British Journal of Pharmacology (1996) 117, 1475–1480

5-HT_4 receptor-mediated modulation of 5-HT release in the rat hippocampus *in vivo*

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1 In the present study, the ability of the 5-hydroxytryptamine$_4$ receptor (5-HT_4 receptor) to modulate the release of 5-HT in the hippocampus of freely-moving rats was investigated by the *in vivo* microdialysis technique.

2 The 5-HT_4 receptor agonist, renzapride (1.0–100 μM, administered via the microdialysis probe) increased extracellular hippocampal levels of 5-HT in a concentration-dependent manner (approximately 200% maximal increase). The ability of renzapride (100 μM, administered via the microdialysis probe) to elevate extracellular levels of 5-HT remained in the presence of the selective 5-HT reuptake blocker, paroxetine (1.0 μM, administered via the microdialysis probe). Furthermore, another 5-HT_4 receptor agonist 5-methoxytryptamine (5-MeOT; 10 μM, administered via the microdialysis probe, in the presence of the non-5-HT_4 5-HT receptor antagonists pindolol (10 μM) and methysergide (10 μM)) maximally elevated extracellular levels of 5-HT by approximately 450% in the rat hippocampus. The elevation of extracellular 5-HT levels induced by either renzapride (100 μM) or 5-MeOT (10 μM) was completely prevented by combined administration of the selective 5-HT_4 receptor antagonist, GR113808 (100 nM, administered via the microdialysis probe). GR113808 (100 nM, administered via the microdialysis probe) administered alone, however, reduced extracellular hippocampal 5-HT levels by some 60%.

3 Systemic administration of the 5-HT_{1A} receptor agonist, 8-OH-DPAT (0.1 mg kg^{-1}, s.c.) reduced extracellular levels of 5-HT in the rat hippocampus by approximately 40%. Prior administration of 8-OH-DPAT (0.1 mg kg^{-1}, s.c.), with an associated reduction of extracellular hippocampal 5-HT levels by approximately 40–50%, however, failed to prevent a subsequent elevation of extracellular levels of 5-HT induced by renzapride (100 μM, administered via the microdialysis probe).

4 Systemic administration of the 5-HT_4 receptor agonist, renzapride (0.25 and 1.0 mg kg^{-1}, i.p.) increased extracellular levels of 5-HT in the hippocampus in a dose-dependent manner. The higher dose of renzapride increasing extracellular 5-HT levels by some 200%. The selective 5-HT_4 receptor antagonist, GR125487D (1.0–100 μg kg^{-1}, i.p.) caused a dose-dependent reduction in extracellular levels of 5-HT in the hippocampus (maximally approximately 80% reduction). Prior administration of GR125487D (10 μg kg^{-1}, i.p.) prevented the elevation of extracellular levels of 5-HT induced by renzapride (1.0 mg kg^{-1}, i.p.).

5 In conclusion, the present study provides evidence that activation of the 5-HT_4 receptor facilitates 5-HT release in the rat hippocampus *in vivo*.

Keywords: 5-Hydroxytryptamine$_4$ receptor; 5-HT release; rat hippocampus; *in vivo* microdialysis; benzamides

Introduction

The 5-hydroxytryptamine$_4$ receptor (5-HT_4 receptor) was first described in primary cultures of mouse colliculi neurones (Dumuis *et al.*, 1988) and guinea-pig hippocampal homogenates (Bockaert *et al.*, 1989). Subsequently, both the receptor protein and mRNA have been demonstrated to be widely distributed in various peripheral tissues e.g. (Craig & Clarke, 1990; Eglen *et al.*, 1990; Baxter *et al.*, 1991; Elswood *et al.*, 1991; Lefebvre *et al.*, 1992; Villalon *et al.*, 1991; Buchheit & Bertholet, 1992; Gerald *et al.*, 1995) and the central nervous system (Grossman *et al.*, 1993; Waeber *et al.*, 1993; Jakeman *et al.*, 1994; Gerald *et al.*, 1995).

