Electrophysiologic Evidence for Desensitization of α_2-Adrenoceptors on Serotonin Terminals Following Long-Term Treatment with Drugs Increasing Norepinephrine Synaptic Concentration

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Previous results from our laboratory have indicated that small intravenous doses of the α_2-adrenergic agonist clonidine increase serotonin (5-HT) neurotransmission by attenuating the release of endogenous norepinephrine (NE), as a result of the activation of α_2-adrenergic autoreceptor on NE neurons, and that high doses of clonidine decrease 5-HT neurotransmission by directly activating α_2-adrenergic heteroreceptors on 5-HT terminals. The aim of the present study was to assess whether antidepressant treatments that increase the synaptic concentration of NE or 5-HT alter the ability of clonidine to modulate 5-HT neurotransmission through these two α_2-adrenoceptors. Rats were treated for 3 weeks with 0.75 mg/kg per day of befloxatone (a reversible inhibitor of monoamine oxidase A), 10 mg/kg per day of nisoxetine (a selective NE reuptake inhibitor), 10 mg/kg per day of paroxetine (a selective 5-HT reuptake inhibitor) or saline using subcutaneous osmotic minipumps (removed 48 hours before the experiment). No significant change in the effect of the small dose of clonidine (10 μg/kg, IV) was found following the befloxatone, the nisoxetine, or the paroxetine treatments. The reduction of 5-HT neurotransmission by the high dose of clonidine (400 μg/kg, IV) was no longer present in rats treated with nisoxetine or befloxatone, but was unaltered in those treated with paroxetine. Furthermore, in rats pretreated with the NE neurotoxin 6-hydroxydopamine, a long-term treatment with befloxatone failed to alter the reducing effect of the high dose of clonidine but abolished the reducing effect of the low dose of clonidine. These results suggest that antidepressant drugs that increase NE synaptic concentration induce a desensitization of α_2-heteroreceptor on 5-HT terminals.

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KEY WORDS: *α_2-Adrenergic autoreceptors; α_2-Adrenergic heteroreceptors; Antidepressant drugs; Norepinephrine reuptake inhibitors; Serotonin reuptake inhibitors; Monoamine oxidase inhibitors*

Several studies have documented the possibility that presynaptic α_2-adrenergic autoreceptors become desensitized following long-term antidepressant treatments that increase the synaptic concentration of norepinephrine (NE) (Crews and Smith 1978; Svensson and Usdin 1978; McMillen et al. 1980; Spyraky and Fibiger 1980; Cohen et al. 1982; Finberg and Tal 1985; Lacroix et al. 1991). Furthermore, the density of high-affinity state α_2-adrenoceptors has been shown to be increased on platelets of depressed patients, a condition normalized by long-term treatment with antidepressant drugs (Garcia-Sevilla et al. 1986, 1987; Doyle et al. 1985;

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Takeda et al. 1989; Piletz et al. 1991). However, not all antidepressant treatments share the property of decreasing these α_2-adrenoceptors (Willner 1985). In contrast, several classes of antidepressant treatments have been shown to alter serotonin (5-HT) neurotransmission (for review, see Blier et al., 1990).

The capacity of α_2-adrenergic ligands to modulate the evoked release of [^{3}H]5-HT in vitro has long been known to be exerted by α_2-adrenoceptors located on 5-HT terminals (Starke and Montel 1973; Göthert and Huth 1980; Frankhuyzen and Mulder 1980,1982; Göthert et al. 1981; Maura et al. 1982). Using an in vivo electrophysiologic paradigm, it was recently shown that these α_2-adrenergic heteroreceptors in the rat hippocampus are tonically activated by endogenous NE (Mongeau et al. 1993), as is the case in the human brain (Galzin et al. 1992; Feuerstein et al. 1993). Small doses (2 μg/kg and 10 μg/kg IV) of the α_2-adrenergic agonist clonidine enhance 5-HT neurotransmission, an effect that is abolished by a 6-hydroxydopamine (6-OHDA) pretreatment and blocked by the α_2-adrenergic antagonist yohimbine (0.1 mg/kg IV). These results indicate that a low dose of clonidine preferentially activates α_2-adrenergic autoreceptors on NE neurons, reducing the tonic inhibitory action of endogenous NE on α_2-adrenergic heteroreceptors on 5-HT terminals and, consequently, increasing 5-HT release. However, at higher doses (100 μg/kg and 400 μg/kg IV), clonidine decreases 5-HT neurotransmission through a direct activation of α_2-adrenergic heteroreceptors, as this effect is not affected by NE denervation and is blocked by yohimbine (1 mg/kg IV; Mongeau et al. 1993).

In the present study, this in vivo modulation of 5-HT neurotransmission by clonidine via α_2-adrenergic auto- and heteroreceptors was used to investigate the effect of long-term treatments that increase the synaptic concentration of NE or 5-HT. The changes in the synaptic concentration of the neurotransmitters were induced by either selective reuptake blockade or monoamine oxidase A (MAO-A) inhibition. This electrophysiologic paradigm was considered most appropriate to investigate the modulation of 5-HT neurotransmission by the NE system because, as described before, it allows the in vivo assessment of the responsiveness of the α_2-adrenergic heteroreceptors on 5-HT terminals and of the level of tonic inhibitory action of NE acting on them.

