British Journal of Pharmacology (1996) 118, 681-689

Functional characterization of 5-HT_{1D} autoreceptors on the modulation of 5-HT release in guinea-pig mesencephalic raphe, hippocampus and frontal cortex

[1]Mostafa El Mansari & Pierre Blier

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

1 The aims of the present study were (i) to characterize further the pharmacology of 5-HT_{1D} autoreceptors modulating 5-HT release in guinea-pig mesencephalic raphe, hippocampus and frontal cortex; (ii) to determine whether 5-HT_{1D} receptors in the mesencephalic raphe are located on 5-HT neurones; (iii) to determine whether 5-HT_{1D} autoreceptors are coupled to G proteins; and (iv) to assess their sensitivity following long-term 5-HT reuptake blockade and inhibition of type-A monoamine oxidase.

2 In mesencephalic raphe, hippocampus and frontal cortex slices, the 5-$HT_{1D/1B}$ receptor agonist, sumatriptan and the 5-HT_1 receptor agonist, 5-methoxytryptamine (5-MeOT) but not the 5-HT_{1B} receptor agonist, CP93129, inhibited electrically the evoked release of [^{3}H]-5-HT in a concentration-dependent manner. This effect was antagonized by the 5-$HT_{1D/1B}$ receptor antagonist GR127935 in the three structures, but not by the 5-HT_{1A} receptor antagonist, (+)-WAY100635 in mesencephalic raphe slices. These results confirm the presence of functional 5-HT_{1D} autoreceptors controlling 5-HT release within the mesencephalic raphe as well as in terminal regions.

3 The inhibitory effect of sumatriptan on K^+-evoked release of [^{3}H]-5-HT was not reduced by the addition of the Na^+ channel blocker, tetrodotoxin to the superfusion medium, suggesting that these 5-HT_{1D} receptors in the mesencephalic raphe are located on 5-HT neurones and may be considered autoreceptors.

4 The *in vitro* treatment with the alkylating agent N-ethylmaleimide (NEM) was used to determine whether these 5-HT_{1D} autoreceptors are coupled to G proteins. The inhibitory effect of sumatriptan on electrically evoked release of [^{3}H]-5-HT was attenuated in NEM-pretreated slices from mesencephalic raphe, hippocampus and frontal cortex, indicating that the 5-HT_{1D} autoreceptors activated by sumatriptan are coupled to G proteins in these three structures. Taken together with our previous results, this suggests that, in addition to the 5-HT_{1D} autoreceptor activated by sumatriptan, another subtype of 5-HT autoreceptor is activated by 5-MeOT in the hippocampus.

5 Following a 3-week treatment with the selective 5-HT reuptake inhibitor, paroxetine (10 mg kg^{-1} day^{-1}) and a 48 h washout period, the electrically evoked release of [^{3}H]-5-HT was enhanced in mesencephalic raphe, hippocampus and frontal cortex slices. There was an attenuation of the capacity of sumatriptan to inhibit the evoked release of [^{3}H]-5-HT from mesencephalic raphe slices but not from frontal cortex and hippocampus slices. Only in the latter structure was the suppressant effect of 5-MeOT attenuated. After a 3-week treatment with the reversible type-A monoamine oxidase inhibitor, befloxatone (0.75 mg kg^{-1} day^{-1}) and 48 h washout period, the effectiveness of sumatriptan and 5-MeOT on the evoked release of [^{3}H]-5-HT was unaltered in the same brain structures.

6 The enhancement of [^{3}H]-5-HT release by long-term paroxetine treatment is possibly due to a desensitization of 5-HT_{1D} autoreceptors activated by sumatriptan in mesencephalic raphe and by terminal 5-HT autoreceptors activated by 5-MeOT in hippocampus. In the case of the frontal cortex, it appears that 5-MeOT and sumatriptan may act on the same 5-HT_{1D} autoreceptor which is not desensitized either after paroxetine or befloxatone treatment, as previously reported.

Keywords: [^{3}H]-5-HT release; 5-HT_{1D} autoreceptors; tetrodotoxin; selective 5-HT reuptake blockade; monoamine oxidase inhibition; N-ethylmaleimide; mesencephalic raphe; hippocampus; frontal cortex

Introduction

Several lines of evidence indicate that 5-hydroxytryptamine (5-HT) release is modulated by somatodendritic and terminal 5-HT autoreceptors. Previous studies have demonstrated that somatodendritic 5-HT_{1A} autoreceptors inhibit the spontaneous firing rate of dorsal 5-HT raphe neurones in rats and guinea-pigs (Aghajanian *et al.*, 1987; Mundey *et al.*, 1994) which in turn results in a reduction of the amount of 5-HT released in the terminal regions of these neurones (Adell & Artigas, 1991). In addition, it has been shown, *in vitro*, that 5-HT_{1D} receptors can also modulate 5-HT release in mesencephalic raphe of the guinea-pig and rat (Piñeyro *et al.*, 1993; Starkey & Skingle, 1994; Piñeyro & Blier, 1996).

Terminal 5-HT autoreceptors in projection areas exert a negative feedback on 5-HT release and have been shown to be of the 5-HT_{1B} receptor subtype in the rat and of the 5-HT_{1D} receptor subtype in the guinea-pig and man (Middlemiss, 1985; Engel *et al.*, 1986; Hoyer & Middlemiss, 1989; Limberger *et al.*, 1991; Galzin *et al.*, 1992; Maura *et al.*, 1993). Pharmacological studies indicate differences in the profile of the cloned 5-HT_{1D} receptors and the existence of two isoforms has been demonstrated: 5-$HT_{1D\alpha}$ and 5-$HT_{1D\beta}$ (Weinshank *et al.*, 1992; Zgombic *et al.*, 1993). Similarly, heterogeneity of the 5-HT

[1] Author for correspondence.

autoreceptors mediating inhibition of [^{3}H]-5-HT in *in vitro* experiments release from the hippocampus of guinea-pigs was postulated based on the potency of methiothepin in antagonizing the inhibitory effect of the 5-HT receptor agonists, sumatriptan, 5-carboxamidotryptamine and 5-HT itself (Wilkinson & Middlemiss, 1992).

The ability of the cloned human 5-HT_{1D} receptor to inhibit the accumulation of forskolin-stimulated cyclicAMP suggests that the 5-HT_{1D} receptors are coupled to G proteins (Schoeffter *et al.*, 1988; Hamblin & Metcalf, 1991). It is possible that differences in mechanisms modulating the function of the autoreceptors in the 5-HT projection areas might constitute a tool to distinguish different 5-HT autoreceptors suggested above.

The present study was undertaken to characterize further the pharmacology of 5-HT_{1D} receptors modulating 5-HT release in the guinea-pig mesencephalic raphe containing the dorsal and median raphe nuclei, the hippocampus and the frontal cortex, and to determine whether these 5-HT_{1D} receptors are in fact located on 5-HT mesencephalic raphe neurones. Then, we determined whether 5-HT_{1D} receptor subtypes in mesencephalic raphe, hippocampus and frontal cortex are sensitive to alteration of the activity of G proteins using *in vitro* exposure to the alkylating agent N-ethylmaleimide (NEM). Finally, we examined the effect of long-term treatment with paroxetine, a selective 5-HT reuptake inhibitor (SSRI), and befloxatone, a selective and reversible type-A monoamine oxidase inhibitor (MAOI) on the sensitivity of 5-HT_{1D} receptors by use of the 5-HT_{1D} receptor agonist, sumatriptan.

Methods

Treatment

Male Hartley guinea-pigs (250–300 g) were anaesthetized with halothane and implanted with an osmotic minipump (Alza Palo Alto, CA, U.S.A.) that delivered the SSRI paroxetine (10 mg kg^{-1} day^{-1}), the reversible MAOI, befloxatone (0.75 mg kg^{-1} day^{-1}), or the vehicle used to dilute these two drugs (50/50 ethanol and water). After 3 weeks of treatment, the minipumps were removed under halothane anaesthesia and the *in vitro* experiments were carried out 48 h later to allow elimination of paroxetine or befloxatone. The rationale for using these treatment regimens is the following. Previous studies have shown that 10 mg kg^{-1} day^{-1} paroxetine or 0.75 mg kg^{-1} day^{-1} befloxatone produced a desensitization of the terminal 5-HT autoreceptors and of the α_2-adrenoceptor heteroreceptors on 5-HT terminals, respectively, in the guinea-pig hippocampus after a 3-week treatment (Blier & Bouchard, 1994).

