Functional characterization of a 5-HT₃ receptor which modulates the release of 5-HT in the guinea-pig brain

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1 The aims of the present study were to confirm the modulation by 5-HT₃ receptors of the electrically evoked release of tritium from slices preloaded with [³H]-5-HT of guinea-pig frontal cortex, hippocampus and hypothalamus, and to assess their functional role in 5-HT release.

2 The selective 5-HT₃ agonist, 2-methyl-5-HT, introduced 8 min before the electrical stimulation, enhanced in a concentration-dependent manner the evoked release of [³H]-5-HT in the three brain regions studied. The 5-HT₃ agonists, phenylbiguanide and *m*-chlorophenyl-biguanide, did not enhance the release of tritium in frontal cortex and hypothalamus slices.

3 In hypothalamus slices, this response was lost when 2-methyl-5-HT was introduced 20 min before the stimulation, thus indicating that these 5-HT₃ receptors desensitize rapidly. When 2-methyl-5-HT was added 20-min before the first stimulation period to desensitize the 5-HT₃ receptors, removed for 24 min, and then re-introduced 8 min before the second stimulation period, the enhancing effect of 2-methyl-5-HT was restored, thus indicating that these 5-HT₃ receptors can rapidly regain normal sensitivity.

4 The enhancing effect of 2-methyl-5-HT was attenuated by the 5-HT₃ receptor antagonists *m*-chlorophenylpiperazine = quipazine = ondansetron ≥ ICS 205-930 = BRL 24924 > MDL 72222 = zacopride.

5 The 5-HT reuptake blocker, paroxetine, enhanced the electrically evoked release of tritium when introduced 8 min before stimulation; this effect of paroxetine was blocked by ICS 205-930, thus indicating that these 5-HT₃ receptors can be activated by endogenous 5-HT.

6 In the absence of electrical stimulation, 2-methyl-5-HT (10 μM) produced a marked enhancement of the basal release of [³H]-5-HT which was calcium-dependent and blocked by S-zacopride but not by paroxetine.

7 The enhancing effect of 2-methyl-5-HT was dependent both on the frequency of stimulation, as indicated by the attenuated effect of 120 stimulations delivered at 1 Hz instead of 5 Hz, and on the duration of the stimulation, as indicated by the more pronounced effect of pulses delivered at 5 Hz for 24 s instead of 72 s or 120 s.

Keywords: 5-HT₃ receptors; [³H]-5-HT release; presynaptic modulation; desensitization; 2-methyl-5-HT; ondansetron; ICS 205-930; zacopride; paroxetine; *m*-chloro-phenylpiperazine (*m*CPP)

Introduction

5-Hydroxytryptamine (5-HT) receptors are now divided into four main classes. Each class is defined by several pharmacological criteria. For instance, whereas 5-HT₁ receptors display nanomolar affinity for 5-HT itself, the 5-HT₂, 5-HT₃ and 5-HT₄ receptors have micromolar affinity for their endogenous neurotransmitter. Molecular biology has now established that the multiple 5-HT binding sites do not merely represent pharmacological artifacts since 5-HT₁, including 5-HT_{1A}, 5-HT_{1B}, 5-HT_{1C}, and 5-HT_{1D}, as well as 5-HT₂ and 5-HT₃ receptors have now been cloned (Maricq *et al.*, 1991; Weinshanck *et al.*, 1992). Most of these receptors have been shown to be coupled to second messenger systems or to ion channels via G proteins, with two possible exceptions: 5-HT_{1B} autoreceptors located on 5-HT terminals in the rat brain, which exert a negative feedback control on 5-HT release, and 5-HT₃ receptors (Blier, 1991; Hoyer & Neijt, 1988). The 5-HT₃ receptors are coupled directly to an ion channel permeable to sodium and potassium (Derkach *et al.*, 1989). Another characteristic of 5-HT₃ receptors is their rapid desensitization following their activation (see Hoyer, 1990 for review).

Activation of 5-HT₃ receptors has been demonstrated to modulate the release of various neurotransmitters in the brain. The selective 5-HT₃ agonist, 2-methyl-5-HT, was

shown to increase the potassium-evoked release of [³H]-noradrenaline from preloaded slices of rabbit hippocampus (Feuerstein & Hertting, 1986). This same 5-HT₃ agonist decreases the potassium-evoked release of [³H]-acetylcholine in slices of the rat cerebral cortex (Barnes *et al.*, 1989). Both in *in vitro* and *in vivo* paradigms, the activation of 5-HT₃ receptors results in an increase of the release of dopamine in the rat brain (Blandina *et al.*, 1989; Chen *et al.*, 1991). The activation of 5-HT₃ receptors enhances the release of cholecystokinin in synaptosomes prepared from the rat nucleus accumbens and cerebral cortex (Paundice & Raiteri, 1991). Finally, Galzin *et al.* (1990) reported on the capacity of 5-HT₃ receptors to increase the electrically evoked release of tritium in guinea-pig frontal cortex slices preloaded with [³H]-5-HT₃. The present study was thus undertaken first to confirm the presence of these 5-HT₃ receptors in three regions of the guinea-pig limbic system, namely the frontal cortex, the hippocampus and the hypothalamus, and, second, to assess their physiological relevance for 5-HT release.

Methods

Male guinea-pigs (300–400 g) 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

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solution containing $0.02 \mu\text{M}$ [^3H]-5-HT creatinine sulphate (specific activity $914 \text{ GBq mmol}^{-1}$; NEN Research Products, Mississauga, Canada) and bubbled with a mixture of 95% O_2 :5% CO_2 . The composition of the Krebs solution in mmol l^{-1} concentration was: 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 with [^3H]-5-HT, one hypothalamic slice or three slices of the hippocampus or two slices of frontal cortex were transferred into each of the 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 8 min (S_1) and 44 (S_2) min 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 5 V cm^{-1} . The following stimulation parameters were generally used to stimulate hypothalamic slices: 20 mA, 2 ms, 3 Hz for 2 min. In some experiments, the pulses were delivered at 1 Hz for 6 min or 5 Hz for 72 s in order to deliver the same number of pulses (360) per stimulation period as in the 3 Hz/2 min condition. 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. These frequencies of stimulation were chosen because they are within the range of the firing rate of 5-HT neurones recorded in freely-moving cats (Jacobs, 1985). The first stimulation period (S_1) was always used as control and the putative antagonists were added 20 min before S_1 and remained present thereafter, whereas the other drugs were added 8 or 20 min before S_2 and also 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 spectrometry. The amount of tritium released per 4-min sample was expressed as a fraction of the total tissue tritium content at the start 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 (S_{p1} or S_{p2}). In order to assess the drug-induced changes of electrically-evoked overflow of radioactivity, S_2/S_1 ratios were calculated, and S_{p2}/S_{p1} ratios were examined to determine whether the drugs 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, 1990; see Results section).

