 8 min after the end of the 90-min washing period. The electrical field was generated in the chambers between two platinum electrodes (30 mA, 2 ms, 3 Hz for 2 min for hippocampal slices; 20 mA, 2 ms, 3 Hz for 2 min for hypothalamic slices), positioned 2 cm apart. The stimulation period S_1 was carried out to ascertain the viability of the slices. Venlafaxine (3 or 10 μM), (±)fenfluramine (10 μM), or tyramine (3 μM) were added to the perfusion medium at fraction #14 for a period of 2 min while increasing the rate of superfusion to 1 ml/min. At the end of the superfusion period, the slices were solubilized with 0.5 ml of Soluene 350 (Packard Instruments, Downers Grove, IL, USA), and the radioactivity in the slices and superfusate samples was determined by liquid scintillation spectrometry. The results were expressed as the fraction of the tritium content present in the time at the onset of the respective collection periods. The overflow of tritium evoked by the electrical stimulation (S_1), as well as that induced by incorporation of the drug (venlafaxine, (±)fenfluramine, or tyramine) to the perfusion medium (S_2), was calculated as the total increase in radioactivity released above the basal outflow of tritium determined in the sample immediately preceding the onset of the electrical- or drug-induced stimulation (i.e., at fraction #2 and #13, respectively).

Drugs

Venlafaxine and WAY 100635 were kindly provided by Wyeth-Ayerst Research (NJ, USA), paroxetine by Smith Kline Beecham (West Sussex, UK), fluoxetine by Lilly Research Laboratories (Indianapolis, IN, USA), piperoxane by Rhône-Poulenc (Vitry, France), (±)fenfluramine by Servier (Courbevoie, France), while desipramine was purchased from RBI (Natick, MA, USA), and tyramine from Aldrich (Milwaukee, WI, USA). LSD was obtained from the Ministry of Health and Welfare (Ottawa, Canada).

Statistical analysis.

In vivo electrophysiological experiments. Correlation coefficients (*r* values) for the dose–response relationship observed in the dorsal raphe were calculated using simple linear regression analysis and in the locus by polynomial regression analysis. The SEM for the ED_{50} values for the dorsal raphe and the linear section of the locus coeruleus dose–response curves were calculated by regression analysis, with the y value of 50 used as the regressor. Differences between two regressions were assessed by comparing their ED_{50} values using the confidence intervals method. The 95% or 99% confidence limit was determined from the Student's *t* distribution.

In vitro superfusion experiments. Results were expressed as means ± SEM. When two means were compared, the statistical significance of the difference was assessed using either a paired or nonpaired Student's *t*-test, as indicated in the Results section. Probability values of $P < 0.05$ were considered statistically significant.

RESULTS

Effect of the acute administration of venlafaxine and paroxetine on the firing activity of dorsal raphe 5-HT neurons

The basal firing activity of the 33 dorsal raphe 5-HT neurons recorded was of 1.32 ± 0.11 Hz. A single i.v. dose of venlafaxine or paroxetine was administered to 14 naive rats via a lateral tail vein while recording a spontaneously active dorsal raphe 5-HT neuron. Both drugs induced a dose-dependent suppression of the firing activity of the dorsal raphe 5-HT neurons (Figs. 1A,B, 2A,B). This suppressant effect was reversed by the subsequent acute i.v. administration of 100 μg/kg of the selective 5-HT_{1A} antagonist WAY 100635. Paroxetine displayed an ED_{50} of 211 ± 11 μg/kg, while that of venlafaxine was 233 ± 12 μg/kg (Table I).

The suppressant effect of venlafaxine on the firing activity of 5-HT neurons was also assessed in rats pretreated with the noradrenergic toxin 6-OHDA. As was the case with naive rats, venlafaxine suppressed the firing activity of dorsal raphe 5-HT neurons in a dose-dependent manner. The ED_{50} was calculated to be 264 ± 43 μg/kg, a value not significantly different from that calculated in intact rats ($P > 0.1$; Table I).

Effect of the acute administration of venlafaxine, desipramine, paroxetine, and fluoxetine on the firing activity of locus coeruleus NE neurons

The basal firing activity of the 56 locus coeruleus NE neurons recorded was 2.6 ± 0.18 Hz. The acute i.v. administration of venlafaxine or desipramine induced a dose-dependent suppression of locus coeruleus NE neu-

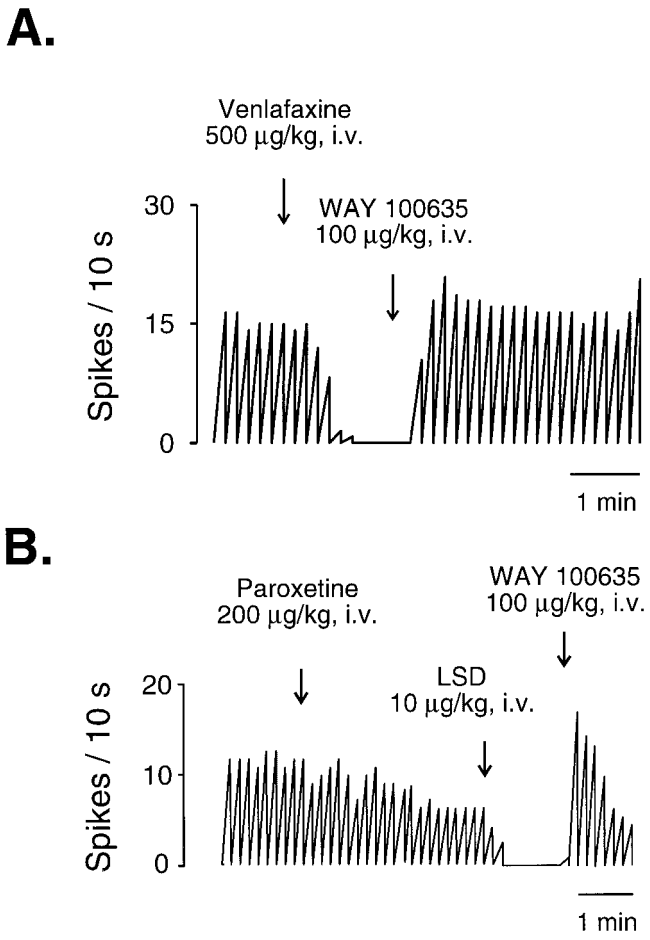


Fig. 1. Integrated firing rate histograms of serotonergic neurons of the dorsal raphe nucleus showing the effects of i.v. administration of 500 µg/kg of venlafaxine (**A**) and 200 µg/kg of paroxetine (**B**) on the spontaneous firing activity of the neuron. LSD (10 µg/kg) was sometimes injected i.v. in order to confirm the serotonergic nature of the neuron recorded. The effect of a subsequent i.v. administration of 100 µg/kg of the selective 5-HT_{1A} antagonist WAY 100635 is also shown.

rons firing activity (Figs. 3A,B, 4A,B). From these dose–response curves, the ED_{50}'s of venlafaxine and desipramine to suppress the firing activity of NE neurons were calculated to be 737 ± 68 µg/kg and 240 ± 54 µg/kg, respectively (Table I). Low doses of venlafaxine did not induce the transient increase of the firing activity of NE neurons previously reported (Haskins et al., 1985). It is noteworthy that the maximal suppressant effect of venlafaxine obtained at a dose of 2 mg/kg i.v. was ca. 90%, i.e., a complete suppression of firing could not be attained even with doses of 3 and 4 mg/kg, i.v. In contrast, desipramine (Figs. 3B, 4B) induced a total (i.e., 100%) suppression of firing activity. The subsequent i.v. administration of the α_2-adrenergic

