Br. J. Pharmacol. (1994), **113**, 485–495 © Macmillan Press Ltd, 1994

Modulation of 5-HT release in the guinea-pig brain following long-term administration of antidepressant drugs

[1]Pierre Blier & Claude Bouchard

Neurobiological Psychiatry Unit, Department of Psychiatry, McGill University, 1033, Pine Avenue West, Montréal, Québec, Canada H3A 1A1

1 The aims of the present study were to determine whether long-term 5-hydroxytryptamine (5-HT) reuptake blockade and inhibition of type-A monoamine oxidase (MAO-A) lead to an enhancement of the electrically evoked release of tritium from guinea-pig brain slices preloaded with [^{3}H]-5-HT, and to assess the sensitivity of the terminal 5-HT$_{1D}$ autoreceptor, the α_2-adrenoceptor also located on 5-HT terminals, and the 5-HT$_3$ receptor that modulates 5-HT release following these two types of antidepressant treatments.

2 The electrically evoked release of tritium was significantly enhanced following a 21-day treatment with the 5-HT reuptake blocker, paroxetine and the reversible MAO-A inhibitor, befloxatone, in preloaded slices of the hypothalamus, hippocampus and frontal cortex 48 h after removal of the osmotic minipumps used to deliver the drugs.

3 The inhibitory effect of the terminal 5-HT autoreceptor agonist, 5-methoxytryptamine, on the evoked release of tritium was attenuated in slices of the hypothalamus, hippocampus, but not frontal cortex, following the paroxetine treatment. In the befloxatone group, the effectiveness of 5-methoxytryptamine was unaltered in the same brain structures.

4 The sensitivity of the α_2-adrenoceptor on 5-HT terminals, assessed using UK 14.304, was attenuated in hypothalamus, hippocampus, but not frontal cortex slices prepared from befloxatone-treated guinea-pigs and preloaded with [^{3}H]-5-HT. The paroxetine treatment did not alter the sensitivity of this α_2-adrenoceptor in the hypothalamus.

5 The sensitivity of the α_2-adrenoceptor on noradrenaline terminals, also assessed using UK 14.304, was not altered in hippocampus and hypothalamus slices preloaded with [^{3}H]-noradrenaline following the long-term befloxatone treatment.

6 In frontal cortex slices, [^{3}H]-5-HT uptake was no longer significantly attenuated after a 21-day treatment with paroxetine, whereas it was still markedly inhibited in hypothalamus slices. The enhancing effect of paroxetine on the evoked release of [^{3}H]-5-HT in the superfusion medium was no longer evident in frontal cortex slices of the paroxetine group. These data indicate that long-term 5-HT reuptake blockade desensitized the 5-HT transporter in the frontal cortex.

7 The capacity of the 5-HT$_3$ receptor agonist, 2-methyl-5-HT, to enhance the electrically evoked release of tritium was not altered in hypothalamus, hippocampus, and frontal cortex slices prepared from befloxatone-treated guinea-pigs, but was significantly attenuated in the paroxetine group also treated for 21 days. Following a 2-day paroxetine treatment, the enhancing effect of 2-methyl-5-HT on tritium release was unaltered in frontal cortex slices.

Keywords: 5-HT reuptake blockade; monoamine oxidase inhibition; 5-HT autoreceptor; α_2-adrenoceptors; 5-HT$_3$ receptors; [^{3}H]-5-HT release; [^{3}H]-noradrenaline release

Introduction

By use of *in vivo* electrophysiological techniques, long-term administration of antidepressant treatments has been shown to increase 5-hydroxytryptamine (5-HT) neurotransmission in the rat hippocampus (Blier & de Montigny, 1994). In the case of electroconvulsive shocks and tricyclic antidepressant (TCA) drugs, this enhancement was attributable to an enhanced responsiveness of the postsynaptic 5-HT$_{1A}$ receptors (Chaput *et al.*, 1991). Long-term administration of TCA drugs has also been shown to induce a progressive increase of the responsiveness of postsynaptic neurones to microiontophoretically-applied 5-HT in other rat brain regions, such as the amygdala, lateral geniculate nucleus and facial motor nucleus via 5-HT receptors which have not yet been fully characterized (de Montigny & Aghajanian, 1978; Menkes *et al.*, 1980; Wang & Aghajanian, 1980; Menkes & Aghajanian, 1981). Selective 5-HT reuptake inhibitors (SSRI) and monoamine oxidase inhibitors (MAOI), in contrast, produce the same net effect on 5-HT neurotransmission but by enhancing the function of 5-HT neurones (Blier & de Montigny, 1994). Both types of drugs produce an initial decrease of 5-HT neuronal firing activity, which plays an important role in controlling 5-HT release in terminal fields, which is followed by a gradual recovery as a result of the desensitization of their somatodendritic 5-HT$_{1A}$ autoreceptors. This normalized firing activity in the presence of an increased neuronal concentration of 5-HT resulting from sustained MAO inhibition could explain, at least in part, the enhancement of 5-HT neurotransmission by MAOI. While the SSRI do not increase the tissue concentration of 5-HT, as MAOI do, they produce a desensitization of the terminal 5-HT autoreceptor, which exerts a potent negative feedback influence on 5-HT release, thus allowing a greater release of 5-HT per action potential.

The present studies were undertaken to determine whether 5-HT release is also increased in brain regions other than the hippocampus following long-term treatment with an SSRI and a MAOI, and to elucidate further the mechanisms by which these two types of drugs enhance 5-HT release. Three receptors were studied: the terminal 5-HT autoreceptor, the α_2-adrenoceptor located on 5-HT terminals, which also inhibits 5-HT release (see Starke *et al.* for a review, 1989), and finally the 5-HT$_3$ receptor that is not located on 5-HT

[1] Author for correspondence.

terminals but exerts a positive feedback influence on 5-HT release (Galzin & Langer, 1991; Blier & Bouchard, 1993; Blier *et al.*, 1993). The experiments were carried out in guinea-pigs for two reasons. First, their terminal 5-HT autoreceptors are of the 5-HT_{1D} subtype, as in man, whereas they are of the 5-HT_{1B} subtype in rats (Hoyer & Middlemiss, 1989; Galzin *et al.*, 1992). Second, the MAO isozyme ratio of the A and B subtypes in guinea-pig is similar to that found in man but different from that in the rat, at least in the basal ganglia (Azzaro *et al.*, 1985).

Methods

Treatments

Male guinea-pigs were implanted, under halothane anaesthesia, with an osmotic minipump (Alza, Palo Alto, CA, U.S.A.) which delivered the SSRI, paroxetine ($10\ mg\ kg^{-1}$, day^{-1}; SmithKline Beecham, Harlow, England), the reversible and selective type A MAOI, befloxatone ($0.75\ mg\ kg^{-1}$ day^{-1}; Delalande, Rueil-Malmaison, France), or the vehicle used to dilute these two drugs (50:50 ethanol and water). After 21 days, the minipumps were removed under halothane anaesthesia and the *in vitro* release experiments were carried out 48 h later to allow elimination of drugs.

Superfusion experiments

The guinea-pigs, weighing 300–400 g at the time of the experiments, were killed by decapitation and their brains rapidly removed and dissected on an ice-cold glass plate. Slices from the hypothalamus, hippocampus, or frontal cortex of 0.4 mm thickness were prepared with a McIlwain chopper and incubated for 30 min at 37°C in Krebs solution containing 20 nM [^{3}H]-5-HT creatinine sulphate (specific activity $1.1\ TBq\ mmol^{-1}$; NEN Research Products, Mississauga, Canada), 100 nM [^{3}H]-5-HT in one series of experiments, or 100 nM [^{3}H]-noradrenaline (specific activity 543 $GBq\ mmol^{-1}$), and bubbled with a mixture of 95% O_2:5% CO_2. The composition of the Krebs solution in $mmol\ l^{-1}$ concentrations were: NaCl 118, KCl 4.7, $CaCl_2$ 1.3, $MgCl_2$ 1.2, NaH_2PO_4 1, $NaHCO_3$ 25, glucose 11.1, Na_2EDTA 0.004 and ascorbic acid 0.11. At the end of the incubation period, one hypothalamic slice, three slices of the hippocampus, or two slices of frontal cortex were transferred into separate glass chambers and superfused continuously at a rate of $0.5\ ml\ min^{-1}$ with Krebs solution maintained at 37°C and saturated with O_2/CO_2. Nineteen consecutive 4-min fractions were collected starting 52, 60 or 90 min after the beginning of superfusion for the hypothalamus, hippocampus, and frontal cortex slices, respectively. The slices were stimulated twice for 2 min, 8 min (S_1) and 44 min (S_2) after the end of the washing period. The electrical field generated in the chambers between two platinum electrodes (2 cm apart) had a voltage drop of about 10 V. The following stimulation parameters were used to elicit [^{3}H]-5-HT and [^{3}H]-NA release in hypothalamic slices: 20 mA, 2 ms, 3 Hz for 2 min. In the experiments carried out with hippocampus and frontal cortex slices, the following parameters were used: 30 mA, 2 ms, 3 Hz for 2 min. This frequency of stimulation was chosen because it is within the range of the firing rate of 5-HT and noradrenergic neurones recorded in freely-moving cats (Jacobs, 1986). The first stimulation period (S_1) was always used as control and the drugs were added 8 or 20 min before S_2 and remained present until the end of the superfusion. At the end of the experiments, the slices were solubilized in 0.5 ml of Soluene 350 (Packard Instruments, Downers grove, Il, U.S.A.) and the radioactivity in the slices and superfusate samples was determined by liquid scintillation spectrometery. The amount of tritium released per 4-min sample was expressed as a fraction of the total tissue tritium content at the beginning of the respective collection period. The overflow of tritium produced by the electrical stimulation was calculated as the total increase in radioactivity above the basal outflow of tritium determined in the sample immediately preceding the start of stimulation (sp_1 or sp_2). In order to assess the drug-induced changes of electrically-evoked overflow of radioactivity, S_2/S_1 ratios were calculated. The sp_2/sp_1 ratios were also calculated to determine whether the drugs had altered the basal outflow of radioactivity. The amount of tritium released by electrical stimulation in rat brain slices under these conditions provides a reliable estimate of the release of tritiated or endogenous 5-HT (Baumann & Waldmeier, 1981; Moret & Briley, 1990b; Blier & Bouchard, 1993).