In some experiments, the superfusate was fractionated to determine the ratio of [^3H]-5-HT to [^3H]-5-hydroxyindoleacetic acid ([^3H]-5-HIAA) during the basal outflow and evoked overflow collection periods. One ml of the superfusate was added to 5 ml of *n*-heptane, 1.8 ml of butanol, and 0.1 ml of 2 N HCl. The mixture was vigorously agitated for 10 min and centrifuged at $2,000 g$ for 10 min. The radioactivity was then counted in 4 ml of the organic phase to determine the concentration of [^3H]-5-HIAA and in 1 ml of the aqueous phase for [^3H]-5-HT (Monroe & Smith, 1985).

The following drugs were used: 2-methyl-5-HT, 5-carboxyamidotryptamine (5-CT), trimethylfluorophenylpiperazine (TFMPP), phenylbiguanide (Research Biochemicals, Natick, MA, U.S.A.), *m*-chloro-phenylbiguanide (Cookson, Southampton, England), ondansetron (Glaxo, Montreal, Canada), ICS 205-930 (3- α -tropanyl)-1H-indole-3-carboxylic ester methiodide; Sandoz, Basel, Switzerland), paroxetine, BRL 24924 ([$(\pm)$ -(endo)-4-amino-N-1-azabicyclo[3.3.1]-non-4-yl-5-chloro-2-methoxybenzamide), BRL 46470A (endo-N-(8-methyl-8-azabicyclo[3.2.1]oct-3-yl)2,3-dihydro-3,3-dimethylindole-1-

carboxamide HCl) (SmithKline Beecham, Harlow, England), *m*-chlorophenylpiperazine (*m*CPP; Aldrich Milwaukee, WI, U.S.A.), quipazine (Miles, Elkhart, IN, U.S.A.), R, S and R/S zacopride (Delalande, Rueil-Malmaison, France), MDL 72222 (endo-8-methyl-8-azabicyclo[3.2.1]oct-3-yl-3,5-dichlorobenzoate HCl; Merrell Dow, Cincinnati, OH, U.S.A.) and (+)-tubocurarine (Sigma, St-Louis, MO, U.S.A.).

The *n*'s refer to the number of superfusion chambers studied. All results are expressed as means $\pm$ s.e. The means were compared by the two-tailed Student's *t* test, and Dunnett's correction for multiple comparisons with a single control group when necessary. Probability (*P*) values smaller than 0.05 were considered as significant.

Results

Effects of 2-methyl-5-HT on the evoked release of tritium

In the *in vitro* paradigm used in the present study, the electrically evoked release of [^3H]-5-HT is entirely calcium-dependent as demonstrated by its complete suppression by the removal of calcium from the superfusion medium (Bliez & Bouchard, 1992). The selective 5-HT₃ receptor agonist, 2-methyl-5-HT (0.1 – $3 \mu\text{M}$), introduced 8 min before S_2 , produced a concentration-dependent enhancement of the electrically evoked tritium release in hypothalamus, hippocampus, and frontal cortex slices (Figures 1 and 2). As illustrated in Figure 1, 2-methyl-5-HT at a concentration of $1 \mu\text{M}$ introduced 8 min before S_2 did not induce any enhancement of the

Figure 1 Basal and electrically evoked release of tritium from hypothalamic slices preloaded with [^3H]-5-hydroxytryptamine ([^3H]-5-HT) in the control condition, and with the introduction of 2-methyl-5-HT 8 min before the second period of electrical stimulation (S_2). Each stimulation period consisted of 360 pulses delivered at an intensity of 20 mA, a duration of 2 ms, and a frequency of 3 Hz. Ordinate scale: amount of tritium released per 4 min sample expressed as a fraction of the total tissue tritium content at the start of the respective collection period. The hatched areas represent the proportion of tritium overflow evoked by the stimulation. Values are expressed as means $\pm$ s.e. (vertical bars) of 9 experiments.

Figure 2 Effect of 2-methyl-5-hydroxytryptamine (2-methyl-5-HT) introduced 8 min before S_2 on the release of tritium elicited by the electrical stimulation of hypothalamus ($\circ$), hippocampus (Δ) and frontal cortex ($\square$) slices preloaded with [3 H]-5-HT, and of 2-methyl-5-HT introduced 20 min before S_2 in hypothalamus slices ($\bullet$). Ordinate scale: fraction of the total tissue radioactivity released by 360 pulses (hypothalamus: 20 mA, 2 ms, 3 Hz; hippocampus and frontal cortex: 30 mA, 2 ms, 3 Hz) expressed as the ratio (S_2/S_1) obtained between the second period of stimulation in the presence of the drug (S_2) and the first period of stimulation carried out in the absence of the drug (S_1). Each point represents the mean $\pm$ s.e. (vertical bars) of 5–10 experiments.

spontaneous outflow of the radioactivity from hypothalamic slices, thus suggesting that 2-methyl-5-HT did not merely act as a displacing agent of the intraneuronal store of tritium under these conditions. In hypothalamic slices, the Sp_2/Sp_1 ratio was 0.79 ± 0.01 ($n = 29$) in the control condition and it was slightly increased with a $3 \mu\text{M}$ concentration of 2-methyl-5-HT (0.86 ± 0.04 , $n = 5$), but this ratio was only significantly enhanced at a concentration of $10 \mu\text{M}$ (0.92 ± 0.03 , $n = 5$). Similar results for the Sp_2/Sp_1 ratio were also obtained in the hippocampus and cortex (data not shown).