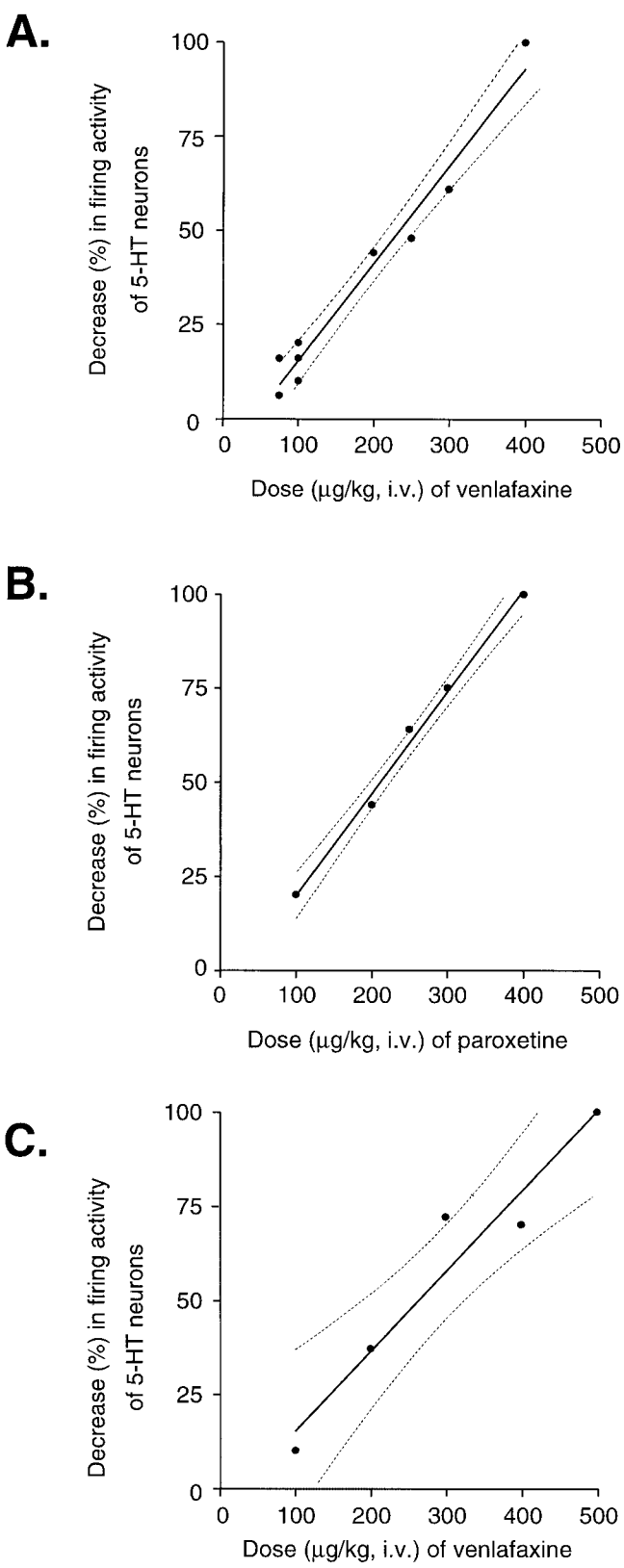


Fig. 2. Dose–response curves showing the effect of i.v. administration of venlafaxine (**A**) and of paroxetine (**B**) on the firing activity of dorsal raphe 5-HT neurons. The dose–response curve of venlafaxine for suppressing the firing activity of dorsal raphe 5-HT neurons of rats that where treated i.c.v. with the neurotoxin 6-OHDA is shown in **C**. In these and subsequent dose–response curves, each dot represent the response of one neuron to a single dose in one rat while the dotted curved lines depict the 95% confidence interval of the regression.

TABLE I. Effects of venlafaxine, paroxetine, desipramine and fluoxetine on the firing activity of dorsal raphe 5-HT and/or locus coeruleus NE neurons[1]

	ED_{50} (µg/kg)	r	n
Dorsal raphe 5-HT neurons			
Venlafaxine	233 ± 12	0.98	9
Venlafaxine (6-OH-DA)[2]	264 ± 43[3]	0.96	5
Paroxetine	211 ± 11	0.99	5
Locus coeruleus NE neurons			
Venlafaxine	737 ± 68	0.96	12
Venlafaxine (5,7-DHT)[2]	285 ± 49*	0.98	15
Desipramine	240 ± 54	0.93	10
Paroxetine	>10 000	~0	11
Fluoxetine	>5 000	~0	5

[1]Data are the regression parameters of the dose-response curves shown in Figure 2, 4 and 5. ED_{50} = predicted dose that would elicit a 50% suppression of the firing activity of the neuron; r = regression coefficient; n = number of neurons; n.d. = not determined.
[2]The experiments were performed at least 14 days after the i.c.v. injection of the toxin.
[3]Not significant when compared to the ED_{50} value for venlafaxine in untreated rats.
*$p < 0.01$ using confidence intervals, compared to venlafaxine in untreated rats.

antagonist piperoxane reversed the suppressant effect of both venlafaxine and desipramine (Fig. 3A,B). Following the restoration of the firing activity by piperoxane, an injection of the selective 5-HT_{1A} antagonist WAY 100635 (100 µg/kg, i.v.) was given to five rats. WAY 100635 induced a total suppression of firing in all neurons tested (Fig. 3A). Thus, the suppressant effect of WAY 100635 appeared unimpaired since, by itself, when administered i.v. to naive rats, it induced, at this dose, a total suppression of the firing activity of NE neurons (data not shown), in keeping with the previous study of Haddjeri et al. (1997).

The effects of paroxetine and fluoxetine were also assessed on the firing activity of locus coeruleus NE neurons. Doses ranging from 0.5 to 10 mg/kg of paroxetine and 0.5 to 5 mg/kg of fluoxetine did not affect the firing activity of these neurons (Fig. 5A,B). The i.v. administration of fluoxetine and paroxetine did not prevent the suppressant effect on the firing activity of the NE neurons induced by the subsequent i.v. administration of 100 µg/kg of WAY 100635 in two and five rats, respectively (Fig. 5A,B).

The suppressant effect of venlafaxine on the firing activity of NE neurons was also assessed in rats pretreated with the serotonergic toxin 5,7-DHT. In these rats, venlafaxine suppressed the firing activity of locus coeruleus NE neurons in a dose-dependent manner (Figs. 3C, 4C). As was the case in naive rats, a full suppression of the firing activity of these neurons was never achieved in these pretreated rats (Fig. 4C). Indeed, venlafaxine induced a suppression of around 80% of the firing activity. The ED_{50} was calculated to be 285 ± 49 µg/kg, 2.6-fold lower than that obtained with intact rats ($P < 0.01$, using confidence intervals; Table I). The 5,7-DHT pretreatment did not alter the capacity of i.v. administered piperoxane (1 mg/kg) to reverse the suppressant effect of venlafaxine (Fig. 3C). In keeping with previous findings (Haddjeri et al., 1997), the suppressant effect elicited by the acute i.v. administration of 100 µg/kg of WAY 100635 was abolished in 5,7-DHT rats. This lack of effect of WAY 100635 was taken as indirect evidence of the effectiveness of the lesion (Haddjeri et al., 1997).