In order to detect treatment effects, concentration-effect curves were constructed by studying simultaneously in the same superfusion apparatus five chambers with slices prepared from a control and five chambers with slices from a treated guinea-pig with the same drug concentration. One to three pairs of guinea-pigs from the same arrival (which were implanted with the minipump on the same day and then housed in the same cages) were used for each drug concentration. This experimental design was deemed optimal to minimize the problem of inter-experimental variations, as indicated by the differences between the control groups, which are inherent to such superfusion studies.

Determination of [^{3}H]-5-HT uptake

For determination of *in vitro* [^{3}H]-5-HT uptake, hypothalamus and frontal cortex slices were prepared as for the superfusion experiments. They were incubated for 3 min at 37°C and continuously bubbled with 5% CO_2:95% O_2. Following this stabilization period, [^{3}H]-5-HT was added to final concentration of 20 nM in all the experiments with the exception of one series in which 100 nM was used. After a 3 min incubation period, uptake was terminated by transferring the slices to 5 ml of ice cold Krebs solution and they were then solubilized in 0.5 ml Soluene 350. Radioactivity in the slices and the incubation medium was determined by liquid spectroscopy. Parallel experiments were carried out at 0°C as control for passive diffusion. The amount of tritium actively captured by the tissue (C_A) was calculated according to the formula: $C_A = C_T - C_P$ where C_T and C_P are the tissue/medium ratios of [^{3}H]-5-HT at 37°C (total) and 0°C (passive), respectively. The percentage inhibition was calculated by comparing the C_A values obtained in the slices prepared from the control and treated guinea-pigs.

The following drugs were used: 2-methyl-5-HT, 5-carboxamidotryptamine (5-CT), UK 14.304 (5-bromo-6-[2-imidazoline-2-ydaminol-quinoxaline]), idazoxan, (Research Biochemicals, Natick, MA, U.S.A.), 5-methoxytryptamine (5-MeOT; Sigma, St-Louis, MO, U.S.A.), paroxetine, methiothepin, S-zacopride and befloxatone.

The means for the spontaneous and evoked fractional releases were compared with Student's two-tailed *t* test. The concentration-effect curves were compared using a three-factor analysis of variance (independent variables: agonist concentration, treatment and animal; dependent variable: S_2/S_1 ratios for the individual chambers). The tissue-medium ratios of radioactivity were compared in the control and treated groups to determine the *t* values. Probability (*P*) values smaller than 0.05 were considered as significant.

Results

Effects of long-term paroxetine and befloxatone administration on tritium release

The electrically evoked release of tritium in the absence of any drug in S_1 was significantly enhanced following the 21-day treatment with paroxetine by 35%, 25% and 31% in the hypothalamus, hippocampus and frontal cortex, respectively, when compared to controls processed in parallel in the same

experiments (Figure 1). The spontaneous outflow of radioactivity in the sample immediately preceding this first period of stimulation (sp_1), was slightly but significantly decreased by 9% in the hypothalamic and hippocampal slices prepared from the paroxetine treated guinea-pigs as compared to the controls (Table 1). These alterations were unlikely to have resulted from the residual 5-HT reuptake blockade following the 48 h washout for two reasons. First, when paroxetine was

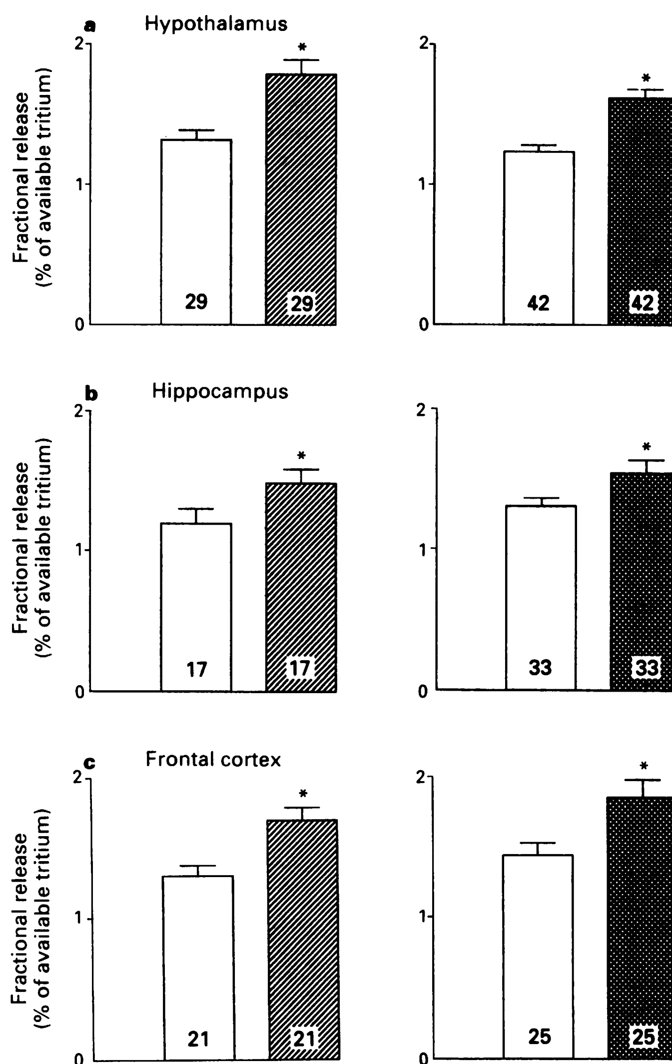

Figure 1 Effect of long-term administration of paroxetine (10 mg kg^{-1} day^{-1} for 21 days) and of befloxatone (0.75 mg kg^{-1} day^{-1} for 21 days) on the electrically stimulated tritium overflow in slices prepared from various regions of the guinea-pig brain and preloaded with [^{3}H]-5-HT. These drugs were administered using osmotic minipumps implanted subcutaneously and removed 48 h before the animals were killed. Ordinates: fraction of the total radioactivity released by 360 pulses (20 mA in hypothalamic and 30 mA in hippocampal and frontal cortex slices, 2 ms, 3 Hz) in the first period of stimulation before the introduction of any drug in the superfusate. Experiments were always carried out in pairs of guinea-pigs, a control and a treated one, processed in parallel in the same superfusion apparatus. The figures within the columns refer to the number of animals studied, and for each animal four to five chambers were used. Open columns, control; hatched columns, paroxetine; cross-hatched columns, befloxatone. *$P<0.05$ when compared with the corresponding control value.

Table 1 Effect of long-term administration of paroxetine or befloxatone on the spontaneous outflow of tritium from guinea-pig brain slices preloaded with [^{3}H]-5-HT[1]

	Hypothalamus	*Hippocampus*	*Frontal cortex*
Control	1.20 ± 0.03% (29)	1.12 ± 0.06% (17)	0.82 ± 0.02% (21)
Paroxetine	1.09 ± 0.03%* (29)	1.04 ± 0.04%* (17)	0.76 ± 0.03% (21)
Control	1.18 ± 0.02% (42)	1.16 ± 0.03% (33)	0.87 ± 0.04% (25)
Befloxatone	1.27 ± 0.02%* (42)	1.28 ± 0.04%* (33)	0.88 ± 0.03% (25)

[1]Percentage of total tissue tritium content present in the slices in the 4-min sample of superfusate collected immediately before the first period of electrical stimulation. The experiments were carried out 48 h after a 21-day treatment with paroxetine (10 mg kg^{-1} day^{-1}, s.c.) or befloxatone (0.75 mg kg^{-1} day^{-1}, s.c.). No drug was present in the superfusate. The figures given in parentheses refer to the number of guinea-pigs studied, and for each animal four to five chambers were studied.
*$P<0.05$ when compared to the controls processed in parallel in the same experiments.

introduced 20 min before S_2 in controls, it produced a significant increase in the spontaneous outflow of tritium from hypothalamic slices ($sp_2/sp_1 = 0.88 \pm 0.02$, $n = 6$, in the presence of 1 μM paroxetine versus 0.79 ± 0.01, $n = 29$, in the absence of paroxetine). Second, when [^{3}H]-5-HT uptake was determined with the same concentration of [^{3}H]-5-HT as that used in the superfusion experiments (20 nM), it was not significantly altered in hypothalamic slices 48 h after a 21-day treatment (Figure 8). The total tissue content of radioactivity at the end of the experiments was not significantly altered in the slices prepared from guinea-pigs treated with paroxetine compared with the controls (data not shown).

In the slices prepared from guinea-pigs treated with befloxatone for 21 days, the electrically evoked release of tritium was also significantly enhanced by 30%, 18% and 29% in the hypothalamus, hippocampus and frontal cortex, respectively, when compared to controls processed in parallel (Figure 1). The spontaneous outflow of radioactivity (sp_1) was slightly but significantly enhanced by 9% and 10% in the hypothalamus and hippocampus, respectively, but unchanged in frontal cortex slices prepared from befloxatone-treated guinea-pigs (Table 1). These modifications of basal outflow and evoked overflow of tritium release are not likely to be attributable to residual inhibition of MAO-A since the introduction of befloxatone (0.1 μM) 20 min before S_2 decreased in hippocampal slices both the electrically evoked release of tritium ($S_2/S_1 = 0.56 \pm 0.06$, $n = 8$, versus the control condition $S_2/S_1 = 1.06 \pm 0.07$, $n = 10$) and the spontaneous outflow of radioactivity ($sp_2/sp_1 = 0.67 \pm 0.02$, $n = 8$ versus controls, 0.80 ± 0.02, $n = 10$). The total tissue content of radioactivity at the end of the experiments was not significantly altered in the slices prepared from guinea-pigs treated with befloxatone than from the controls (data not shown).