In order to ascertain that the enhanced evoked release of tritium produced by 2-methyl-5-HT was due at least in part to an increased release of [3 H]-5-HT, the superfusate was fractionated into [3 H]-5-HT and [3 H]-5-HIAA. In hypothalamic and hippocampal slices, the proportion of [3 H]-5-HT in the superfusate was constant at about 40% with the exception of the two fractions when stimulation was applied in which it increased to 50% (Figure 3).

When 2-methyl-5-HT was introduced 20 min before S_2 , its enhancing effect on the electrically evoked release of tritium was totally abolished (Figure 2), thus indicating that the additional 12 min exposure to the agonist was sufficient to desensitize the response. Given this phenomenon, it was deemed crucial to assess whether the response could be restored. To this end, 2-methyl-5-HT ($1 \mu\text{M}$) was introduced 20 min before S_1 in order to obtain an electrically evoked release of tritium during the desensitized state of the receptor. The slices were then superfused with normal Krebs from the 12th min after the beginning of the first stimulation period (S_1) until 8 min prior to S_2 in order to remove the activation of the 5-HT₃ receptors by 2-methyl-5-HT. When 2-methyl-5-HT was again introduced 8 min before S_2 , the electrically evoked release was enhanced to approximately the same degree ($S_2/S_1 = 1.82 \pm 0.12$, $n = 9$) as observed when S_1 was carried out in the absence of 2-methyl-5-HT ($S_2/S_1 = 2.25 \pm 0.23$, $n = 8$).

The release of tritium from brain slices preloaded with

Figure 3 Basal and electrically evoked release of tritium (top line) and [3 H]-5-hydroxytryptamine ([3 H]-5-HT) (bottom dotted line) from hippocampal slices preloaded with [3 H]-5-HT with the introduction of 2-methyl-5-HT 8 min before the second period of electrical stimulation (S_2). Each stimulation period consisted of delivering 360 pulses at an intensity of 30 mA, a duration of 2 ms, and a frequency of 3 Hz. Ordinate scale: amount of tritium released per 4 min sample as a fraction of the total tissue tritium content at the start of the respective collection period. The hatched areas represent the proportion of tritium overflow evoked by the stimulation and the shaded areas represent the proportion of [3 H]-5-HT overflow in the corresponding samples. Values represent the mean of two separate experiments, each involving a total of 10 slices.

[3 H]-5-HT can also be elicited with fenfluramine which acts by a calcium-independent mechanism (Langer & Moret, 1982). In hypothalamic slices, a $7 \mu\text{M}$ concentration of fenfluramine superfused for 2 min was required to induce a fractional release of tritium approximately equivalent ($S_1 = 1.77 \pm 0.22\%$, $n = 9$) to that produced by electrical stimulation ($S_1 = 1.48 \pm 0.15\%$, $n = 29$). Under these conditions, 2-methyl-5-HT ($1 \mu\text{M}$) introduced 8 min before S_2 did not produce a significant alteration of the fenfluramine-evoked release of tritium (control: $S_2/S_1 = 0.97 \pm 0.10$, $n = 4$; 2-methyl-5-HT: $S_2/S_1 = 0.98 \pm 0.07$, $n = 5$).

Effects of phenylbiguanide and m-chloro-phenylbiguanide on the electrically evoked release of tritium

Phenylbiguanide and *m*-chloro-phenylbiguanide have been shown to be effective 5-HT₃ receptor agonists in rat, but not guinea-pig tissues (see Kilpatrick *et al.*, 1991; Robertson & Bevan 1992; Butler *et al.*, 1990). In frontal cortex slices, phenylbiguanide (0.01 and $0.1 \mu\text{M}$), introduced 8 min before S_2 , did not significantly alter the electrically evoked release of tritium. At greater concentrations, phenylbiguanide produced a concentration-dependent decrease of the evoked release of tritium ($1 \mu\text{M}$: $S_2/S_1 = 0.61 \pm 0.07$, $n = 5$; $10 \mu\text{M}$: $S_2/S_1 = 0.37 \pm 0.04$, $n = 5$), without altering spontaneous outflow of radioactivity. In hypothalamic slices at concentrations of 0.1 and $1 \mu\text{M}$ (Figure 4), phenylbiguanide did not alter the electrically evoked release of tritium. In an attempt to reconcile the high affinity of phenylbiguanide for 5-HT₃ sites with its lack of effect in the present paradigm, it was then tested as an antagonist in hypothalamic slices. When phenylbiguanide ($1 \mu\text{M}$) was introduced 20 min before S_1 and remained present until the end of the experiment, it partially blocked the enhancing effect of $1 \mu\text{M}$ 2-methyl-5-HT introduced 8 min before S_2 (Figure 4). *m*-Chloro-phenylbiguanide, which displays greater affinity for rat 5-HT₃ receptors than phenylbiguanide (Kilpatrick *et al.*, 1990), also did not modify the

Figure 4 Effect of phenylbiguanide (PBG, 1 μ M), introduced 8 min before S₂ or 20 min before S₁ and that of 2-methyl-5-hydroxytryptamine (2-methyl-5-HT, 1 μ M) introduced 8 min before S₂ alone or in the presence of phenylbiguanide, on the electrically evoked release of tritium from hypothalamic slices preloaded with [³H]-5-HT. Values are expressed as means $\pm$ s.e. (vertical bars) for which the number of experiments per group is given at the bottom of each column.

* $P < 0.05$ when compared with the control value; † $P < 0.05$ when compared to the value obtained with 2-methyl-5-HT alone.

evoked release of tritium from guinea-pig hypothalamic slices at a concentration of 0.1 μ M introduced 8 min before S₂ (S₂/S₁ = 0.95 $\pm$ 0.13, n = 5); however, a significant attenuation was obtained at a 1 μ M concentration of *m*-chlorophenylbiguanide (S₂/S₁ = 0.51 $\pm$ 0.16, n = 5), but in these conditions the spontaneous outflow was markedly increased.