At the molecular level, the 5-HT_4 receptor is a member of the putative seven transmembrane domain G protein coupled receptor superfamily (Gerald *et al.*, 1995); consistent with functional responses demonstrating that 5-HT_4 receptor activation elevates adenylate cyclase activity with a subsequent increase in cyclic AMP levels (Dumuis *et al.*, 1988; 1989; Bockaert *et al.*, 1989; Fagni *et al.*, 1992). In neurones, activation of the 5-HT_4 receptor results in increased excitability and a slowing of repolarization (Chaput *et al.*, 1990; Andrade & Chaput, 1991; Torres *et al.*, 1995) which is likely to underlie the ability of the 5-HT_4 receptor to enhance neurotransmitter release (e.g. Consolo *et al.*, 1994; Steward *et al.*, 1996).

We have previously shown that the 5-HT_4 receptor agonist/5-HT_3 receptor antagonist, S(-)-zacopride elevates 5-HT release in the rat frontal cortex estimated by the *in vivo* microdialysis technique (Barnes *et al.*, 1992). This response is not mimicked by the selective 5-HT_3 receptor antagonist, ondansetron (Butler *et al.*, 1988), indicating that the 5-HT_4 receptor may mediate the response. However, this putative 5-HT_4 receptor-mediated response is evident only following a prior reduction in extracellular 5-HT levels (Barnes *et al.*, 1992) which may indicate a high level of endogenous tone on the receptor in this preparation.

In the present study, we investigated the ability of the 5-HT_4 receptor to modulate basal 5-HT release in the rat hippocampus estimated using the *in vivo* microdialysis technique. A preliminary account of this work was presented to the British Pharmacological Society (Ge & Barnes, 1995).

Methods

Animals

Female Wistar rats (150–250 g; Birmingham bred) were housed in groups of around 5 in a temperature and humidity-controlled environment with free access to food and water.

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Stereotaxic implantation of chronic indwelling guide cannulae for microdialysis studies

Rats were anaesthetized with ketamine (60 mg kg^{-1}, i.p.) and medetomidine (250 μg kg^{-1}, i.p.) before 5 mm chronically indwelling guide cannulae (19 guage stainless steel tubing; Coopers Needle Work Ltd) were stereotaxically inserted (the tip of the indwelling guide cannulae were in the cerebral cortex overlying the hippocampus; final microdialysis probe tip location, A -4.5, V -7.9, L -4.9 relative to Bregma; Paxinos & Watson, 1986) and secured to the skull with screws and dental cement. The guide cannulae were kept patent with stylets which protruded 0.5 mm from the cannulae.

Assessment of extracellular 5-HT levels in the rat hippocampus using the in vivo *microdialysis technique*

At least 14 days after stereotaxic location of the guide cannulae, rats were individually placed in a single animal test cage (with free access to food and water) for approximately 12 h before the rat was immobilized with a soft-cloth wrapping technique and the microdialysis probe (4 mm AN69 dialysis membrane, external/internal diameter 310/220 μm, molecular weight cut off 40,000; Hospital Medical; for probe construction see Barnes *et al.*, 1992) was gently implanted into the hippocampus and secured with cyanoacrylate adhesive. After at least 1 h, the microdialysis probe was perfused with artificial cerebro-spinal fluid (aCSF; mM; NaCl 126.6, KCl 2.4, KH_2PO_4 0.49, $MgCl_2$ 1.28, $CaCl_2$ 1.1, $NaHCO_3$ 27.4, Na_2HPO_4 0.48, glucose 7.1, pH 7.4) at 2 μl min^{-1}. Dialysate samples collected for at least the first 100 min were discarded and subsequent samples were collected every 20 min. After the establishment of a reproducible baseline of dialysate 5-HT levels, drugs (or vehicle) were either administered via the perfusing aCSF (by using a liquid switch) or administered systemically. Dialysate 5-HT levels were quantified immediately by high performance liquid chromotography - electrochemical detection (h.p.l.c. - e.c.d).

At the end of each experiment, microdialysis probe placement was verified visually by coronal slicing of the brain with a freezing microtome. Data from animals where the microdialysis probes were not correctly located within the hippocampus were not included in the present report.