In order to study the coupling of the 5-HT receptors mediating inhibition of [^{3}H]-5-HT release to G proteins, sumatriptan and the 5-HT analogue, 5-methoxytryptamine, were used as agonists; 5-HT itself could not be used since it produces a marked increase in basal ^{3}H efflux in the absence of a SSRI. Moreover, in the presence of a SSRI in the superfusion medium, the concentration-effect curves for 5-HT autoreceptor agonists are shifted to the right (Galzin *et al.*, 1985).

Superfusion experiments

Guinea-pigs were killed by decapitation and the brain immediately removed and rapidly dissected on an ice-cold glass plate. Slices, 400 μm thick, from the mesencephalic raphe, hippocampus and frontal cortex were prepared with a McIlwain tissue chopper. The slices were then incubated for 30 min at 37°C in Krebs buffer containing 100 nM [^{3}H]-5-HT creatinine sulphate for mesencephalic raphe and 20 nM for hippocampus and frontal cortex (specific activity 1.1 TBq $mmol^{-1}$; NEN Research Products, Mississauga, Canada) and bubbled with a mixture of 95% O_2/5% CO_2. The composition of the Krebs solution was the following (in mM): NaCl 118, KCl 4.7, $CaCl_2$ 1.3, $MgCl_2$ 1.2, NaH_2PO_4 1, $NaHCO_3$ 25, glucose 11.1, Na_2 EDTA 0.004 and ascorbic acid 0.11. At the end of the incubation period, the slices were washed, transferred to glass superfusion chambers, and superfused at a rate of 0.5 ml min^{-1} with oxygenated Krebs solution maintained at 37°C. Nineteen consecutive 4-min fractions were collected starting 90 min after the beginning of superfusion for the three structures. Two periods of stimulation, S_1 and S_2, were carried out within the same experiment at 8 min and 52 min, respectively, after the end of the 90-min washing period. The electrical field was generated in the chambers between 2 platinum electrodes (30 mA, 2 ms, 3 Hz for 2 min) positioned 2 cm apart. The frequency of stimulation was chosen because it was within the range of the firing rate of 5-HT neurones recorded in freely moving cats (Jacobs, 1986). To determine whether 5-HT_{1D} receptors are autoreceptors, tetrodotoxin was used to prevent the occurrence of Na^+-dependent action potentials, by blocking Na^+ channels. The slices were stimulated twice (S_1 and S_2) for 4 min with Krebs solution containing 25 mM KCl with equimolar reduction of NaCl to maintain isotonicity. In this case when [^{3}H]-5-HT release is evoked with high concentration of potassium, only 5-HT cell bodies or 5-HT axon terminals remain potential targets of drug action (Starke *et al.*, 1989). In support of this, it was shown that the 5-HT_3 receptors that increase the evoked release of [^{3}H]-5-HT in preloaded guinea-pig hypothalamic slices are not located on 5-HT terminals because TTX abolished the enhancing effect of the 5-HT_3 agonist, 2-methyl-5-HT (Blier *et al.*, 1993). The first stimulation period (S_1) was used as control and the drugs were added 20 min before S_2 and remained present throughout the rest of the experiment. At the end of superfusion period, the slices were solubilized with 0.5 ml Soluene 350 (Packard Instruments, Downers grove, IL, U.S.A.), and the radioactivity in the slices and superfusate samples was determined by liquid scintillation spectrometry. The results are expressed as the fraction of the tritium content present at the time of the onset of the respective collection periods. The fractional release evoked by electrical stimulation was calculated as the difference between the total amount of radioactivity released during stimulation and the basal outflow obtained in the sample preceding the onset of stimulation (Sp_1 or Sp_2). To assess the drug-induced changes of electrically evoked release of tritium from the slices preloaded with [^{3}H]-5-HT, the ratio of fractional release between the second and the first period of stimulation (S_2/S_1) was calculated. The Sp_2/Sp_1 ratios were also calculated to determine whether the drugs altered the basal outflow of radioactivity. The amount of tritium released by electrical stimulation under these conditions provides a reliable estimate of the release of tritiated or endogenous 5-HT (Baumann *et al.*, 1981; Blier & Bouchard, 1993).

Results are expressed as mean ± s.e.mean. Differences between the controls and the treated groups were compared with Student's two-tailed *t* test or the Mann-Whitney test. In order to detect treatment effects, concentration-effect curves were constructed by studying simultaneously in the same superfusion apparatus slices prepared from a control and slices from a treated guinea-pig with the same drug solution. The entire concentration-effect curves, rather than the means for each concentration, was compared using 3-factor analysis of variance (independent variables: agonist concentrations, treatment and animal; dependent variable: S_2/S_1 ratio for the individual chambers). This experimental design was deemed optimal to minimize the problem of inter-experimental variations. Probability values (P) <0.05 were considered as significant.

The following drugs were used: 5-carboxamidotryptamine (Research Biochemicals International, Natick, MA, U.S.A.), 5-methoxytryptamine (Sigma, St-Louis, MO, U.S.A.), befloxatone (Delalande, Rueil-Malmaison, France), CP93129 (Pfizer, Groton, CT, U.S.A.), GR127935 and sumatriptan (Glaxo, Greenford, U.K.), methiothepin maleate (Hoffman La Roche, Basel, Switzerland), N-ethylmaleimide (Sigma, St-

Louis, MO, U.S.A.), paroxetine (SmithKline Beecham, Harlow, England), tetrodotoxin (Research Biochemicals International, Natick, MA, U.S.A.), (+)-WAY100635 (Wyeth Research, Berkshire, U.K.).

Results

Effect of 5-HT receptor ligands on the electrically evoked release of tritium from [^{3}H]-5-HT preloaded slices of mesencephalic raphe, hippocampus and frontal cortex

In guinea-pig mesencephalic raphe, hippocampus and frontal cortex slices, the 5-HT$_{1D/1B}$ receptor agonist, sumatriptan and 5-MeOT, introduced 20 min before S_2, inhibited the electrically evoked release of tritium in a concentration-dependent manner (Figures 5, 6, 7). The spontaneous outflow of radioactivity was not altered with any of the concentrations used (data not shown). The 5-HT$_{1D/1B}$ receptor antagonist GR127935 (0.3 μM) introduced 20 min before S_1 significantly increased [^{3}H]-5-HT release in slices prepared from mesencephalic raphe (43%), hippocampus (50%) and frontal cortex (37%). When introduced 20 min before S_1, GR127935 (0.3 μM) did not modify the S_2/S_1 ratio of [^{3}H]-5-HT release in mesencephalic raphe (S_2/S_1: 1.19±0.09, $n=4$ versus 1.00±0.06, $n=6$ in controls), in hippocampus (S_2/S_1: 1.00±0.10, $n=5$ versus 1.08±0.03, $n=5$ in controls), and in frontal cortex slices (S_2/S_1: 0.90±0.09, $n=5$ versus 1.02±0.05, $n=7$ in controls). The spontaneous outflow of radioactivity was not altered by 0.3 μM GR127935 but significantly enhanced at concentration of 1 μM (data not shown). Under these conditions, GR127935 (0.3 μM) introduced 20 min before S_1 and maintained in the superfusion medium until the end of the experiment prevented the inhibition produced by 1 μM sumatriptan and 0.1 μM 5-MeOT in mesencephalic raphe, hippocampus and frontal cortex slices (Figures 1, 2, 3). In the mesencephalic raphe slices, when the 5-HT$_{1A}$ antagonist (+)-WAY100635 (1 μM) was added 20 min before S_1 and maintained in the superfusion medium until the end of experiment, the ratio S_2/S_1 was slightly but significantly increased (S_2/S_1 1.24±0.06, $n=4$ versus 1.03±0.06, $n=10$ in controls) without altering the spontaneous outflow. Under these conditions, (+)-WAY100635 antagonized the inhibitory effect of 0.1 μM 5-MeOT but not of 1 μM sumatriptan introduced 20 min before S_2 (Figure 1). The 5-HT$_{1B}$ receptor agonist, CP93129 (0.1 μM), introduced 20 min before S_2, had no effect on evoked [^{3}H]-5-HT release in mesencephalic raphe, hippocampus and frontal cortex slices (Figures 1, 2, 3). At a concentration of 1 μM, CP93129 increased spontaneous tritium outflow in the 3 structures studied and this effect was not prevented by the SSRI, paroxetine (data not shown).

Effect of tetrodotoxin on the modulation of K$^+$-release of tritium from [^{3}H]-5-HT preloaded slices of mesencephalic raphe

When added to the medium superfusing mesencephalic raphe slices preloaded with [^{3}H]-5-HT, 1 μM sumatriptan inhibited by 42% the overflow of tritium evoked by 25 mM K$^+$ (Figure 4). This inhibition was significantly smaller than that produced by the same concentration of sumatriptan on the electrically evoked release of [^{3}H]-5-HT.