Effect of 5-HT₃ antagonists on the enhancement of the electrically-evoked release of tritium by 2-methyl-5-HT

Few 5-HT₃ receptor agonists are available at present while a good number of 5-HT₃ antagonists are. Seven compounds with documented 5-HT₃ antagonistic properties were tested in hypothalamic slices. These drugs were introduced 20 min before S₁, and 2-methyl-5-HT was introduced 8 min before S₂, both agents remaining present in the superfusion medium from the time of their introduction until the end of the experiment. All 5-HT₃ receptor antagonists (1 μ M) tested produced a significant attenuation of the enhancing effect of 2-methyl-5-HT (1 μ M) with the following order of potency: *m*CPP = quipazine = ondansetron $\geq$ ICS 205-930 = BRL 24924 > MDL 72222 = zacopride (Figure 5). The R- and S-enantiomers of zacopride (1 and 0.1 μ M) were also effective in blocking the effect of 2-methyl-5-HT and were equipotent with 1 μ M of the racemic compound (data not shown). In contrast, ICS 205-930 was ineffective at 0.1 μ M (S₂/S₁ = 2.18 $\pm$ 0.32, n = 5). It is noteworthy that all these antagonists did not attenuate the electrically-evoked release of tritium when they were superfused 8 min before S₂ in the absence of 2-methyl-5-HT (data not shown), with the exception of 1 μ M S-zacopride (S₂/S₁ = 0.68 $\pm$ 0.09, n = 5) and *m*CPP (S₂/S₁ = 0.65 $\pm$ 0.10, n = 5). The latter drug is a piperazine derivative which is also a 5-HT_{1D} receptor agonist and thus may have directly activated the terminal 5-HT autoreceptor, which is a 5-HT_{1D} receptor in the guinea-pig brain (Hoyer & Middlemiss, 1989).

The capacity of the following drugs to block the enhancement by 2-methyl-5-HT (3 μ M) of the electrically-evoked overflow of tritium was also studied in hippocampal slices. The novel 5-HT₃ antagonist, BRL 46470A (Newberry *et al.*, 1992), at 1 μ M, and R- and S-zacopride at 0.01 μ M blocked

the effect of 2-methyl-5-HT (Figure 6). At 0.001 μ M, the two stereoisomers of zacopride were ineffective (Figure 6). (+)-Tubocurarine, which effectively blocks 5-HT₃ receptors in the nanomolar range in mouse hippocampal preparations (Peters

Figure 5 Effect of seven 5-HT₃ antagonists on the enhancement of the electrically evoked release of tritium produced by 2-methyl-5-hydroxytryptamine (2-methyl-5-HT) in hypothalamic slices preloaded with [³H]-5-HT. The antagonists (1 μ M) were introduced 20 min before the first stimulation period and 2-methyl-5-HT was introduced 8 min before S₂, both agents remaining present in the superfusate until the end of superfusion. Ordinate scale: fraction of the total tissue radioactivity released by 360 pulses expressed as the ratio (S₂/S₁) obtained between the second period of stimulation (S₂) and the first one (S₁). Values are expressed as means $\pm$ s.e. (vertical bars) for which the number of experiments per group is given at the bottom of each column.

* $P < 0.05$ when compared with the control value; † $P < 0.05$ when compared to the value obtained with 2-methyl-5-HT alone.

Figure 6 Effect of four compounds with documented 5-HT₃ antagonistic properties and of trifluoro-methyl-phenylpiperazine (TFMPP) on the enhancement of the electrically evoked release of tritium produced by 2-methyl-5-hydroxytryptamine (2-methyl-5-HT) in hippocampal slices preloaded with [³H]-5-HT. The compounds tested on the enhancing effect of 2-methyl-5-HT were introduced 20 min before the first stimulation period and remained present in the superfusate until the end of the experiment. Ordinate scale: fraction of the total tissue radioactivity released by 360 pulses expressed as the ratio (S₂/S₁) obtained between the second period of stimulation (S₂) and the first one (S₁). Values are expressed as means $\pm$ s.e. (vertical bars) for which the number of experiments per group is given at the bottom of each column.

* $P < 0.05$ when compared with the control value; † $P < 0.05$ when compared to the value obtained with 2-methyl-5-HT alone. Note that (+)-tubocurarine [(+)-Tubo] was ineffective.

et al., 1990; Yakel & Jackson, 1988) but is virtually inactive at guinea-pig 5-HT₃ receptors (Malone *et al.*, 1991; Newberry *et al.*, 1991), did not block the enhancing effect of 2-methyl-5-HT (Figure 6). TFMPP, which like mCPP is a piperazine but is devoid of significant affinity for 5-HT₃ binding sites (Hoyer, 1991; Robertson *et al.*, 1992), was also ineffective in blocking the effect of 2-methyl-5-HT (Figure 6), despite the fact that by itself it decreased the evoked release by about 40% as was the case with mCPP.

Effect of the 5-HT reuptake blocker, paroxetine, on the electrically-evoked release of tritium

In rat hypothalamic slices, selective 5-HT reuptake blockers, introduced 20 min before S₂, do not enhance the electrically evoked overflow of tritium, although increasing the proportion of [³H]-5-HT relative to [³H]-5-HIAA in the superfusate; this is presumably attributable to the potent inhibitory feedback exerted by the enhanced activation of the 5-HT autoreceptor resulting from 5-HT reuptake blockade (Galzin *et al.*, 1985; Blier *et al.*, 1989; Moret & Briley, 1990). In the present experiments carried out under identical conditions in guinea-pig hypothalamic slices, the same lack of effect of the selective 5-HT reuptake blocker, paroxetine, was observed (Figure 7a). However, given the rapid desensitization of the 5-HT₃ receptor observed in the present study, it was thus possible that the prolonged 20 min exposure to an enhanced synaptic concentration of 5-HT was sufficient to desensitize the facilitatory 5-HT₃ receptors. Paroxetine was then introduced only 8 min before S₂ and it produced a 31% increase of the electrically-evoked overflow of tritium (Figure 7a). In order to ascertain that this moderate, albeit significant, increase was attributable to an enhanced activation of 5-HT₃ receptors by endogenous 5-HT, the same experiment was repeated in the presence of the 5-HT₃ antagonist, ICS 205-930, introduced 20 min before S₁ and maintained in the superfusion medium until the end of the experiment. Under these conditions, the enhancing effect of paroxetine was blocked (Figure 7a). In the rat hippocampus, paroxetine approximately doubles the evoked overflow of tritium when introduced 20 min before S₂ (Blier, 1991). In the guinea-pig hippocampal slices, paroxetine increased the evoked overflow of tritium by 40% when introduced 20 min before S₂. When introduced 8 min before S₂, paroxetine enhanced the evoked overflow two fold (Figure 7b). This potentiation of the evoked overflow of [³H]-5-HT by paroxetine was completely blocked by ICS 205-930 introduced 20 min before S₁ (Figure 7b). Fractionation of the superfusate from hippocampal slices showed that, in the presence of paroxetine introduced before S₂, the proportion of [³H]-5-HT in the fractions following electrical stimulation was markedly increased (S₂/S₁ for total tritium: 1.45 versus 2.09 for [³H]-5-HT). These results, which are consistent with those observed in hypothalamic slices (Moret & Briley, 1990), indicate that in the absence of a reuptake blocker, an important proportion of the [³H]-5-HT that is released by electrical stimulation is taken back up into 5-HT terminals. In order to provide an additional line of evidence for the desensitization of these 5-HT₃ receptors in hypothalamic slices, paroxetine was introduced before S₁ and superfused throughout the experiment in order to increase the tonic activation of these 5-HT₃ receptors by 5-HT. Under these conditions, 2-methyl-5-HT (1 µM) did not enhance the electrically evoked release of tritium (paroxetine before S₁ alone: S₂/S₁ = 0.97 ± 0.07, n = 7; paroxetine before S₁ and 2-methyl-5-HT before S₂: S₂/S₁ = 1.04 ± 0.11, n = 8).

