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Effect of the reversible monoamine oxidase-A inhibitor befloxatone on the rat 5-hydroxytryptamine neurotransmission

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Abstract

The aim of the present study was to assess, using in vivo electrophysiological paradigms, the effect of sustained administration of the selective and reversible monoamine oxidase-A inhibitor beflotaxone on serotonin (5-hydroxytryptamine, 5-HT) neurotransmission. In male Sprague–Dawley rats with the osmotic minipumps in place, a treatment with befloxatone (0.75 mg/kg per day, s.c.) for 2 days decreased the spontaneous firing activity of dorsal raphe 5-HT neurons. The combination of befloxatone and the 5-$HT_{1A/1B}$ receptor antagonist (−)-pindolol (15 mg/kg per day, s.c.) for 2 days slightly increased the firing activity of 5-HT neurons, whereas a treatment with (−)-pindolol alone for 2 days did not modify this parameter. The suppressant effects on the firing activity of 5-HT neurons of the 5-HT autoreceptor agonist lysergic acid diethylamide (LSD), injected intravenously, and of both 5-HT and the 5-HT_{1A} receptor agonist 8-hydroxy-2-(di-*n*-propylamino)-tetralin (8-OH-DPAT), applied by microiontophoresis, were attenuated in rats treated with befloxatone for 2 days, suggesting an early desensitization of the somatodendritic 5-HT_{1A} receptors. The firing activity of 5-HT neurons was back to normal after a treatment for 21 days with befloxatone but the suppressant effects of LSD, 5-HT or 8-OH-DPAT was the same as in controls. In contrast, the suppressant effect of the α_2-adrenoceptor agonist clonidine on the firing activity of 5-HT neurons was significantly attenuated after the treatment with befloxatone for 21 days. At the postsynaptic level, the administration of the selective 5-HT_{1A} receptor antagonist (*N*-{2-[4(2-methoxyphenyl)-1-piperazinyl]ethyl}-*N*-(2-pyridinyl)cyclohexanecarboxamide trihydroxychloride (WAY 100635, 100 μg/kg, i.v.) did not modify the firing activity of quisqualate-activated dorsal hippocampus CA_3 pyramidal neurons in control rats. In contrast, in rats treated with befloxatone in combination with (−)-pindolol for 2 days as well as with befloxatone alone for 21 days, WAY 100635 significantly increased the firing of CA_3 pyramidal neurons. In conclusion, these data suggest that when the firing activity of 5-HT neurons is normal in the presence of befloxatone, either after a two-day treatment together with (−)-pindolol or alone for 21 days, the tonic activation of postsynaptic 5-HT_{1A} receptors is enhanced. © 1998 Elsevier Science B.V.

Keywords: Befloxatone; Monoamine oxidase-A; (5-hydroxytryptamine, 5-HT); Hippocampus; Dorsal raphe; 5-HT_{1A} receptor; WAY 100635

1. Introduction

Catecholamines and serotonin (5-hydroxytryptamine, 5-HT) can be metabolized by the two isoenzymes of monoamine oxidase denoted A and B (Johnston, 1968). The oxidative deamination of 5-HT, norepinephrine and epinephrine is preferentially mediated by monoamine oxidase-A whereas the monoamine oxidase-B isoenzyme preferentially deaminates phenylethylamine and benzylamine, while dopamine is deaminated by both isoforms (Yang and Neff, 1973; Denney and Denney, 1985; Westlund et al., 1985; Youdim and Finberg, 1991; Saura et al., 1992). Because of the important side effects of classical monoamine oxidase inhibitors (e.g. the hypertensive crises triggered by the ingestion of tyramine-containing foods, Murphy et al., 1987), a second-generation of reversible monoamine oxidase-A inhibitors have been developed (see Thase et al., 1995; Youdim, 1995, for review). Among the latter, befloxatone, an oxazolidinone derivative which is a selective and reversible monoamine oxidase-A inhibitor (Curet et al., 1995), seems to be a promising and effective antidepressant in experimental models (Caille et al., 1996). The pharmacological profile of befloxatone reveals that it has a much higher affinity for monoamine oxidase-A ($K_i = 2.8 \pm 0.4$ nM) than moclobemide ($K_i = 14\,000 \pm 1250$ nM) in rat brain homogenates (Curet et al., 1995).

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Table 1
Brain concentrations of monoamines in control and befloxatone (0.75 mg/kg per day, s.c.) treated rats. Results represent the means ± S.E.M. obtained from group of 6–7 animals

	NE	DA (ng/g of tissue)	5-HT
Control	328 ± 17	1003 ± 34	810 ± 45
Befloxatone 2 days	472 ± 33[b] (+43%)	1384 ± 58[b] (+38%)	1236 ± 56[b] (+52%)
21 Days	436 ± 25[a] (+33%)	1373 ± 58[b] (+37%)	1119 ± 77[b] (38%)

% of variation versus control values were given in parentheses.
[a] $p < 0.05$.
[b] $p < 0.01$, using Dunett's test.

Moreover, befloxatone does not affect the binding of radioligands acting on the norepinephrine, dopamine, 5-HT, muscarinic, histamine, opioid or sigma receptor subtypes nor inhibits the uptake of monoamines in rat brain synaptosomes (Curet et al., 1996a). In vivo, befloxatone (1–750 μg/kg, p.o.) increases rat whole brain levels of norepinephrine, dopamine and 5-HT and decreases the levels of corresponding deaminated metabolites dihydroxyphenylacetic acid (DOPAC) and 5-hydroxyindolacetic acid (5-HIAA) (Curet et al., 1996a). In microdialysis studies performed in freely moving rats, befloxatone (0.75 mg/kg, i.p.) increases striatal dopamine and decreases DOPAC, homovanillic acid (HVA) and 5-HIAA extracellular levels (Curet et al., 1994). Befloxatone (0.75 mg/kg, i.p.) also increases the extracellular levels of norepinephrine but not those of 5-HT in the frontal cortex of freely moving rats (Curet et al., 1994). An autoradiographic study on rat brain sections showed that befloxatone binds selectively to monoamine oxidase-A, with a very high labelling density of [^{3}H]befloxatone being found in the locus coeruleus and a high level being observed in the dorsal raphe (Curet et al., 1995). It is important to mention that recent studies have shown that both MAO-A and its mRNA are detectable in the rat dorsal raphe nucleus (Saura et al., 1992; Jahng et al., 1997). It has also been shown that long-term treatment with befloxatone (0.75 mg/kg per day, s.c. × 21 days) desensitizes α_2-adrenergic heteroreceptors on 5-HT fibers in rats and in guinea-pigs (Blier and Bouchard, 1994; Mongeau et al., 1994). Previous studies from our laboratory have also revealed that monoamine oxidase-A inhibitors affect central norepinephrine and 5-HT neurotransmission in the rat brain (see Blier and De Montigny,