Assessment of the responsiveness of the terminal 5-HT autoreceptor

In order to assess the sensitivity of the terminal 5-HT autoreceptor following a 21-day treatment with paroxetine or befloxatone, concentration-effect curves were constructed using the potent 5-HT$_1$ agonist, 5-CT (1–100 nM). In the paroxetine group, there was an attenuation of the capacity of 5-CT to inhibit the electrically evoked release of tritium from preloaded hypothalamic slices, whereas in the befloxatone group there was no difference versus the control groups (Figure 2). These results indicate that the paroxetine, but not the befloxatone treatment, produced a desensitization of the terminal 5-HT autoreceptor in the hypothalamus. However, given the marked inhibition of 5-CT already present at concentrations as low as 1 and 3 nM, another 5-HT autoreceptor agonist, 5-MeOT, was used. 5-MeOT produced a more gradual inhibitory effect of the electrically evoked release of tritium in hypothalamic slices over a concentration-range of three orders of magnitude (Figure 3). In the paroxetine group, there was again an attenuation of the inhibitory effect of 5-MeOT on the electrically evoked release of tritium. This attenuation was greater than that obtained with 5-CT. Consistent with the results obtained with 5-CT, the inhibitory effect of 5-MeOT was unaltered in the befloxatone group. In order to demonstrate further the attenuated function of the terminal 5-HT autoreceptor following the long-term paroxetine treatment, the efficacy of the 5-HT autoreceptor antagonist, methiothepin, was examined in hypothalamic slices prepared from control and treated guinea-pigs. The rationale for this approach was that if indeed the paroxetine, but not the befloxatone, treatment desensitized the 5-HT autoreceptor, then blocking it should produce a smaller effect in the treated group. At a 0.3 μM concentration, methiothepin did in fact produce a lesser enhancement of the evoked release of tritium in hypothalamic slices prepared from the paroxetine-treated guinea-pigs than in those from the control group. ($S_2/S_1 = 2.04 \pm 0.10$, $n = 5$, vs controls 2.52 ± 0.07, $n = 5$). However, in the befloxatone-treated guinea-pigs, the effect of methiothepin was unaltered ($S_2/S_1 = 1.76 \pm 0.15$, $n = 4$, vs controls 1.62 ± 0.08, $n = 5$).

In hippocampal slices, there was a small, albeit significant, attenuation of the inhibitory effect of 5-MeOT on the electrically evoked release of tritium in the paroxetine group, while befloxatone treatment did not have such an effect (Figure 3b). As with hypothalamic slices, the enhancing effect of 0.3 μM methiothepin was significantly less pronounced in the paroxetine group ($S_2/S_1 = 2.11 \pm 0.08$, $n = 10$) than in the control group ($S_2/S_1 = 2.62 \pm 0.21$, $n = 10$). As was also the case for hypothalamic slices, in hippocampal slices prepared from befloxatone-treated guinea-pigs, the enhancing effect of

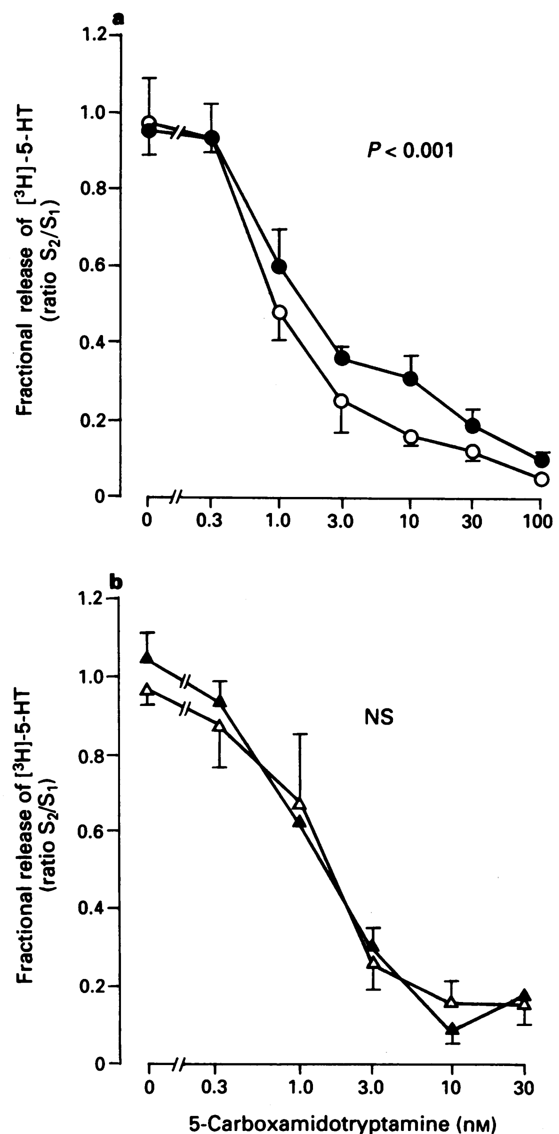

Figure 2 Inhibition produced by the 5-HT autoreceptor agonist 5-carboxamidotryptamine of the evoked release of tritium elicited by electrical stimulation in preloaded hypothalamus slices prepared from control (open symbols) and treated (solid symbols) guinea-pigs with paroxetine (a) or befloxatone (b) for 21 days. The experiments were carried out 48 h after removal of the osmotic minipump used to deliver the drugs. Ordinates: fraction of the total tissue radioactivity released by 360 pulses (30 mA, 2 ms, 3 Hz). Values are expressed as the ratio (S_2/S_1) obtained between the second period of stimulation in the presence of the agonist (S_2) and the first one carried out in the absence of the agonist (S_1). Each point represents the mean ± s.e. of the results obtained with superfusion chambers in the same experiments in pairs of control and treated guinea-pigs. The level of statistical significance, calculated using analysis of variance, between the curves obtained in the control and the treated groups is indicated in the graphs; NS indicates a $P > 0.05$.

0.3 μM methiothepin remained unaltered (control: $S_2/S_1 = 2.48 \pm 0.20$, $n = 5$; befloxatone: $S_2/S_1 = 2.50 \pm 0.20$, $n = 4$).

In frontal cortex slices prepared from paroxetine-treated guinea-pigs, although there was a significant difference in the inhibitory effect of 0.1 μM 5-MeOT on the electrically evoked release of tritium, analysis of the concentration-effect curves did not yield a statistically significant difference between the control and the paroxetine group. As with hypothalamic and hippocampal slices prepared from befloxatone-treated guinea-pigs, the inhibitory effect of 5-MeOT was also unaltered in frontal cortex slices (Figure 3c).

Assessment of the responsiveness of the α_2-adrenoceptor located on 5-HT terminals

Since the inhibition of MAO-A by befloxatone increases brain noradrenaline concentration and since acute 5-HT reuptake blockade attenuates the capacity of the α_2-adreno-