Effects of 2-methyl-5-HT on the spontaneous outflow of tritium

Previous *in vitro* experiments examining the release of tritium in rat spinal cord synaptosomes preloaded with [³H]-5-HT have shown that a 10 µM concentration of 2-methyl-5-HT produced an outflow of [³H]-5-HIAA which was blocked by

Figure 7 Effect of the selective 5-hydroxytryptamine (5-HT) reuptake blocker paroxetine (1 µM), introduced 20 or 8-min before S₂ and 8 min before S₂ in the presence of ICS 205-930, on the electrically evoked release of tritium from hypothalamic slices preloaded with [³H]-5-HT in (a) and hippocampal slices in (b). Values are expressed as means ± s.e. (vertical bars) for which the number of experiments per group is given at the bottom of each column.

**P* < 0.05 when compared with the control value; †*P* < 0.05 when compared to the value obtained with paroxetine alone introduced 8 min before S₂.

the selective 5-HT reuptake blocker, fluoxetine, while leaving unaltered the release of [³H]-5-HT (Williams *et al.*, 1992). In order to assess whether the same phenomenon was present in guinea-pig hypothalamic slices, the effect of 2-methyl-5-HT on the outflow of radioactivity was examined by introducing it 8 min before the normal time of the S₂ stimulation period, but without subsequently delivering the stimulations. The amount of radioactivity released in such a sham S₂ period was compared to that released in S₁ produced by delivering 360 pulses at 3 Hz for 2 min. Under these conditions, the 0.3 µM concentration of 2-methyl-5-HT by itself in the absence of electrical stimulations did not produce any effect (Table 1). At 1 µM, however, 2-methyl-5-HT did produce an outflow of tritium which amounted to a fractional release of about 0.3%. Since the spontaneous outflow was not altered in the tube immediately preceding the usual time of the delivery of electrical stimulation (8 min after the introduction of 2-methyl-5-HT, see Figure 1b and Table 1), the 5-HT₃ agonist therefore exerted this effect with a delay of at least 12 min, and is thus inconsistent with a simple displacement mechanism of action. When a 10 µM concentration was used,

Table 1 Effect of 2-methyl-5-hydroxytryptamine (2-methyl-5-HT) on the basal release of [³H]-5-HT as compared to the electrically-evoked release of [³H]-5-HT in the same hypothalamic slices^{1,2}

	<i>S</i> ₁	<i>Sham S</i> ₂	<i>Sham S</i> ₂ / <i>S</i> ₁
2-Methyl-5-HT			
0.3 μM	0.89 ± 0.17 (5)	0.08 ± 0.08	0.07 ± 0.06
1.0 μM	0.84 ± 0.10 (5)	0.30 ± 0.06*	0.39 ± 0.10
10 μM	0.85 ± 0.14 (5)	1.00 ± 0.23*	1.14 ± 0.03
2-Methyl-5-HT (10 μM)			
+0 Ca ²⁺ ³	0.75 ± 0.12 (5)	0.16 ± 0.05	0.21 ± 0.07†
+S-Zacopride ⁴ (0.1 μM)	0.90 ± 0.13 (5)	0.34 ± 0.13	0.37 ± 0.11†
+Paroxetine ⁴ (1 μM)	1.03 ± 0.18 (4)	0.88 ± 0.14	0.87 ± 0.10

¹Electrical stimulations (360 pulses: 3 Hz, 0.5 ms, 20 mA) were delivered in *S*₁ after 60 min of superfusion.

²2-Methyl-5-HT was introduced 8 min before the usual time of the *S*₂ stimulation period, but the electrical pulses were not delivered. The overflow of radioactivity in this sham *S*₂ period was calculated as the amount of radioactivity released above that released in the sample immediately preceding the introduction of 2-methyl-5-HT.

³A Ca²⁺-free Krebs solution was superfused starting 20 min before the introduction of 2-methyl 5-HT.

⁴S-zacopride and paroxetine were superfused starting 12 min before the introduction of 2-methyl-5-HT.

**P* < 0.05 when compared to the basal outflow of radioactivity; †*P* < 0.05 when compared to the sham *S*₂/*S*₁ value obtained in the presence of 2-methyl-5-HT (10 μM) alone.

the amount of tritium was about the same as that released in *S*₁ (Figure 8). This marked enhancement of the spontaneous outflow of tritium by 10 μM 2-methyl-5-HT consisted of about the same proportion of [³H]-5-HT as that released during electrical stimulation (data not shown). Furthermore, it was markedly reduced in slices superfused with a Ca²⁺-free Krebs solution 20 min before the introduction of 2-methyl-5-HT and also by S-zacopride (0.1 μM) introduced 20 min before *S*₁ and maintained until the end of the experiment. In contrast with the results obtained in the rat spinal cord synaptosomes (Williams *et al.*, 1992), the selective 5-HT reuptake blocker, paroxetine (1 μM) did not prevent the enhancing effect of 10 μM 2-methyl-5-HT on the spontaneous outflow of tritium (Table 1).