H.p.l.c. - e.c.d. system for the quantification of 5-HT

For the determination of 5-HT levels in dialysates, the h.p.l.c. - e.c.d. system comprised of an isocratic pump (Gynkotek model 300 Solvent Delivery System) which was connected to an analytical column (Hypersil 5ODS; 150 × 4.6 mm; HPLC Technology) via a Rheodyne injector. The eluate from the analytical column was passed into an electrochemical detector (ANTEC working electrode +700 mV versus Ag/AgCl reference electrode), the output from which was monitored using a recording integrator (MacIntegrator). The h.p.l.c. - e.c.d. system, with the exception of the integrator, was maintained at a constant temperature of 4°C inside a glass-fronted cool cabinet. The optimized mobile phase (methanol 11% v/v, disodium hydrogen orthophosphate 106 mM, citric acid 36 mM, tetraethylammonium bromide 2 mM, pH 6.2–6.3; slight adjustments to the pH and/or methanol concentration were made to overcome variations in the chromatography) was delivered to the analytical column at a rate of 1.4 ml min^{-1}. Injections of external standards were made in order to identify and calibrate the peaks resulting from the injection of the dialysates.

Drugs

GR113808 ([1-[2-(methylsulphonylamino)ethyl]-4-piperidinyl] methyl 1-methyl-1*H*-indole-3-carboxylate maleate; Glaxo), GR125487D ([1-[2-(methylsulphonyl)amino]ethyl]-4-piperidinyl-methyl 5-fluoro-2-methoxy-1H-indole-3-carboxylate, sulphamate; Glaxo), 5-HT (maleate; Sigma), 5-methoxytryptamine (5-MeOT; HCl; Sigma), methysergide (maleate; Sandoz), 8-OH-DPAT (8-hydroxy-2-(di-n-propylamino) tetralin hydrobromide; Research Biochemicals Inc.), paroxetine (HCl; SmithKline Beecham Pharmaceuticals), (±)-pindolol (Sandoz), renzapride (HCl; SmithKline Beecham Pharmaceuticals) and tetrodotoxin (Sigma) were dissolved in a minimum quantity of distilled water and made to volume in either 0.9% w/v aCSF or NaCl as appropriate. All drugs were used as received and were freshly prepared immediately before use.

Results

Basal extracellular levels of 5-HT and validation of their neuronal origin

The *in vitro* recovery of 5-HT with the home made microdialysis probes (4 mm dialysis membrane) was 18±2% (mean±s.e.mean, 8 probes). The limit of detection for 5-HT was routinely between 1–3 fmol on column, respectively (injection volume 40 μl; signal to noise ratio = 3 : 1). Basal extracellular levels of 5-HT in the rat hippocampus were stable throughout the time course of the experiments (typically 260 min; basal extracellular 5-HT levels = 8.25±0.41 fmol 20 min^{-1}, mean±s.e.mean, n=34) and were not altered by perfusion of vehicle (aCSF) or the peripheral injection of saline (0.9% w/v NaCl 1.0 ml kg^{-1}, i.p.; data not shown).

The perfusion of tetrodotoxin (1.0 μM, via the microdialysis probe) maximally reduced hippocampal extracellular 5-HT levels by some 60% (Figure 1).

Ability of centrally administered 5-HT$_4$ receptor ligands to modify extracellular levels of 5-HT in the rat hippocampus

Administration of the 5-HT$_4$ receptor agonist, renzapride (1.0–100 μM, administered via the microdialysis probe) increased extracellular levels of 5-HT in the rat hippocampus in a concentration-dependent manner (Figure 2a).