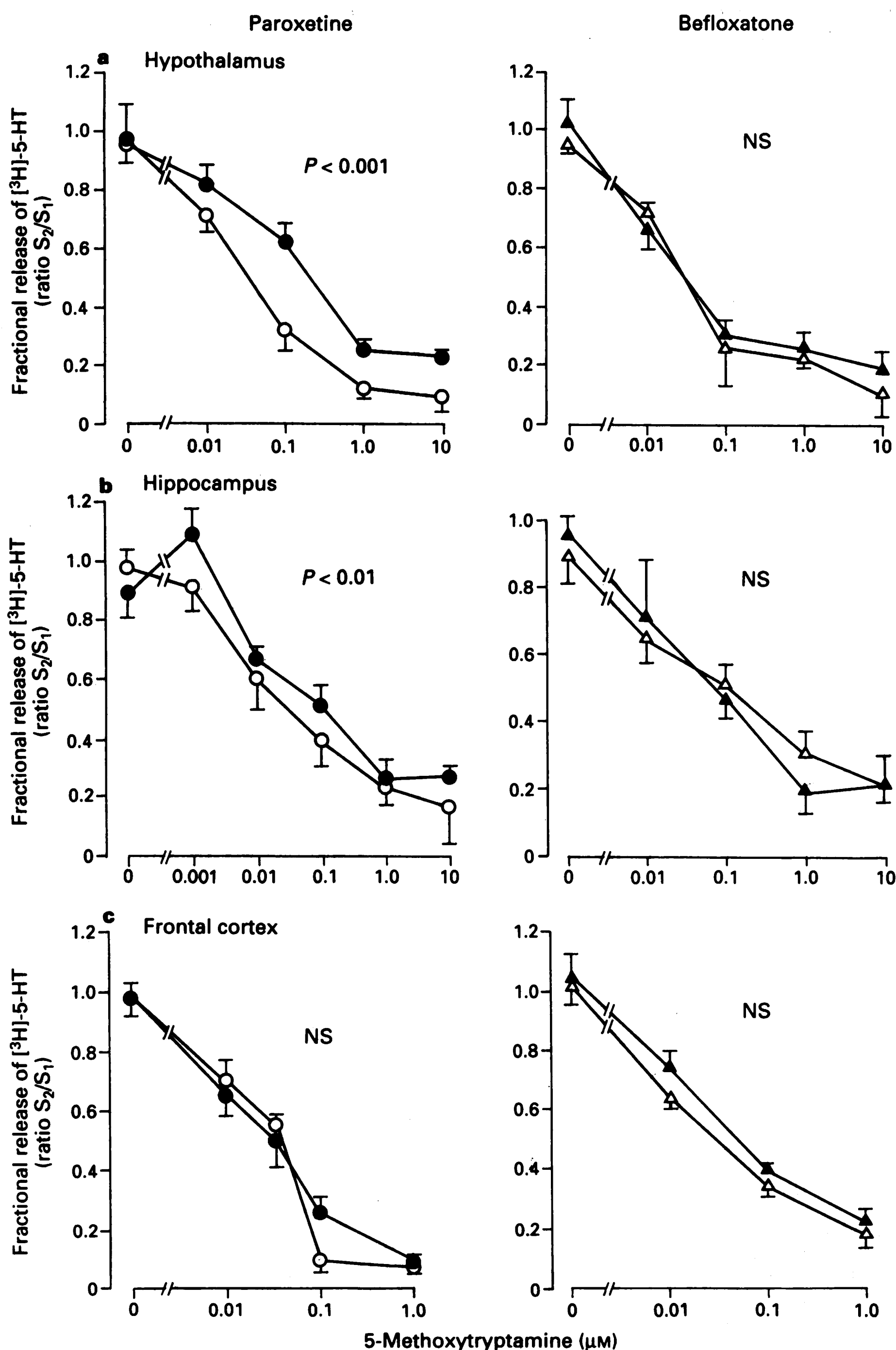

Figure 3 Inhibition produced by the 5-HT autoreceptor agonist, 5-methoxytryptamine, of the evoked release of radioactivity elicited by electrical stimulation in preloaded hypothalamic, hippocampal, and frontal cortex slices prepared from control (open symbols) and treated guinea-pigs (solid symbols) paroxetine or befloxatone for 21 days. The experiments were carried out 48 h after removal of the osmotic minipump used to deliver the drugs. Ordinates: fraction of the total tissue radioactivity released by 360 pulses (20 mA for hypothalamus and 30 mA for hippocampus and frontal cortex slices, 2 ms, 3 Hz). Values are expressed as the ratio (S_2/S_1) obtained between the second period of stimulation in the presence of the agonist (S_2) and the first one carried out in the absence of the agonist (S_1). Each point represents the mean ± s.e. of the results obtained with superfusion chambers in the same experiments in pairs of control and treated guinea-pigs. The level of statistical significance, calculated by analysis of variance, between the control and curves obtained in the treated group is indicated in the graphs; NS indicates a $P > 0.05$.

ceptor to decrease 5-HT release (Blier *et al.*, 1990), it was deemed crucial to examine the sensitivity of the α_2-adrenoceptor following a long-term treatment with drugs which were shown to alter its degree of activation, or its function, when given acutely. In addition, the function of this α_2-adrenoceptor was also examined using the α_2-adrenoceptor antagonist, idazoxan. In hypothalamic slices, the inhibitory effect of the α_2-adrenoceptor agonist, UK 14.304, on the electrically-evoked release of tritium was identical in the control group and in the group treated for 21 days with paroxetine (Figure 4a). In contrast, the inhibitory effect of UK 14.304 was significantly attenuated in the befloxatone group, as was the enhancing effect of a 1 μM concentration of

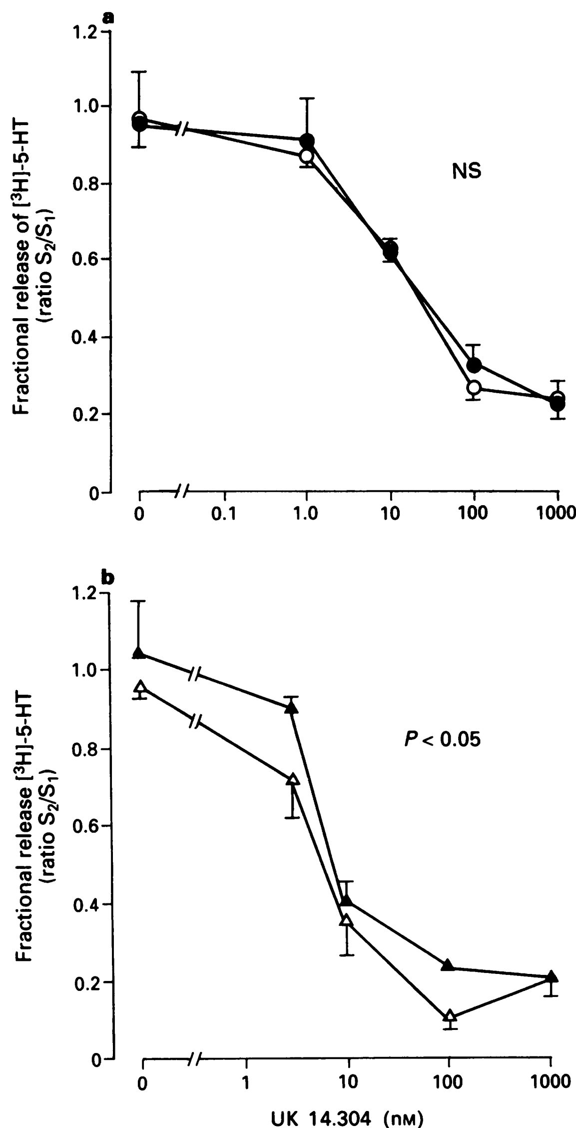

Figure 4 Inhibition produced by the α_2-adrenoceptor agonist, UK 14.304, of the evoked release of radioactivity elicited by electrical stimulations in preloaded hypothalamic slices prepared from control (open symbols) and treated (solid symbols) guinea-pigs with paroxetine (a) or befloxatone (b) for 21 days. The experiments were carried out 48 h after removal of the osmotic minipump used to deliver the drugs. Ordinates: fraction of the total tissue radioactivity released by 360 pulses (30 mA, 2 ms, 3 Hz). Values are expressed as the ratio (S_2/S_1) obtained between the second period of stimulation in the presence of the agonist (S_2) and the first one carried out in the absence of the agonist (S_1). Each point represents the mean ± s.e. of the results obtained with superfusion chambers in the same experiments in pairs of control and treated guinea-pigs. The level of statistical significance, calculated by analysis of variance, between the control and the curves obtained in the treated group is indicated in the graphs; NS indicates a $P > 0.05$.

the α_2-adrenoceptor antagonist, idazoxan (Figures 4b and 6). In hippocampal slices, the inhibitory effect of UK 14.304 on the electrically evoked release of tritium was also reduced in the befloxatone group (although statistical significance was not quite reached; Figure 5a), but the enhancing effect of 1 μM idazoxan was similar in both groups (Figure 6). In frontal cortex slices, neither the inhibitory effect of UK 14.304 nor the effect of 1 μM idazoxan was significantly altered by the befloxatone treatment (Figures 6 and 7).

Assessment of the sensitivity of the α_2-adrenoceptor located on NA terminals following long-term befloxatone administration

Since the long-term befloxatone treatment induced a desensitization of the α_2-adrenoceptor located on 5-HT terminals in the hypothalamus, the sensitivity of the α_2-adrenoceptor on noradrenaline terminals was then investigated in the hypothalamus and hippocampus to determine whether a sustained increase of endogenous NA could also alter the responsiveness of the latter presynaptic α_2-adrenoceptor. The concentration-effect curves of the UK 14.304-induced inhibition of the electrically evoked release of tritium in hippocampal and hypothalamic slices preloaded with [^{3}H]-NA were identical in the control and the befloxatone group (Figure 5b; data not shown for hypothalamic slices). It is noteworthy that in hippocampal slices, the evoked release of tritium and the spontaneous outflow of radioactivity were significantly enhanced in four pairs of controls and befloxatone-treated guinea-pigs (control: $S_1 = 1.56 \pm 0.06\%$, befloxatone: $S_1 = 1.92 \pm 0.16\%$; control: $sp_1 = 0.80 \pm 0.05\%$, befloxatone: $sp_1 = 0.98 \pm 0.05\%$) and the tissue content of radioactivity decreased (control: 108 ± 6 nCi, befloxatone: 86 ± 4 nCi). In hypothalamic slices, none of these parameters were significantly altered (data not shown).

Determination of [^{3}H]-5-HT uptake and of the function of the 5-HT transporter

Using a final concentration of 20 nM [^{3}H]-5-HT for incubation, uptake was determined in control and paroxetine-treated guinea-pigs. Following a 2-day treatment with paroxetine, [^{3}H]-5-HT uptake was inhibited by approximately 50% in hypothalamic and frontal slices, with the minipump delivering the drug at the time the guinea-pigs were killed, when compared to control slices processed in parallel in the same experiments (Figure 8). There was still a significant attenuation of the degree of [^{3}H]-5-HT uptake in hypothalamic slices after the 21-day paroxetine treatment when the animals were killed with the minipump in place, but it was smaller than that observed after the 2-day treatment. This could be attributable, in part, to the fact that the guinea-pigs received more than 10 mg kg^{-1} day^{-1} of paroxetine at the beginning of the treatment and less at the end of the 21-day period since the dose was based on the mean weight of the animal during this period. The decreasing dose of paroxetine that the guinea-pigs were receiving during the 21-day treatment could not, however, explain the lack of significant inhibition of [^{3}H]-5-HT uptake in frontal cortex (Figure 8). Furthermore, the [^{3}H]-5-HT uptake values obtained after the 21-day treatment with paroxetine were nearly identical whether or not the pump was in the animal delivering the drug (Figure 8). These results suggest that the 5-HT transporter had desensitized during the 21-day treatment, at least in the frontal cortex, as previously described in the rat brain subjected to the same treatment (Piñeyro *et al.*, 1994). In these previous experiments, the desensitization of the transporter was evidenced by incubating the slices with a 100 nM, but not with a 20 or a 5 nM concentration of [^{3}H]-5-HT after a 48 h washout period. This was probably attributable to the 5-HT transporters being more saturated at the 100 nM concentration. Under the latter condition in the present experiments, there was a significant inhibition of the uptake of [^{3}H]-5-HT

(23 ± 4%, $P = 0.002$) in frontal cortex slices prepared from five paroxetine-treated guinea-pigs (after a 48 h washout period) when compared to five controls processed at the same time.