Frequency- and time-dependence of the facilitatory role of the 5-HT₃ receptor on the electrically evoked release of tritium

Previous studies have shown that the modulation of 5-HT release by the inhibitory effect of 5-HT autoreceptors located on 5-HT terminals is dependent on the synaptic concentration of 5-HT which varies with the frequency of depolarization (Göthert, 1980; Baumann & Waldmeier, 1981). The effectiveness of an exogenous agonist in suppressing 5-HT release is therefore attenuated when determined at high frequencies of stimulation since it has to compete with a greater amount of endogenous 5-HT resulting from the shortening of the interval between each impulse (Chaput *et al.*, 1986; Bluer *et al.*, 1989). In order to test this hypothesis on the function of the 5-HT₃ receptors, the effectiveness of the agonist, 2-methyl-5-HT, in enhancing tritium release at 3 Hz for 2 min was compared to that at 5 Hz for 72 s and at 1 Hz for 6 min in order to deliver the same number of pulses (360) during each stimulation period. In keeping with the above-mentioned data, the effectiveness of 2-methyl-5-HT was

Figure 8 Basal and electrically evoked release of tritium (top line) and [³H]-5-hydroxytryptamine ([³H]-5-HT) (bottom dotted line) from hypothalamic slices preloaded with [³H]-5-HT with the introduction of 2-methyl-5-HT 8 min before the usual time of the second period of stimulation. The stimulation period (*S*₁) after 60 min of superfusion consisted of delivering 360 pulses at an intensity of 20 mA, a duration of 2 ms, and a frequency of 3 Hz. Note that no electrical stimulation was applied at minute 104. Ordinate scale: amount of tritium released per 4 min sample as a fraction of the total tissue tritium content at the onset of the respective collection period. The hatched areas represent the proportion of tritium released by the electrical stimulations in *S*₁ and by 2-methyl-5-HT alone. The shaded areas represent the proportion of [³H]-5-HT overflow in the corresponding samples. Values represent the mean of five experiments.

attenuated when the pulses were delivered at 5 Hz (Figure 9). However, when the pulses were delivered at 1 Hz, a decrease was also obtained instead of the expected enhanced efficacy (Figure 9). Since it was observed in a previous series of experiments that this 5-HT₃ receptor can desensitize within a few minutes, it was thus possible that stimulations delivered over a period of 6 min were sufficient to produce a partial desensitization. In order to test this possibility, 1 Hz pulses were delivered but for only 2 min. As illustrated in Figure 10, 2-methyl-5-HT was significantly more effective in enhancing tritium release when the stimulation period lasted 2 instead of 6 min. Since it would be expected that 5 Hz stimulations would lead to a greater degree of activation of 5-HT₃ receptors than 3 and 1 Hz stimulations, it was thus possible that a desensitization could also occur over a period of stimulation lasting 72 s. To address this issue, the effectiveness of 2-methyl-5-HT was studied with 5 Hz stimulations delivered for 120 and 24 s. As shown in Figure 11, 2-methyl-5-HT had approximately the same effect when the 5 Hz stimulations were delivered for 72 and 120 s, despite releasing considerably less tritium in the former condition (*S*₁ = 1.34 ± 0.15%, *n* = 10) than in the latter (*S*₁ = 2.42 ± 0.15%, *n* = 8). In contrast, when the 5 Hz stimulations were delivered for only 24 s, 2-methyl-5-HT (1 μM) actually produced a three fold increase in the release of tritium (Figure 11), despite releasing approximately the same amount of tritium in *S*₁ (0.68 ± 0.13%, *n* = 15) as in the 1 Hz/2 min condition (0.77 ± 0.11%, *n* = 13) when only a two fold increase was observed (Figure 10). It is noteworthy that a two fold increase was obtained with 0.3 μM 2-methyl-5-HT in the 5 Hz/24 s condition (*S*₂/*S*₁ = 1.90 ± 0.32, *n* = 5), a concentration of 2-methyl-5-HT that was completely devoid of effect on the basal tritium release in the absence of electrical stimulation (Table 1).

Figure 9 Effect of 2-methyl-5-hydroxytryptamine (2-methyl-5-HT) on the evoked release of tritium from hypothalamic slices, preloaded with [³H]-5-HT, produced by 360 electrical pulses delivered at three frequencies of stimulation. The drug was introduced 8 min before S₂. Ordinate scale: fraction of the total tissue radioactivity expressed as the ratio (S₂/S₁) obtained between the second period of stimulation (S₂) and the first one (S₁) in the absence of the drug. The duration of the stimulation periods is indicated in seconds above the histograms. Values are expressed as means ± s.e. (vertical bars) for which the number of experiments per group is given at the bottom of each column.

**P* < 0.05 when compared with the corresponding control value; open columns, control; hatched columns, 2-methyl-5-HT (1 μM) before S₂.

Effect of 5-HT₃ receptor blockade on the modulation of tritium release by the inhibitory 5-HT autoreceptor

In guinea-pig hypothalamic slices, as in rat hypothalamic slices (Blair *et al.*, 1989), changing the frequency of stimulation from 1 Hz to 3 Hz yielded a smaller fractional release of tritium (1 Hz: S₁ = 1.94 ± 0.11%, *n* = 10; 3 Hz: S₁ = 1.14 ± 0.12%, *n* = 10). Also consistent with the hypothesis mentioned in the previous section, the exogenous 5-HT autoreceptor agonist 5-CT was significantly less effective in decreasing tritium release at 3 Hz for 2 min than at 1 Hz for 6 min (Figure 12). When the 5-HT₃ antagonist, ICS 205-930, was superfused starting 20 min before S₁ until the end of the experiment, the effectiveness of 5-CT was not altered at either frequency of stimulation (Figure 12), thus indicating that the facilitatory 5-HT₃ receptor does not significantly modulate the frequency-dependence effect of the inhibitory 5-HT_{1D} autoreceptor on tritium release in guinea-pig hypothalamic slices.