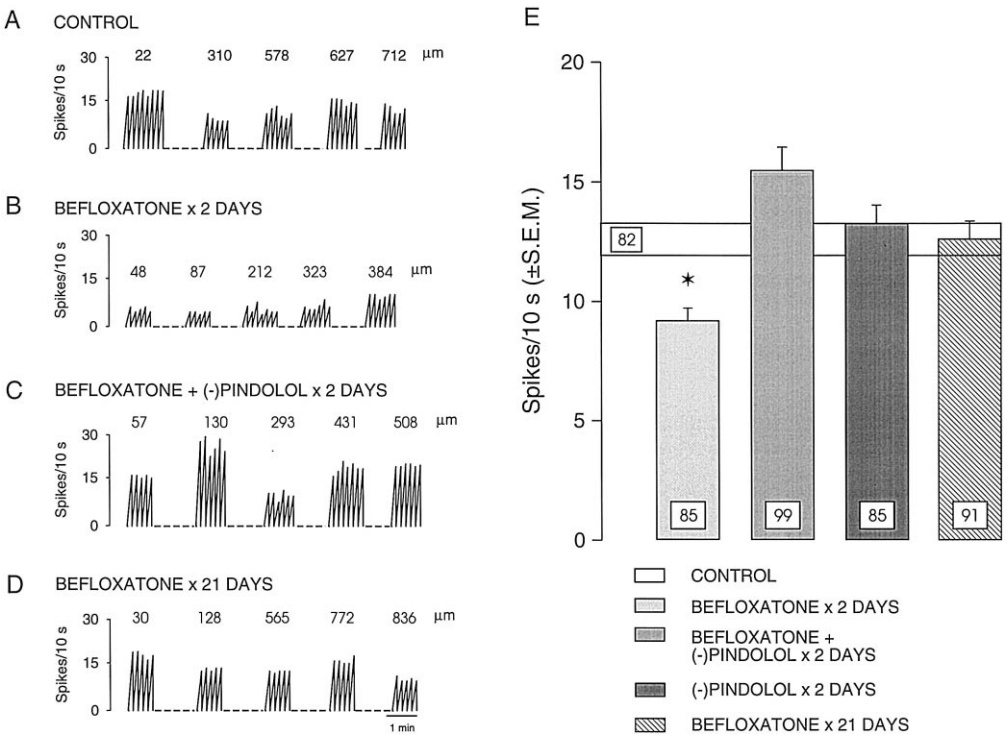

Fig. 1. Integrated firing rate histograms of dorsal raphe 5-HT neurons showing their spontaneous firing activity in a control rat (A, range of 1.26 ± 0.07 Hz; 4.1 ± 0.4 cells/descent for 7 rats), a rat treated with befloxatone (0.75 mg/kg per day, s.c.) for 2 days (B, 4.2 ± 0.6 cells/descent for 6 rats), a rat treated with both befloxatone and (−)-pindolol (15 mg/kg/day, s.c.) for 2 days (C, 4.9 ± 0.2 cells/descent for 7 rats) and rat treated for 21 days with befloxatone (0.75 mg/kg per day, s.c.) (D, 4.5 ± 0.4 cells/descent for 7 rats). The number above each recording indicates the depth from the floor of the sylvius aqueduct at which it was recorded. Mean results ± S.E.M. are presented in (E). Note the unchanged firing activity of 5-HT neurons in the group treated with (−)-pindolol alone (4 ± 0.3 cells/descent for 8 rats). The numbers at the bottom of the columns indicate the number of neurons tested * $P < 0.05$ (using unpaired Student's *t*-test or Kruskal–Wallis one way ANOVA). Note that the number of 5-HT cells found per descent in the dorsal raphe is not significantly different between treatments.

1994). In fact, it has been demonstrated that repeated administration of the nonselective monoamine oxidase inhibitor phenelzine and of the irreversible monoamine oxidase-A inhibitor clorgyline produces an early and sustained decrease of the firing activity of rat locus coeruleus norepinephrine neurons and also a transient decrease of the firing activity of dorsal raphe 5-HT neurons. In contrast, deprenyl (a monoamine oxidase-B inhibitor ineffective in endogenous depression) does not affect the firing activity of 5-HT or norepinephrine neurons (Blier and De Montigny, 1985).

The aim of the present study was to assess the effect of sustained treatment with befloxatone on the efficacy of brain 5-HT neurotransmission and on the sensitivity of pre- and postsynaptic 5-HT_{1A} receptors, using in vivo electrophysiological paradigms in the rat dorsal raphe and dorsal hippocampus.

2. Materials and methods

The experiments were carried out in male Sprague–Dawley rats (Charles River, St. Contant, Quebec) weighing 250 to 300 g which were kept under standard laboratory conditions (12:12 light–dark cycle with free access to food and water). Four groups of rat were treated for 2 days with either befloxatone alone (0.75 mg/kg per day), (−)-pindolol (15 mg/kg/day) alone, both befloxatone (0.75 mg/kg per day) and (−)-pindolol (15 mg/kg per day) or vehicle (a 50% ethanol water solution) delivered by osmotic minipumps (ALZA, Palo Alto, CA) inserted subcutaneously and one group of rat was treated for 21 days with befloxatone alone (0.75 mg/kg per day). The rats were tested with the minipumps on board. The animals were anesthetized with chloral hydrate (400 mg/kg, i.p.). Supplemental doses were given to maintain constant anesthesia and to prevent any nociceptive reaction to a tail pinch.

2.1. Determination of monoamine oxidase activities and assay of monoamines in the rat brain

These experiments were carried out in Synthélabo Recherche Laboratories. For the determination of the monoamine oxidase activity, rats were decapitated and brains were dissected out and rapidly frozen. The samples were kept at −80°C until the monoamine oxidase-A and monoamine oxidase-B assay. The different tissues were homogenised in 20 volumes of ice-cold 0.1 M sodium phosphate buffer (pH 7.4). After a preincubation time of 20 min, aliquots (0.1 ml) of crude membrane suspensions were incubated with [^{14}C]5-HT (final concentration 125 μM) for 5 min and with [^{14}C]phenylethylamine (final concentration 8 μM) for 1 min in a total volume of 0.5 ml at 37°C. The reaction was stopped with 200 μl of 4 M HCl and deaminated metabolites were extracted by vigourous shaking for 10 min with 7 ml of toluene/ethyl acetate (v/v) and quantified by liquid scintillation counting.

For the assay of monoamines, whole brain (minus cerebellum) was removed, frozen, weighed and stored at −80°C until analysis. Norepinephrine, 5-HT and dopamine were measured by high pressure liquid chromatography (HPLC) with electrochemical detection. Frozen tissues were sonicated in 10 volumes of 0.1 M $HClO_4$ containing 0.6 mM of ethylenediaminetetraacetic acid and 3,4-dihydroxybenzylamine (final concentration 1 ng/60 μl) as internal standards. After centrifugation, 60 μl of the supernatant were injected onto the liquid chromatographic column using a refrigerated (4°C) autoinjector Wisp 510 (Waters, Milford, MA). Separation was achieved at room temperature. The HPLC system consisted of a pump, a stainless steel separation column (0.45 × 25 cm) packed

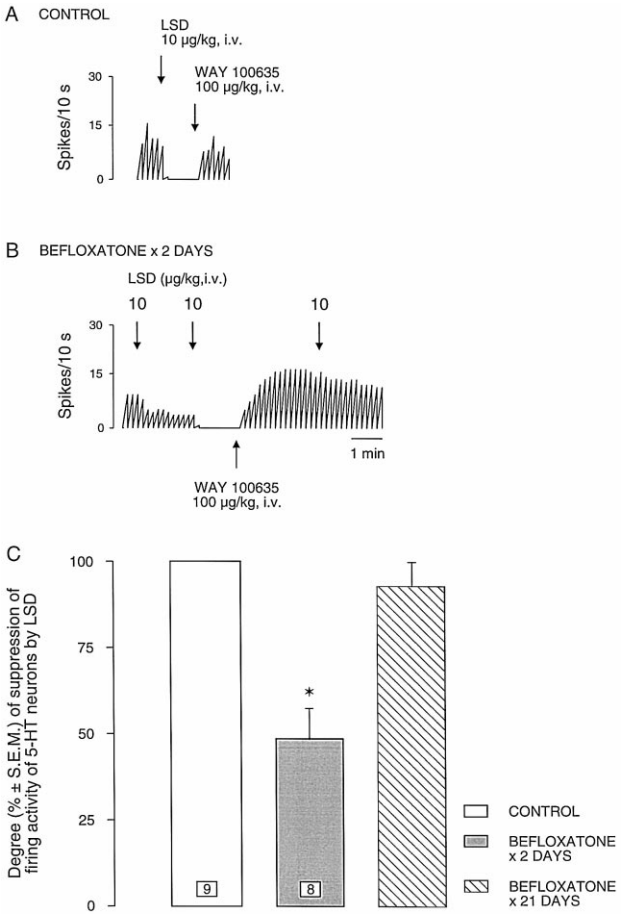

Fig. 2. Integrated firing rate histograms of dorsal raphe 5-HT neurons showing their response to LSD and WAY 100635 in a control (A) (% of recovery after subsequent injection of WAY 100635 = 57 ± 13, $n = 4$) and in a rat treated with befloxatone for 2 days (B) (% of recovery after subsequent injection of WAY 100635 = 168 ± 18, $n = 7$). Mean results ± S.E.M. of the suppressant effect of LSD (10 μg/kg, i.v.) on the firing activity of 5-HT neurons in controls and rats treated with befloxatone for 2 and 21 ($n = 8$) days are presented in (C). The numbers at the bottom of the columns indicate the number of rats tested. $^{*}P < 0.05$ (unpaired Student's t-test).

with Ultrasphere ODS C18, 5 μm particle size (Beckman, Fullerton, CA). The mobile phase contained 0.1 M NaH_2PO_4, 1 mM ethylenediaminetetraacetic acid, 2.5 mM octane sulphonic acid, 7% CH_3CN, pH 3.4. The flow rate was 1 ml/min. Electrochemical detection was carried out by means of an amperometric detector (model 460 Waters) and achieved by setting the glassy carbon working electrode at +800 mV (with respect to a Ag/AgCl reference electrode). The chromatograms were registered on a Baseline 810 system (Waters). The retention times for norepinephrine, dopamine and 5-HT were 5, 13 and 36 min, respectively. Concentrations of each compound were calculated with reference to standards.