In a final series of experiments, the effect of 1 μM paroxetine on the evoked release of [^{3}H]-5-HT was examined in frontal cortex slices to provide further functional evidence for the attenuated efficacy of the 5-HT transporter. The enhancing effect of paroxetine, introduced 20 min before S_2, was significantly attenuated in the paroxetine group (S_2/S_1 = 1.12 ± 0.07, $n = 10$) when compared to the controls (S_2/S_1 = 1.46 ± 0.11, $n = 15$). In contrast, 1 μM paroxetine altered the S_2/S_1 ratios in hypothalamic slices neither in the control

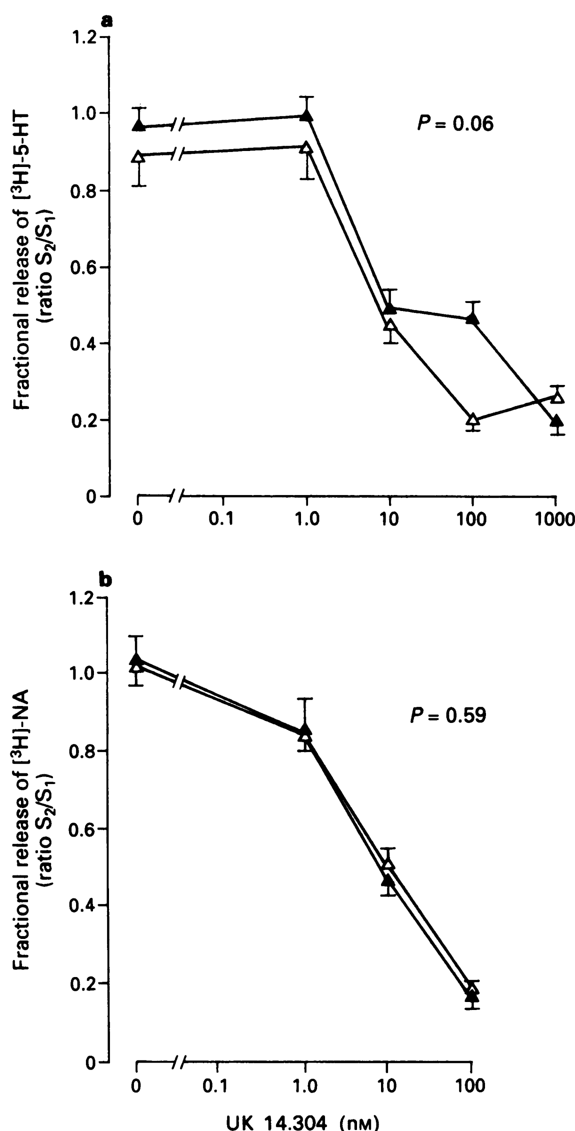

Figure 5 Inhibition produced by the α_2-adrenoceptor agonist, UK 14.304, of the evoked release of radioactivity elicited by electrical stimulations in hippocampal slices preloaded with either [^{3}H]-5-HT (a) or [^{3}H]-noradrenaline ([^{3}H]-NA) (b) in controls (Δ) and guinea-pigs treated with befloxatone for 21 days (▲). The experiments were carried out 48 h after removal of the osmotic minipump used to deliver the drugs. Ordinates: fraction of the total tissue radioactivity released by 360 pulses (30 mA, 2 ms, 3 Hz). Values are expressed as the ratio (S_2/S_1) obtained between the second period of stimulation in the presence of the agonist (S_2) and the first one carried out in the absence of the agonist (S_1). Each point represents the mean ± s.e. of the results obtained with superfusion chambers in the same experiments in pairs of control and befloxatone-treated guinea-pigs. The level of statistical significance, calculated by analysis of variance, between the control and the curves obtained in the treated group is indicated in the graphs.

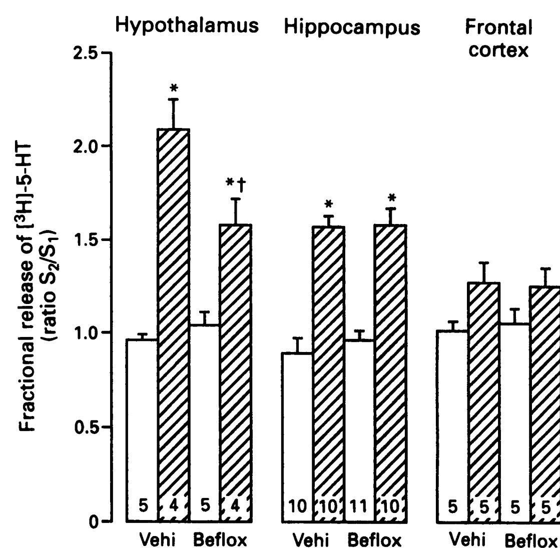

Figure 6 Effect of the α_2-adrenoceptor antagonist, idazoxan, on the evoked release of radioactivity elicited by electrical stimulations in preloaded hypothalamic hippocampal, and frontal cortex slices prepared from control and treated guinea-pigs with befloxatone for 21 days. The experiments were carried out 48 h after removal of the osmotic minipump used to deliver the drug. Open columns, control; hatched columns, idazoxan, 1 μM before S_2. Ordinates: fraction of the total tissue radioactivity released by 360 pulses (30 mA, 2 ms, 3 Hz). Values are expressed as the ratio (S_2/S_1) obtained between the second period of stimulation in the presence of the antagonist (S_2) and the first one carried out in the absence of the antagonist (S_1). Values are expressed as mean ± s.e. of the results obtained in the same experiments with control and treated guinea-pigs. *$P < 0.05$ when compared with the corresponding control value; †$P < 0.05$ when compared to the value obtained with idazoxan in the control group.

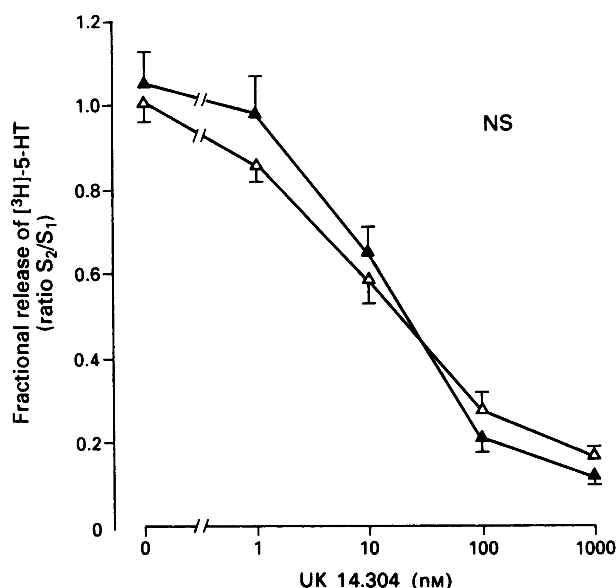

Figure 7 Inhibition produced by the α_2-adrenoceptor agonist, UK 14.304, of the evoked release of radioactivity elicited by electrical stimulations in preloaded frontal cortex slices prepared from control (Δ) and treated guinea-pigs (▲) with befloxatone for 21 days. The experiments were carried out 48 h after removal of the osmotic minipump used to deliver the drugs. Ordinates: fraction of the total tissue radioactivity released by 360 pulses (30 mA, 2 ms, 3 Hz). Values are expressed as the ratio (S_2/S_1) obtained between the second period of stimulation in the presence of the agonist (S_2) and the first one carried out in the absence of the agonist (S_1). Each point represents the mean ± s.e. of the results obtained with superfusion chambers in the same experiments with control and treated guinea-pigs. The level of statistical significance, calculated by analysis of variance, between the control and the curves obtained in the treated group is indicated in the graphs; NS indicates a $P > 0.05$.

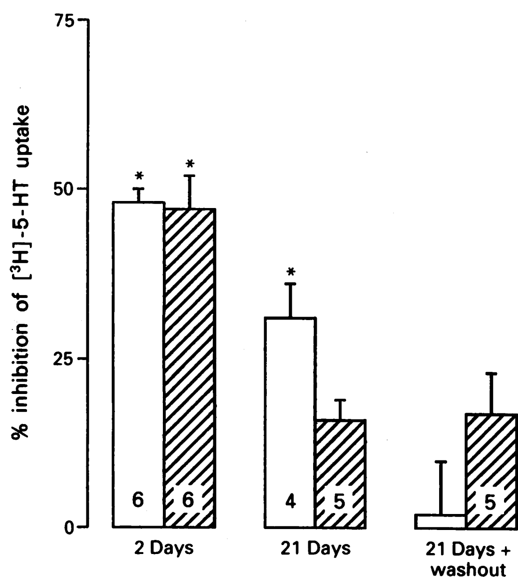

Figure 8 Inhibition of [^{3}H]-5-HT uptake in hypothalamus (open columns) and frontal cortex (hatched columns) slices of guinea-pigs during and following the administration of the 5-HT reuptake blocker, paroxetine. Paroxetine was administered via an osmotic minipump implanted subcutaneously which delivered 10 mg kg^{-1} day^{-1}. Inhibition of [^{3}H]-5-HT uptake was determined by incubating the slices with 20 nM [^{3}H]-5-HT for 3 min. The percentage inhibition was calculated by comparing the tissue/medium ratios of radioactivity in the control and the treated groups. The figures given in the histograms refer to the number of pairs of guinea-pigs studied: a control and a treated one done in quintuplets. Five pairs were tested in the 21 days + washout condition for the hypothalamus. The washout period consisted of removing the minipump for 48 h prior the experiment. *$P < 0.05$, when compared to the tissue-medium ratios obtained in the controls processed in parallel in the same experiments.

(0.97 ± 0.12, $n = 4$) nor in the paroxetine group (0.95 ± 0.06, $n = 5$).