METHODS

Animals and Treatments

Male Sprague-Dawley rats, maintained on a 12-hour/12-hour light–dark cycle with free access to food and water, were treated over a course of 3 weeks with 0.75 mg/kg per day of the reversible MAO-A inhibitor befloxatone (Curet et al. 1992a), 10 mg/kg per day of the selective 5-HT reuptake inhibitor paroxetine (Thomas et al. 1987), 10 mg/kg per day of the selective NE reuptake inhibitor nisoxetine (Fuller et al. 1979), or saline delivered by osmotic minipumps (ALZA, Palo Alto, CA) inserted subcutaneously. The minipumps were removed 48 hours before the experiments began to allow elimination of the drugs. Lesions of NE neurons were performed under chloral hydrate anesthesia (400 mg/kg IP) by injecting 6-OHDA intracerebroventricularly (120 μg free base in 20 μl of 0.9% NaCl and 0.1% ascorbic acid) 1 hour after an injection of fluoxetine (10 mg/kg, IP) administered to protect the 5-HT system from the neurotoxic action of 6-OHDA. One week after this procedure, the rats were treated with 0.75 mg/kg per day of befloxatone for 3 weeks. At the time of the experiments, the rats (weighing between 325 and 375 g) were anesthetized with an injection of 400 mg/kg (IP) of chloral hydrate.

Recording from Dorsal Hippocampus CA_3 Pyramidal Neurons

Extracellular unitary recording and microiontophoresis onto pyramidal neurons in the CA_3 region of the dorsal hippocampus were conducted with five-barrelled micropipettes, pulled conventionally with the tip broken to a diameter of 9 μm to 11 μm. The central barrel, used for recording, was filled with a 2 mol/L NaCl solution. The side barrels contained the following solutions: 5-HT creatinine sulfate (0.5 mmol/L in 200 mmol/L NaCl, pH 4), NE bitartrate (20 mmol/L in 200 mmol/L NaCl, pH 4), acetylcholine chloride (20 mmol/L in 200 mmol/L NaCl, pH 4), and a 2-mol/L NaCl solution used for automatic current balancing. The 5-HT and NE solutions were retained with a −7 nA current between ejections. Pyramidal neurons were identified by their large amplitude (0.5 mV to 1.2 mV) and long-duration (0.8 msec to 1.2 msec) simple spikes alternating with complex spike discharges (Kandel and Spencer 1961). These characteristics readily allow the differentiation of pyramidal neurons from interneurons. A small current or leak of acetylcholine (− 1 nA to 5 nA) was used to activate silent or slowly discharging pyramidal neurons to a physiologic firing rate (8 Hz to 12 Hz), because most of these cells do not discharge spontaneously in chloral hydrate–anesthetized rats. The responsiveness to microiontophoretic application of 5-HT and NE was evaluated from the number of spikes suppressed/nA calculated by an on-line computer with a 100-millisecond discrimination.

To evaluate the effectiveness of the 6-OHDA lesion, the recovery of the firing activity of the pyramidal neurons following the microiontophoretic application of NE was assessed by determining the recovery time 50

(RT_{50}). This parameter is defined by the time (in seconds) required for the firing rate to recover by 50% from the termination of the microiontophoretic application (de Montigny et al. 1980).

Stimulation of the 5-HT Pathway

To activate the 5-HT projections to the dorsal hippocampus, a bipolar electrode (NE-100; David Kopf, Tujunga, CA) was implanted on the midline with a 10° backward angle in the ventromedial tegmentum, 1 mm anterior to lambda, and 8.3 mm below the cortical surface. Two hundreds square pulses of 0.5 milliseconds were delivered by a stimulator (Model S8800; Grass, Quincy, MA) at a frequency of 1 Hz and at an intensity of 300 μA. The stimulation pulses and the firing activity of the neuron recorded were fed to a computer equipped with a Tecmar interface. Peristimulus time histograms were generated to determine the duration of suppression of firing, measured to absolute silence value (SIL, in milliseconds; Chaput et al. 1986). This parameter is obtained by dividing the total number of events suppressed with the stimulation by the mean frequency of firing of the neuron recorded. It thus represents an estimate of the duration of the suppression of firing corrected for the mean prestimulation firing frequency. Several lines of evidence indicate that the effect of the electrical stimulation of the ascending 5-HT pathway is due to the release of 5-HT into the synaptic cleft. First, it is virtually abolished by a pretreatment with the 5-HT neurotoxin 5,7-dihydroxytryptamine (Blier and de Montigny 1983, 1985). Second, it is blocked by the acute intravenous injection of the 5-HT_{1A} receptor antagonist BMY 7378 (Yocca et al. 1987; Chaput and de Montigny 1988). Third, it is enhanced by terminal 5-HT autoreceptor blockade (Chaput et al. 1986). Fourth, it is reduced by terminal 5-HT autoreceptor activation (Chaput and de Montigny 1988).

In most neurons, following the suppression of firing induced by the electrical stimulation there is a transient increase of the probability of firing, the exact nature of which is presently unknown. However, this transient increase was shown to be unaffected by 5-HT reuptake inhibitors or MAO inhibitors (Blier et al. 1988). The relative activation was calculated by dividing the total number of supplementary events by the mean prestimulation firing frequency.

The effect of stimulating the ascending 5-HT fibers was determined while recording from the same neuron before and after the successive intravenous injections of 10 μg/kg and 400 μg/kg of clonidine. The time elapsed between the control stimulation periods and those following the administration of clonidine was approximately 15 minutes.

Drugs

The following drugs were used: befloxatone (Delalande, Rueil-Malmaison, France); paroxetine (SmithKline Beecham, Harlow, England); nisoxetine and fluoxetine (Eli Lilly, Indianapolis, IN); clonidine HCl, 5-HT creatinine sulfate, NE bitartrate, 6-OHDA HCl, and acetylcholine chloride (Sigma Chemical, St. Louis, MO); and chloral hydrate (American Chemicals, Montréal, Québec, Canada).

Statistical Analyses

All results are expressed as means plus/minus standard error of the mean. The statistical significance of the difference between the effects of the stimulation of the 5-HT pathway before and after the administration of a drug in the same rat was assessed using the paired Student's *t*-test. Possible differences in the magnitude of the effects of clonidine between saline- and drug-treated rats were tested for statistical significance by covariance analysis. Differences in the efficacy of the stimulation of the 5-HT pathway and neuronal responsiveness to microiontophoretic application of NE and 5-HT between saline- and drug-treated rats were assessed for statistical significance using the unpaired Student's *t*-test.