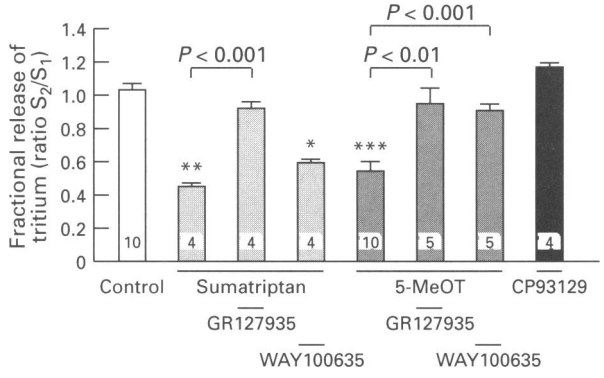

Figure 1 Effect of sumatriptan (a 5-HT$_{1D/1B}$ receptor agonist), 5-MeOT (a 5-HT$_1$ receptor agonist) and CP93129 (a 5-HT$_{1B}$ receptor agonist) on the electrically evoked release of tritium in mesencephalic raphe slices preloaded with [^{3}H]-5-HT. The inhibitory effect of sumatriptan (1 μM) was antagonized by GR127935 (0.3 μM), a 5-HT$_{1D/1B}$ receptor antagonist but not by the 5-HT$_{1A}$ receptor antagonist, (+)-WAY100635 (1 μM). The inhibitory effect of 5-MeOT (0.1 μM) was antagonized by either GR127935 (0.3 μM) or (+)-WAY100635 (1 μM). The antagonists were introduced 20 min before S_1 and remained present in the superfusate until the end of the experiment. The agonists were introduced 20 min before S_2. Values are expressed as means±s.e.mean for which the number of the experiments per group is given at the bottom of each column.

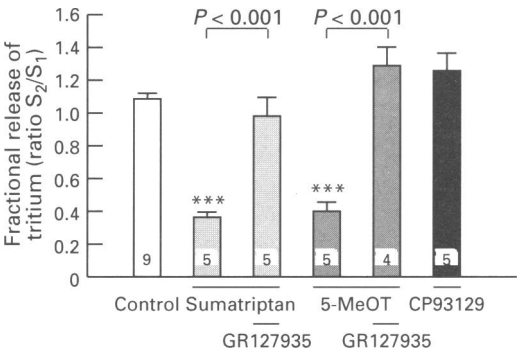

Figure 2 Effect of sumatriptan (a 5-HT$_{1D/1B}$ receptor agonist), 5-MeOT (a 5-HT$_1$ receptor agonist) and CP93129 (a 5-HT$_{1B}$ receptor agonist) on the electrically evoked release of tritium in hippocampus slices preloaded with [^{3}H]-5-HT. The inhibitory effect of sumatriptan (1 μM) and 5-MeOT (0.1 μM) was antagonized by GR127935 (0.3 μM), a 5-HT$_{1D/1B}$ receptor antagonist. The antagonist was introduced 20 min before S_1 and remained present in the superfusate until the end of the experiment. The agonists were introduced 20 min before S_2. Values are expressed as means±s.e.mean for which the number of the experiments per group is given at the bottom of each column.

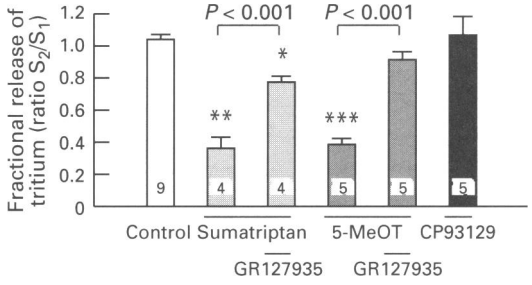

Figure 3 Effect of sumatriptan (a 5-HT$_{1D/1B}$ receptor agonist), 5-MeOT (a 5-HT$_1$ receptor agonist) and CP93129 (a 5-HT$_{1B}$ receptor agonist) on the electrically evoked release of tritium in frontal cortex slices preloaded with [^{3}H]-5-HT. The inhibitory effect of sumatriptan (1 μM) and 5-MeOT (0.1 μM) was antagonized by GR127935 (0.3 μM), a 5-HT$_{1D/1B}$ receptor antagonist. The antagonist was introduced 20 min before S_1 and remained present in the superfusate until the end of the experiment. The agonists were introduced 20 min before S_2. Values are expressed as means±s.e.mean for which the number of the experiments per group is given at the bottom of each column.

To determine whether the inhibitory 5-HT$_{1D}$ receptors are located on 5-HT neurones, tetrodotoxin (TTX, 1 μM) which blocks Na_+ channels (see Starke *et al.*, 1989) was added to the superfusion medium 20 min before S_1. The Sp_2/Sp_1 ratio was not altered by exposure to TTX, indicating that this drug did not interfere with the spontaneous tritium outflow. In the presence of TTX, the inhibitory effect of 1 μM sumatriptan, introduced 20 min before S_2, was not altered (Figure 4), indicating that 5-HT$_{1D}$ are autoreceptors in mesencephalic raphe.

Effect of in vitro *N-ethylmaleimide exposure on the modulation of tritium release by the 5-HT$_{1D}$ autoreceptor from [^{3}H]-5-HT preloaded slices*

An *in vitro* pretreatment with the alkylating agent, NEM, was used to assess whether the 5-HT$_{1D}$ autoreceptors are coupled to G proteins in mesencephalic raphe, hippocampus and frontal cortex. In these experiments, the slices were incubated with 30 μM of NEM for 30 min. At this concentration, Blier (1991) has shown that NEM, which inactivates G proteins through the alkylation of the sulphydryl group of their α subunits (Hertting *et al.*, 1988) did not alter the basal outflow (Sp_2/Sp_1) nor the function of the 5-HT reuptake carrier, indicating that this concentration produced minimal non-specific effects. Under these conditions, the fractional release of [^{3}H]-5-HT induced by electrical stimulation during S_1 was significantly increased in slices prepared from mesencephalic raphe (44%), hippocampus (50%) and frontal cortex (39%) pretreated with NEM, consistent with a negative feedback role of G protein coupled 5-HT$_{1D}$ autoreceptors controlling [^{3}H]-5-HT release (Table 1).

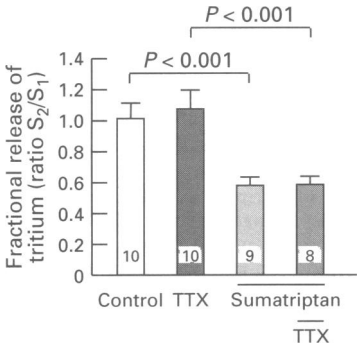

Figure 4 Effect of sumatriptan (a 5-HT$_{1D/1B}$ receptor agonist) on the electrically evoked release of tritium in mesencephalic raphe slices preloaded with [^{3}H]-5-HT. The inhibitory effect of sumatriptan was not antagonized by tetrodotoxin, a Na^+ channel blocker, in mesencephalic raphe. The tetrodotoxin was introduced 20 min before S_1 and remained present in the superfusate until the end of the experiment. The agonist sumatriptan was introduced 20 min before S_2. Values are expressed as means ± s.e.mean for which the number of the experiments per group is given at the bottom of each column.

In order to assess the sensitivity of the 5-HT$_{1D}$ autoreceptors following a 30 min *in vitro* treatment with NEM, concentration-effect curves were constructed with the 5-HT$_{1D/1B}$ agonist, sumatriptan, to inhibit electrically evoked release of [^{3}H]-5-HT. The inhibitory effect of sumatriptan on evoked release of [^{3}H]-5-HT was attenuated in NEM-pretreated slices from mesencephalic raphe, hippocampus and frontal cortex (Figure 5). These observations indicate that 5-HT$_{1D}$ autoreceptors activated by sumatriptan are coupled to G proteins in the three structures studied.

Given the results of Wilkinson & Middlemiss (1992) showing the existence of two 5-HT receptors, one activated by sumatriptan and 5-CT and another by 5-HT, the present experiments were carried out using 5-MeOT instead of 5-HT for the reasons cited in Methods. In mesencephalic raphe and frontal cortex slices pretreated with NEM, there was a significant attenuation in the inhibitory effect of 5-MeOT on the electrically evoked release of tritium (Figure 5a, c). Following such a treatment, however, the effectiveness of 5-MeOT in decreasing the evoked release of [^{3}H]-5-HT was unaltered in hippocampal slices (Figure 5b). These results indicate that 5-HT autoreceptors activated by 5-MeOT are coupled to G proteins in the mesencephalic raphe and frontal cortex, but not in the hippocampus.

