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Ability of 5-HT_4 receptor ligands to modulate rat striatal dopamine release *in vitro* and *in vivo*

Lucinda J. Steward, Jian Ge, Rebecca L. Stowe, D. Charles Brown, Rachel K. Bruton, Paul R. A. Stokes & [1]Nicholas M. Barnes

Department of Pharmacology, The Medical School, University of Birmingham, Edgbaston, Birmingham, B15 2TT

1 The ability of 5-HT_4 (5-hydroxytryptamine$_4$) receptor ligands to modify dopamine release from rat striatal slices *in vitro* and in the striatum of freely moving rats was assessed by the microdialysis technique.

2 The release of dopamine from slices of rat striatum continually perfused with Krebs buffer was enhanced by 5-HT_4 receptor agonists; 5-HT (10 μM), 5-methoxytryptamine (5-MeOT; 10 μM), renzapride (10 μM) and (S)-zacopride (10 μM) maximally increased dopamine release by 133±5, 214±25, 232±29 and 264±69%, respectively (mean±s.e.mean, $n=3-8$). The drug-induced responses were maximal within the first 2 min of drug application, and subsequently declined. The non-selective 5-HT_3/5-HT_4 receptor antagonist, SDZ205-557 (10 μM), failed to modify basal dopamine release from striatal slices but completely antagonized the (S)-zacopride (10 μM)-induced increase in dopamine release.

3 To allow faster drug application, the modulation of dopamine release from rat striatal slices in a static release preparation was also investigated. The 5-HT_4 receptor agonist, renzapride (10 μM) also enhanced dopamine release in this preparation (maximal increase=214±35%, mean±s.e.mean, $n=14$), whilst a lower concentration of renzapride (3 μM) was less effective. The renzapride-induced response was maximal within the first 2 min of drug application, before declining. In this preparation, the stimulation of dopamine release by renzapride (10 μM), was completely antagonized by the selective 5-HT_4 receptor antagonist, GR113808 (100 nM). In addition, both the Na^+ channel blocker, tetrodotoxin (100 nM) and the non-selective protein kinase A inhibitor, H7 (100 nM) completely prevented the stimulation of dopamine release induced by renzapride (10 μM).

4 *In vivo* microdialysis studies demonstrated that the 5-HT_4 receptor agonists, 5-MeOT (10 μM), renzapride (100 μM) and (S)-zacopride (100 μM) maximally elevated extracellular levels of dopamine in the striatum by 220±20, 161±10 and 189±53%, respectively (mean±s.e.mean, $n=5-9$). A lower concentration of renzapride (10 μM) was less effective. The elevation of extracellular striatal dopamine levels induced by either renzapride (100 μM) or (S)-zacopride (100 μM) were completely antagonized by the non-selective 5-HT_3/5-HT_4 receptor antagonist, SDZ205-557 (100 μM). In addition, the elevation of extracellular levels of dopamine induced by either 5-MeOT (10 μM) or renzapride (100 μM) was completely prevented by the selective 5-HT_4 receptor antagonist, GR113808 (1 μM) and the renzapride (100 μM)-induced response was also completely prevented by the non-selective protein kinase A inhibitor, H7 (1 μM). In this *in vivo* preparation, both GR113808 (1 μM) and H7 (1 μM), when perfused alone, reduced extracellular levels of dopamine.

5 In conclusion, the present study provides evidence that the 5-HT_4 receptor facilitates rat striatal dopamine release *in vitro* and *in vivo*.

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

Introduction

Numerous studies have demonstrated the ability of the central 5-hydroxytryptamine (5-HT serotonin) system to modulate the activity of dopaminergic neurones in the nigrostriatal pathway via a number of 5-HT receptor subtypes (e.g. Benloucif *et al.*, 1993; for review see Kelland *et al.*, 1990). Recently, the 5-HT_4 receptor has been identified in the nigrostriatal and mesolimbic systems (e.g. Grossman *et al.*, 1993; Waeber *et al.*, 1993; Jakeman *et al.*, 1994), suggesting that this receptor may also play a modulatory role upon neurotransmission in the striatum. The predicted molecular structure of the 5-HT_4 receptor categorizes it as a member of the putative 7 transmembrane domain, G protein-coupled receptor superfamily (Gerald *et al.*, 1995). This classification is consistent with second messenger studies with both native and recombinant 5-HT_4 receptors which have demonstrated that receptor activation elevates adenylate cyclase activity (e.g. Ford & Clarke, 1993; Gerald *et al.*, 1995). In neurones, the receptor-induced elevation in adenosine 3′: 5′-cyclic monophosphate (cyclic AMP) levels increases the activity of cyclic AMP-dependent protein kinase A with a subsequent closing of voltage-gated K^+ channels (Fagni *et al.*, 1992). The predicted subsequent increase in neuronal excitability and slowing of repolarization can be detected electrophysiologically (Chaput *et al.*, 1990; Andrade and Chaput, 1991; Roychowdhury *et al.*, 1994) and is consistent with the ability of the 5-HT_4 receptor to enhance neurotransmitter release. Currently, the modulation of acetylcholine release via the 5-HT_4 receptor has received most attention with the demonstration that the 5-HT_4 receptor facilitates the release of this neurotransmitter centrally (Boddeke & Kalkman, 1990; 1992; Consolo *et al.*, 1994) and from peripheral neurones (e.g. Tonini *et al.*, 1989; 1992; Craig & Clarke, 1990; Eglen *et al.*, 1990; Elswood *et al.*, 1991; Kilbinger *et al.*, 1995). In addition, there is also some evidence that the 5-HT_4 receptor may modulate dopamine release. Thus, Benloucif and colleagues (1993) have demonstrated, using the *in vivo* microdialysis technique, that the non-selective 5-HT_4 receptor agonist, 5-methoxytryptamine (5-MeOT) increased striatal dopamine release; the response being partially blocked by high con-