2.2. Recordings of dorsal raphe 5-HT neurons

Extracellular recordings were performed with single-barrelled glass micropipettes preloaded with fibreglass filaments in order to facilitate filling. The tip was broken back to 1 to 4 μm and filled with a 2 M NaCl solution saturated with Fast Green FCF. The rats (control or treated rats) were placed in a stereotaxic frame and a burr hole was drilled on the midline 1 mm anterior to lambda. Dorsal raphe 5-HT neurons were encountered over a distance of 1 mm starting immediately below the ventral border of the Sylvius aqueduct. These neurons were identified using the criteria of Aghajanian (1978): a slow (0.5–2.5 Hz) and regular firing rate and long-duration (0.8–1.2 ms) positive action potentials. In order to determine the possible changes of the spontaneous firing activity of dorsal raphe 5-HT neurons, five to six electrode descents were carried out through this nucleus in controls and rats with minipumps on board. For the control and treated groups of rats, the responsiveness of somatodendritic 5-HT_{1A} receptors was assessed after the intravenous injection of lysergic acid diethylamide (LSD, 10 μg/kg). In both the dorsal raphe and the dorsal hippocampus, the change of firing activity was assessed by calculating the mean of firing rate of cells from about 1 to 2 min prior to and after (until a 'plateau') the i.v. administration of the drugs and a percentage of change was obtained. Microiontophoresis was performed with five-barrelled micropipettes preloaded (R&D Scientific Glass Co., Spencerville, MD) with fibreglass filaments in order to facilitate filling and the tip was broken back to 4 to 8 μm. The central barrel was used for recording and filled with a 2 M NaCl solution. The side barrels contained the following solutions: 5-HT creatinine sulphate (20 mM in 200 mM NaCl, pH 4), 8-hydroxy-2-(di-*n*-propylamino)-tetralin (8-OH-DPAT, 1 mM in 100 mM NaCl, pH 4) and 2 M NaCl used for automatic current

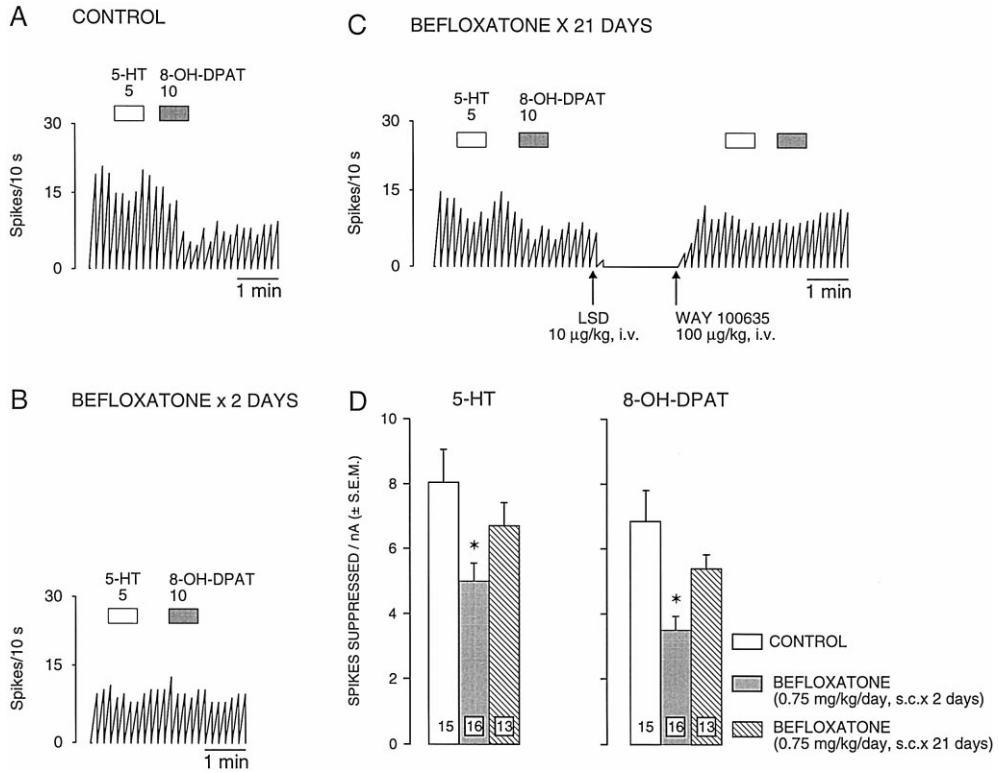

Fig. 3. (A), (B) and (C) represent integrated firing rate histograms of three dorsal raphe 5-HT neurons showing their responsiveness to microiontophoretic application of 5-HT and 8-OH-DPAT in a control rat (A), a rat treated with befloxatone for 2 days (B) and rat treated with befloxatone for 21 days (C). Horizontal bars indicate the duration of the applications for which the current is given in nA. In D, the responsiveness to 5-HT and 8-OH-DPAT is expressed as the number of spikes per nA (means ± S.E.M.). The numbers at the bottom of the columns indicate the number of neurons tested. $^{*}P < 0.05$ (unpaired Student's *t* test).

balancing. Finally, the suppressant effect of the α_2-adrenoceptor agonist clonidine (10 μg/kg, i.v.) on the firing activity of 5-HT neurons was assessed in controls and rats treated with befloxatone for 21 days.

2.3. Extracellular recordings from locus coeruleus norepinephrine neurons

Unitary extracellular recordings were performed with single-barrelled glass micropipettes preloaded with fibreglass filaments in order to facilitate filling. The tip was broken back to 1 to 4 μm and filled with a 2 M NaCl solution saturated with Fast Green FCF. Locus coeruleus norepinephrine neurons were recorded at the following stereotaxic coordinates: −0.7 mm posterior to lambda and 1.1 to 1.4 mm lateral (Paxinos and Watson, 1982). Norepinephrine neurons were identified by their regular firing rate (1–5 Hz), long-duration (0.8–1.2 ms) positive action potentials and their characteristic burst discharge in response to nociceptive pinch of the contralateral hind paw (Aghajanian, 1978). In each rat (control and treated), the spontaneous firing activity of locus coeruleus norepinephrine neurons was determined from four to five electrode descents through this nucleus. In rats treated with befloxatone (0.75 mg/kg per day, s.c.) for 21 days, their spontaneous firing activity was assessed prior and after the i.v. injection of the α_2-adrenoceptor antagonist idazoxan (1 mg/kg, i.v.).

2.4. Recordings from CA_3 dorsal hippocampus pyramidal neurons

Recording and microiontophoresis were performed with five-barrelled glass micropipettes broken back to 8–12 μm under microscopic control (ASI Instruments, Warren, MI). The central barrel was filled with a 2 M NaCl solution and used for extracellular unitary recordings. The pyramidal neurons were identified by their large amplitude (0.5–1.2 mV) and long-duration (0.8–1.2 ms) simple spikes alternating with complex spike discharges (Kandel and Spencer, 1961). The side barrels contained the following solutions: 5-HT creatinine sulphate (20 mM in 200 mM NaCl, pH 4), quisqualate (1.5 mM in 200 mM NaCl, pH 8) and 2 M NaCl used for automatic current balancing. The rats were mounted on a stereotaxic apparatus and the microelectrodes were lowered at 4.2 mm lateral and 4.2 anterior to lambda into the CA_3 region of the dorsal hippocampus. Since most hippocampus pyramidal neurons are not spontaneously active under chloral hydrate anaesthesia, a leak or a small ejection current of quisqualate (+1 to −6 nA) was used to activate them within their physiological firing range (10–15 Hz; Ranck, 1975). Neuronal responsiveness to the microiontophoretic application of 5-HT were assessed by determining the number of spikes suppressed/nA. The duration of the micro-iontophoretic applications of the agonist was 50 s. The same current of ejection was always used before and after the i.v. injection of the selective 5-HT_{1A} receptor antagonist WAY 100635 (*N*-{2-[4(2-methoxyphenyl)-1-piperazinyl]ethyl}-*N*-(2-pyridinyl)cyclohexanecarboxamide trihydroxychloride, 100 μg/kg). In order to assess the degree of activation of the postsynaptic 5-HT_{1A} receptors exerting an inhibitory influence on the firing activity of quisqualate-activated CA_3 pyramidal neurons, the firing was reduced to a frequency of about 5 Hz 3 min before the i.v. injection of WAY 100635.