Assessment of the sensitivity of the 5-HT$_3$ receptor which enhances the evoked release of 5-HT

The 5-HT$_3$ receptors which modulate 5-HT release desensitize rapidly *in vitro* (Galzin & Langer, 1991; Blier & Bouchard, 1993). The sensitivity of the latter receptors was therefore also studied following long-term treatment with paroxetine and befloxatone. As previously reported by Galzin & Langer (1991) and our group (Blier & Bouchard, 1993), the 5-HT$_3$ receptor agonist, 2-methyl-5-HT, produced a concentration-dependent increase of the electrically-evoked release of tritium in the hypothalamic, hippocampal and frontal cortex slices prepared from control guinea-pigs (Figure 9). In slices prepared from paroxetine-treated guinea-pigs following a 48 h wash-out period, there was a significant attenuation of the enhancing effect of 2-methyl-5-HT on the evoked release of tritium in all three brain regions (Figure 9). In order to determine whether this desensitization induced by paroxetine supervenes only following long-term 5-HT reuptake blockade, the effect of 2-methyl-5-HT was studied in frontal cortex slices prepared from guinea-pigs treated for 2 days with paroxetine, also following a 48-h washout period: the enhancing effect of 2-methyl-5-HT was unchanged (Figure 10). In contrast with the results obtained with the 21-day treatment with paroxetine, the enhancing effect of 2-methyl-5-HT was unaltered following a 21-day treatment with befloxatone (Figure 9). Given the unmodified responsiveness of this 5-HT$_3$ receptor in befloxatone-treated animals, we then assessed whether an enhanced activation of this receptor could contribute in frontal cortex slices to the enhanced [^{3}H]-5-HT release consistently observed in the befloxatone group. To this end, the 5-HT$_3$ antagonist S-zacopride (0.1 μM) was superfused, starting 8 min before S$_2$. In the control as well as in the befloxatone group, this antagonist did not significantly alter [^{3}H]-5-HT release (control: $S_2/S_1 = 1.11 \pm 0.06$, $n = 5$; befloxatone: $S_2/S_1 = 1.11 \pm 0.09$, $n = 5$), thus indicating that under these *in vitro* conditions, this 5-HT$_3$ receptor is not tonically activated.

Discussion

The results of the present study indicate that long-term 5-HT reuptake blockade and type A-MAO inhibition lead to enhanced 5-HT release (Figure 1). In the case of paroxetine, this effect appears to be due to an attenuation of the capacity of the terminal 5-HT autoreceptor to inhibit 5-HT release in the hypothalamus and hippocampus. This is suggested by the lesser effectiveness of the 5-HT autoreceptor antagonist, methiothepin, to increase [^{3}H]-5-HT release and by the attenuation of the inhibitory effect of the 5-HT receptor agonist, 5-MeOT (Figure 3), following a long-term paroxetine treatment. It is conceivable that the attenuated effect of 5-MeOT might have been due to competition of this exogenous agonist with endogenous 5-HT, the availability of which was increased by the paroxetine treatment. However, 5-HT release was enhanced to a similar level in the befloxatone group in the hypothalamic and hippocampal slices and yet, the effectiveness of 5-MeOT was not altered following the befloxatone treatment (Figures 1 and 3). This desensitization of the terminal 5-HT autoreceptor in the guinea-pig hypothalamus is fully consistent with that observed in the study of Moret & Briley (1990a) using the same *in vitro* methodology in hypothalamic slices prepared from rats treated with the SSRI, citalopram. Similarly, previous results obtained using *in vivo* electrophysiological techniques in our laboratory indicated the capacity of the terminal 5-HT autoreceptor to desensitize in the rat hippocampus following a 2 to 3 week treatment with SSRI, including paroxetine (Chaput *et al.*, 1986; 1991; Blier *et al.*, 1988). Taken together, these results show that the terminal 5-HT autoreceptor in the hypothalamus and hippocampus, whether it be of the 5-HT$_{1B}$ subtype in the rat brain or of the 5-HT$_{1D}$ subtype in the guinea-pig brain, desensitizes following long-term 5-HT reuptake blockade.

In frontal cortex slices, however, the evoked [^{3}H]-5-HT release was increased despite the absence of a desensitization of the terminal 5-HT autoreceptor (Figures 1 and 3). The most likely explanation for this enhanced release is a down-regulation of the 5-HT transporter (Figure 8). Indeed, it has been reported that following a 21-day treatment with paroxetine, there was a 60% decrease in the B_{max} value for the 5-HT transporter in the rat frontal cortex, using [^{3}H]-paroxetine as a ligand, after a 48 h washout period (Piñeyro *et al.*, 1994). This decrease in the density of [^{3}H]-5-HT uptake sites could be attributed to a decrease in their mRNA level as documented by Lesch *et al.* (1993). Since, in the present experiments the addition of 1 μM paroxetine in the superfusate did not increase the electrically evoked release of tritium in frontal cortex slices in the paroxetine, it may be assumed that a decreased number of 5-HT transporter sites was the underlying cause of the 31% increase in [^{3}H]-5-HT release (Figure 1c). This, raises the possibility that such a down-regulation of the 5-HT transporter may have contributed to the enhancement of the electrically evoked [^{3}H]-5-HT release observed in hypothalamic and hippocampal slices following the long-term treatment. In the guinea-pig and rat hypothalamus, a complete blockade of 5-HT reuptake does not alter the evoked release of [^{3}H]-5-HT (Passarelli *et al.*, 1987; Blier & Bouchard, 1993; present results). It may thus be concluded that this enhanced 5-HT release in the hypothalamus was entirely attributable to the desensitization of the 5-HT autoreceptor. In the hippocampus, however,

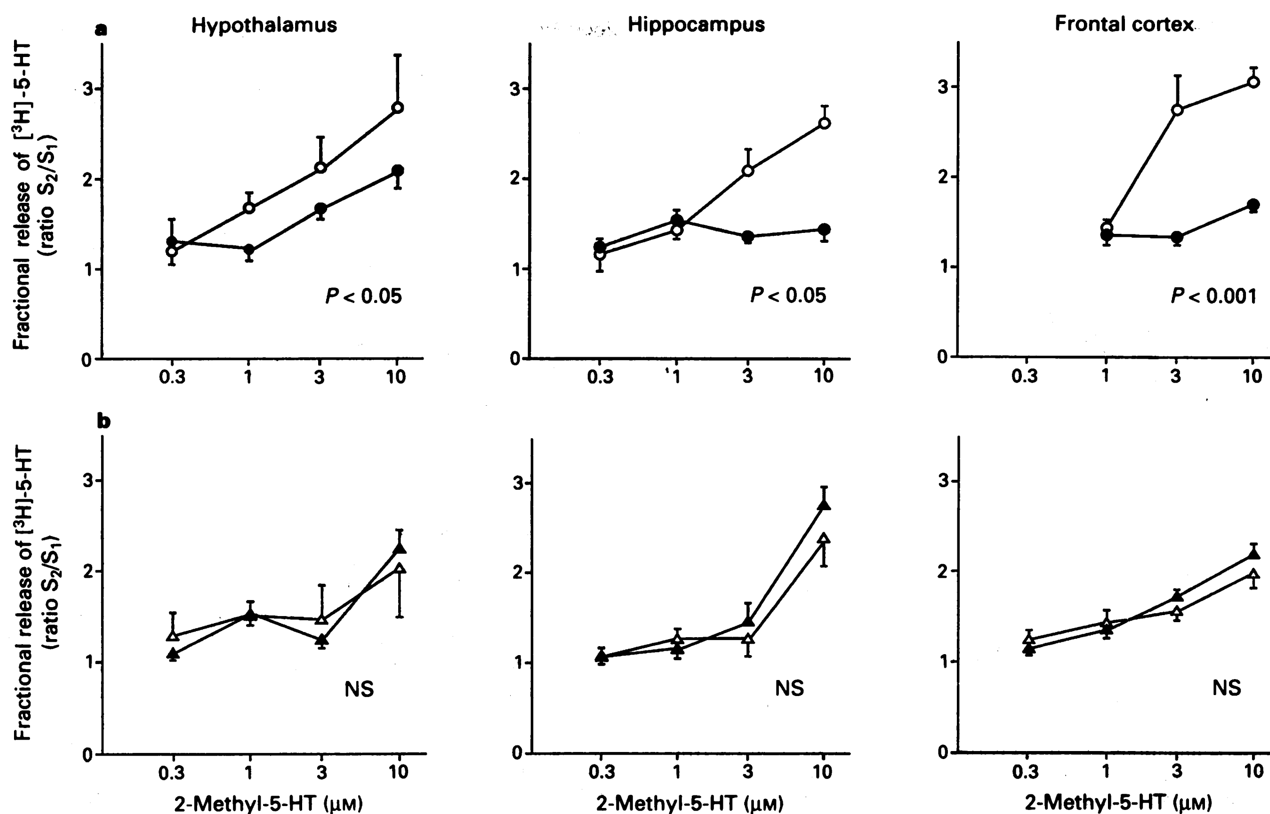

Figure 9 Enhancement produced by the 5-HT_3 receptor agonist, 2-methyl-5-HT, of the evoked release of radioactivity elicited by electrical stimulations in preloaded hypothalamic, hippocampal, and frontal cortex slices prepared from control (open symbols) and treated guinea-pig (filled symbols) with paroxetine (a) or befloxatone (b) for 21 days. The experiments were carried out 48 h after removal of the osmotic minipump used to deliver the drugs. Ordinates: fraction of the total tissue radioactivity release by 360 pulses (20 mA for hypothalamus and 30 mA for hippocampus and frontal cortex slices, 2 ms, 3 Hz). Values are expressed as the ratio (S_2/S_1) obtained between the second period of stimulation in the presence of the agonist introduced 8 min before S_2 and the first one carried out in the absence of the agonist (S_1). Each point represents the mean ± s.e. of the results obtained with superfusion chambers in the same experiments with control and treated guinea-pigs. The level of statistical significance, calculated by analysis of variance, between the control and the curves obtained in the treated group is indicated in the graphs; NS indicates a $P > 0.05$.