[1] Author for correspondence.

centrations of the weak 5-HT$_4$ receptor antagonist, tropisetron. In the present study, we assess directly the ability of the 5-HT$_4$ receptor to modulate striatal dopamine release *in vitro* and *in vivo*. Some of these results have been reported in abstract form to the British Pharmacological Society (Steward & Barnes, 1994; Steward *et al.*, 1995).

Methods

Assessment of dopamine release from perfused rat striatal slices

Female Wistar rats (150–220 g) were killed by cervical dislocation. The dissected striata were chopped (400 × 400 μm; McIlwain Tissue Chopper) before being continually perfused (0.5 ml min^{-1}) with gassed (95/5%O_2/CO_2) Krebs buffer (mM: NaCl 120, KCl 4.75, KH_2PO_4 1.2, $MgSO_4$ 1.2, $CaCl_2$ 2.5, $NaHCO_3$ 25, glucose 11; plus tyrosine 50 μM, GBR 12909 1 μM and (−)-sulpiride 1 μM; 37°C) for 123 min prior to collection of 2 min perfusate samples. Perfusate dopamine levels were quantitated by high-performance liquid chromatography with electrochemical detection (h.p.l.c.-e.c.d.); ANTEC working electrode +700 mV versus Ag/AgCl reference electrode. The dopamine peak was identified by its retention time corresponding to the retention time of a dopamine standard. The optimised mobile phase (methanol 10% v/v, sodium dihydrogen phosphate 0.12 M; octane sulphonic acid 0.55 mM; EDTA 0.1 mM; pH 5.4; slight adjustments to the methanol concentration and/or octane sulphonic acid concentration and/or pH were made to overcome changes in the chromatography) was delivered to the analytical column (Spherisorb or Hypersil 5 ODS; 150 × 4.6 mm) at a rate of 0.9 ml min^{-1}. Protein content of the perfused striatal slices was assayed by the method of Bradford (1976) with bovine serum albumin as the standard.

Assessment of dopamine release from rat striatal slices using a static release preparation

Female Wistar rats (150–200 g) were killed by cervical dislocation. The dissected striata were sliced (400 μm; Vibratome) before being placed in gassed (95/5%O_2/CO_2) Krebs buffer (which included tyrosine (50 μM), GBR 12909 (1 μM) and (−)-sulpiride (1 μM)) at 37°C for at least 60 min. Striatal slices (2 or 3) were carefully placed in wells containing 120 μl of Krebs buffer which was exchanged for fresh Krebs buffer every 2 min for 16 min (compared to the perfusion *in vitro* release preparation, this protocol allowed a faster application of the drug to the striatal slice at the applied concentration since the drug was not initially diluted in the perfusion chamber which had a dead volume of approximately 0.5 ml). Drugs were usually applied after 8 min and were subsequently present for the remainder of the experiment (GR113808, H7 or tetrodotoxin were present throughout the 16 min collection period in experiments to assess the ability of these compounds to prevent agonist-induced responses). Dopamine levels and protein content of the striatal slices were assayed as for the perfused *in vitro* release preparation.

Stereotaxic implantation of chronic indwelling guide cannulae for microdialysis studies

Female Wistar rats (150–250 g) 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 striatum; final microdialysis probe tip location, A+0.2, V−7.5, L−2.5 relative to bregma; Paxinos & Watson, 1986) and secured to the skull with screws and dental cement. The guide cannulae were kept patent with stylets.

Assessment of dopamine release in the rat striatum by the in vivo *microdialysis technique*

At least 14 days after stereotaxic location of the guide cannulae, a rat was placed in a single animal test cage (with free access to food and water) for approximately 12 h before it was immobilised by 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; Hospal Medical; for probe construction see Barnes *et al.*, 1992) was gently implanted into the striatum and secured with cyanoacrylate adhesive (Permabond C2). After at least an hour, the microdialysis probe was perfused with artificial cerebrospinal fluid (aCSF; mM: NaCl 126, 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 dopamine levels, drugs (or vehicle) were administered via the perfusing aCSF by using a liquid switch. Dialysate dopamine levels were quantified immediately by h.p.l.c.-e.c.d. as described above.