The selective 5-HT re-uptake inhibitor, paroxetine (1.0 μM, administered via the microdialysis probe) elevated the extracellular levels of 5-HT (Figure 2b). Subsequent administration

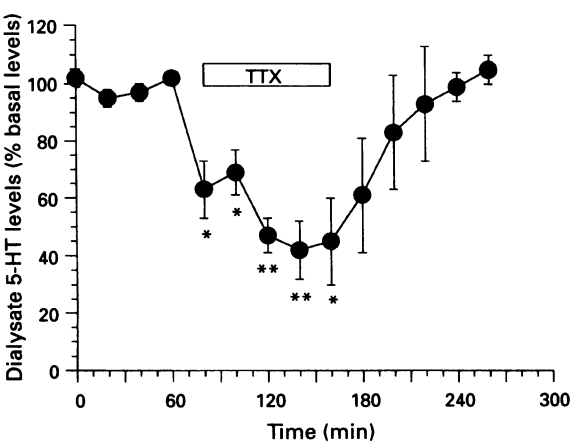

Figure 1 Effect of tetrodotoxin (TTX: 1.0 μM, administered via the perfusing aCSF) on extracellular 5-HT levels in rat hippocampus assessed using the *in vivo* microdialysis technique. Extracellular 5-HT levels are expressed as a percentage of the meaned absolute amount in the 4 collections preceding the tetrodotoxin treatment. The horizontal bar represents application of tetrodotoxin, corrected for void volume. Data represent the mean±s.e.mean, n=4. ANOVA $P<0.05$, *$P<0.05$, **$P<0.01$ (Dunnett's t test).

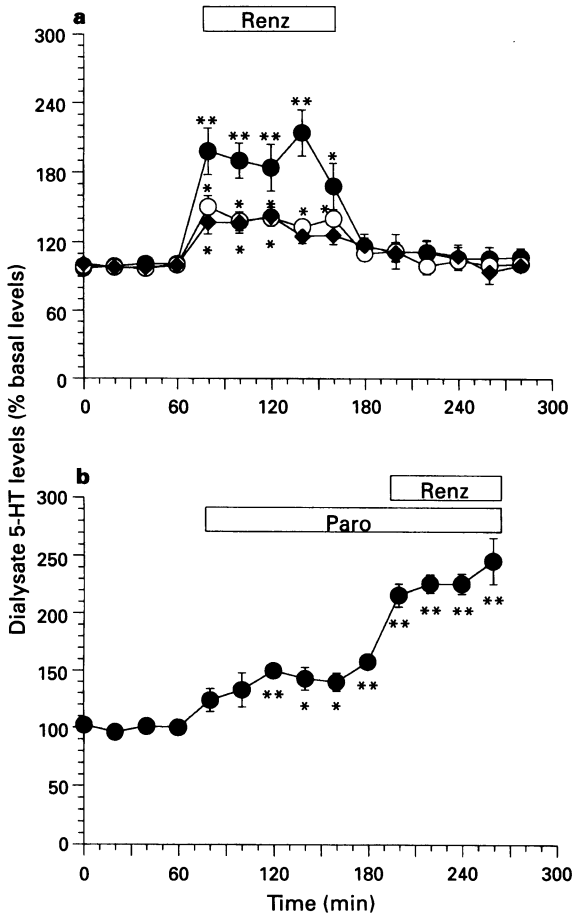

Figure 2 Ability of the 5-HT_4 receptor agonist, renzapride and the selective 5-HT uptake inhibitor, paroxetine, to modulate the extracellular levels of 5-HT in the rat hippocampus assessed by the *in vivo* microdialysis technique. (a) Renzapride (Renz: ◆, 1.0 μM; ○, 10 μM; ●, 100 μM; administered via the perfusing aCSF) and (b) paroxetine (Paro: 1.0 μM, administered via the perfusing aCSF) and co-perfusion of paroxetine (1.0 μM) and renzapride (Renz: 100 μM, administered via the perfusing aCSF). Extracellular 5-HT levels are expressed as a percentage of the meaned absolute amount in the 4 collections preceding the drug treatment. The horizontal bars represent application of the indicated drugs, corrected for void volume. Data represent the mean ± s.e.mean, n=4-6. ANOVA $P<0.05$, $^{*}P<0.05$, $^{**}P<0.01$ (Dunnett's t test).