Correlation between the potency of reuptake inhibitors to suppress the firing activity of 5-HT and/or NE neurons with their affinity for the 5-HT and/or NE transporters

In order to further evaluate the potencies of venlafaxine as a dual 5-HT/NE reuptake inhibitor, the potencies with which it suppressed the firing activity of both dorsal raphe 5-HT and locus coeruleus NE neurons were compared with those, taken from previous studies, of other monoamine reuptake inhibitors. These potencies were further related to previously published affinities of these same reuptake inhibitors for the 5-HT and/or NE transporters. The values for the suppression of firing activity of 5-HT and NE neurons (ED_{50} values) for reuptake inhibitors other than venlafaxine, desipramine, and paroxetine were taken from previously published electrophysiological studies that used an in vivo electrophysiological paradigm essentially similar to that used in the present study. As for the affinities of the various reuptake inhibitors for both transporters (K_i values), they were obtained from studies that have used either [^{3}H]cyanoimipramine or [^{3}H]paroxetine as a marker for the 5-HT transporter and of [^{3}H]nisoxetine for the NE transporter (for references for both the ED_{50} and K_i values, see legend to Fig. 6). From these data it was thus calculated, on the one hand, that the potencies, expressed as ED_{50} values, of eight 5-HT reuptake inhibitors (labeled 1 to 8, Fig. 6A) for suppressing the firing activity of 5-HT neurons are highly correlated with their affinities for the 5-HT transporter, expressed as K_i values ($r = 0.89$). On the other hand, the potencies, expressed as ED_{50} values, of seven NE reuptake inhibitors (labeled 1 to 7, Fig. 6B) for suppressing the firing activity of NE neurons are also highly correlated with their affinities for the NE transporter, expressed as K_i values ($r = 0.91$). However, and most interestingly, the affinities of both venlafaxine and zimelidine for the 5-HT transporters were out of keeping with their potencies for suppressing the firing activity of dorsal raphe 5-HT neurons. However, the affinity of norzimelidine (zimelidine's major metabolite; compound #6, Fig. 6A) is well in keeping with its potency for suppressing the firing activity of dorsal raphe 5-HT neurons. Similarly, the affinity of venlafaxine for the NE transporter was out of keeping with its potency to suppress the firing activity of locus coeruleus NE neurons.

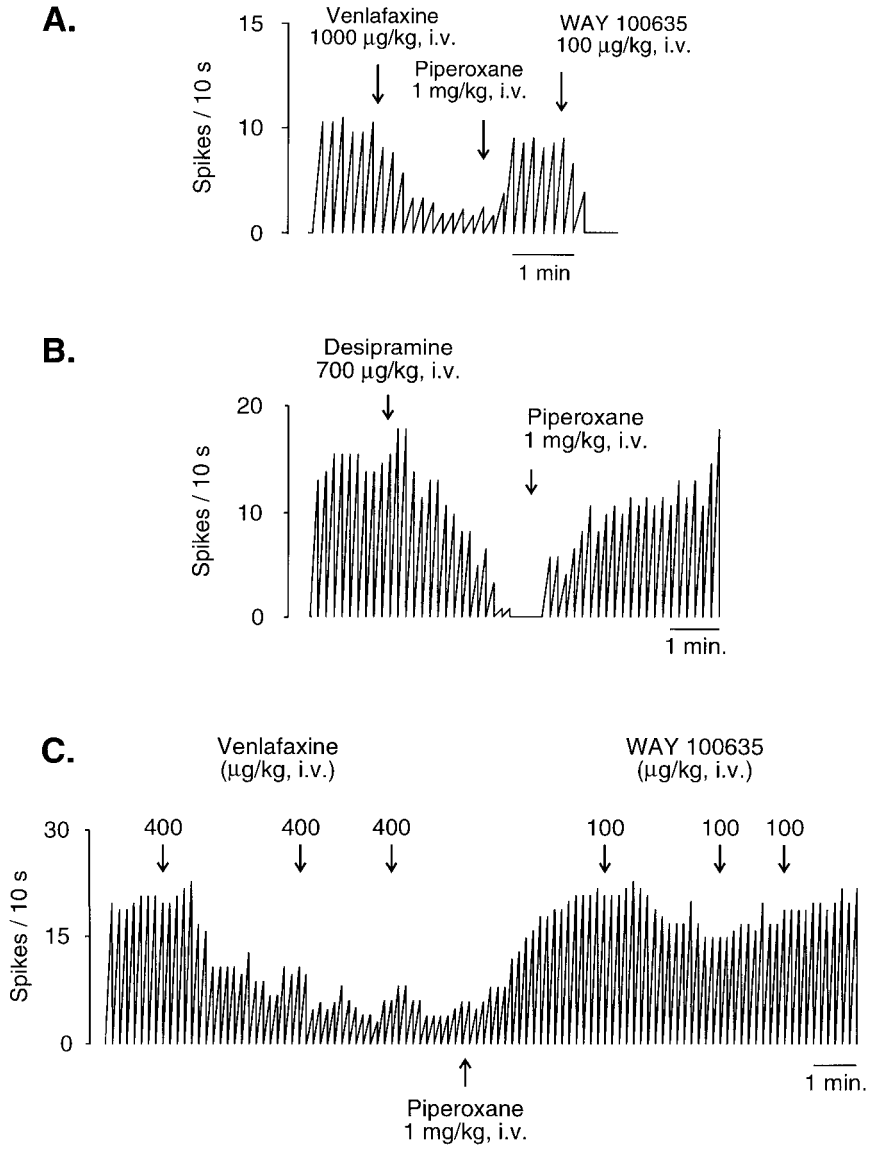


Fig. 3. Integrated firing rate histograms of NE neurons of the locus coeruleus showing the effect of i.v. administration of 1 mg/kg of venlafaxine (**A**), 700 µg/kg of desipramine (**B**), and multiple doses of venlafaxine in rats treated i.c.v. with the neurotoxin 5,7-DHT (**C**) on the spontaneous firing activity of the neuron. The effect of the subsequent intravenous administration of the α_2-antagonist piperoxane (1 mg/kg) and of WAY 100635 (100 µg/kg) is also shown.

Effects of (±)fenfluramine and venlafaxine on the spontaneous release of tritium from hypothalamic slices preloaded with [^{3}H]5-HT

These in vitro experiments were carried out to rule out a possible 5-HT releasing property for venlafaxine by comparing its effect on the spontaneous release of [^{3}H]5-HT from preloaded hypothalamic slices with those of the 5-HT releaser (±)fenfluramine (Kannengiesser et al., 1976; Carruba et al., 1977). In every experiment the slices were first electrically stimulated to ascertain their viability. As shown in Figure 7, the electrical stimulation of hypothalamic slices induced a release of tritium (S_1; see Table II). The S_1 obtained from slices that were to subsequently receive (±)fenfluramine was not statistically different from that obtained from slices that were to receive venlafaxine (see Fig. 7A vs. B, using the nonpaired Student's *t*-test). The 5-HT releasing agent (±)fenfluramine (10 µM), when superfused for 2 min (at 1 ml/min), induced a massive release of tritium, consistent with previous findings (Ramdine et al., 1989; Blier and Bouchard, 1993). The (±)fenfluramine-induced release of tritium was greater than that produced by the electrical stimulation of the same slices ($P < 0.001$, using the paired Student's *t*-test; see Fig. 7A, Table II). Conversely, when venlafaxine (10 µM) was superfused in a fashion similar to that of (±)fenfluramine, only a minimal enhancement of tritium outflow over baseline could be detected, smaller than that induced by fenfluramine ($P < 0.001$ using the nonpaired Student's *t*-test) and by the electrical stimulation of the same slices ($P < 0.001$, using the paired Student's *t*-test).