5-HT reuptake blockade does lead to enhanced [^{3}H]-5-HT collected in the superfusate (Blier & Bouchard, 1993). Therefore, based on this acute effect of paroxetine and on the down-regulation of the 5-HT transporter reported in the rat hippocampus following long-term paroxetine administration (Piñeyro *et al.*, 1994), the contribution of such an alteration of the 5-HT transporter to the enhancement of [^{3}H]-5-HT release observed in this brain region cannot be ruled out. It is noteworthy that an upregulation of the 5-HT reuptake sites was recently reported to occur in the rat brain following long-term fluoxetine administration (Hrdina & Vene, 1993). However, these results were obtained with a single concentration of [^{3}H]-paroxetine.

In the case of the befloxatone treatment, the enhanced [^{3}H]-5-HT release after a 48-h washout period is not likely to result from residual inhibition of MAO-A activity since the activity of the enzyme is back to normal 24 h after a supramaximal dose of befloxatone (Curet *et al.*, 1992), consistent with the fact that endogenous 5-HT levels in the rat brain return to normal within 24 h after a supramaximal dose of befloxatone (Curet *et al.*, 1992), and that the content of tritium in the slices prepared from the treated and control guinea-pigs remained unaltered (present study). In the hypothalamus and the hippocampus, the enhancement of the evoked release of [^{3}H]-5-HT was possibly due to a desensitization of the α_2-adrenoceptor on 5-HT terminals, as previously documented in the rat hippocampus (Mongeau *et al.*, 1994). Indeed, when maximally activated by the α_2-adrenoceptor agonist UK 14.304, this presynaptic receptor produces the same degree of inhibition (>80%) of the electrically-evoked release of [^{3}H]-5-HT as that mediated by the 5-HT autoreceptor (Figures 1, 2 and 5), consistent with previous results obtained in the rat brain (Blier & de Montigny, 1994). Furthermore, since the α_2-adrenoceptor antagonist, idazoxan, enhances the electrically evoked release of [^{3}H]-5-HT, this α_2-heteroreceptor appears to be tonically activated in the guinea-pig brain as also appears to be the case in the rat and the human brain (Raiteri *et al.*, 1990; Galzin *et al.*, 1992; Feuerstein *et al.*, 1993; Mongeau *et al.*, 1993). The possibility that, in the hypothalamus, the α_2-heteroreceptor plays a significant role in the enhancement of [^{3}H]-5-HT release in the befloxatone group is further supported by the observation that the α_2-adrenoceptor antagonist, idazoxan, exerted a smaller enhancing effect on the evoked release of [^{3}H]-5-HT from slices prepared from befloxatone-treated guinea-pigs.

In frontal cortex slices, however, neither the inhibitory effect of UK 14.304 nor the enhancing effect of idazoxan was significantly altered following the befloxatone treatment (Figures 6 and 7). Yet, the electrically evoked release of [^{3}H]-5-HT was enhanced to the same degree (30%) as in the hypothalamic slices. Taken together, these results suggest that 48 h following a 21-day treatment with befloxatone, the releasable pool of 5-HT may still be enhanced despite the total concentration of 5-HT in the brain being back to normal. It is interesting to note that the degree of enhancement of [^{3}H]-5-HT release was smaller in the hippocampus (+18%) than in the hypothalamus (+30%). This could well result from a greater inhibitory tonus by endogenous and tritiated noradrenaline on the α_2-adrenoceptors on 5-HT

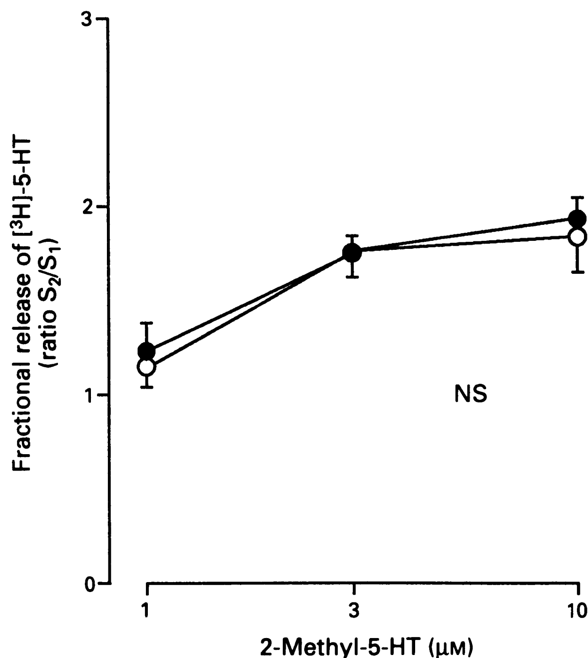

Figure 10 Enhancement produced by the 5-HT_3 receptor agonist, 2-methyl-5-HT, of the evoked release of radioactivity elicited by electrical stimulations in frontal cortex slices prepared from control (○) and treated guinea-pigs (●) with paroxetine for 2 days. The experiments were carried out 48 h after removal of the osmotic minipump used to deliver the drug. Ordinates: fraction of the total tissue radioactivity release by 360 pulses (30 mA, 2 ms, 3 Hz). Values are expressed as the ratio (S_2/S_1) obtained between the second period of stimulation in the presence of the agonist introduced 8 min before S_2 and the first one carried out in the absence of the agonist (S_1). Each point represents the mean ± s.e. of the results obtained with chambers in the same experiments with control and treated guinea-pigs. The level of statistical significance, calculated by analysis of variance, between the control and the curves obtained in the treated animals is indicated in the graphs; NS indicates a $P > 0.05$.

terminals in the hippocampus than in the hypothalamus as suggested by the observation that the electrically evoked release of [^{3}H]-noradrenaline was enhanced in the former but not in the latter structure. This enhanced [^{3}H]-noradrenaline release could also explain why the enhancing effect of idazoxan on [^{3}H]-5-HT release was not attenuated in hippocampal slices prepared from befloxatone-treated guinea-pigs, and also the clear trend toward a desensitization of the α_2-adrenoceptor on 5-HT terminals. Indeed, it is expected that, through α_2-autoreceptor antagonism, idazoxan further enhanced noradrenaline release thus resulting in an increased activation of α_2-adrenoceptor on 5-HT terminals in the hippocampus.

Following long-term MAO-A inhibition, the α_2-adrenoceptor on 5-HT terminals was desensitized in the hypothalamus whereas, in the same structure, the α_2-autoreceptor remained normosensitive. It seems that the terminal 5-HT autoreceptor also would not desensitize as a consequence of sustained agonist occupation because long-term administration of befloxatone did not produce this adaptative phenomenon. Since the paroxetine treatment induced a desensitization of the 5-HT autoreceptor, it is nevertheless possible that these two drugs do not alter the concentration of 5-HT in the biophase of the 5-HT autoreceptor to the same extent. Another possibility to account for this differential adaptive capacity would be that sustained blockade of the 5-HT transporter results in an alteration of signal transduction from the 5-HT autoreceptor sites.

The results of this study indicate that the 5-HT_3 receptors which enhance [^{3}H]-5-HT release were desensitized following the paroxetine, but not the befloxatone, treatment (Figure 9). Since it was demonstrated that this receptor is not located on 5-HT terminals at least in the hypothalamus (Blier *et al.*, 1993), it is unlikely that this desensitization resulted from a functional interaction between the 5-HT transporter and this 5-HT_3 receptor. Rather, it appears that 5-HT reuptake blockade and MAO-A inhibition do not alter the level of 5-HT to the same degree in the biophase of this 5-HT_3 receptor. Since this receptor exerts a positive feedback role on 5-HT release and since it is desensitized following long-term paroxetine administration, it cannot contribute to the enhancement of [^{3}H]-5-HT release observed in this treatment group. 5-HT_3 receptors, in the area postrema and/or on vagal afferents, are believed to mediate nausea and emesis following administration of chemotherapeutic agents because these side effects can be blocked with selective 5-HT_3 antagonists. Since 5-HT reuptake blockers can enhance the availability of 5-HT at all 5-HT receptor subtypes, it is thus possible that the nausea they often produce is the result of enhanced activation of 5-HT_3 receptors. In support of this possibility, we have recently provided evidence that the nausea produced by the administration of SSRI in man can be blocked by cisapride (Bergeron & Blier, 1994), a benzamide derivative with moderate affinity for 5-HT_3 receptors, which blocks the enhancement of the electrically evoked release of [^{3}H]-5-HT produced by 2-methyl-5-HT in preloaded slices of the guinea-pig brain. If the 5-HT_3 receptors which mediate nausea and emesis had characteristics similar to those which can modulate 5-HT release, then their desensitization observed after the 21-day, but not the 2-day, treatment with paroxetine could explain the well-known disappearance of nausea following prolongation of a treatment with SSRI in man.

In conclusion, long-term 5-HT reuptake blockade leads to an enhancement of the evoked [^{3}H]-5-HT release which can be attributable, at least in the hypothalamus and hippocampus, to a desensitization of the terminal 5-HT_{1D} autoreceptor. It can be presumed that in depressed patients, long-term administration of an SSRI also produces a desensitization of the terminal 5-HT_{1D} autoreceptor and results in an enhanced 5-HT release. The possibility that the ensuing increased 5-HT neurotransmission is responsible for the antidepressant response is supported by the reappearance of depressive symptoms observed after tryptophan depletion in patients successfully treated with the SSRI fluvoxamine or fluoxetine (Delgado *et al.*, 1990; 1992). In the frontal cortex, other factors such as the down-regulation of the 5-HT transporter may account for this enhanced release. Inhibition of type-A MAO also produces an enhancement of the evoked [^{3}H]-5-HT release which could result from a desensitization of α_2-heteroreceptors, at least in the hypothalamus. Other factors, such as the increase in the releasable pool of 5-HT, may also contribute to this effect of MAOI. Again it can be presumed that an enhancement of 5-HT neurotransmission is important in the therapeutic response produced by MAOI in depressed patients because tryptophan depletion, as well as 5-HT synthesis inhibition, produced a rapid relapse in remitted patients treated with an MAOI (Shopsin *et al.*, 1976; Delgado *et al.*, 1990).