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

Drugs

GBR 12909 (1-[2-[*bis*(4-fluorophenyl)methoxy]ethyl]-4-[3-phenylpropyl]piperazine dihydrochloride; RBI), GR113808 ([1-[2-(methylsulphonylamino)ethyl]-4-piperidinyl]methyl 1-methyl-1*H*-indole-3-carboxylate maleate; Glaxo), H7 (1-(5-isoquinolinesulphonyl)-2-methylpiperazinedihydrochloride; RBI), 5-HT (maleate; Sigma), 5-MeOT (HCl; Sigma), methysergide (maleate; Sandoz), pindolol (Sandoz), renzapride (HCl; SmithKline Beecham), SDZ 205-557 (2-methoxy-4-amino-5-chloro-benzoic acid 2(diethylamino)ethyl ester; Sandoz), (−)-sulpiride (RBI), tetrodotoxin (Sigma) and (S)-zacopride (HCl; Delalande) were dissolved in a minimum quantity of distilled water and made to volume in either Krebs buffer or aCSF as appropriate. Medetomidine (SmithKline Beecham) and ketamine (Parke-Davis) were dissolved in 0.9% wt/vol NaCl. Ondansetron (hydrochloride dihydrate, Glaxo Laboratories) was supplied in aqueous solution (2 mg ml^{-1}) and diluted with Krebs buffer. All drugs were used as received and were freshly prepared immediately before use.

Results

Modulation of dopamine release from perfused rat striatal slices

In the absence of tyrosine in the Krebs buffer, the perfusate dopamine levels gradually declined (data not shown). The presence of tyrosine (50 μM) in the Krebs buffer, in addition to maximal effective concentrations of the dopamine uptake blocker GBR12909 (1.0 μM) and the dopamine D_2 receptor blocker (−)-sulpiride (1.0 μM), allowed a steady basal perfusate dopamine level to be achieved (basal dopamine release = 1.57 ± 0.45 pmol min^{-1} mg^{-1} protein, mean ± s.e. mean, n = 30; Figure 1). The GBR12909 and (−)-sulpiride were included in the perfusing Krebs buffer to maximize dopamine levels in the perfusate and to prevent the inhibitory presynaptic dopamine D_2 receptor from 'dampening' any drug induced facilitation of dopamine release by increasing the tone on the dopamine D_2 autoreceptor.