RESULTS

Effect of Long-Term MAO Inhibition on the Modulation of 5-HT Neurotransmission by Clonidine

The suppressant effect of the electrical stimulation of the ascending 5-HT pathway on the firing rate of CA_3 pyramidal neurons of the dorsal hippocampus was assessed following long-term drug treatments. The peristimulus time histograms in Figure 1A show that 10 μg/kg IV of clonidine enhanced, while 400 μg/kg IV reduced, the duration of suppression of firing in the same neuron of a saline-treated rat. Following the administration of 10 μg/kg and 400 μg/kg IV of clonidine, the mean duration of suppression of firing of the saline-treated group (Fig. 1B) was increased by 37% ± 8% and decreased by 25% ± 7%, respectively. These incremental and decremental effects of clonidine were statistically significant at $p < .01$ and $p < .001$, respectively, using the paired Student's *t*-test. In contrast, the facilitatory effect on the firing rate of pyramidal neurons induced by the electrical stimulation was not significantly changed by either dose of clonidine (Table 1).

To induce a sustained increase in the synaptic concentration of 5-HT and NE, MAO-A was blocked over a 3-week period with 0.75 mg/kg per day of befloxatone. The minipumps were removed 48 hours before

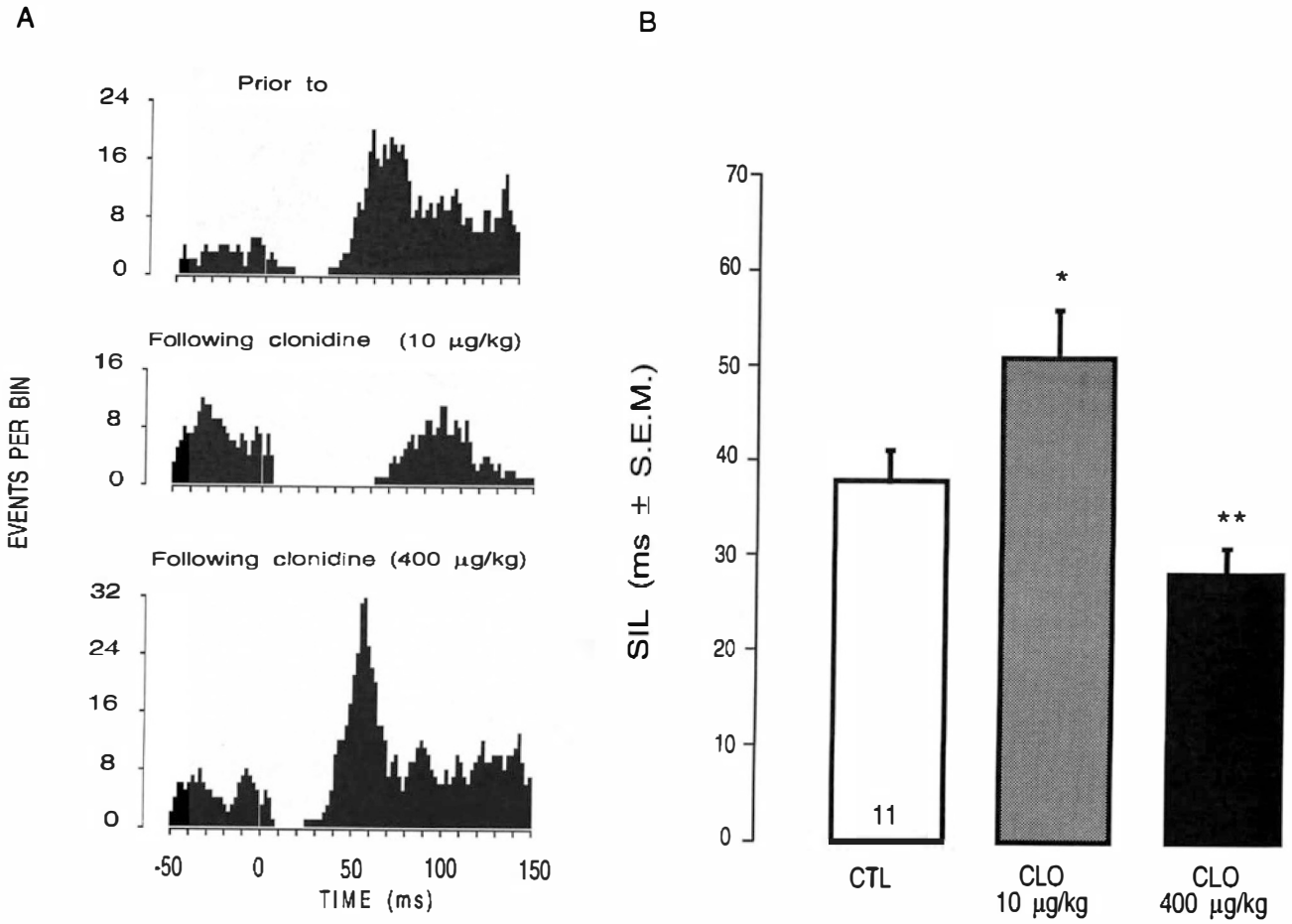

Figure 1. Effect of systemic injections of clonidine on the efficacy of the electrical stimulation of the ascending 5-HT pathway in suppressing the firing activity of CA_3 pyramidal neurons of hippocampus in the saline-treated group. **A**: Representative peristimulus time histograms illustrating the effects of clonidine (10 µg/kg and 400 µg/kg IV) in a single experiment. Each peristimulus time histogram was constructed from 200 pulses of 0.5 msec delivered at 1 Hz at time 0 with an intensity of 300 µA. Bin width is 2 msec. **B**: Histograms showing the effects of the high and the low dose of clonidine in saline-treated rats. The number of neurons tested is given at the bottom of the open column. * $p < .01$, ** $p < .001$, using a two-tailed paired Student's *t*-test, comparing prior to and following clonidine administration.