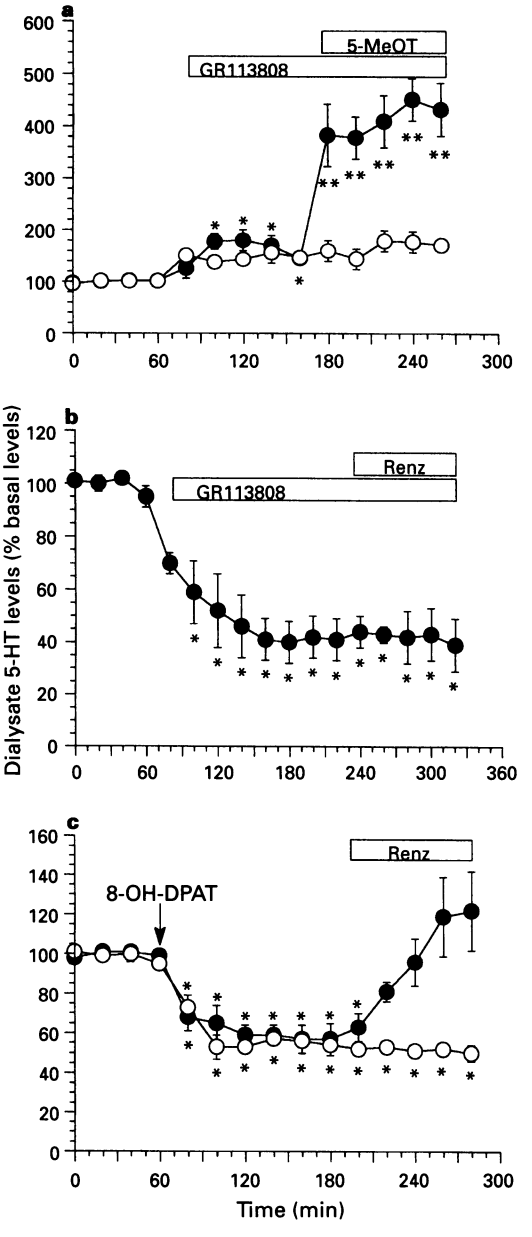

Figure 3 Ability of 5-HT_4 receptor ligands and the 5-HT_{1A} receptor agonist, 8-OH-DPAT to modulate the extracellular levels of 5-HT in the rat hippocampus assessed by the *in vivo* microdialysis technique. (a) 5-MeOT (●, 10 μM, administered via the perfusing aCSF, in the presence of pindolol (10 μM) and methysergide (10 μM)) and co-perfusion of 5-MeOT (○, 10 μM, in the presence of pindolol (10 μM) and methysergide (10 μM)) and GR113808 (100 nM, administered via the perfusing aCSF). (b) GR113808 (100 nM, administered via the perfusing aCSF) and co-perfusion of renzapride (Renz: 100 μM, administered via the perfusing aCSF) and GR113808 (100 nM, administered via the perfusing aCSF) and (c) 8-OH-DPAT (○, 0.1 mg kg^{-1}, s.c.) and renzapride (Renz: 100 μM, administered via the perfusing aCSF, 120 min after the administration of 8-OH-DPAT). Extracellular 5-HT levels are expressed as a percentage of the meaned absolute amount in the 4 collections preceding the drug treatment. The horizontal bars represent application of the indicated drugs, corrected for void volume. Data represent the mean ± s.e.mean, n=3-6. ANOVA $P<0.05$, $^{*}P<0.05$, $^{**}P<0.01$ (Dunnett's t test).

of renzapride (100 μM, in the continued presence of paroxetine, 1.0 μM) further enhanced extracellular levels of 5-HT (Figure 2b).

Administration of another, structurally different, 5-HT_4 receptor agonist, 5-MeOT (10 μM) in the continued presence of, and 80 min following, the perfusion of the non-5-HT_4 5-HT receptor antagonists, methysergide (10 μM) and pindolol (10 μM), also enhanced extracellular levels of 5-HT (Figure 3a).