Supported by the Medical Research Council of Canada Grant no. MA-11014 and Scholarship to P.B., the Fonds de la Recherche en Santé du Québec, and Centre de Recherche Delalande. We would like to thank T. Vo and L. Caillé for their help with the statistical analyses, and H. Cameron for secretarial assistance.

References

AZZARO, A.J., KING, J., KOTZUK, J., SCHOEPP, D.D., FROST, J. & SCHOCHET, S. (1985). Guinea pig striatum as a model of human dopamine distribution: the role of monoamine oxidase isozyme ratio, localization and affinity for substrate in synaptic dopamine metabolism. *J. Neurochem.*, **45**, 949–956.

BAUMANN, P.A. & WALDMEIER, P.C. (1981). Further evidence for negative feedback control of serotonin release in the central nervous system. *Naunyn-Schmied. Arch. Pharmacol.*, **317**, 36–43.

BERGERON, R. & BLIER, P. (1994). Cisapride for the treatment of nausea produced by selective serotonin reuptake inhibitors. *Am. J. Psychiat.*, **151**, 1084–1086.

BLIER, P. & BOUCHARD, C. (1993). Functional characterization of a 5-HT_3 receptor which modulates the release of 5-HT in the guinea-pig brain. *Br. J. Pharmacol.*, **108**, 13–22.

BLIER, P., CHAPUT, Y. & DE MONTIGNY, C. (1988). Long-term 5-HT reuptake blockade, but not monoamine oxidase inhibition, decreases the function of terminal 5-HT autoreceptors: an electrophysiological study in the rat brain. *Naunyn-Schmied. Arch. Pharmacol.*, **337**, 246–254.

BLIER, P. & DE MONTIGNY, C. (1994). Current advances and trends in the treatment of depression. *Trends Pharmacol. Sci.*, **15**, 220–226.

BLIER, P., GALZIN, A.-M. & LANGER, S.Z. (1990). Interaction between serotonin uptake inhibitors and alpha-2 adrenergic heteroreceptors in the rat hypothalamus. *J. Pharmacol. Exp. Ther.*, **254**, 236–244.

BLIER, P., MONROE, P.J., BOUCHARD, C., SMITH, D.C. & SMITH, D.J. (1993). 5-HT_3 receptors which modulate $[^3H]$-5-HT release in the guinea pig hypothalamus are not autoreceptors. *Synapse*, **15**, 143–148.

CHAPUT, Y., DE MONTIGNY, C. & BLIER, P. (1986). Effects of a selective 5-HT reuptake blocker, citalopram, on the sensitivity of 5-HT autoreceptors: electrophysiological studies in the rat. *Naunyn-Schmied. Arch. Pharmacol.*, **33**, 342–349.

CHAPUT, Y., DE MONTIGNY, C. & BLIER, P. (1991). Pre- and postsynaptic modifications of the serotonin system by long-term administration of antidepressant treatments: an *in vivo* electrophysiological study in the rat. *Neuropsychopharmacol.*, **5**, 219–229.

CURET, O., DAMOISEAU, G. & AUBIN, N. (1992). Biochemical profile of befloxatone, a new reversible MAO-A inhibitor. *Clin. Neuropharmacol.*, **15** (Suppl. 1B), 424.

DE MONTIGNY, C. & AGHAJANIAN, G.K. (1978). Tricyclic antidepressants: long-term treatment increases responsitivity of rat forebrain neurons to serotonin. *Science*, **202**, 1303–1306.

DELGADO, P.L., CHARNEY, D.S., PRICE, L.H., AGHAJANIAN, G.K., LANDIS, H. & HENINGER, G.R. (1990). Serotonin function and the mechanism of antidepressant action: reversal of antidepressant-induced remission by rapid depletion of plasma tryptophan. *Arch. Gen. Psychiatry*, **47**, 411–418.

DELGADO, P.L., MILLER, H.L., SALOMON, R.M., LICINIO, J., PRICE, L.H., HENINGER, G.R. & CHARNEY, D.S. (1992). The mechanism of antidepressant action in depression. *Soc. Neurosci.*, Abst 18, p. 727.

FEUERSTEIN, T.J., MUTSCHLER, A., LUPP, A., VAN VILTHOVEN, V., SCHLICKER, E. & GÖTHERT, M. (1993). Endogenous noradrenaline activates α_2-adrenoceptors on serotonergic nerve endings in human and rat neocortex. *J. Neurochem.*, **61**, 474–480.

GALZIN, A.M. & LANGER, S.Z. (1991). Modulation of 5-HT release by presynaptic inhibitory and facilitatory 5-HT receptors in brain slices. *Adv. Biosci.*, **82**, 59–62.

GALZIN, A.M., POIRIER, M.F., LISTA, A., CHODKIEWIECZ, J.P., BLIER, P., RAMDINE, R., LOO, H., ROUX, F.X., REDONDO, A. & LANGER, S.Z. (1992). Characterization of the 5-hydroxytryptamine autoreceptor modulating the release of $[^3H]$-5-hydroxytryptamine in slices of the human neocortex. *J. Neurochem.*, **59**, 1293–1301.

HRDINA, P.D. & VENE, T.B. (1993). Chronic fluoxetine treatment upregulates 5-HT uptake sites and 5-HT_2 receptors in rat brain: an autoradiographic study. *Synapse*, **14**, 324–331.

HOYER, D. & MIDDLEMISS, D.K. (1989). The pharmacology of terminal 5-HT autoreceptors in mammalian brain: evidence for species differences. *Trends Pharmacol. Sci.*, **10**, 130–132.

JACOBS, B.L. (1986). Single unit activity of brain monoamine-containing neurons in freely moving animals. *N.Y. Acad. Sci.*, **473**, 70–77.

LESCH, K.P., AUKLAKH, C.S., WOLOZIN, B.J., TOLLIVER, T.J., HILL, J.L. & MURPHY, D.L. (1993). Regional brain expression of serotonin transporter mRNA and its regulation by reuptake inhibiting antidepressants. *Mol. Brain Res.*, **17**, 31–35.

MENKES, D.B. & AGHAJANIAN, G.K. (1981). α_1-Adrenoceptor-mediated responses in the lateral geniculate nucleus are enhanced by chronic antidepressant treatment. *Eur. J. Pharmacol.*, **74**, 27–35.

MENKES, D.B., AGHAJANIAN, G.K. & MCCALL, R.B. (1980). Chronic antidepressant treatment enhances α-adrenergic and serotonergic responses in the facial nucleus. *Life Sci.*, **27**, 45–55.

MONGEAU, R., BLIER, P. & DE MONTIGNY, C. (1993). *In vivo* electrophysiological evidence for a tonic inhibitory action of endogenous norepinephrine on α_2-adrenergic heteroreceptors on 5-hydroxytryptamine terminals in the rat hippocampus. *Naunyn Schmied. Arch. Pharmacol.*, **347**, 266–272.

MONGEAU, R., DE MONTIGNY, C. & BLIER, P. (1994). Electrophysiological evidence for desensitization of α_2-adrenoceptors on 5-HT terminals following long-term treatment with drugs that increase noradrenaline synaptic availability. *Neuropsychopharmacol.*, **10**, 41–51.

MORET, C. & BRILEY, M. (1990a). Serotonin autoreceptor subsensitivity and antidepressant activity. *Eur. J. Pharmacol.*, **180**, 351–356.

MORET, C. & BRILEY, M. (1990b). Modulation by drugs of the release of total tritium and ^{3}H5-HT from rat hypothalamic slices. *Naunyn-Schmied. Arch. Pharmacol.*, **341**, 398–403.

PASSARELLI, F., GALZIN, A.-M. & LANGER, S.Z. (1987). Interaction between neuronal uptake inhibitors and presynaptic serotonin autoreceptors in rat hypothalamic slices: comparison of K^+ and electrical depolarization. *J. Pharmacol. Exp. Ther.*, **242**, 1056–1063.

PIÑEYRO, G., BLIER, P., DENNIS, T. & DE MONTIGNY, C. (1994). Desensitization of the neuronal 5-HT reuptake carrier following its long-term blockade. *J. Neurosci.*, **14**, 3036–3047.

RAITERI, M., MAURA, G., FOLGHERA, S., CAVAZZANI, P., ANDRIOLI, G.C., SCHLICKER, E., SCHALNUS, R. & GÖTHERT, M. (1990). Modulation of 5-hydroxytryptamine release by presynaptic inhibitory α_2-adrenoceptors in the human cerebral cortex. *Naunyn-Schmied. Arch. Pharmacol.*, **342**, 508–512.

SHOPSIN, B., FRIEDMAN, E. & GERSHON, S. (1976). Parachlorophenylalanine reversal of tranylcypyromine effects in depressed patients. *Arch. Gen. Psychiatry*, **33**, 811–819.

STARKE, K., GÖTHERT, M. & KILBINGER, H. (1989). Modulation of neurotransmitter release by presynaptic autoreceptors. *Physiol. Rev.*, **69**, 864–969.

WANG, R.Y. & AGHAJANIAN, G.K. (1980). Enhanced sensitivity of amygdaloid neurons to serotonin and norepinephrine by antidepressant treatment. *Comm. Psychopharmacol.*, **4**, 83–90.

(Received May 31, 1994
Accepted June 13, 1994)