Table 1. Effect of Long-Term Drug Treatments with Befloxatone, Nisoxetine, or Paroxetine on the Late Excitatory Effect of the Firing Activity of Dorsal Hippocampus Pyramidal Neurons Induced by the Electrical Stimulation Before and After the Intravenous Administration of Clonidine[a]

Treatment[b]	Before Clonidine[c]	After Clonidine		No. of Neurons
		10 µg/kg	400 µg/kg	
Saline	102 ± 25	97 ± 23	140 ± 25	11
Befloxatone	89 ± 22	117 ± 15	167 ± 61	9
6-OHDA[d] + befloxatone	135 ± 37	171 ± 34	162 ± 42	6
Nisoxetine	130 ± 54	131 ± 35	155 ± 42	9
Paroxetine	172 ± 69	134 ± 37	123 ± 36	8

[a] One neuron per rat was studied before and after the administration of clonidine.
[b] Befloxatone: 0.75 mg/kg per day; nisoxetine and paroxetine: 10 mg/kg per day.
[c] Data are expressed as the relative activation in milliseconds ± the standard error of the mean.
[d] One-hundred twenty micrograms free base of 6-OHDA was injected intracerebroventricularly 1 week before the initiation of the befloxatone treatment.

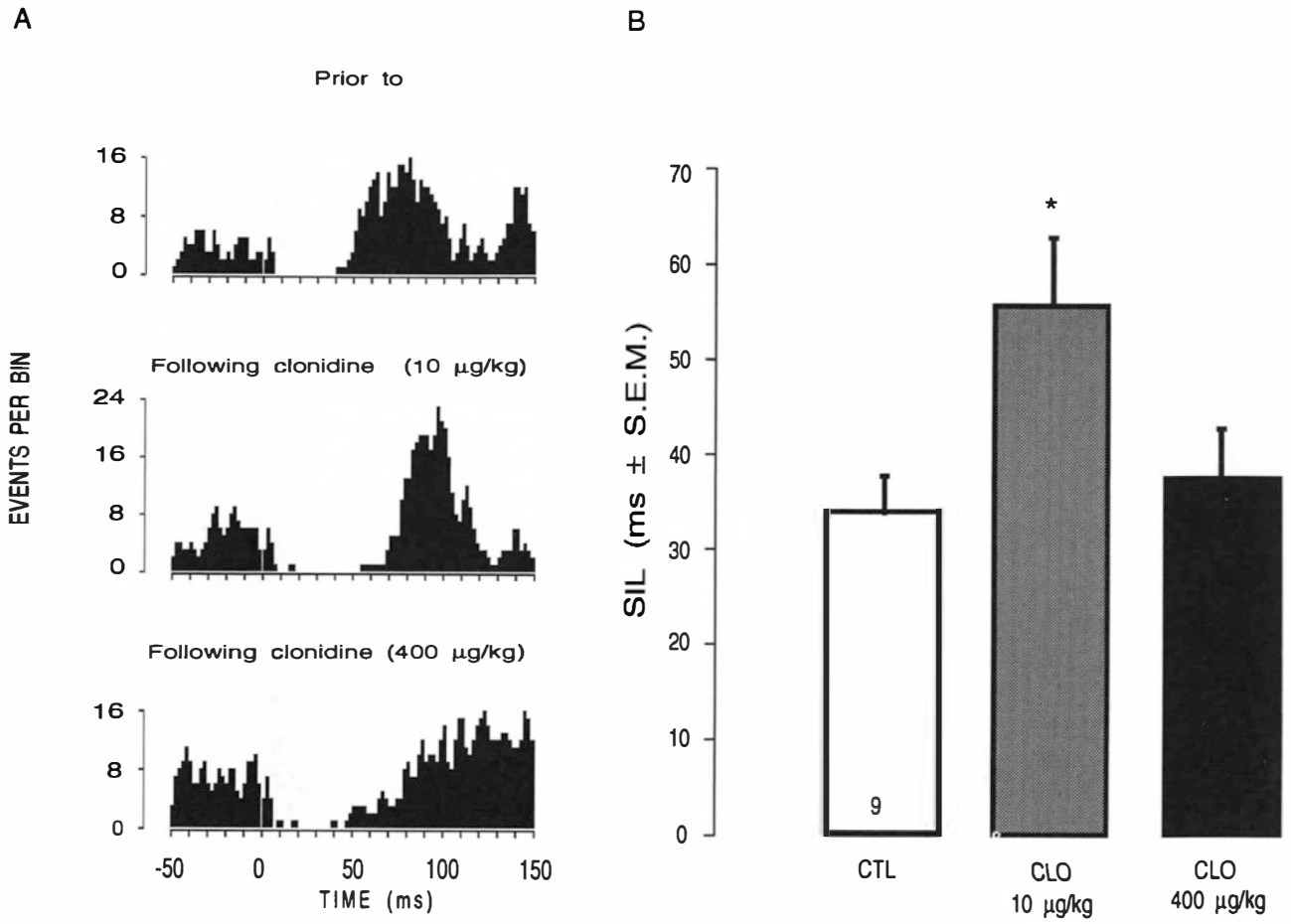

Figure 2. Effect of systemic injections of clonidine on the efficacy of the electrical stimulation of the ascending 5-HT pathway after a long-term treatment with 0.75 mg/kg per day of befloxatone. The minipumps containing the drug were removed 48 hours before the experiment began. **A**: Representative peristimulus time histograms illustrating the effects of clonidine (10 µg/kg and 400 µg/kg IV) in a single experiment. **B**: Histograms showing the effects of the high and the low dose of clonidine in befloxatone-treated rats. The number of neurons tested is given at the bottom of the open column. $p < .01$, using a two-tailed paired Student's *t*-test, comparing prior to and following clonidine administration.