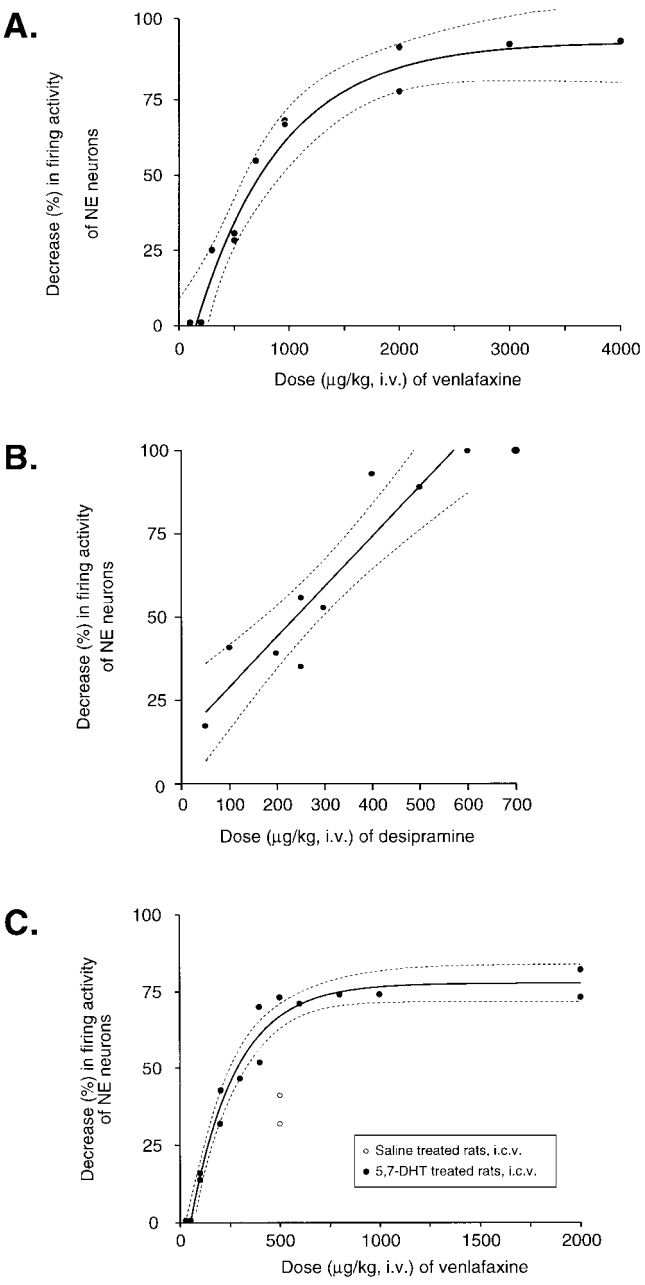


Fig. 4. Dose–response curves showing the effect of i.v. administration of venlafaxine (**A**), desipramine (**B**), and venlafaxine in rats treated i.c.v. with the neurotoxin 5,7-DHT (**C**) on the firing activity of locus coeruleus NE neurons. The open circles in **C** represent the effect of venlafaxine on the firing activity of locus coeruleus NE neurons of rats treated i.c.v. with saline.

Effects of tyramine and venlafaxine on the spontaneous release of tritium from hippocampal slices preloaded with [^{3}H]NE

The existence of a NE releasing effect of venlafaxine was searched for by comparing the effect of venlafaxine on the spontaneous release of [^{3}H]NE from preloaded hippocampal slices with that of the NE releaser tyramine (Pylatuk and McNeil, 1976). In every experiment,

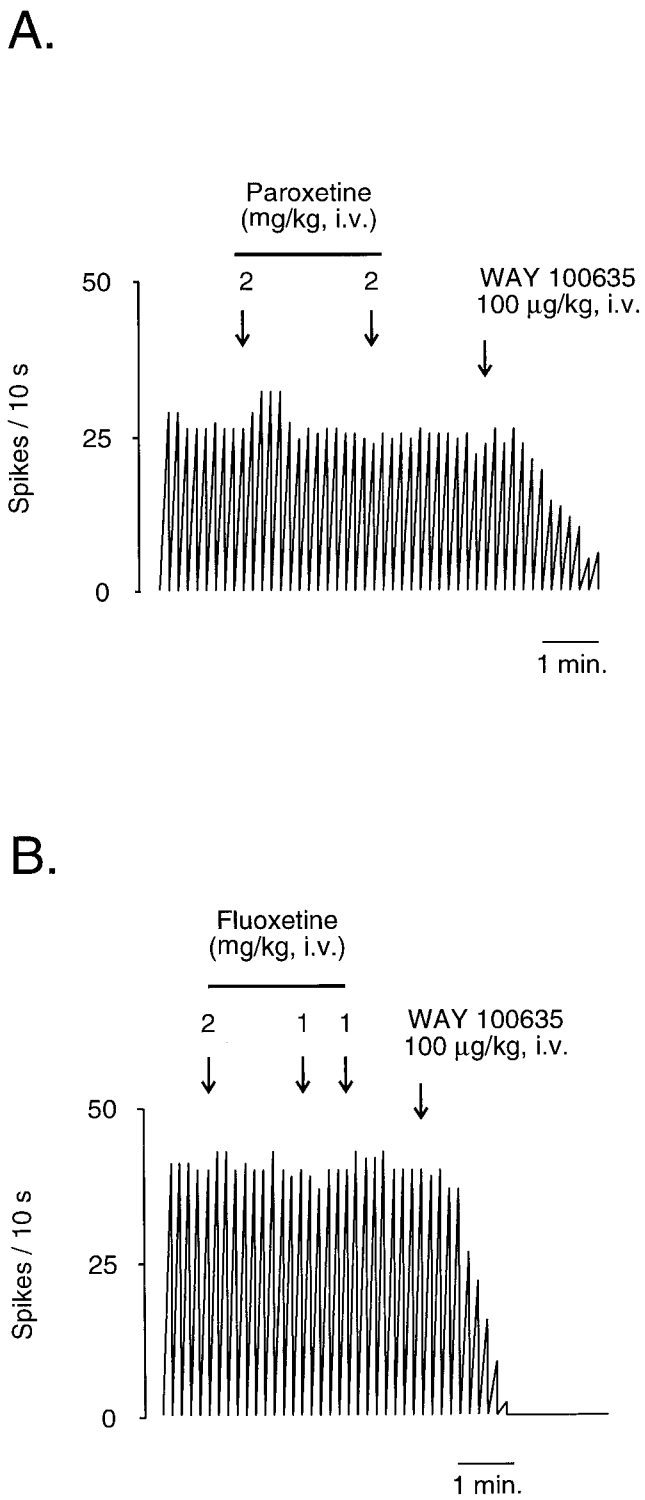
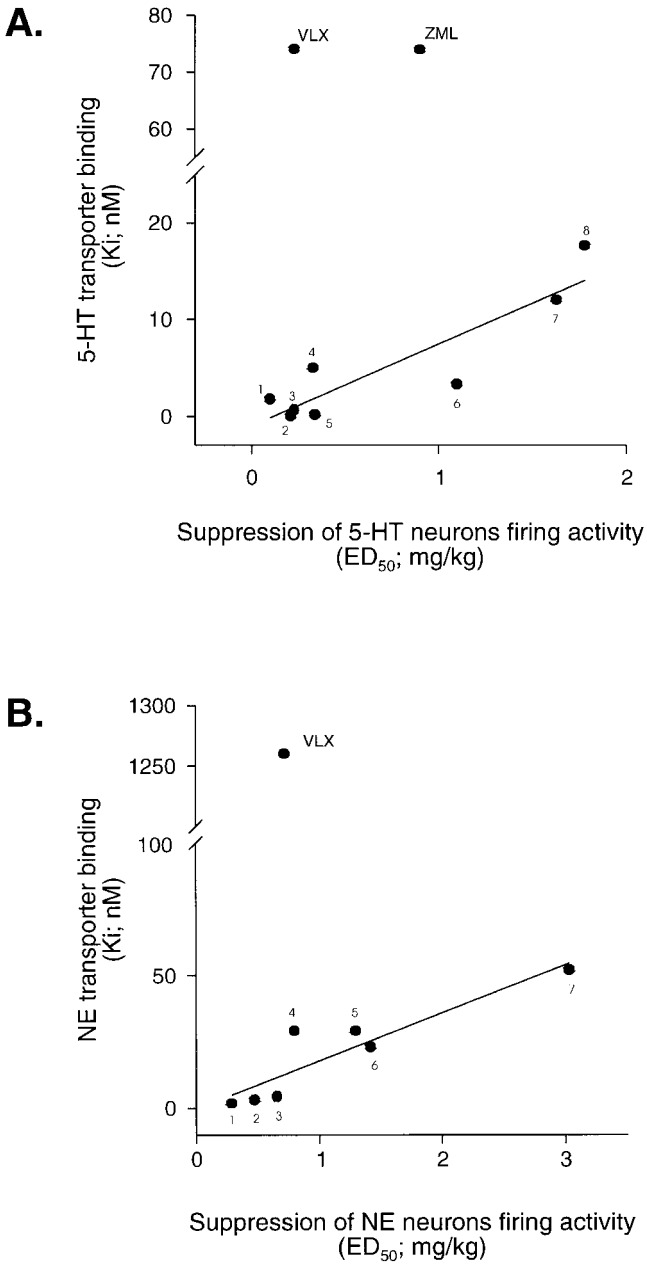