Addition of KCl (20 mM) to the perfusing Krebs buffer

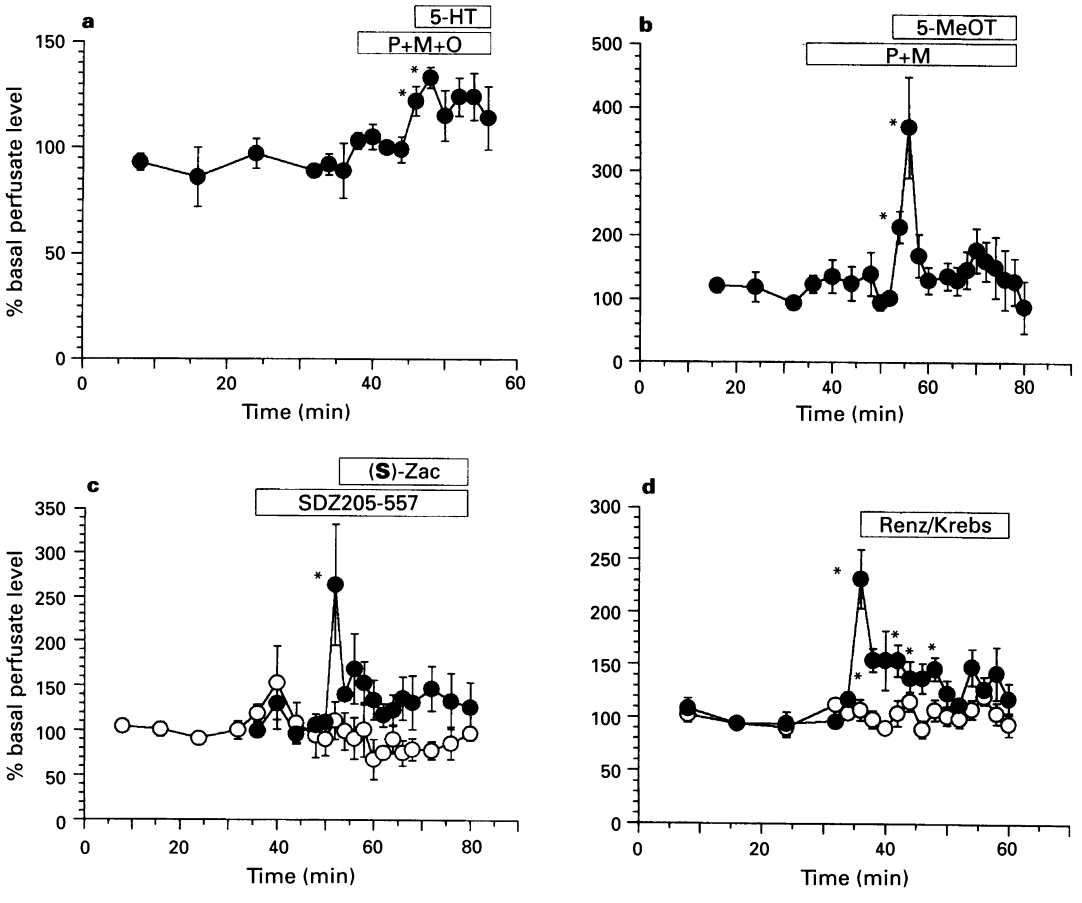

Figure 1 Ability of 5-HT$_4$ receptor ligands to modulate dopamine release from rat striatal slices perfused with Krebs buffer. (a) 5-HT (10 μM; in the presence of pindolol (P; 10 μM), methysergide (M; 10 μM) and ondansetron (1 μM; O)); (b) 5-MeOT (10 μM; in the presence of pindolol (P; 1 μM) and methysergide (M; 1 μM)); (c) (S)-zacopride (●; 10 μM; (S)-Zac) and (S)-zacopride (10 μM) plus SDZ 205-557 (○; 10 μM); (d) renzapride (●; 10 μM; Renz) or vehicle (○; Krebs buffer). Dopamine levels in perfusates are expressed as the percentage of the meaned absolute amount in the 4 collections preceding the drug treatment. Data represent mean ± s.e.mean, $n = 3-8$. Horizontal bars represent application of the indicated drug (corrected for the void volume). ANOVA < 0.05, $^*P < 0.05$ (Dunnett's t test).

increased dopamine levels in the perfusate by some 1600%, whereas an identical KCl treatment in the absence of Ca^{2+} ions in the perfusing Krebs buffer (additional presence of EGTA, 1 mM) reduced the stimulation in dopamine levels by some 75–80% (data not shown). Basal dopamine levels in the perfusates were not significantly reduced following removal of Ca^{2+} ions from the perfusing Krebs buffer (additional presence of EGTA, 1 mM) or addition of tetrodotoxin (100 nM) to the perfusing Krebs buffer (data not shown).

Administration of the 5-HT$_4$ receptor agonists 5-HT (10 μM, in the presence of pindolol (10 μM), methysergide (10 μM) and ondansetron (1 μM) to block 5-HT$_1$, 5-HT$_2$ and 5-HT$_3$ receptors), 5-MeOT (10 μM; in the presence of pindolol (1 μM) and methysergide (1 μM)), renzapride (10 μM) and (S)-zacopride (10 μM) via the perfusing Krebs buffer enhanced the release of dopamine from rat striatal slices (Figure 1). Renzapride (10 μM) failed to increase dopamine release from striatal slices perfused with calcium-free Krebs buffer plus EGTA (1 mM; 76 ± 3% first 2 min collection period after the addition of renzapride, mean ± s.e.mean, $n = 3$). The 5-HT$_4$ receptor antagonist, SDZ 205-557 (10 μM) antagonized the increase in dopamine release induced by (S)-zacopride (10 μM; Figure 1).

Modulation of dopamine release from rat striatal slices using a static release preparation

Basal dopamine release was consistent over the time course of the experiment (basal dopamine release = 2.78 ± 0.29 pmol min^{-1} mg^{-1} protein, mean ± s.e.mean, $n = 62$; Figure 2). Addition of KCl (20 mM) or glutamate (10 mM) to the Krebs buffer maximally increased dopamine levels in the Krebs buffer by 332 ± 131 and 343 ± 44%, respectively (mean ± s.e.mean, $n = 3$).

Administration of the 5-HT$_4$ receptor agonist, renzapride (10 μM) enhanced the release of dopamine (Figure 2). A lower concentration of renzapride (3 μM) was less effective (maximal stimulation detected after 2 min = 137 ± 15%, mean ± s.e. mean, $n = 3$). The stimulation of dopamine release by renzapride (10 μM), was completely antagonized by the selective 5-HT$_4$ receptor antagonist GR113808 (100 nM; Figure 2) and the non-selective protein kinase A inhibitor, H7 (100 nM; Figure 2). Renzapride (10 μM) failed to increase dopamine release from rat striatal slices in the presence of the Na^+ channel blocker, tetrodotoxin (100 nM; Figure 2). GR113808 (100 nM), H7 (100 nM) or tetrodotoxin (100 nM) did not alter the basal release of dopamine (Figure 2).

Modulation of dopamine release in rat striatum assessed by the in vivo *microdialysis technique*

The *in vitro* recovery of dopamine using the 'in house' microdialysis probes was approximately 7–10% and the limit of detection for dopamine was routinely between 4–20 fmol on column (SNR = 3:1). Basal dialysate levels of dopamine were 66 ± 8 fmol 20 min^{-1}, mean ± s.e.mean, $n = 62$. Extracellular dopamine levels were not modified by the administration of vehicle (Figure 3), whereas perfusion of tetrodotoxin (1 μM), maximally reduced extracellular dopamine levels by some 60% (Figure 3).