the experiments began to regain full enzymatic activity (Curet et al. 1992a). The results in Figure 2A are representative peristimulus time histograms of the effect of clonidine in befloxatone-treated rats. The small dose of clonidine (10 µg/kg IV) produced a 72% ± 21% increase of the duration of the suppression of firing in the befloxatone-treated group (Fig. 2B). Although the amplitude of the effect of the small dose of clonidine appeared greater than that of the saline group (37% ± 8%; Fig. 1B), the difference between the two groups did not reach statistical significance using covariance analysis. The subsequent administration of the high dose of clonidine (400 µg/kg IV) decreased the duration of suppression of firing (Fig. 2B); however, unlike the controls (Fig. 1B), it was not reduced below the initial value. This decreased responsiveness of the α_2-adrenoceptors mediating the effect of the high dose of clonidine was not accompanied by any change in the responsiveness of pyramidal neurons of the CA_3 region of hippocampus to microiontophoretic applications of 5-HT and NE. The suppressant effects of the microiontophoretic applications of 5-HT and NE on the firing rate of the pyramidal cells after the befloxatone treatment (5-HT: 322 ± 37 spikes/nA, $n = 13$; NE: 407 ± 43 spikes/nA; $n = 13$) were not different from those of the saline-treated group (5-HT: 376 ± 62 spikes/nA, $n = 11$; NE: 400 ± 71 spikes/nA, $n = 11$).

The assumption that the increased synaptic concentration of NE, but not that of 5-HT, is responsible for the decrease in the responsiveness of the α_2-adrenoceptors mediating the effect of the high dose of clonidine was assessed by investigating the effect of the same befloxatone treatment in NE-denervated rats. Intracerebroventricular injections of the neurotoxin 6-OHDA (1

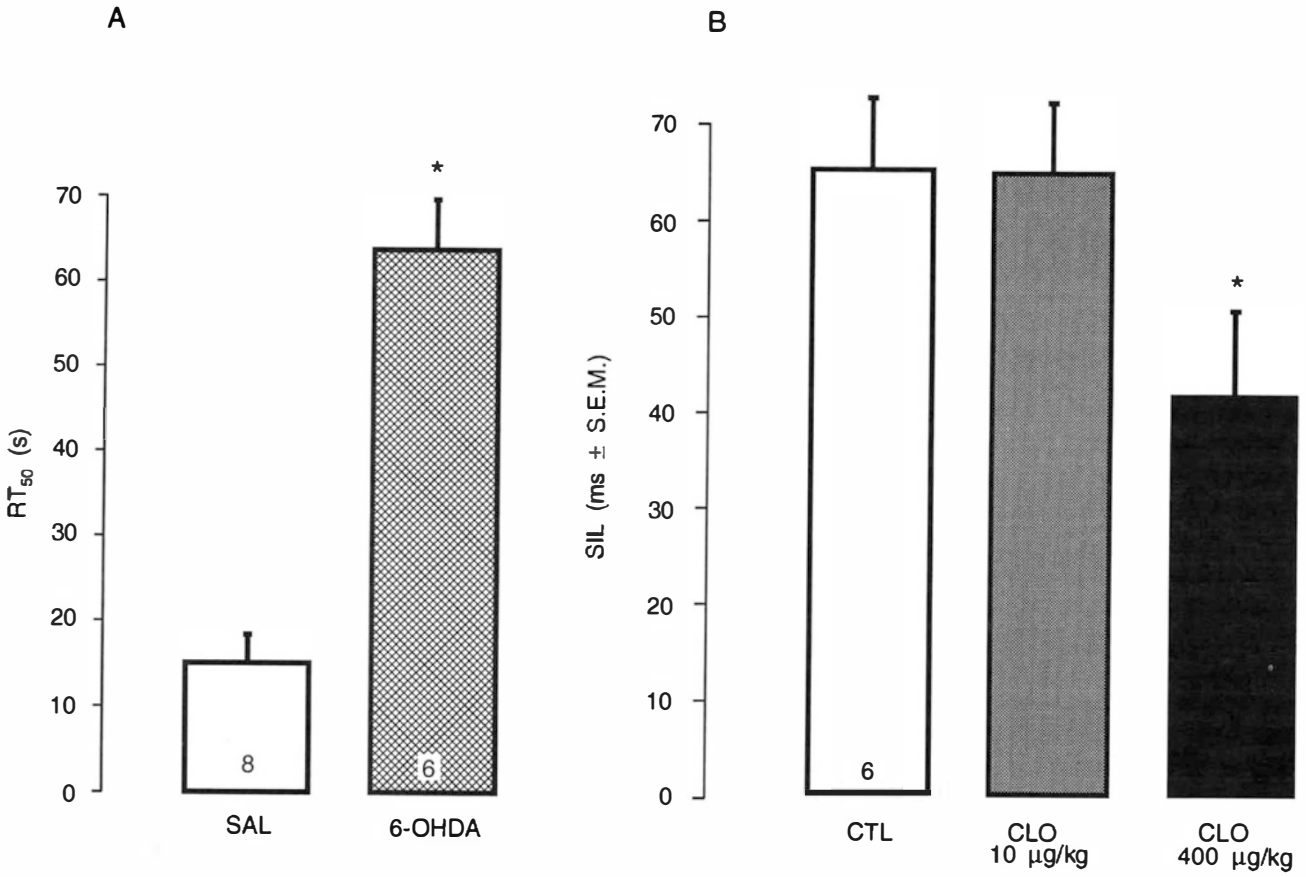

Figure 3. Effect of long-term treatment with 0.75 mg/kg per day of befloxatone in NE-denervated rats. The intracerebroventricular injection of 120 µg of 6-OHDA was given 1 week before the minipump containing the drug was installed. The minipumps were removed 48 hours before the experiment began. **A**: Neuronal recovery from the microiontophoretic application of NE in saline (SAL) and 6-OHDA + befloxatone-pretreated rats. The RT_{50} value is the time required for the neuron to recover its firing activity by 50% from the end of the application. The number of neurons tested is given at the bottom of each column. Data are expressed as mean ± SEM. * $p < .001$, two-tailed Student's *t*-test comparing control and 6-OHDA-pretreated rats. **B**: Histograms showing the effects of systemic injections of clonidine on the efficacy of the electrical stimulation of the ascending 5-HT pathway after a long-term treatment with befloxatone in six 6-OHDA-pretreated rats. * $p < .01$, using a two-tailed paired Student's *t*-test, comparing prior to and following clonidine administration.