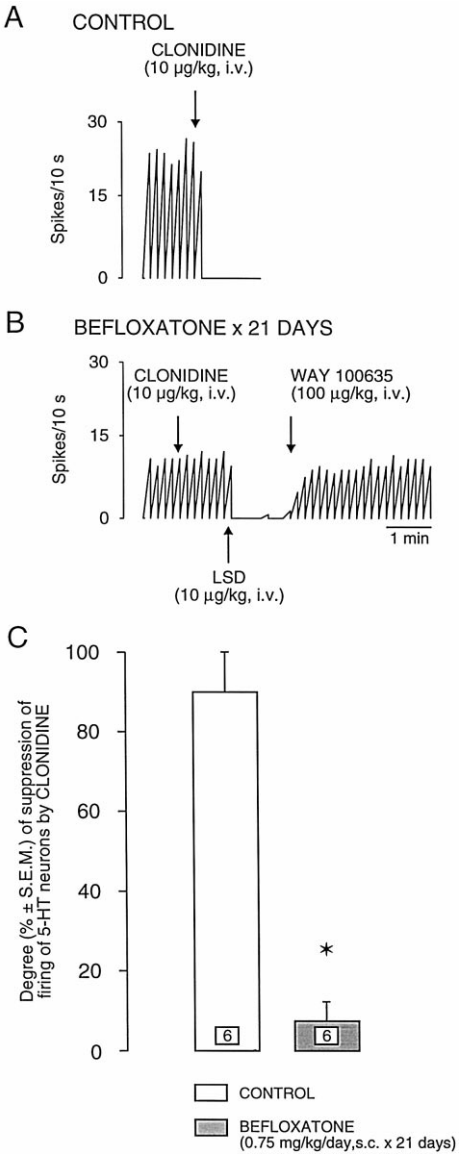

Fig. 4. Integrated firing rate histograms of dorsal raphe 5-HT neurons showing their response to clonidine in a control rat (A) and a rat treated with befloxatone for 21 days (B). Mean results ± S.E.M. of the suppressant effect of clonidine (10 μg/kg, i.v.) on the firing activity of 5-HT neurons in controls and rats treated with befloxatone for 21 days are presented in C. The number at the bottom of the columns indicates the number of rats tested. $^{*}P < 0.01$ (unpaired Student's *t*-test).

2.5. Electrical activation of the afferent 5-HT fibers to the hippocampus

A bipolar electrode (NE-110; 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. A stimulator (S8800; Grass Instruments, Quincey, MA) delivered two hundred square pulses of 0.5 ms at a frequency of 1 Hz and an intensity of 300 μA. The stimulation pulses and the firing activity of the neuron recorded were fed to an IBM-PC computer equipped with a Tecmar interface. Peristimulus time histograms were generated to determine the duration of suppression of firing activity of the CA3 pyramidal neuron, measured as absolute silence value (ms). This value is obtained by dividing the total number of events suppressed following the stimulation by the mean frequency of firing of the neuron recorded (Chaput et al., 1986). The CA_3 region of the hippocampus receives extensive innervation from 5-HT neurons of the dorsal and median raphe nuclei (Hensler et al., 1994). This brief suppressant effect ($\approx$ 50 ms) resulting from the electrical stimulation of the ascending 5-HT pathway is due to the release of 5-HT for each impulse applied to the 5-HT axons and it is mediated by postsynaptic 5-HT_{1A} receptors (Chaput et al., 1986). Thus, for the CA3 neurons tested in control rats, the effect of the stimulation of the ascending 5-HT pathway was first determined prior to and following the intravenous injection of WAY 100635 (100 μg/kg). The effect of the stimulation was then assessed prior to and following the injection of WAY 100635 (100 μg/kg, i.v.) in treated rats with minipumps on board. In order to determine the possible changes of the responsiveness of the terminal 5-HT autoreceptors, in each group, two series of stimulations (1 and 5 Hz) were carried out, while recording the same neuron, since it has previously been demonstrated in vitro and in vivo that the activation of terminal 5-HT autoreceptors decreases the release of 5-HT and that this reduction is enhanced by increasing the frequency of the stimulations (Göthert, 1980; Chaput et al., 1986, Blier et al., 1989b).

2.6. Drugs

The drugs used were befloxatone (Synthélabo Recherche, Rueil-Malmaison, France); 5-HT creatinine sulphate, quisqualate and clonidine (Sigma Chemical, St. Louis, MO); WAY 100635 (Wyeth Research, Bershire, UK); 8-OH-DPAT (Research Biochemical, Natick, MA) and LSD (Ministry of Health and Welfare, Ottawa, ON). The concentrations and the doses used for these compounds were chosen on the basis of previous experiments carried out in our and other laboratories.

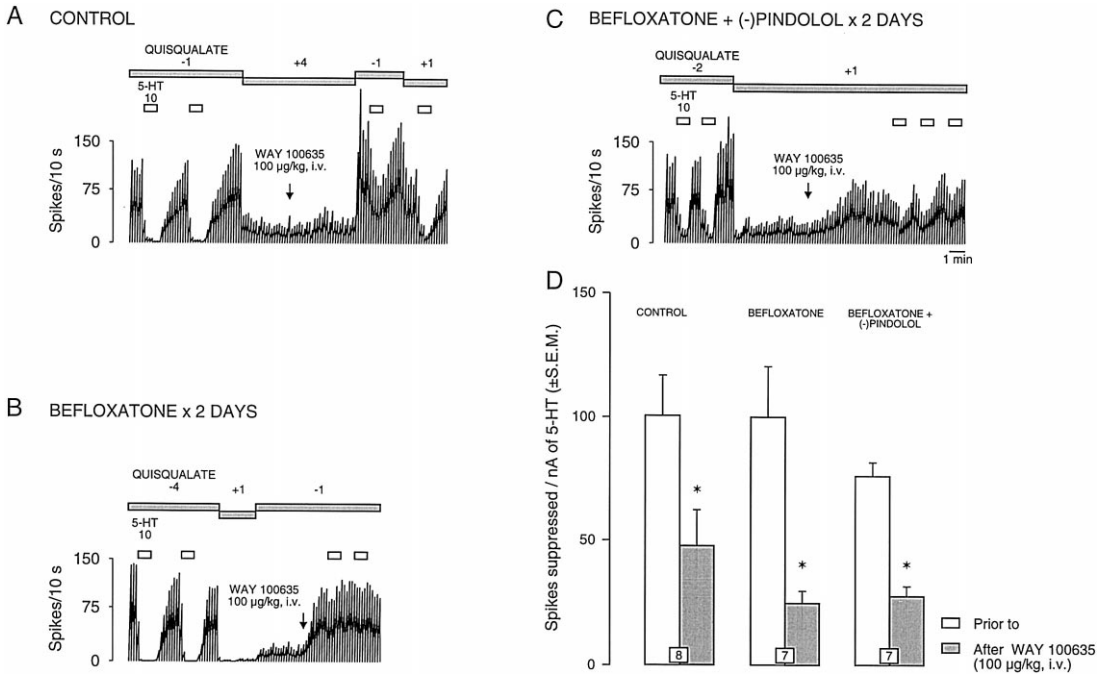

Fig. 5. (A), (B) and (C) represent integrated firing rate histograms of three dorsal hippocampus CA_3 pyramidal neurons showing their responsiveness to the microiontophoretic application of 5-HT prior to and after the i.v. administration of WAY 100635 in a control rat (A) and a rat treated for 2 days with befloxatone alone (0.75 mg/kg per day, s.c.) (B) and in combination with (−)-pindolol (0.75 mg/kg per day, s.c.) (C). These neurons were activated with quisqualate. Horizontal bars indicate the duration of the applications for which the current is given in nA. In D, the responsiveness to 5-HT is expressed as the number of spikes suppressed per nA (means ± S.E.M.). The numbers at the bottom of the columns indicate the number of neurons tested. Note the altered effectiveness of 5-HT in suppressing firing activity after WAY 100635 administration in each group. $^{*}P < 0.05$ (unpaired Student's *t*-test).