Fig. 5. Integrated firing rate histograms of NE neurons of the locus coeruleus showing the effects of intravenous administration of paroxetine (**A**) and fluoxetine (**B**) on the spontaneous firing activity of the neuron. These two drugs did not induce any significant suppression of the firing activity of these neurons nor did they prevent the suppressant effect of WAY 100635 (100 µg/kg, i.v.).

the slices were first electrically stimulated to ascertain their viability. As shown in Figure 8, the electrical stimulation of hippocampal slices induced the release of tritium (S_1; see Table II). The S_1 obtained from slices that were to subsequently receive tyramine was not statistically different from that obtained from slices that were to receive venlafaxine (see Fig. 8A vs. B, using the nonpaired Student's *t*-test). The NE-releasing agent tyramine (3 μM), when superfused for 2 min (at 1 ml/min), induced a massive release of tritium, consistent with the finding of Ramdine et al. (1989). The tyramine-induced release of tritium was greater than that produced by the electrical stimulation of the same slices ($P < 0.05$, using the paired Student's *t*-test; see Fig. 7A, Table II). Venlafaxine (3 μM), when superfused to slices in a fashion similar to that for tyramine, produced only a minimal release of tritium over baseline, which was smaller than that produced by tyramine ($P < 0.001$, using the nonpaired Student's *t*-test), and by the electrical stimulation of the same slices ($P < 0.001$, using the paired Student's *t*-test).



DISCUSSION

The potencies of reuptake inhibitors for suppressing the firing activity of dorsal raphe 5-HT and locus coeruleus NE neurons can be taken as an in vivo index of 5-HT and NE reuptake blocking potencies, respectively (Quinaux et al., 1982). Hence, the results presented here confirm and extend previous studies (Muth et al., 1986a; Bolden-Watson and Richelson, 1993; Béïque et al., 1998a,b) that have demonstrated a dual 5-HT/NE reuptake blocking properties of venlafaxine, its potency being greater to block the 5-HT reuptake process than that for NE. Venlafaxine and paroxetine were equipotent in suppressing the firing activity of dorsal raphe 5-HT neurons, while venlafaxine was about 3 times less potent than desipramine in suppressing that of locus coeruleus NE neurons (see Table I). The potencies of venlafaxine to suppress the firing activity of both dorsal raphe 5-HT and locus coeruleus NE neurons are nonetheless out of keeping with its affinities for both the 5-HT and NE transporters (Fig. 6). This raises issues with regard to the exact mechanism by which venlafaxine exerts its in vivo 5-HT and NE reuptake blockade.

Fig. 6. (**A**) Correlation (r = 0.89) between the potency, expressed as ED_{50} values, of various compounds to suppress the firing activity of dorsal raphe 5-HT neurons and their affinity, expressed as K_i values, for the 5-HT transporter (taken from either [^{3}H]cyanoimipramine or [^{3}H]paroxetine displacement studies). (**B**) Correlation (r = 0.91) between the potency, expressed as ED_{50} values, of various compounds to suppress the firing activity of locus coeruleus NE neurons and their affinity, expressed as K_i values, for the NE transporter (taken from [^{3}H]nisoxetine displacement studies). The values reported for venlafaxine (VLX; **A** and **B**) and zimelidine (ZML; **A**) were not taken into account for the calculation of the correlation coefficient (r) of the two linear regressions. The numbers on **A** and **B** correspond to the following compounds followed by the study from which the data have been obtained (ED_{50}; K_i). **A**: 1) duloxetine (Kasamo et al., 1996; Béïque et al., 1998b); 2) paroxetine (present study; Béïque et al., 1998b); 3) citalopram (de Montigny et al., 1990; Cheetham et al., 1993); 4) indalpine (Blier et al., 1984; Béïque et al., 1998b); 5) chlorimipramine (Scuvée-Moreau and Dresse, 1979; Cheetham et al., 1993); 6) norzimelidine (de Montigny et al., 1981; Kovachich et al., 1988); 7) imipramine (Scuvée-Moreau and Dresse, 1979; Cheetham et al., 1993); 8) amitriptyline (Scuvée-Moreau and Dresse, 1979; Cheetham et al., 1993); VLX = venlafaxine (present study; Béïque et al., 1998b); ZML = zimelidine (de Montigny et al., 1981; Kovachich et al., 1988). **B**: 1) desipramine (present study; Béïque et al., 1998b); 2) duloxetine (Kasamo et al., 1996; Béïque et al., 1998b); 3) nortriptyline (Scuvée-Moreau and Dresse, 1979; Cheetham et al., 1996); 4) tranylcypromine (Scuvée-Moreau and Dresse, 1979; Cheetham et al., 1996); 5) imipramine (Scuvée-Moreau and Dresse, 1979; Cheetham et al., 1996); 6) amitriptyline (Scuvée-Moreau and Dresse, 1979; Cheetham et al., 1996); 7) chlorimipramine (Scuvée-Moreau and Dresse, 1979; Cheetham et al., 1996); VLX = venlafaxine (present study; Béïque et al., 1998b).