hour after a pretreatment with the 5-HT reuptake inhibitor fluoxetine) were performed 1 week before initiating the befloxatone treatment. The effectiveness of the lesions was verified by assessing the RT_{50} of the firing rate of CA_3 pyramidal neurons following the microiontophoretic application of NE. In keeping with the notion that intact NE fibers are required for the NE reuptake process to allow a prompt recovery of the firing activity of these neurons (de Montigny et al. 1980), the marked increased (327%) in the RT_{50} of 6-OHDA-treated rats (Fig. 3A) confirmed an adequate lesioning of the NE terminals (except in two rats that were excluded from statistical analyses). In these conditions, the enhancing effect of the small dose of clonidine (10 µg/kg IV) was completely abolished, as previously observed (Mongeau et al. 1993). On the other hand, the decreasing effect of the high dose of clonidine (400 µg/kg IV) was still present and was even of a greater amplitude than that in the saline group (saline: 25% ± 5%, $n = 11$; 6-OHDA: 39% ± 9%, $n = 6$; $p = .06$, using covariance analysis).

The efficacy of the stimulation of the 5-HT pathway in the saline group was compared to those in the drug-treated groups using the unpaired Student's *t* test. The duration of suppression of firing in the 6-OHDA plus befloxatone group was increased by 71% (SIL values: saline 38 msec ± 3 msec, $n = 11$; 6-OHDA + befloxatone 65 msec ± 7 msec, $n = 6$; $p < .01$; Figs. 1 and 3). However, the befloxatone treatment did not by itself change the efficacy of the stimulation in suppressing the firing activity of the pyramidal neurons (Figs. 1 and 2). It is noteworthy that in the two rats pretreated with 6-OHDA plus befloxatone for which the NE denervation failed (as indicated by the unchanged RT_{50}), the

increase in 5-HT transmission was not observed, and the effects of clonidine were similar to those observed for the befloxatone group (data not shown). Finally, none of the above treatments significantly changed the late excitatory effect on the firing rate induced by the electrical stimulation (Table 1).

Effect of Long-Term Monoamine Uptake Inhibition on the Modulation of 5-HT Neurotransmission by Clonidine

For two reasons, it was deemed crucial to test whether monoamine reuptake inhibitors could also affect the α_2-adrenoceptors that modulate 5-HT transmission. First, selective 5-HT reuptake inhibitors have long been known to have antidepressant efficacy, and, second, 5-HT and NE reuptake inhibitors have been shown to increase the synaptic concentration of 5-HT and NE, respectively (L'Heureux et al. 1986; Bel and Artigas 1992; Curet et al. 1992b). The effect of a sustained increase in the synaptic concentration of either NE or 5-HT was investigated using specific reuptake inhibitors. Following long-term treatment with the selective 5-HT reuptake inhibitor paroxetine (10 mg/kg per day for 21 days), the effects of the low and the high dose of clonidine on 5-HT transmission were unchanged. After the paroxetine treatment, the small dose of clonidine (10 μg/kg IV) increased the efficacy of the stimulation of the 5-HT pathway by 29% ± 9% whereas the high dose (400 μg/kg IV) decreased it by 25% ± 7% (Fig. 4A).

In contrast, the 21-day treatment with the selective NE reuptake inhibitor nisoxetine (10 mg/kg per day) produced effects similar to those obtained in the befloxatone-treated rats. The small dose of clonidine produced a 30% ± 8% increase (Fig. 4B), which was similar to that of the saline group (Fig. 1B). The subsequent administration of the high dose of clonidine decreased the duration of suppression of firing; however, it was not reduced below the initial value (Fig. 4B). Furthermore, as observed in the befloxatone group (Fig. 2B), there was no change in the responsiveness of pyramidal neurons of the CA_3 region of hippocampus to microiontophoretic applications of 5-HT and NE in the nisoxetine-treated rats. The suppressant effects of the microiontophoretic applications of 5-HT and NE on the firing rate of the pyramidal neurons after the nisoxetine treatment (5-HT: 332 ± 66 spikes/nA, n = 11; NE: 378 ± 71 spikes/nA, n = 11) were not different from those of the saline-treated group (5-HT: 376 ± 62 spikes/nA, n = 11; NE: 400 ± 71 spikes/nA, n = 11).