The coupling of terminal 5-HT$_{1D}$ autoreceptors to G proteins was also assessed using the 5-HT receptor antagonist, methiothepin, which would be expected to exert a smaller effect on [^{3}H]-5-HT overflow than in controls, should a pretreatment reduce the function of the terminal 5-HT autoreceptor. Exposure to 0.3 μM methiothepin, 20 min before S_2 enhanced the electrically evoked release of [^{3}H]-5-HT in control slices from hippocampus (S_2/S_1 : 1.44 ± 0.10 versus 1.01 ± 0.06 in controls, $n=5$) and frontal cortex (S_2/S_1 : 1.44 ± 0.06 versus 1.00 ± 0.07 in controls, $n=8$), but this effect was no longer present after a pretreatment with NEM in these two structures (S_2/S_1 : hippocampus 1.09 ± 0.10, $n=4$; frontal cortex $S_2/S_1 = 1.15 \pm 0.09$, $n=8$).

Effect of long-term paroxetine and befloxatone administration on the electrically evoked release of tritium from [^{3}H]-5-HT preloaded slices

The electrically evoked release of [^{3}H]-5-HT in the absence of any drug in S_1 was significantly enhanced following the 3-week treatment with paroxetine by 17%, 40% and 28% in the mesencephalic raphe, hippocampus and frontal cortex slices, respectively, when compared to controls processed in parallel in the same experiments (Table 1). The spontaneous outflow of radioactivity in the sample immediately preceding this first

Table 1 Effect of N-ethylmaleimide, long-term paroxetine and befloxatone treatment on the electrically evoked release (S_1[a]) and spontaneous outflow (Sp_1[b]) of tritium from guinea-pig brain slices preloaded with [^{3}H]-5-HT

	Mesencephalic raphe		*Hippocampus*		*Frontal cortex*	
	S_1	Sp_1	S_1	Sp_1	S_1	Sp_1
Control	1.36 ± 0.16	1.43 ± 0.09	2.01 ± 0.10	1.43 ± 0.08	2.39 ± 0.13	1.08 ± 0.10
N-ethylmaleimide	1.96 ± 0.19*	1.51 ± 0.10	3.01 ± 0.26**	2.02 ± 0.09***	3.32 ± 0.16***	1.62 ± 0.07***
Control	0.98 ± 0.05	1.30 ± 0.05	1.48 ± 0.09	1.12 ± 0.06	2.36 ± 0.20	0.95 ± 0.04
Paroxetine	1.15 ± 0.06*	1.29 ± 0.06	2.08 ± 0.10***	1.12 ± 0.05	3.03 ± 0.17*	0.86 ± 0.05
Control	0.90 ± 0.06	1.41 ± 0.08	1.62 ± 0.08	1.25 ± 0.04	1.68 ± 0.10	1.10 ± 0.04
Befloxatone	1.01 ± 0.07	1.36 ± 0.04	2.21 ± 0.12***	1.31 ± 0.04	2.34 ± 0.10***	1.24 ± 0.04*

[a] Fraction of the total radioactivity released by 360 pulses (30 mA, 2 ms, 3 Hz) in the first period of stimulation before the introduction of any drug in the superfusate. [b] Percentage of total tritium content present in the slices in the 4-min sample of perfusate collected immediately before the first period of electrical stimulation. The number given in parentheses refers to the number of experiments. *$P<0.05$, **$P<0.01$, ***$P<0.001$.

period of stimulation (Sp_1) was unaltered in mesencephalic raphe, hippocampus and frontal cortex slices prepared from the paroxetine-treated guinea-pigs as compared to controls (Table 1).

In the slices prepared from guinea-pigs treated with befloxatone for 3 weeks, the electrically evoked release of [^{3}H]-5-HT was also significantly enhanced by 36% and 39% hippocampus and frontal cortex slices, respectively, but not significantly so in the mesencephalic raphe slices (12%) when compared with the corresponding control values (Table 1). The spontaneous outflow of radioactivity was unchanged in mesencephalic raphe, hippocampus and frontal cortex slices prepared from befloxatone-treated guinea-pigs (Table 1).

Effect of long-term paroxetine and befloxatone treatment on the modulation of tritium release by the somatodendritic and terminal 5-HT$_{1D}$ autoreceptors from [^{3}H]-5-HT preloaded slices

In previous studies, the inhibitory effect of 5-methoxytryptamine was attenuated in hippocampus but not in frontal cortex after a 3-week paroxetine treatment, suggesting that the terminal 5-HT autoreceptor was only desensitized in hippocampus (Blier & Bouchard, 1994; El Mansari *et al.*, 1995). However, following a 3-week befloxatone treatment, the 5-HT autoreceptor was not desensitized in the hippocampus nor in the frontal cortex (Blier & Bouchard, 1994).

In the present study, there was an attenuation of the ca-

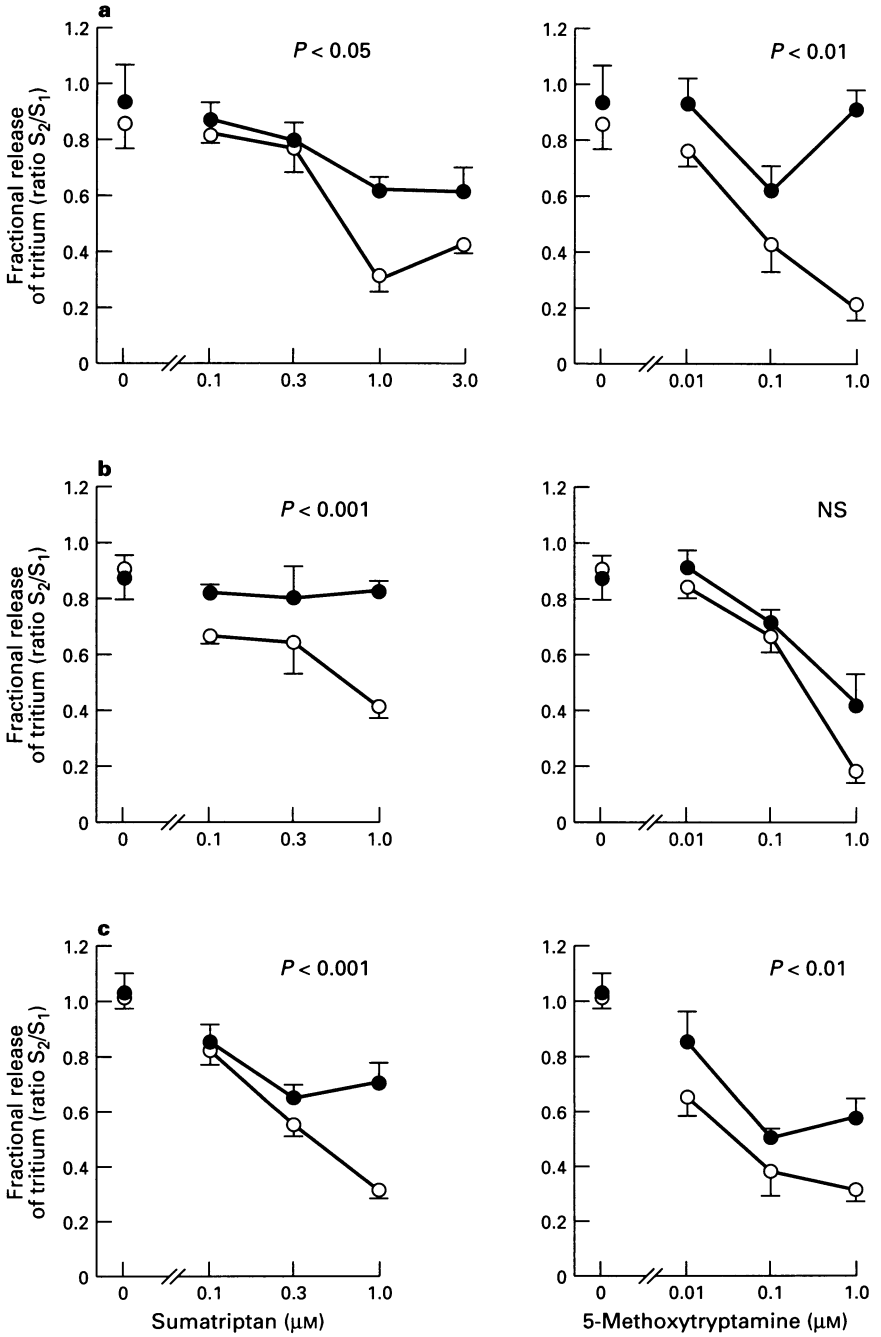

Figure 5 Concentration-effect curves of the 5-HT$_{1D/1B}$ receptor agonist, sumatriptan, introduced 20 min before S_2, on the release of tritium elicited by the electrical stimulation of mesencephalic raphe (a), hippocampus (b) and frontal cortex (c) slices treated with (●) or without (○) NEM. Ordinate scale is the fraction of total tissue radioactivity released by 360 pulses (30 mA, 2 ms, 3 Hz for 2 min) expressed as the S_2/S_1 ratio. Each point represents the mean ± s.e.mean of at least five experiments per group. The level of statistical significance, calculated by three-factor analysis of variance between the curves obtained in the control and NEM-pretreated slices is indicated in the graphs (NS: non-significant).