Discussion

The results of the present study confirm the capacity of 5-HT₃ receptors to enhance the electrically-evoked release of [³H]-5-HT from preloaded slices of the frontal cortex (Galzin *et al.*, 1990) and hypothalamus (Blair & Bouchard, 1992) of the guinea-pig, and demonstrate the presence of such receptors in the hippocampus as well. The selective agonist, 2-methyl-5-HT, did not alter the fenfluramine-induced release

Figure 10 Effect of 2-methyl-5-hydroxytryptamine (2-methyl-5-HT) on the evoked release of tritium from hypothalamic slices, preloaded with [³H]-5-HT, produced by electrical pulses delivered at a 1 Hz frequency of stimulation. The drug was introduced 8 min before S₂. Ordinate scale: fraction of the total tissue radioactivity expressed as the ratio (S₂/S₁) obtained between the second period of stimulation (S₂) and the first one (S₁) in the absence of the drug. The duration of the stimulation periods is indicated in seconds above the histograms. Values are expressed as means ± s.e. (vertical bars) for which the number of experiments per group is given at the bottom of each column.

**P* < 0.05 when compared with the control value, open columns; control; hatched columns: 2-methyl-5-HT (1 μM) before S₂.

of [³H]-5-HT, thus indicating that the activation of these facilitatory 5-HT₃ receptors cannot modulate the release of 5-HT by this calcium-independent mechanism. As would be expected from such a positive feedback system, it was also shown to be inactivated by prolonged exposure (20 min) to an exogenous agonist in slices of the frontal cortex (Galzin *et al.*, 1990) and the hypothalamus (Figure 2). It is important to note that this 5-HT₃ response had recovered 24 min after superfusion of a medium not containing 2-methyl-5-HT. In *in vitro* electrophysiological experiments carried out in hippocampal neurones (Yakel & Jackson, 1988), however, the desensitization occurred within a few seconds instead of a few minutes as in the present experiments as suggested by the results presented in Figure 2. Nevertheless, the results obtained with the 5 Hz frequency of stimulation suggest that the desensitization of these 5-HT₃ receptors may also begin to occur within a matter of seconds, as the effect of 2-methyl-5-HT was markedly attenuated when the stimulations were delivered for 72 s instead of 24 s (Figure 11). The activation of these 5-HT₃ receptors by 5-HT itself, as well as their rapid desensitization, was also shown when paroxetine was used to increase the synaptic availability of 5-HT by blocking the 5-HT reuptake carrier (Figure 7). As was the case with the exogenous agonist, 2-methyl-5-HT, blocking the reuptake process for an additional 12 min attenuated the effect of paroxetine on the evoked [³H]-5-HT overflow. Furthermore,

Figure 11 Effect of 2-methyl-5-hydroxytryptamine (2-methyl-5-HT) on the evoked release of tritium from hypothalamic slices, preloaded with [³H]-5-HT, produced by electrical pulses delivered at a 5 Hz frequency of stimulation. The drug was introduced 8 min before S₂. Ordinate scale: fraction of the total tissue radioactivity expressed as the ratio (S₂/S₁) obtained between the second period of stimulation (S₂) and the first one (S₁) in the absence of the drug. The duration of the stimulation periods is indicated in seconds above the histograms. Values are expressed as means ± s.e. (vertical bars) for which the number of experiments per group is given at the bottom of each column.

**P* < 0.05 when compared with the control value; open column: control; hatched columns: 2-methyl-5-HT (1 μM) before S₂.

when paroxetine was present throughout the superfusion experiment, 2-methyl-5-HT no longer enhanced the electrically evoked overflow of [³H]-5-HT. These latter two series of experiments constitute further evidence for the capacity of these 5-HT₃ receptors to desensitize.

The purpose of the present study was not to trace a precise pharmacological profile of these 5-HT₃ receptors involved in the release of 5-HT. The existence of several subtypes of 5-HT₃ receptors is still controversial (Butler *et al.*, 1990; Fozard, 1992; Newberry *et al.*, 1991). Nevertheless, the following points deserve to be emphasized. First, the low micromolar concentrations of the 5-HT₃ agonist, 2-methyl-5-HT, required to detect a significant effect on the evoked release of [³H]-5-HT are fully consistent with the micromolar affinity of this drug for 5-HT₃ binding sites and with previous results obtained in *in vitro* studies assessing the effect of this 5-HT₃ agonist on dopamine and acetylcholine release (Barnes *et al.*, 1989; Blandina *et al.*, 1989; Hoyer, 1990). Furthermore, consistent with previous reports that 2-methyl-5-HT is more potent in rat than in identical guinea-pig preparations (Newberry *et al.*, 1991), preliminary observations from our laboratory indicate that this drug is about 10 fold more potent in enhancing the electrically evoked release of [³H]-5-HT from rat than from guinea-pig frontal cortex slices. Second, the selective 5-HT₃ ligand, phenylbiguanide, was ineffective in enhancing the electrically-evoked release of [³H]-5-HT, consistent with previous reports on its lack of agonistic activity in guinea-pig tissues (Butler *et al.*, 1990; Newberry *et al.*, 1991). It is noteworthy that in the present study, phenylbiguanide appeared to act as an antagonist whereas in the study by Butler *et al.* (1990), it did not. This is perhaps attributable to phenylbiguanide having a very weak partial agonist activity at the present 5-HT₃ receptors. It is not clear, however, why in the preliminary experiments reported by Galzin *et al.* (1990), phenylbiguanide appeared to be an effective, although weak, 5-HT₃ agonist in frontal cortex slices of guinea-pigs. Third, although a 1 μM concentration

Figure 12 Effect of the 5-hydroxytryptamine (5-HT) autoreceptor agonist 5-carboxyamidotryptamine (5-CT) on the evoked release of tritium from hypothalamic slices, preloaded with [³H]-5-HT, produced by 360 electrical pulses delivered at two frequencies of stimulation (a) at 1 Hz and (b) at 3 Hz. 5-CT (0.003 μM) was introduced 20 min before S₂ and ICS 205-930 (1 μM) was introduced 20 min before S₁ and remained present until the end of the experiment. Ordinate scale: fraction of the total tissue radioactivity expressed as the ratio (S₂/S₁) obtained between the second period of stimulation (S₂) and the first one (S₁). Values are expressed as means ± s.e. (vertical bars) for which the number of experiments per group is given at the bottom of each column.