The paroxetine and nisoxetine treatments did not significantly modify the efficacy of the stimulation of the 5-HT pathway in suppressing the firing activity of pyramidal neurons of the dorsal hippocampus (Fig. 4). In addition, the late excitatory effect of the stimulation

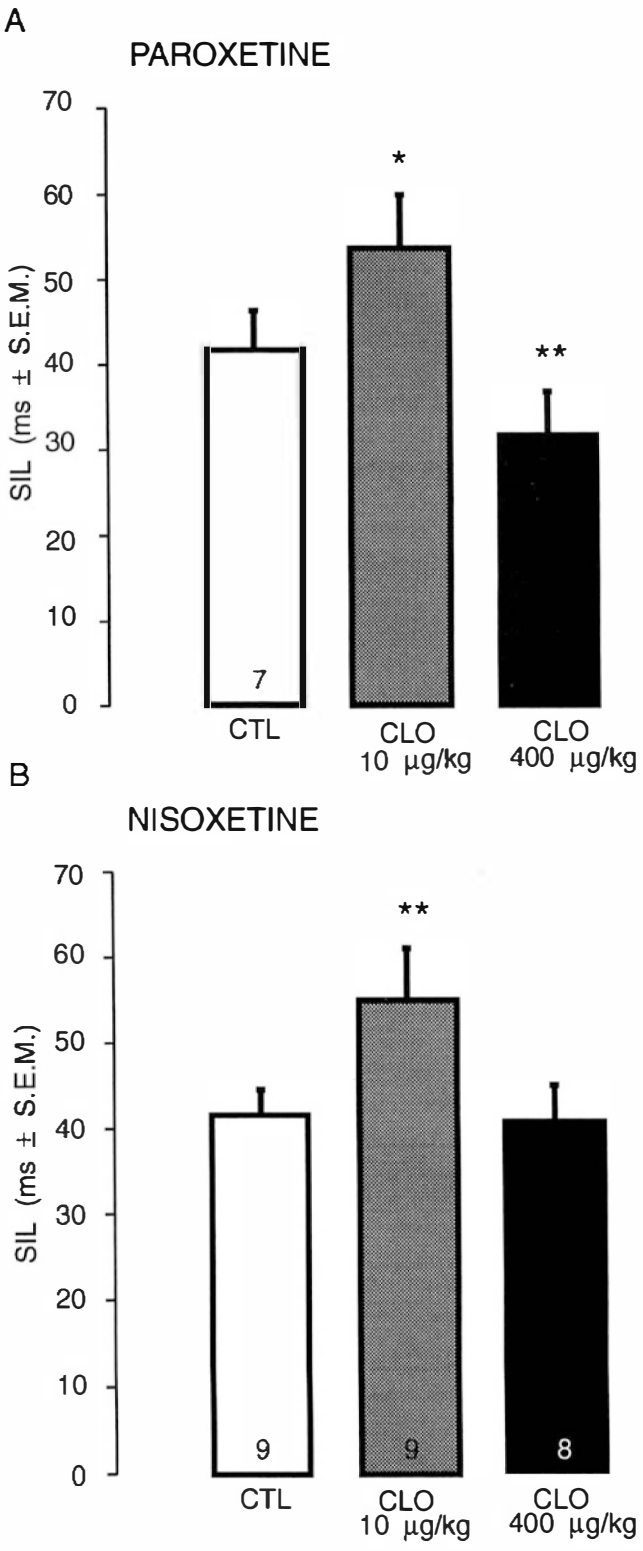

Figure 4. Histograms showing the effect of systemic injections of clonidine on the efficacy of the electrical stimulation of the ascending 5-HT pathway after a long-term treatment with 10 mg/kg per day of paroxetine (**A**) and 10 mg/kg per day of nisoxetine (**B**). The number of rats tested is given inside the columns. * $p < .05$, ** $p < .01$ using a two-tailed paired Student's t-test, comparing prior to and following clonidine administration.

observed on these same neurons was not changed by these treatments (Table 1).

DISCUSSION

The present electrophysiologic data show that long-term treatment with pharmacologic agents that increase the synaptic concentration of NE, but not that of 5-HT, decrease the inhibitory action of a high dose of clonidine (400 μg/kg IV) on 5-HT neurotransmission and that the enhancing effect of a low dose of clonidine (10 μg/kg IV) is not affected by such treatments (Figs. 2, and 4B). These changes observed in the befloxatone and the nisoxetine groups are most likely related to the sustained increase in NE concentration in the synapse, because similar changes were not produced either by long-term 5-HT reuptake blockade or by long-term MAO-A inhibition in NE-denervated rats (Figs. 3B and 4B). None of the long-term treatments produced any significant changes in the enhancing effect of the low dose of clonidine (Figs. 2 and 4). However, the lesion of the NE neurons abolished, as expected, this enhancing effect of clonidine (Fig. 3B).

In a previous study, the incremental and decremental effects of clonidine have been fully characterized using the present electrophysiologic paradigm (Mongeau et al. 1993). On the one hand, it was concluded that the reducing effect of the high dose of clonidine is most likely mediated by the activation of α_2-adrenergic heteroreceptors on 5-HT terminals, which results in a decrease in the amount of 5-HT released per action potential. On the other hand, low doses of clonidine enhance the suppression of firing by preferentially activating α_2-adrenergic autoreceptors, thereby reducing the concentration of endogenous NE tonically activating α_2-adrenergic heteroreceptors on 5-HT terminals (Mongeau et al. 1993). Thus, the present results suggest that although the sensitivity of α_2-adrenergic heteroreceptors is reduced by long-term treatment with nisoxetine and befloxatone, an inhibitory action of endogenous NE on 5-HT neurotransmission still appears to be present.

The magnitude of the effect of the small dose of clonidine on 5-HT neurotransmission does not provide a direct estimate of the responsiveness of the α_2-adrenergic autoreceptors, because its magnitude is also determined by the level of activation of α_2-adrenergic heteroreceptors by endogenous NE. Thus, the unaltered effect of the low dose of clonidine after these treatments is not an indication that the α_2-adrenergic autoreceptors on the cell body and terminals of NE neurons are normosensitive. Previous investigations performed in our laboratory have shown that terminal α_2-adrenergic autoreceptors are desensitized following a long-term treatment with the NE reuptake inhibitor desipramine (Lacroix et al. 1991). However, despite an attenuated responsiveness of the somatodendritic NE autoreceptors of locus coeruleus neurons to clonidine following this treatment, a 10-μg/kg IV dose of clonidine still substantially decreased (85% instead of 100%) the firing rate of these neurons. It can thus be concluded that the desensitization of the α_2-adrenergic autoreceptors would not hinder the enhancement of 5-HT neurotransmission that results from a decrease in the synaptic concentration of NE when these autoreceptors are activated by the 10-μg/kg IV dose of clonidine. In fact, a desensitization of α_2-adrenergic autoreceptors could even increase the inhibitory tone of NE on 5-HT neurotransmission, because the synaptic concentration of NE is normally expected to be increased when the negative feedback autoregulation is decreased.