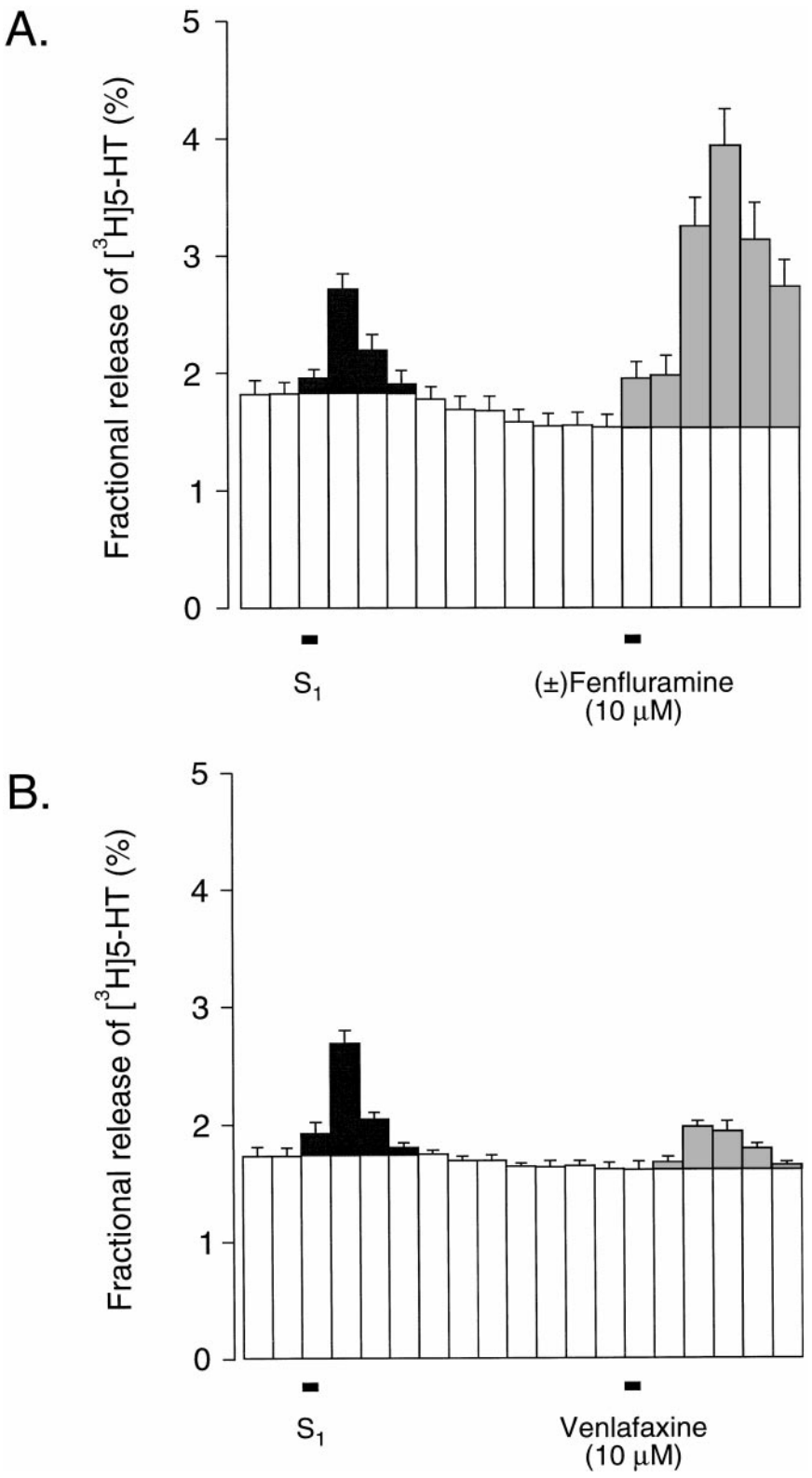


Fig. 7. Basal, electrically evoked (S_1), and drug-induced (S_2) release of tritium from hypothalamic slices preloaded with [^{3}H]5-HT. The electrical stimulation (S_1) was delivered at fraction #3 to ascertain the viability of the slices and consisted of 360 pulses delivered at an intensity of 20 mA, a duration of 2 ms, and a frequency of 3 Hz. The drug-induced (S_2) release of tritium was achieved by incorporating into the Krebs solution either (±)fenfluramine (**A**; 10 μM) or venlafaxine (**B**; 10 μM), and by perfusing the slices at 1 ml/min for 2 min. Ordinate scale: amount of tritium released per 4-min sample expressed as a fraction of total tissue tritium content of the slices at the start of the respective collection period. The gray area represents the proportion of tritium overflow evoked by either the electrical stimulation or drug superfusion. Values are expressed as means ± SEM of ten experiments.

Venlafaxine dose-dependently suppressed the firing activity of dorsal raphe 5-HT neurons (Figs. 2, 4). It is well established that the acute administration of nonselective (Sheard et al., 1972; Scuvée-Moreau and Dresse, 1979) as well as selective 5-HT reuptake inhibitors (SSRI's) such as indalpine, citalopram, zimelidine, or paroxetine (de Montigny et al., 1981; Blier et al., 1984; Chaput et al., 1986; Hajós et al., 1995) induces a

TABLE II. Effects of venlafaxine, fenfluramine and tyramine on the spontaneous release of tritium from brain slices preloaded with either [^{3}H]5-HT or [^{3}H]NE[1]

Hypothalamic slices preloaded with [^{3}H]5-HT	S_1 Electrical stimulation	S_2 Venlafaxine (10 µM)	S_1 Electrical stimulation	S_2 Fenfluramine (10 µM)
	1.98 ± 0.24 (n = 10)	0.64 ± 0.18	1.49 ± 0.16[2] (n = 10)	7.7 ± 0.8*
Hippocampal slices preloaded with [^{3}H]NE	**S_1 Electrical stimulation**	**S_2 Venlafaxine (3 µM)**	**S_1 Electrical stimulation**	**S_2 Tyramine (3 µM)**
	3.63 ± 0.56 (n = 5)	0.35 ± 0.30	3.31 ± 0.5[2] (n = 5)	6.4 ± 0.75*

[1]Data represent the total increase in radioactivity released above resting outflow by either electrical stimulation (S_1) or drug perfusion (S_2), as calculated from figure 7 and 8.
[2]The electrically evoked release (S_1) of tritium is not statistically different from that induced in slices that were subsequently exposed to venlafaxine (processed in parallel).
*$p < 0.001$ when compared to venlafaxine-induced release of tritium.

suppression of the firing activity of dorsal raphe 5-HT neurons. Given that: 1) the suppressant effects of 5-HT reuptake inhibitors are dose-dependent; 2) neither paroxetine nor venlafaxine have appreciable affinity for any receptors known to modulate the firing activity of dorsal raphe 5-HT neurons (Muth et al., 1986b; Thomas et al., 1987; Cusack et al., 1994); and 3) that the selective 5-HT_{1A} antagonist WAY 100635 reversed the suppressant effect of both paroxetine and venlafaxine, it can be assumed that the potency of the latter drugs for suppressing the firing activity of dorsal raphe 5-HT neurons is a reliable index to assess their potencies as 5-HT reuptake inhibitors. According to the latter assumption, the present results suggest that venlafaxine and paroxetine are equipotent (ED_{50}: 233 and 211 µg/kg, respectively; Table I) in blocking 5-HT reuptake in the dorsal raphe. In support of this assertion, a previous study in the dorsal hippocampus using an in vivo electrophysiological paradigm has shown that paroxetine and venlafaxine inhibit the reuptake of 5-HT by 5-HT terminals with an equal potency (Béïque et al., 1998a). The suppressant effect of paroxetine and venlafaxine on the firing activity of dorsal raphe 5-HT neurons is in keeping with previous studies (Hajós et al., 1995; Gartside et al., 1997).

The acute i.v. administration of NE reuptake inhibitors suppresses the firing activity of locus coeruleus NE neurons (Nyback et al., 1975; Scuvée-Moreau and Dresse, 1979; Haskins et al., 1985; Ceci and Borsini, 1996), which effect was shown to correlate with the NE reuptake blocking potencies of these compounds (Quinaux et al., 1982). Should this contention be also true for venlafaxine (i.e., that the potency of venlafaxine for suppressing the firing activity of locus coeruleus NE neurons can be taken as an index of its NE reuptake inhibition potency), the potency of venlafaxine obtained in this study (ED_{50}: 737 µg/kg) would suggest that it is three times less potent than desipramine (ED_{50}: 240 µg/kg) as an NE reuptake blocker, consistent with ED_{50} values previously published (Nyback et al., 1975; Scuvée-Moreau and Dresse, 1979; Haskins et al., 1985; Ceci and Borsini, 1996). It is striking, however, that venlafaxine failed to induce a complete suppression of firing of NE neurons, unlike other NE reuptake inhibitors (Fig. 4A,B).