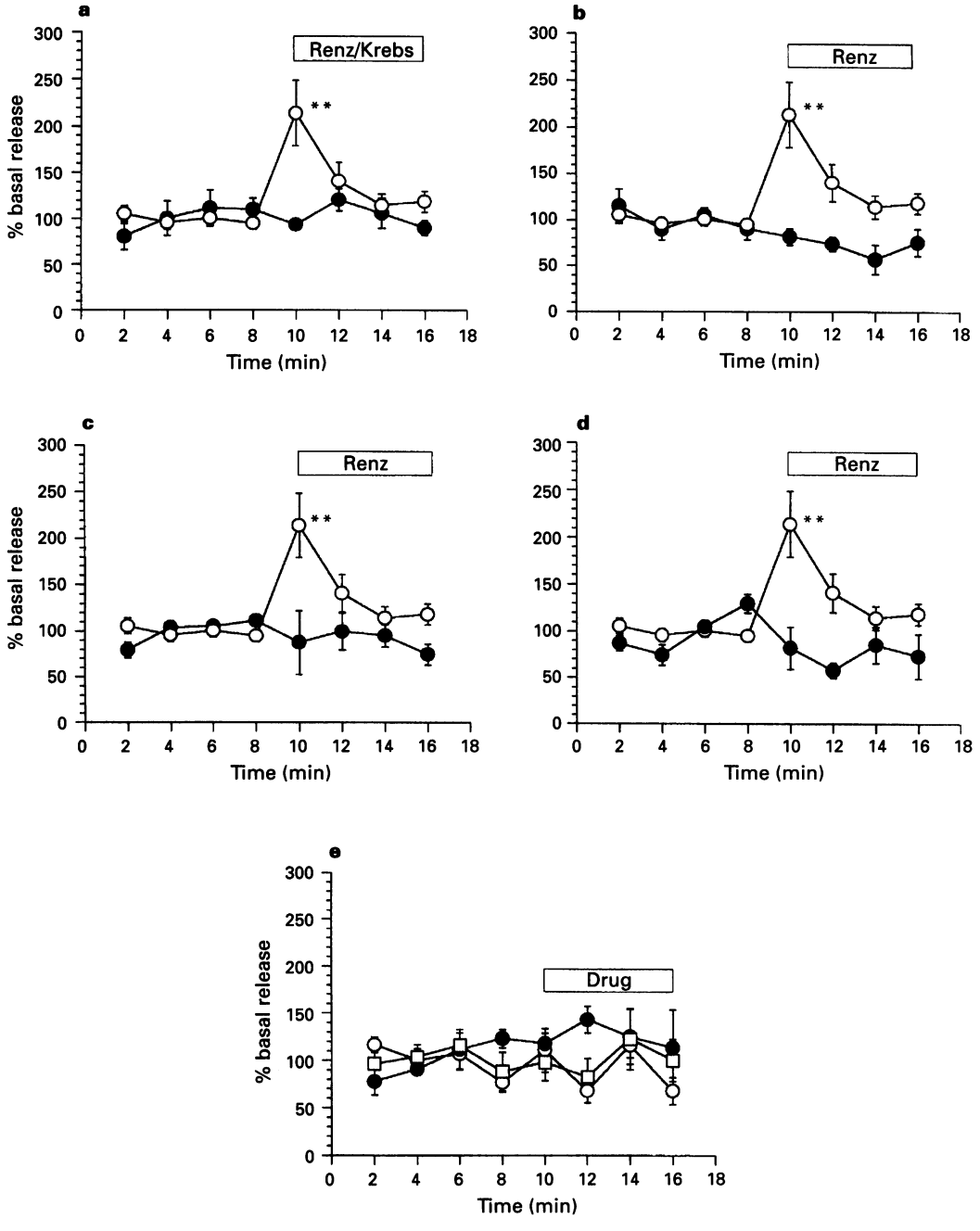

Figure 2 Ability of 5-HT$_4$ receptor ligands to modulate dopamine release from rat striatal slices in the static release preparation. (a) Renzapride (○; 10 μM; Renz) or vehicle (●; Krebs buffer); (b) renzapride (○; 10 μM; Renz) and renzapride (10 μM) plus GR113808 (●; 100 nM present throughout the collection period); (c) renzapride (○; 10 μM; Renz) and renzapride (10 μM) plus H7 (●; 100 nM present throughout the collection period); (d) renzapride (○; 10 μM; Renz) and renzapride (10 μM) plus tetrodotoxin (●; 100 nM present throughout the collection period); (e) GR113808 (○; 100 nM), H7 (●, 100 mM) or tetrodotoxin (□; 100 nM). Dopamine levels in the Krebs buffer are expressed as the percentage of the meaned absolute amount in the 4 collections proceeding the drug treatment. Data represent mean ± s.e.mean, $n = 3-14$. Horizontal bars represent application of renzapride (a–d), vehicle (a) or either GR113808, H7 or tetrodotoxin (e). ANOVA < 0.05, $^{**}P < 0.01$ (Dunnett's t test).

Administration of either 5-MeOT (10 μM; in the presence of pindolol (10 μM) and methysergide (10 μM) to block 5-HT$_1$ and 5-HT$_2$ receptors), renzapride (100 μM) or (S)-zacopride (100 μM) elevated extracellular levels of dopamine (Figure 4), whilst a lower concentration of renzapride (10 μM) induced a more modest stimulation (Figure 4).