3. Results

3.1. Determination of monoamine oxidase activities and assay of monoamines in the rat brain

The assessment of monoamine oxidase-A and B activities from whole brain homogenates revealed that monoamine oxidase-B activity was unchanged after 2 and 21 days of treatment with befloxatone (monoamine oxidase-B activity in controls: 0.104 ± 0.002 nmol · min/mg of tissue) whereas monoamine oxidase-A activity was inhibited by 80% compared to controls both after 2 and 21 days of treatment with befloxatone (monoamine oxidase-A activity in controls: 0.236 ± 0.002 nmol · min/mg of tissue). Furthermore, similarly to other monoamine oxidase inhibitors (Blier and De Montigny, 1985; Blier et al., 1986a,b; Da Prada et al., 1989), the brain concentration of norepinephrine, dopamine and 5-HT were significantly increased in rats treated with befloxatone for 2 and 21 days compared to controls (see Table 1).

3.2. Effect of sustained treatment with befloxatone alone and in combination with (−)-pindolol on the firing activity of dorsal raphe 5-HT neurons

It has been shown that the administration of befloxatone (1 mg/kg, i.p.) suppressed, with a short latency, the firing activity of rat dorsal raphe 5-HT neurons (Curet et al., 1996b). Consistent with these results, short-term treatment with befloxatone (0.75 mg/kg per day, s.c. × 2 days) decreased of the spontaneous firing activity of dorsal raphe 5-HT neurons by 27% (Fig. 1B and E). This decrease of firing activity of 5-HT neurons observed in befloxatone-treated rats was no longer present in rats treated with both befloxatone and the 5-HT_{1A} autoreceptor antagonist (−)-pindolol. In fact, the combination of the two drugs increased the firing activity of 5-HT neurons by 23% (Fig. 1C and E). In rats treated with (−)-pindolol (15 mg/kg per day, s.c.) for 2 days or with befloxatone (0.75 mg/kg per day, s.c.) for 21 days, the firing activity of 5-HT was not significantly different from that measured in controls (Fig. 1D and E).

As illustrated in Fig. 2, the i.v. administration of LSD (10 μg/kg) suppressed the firing activity of 5-HT neurons in control rats and this effect of LSD was reversed by the administration of the selective 5-HT_{1A} receptor antagonist WAY 100635 (100 μg/kg, i.v). Interestingly, the short-term befloxatone treatment (0.75 mg/kg per day, s.c. × 2 days) attenuated the suppressant effect of LSD on the firing activity of 5-HT neurons (Fig. 2B). As illustrated in Fig. 2C, a treatment with befloxatone for 2 days decreased the suppressant effect of LSD on the firing activity of dorsal raphe 5-HT neurons by 52%, and this suppressant effect of LSD on the firing activity of 5-HT neurons was

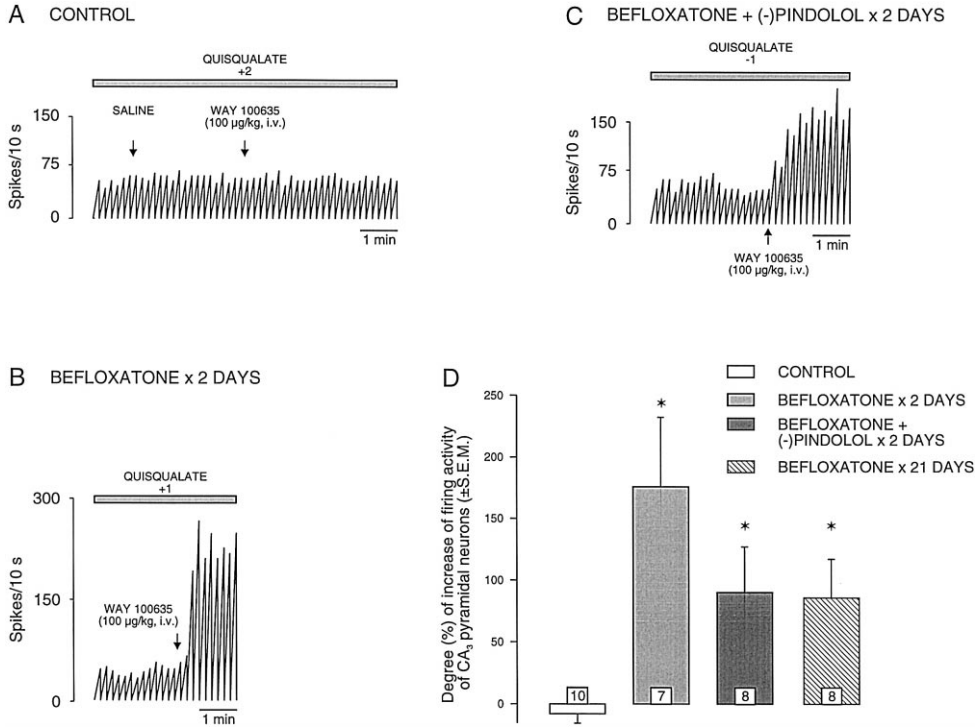

Fig. 6. (A), (B) and (C) represent integrated firing rate histograms of three dorsal hippocampus CA_3 pyramidal neurons showing their responsiveness to the i.v. administration of WAY 100635 (100 μg/kg, i.v.) in a control rat (A) and in a rat treated for 2 days with befloxatone alone (0.75 mg/kg per day, s.c.) (B) and in combination with (−)-pindolol (0.75 mg/kg per day, s.c.) (C). These neurons were activated with quisqualate. In D, the responsiveness to WAY 100635 is expressed as degree (%) of increase of the firing of CA_3 pyramidal neurons (means ± S.E.M.). The number at the bottom of the columns indicates the number of neurons tested. * $P < 0.05$ (unpaired Student's *t*-test).

back to normal after a treatment with befloxatone for 21 days (Fig. 2C). In order to confirm this apparent early desensitization of the somatodendritic 5-HT_{1A} receptors on 5-HT neurons, both 5-HT and 8-OH-DPAT were applied microiontophoretically directly onto dorsal raphe 5-HT neurons. As illustrated in Fig. 3B and D, the suppressant effects of the two 5-HT receptor agonists were significantly reduced after the treatment with befloxatone for 2 days. It has been shown previously that a treatment with (−)-pindolol (15 mg/kg per day, s.c.) for 2 days reduces the suppressant effect of LSD on the firing activity of dorsal raphe 5-HT neurons (Romero et al., 1996). In the present study, the combination of befloxatone and (−)-pindolol treatment also attenuated this reducing effect of LSD on the firing activity of dorsal raphe 5-HT neurons (inhibition of $10 \pm 6\%$ with 10 μg/kg, i.v. of LSD in 3 rats). However, in rats treated with befloxatone for 21 days (0.75 mg/kg, s.c.), both the spontaneous firing activity of 5-HT neurons (Fig. 1E and F) and the suppressant effects of the i.v. injection of LSD as well as the microiontophoretic applications of 5-HT and 8-OH-DPAT (Fig. 2C and D and Fig. 3C and D) on the firing activity of these neurons were similar to controls. The i.v. injection of the α_2-adrenoceptor agonist clonidine (10 μg/kg, i.v.) suppressed the firing activity of 5-HT neurons in controls and this effect of clonidine was markedly attenuated in rats treated for 21 days with befloxatone (0.75 mg/kg per day, s.c., Fig. 4).

The effect of the treatment with befloxatone for 21 days on the spontaneous firing activity of locus coeruleus norepinephrine neurons was assessed. It was found that this long-term treatment significantly ($P < 0.01$, using unpaired Student's *t*-test) decreased the spontaneous firing activity of locus coeruleus norepinephrine neurons. The spontaneous firing activity of these neurons in control rats was 2.04 ± 0.2 Hz ($n = 59$ neurons in 7 rats with 4.1 ± 0.4 cells/descent) and in treated rats was 0.4 ± 0.08 Hz ($n = 118$ neurons in 7 rats with 5.3 ± 0.5 cells/descent prior to idazoxan administration) and was 3.2 ± 0.4 Hz ($n = 28$ neurons in 5 rats with 5.6 ± 1.1 cells/descent) after idazoxan administration.