The selective 5-HT_4 receptor antagonist, GR113808 (100 nM, administered via the microdialysis probe), significantly reduced extracellular levels of 5-HT (Figure 3b). Prior administration of GR113808 (100 nM, administered via the microdialysis probe) prevented the elevation of extracellular 5-HT levels induced by renzapride (100 μM; Figure 3b) or 5-MeOT (10 μM; in the continued presence of, and 80 min following, the perfusion of methysergide (10 μM) and pindolol (10 μM; Figure 3a)).

Systemic administration of the 5-HT_{1A} receptor agonist, 8-OH-DPAT (0.1 mg kg^{-1}, s.c.) reduced extracellular levels of 5-HT with maximal reduction being reached within 40–60 min and levels remained reduced for the remainder of the experiment (approximately 3 h, Figure 3c). Central administration of renzapride (100 μM, administered via the microdialysis probe), 2 h after peripheral injection of 8-OH-DPAT (0.1 mg kg^{-1}, s.c.), elevated the extracellular levels of 5-HT (Figure 3c).

Ability of systemic administration of 5-HT$_4$ receptor ligands to modify extracellular levels of 5-HT in the rat hippocampus

Renzapride (0.25–1.0 mg kg^{-1}, i.p.) enhanced extracellular levels of 5-HT in rat hippocampus in a dose-dependent manner (Figure 4a). Systemic administration of the selective 5-HT$_4$ receptor antagonist, GR125487D (1.0–100 μg kg^{-1}, i.p.) dose-dependently reduced extracellular levels of 5-HT in the rat hippocampus (Figure 4b).

GR125487D (10 μg kg^{-1}, i.p.) administered 60 min prior to the injection of renzapride (1.0 mg kg^{-1}, i.p.) prevented the renzapride-induced increase in extracellular levels of 5-HT (Figure 4c).