pacity of sumatriptan to inhibit the electrically evoked release of tritium from preloaded mesencephalic raphe slices but not from hippocampus and frontal cortex slices after a 3-week paroxetine treatment (Figure 6a, b, c). Similarly, 5-CT (a 5-HT autoreceptor agonist; 3 and 30 nM) added 20 min before S_2, inhibited the electrically evoked overflow of [^{3}H]-5-HT in the hippocampus of control guinea-pigs (data not shown). In the latter structure, the effect of 5-CT in the 3-week paroxetine group was similar to that obtained in the controls.

There was no difference in the inhibitory effect of sumatriptan in mesencephalic raphe, hippocampus and frontal cortex in 3-week befloxatone-treated group versus that in the corresponding control group (Figure 7a, b, c).

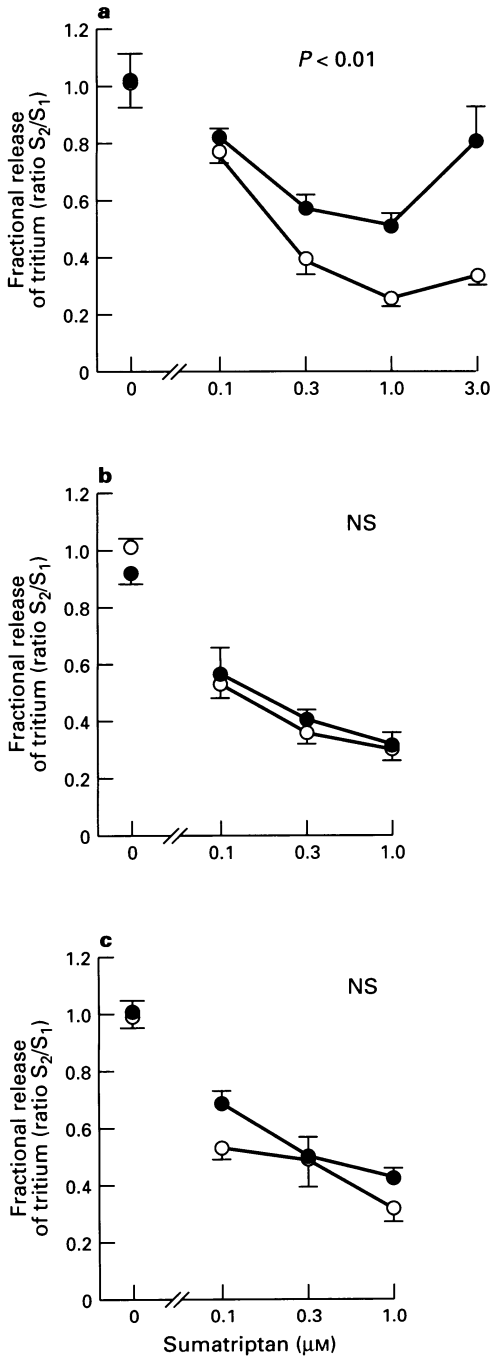

Figure 6 Concentration-effect curves of the 5-HT$_{1D/1B}$ receptor agonist, sumatriptan, introduced 20 min before S_2, on the release of tritium elicited by the electrical stimulation of mesencephalic raphe (a), hippocampus (b) and frontal cortex (c) slices prepared from control (○) and treated (●) guinea-pigs with paroxetine for 3 weeks. Ordinate scale is the fraction of total tissue radioactivity released by 360 pulses (30 mA, 2 ms, 3 Hz for 2 min) expressed as the S_2/S_1 ration. Each point represents the mean ± s.e.mean of at least five experiments per group in pairs of control and treated guinea-pigs. The level of statistical significance, calculated by three-factor analysis of variance between the curves obtained in the control and treated group is indicated in the graphs (NS: non-significant).

Discussion

The present study confirms the presence of functional 5-HT$_{1D}$ autoreceptors within mesencephalic raphe using the 5-HT$_{1D/1B}$ receptor antagonist, GR127935, to attenuate the inhibitory action of sumatriptan (5-HT$_{1D/1B}$ receptor agonist) on electrically evoked release of [^{3}H]-5-HT (Piñeyro *et al.*, 1993; Starkey & Skingle, 1994; Davidson & Stamford, 1995; Huston *et al.*, 1995; Skingle *et al.*, 1995; Piñeyro & Blier, 1996). The effect of 5-MeOT was blocked either by GR127935 or by (+)-WAY100635 indicating that the effect of 5-MeOT is mediated by 5-HT$_{1A}$ and 5-HT$_{1D}$ autoreceptors in mesencephalic raphe. The observation that 5-HT$_{1D}$ receptors control extracellular somatodendritic availability of 5-HT is also supported by the results showing the presence of 5-HT$_{1D}$ binding sites and specific mRNA in mesencephalic raphe nuclei (Bruinvels *et al.*, 1994; Neumaier *et al.*, 1994). However, it was reported that sumatriptan binds with nanomolar affinity for 5-HT$_{1D}$ sites but only has a ten fold selectivity for 5-HT$_{1D}$ with respect to 5-HT$_{1B}$ and 5-HT$_{1A}$ sites (Hoyer, 1991). The involvement of 5-HT$_{1B}$ receptors may be ruled out on the basis of the present results since the 5-HT$_{1B}$ receptor agonist, CP93129 (Koe *et al.*, 1992), at a concentration (0.1 μM) which does not affect the basal outflow of tritium, had no effect on the electrically evoked release of [^{3}H]-5-HT from mesencephalic raphe as well as hippocampus and frontal cortex (Figures 1, 2, 3). In identical experiments in rat mesencephalic raphe slices (Piñeyro *et al.*, 1995), the inhibitory effect of sumatriptan was also prevented by mianserin, a drug which has high affinity for rat 5-HT$_{1D}$ but not 5-HT$_{1B}$ receptors (Hamblin *et al.*, 1992). Though the 5-HT$_{1A}$ receptors negatively regulate the somatodendritic release of 5-HT, the role of 5-HT$_{1A}$ in mediating the effect of sumatriptan can be excluded because the 5-HT$_{1A}$ receptor antagonist, (+)-WAY100135, at a concentration which blocks 5-HT$_{1A}$ receptors (Fletcher *et al.*, 1993). did not block the effect of sumatriptan in the present experiments (Figure 1). This contention is in keeping with recent results in our laboratory showing that the inhibitory effect of sumatriptan on [^{3}H]-5-HT release in rat mesencephalic raphe is not blocked by the 5-HT$_{1A}$ receptor antagonist, (S)-UH-301 (Piñeyro *et al.*, 1995). Moreover, it was also shown that GR127935 does not block the inhibitory effect of the 5-HT$_{1A}$ receptor agonist, 8-OH-DPAT, on the evoked release of [^{3}H]-5-HT from rat mesencephalic raphe (Piñeyro *et al.*, 1995). The 5-HT$_{1E}$ subtype is unlikely to explain the action of sumatriptan and GR127935 in the present study since their affinity at this site is about two orders of magnitude lower than for the 5-HT$_{1D}$ receptors (p*K*i of sumatriptan for 5-HT$_{1D}$ sites 7.5 and for 5-HT$_{1E}$ sites 5.6; Starkey & Skingle, 1994).