3.3. Effect of sustained treatment with befloxatone alone and in combination with (−)-pindolol on the CA3 dorsal hippocampus pyramidal neurons responsiveness to 5-HT

It has been demonstrated previously that the microiontophoretic application of 5-HT onto rat dorsal hippocampus pyramidal neurons produces a suppressant effect on their firing activity and that this effect is mediated by postsynaptic 5-HT_{1A} receptors (Blier and De Montigny, 1987; Chaput and De Montigny, 1988; Blier et al., 1993b). For all CA_3 hippocampus pyramidal neurons tested, 5-HT induced a reduction of firing activity, generally 100% with a 10 nA current applied for 50 s (Fig. 5A, B and C). This suppressant effect occurred in the absence of any alteration of the shape of the action potentials. As illustrated in Fig. 5B, C and D, short-term treatment with befloxatone alone and in combination with (−)-pindolol did not modify the suppressant effect of microiontophoretically-applied 5-HT on the firing activity of CA_3 dorsal hippocampus pyramidal neurons. Fig. 5D illustrates the mean suppressant action of 5-HT microiontophoretically-applied onto CA_3 pyramidal neurons and the antagonistic effect of WAY 100635 on the responsiveness of these postsynaptic 5-HT_{1A} receptors in control and treated rats. In the present study, WAY 100635 (100 μg/kg, i.v.) significantly reduced the suppressant effect of 5-HT on CA_3 hippocampus pyramidal neurons firing by 52% in controls, by 75% in befloxa-

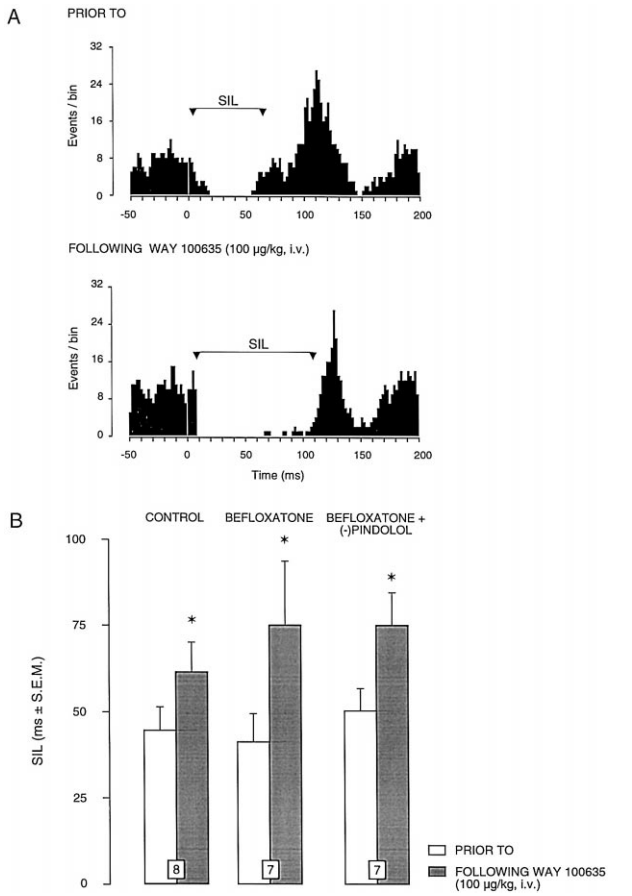

Fig. 7. (A) Peristimulus time histograms illustrating the effect of the electrical stimulation of the ascending 5-HT pathway at the level of the ventro-medial tegmentum on the firing activity of dorsal hippocampus CA_3 pyramidal neurons in a control rat prior to and after WAY 100635 (100 μg/kg, i.v.). The silence value represents the duration of suppression of firing (see Section 2). Each histogram was constructed from 200 consecutive stimulations (300 μA, 0.5 ms, 1 Hz) with a bin width of 2 ms. (B) Duration of suppression of firing activity (means ± S.E.M) of CA_3 hippocampus pyramidal neurons produced by the stimulation of the ascending 5-HT pathway, before and after the administration of WAY 100635 (100 μg/kg, i.v.) in controls and rats treated for 2 days with befloxatone alone (0.75 mg/kg per day, s.c.) and in combination with (−)-pindolol (0.75 mg/kg per day, s.c.). The number in the columns indicates the number of neurons tested. $^{*}P < 0.05$, using the paired Student *t*-test.

tone-treated rats and by 64% in befloxatone and (−)-pindolol-treated rats (Fig. 5D). The effect of WAY 100635 (100 μg/kg, i.v.) on the quisqualate-activated firing activity of CA_3 pyramidal neurons was assessed in control rats, in rats treated with befloxatone alone and in rats treated with both befloxatone and (−)-pindolol. In control rats i.e. in rats with minipumps delivering 50% ethanol for 2 (Fig. 6A) and 21 days (firing activity prior to WAY 100635 = 5.1 ± 0.3 Hz; firing activity after WAY 100635 = 4.8 ± 0.5 Hz; $n = 9$), the i.v. administration of WAY 100635 did not modify the firing activity of dorsal hippocampus CA_3 pyramidal neurons indicating a minimal activation of postsynaptic $5\text{-}HT_{1A}$ receptors by endogenous 5-HT under these experimental conditions (Fig. 6A and D). In the treated groups, the firing activity of these neurons was significantly increased by the same dose of WAY 100635, thus indicating an increased tonic activation of postsynaptic $5\text{-}HT_{1A}$ receptors by the treatment regimens (Fig. 6B, C and D), although these enhancements were not significantly different from each other ($P = 0.16$ using an unpaired Student's *t*-test).

3.4. Electrical stimulation of the afferent 5-HT fibers to the hippocampus

In order to determine whether treatments with befloxatone alone and in combination with (−)-pindolol could modulate in vivo the release of 5-HT per electrical impulse reaching 5-HT terminals and to assess the effect of the $5\text{-}HT_{1A}$ receptor antagonist WAY 100635 on the effectiveness of the stimulation of the 5-HT pathway, the capacity to modify the duration of the suppression of the firing activity of CA_3 hippocampus pyramidal neurons produced by the electrical activation of the ascending 5-HT pathway was examined in control rats and in rats with the minipump in place delivering the drugs. Neither befloxatone (0.75 mg/kg per day, s.c. × 2 days) nor befloxatone (0.75 mg/kg per day, s.c. × 2 days) plus (−)-pindolol (15 mg/kg per day, s.c. × 2 days) modified the effectiveness of the stimulation of the 5-HT pathway (Fig. 7B). However, in control rats, the i.v. administration of WAY 100635 (100 μg/kg, i.v.) unexpectedly increased the efficacy of the stimulation of the 5-HT pathway (Fig. 7A and B). This enhancing effect of WAY 100635 on the effectiveness of the stimulation was also present in rats treated with befloxatone alone and in rats treated with both befloxatone and (−)-pindolol for 2 days (Fig. 7B).

The responsiveness of terminal 5-HT autoreceptors was evaluated by increasing the frequency of the stimulation from 1 to 5 Hz. In control rats, the 5 Hz stimulation was 23% less effective than the 1 Hz stimulation. However, in rats treated with befloxatone and treated with both befloxatone and (−)-pindolol for 2 days, the decremental effect obtained by increasing the frequency stimulation of the 5-HT pathway from 1 to 5 Hz was absent (Fig. 8B). In addition, in rats treated with befloxatone for 2 days, the enhancing effect of the terminal 5-HT autoreceptor antagonist metergoline (1 mg/kg, i.v.) on the efficacy of the