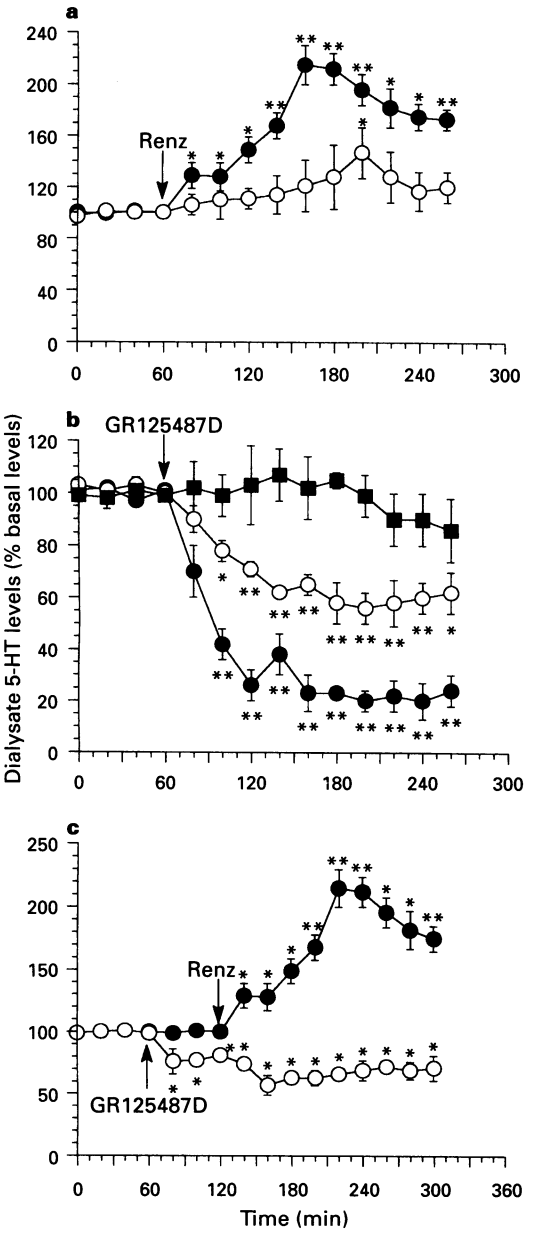

Figure 4 Ability of systemic administration of the 5-HT$_4$ receptor agonist, renzapride and antagonist, GR125487D, to modulate the extracellular levels of 5-HT in the rat hippocampus assessed by the *in vivo* microdialysis technique. (a) Renzapride (Renz: ○, 0.25; ●, 1 mg kg^{-1}, i.p.); (b) GR125487D (■, 1.0; ○, 10; ●, 100 μg kg^{-1}, i.p.) and (c) Renzapride (Renz: ●, 1 mg kg^{-1}, i.p.) and GR125487D (○, 10 μg kg^{-1}, i.p., administered 60 min prior to renzapride) plus renzapride (1 mg kg^{-1}, i.p.). Extracellular 5-HT levels are expressed as a percentage of the meaned absolute amount in the 4 collections preceding the drug treatment. Data represent the mean ± s.e.mean, n = 5-6. ANOVA $P<0.05$, $^*P<0.05$, $^{**}P<0.01$ (Dunnett's t test).

Discussion

In the present studies, the ability of the 5-HT$_4$ receptor to modulate 5-HT release in the hippocampus of freely-moving rats was assessed by the *in vivo* microdialysis technique.

The finding that the majority of the extracellular levels of 5-HT were sensitive to the sodium channel blocker, tetrodotoxin, indicates that at least the majority of the measured 5-HT was neuronal in origin rather than from other sources (e.g. platelets).

The major finding of the present studies was that the 5-HT$_4$ receptor agonists, 5-MeOT and renzapride (for reviews see Bockaert *et al.*, 1992; Ford & Clarke, 1993) increased hippocampal extracellular levels of 5-HT in freely-moving rats. Initially 5-HT$_4$ receptor agonists were directly applied to the hippocampus via the perfusing aCSF although subsequent experiments demonstrated that peripheral administration of the 5-HT$_4$ receptor agonist, renzapride was also able to increase extracellular hippocampal 5-HT levels in a dose-dependent manner.

In the present study, similar concentrations of either renzapride (100 μM) or 5-MeOT (10 μM) were required to increase extracellular 5-HT levels in the hippocampus of freely-moving rats as were required to evoke the 5-HT$_4$ receptor-mediated increase of dopamine release in the rat striatum *in vivo* (Steward *et al.*, 1996). Furthermore if an approximate estimation of 10–20% of renzapride or 5-MeOT crosses the dialysis membrane to the hippocampus, then the effective concentrations of renzapride and 5-MeOT were consistent with effective concentrations of these compounds required to activate the 5-HT$_4$ receptor in *in vitro* preparations (for reviews see Bockaert *et al.*, 1992; Ford & Clarke, 1993).

The renzapride-induced elevation of extracellular hippocampal 5-HT levels are unlikely to result from the ability of this compound to antagonize the 5-HT$_3$ receptor (e.g. Hoyer *et al.*, 1994) since previous studies indicate that 5-HT$_3$ receptor activation enhances 5-HT release in the brain (Galzin *et al.*, 1990; Martin *et al.*, 1992; Blier & Bouchard, 1993) and the administration of a selective 5-HT$_3$ receptor antagonist fails to modify 5-HT release in the rat hippocampus, *in vivo*, indicating the absence of tone on this receptor (Martin *et al.*, 1992).

In addition to renzapride, another 5-HT$_4$ receptor agonist, 5-MeOT was also able to evoke a similar response in the rat hippocampus. 5-MeOT fails to interact with the 5-HT$_3$ receptor (e.g. Hoyer *et al.*, 1994), although it does display efficacy at a number of other 5-HT receptor subtypes which may influence the release of 5-HT. For this reason, 5-MeOT was administered subsequent to, and during, the administration of the non-5-HT$_4$ 5-HT receptor antagonists, methysergide and pindolol. It was of interest, however, that administration of methysergide and pindolol elevated extracellular 5-HT levels. The precise mechanism underlying this response was not investigated but is consistent with the ability of the presynaptic 5-HT$_{1B}$ autoreceptor to inhibit 5-HT release (Brazell *et al.*, 1985; Sharp *et al.*, 1989).