By use of K^+ stimulation to induce [^{3}H]-5-HT release, it was shown in the present *in vitro* experiments that 5-HT$_{1D}$ receptors are autoreceptors in mesencephalic raphe, as the Na^+ channel blocker, TTX, did not prevent the inhibitory effect of sumatriptan in the mesencephalic raphe slices (Figure 4). These results are in keeping with those obtained in midbrain raphe of rat using the same paradigm (Piñeyro & Blier, 1996). This inhibitory effect of sumatriptan and 5-CT was not reduced by TTX, thus reinforcing the contention that 5-HT$_{1D}$ receptors, like 5-HT$_{1A}$ receptors, are located on 5-HT neurones of rat mesencephalic raphe. The 5-HT$_{1A}$ and 5-HT$_{1D}$ receptors are functionally different since 5-HT$_{1A}$ autoreceptors control 5-HT release in mesencephalic raphe 5-HT neurones by regulating

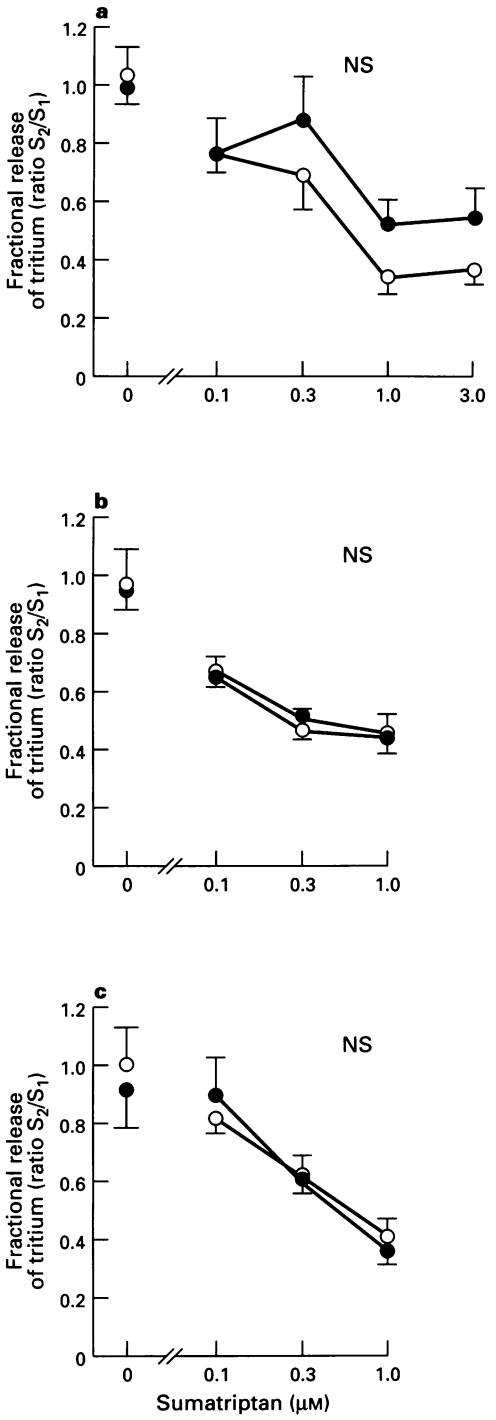

Figure 7 Concentration-effect curves of the 5-HT$_{1D/1B}$ receptor agonist, sumatriptan introduced 20 min before S_2, on the release of tritium elicited by the electrical stimulation of mesencephalic raphe (a), hippocampus (b) and frontal cortex (c) slices prepared from control (○) and treated (●) guinea-pigs with befloxatone for 3 weeks. Ordinate scale is the fraction of total tissue radioactivity released by 360 pulses (30 mA, 2 ms, 3 Hz for 2 min) expressed as the S_2/S_1 ratio. Each point represents the mean ± s.e.mean of at least five experiments per group in pairs of control and treated guinea-pigs. The level of statistical significance, calculated by three-factor analysis of variance between the curves obtained in the control and treated group is indicated in the graphs (NS: non-significant).

the firing activity, while 5-HT$_{1D}$ autoreceptors control 5-HT release in a firing-independent manner. Consistent with this, Craven *et al.* (1994), using guinea-pig midbrain slices, have reported that the inhibitory effect of 5-HT, 5-CT and 8-OH-DPAT on 5-HT neuronal firing activity is antagonized by (+)-WAY100635 but not by GR127935.

Several studies have suggested that 5-HT$_{1D}$ receptors inhibit adenylyl cyclase which is G protein coupled (Herrick-Davis & Titler, 1988; Schoeffter *et al.*, 1988; Waeber *et al.*, 1989). In keeping with the latter assumption, the present experiments indicate that 5-HT$_{1D}$ autoreceptors in the guinea-pig mesencephalic raphe are also coupled to G proteins, because the alkylating agent, NEM, attenuated the suppressant effect of sumatriptan on [^{3}H]-5-HT overflow (Figure 5). This effect is most probably mediated by 5-HT$_{1D}$ but not by 5-HT$_{1A}$ autoreceptors, as discussed above. However, the reduced efficacy of the non-specific 5-HT agonist, 5-MeOT to inhibit [^{3}H]-5-HT release from mesencephalic slices pretreated with NEM could be explained by its action on 5-HT$_{1A}$ and/or 5-HT$_{1D}$, because 5-HT$_{1A}$ autoreceptors are coupled to G proteins in mesencephalic raphe. Indeed, in *in vivo* electrophysiological experiments, it was previously reported that an intra-raphe injection of pertussis toxin (a $G_{i/o}$ protein inactivator) completely inactivates 5-HT$_{1A}$ autoreceptors located on 5-HT neurones in the rat mesencephalic dorsal raphe (Innis & Aghajanian, 1987; Innis *et al.*, 1988). In addition, an attenuation of the inhibitory effect of 8-OH-DPAT on electrically evoked release of [^{3}H]-5-HT from NEM-pretreated slices of rat mesencephalic raphe was recently documented (Piñeyro & Blier, 1996).

The inhibition of [^{3}H]-5-HT release observed with sumatriptan and 5-MeOT in hippocampus and frontal cortex is consistent with previous findings in several animal species (see Hoyer & Middlemiss, 1989) and has been attributed to 5-HT$_{1D}$ autoreceptor activation. Strong support for this view comes from the present results obtained with GR127935 showing that the inhibitory effect of sumatriptan and 5-MeOT are antagonized. Recently, heterogeneity of 5-HT receptors mediating inhibition of [^{3}H]-5-HT release in guinea-pig hippocampus was reported (Wilkinson & Middlemiss, 1992), but not in the frontal cortex (Wilkinson *et al.*, 1993). Indeed, based upon the differential potency of methiothepin against 5-HT versus that against the 5-HT receptor agonists sumatriptan and 5-CT, Wilkinson & Middlemiss (1992) suggested the existence of two receptors mediating the inhibition of [^{3}H]-5-HT release in guinea-pig hippocampus. One would have high affinity for methiothepin and would be preferentially activated by 5-HT, and the other would have less affinity for methiothepin and would be preferentially activated by sumatriptan and 5-CT. In the present studies, we have used 5-MeOT instead of 5-HT in an attempt to provide further evidence for the existence of these two receptors in the absence of an SSRI. Following an NEM pretreatment of hippocampal slices, the inhibitory effect of sumatriptan but not that of 5-MeOT on electrically evoked release of [^{3}H]-5-HT was abolished (Figure 5). This result together with the fact that the NEM pretreatment prevented the enhancing effect of methiothepin suggest that the 5-HT$_{1D}$ autoreceptor subtype activated by sumatriptan, is coupled to G proteins in hippocampus, whereas the one activated by 5-MeOT is not. The possibility that distinct terminal 5-HT autoreceptors might actually exist in hippocampus is compatible with recent pharmacological studies in guinea-pig (Wilkinson & Middlemiss, 1992). Our results, together with the latter observations, suggest that the terminal 5-HT autoreceptor mediating the inhibition of [^{3}H]-5-HT release in guinea-pig hippocampus which is less sensitive to methiothepin and preferentially activated by sumatriptan and 5-CT might correspond to the receptor coupled to G proteins in this study. The terminal 5-HT receptor which has a high affinity for methiothepin and is preferentially activated by 5-HT might correspond to the one which is not coupled to G proteins when activated by 5-MeOT in the present experiments. However, these results stand in contrast with data previously reported for the rat hippocampus showing, in the same paradigm, that these terminal 5-HT autoreceptors are not coupled to G proteins when either 5-MeOT or 5-CT are used as agonists (Blier, 1991) perhaps being attributable to different receptor subtypes (5-HT$_{1B}$ in rat vs 5-HT$_{1D}$ in guinea-pig).

In the case of the frontal cortex, the present results indicated an attenuation of the inhibitory effect of sumatriptan, 5-MeOT

and a prevention of the enhancing effect of methiothepin in NEM-pretreated slices. This suggests that there would be a single subtype of terminal 5-HT$_{1D}$ autoreceptors activated by both sumatriptan and 5-MeOT in this brain region, which would be coupled to G proteins.