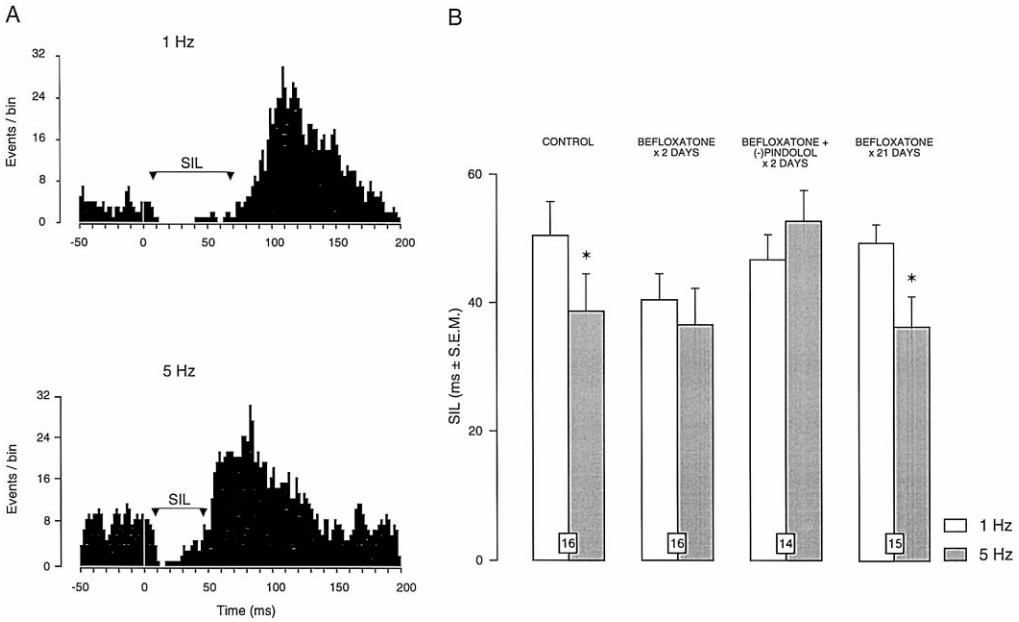

Fig. 8. (A) Peristimulus time histograms illustrating the effect of the electrical stimulation of the ascending 5-HT pathway at the level of the ventro-medial tegmentum on the firing activity of dorsal hippocampus CA_3 pyramidal neurons for the same neurons at 1 and 5 Hz in a control rat. Note the reduction of the silence value which represents the duration of suppression of firing (see Section 2) by increasing frequency. (B) Histograms illustrating the effects of treatments on the efficacy of the stimulation of the ascending 5-HT pathway from 1 to 5 Hz (means ± S.E.M.). The number in the columns indicate the number of neurons tested. $^{*}P < 0.05$ using a paired Student *t*-test or a Kruskal–Wallis one way ANOVA. The decremental effect of increasing the frequency of the stimulation from 1 to 5 Hz in rats treated with befloxatone for 21 days was not significantly different from that in controls rats.

stimulation of the 5-HT pathway (control silence value prior to: 38 ± 4 ms; silence value after metergoline = 49 ± 7 ms, $n = 7$; Haddjeri et al., 1996) was no longer present, thus suggesting an desensitization of terminal 5-HT autoreceptors (silence value prior to: 35 ± 4 ms; silence value after metergoline = 31 ± 5 ms, $n = 4$). This desensitization of 5-HT autoreceptors was no longer present in rats treated with befloxatone for 21 days, as the sensitivity of these receptors was similar to controls (Fig. 8B).

4. Discussion

In the present study, befloxatone (0.75 mg/kg per day × 2 days) decreased the firing activity of dorsal raphe 5-HT neurons. This suggests that short-term treatment with befloxatone produces an inhibition of monoamine oxidase-A in the dorsal raphe and consequently enhances the synaptic availability of 5-HT, as previously demonstrated with other monoamine oxidase inhibitors (Aghajanian et al., 1970). The release of 5-HT from the dendrites or recurrent collaterals in the dorsal raphe has been well documented (Héry et al., 1982; Piñeyro et al., 1995; Davidson and Stamford, 1995; Piñeyro and Blier, 1996) and since the firing activity of 5-HT neurons is in part controlled by somatodendritic 5-HT_{1A} autoreceptors, one may assume that the decrease of the firing activity occurring after treatment with befloxatone for 2 days was due to an increase of the availability of 5-HT with subsequent activation of somatodendritic 5-HT_{1A} autoreceptors. Indeed, an increase in the firing activity of 5-HT neurons in rats treated with both befloxatone and the 5-$HT_{1A/1B}$ autoreceptor antagonist (−)-pindolol was observed as well as a marked increase of the firing activity of 5-HT neurons after i.v administration of the 5-HT_{1A} autoreceptor antagonist WAY 100635 in rats treated with befloxatone alone for 2 days (Fig. 2B). Although, the reason for the increase of the firing activity above the baseline is still unclear, preliminary data from Fornal et al. (1995) revealed that WAY 100635 blocks the action of endogenous 5-HT at 5-HT_{1A} autoreceptors in the cat dorsal raphe and also reverses, above baseline value, the firing rate of 5-HT neurons following the inhibitory action of the 5-HT reuptake blocker fluoxetine.

In the present study, after a treatment with befloxatone for 2 days, the decrease of the firing activity of 5-HT neurons was only 27% which represents a small reduction in comparison with the approximate 50% reduction of 5-HT neuronal firing activity using either phenelzine (non-selective monoamine oxidase inhibitor), clorgyline (irreversible monoamine oxidase-A inhibitor) or amiflamine (reversible monoamine oxidase-A inhibitor) (Blier and De Montigny, 1985, Blier et al., 1986a,b). Moreover, a single administration of befloxatone (1 mg/kg, i.p.) has been shown to cause a total cessation of the firing activity of rat dorsal raphe 5-HT neurons (Curet et al., 1996b). Such results might be related to the fact that 5-HT_{1A} autoreceptors on 5-HT neurons of the dorsal raphe nucleus could already be inactivated after a treatment with befloxatone for only 2 days. In fact, in rats treated with befloxatone for 2 days, the responsiveness of the somatodendritic 5-HT_{1A} autoreceptors to the systemic administration of LSD and to the microiontophoretic application of 5-HT and 8-OH-DPAT, was decreased by about 50%. Similar results have also been obtained by Le Poul et al. (1995) using two selective 5-HT reuptake inhibitors. This group has shown that after 3 days of treatment with fluoxetine or paroxetine, followed by a one day washout period, the ability of 8-OH-DPAT to suppress the firing of dorsal raphe 5-HT neurons recorded from brain slices was already significantly reduced. Moreover, these treatments failed to modify the specific 5-HT_{1A} binding sites in the dorsal raphe or in other brain areas as measured with either [^{3}H]8-OH-DPAT or with [^{3}H]WAY 100635. The authors have suggested that, already after a 3-day treatment with selective 5-HT reuptake inhibitors, an adaptative desensitization of the somatodendritic 5-HT_{1A} autoreceptors within the dorsal raphe nucleus had taken place without any change in the number of 5-HT_{1A} binding sites. The latter phenomenon (i.e. desensitization of dorsal raphe 5-HT_{1A} autoreceptors without any reduction in the number of the 5-HT_{1A} binding sites which is likely due to uncoupling of the receptor from its transduction system) has also been observed following long-term treatment with the partial 5-HT_{1A} receptor agonist ipsapirone (Schechter et al., 1990). In contrast to long-term treatment with selective 5-HT reuptake inhibitors (see Blier and De Montigny, 1994), the early desensitization of the somatodendritic 5-HT_{1A} autoreceptors observed in the present study did not persist since the long-term treatment with befloxatone modified neither the suppressant effect of the autoreceptor agonist LSD administered intravenously, nor those of 5-HT and 8-OH-DPAT applied by microiontophoresis. This unexpected result is not due to the incomplete inhibition of monoamine oxidase-A by befloxatone since the extent of monoamine oxidase inhibition was similar in rats treated for 2 and 21 days with the drug (see Section 3). Furthermore, an increase in brain levels of 5-HT was also present in rats treated for 21 days with befloxatone, although the magnitude of this increase was somewhat lower than after a two-day treatment (see Table 1). One possible explanation for such normalized 5-HT neuronal firing activity and for such normosensitive 5-HT_{1A} autoreceptors following sustained monoamine oxidase inhibition, might be the adaptive change of locus coeruleus norepinephrine neurons which exert a major influence on the firing activity of 5-HT neurons. In fact, dorsal raphe 5-HT neurons receive norepinephrine projections from the locus coeruleus (Loizou, 1969; Anderson et al., 1977; Baraban and Aghajanian, 1981; Jones and Yang, 1985; Luppi et al., 1995). Pharmacological studies have indicated that the firing activity of 5-HT neurons in the dorsal raphe is dependent