The observations that, first, paroxetine and venlafaxine were equipotent in suppressing the firing activity of dorsal raphe 5-HT neurons and, second, that desipramine was three times more potent than venlafaxine to suppress that of locus coeruleus NE neurons is highly interesting, given results from previous studies that have used different experimental approaches. For instance, a synaptosomal uptake study has shown that venlafaxine is 53 times less potent than paroxetine to inhibit the uptake of [^{3}H]5-HT and that venlafaxine is 344 times less potent than desipramine to inhibit that of [^{3}H]NE (Bolden-Watson and Richelson, 1993). Moreover, in a recent radioligand binding study, venlafaxine displayed an affinity for the 5-HT transporter about 2,000 times lower than that of paroxetine (K_i of 74 and 0.04 nM, respectively), as assessed with [^{3}H]cyanoimipramine displacement, while venlafaxine also displayed 2,000 times lesser affinity for the NE transporter compared to desipramine (K_i of 1,260 and 0.551 nM, respectively), as assessed with [^{3}H]nisoxetine displacement (Béïque et al., 1998b). The relative affinities and potencies derived from the latter in vitro studies are thus totally incongruent with the in vivo potencies reported here. This discrepancy is nonetheless best demonstrated by the correlations depicted in Figure 6A,B: the potencies of reuptake inhibitors for suppressing the firing activity of dorsal raphe 5-HT and/or locus coeruleus NE neurons are tightly correlated with their respective affinities for both transporters. Of utmost interest is the fact that the suppression of the firing activity of dorsal raphe 5-HT neurons achieved by venlafaxine, as well as by zimelidine, does not conform to this correlation (Fig. 6A). As for zimelidine, its suppressant effect on the firing activity of dorsal raphe 5-HT neurons is most probably mediated by its active metabolite, norzimelidine, since zimelidine itself displays a low potency to inhibit [^{3}H]5-HT uptake (Hyttel, 1982), and has low affinity for the 5-HT transporter (Kovachich et al., 1988; Marcusson et al., 1992), whereas norzimelidine (compound #6 in Fig. 6A) has potent 5-HT reuptake inhibition properties (Hyttel, 1982), and displays a high affinity for the 5-HT transporter, well in keeping with its potency to suppress the firing activity of dorsal raphe 5-HT neurons (Fig. 6A). Nonetheless, the most intriguing and perhaps the most convincing assertion derived from the present study is the fact that the potencies of venlafaxine for suppressing the firing activity of both 5-HT and NE neurons is out of keeping with its affinities for both transporters (Fig. 6A,B). The

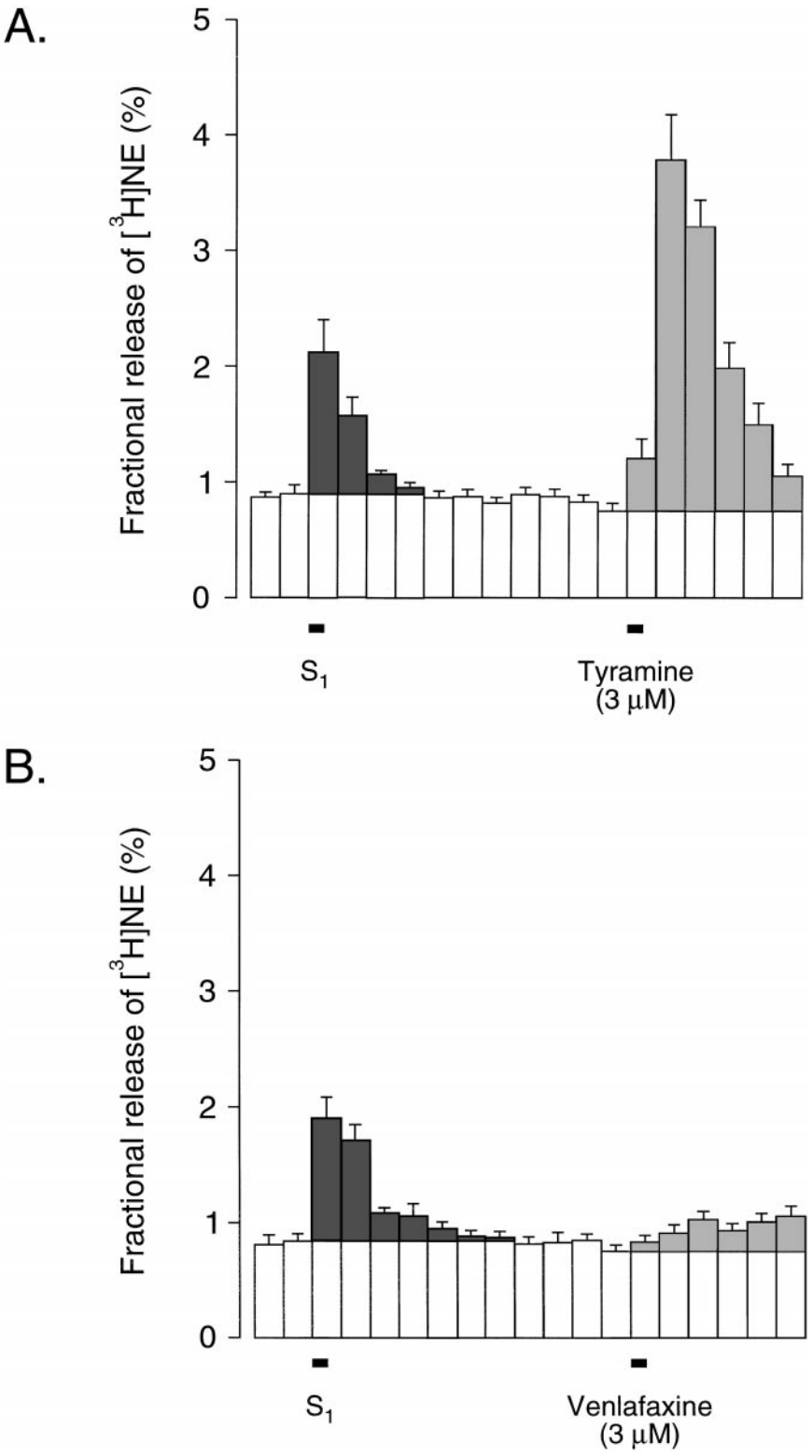


Fig. 8. Basal, electrically evoked (S_1), and drug-induced (S_2) release of tritium from hippocampus slices preloaded with [^{3}H]NE. The electrical stimulation (S_1) was delivered at fraction #3 to ascertain the viability of the slices and consisted of 360 pulses delivered at an intensity of 30 mA, a duration of 2 ms, and a frequency of 3 Hz. The drug-induced release of tritium was achieved by incorporating into the Krebs solution either tyramine (**A**; 3 µM) or venlafaxine (**B**; 3 µM), and by perfusing the slices at 1 ml/min for 2 min. Ordinate scale: amount of tritium released per 4 min sample expressed as a fraction of total tissue tritium content of the slices at the start of the respective collection period. The gray area represents the proportion of tritium overflow evoked by either the electrical stimulation or drug superfusion. Values are expressed as means ± SEM of five experiments.

underlying reasons for this discrepancy are yet to be resolved, although some possibilities can be readily excluded. For instance, venlafaxine's major metabolite, O-desmethyl-venlafaxine (WY-45,233; Howell et al., 1993), was shown to bear equal 5-HT reuptake blocking potencies in vitro (IC_{50} 0.18 and 0.21 µM, respectively) and lower NE reuptake potencies (IC_{50} 1.6 and 0.64 µM, respectively) than venlafaxine itself (Muth et al., 1986b), and thus suggests that this metabolite is not the mediator of the high in vivo potencies observed with

venlafaxine, as is the case for norzimelidine's action on the firing activity of dorsal raphe 5-HT neurons.

Furthermore, given that venlafaxine is a dual 5-HT/NE reuptake inhibitor and that both systems are known to be functionally interconnected, it is possible that the effects of venlafaxine on the firing activity of both dorsal raphe 5-HT and locus coeruleus NE neurons may not be mediated by its sole and independent effect on these neurons per se, but via an action on both these systems together. In order to assess the latter possibility, the effect of venlafaxine on the firing activity of dorsal raphe 5-HT neurons of rats treated with the noradrenergic toxin 6-OHDA was assessed given that an important noradrenergic input to the dorsal raphe has been described both anatomically (Anderson et al., 1977; Sakai et al., 1977) and functionally (Baraban and Aghajanian, 1980a,b). Since the 6-OHDA lesion did not have any significant effect on the dose–response curve of the suppressant effect of venlafaxine on the firing activity of 5-HT dorsal raphe neurons (Fig. 2A,C, Table I), it can be concluded that the suppressant effect of venlafaxine on the firing activity of dorsal raphe 5-HT neurons is solely attributable to a direct action on the 5-HT system, i.e., 5-HT reuptake blockade. Moreover, this contention is consistent with the fact that NE reuptake inhibition, achieved with i.v. administration of desipramine, does not influence the firing activity of 5-HT neurons ($ED_{50} > 12$ mg/kg; Scuvée-Moreau and Dresse, 1979).