Long-term treatment with paroxetine, but not with befloxatone, led to an enhancement of the electrically evoked release of [^{3}H]-5-HT in mesencephalic raphe. A desensitization of somatodendritic 5-HT$_{1A}$ autoreceptors has been reported following such a treatment and could thus account at least in part for the enhanced [^{3}H]-5-HT release (Piñeyro & Blier, 1996). In addition, this increase in [^{3}H]-5-HT could also be due to a desensitization of 5-HT$_{1D}$ autoreceptors in mesencephalic raphe. This is indicated by a decrease in the effectiveness of sumatriptan in inhibiting evoked release of [^{3}H]-5-HT following long-term treatment with paroxetine (Figure 6a). These results are in keeping with recent *in vitro* studies showing a desensitization of 5-HT$_{1D}$ autoreceptors in rat mesencephalic raphe after prolonged paroxetine treatment (Piñeyro & Blier, 1996). In the latter study, it has been shown that the mesencephalic terminal 5-HT$_{1D}$ autoreceptors are also desensitized after a 3-week befloxatone treatment (Piñeyro & Blier, 1996) in contrast to the results obtained in this study. These divergent results may stem from the difference in the species used.

The present results confirm previous data showing enhanced [^{3}H]-5-HT release in frontal cortex after 3 and 8 weeks of paroxetine or 3 weeks of befloxatone treatment (Blier & Bouchard, 1994; El Mansari *et al.*, 1995). In the absence of terminal 5-HT$_{1D}$ autoreceptor desensitization in the frontal cortex after these treatment regimens, it was suggested that this enhancement presumably resulted from a down-regulation of the 5-HT transporter in the paroxetine group and a desensitization of α_2-adrenoceptors located on 5-HT terminals in the befloxatone group (Mongeau *et al.*, 1994; Blier & Bouchard, 1994; El Mansari *et al.*, 1995). The 5-HT autoreceptors activated by sumatriptan and 5-MeOT are not desensitized by 3-week paroxetine treatment reinforcing the assertion that sumatriptan and 5-MeOT act at the same receptor in the frontal cortex.

In hippocampus, long-term 5-HT reuptake blockade and type A-MAO inhibition lead to enhanced [^{3}H]-5-HT release despite the absence of a desensitization of the terminal 5-HT autoreceptors when activated by sumatriptan or 5-CT in this study. However, we have shown, using 5-MeOT, an attenuation of the capacity of the terminal 5-HT autoreceptors to inhibit [^{3}H]-5-HT release and a desensitization of the 5-HT carriers in the hippocampus occurring after a 3-week paroxetine treatment (Blier & Bouchard, 1994). Therefore, the increase of the evoked [^{3}H]-5-HT release was possibly due to a desensitization of the terminal 5-HT autoreceptors that are preferentially activated by 5-MeOT in hippocampus. Since the conditions used in the present *in vitro* studies were identical to those used in the above mentioned previous experiments, with the exception of the 5-HT agonist, it is thus conceivable that the occurrence or absence of the desensitization in the hippocampus reflects different subtypes of the terminal 5-HT$_{1D}$ autoreceptors activated by sumatriptan or 5-MeOT or differences in signal transducing mechanisms (i.e. G protein coupling) mediating the effect of the terminal 5-HT autoreceptor on [^{3}H]-5-HT release. Yet, in the present study, we report different coupling of the terminal 5-HT autoreceptors to G proteins in hippocampus according to their activation by sumatriptan or 5-MeOT. It is thus possible that the enhanced 5-HT release in the guinea-pig hippocampus, after a 3-week paroxetine treatment, could be attributable to the desensitization of the 5-HT autoreceptors preferentially activated by 5-MeOT which are not coupled to G proteins.

In conclusion, the present results confirm the presence of functional 5-HT$_{1D}$ autoreceptors controlling 5-HT release within the mesencephalic raphe as well as in hippocampus and frontal cortex. The inhibitory effect of sumatriptan persisted in the presence of tetrodotoxin in mesencephalic raphe indicating that these 5-HT$_{1D}$ receptors are autoreceptors. The possibility of the existence of two different 5-HT autoreceptors in hippocampus is strengthened by the differential effect of G protein inactivation on their response to two 5-HT agonists. Moreover, the increase in [^{3}H]-5-HT release in hippocampus after the 3-week paroxetine treatment was not attributable to a desensitization of 5-HT autoreceptors activated by sumatriptan but possibly to autoreceptors activated by 5-MeOT, thus providing further evidence for the existence of the two 5-HT autoreceptors in this structure.

This study was supported by the Medical Research Council of Canada grant MA-11014 and a Scientist award to P.B. and the Fonds de la Recherche en Santé du Québec (FRSQ). M.E. was in receipt of postdoctoral fellowship from the FRSQ. We thank C. Bouchard for technical assistance.

References

ADELL, A. & ARTIGAS, F. (1991). Differential effects of clomiparamine given locally or systemically on extracellular 5-hydroxytryptamine in raphe nuclei and frontal cortex. *Naunyn-Schmiedeberg's. Arch. Pharmacol.*, **343,** 237–244.

AGHAJANIAN, G., SPROUSE, J. & RASMUSSEN, K. (1987). Physiology of the mesencephalic serotonin system. In *Psychopharmacology: The Third Generation of Progress*. ed. Meltzer, H.Y. pp 141–149. New York: Raven Press.

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

BLIER, P. (1991). Terminal serotonin autoreceptor function in rat hippocampus is not modified by pertussis and cholera toxins. *Nauny-Schmiedeberg's. Arch. Pharmacol.*, **344,** 160–166.

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. & BOUCHARD, C. (1994). Modulation of 5-HT release in the guinea-pig brain following long-term administration of antidepressant drugs. *Br. J. Pharmacol.*, **113,** 485–495.

BLIER, P., MONROE, P.J., BOUCHARD, C., SMITH, D.L. & SMITH, D.J. (1993). 5-HT$_3$ receptors which modulate [^{3}H]-5-HT release in the guinea pig hypothalamus are not autoreceptors. *Synapse*, **15,** 143–148.

BRUINVELS, A.T., LANDWEHRMEYER, B., GUSTAFSON, E.L., DURKIN, M.M., MENGOD, G., BRANTCHEK, T.A., HOYER, D. & PALACIOS, J.M. (1994). Localization of 5-HT$_{1B}$, 5-HT$_{1D\alpha}$, 5-HT$_{1E}$ and 5-HT$_{1F}$ receptor mesenger RNA in rodent and primate brain. *Neuropharmacol.*, **33,** 367–386.

CRAVEN, R., GRAHAME-SMITH, D. & NEWBERRY, N. (1994). WAY-100635 and GR 127935: Effects on 5-hydroxytryptamine-containing neurones. *Eur. J. Pharmacol.*, **271,** R1–R3.

DAVIDSON, C. & STAMFORD, J.A. (1995). Evidence that 5-hydroxytryptamine release in rat dorsal raphé nucleus is controlled by 5-HT$_{1A}$, 5-HT$_{1B}$ and 5-HT$_{1D}$ autoreceptors. *Br. J. Pharmacol.*, **114,** 1107–1109.

EL MANSARI, M., BOUCHARD, C. & BLIER, P. (1995). Alteration of serotonin release in the guinea-pig orbito-frontal cortex by selective serotonin reuptake inhibitors. Relevance to treatment of obsessive-compulsive disorder. *Neuropsychopharmacol.*, **13,** 117–127.

ENGEL, G., GÖTHERT, M., HOYER, D., SCHLICKER, E. & HILLENBRAND, K. (1986). Identity of inhibitory presynaptic 5-hydroxytryptamine (5-HT) autoreceptors in the rat brain cortex with 5-HT$_{1B}$ binding sites. *Naunyn-Schmiedeberg's. Arch. Pharmacol.*, **332,** 1–7.

FLETCHER, A., BILL, D., CLIFFE, I., DOVER, G.M., FOSTER, E., HASKINS, T.J., JONES, D., MANSELL, H.L. & REILLY, Y. (1993). WAY100135: a novel selective antagonist at presynaptic and postsynaptic 5-HT$_{1A}$ receptors. *Eur. J. Pharmacol.*, **237**, 283–291.

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

GALZIN, A.M., MORET, C., VERZIER, B. & LANGER, S.Z. (1985). Interaction between tricyclic and notricyclic 5-hydroxytryptamine uptake inhibitors and the presynaptic 5-hydroxytryptamine inhibitory autoreceptors in the rat hypothalamus. *J. Pharmacol. Exp. Ther.*, **235**, 200–210.