It is also interesting that the high dose of clonidine in both nisoxetine- and befloxatone-treated rats brought back the effectiveness of the stimulation to initial value (Figs. 2B and 4B), suggesting that these receptors still possess residual capacity to modulate 5-HT release after the befloxatone or the nisoxetine treatment. The tonic inhibitory action of endogenous NE on the α_2-adrenergic heteroreceptors at baseline was probably maximal because the high dose of clonidine merely reestablished the initial duration of suppression of firing (Figs. 2B and 4B).

These observations lead us to the following interpretation: normally 5-HT neurotransmission is decreased in the presence of an increased NE output. After long-term exposure to a high concentration of synaptic NE, there is a desensitization of α_2-adrenergic heteroreceptors. Although the paradigm used in the present study does not provide clues about the cellular mechanisms involved, it might result from a decrease in the density of the receptors, a change in the coupling of the receptors with their G proteins, or an alteration in second messenger function. This desensitization would hinder the tonic inhibitory action of the NE system on 5-HT neurotransmission, more so in the condition of a high NE output.

No change in the efficacy of 5-HT neurotransmission was observed with long-term treatment with befloxatone, paroxetine, or nisoxetine. However, in the 6-OHDA-pretreated rats, the long-term befloxatone treatment significantly increased the duration of suppression of firing. This increase most likely resulted from the depletion of the endogenous NE that would normally exert a tonic inhibitory action on 5-HT neurotransmission through α_2-adrenergic heteroreceptors. Finally, the increase in the magnitude of the effect of the high dose of clonidine in these rats was expected and is consistent with our previous study (Mongeau et al. 1993). It was most likely related to a supersensitivity of the α_2-adrenergic heteroreceptors resulting from the NE depletion.

To our knowledge, no study has directly assessed the modulation of 5-HT neurotransmission by α_2-adrenergic ligands following antidepressant treatments in an in vivo paradigm. Nevertheless, this has been previously investigated in in vitro studies. Some have failed to detect a reduction in the efficacy of α_2-adrenergic heteroreceptors function following MAO inhibition of NE reuptake blockade. Groß et al. (1987) reported no change in the potency of clonidine to inhibit the electrically evoked overflow of [^{3}H]5-HT in preloaded rat brain cerebral cortex slices when superfusing throughout the experiment 1 μM of the NE reuptake inhibitor viloxazine. This result suggested that short-term exposure to an elevated synaptic concentration of NE does not desensitize α_2-adrenergic heteroreceptors on 5-HT terminals. However, long-term NE reuptake blockade with desipramine or long-term MAO inhibition with MDL 72394 or pargyline has not been reported to change the ability of α_2-adrenergic heteroreceptors to reduce the electrically induced release of [^{3}H]5-HT in rat cortical and hippocampal slices (Schlicker et al. 1982; Schoffelmeer and Mulder 1982; Palfreyman et al. 1986). Finally, another study using rat brain synaptosomes revealed that the attenuation of K^+-stimulated release of [^{3}H]5-HT by clonidine was diminished following a long-term treatment with the irreversible MAO inhibitor clorgyline but not by desipramine (Ellison and Campbell 1986). This effect of clorgyline was, however, attributable to a competition between clonidine and the increased NE in the biophase. Such a competition, however, cannot account for the decreased sensitivity of α_2-adrenergic heteroreceptors to the high dose of clonidine observed in the present study after the befloxatone treatment, because this drug is a reversible MAO inhibitor and the minipumps were removed 48 hours before the experiments. The method of administration of the drugs might explain the absence of desensitization of α_2-adrenergic heteroreceptors in the abovementioned studies. In the present study, the drugs were continuously delivered through osmotic minipumps inserted subcutaneously, whereas desipramine was administered orally in the studies by Schlicker et al. (1982) and Ellison and Campbell (1986) and intraperitoneally in the study of Schoffelmeer and Mulder (1982). The oral or intraperitoneal administration of desipramine in rats might not produce sustained reuptake blockade, considering the important first-pass hepatic metabolism and also the much faster catabolism of such drugs in rats than in humans.

In vitro studies performed in our laboratory using osmotic minipumps provided results consistent with the desensitization of α_2-adrenergic heteroreceptors. Long-term treatment with minalcipran, a NE reuptake inhibitor, reduced the NE inhibition of the electrically induced release of [^{3}H]5-HT in the rat hippocampus, whereas long-term treatment with befloxatone shifted the concentration–effect curve to the right of the α_2-adrenergic agonist UK 14.304 on electrically induced release of [^{3}H]5-HT in guinea pig hypothalamus slices (Blier and Bouchard 1992; Blier et al. 1993). Hence, these results suggest that α_2-adrenergic heteroreceptors are endowed with the capacity to become desensitized after a sustained increase in NE synaptic concentration.

Although the antidepressant property of befloxatone has not yet been documented in humans, all potent MAO-A inhibitors, specific or not, that bind irreversibly or not to the enzyme (clorgyline, phenelzine, tranylcypromine, moclobemide, brofaromine, and toloxatone) increase 5-HT and NE synaptic concentration and are effective in major depression. Furthermore, minalcipran which, like nisoxetine, selectively blocks the reuptake of NE (Blier et al. 1993), has been reported to be an effective antidepressant in humans (Macher et al. 1989; von Frenckell et al. 1990). It is thus possible that the desensitization of α_2-adrenergic heteroreceptors on 5-HT terminals contributes to the therapeutic action of these drugs.

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