Co-perfusion of the 5-HT$_3$/5-HT$_4$ receptor antagonist, SDZ 205–557 (100 μM) antagonized the increase in dialysate dopamine levels induced by (S)-zacopride (100 μM; Figure 5). The selective 5-HT$_4$ receptor antagonist, GR113808 (1.0 μM) reduced the dialysate dopamine levels by some 10–20% (Figure 5) and prevented the elevation of dialysate dopamine levels following the co-perfusion of either 5-MeOT (10 μM; additional presence of pindolol (10 μM) and methysergide (10 μM) administered with the GR113808) or renzapride (100 μM; Figure 5).

The non-selective protein kinase A inhibitor, H7 (1 μM) reduced the dialysate dopamine levels by some 40–50% (Figure 5) and prevented the elevation of dialysate dopamine levels following co-perfusion of renzapride (100 μM; Figure 5).

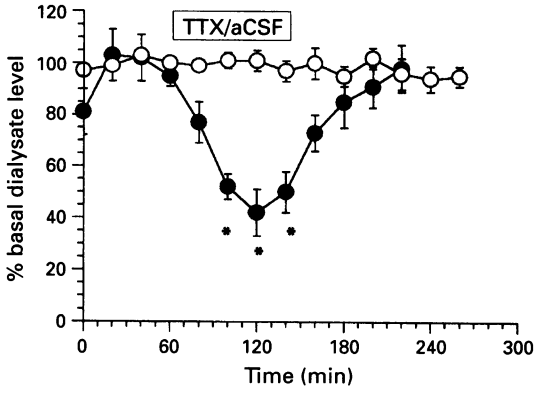

Figure 3 Ability of tetrodotoxin (●; 1.0 μM; TTX) or vehicle (○; aCSF) in the perfusing aCSF to alter extracellular dopamine levels in the striatum of freely moving rats assessed by the *in vivo* microdialysis technique. Dopamine levels in dialysates are expressed as the percentage of the meaned absolute amount in the 4 collections preceding the drug or vehicle treatment. Data represent the mean ± s.e.mean, $n = 3-5$. Horizontal bars represent application of the indicated drug (corrected for the void volume). ANOVA $P < 0.05$, $^*P < 0.05$ (Dunnett's t test).

Discussion

The present studies used two *in vitro* neurotransmitter release preparations and one *in vivo* neurotransmitter release preparation to investigate the modulation of dopamine release in the rat striatum via the 5-HT_4 receptor. Initial characterization experiments were performed to validate the release preparations. Thus the release of dopamine from rat striatal slices assesed by either the perfusion or static system was enhanced by potassium chloride and this stimulation was prevented in the absence of Ca^{2+} ions in the Krebs buffer, indicating that the stimulation of dopamine release was neuronally mediated. Similarly, the extracellular levels of dopamine in the striatum of freely moving rats, sampled by the microdialysis technique, were dramatically reduced by the inclusion of tetrodotoxin in the perfusing aCSF, suggesting that at least a major proportion of the quantified dopamine was neuronal in origin.

In both the *in vitro* and *in vivo* preparations, 5-HT_4 receptor agonists increased the apparent release of dopamine. For example the benzamide derivative 5-HT_4 receptor agonist, renzapride, increased the release of dopamine from rat striatal slices, using the static release preparation, in a concentration-dependent manner. Effective concentrations of renzapride were in the micromolar range which is consistent with the potency of renzapride in activating 5-HT_4 receptors in other preparations (for reviews see Bockaert *et al.*, 1992; Ford & Clarke, 1993). Higher concentrations of renzapride were required to evoke dopamine release in the striatum of freely moving rats assessed by the *in vivo* microdialysis technique. This discrepancy, however, may be explained by the semi-permeable barrier imposed by the microdialysis membrane. If an approximate estimation of 10% of the renzapride crosses the dialysis membrane to the striatum, then the effective concentrations of renzapride in both the *in vitro* and *in vivo* preparations correlate. In addition to renzapride, another benzamide derivative, (S)-zacopride, and the indoleamine 5-HT_4 receptor agonist, 5-MeOT (for reviews see Bockaert *et al.*, 1992; Ford & Clarke, 1993), were also able to enhance dopamine release when investigated with the perfusion *in vitro* release and the *in vivo* release preparations. Effective concentrations of (S)-zacopride and 5-MeOT in the two preparations corresponded when the estimated recovery across the dialysis membrane is taken into consideration and were consistent with effective concentrations of these compounds required to activate the 5-HT_4 receptor (for reviews see Bockaert *et al.*, 1992; Ford & Clarke, 1993).

In the present study, the 5-HT_4 receptor agonists, 5-MeOT, renzapride and (S)-zacopride, induced a greater stimulation of