Because relatively high concentrations of 5-HT$_4$ receptor agonists have been used in the present studies, it may have been possible that the apparent enhancement of 5-HT release-induced by either renzapride or 5-MeOT was the result of an interaction with the high affinity 5-HT uptake system. This possibility, however, was unlikely since the renzapride-induced response remained in the presence of the high affinity selective 5-HT uptake inhibitor, paroxetine (for review see Johnson, 1992). Consistent with previous studies, local perfusion of paroxetine alone increased extracellular levels of 5-HT (e.g. Blier & Bouchard, 1993).

The availability of the selective high affinity 5-HT$_4$ receptor antagonist, GR118303 (Gale *et al.*, 1994c) allowed a more

precise definition of the receptor modulating the release of 5-HT in the rat hippocampus. Our finding that nanomolar concentrations of GR113808, administered via the perfusing aCSF, prevented both the renzapride and 5-MeOT-induced increase in the extracellular levels of 5-HT from the rat hippocampus, indicated that these responses were mediated via the 5-HT$_4$ receptor.

However, administration of GR113808 alone reduced extracellular hippocampal levels of 5-HT, although this reduction in 5-HT release was not apparent when GR113808 was combined with pindolol and methysergide. This latter response was somewhat unexpected and the underlying mechanism warrants further investigations. The ability of GR113808, administered alone, to reduce extracellular levels of 5-HT may indicate that there is a facilitatory endogenous tone on the 5-HT$_4$ receptor in our preparation, antagonism of which reduces the basal extracellular 5-HT levels. It remained possible that this compound reduced extracellular 5-HT levels via some other mechanisms and that the prevention of the renzapride- and 5-MeOT-induced responses was due to physiological rather than pharmacological antagonism. To test this possibility further, extracellular hippocampal levels of 5-HT were reduced by peripheral injection of the 5-HT$_{1A}$ receptor agonist, 8-OH-DPAT. This compound stimulates the somatodendritic 5-HT autoreceptor in the raphe nuclei to reduce terminal release of 5-HT in the forebrain (Maidment *et al.*, 1986; Auerbach *et al.*, 1989; Hutson *et al.*, 1989; Sharp *et al.*, 1989). The ability of renzapride still to enhance extracellular hippocampal 5-HT levels, which had been reduced beforehand with 8-OH-DPAT, suggests that the prevention of the renzapride-induced response by GR113808 was due to pharmacological antagonism of the 5-HT$_4$ receptor. Furthermore, since GR113808 displays considerable selectivity for the 5-HT$_4$ receptor versus a range of other neurotransmitter receptors (Gale *et al.*, 1994c), an interaction with another pharmacological recognition site was unlikely.

In addition to GR113808, another selective high affinity 5-HT$_4$ receptor antagonist, GR125487D (Gale *et al.*, 1994a, b) was used in the present studies. This compound displays a relatively long half-life compared to GR113808 (Gale *et al.*, 1994a) and therefore was selected to antagonize responses following peripheral acute administration. Similar to the central perfusion of GR113808, peripheral administration of GR125487D reduced extracellular hippocampal 5-HT levels in a dose-dependent manner. Presumably, this may also be explained by the antagonism of an endogenous tone on the 5-HT$_4$ receptor. Furthermore, prior administration of GR125487D prevented a subsequent elevation of extracellular hippocampal 5-HT levels induced by peripheral administration of renzapride, consistent with the hypothesis that peripheral administration of renzapride elevates 5-HT release via interaction with the 5-HT$_4$ receptor.

In summary, the present study indicates that the 5-HT$_4$ receptor facilitates 5-HT release in the rat hippocampus.

We are grateful to Drs T.P. Blackburn (SmithKline Beecham Pharmaceuticals), G.J. Kilpatrick (Glaxo) and K. Phillips (Sandoz) for the gifts of drugs. Supported by the Wellcome Trust.

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(Received December 5, 1995
Accepted December 18, 1995)