**P* < 0.05 when compared with the corresponding control value; †*P* < 0.05 when compared to the value obtained with the inhibitory effect of 5-CT at the 1 Hz frequency of stimulation. There was no statistically significant difference in the effect of 5-CT in the presence and in the absence of ICS 205-930. Open columns: control; hatched columns: 5-CT before S₂; cross-hatched column: ICS 205-930 before S₁ + 5-CT before S₂.

for the 5-HT₃ antagonists is greater than that used in previous studies carried out in the rat, the present results are nevertheless fully congruent with these drugs being one to two log units less potent in guinea-pig preparations than in the rabbit or the rat (Butler *et al.*, 1990; Newberry *et al.*, 1991). Nevertheless, R- and S-zacopride were effective down to a concentration of 10 nM. The relative potency order of the antagonists observed in the present study relates fairly well to the data obtained in the guinea-pig vagus preparation (see Hoyer, 1990). Finally, (+)-tubocurarine was ineffective in the present experiments at a 1 μM concentration. This is also fully consistent with the estimated IC₅₀ value of about 10 μM for this drug required to block 5-HT₃ responses in guinea-pig ganglionic neurones, as opposed to nanomolar concentrations necessary to block 5-HT₃ responses in mouse tissue preparations (Peters *et al.*, 1990; Newberry *et al.*, 1991; Malone *et al.*, 1991).

Since the 5-HT₃ antagonist, ICS 205-930, blocked the enhancement of the evoked release of [³H]-5-HT produced by the selective 5-HT reuptake blocker, paroxetine, it thus appears that endogenous 5-HT can activate this facilitatory

5-HT₃ receptor. An endogenous activation of this receptor could not be shown in hypothalamic slices by studying the effect of antagonists superfused alone, with the exception of 1 μ M S-zacopride which did produce an inhibition of the electrically-evoked release of [³H]-5-HT. This is probably attributable to a property of this drug other than its capacity to block 5-HT₃ receptors. Such an endogenous tone also does not seem to be present *in vivo* in the rat frontal cortex and hippocampus, as indicated by the lack of effect of ondansetron and MDL 72222, respectively, on the extracellular level of 5-HT measured by microdialysis (Ge *et al.*, 1991; Martin *et al.*, 1992). It is noteworthy that in the latter report, 2-methyl-5-HT enhanced the extracellular level of 5-HT, and this enhancing effect was prevented by MDL 72222 (Martin *et al.*, 1992). However, given the capacity of this 5-HT₃ receptor to desensitize rapidly and the markedly different quantitative effect it can exert over a very narrow range of 5-HT release, it would seem that microdialysis may not be ideally suited to study its influence on the modulation of 5-HT release *in vivo*. A technique which monitors 5-HT release over much shorter time periods would appear more appropriate.

5-HT₃ receptors share several characteristics with the nicotinic acetylcholine receptor. For instance, they both belong to the family of ligand-gated ion channels, and a recent molecular biology study has shown that they have an important degree of similarity in their amino acid sequences (Maricq *et al.*, 1991). On a functional basis, the facilitatory 5-HT₃ receptors characterized in the present study also present common features with the presynaptic nicotinic receptor that was shown to modulate the release of [³H]-acetylcholine in the rat hippocampus and cerebral cortex (Rowell & Winkler, 1984; Aurajo *et al.*, 1988). First, they both subserve the role of enhancing the release of their respective neurotransmitter even in the absence of depolarizing stimuli. Second, they both appear to desensitize rapidly. However, these nicotinic receptors seem to be located on cholinergic terminals, and are thus autoreceptors, whereas the facilitatory 5-HT₃ receptors are not. This latter contention was derived from the lack of effect of 2-methyl-5-HT in synaptosomes of the guinea-pig hypothalamus (Blieier *et al.*, unpublished). Evidence was recently provided that the 5-HT₃ receptors which modulate the release of dopamine are also not located on 5-HT terminals and that the 5-HT₃ receptors which modulate the release of cholecystikinin are located on cholecystikinin-containing terminals in the rat nucleus accumbens (Chen *et al.*, 1991; Paundice & Raiteri, 1991). In

addition, the 5-HT₃ receptors which inhibit the release of [³H]-acetylcholine in the cerebral cortex are located on cholinergic terminals (Barnes *et al.*, 1989; Maura *et al.*, 1992).

Previous studies have documented the capacity of various types of presynaptic receptors to modulate 5-HT release, such as the benzodiazepine type 1 receptor (Lista *et al.*, 1988), and also interactions between receptors demonstrated to be located on the same nerve terminals, such as between the α_2 -adrenoceptor and the 5-HT autoreceptor (Blieier *et al.*, 1990). In the present study, the blockade of 5-HT₃ receptors with ICS 205-930 did not alter the differential effectiveness of an agonist of the terminal 5-HT_{1D} autoreceptor in attenuating the release of [³H]-5-HT at two frequencies of stimulation. Conversely, in the presence of a sustained activation of the terminal 5-HT_{1D} autoreceptor with the piperazine TFMPP, which is devoid of affinity for 5-HT₃ binding sites (Hoyer, 1991; Robertson *et al.*, 1992), the effectiveness of 2-methyl-5-HT in enhancing the electrically evoked overflow of [³H]-5-HT was not altered (Figure 6). Taken together, these results indicate that, under the conditions used in the present study, there appeared to be no direct interaction between the facilitatory 5-HT₃ receptor and the inhibitory 5-HT_{1D} autoreceptor.

In conclusion, facilitatory 5-HT₃ receptors appeared to be present in the frontal cortex, hypothalamus, and hippocampus of the guinea-pig. These receptors can be activated by endogenous 5-HT. Given their capacity to desensitize rapidly and their efficacy in enhancing 5-HT release in conditions when synaptic availability of 5-HT is low, it would appear that these receptors subserve the role of increasing synaptic 5-HT when 5-HT release is at its lowest, even in the absence of impulse flow in 5-HT terminals, as shown by the enhancing effect of a large concentration of 2-methyl-5-HT in the absence of electrical stimulations. Although repeated electroconvulsive shocks did not alter the capacity of these 5-HT₃ receptors to modulate the electrically-evoked release of [³H]-5-HT (Blieier & Bouchard, 1992), it will be interesting to assess their sensitivity following long-term administration of other antidepressant treatments that have been shown to increase the synaptic availability of 5-HT, such as selective 5-HT reuptake blockers and monoamine oxidase inhibitors.

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