HAMBLIN, M.W., MCGUFFIN, R.W., METCALF, M.A., DORSA, D.M. & MERCHANT, K.M. (1992). Distinct 5-HT$_{1B}$ and 5-HT$_{1D}$ serotonin receptors in rat: Structural and pharmacological comparison of the two cloned receptors. *Mol. Cell. Neurosci.*, **3**, 578–587.

HAMBLIN, H.W. & METCALF, M.A. (1991). Primary structure and functional characterization of a human 5-HT$_{1D}$-type serotonin receptor. *Mol. Pharmacol.*, **40**, 143–148.

HERRICK-DAVIS, K. & TITLER, M. (1988). Serotonin 5-HT$_{1D}$ receptors in human prefrontal cortex and caudate: interaction with a GTP binding protein. *J. Neurochem.*, **51**, 1906–1912.

HERTTING, G., WURSTER, S., GEBICKE-HÄRTER, S., ALLGAIER, C. (1988). Participation of regulatory G-proteins and protein kinase C in the modulation of transmitter release in hippocampus. In *Modulation of Synaptic Transmission and Plasticity in Nervous System*. ed. Hertting, G. & Spatz H.-C. pp. 147–164. Berlin-Heidelberg: Springer Verlag.

HOYER, D. (1991). The 5-HT receptor family: ligands, distribution and receptor-effector coupling. In *5-HT$_{1A}$ Agonists, 5-HT$_3$ Antagonists and Benzodiazepines: their Comparative Behavioural Pharmacology*. ed. Rodgers, R.J. & Cooper, S.J. pp. 31–57. Chichester: John Wiley.

HOYER, D. & MIDDLEMISS, D.N. (1989). Species differences in the pharmacology of terminal 5-HT autoreceptor in mammalian brain. *Trends Pharmacol. Sci.*, **10**, 130–132.

HUSTON, P.H., BRISTOW, L.J., CUNNINGHAM, J.R., HOGG, J.E., LONGMORE, J., MURRAY, F., PEARSE, D., RAZZAQUE, Z., SAYWELL, K., TRICKLEBANK, M.D. & YOUNG, L. (1995). The effects of GR127935, a putative 5-HT$_{1D}$ receptor antagonist, on brain 5-HT metabolism, extracellular 5-HT concentration and behavior in the guinea-pig. *Neuropharmacol.*, **34**, 383–392.

INNIS, R.B. & AGHAJANIAN, G.K. (1987). Pertussis toxin blocks autoreceptor-mediated inhibition of dopaminergic neurones in rat substancia nigra. *Brain Res.*, **411**, 139–143.

INNIS, R., NESTLER, E. & AGHAJANIAN, G.K. (1988). Evidence for G protein mediation of serotonin and GABA$_B$-induced hyperpolarization of rat dorsal neurons. *Brain Res.*, **459**, 27–36.

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

KOE, B.K., NIELSSEN, J.A., MACOR, J.E. & HYEM, J. (1992). Biochemical and behavioural studies of the 5-HT$_{1B}$ receptor agonist, CP 94253. *Drug Dev. Res.*, **26**, 241–250.

LIMBERGER, N., DICHER, R. & STARKE, K. (1991). Species differences in presynaptic serotonin autoreceptors: mainly 5-HT$_{1B}$ but possibly in addition 5-HT$_{1D}$ in the rat, 5-HT$_{1D}$ in the rabbit and guinea pig brain cortex. *Naunyn-Schmiedeberg's. Arch. Pharmacol.*, **343**, 353–364.

MAURA, G., THELLUNG, S., ANDRIOLI, G.C., RUELLE, A. & RAITERI, M. (1993). Release-regulating serotonin 5-HT$_{1D}$ autoreceptors in human cerebral cortex. *J. Neurochem.*, **60**, 1179–1182.

MIDDLEMISS, D.N. (1985). The putative 5-HT$_1$ receptor agonist, RU24969, inhibits the efflux of 5-hydroxytryptamine from rat frontal cortex slices by stimulation of the 5-HT autoreceptor. *J. Pharm. Pharmacol.*, **37**, 434–437.

MONGEAU, R., DE MONTIGNY, C. & BLIER, P. (1994). Electrophysiologic 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.

MUNDEY, M.K., FLETCHER, A. & MARSDEN, C.A. (1994). Effect of the putative 5-HT$_{1A}$ WAY100135 and SDZ 216-525 on 5-HT neuronal firing in the guinea-pig dorsal raphe nucleus. *Neuropharmacol.*, **33**, 61–66.

NEUMAIER, J., SZOT, P., DORSA, D. & HAMBLIN, M. (1994). 5,7-Dihydroxytryptamine alters 5-HT$_{1B}$ and 5-HT$_{1D}$ receptor mRNA expression in the rat brain. *Soc. Neurosci. Abst.*, **20**, 1163.

PIÑEYRO, G. & BLIER, P. (1996). Regulation of [^{3}H]5-HT release from rat midbrain raphe nuclei by 5-HT$_{1D}$ receptors: Effect of tetrodotoxin, G protein inactivation and long-term antidepressant administration. *J. Pharmacol. Exp. Ther.*, **276**, 697–707.

PIÑEYRO, G., DE MONTIGNY, C. & BLIER, P. (1993). Regulation of somatodendritic release of 5-HT in the rat dorsal raphe by 5-HT$_{1D}$ receptors. *Soc. Neurosc. Abst.*, **19**, 216.

PIÑEYRO, G., DE MONTIGNY C. & BLIER, P. (1995). 5-HT$_{1D}$ receptors regulate 5-HT release in the rat raphe nuclei: *in vivo* voltammetry and *in vitro* superfusion studies. *Neuropsychopharmacol.*, **13**, 249–260.

SCHOEFFTER, P., WAEBER, C., PALACIOS, J. & HOYER, D. (1988). The serotonin 5-HT$_{1D}$ subtype is negatively coupled to adenylate cyclase in calf sustantia nigra. *Naunyn-Schmiedeberg's. Arch. Pharmacol.*, **337**, 602–608.

SKINGLE, M., SLEIGHT, A.J. & FENIUK, W. (1995). Effects of the 5-HT$_{1D}$ receptor antagonist GR127935 on extracellular levels of 5-HT in the guinea-pig frontal cortex as measured by microdialysis. *Neuropharmacol.*, **34**, 377–382.

STARKE, K., GÖTHERT, M. & KILBINGER, H. (1989). Modulation of neurotransmitter release by presynaptic autoreceptors. *Physiol. Rev.*, **69**, 864–988.

STARKEY, S. & SKINGLE, M. (1994). 5-HT$_{1D}$ as well as 5HT$_{1A}$ autoreceptors modulate 5-HT release in the guinea-pig dorsal raphe nucleus. *Neuropharmacol.*, **33**, 393–402.

WAEBER, C., SCHOEFFTER, P., PALACIOS, J.M. & HOYER, D. (1989). 5-HT$_{1D}$ receptors in guinea-pig and pigeon brain, Radioligand binding and biochemical studies. *Naunyn-Schmiedeberg's. Arch. Pharmacol.*, **340**, 479–485.

WEINSHANK, R., ZGOMBICK, J., MACCHI, M. & BRANCHEK, T. (1992). Human serotonin$_{1D}$ receptor is encoded by a subfamily of two distinct genes: 5-HT$_{1D\alpha}$ and 5-HT$_{1D\beta}$. *Proc. Natl. Acad. Sci. U.S.A.*, **89**, 3630–3634.

WILKINSON, L.O., HAWKINS, L.M., BEER, M.S., HIBERT, M.F. & MIDDLEMISS, D.N. (1993). Stereoselective actions of the isomers of metitepine at 5-HT$_{1D}$ receptors in the guinea-pig brain. *Neuropharmacol.*, **32**, 205–208.

WILKINSON, L.O. & MIDDLEMISS, D.N. (1992). Metitepine distinguishes two receptors mediating inhibition of [^{3}H]-5-hydroxytryptamine release in guinea-pig hippocampus. *Naunyn-Schmiedeberg's. Arch. Pharmacol.*, **345**, 696–699.

ZGOMBICK, J., BORDEN, L., COCHRAN, T., KUCHAREWICZ, S., WEINSHANK, R. & BRANCHEK, T. (1993). Dual coupling of cloned human 5-hydroxytryptamine$_{1D\alpha}$ and 5-hydroxytryptamine$_{1D\beta}$ receptors stably expressed in murine fibroblasts: inhibition of adenylate cyclase and elevation of intracellular calcium concentrations via pertussis toxin-sensitive G protein(s). *Mol. Pharmacol.*, **44**, 575–582.

(Received November 13, 1995
Revised February 7, 1996
Accepted February 12, 1996)