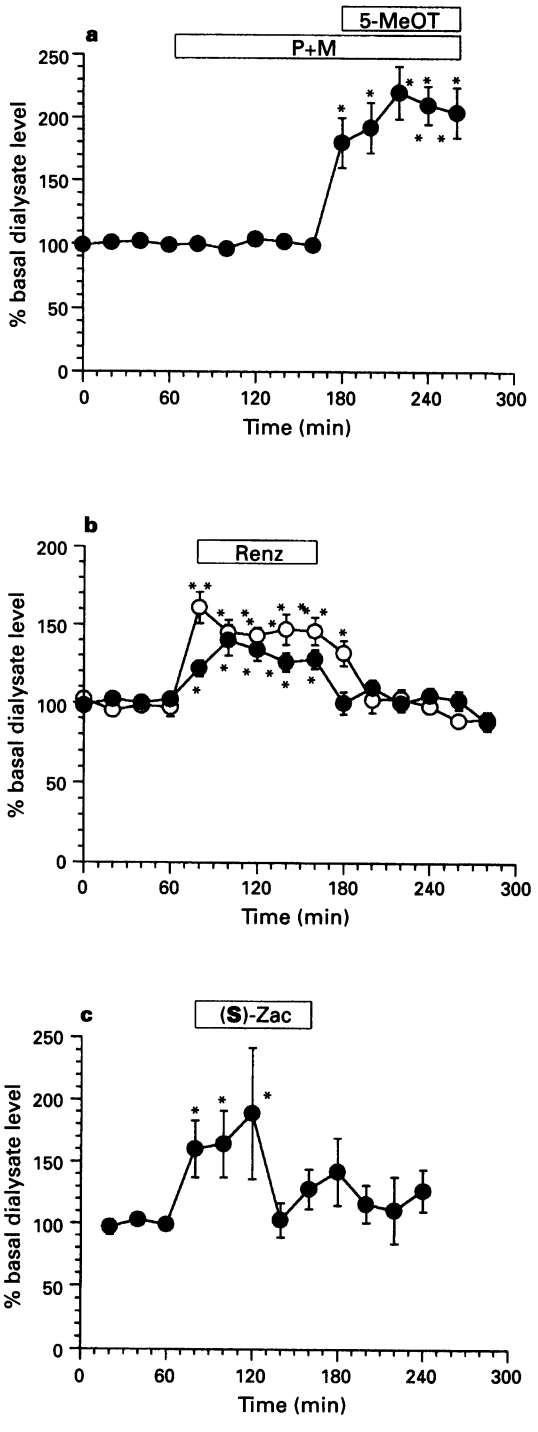

Figure 4 Ability of 5-HT_4 receptor agonists to modulate extracellular levels of dopamine in the striatum of freely moving rats assessed by the *in vivo* microdialysis technique. (a) 5-MeOT (10 μM; in the presence of pindolol (*P*; 10 μM) and methysergide (M; 10 μM)); (b) renzapride (10 (●) and 100 (○) μM; Renz) and (c) (S)-zacopride (100 μM; (S)-Zac). Dopamine levels in dialysates are expressed as the percentage of the meaned absolute amount in the 4 collections preceding the drug treatment. Data represent mean ± s.e.mean, $n = 5-9$. Horizontal bars represent application of the indicated drug (corrected for the void volume). ANOVA < 0.05, $^*P < 0.05$, $^{**}P < 0.01$ (Dunnett's t test). ANOVA < 0.05, $P < 0.05$ (Dunnett's t test) comparing the area under the curve responses evoked by the two concentrations of renzapride.

dopamine release, when compared to 5-HT. Although detailed concentration-response studies may allow a more precise investigation of this phenomenon, it would have been anticipated that the concentration of 5-HT used in the present study