upon a tonic activation by its noradrenergic input (Svensson et al., 1975; Baraban and Aghajanian, 1980). The inhibitory action of the α_2-adrenoceptor agonist clonidine on the firing activity of 5-HT neurons was thus suggested to be due to the activation of somatodendritic and terminal α_2-adrenergic autoreceptors decreasing the endogenous norepinephrine excitatory drive mediated by α_1-adrenoceptors located on 5-HT neurons (Svensson et al., 1975, Clement et al., 1992a,b). In keeping with these observations, α_2-adrenoceptor antagonists enhance the firing activity of dorsal raphe 5-HT neurons (Freedman and Aghajanian, 1984; Garrat et al., 1991; Haddjeri et al., 1996). In the present study, in rats treated with befloxatone for 21 days, the suppressant effect of clonidine on the firing activity of 5-HT neurons was markedly attenuated. This may reflect a desensitization of α_2-adrenergic autoreceptors. However, it was shown previously that the monoamine oxidase-A inhibitors clorgyline and amiflamine do not alter the responsiveness of norepinephrine neurons in the locus coeruleus to the systemic administration of clonidine (Blier et al., 1986a,b). This suggests that the sustained blockade of monoamine oxidase-A activity by befloxatone, as is the case with clorgyline and amiflamine, decreased the firing activity of norepinephrine neurons by a maximum of 40% (Curet, personal communication), consequently leaving little modulation to be exerted by clonidine. The latter possibility is all the more likely as an attenuated norepinephrine activation of 5-HT neurons, resulting from the lesioning of norepinephrine neurons or the blockade of α_1-adrenoceptors, initially suppresses the firing activity of 5-HT neurons but this parameter returns to normal within a few days (Baraban and Aghajanian, 1980; Blier et al., 1989a). To support this assumption we found that the firing activity of locus coeruleus norepinephrine neurons was markedly and significantly decreased after long-term befloxatone treatment.

Among the 5-HT_{1A} receptor antagonists available, WAY 100635 is the most potent and selective antagonist acting at both pre- and postsynaptic 5-HT_{1A} receptors (Fletcher et al., 1996). In the CA_3 region of the dorsal hippocampus, the short- and long-term treatment with befloxatone, as well as the treatment for 2 days with both befloxatone and (−)-pindolol, did not modify the responsiveness of postsynaptic 5-HT_{1A} receptors to microiontophoretic application of 5-HT, indicating that the sensitivity of these receptors remains unchanged following such treatments. However, WAY 100635 (100 μg/kg, i.v.), in contrast to (−)-pindolol (Romero et al., 1996), antagonized the suppressant effect of microiontophoretically-applied 5-HT onto CA_3 pyramidal neurons in both control and treated rats, thus showing its capacity to block 5-HT_{1A} receptors on the cell body of CA_3 pyramidal neurons.

It has been shown that 5-HT terminals are almost exclusively located on the dendritic tree firing of hippocampus pyramidal neurons (Oleskovich and Descarries, 1990) and that the endogenous 5-HT, released by the electrical stimulation of the ascending 5-HT pathway, activates the intrasynaptic 5-HT_{1A} receptors located on dendrites of hippocampus pyramidal neurons (Chaput and De Montigny, 1988; Blier et al., 1993a). In the CA_3 region of the dorsal hippocampus, previous studies from our laboratory have also shown that pertussis toxin nearly abolishes the responsiveness of extrasynaptic 5-HT_{1A} receptors located on the cell body of CA_3 pyramidal neurons, but not of intrasynaptic 5-HT_{1A} receptors located on the dendritic tree of the same neurons (Blier et al., 1993b). Moreover, Hadrava et al. (1994) have demonstrated that the sustained activation of the extrasynaptic, but not of the intrasynaptic, 5-HT_{1A} receptors of CA_3 pyramidal neurons, achieved with treatments with 5-HT_{1A} receptors agonists flesinoxan and BMY 42568 (3-[3-[4-(5-methoxy-4-pyrimidinyl)-1-(2-methylpiperazinyl)]propyl]-5-fluoroindole dihydrochloride), prevents their inactivation by pertussis toxin. These data further suggest that these populations of postsynaptic 5-HT_{1A} receptors located on the same neurons are functionally distinct. In the present study, WAY 100635 (100 μg/kg, i.v.) failed to decrease the duration of suppression of firing of dorsal hippocampus CA_3 pyramidal neurons induced by the electrical stimulation of the ascending 5-HT pathway in control rats and in rats treated with befloxatone alone and in combination with (−)-pindolol for 2 days, as well as after a long-term treatment with befloxatone. This suggests that WAY 100635 failed to block the intrasynaptic 5-HT_{1A} receptors of CA_3 pyramidal neurons, as is the case with the lack of effect of pertussis toxin on this population of postsynaptic 5-HT_{1A} receptors (Blier et al., 1993b; Hadrava et al., 1994). WAY 100635 (100 μg/kg, i.v.), in fact, even increased the efficacy of the stimulation. Another contributory factor to this unexpected effect of WAY 100635 could be due to its suppressant effect on the spontaneous firing activity of locus coeruleus norepinephrine neurons (Haddjeri et al., 1997). This suppressant effect of WAY 100635 on the firing of locus coeruleus norepinephrine neurons presumably induces a decrease of norepinephrine release in the synaptic cleft in the dorsal hippocampus. Consequently, as demonstrated in previous studies (Mongeau et al., 1993, 1994), the decrease of endogenous norepinephrine release reduces the tonic activation of α_2-adrenergic heteroreceptors, located on 5-HT fibers in the dorsal hippocampus, which in turn leads to an enhancement of the endogenous release of 5-HT induced by the electrical stimulation of the ascending 5-HT pathway.

It has previously been demonstrated in vitro and in vivo that the activation of terminal 5-HT autoreceptors decreases the release of 5-HT and that this reduction is even more evident at higher frequencies of stimulation of the 5-HT axons (Göthert, 1980; Chaput et al., 1986; Blier et al., 1989a,b). Long-term treatment with befloxatone does not affect the responsiveness of terminal 5-HT autoreceptors in the hippocampus and hypothalamus of guinea-pigs measured in vitro in brain slices (Blier and Bouchard,

1994). In the present study, two days of treatment with befloxatone alone did not modify the effectiveness of the stimulation of the 5-HT pathway. In fact, the decrease in the effectiveness of the stimulation upon increasing its frequency from 1 to 5 Hz (due to the activation of terminal 5-HT autoreceptors) was attenuated after a treatment for 2 days with befloxatone alone. As was the case with the somatodendritic 5-HT_{1A} autoreceptors, these results suggest that befloxatone administration produced an early desensitization of terminal 5-HT_{1B} autoreceptors. This contention is further supported by the loss of the enhancing effect of the 5-HT autoreceptor antagonist metergoline on the efficacy of the stimulation in rats treated with befloxatone for 2 days. This desensitization, however, was transient as indicated by the return to a normal pattern to the 5 Hz and the 1 Hz stimulations after 21 days of treatment with befloxatone.

In rats treated for 2 days with befloxatone alone or in combination with (−)-pindolol, as well as in rats treated with befloxatone for 21 days, the i.v. administration of WAY 100635 enhanced the firing activity of CA_3 pyramidal neurons. When WAY 100635 is injected systemically, it does not enhance the firing rate of 5-HT neurons in anesthetized rats (Fletcher et al., 1994, 1996; Haddjeri et al., unpublished observation), but it will reverse the suppressant effect of an enhanced activation of 5-HT_{1A} autoreceptors resulting from an increased synaptic availability of 5-HT itself or of an exogenous 5-HT_{1A} receptor agonist. However, in rats treated with befloxatone and (−)-pindolol for two days, as well as in rats treated with befloxatone alone for 21 days, the firing activity of 5-HT neurons is not affected, and WAY 100635 markedly enhanced the firing activity of CA_3 pyramidal neurons. Therefore, it can be concluded that the latter two treatments produced an enhanced tonic activation of postsynaptic 5-HT_{1A} and possibly of other 5-HT receptors.

In conclusion, the present results show that the short-term administration of befloxatone reduces the firing activity of 5-HT neurons which is followed by a recovery after 21 days of sustained administration. This sequence of events correlates well with the delayed onset of action of monoamine oxidase inhibitors in major depression. Given that the firing activity of 5-HT neurons is even above the normal value following a two-day treatment with befloxatone and (−)-pindolol, this combined treatment could be expected to produce the same enhancing effect on 5-HT neurotransmission as after a long-term treatment with befloxatone alone.

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