The 5-HT reuptake inhibition property of venlafaxine is probably not involved in its capacity to suppress the firing activity of locus coeruleus NE neurons for the following reasons. First, 5-HT reuptake inhibition, achieved with the i.v. administration of the two prototypical SSRIs paroxetine and fluoxetine, did not induce any changes in the firing activity of locus coeruleus NE neurons (Fig. 5A,B). Inasmuch as it is well documented that the acute systemic administration of 5-HT reuptake inhibitors induce an elevation in the extracellular concentration of 5-HT not only in the raphe area (Adell and Artigas, 1991; Rutter et al., 1995) but also in terminal areas such as the striatum, hypothalamus, hippocampus, diencephalon, and frontal cortex (Invernizzi et al., 1995; Kreiss and Lucki, 1995; Rutter et al., 1995; Romero et al., 1996a,b; Romero and Artigas, 1997), it is likely that the acute administration of paroxetine and fluoxetine would increase the extracellular concentration of 5-HT in the locus coeruleus. Although the exact nature of the serotonergic modulation of the NE locus coeruleus neurons has not been fully elucidated, nor has the exact nature of the receptors involved in the circuitry, several lines of evidence point toward an inhibitory role for 5-HT on the firing activity of NE neurons of the locus coeruleus (Segal, 1979; Rasmussen and Aghajanian, 1986; Aston-Jones et al., 1991a; Haddjeri et al., 1997). In light of these considerations, the lack of effect of paroxetine and fluoxetine on the firing activity of locus coeruleus NE neurons is somewhat intriguing. At any rate, the lack of effect of these two SSRIs on the firing activity of locus coeruleus NE neurons supports the notion that the 5-HT reuptake inhibition property of venlafaxine cannot be held responsible for the unexpectedly high potency of venlafaxine for suppressing the firing activity of locus coeruleus NE neurons.

If the 5-HT reuptake blocking properties of venlafaxine were to be involved in the surprisingly high potency of venlafaxine to suppress the firing activity of locus coeruleus NE neurons, the lesioning of 5-HT neurons would have induced a shift to the right of the dose–response curve of the suppression by venlafaxine of the firing activity of locus coeruleus NE neurons, indicative of a dampened effect. Our findings, on the contrary, indicate that the dose–response curve of venlafaxine in the locus coeruleus was actually shifted to the left (ED_{50}: 285 vs. 737 μg/kg; Table I) in 5,7-DHT lesioned rats. This finding was unexpected given the presumed inhibitory role of 5-HT in the locus coeruleus. Based on the assumption that the 5-HT system would tonically suppress locus coeruleus NE neurons (Haddjeri et al., 1997), it is conceivable that compensatory homeostatic mechanisms have developed following the lesion with 5,7-DHT. In support of this assertion, it was observed in the amygdala that a 5,7-DHT lesion not only induced a supersensitivity to microiontophoretically applied 5-HT but also to NE (Wang et al., 1979). Given that venlafaxine, as well as other NE reuptake blockers, suppresses the firing activity of locus coeruleus NE neurons ultimately through activation of somatodendritic α_2-adrenergic autoreceptors, the potentiation of the effect of venlafaxine on the firing activity of locus coeruleus NE neurons induced by the serotonergic lesion may be attributable to a supersensitivity of these somatodendritic α_2-adrenergic autoreceptors. This hypothesis seems at first incongruent with the study of Kasamo et al. (1996), who reported an unchanged capacity of duloxetine, another dual 5-HT/NE reuptake inhibitor, to suppress the firing activity of locus coeruleus NE neurons in 5,7-DHT-treated rats. This discrepancy could nevertheless be explained, at least in part, by methodological considerations. Indeed, as the lesioning in that latter study were performed by injecting the toxin directly in the dorsal raphe, our lesioning was made i.c.v. Although the serotonergic input to the locus coeruleus was originally believed to arise predominantly from the raphe nuclei (Pickel et al., 1977; Cedarbaum and Aghajanian, 1978), it has been more recently suggested that an important 5-HT input to the locus arises from the nucleus paragigantocellularis and from pericoerulear area (Pieribone et al., 1989; see Aston-Jones et al., 1991b for review). It is thus possible that, in the study of Kasamo et al. (1996), some relevant serotonergic input to the locus coeruleus may have been spared by the injection of the toxin in the dorsal raphe, whose sole presence may have prevented the development of NE supersensitivity.

The last possibility dealt with in the present study regarding a plausible mechanism that might have underlain the peculiar high in vivo potencies of venlafaxine was that of a putative 5-HT and/or NE releasing property. The rationale being that releasers, by increasing the extracellular concentrations of 5-HT and/or NE, would decrease the firing activity of 5-HT and/or NE neurons via somatodendritic autoreceptor activation. Indeed, as for the 5-HT system, while it was shown that the 5-HT releaser (±)fenfluramine suppressed in vivo the firing activity of 5-HT neurons in the dorsal raphe (Scuvée-Moreau and Dresse, 1990), the suppressant effect of 3,4-methylenedioxymethamphetamine (MDMA) on the firing activity of 5-HT neurons, as assessed in vitro on mesencephalic slices, was shown to be mediated by the release of 5-HT (Sprouse et al., 1989). For the NE system, the NE releaser amphetamine was shown to suppress the firing activity of locus coeruleus NE neurons and this, via an activation of α_2-adrenergic autoreceptors (Rasmussen and Aghajanian, 1986). Therefore, in light of the fact that venlafaxine shares a phenylethylamine moiety with some monoamine releasers such as tyramine, amphetamine, methamphetamine, or (±)fenfluramine, it was deemed necessary to investigate any putative 5-HT- and/or NE-releasing properties for venlafaxine. As illustrated in Figures 7 and 8, venlafaxine was devoid of any 5-HT- and/or NE-releasing properties in sharp contrast to (±)fenfluramine and tyramine, respectively. It is noteworthy that superfusion of the slices with venlafaxine induced a small increase of the spontaneous outflow of both [^{3}H]5-HT and [^{3}H]NE. The latter most probably reflects the 5-HT and NE reuptake blocking properties of venlafaxine, rather than one of releasing. This is consistent with previous studies showing that superfusion with paroxetine and citalopram induce a small increase of the spontaneous outflow of [^{3}H]5-HT (Blier et al., 1990), while superfusion with desipramine induces a small increase of the spontaneous outflow of [^{3}H]NE (Mongeau et al., 1998). Therefore, the present results rule out the possibility that venlafaxine might act via releasing 5-HT or NE.

In summary, if one assumes that suppression of firing activity of monoaminergic neurons by reuptake blockers can be taken as an index of their reuptake inhibition potencies, the present study suggests that venlafaxine has potent 5-HT and NE reuptake inhibition potencies when assessed using an in vivo electrophysiological paradigm. Since venlafaxine is more potent in inhibiting the reuptake of 5-HT compared to that for NE, it is possible that the seemingly unique clinical properties seen with high doses of venlafaxine may be mediated by the recruitment of the NE system by these higher doses. Nonetheless, the fact that the in vivo reuptake blocking properties of venlafaxine does not seem to be solely mediated by an action on the [^{3}H]cyanoimipramine or [^{3}H]nisoxetine binding sites on the 5-HT and NE transporters, respectively, may cast doubt on the latter hypothesis and thus raises important considerations with regard to the exact mechanism by which venlafaxine exerts its antidepressant action.

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