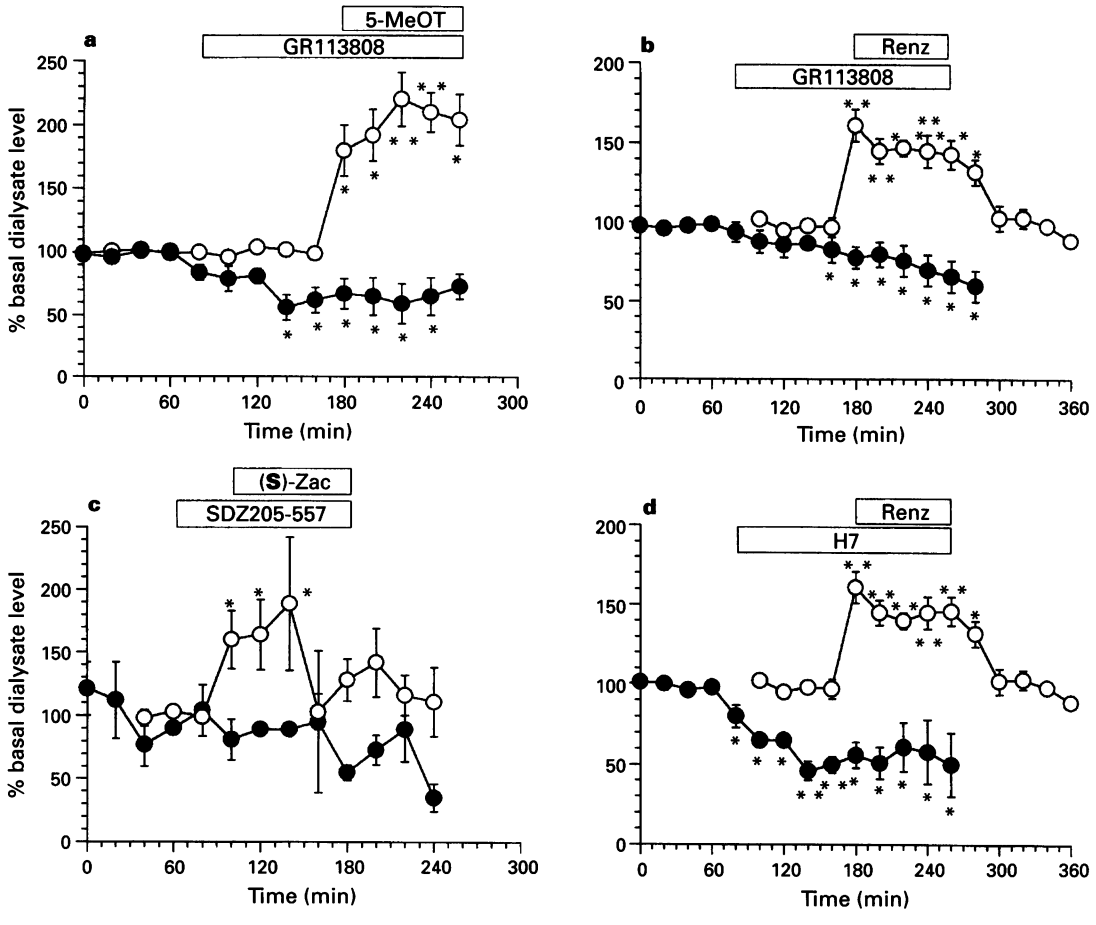

Figure 5 Ability of 5-HT$_4$ receptor ligands and the non-selective cyclic AMP-dependent protein kinase A inhibitor, H7, to modulate extracellular levels of dopamine in the striatum of freely moving rats assessed by the *in vivo* microdialysis technique. (a) 5-MeOT (○; 10 μM; in the presence of pindolol (10 μM) and methysergide (10 μM)) and 5-MeOT (10 μM; in the presence of pindolol (1 μM) and methysergide (1 μM)) plus GR113808 (●; 1 μM); (b) renzapride (○; 100 μM; Renz) and renzapride (100 μM) plus GR113808 (●; 1 μM); (c) (S)-zacopride (○; 100 μM; (S)-Zac) and (S)-zacopride (100 μM) plus SDZ205-557 (●; 100 μM) and (d) renzapride (○; 100 μM; Renz) and renzapride (100 μM) plus H7 (●; 1.0 μM). Dopamine levels in dialysates are expressed as the percentage of the meaned absolute amount in the 4 collections preceding the drug treatment. Data represent mean ± s.e.mean, $n=4-6$. Horizontal bars represent application of the indicated drug (corrected for the void volume). ANOVA <0.05, $^*P<0.05$, $^{**}P<0.01$ (Dunnett's t test).

would have evoked a near maximal response via the 5-HT$_4$ receptor. The apparently smaller response induced by 5-HT may be explained by a number of factors. For example, the effective concentration of 5-HT may have been substantially reduced by high affinity uptake or metabolism in the slice preparation or 5-HT may have been interacting with other receptors within the striatum. Given that 5-HT was able, at least in part, to mimic the actions of the other 5-HT$_4$ receptor agonists and the potential problems associated with using the endogenous ligand (e.g. removal by high affinity uptake and metabolism, lack of selectivity), in subsequent investigations exogenous 5-HT$_4$ receptor agonists were used to evoke the response. It was also of interest, however, that 5-MeOT induced a larger increase in dopamine release, compared to either renzapride or (S)-zacopride, with the perfused striatal slice preparation. Again it is difficult to draw conclusions without data from detailed concentration-response studies; however, the differing levels of efficacy associated with these agonists, as revealed in the 5-HT$_4$ receptor mediated relaxation of the rat oesophagus preparation (for review see Bockaert *et al.*, 1992), may contribute to some of the apparent different levels of efficacy of these agonists detected in the present studies.

In addition to the ability of 5-HT$_4$ receptor agonists to modify striatal dopamine release *in vitro* and *in vivo*, the pharmacology of the receptor mediating the response was further characterized with two 5-HT$_4$ receptor antagonists. The non-selective 5-HT$_3$/5-HT$_4$ receptor antagonist, SDZ 205-557 (Buchheit *et al.*, 1992) prevented the (S)-zacopride-induced response in both the perfusion *in vitro* and in the *in vivo* release preparations. Furthermore, the availability of the selective high affinity 5-HT$_4$ receptor antagonist, GR113808 (Gale *et al.*, 1994), allowed a more precise definition of the 5-HT receptor modulating the release of dopamine in the rat striatum. Our findings that nanomolar concentrations of GR113808 completely antagonized the renzapride-induced increase in dopamine release from rat striatal slices provides strong evidence that this response was mediated via the 5-HT$_4$ receptor. Similarly, the ability of GR113808 (1 μM) to antagonize completely both the 5-MeOT- and renzapride-induced increase in extracellular dopamine levels when administered to the striatum *in vivo*, via the perfusing aCSF (and hence necessitating a 'recovery' factor to be taken into consideration), implies that the 5-HT$_4$ receptor mediates the response. It was of interest that in the *in vivo* release preparation, GR113808 tended to reduce the levels of dopamine. It is unlikely that GR113808 reduced the extracellular levels of dopamine by antagonizing a facilitatory 5-HT$_3$ receptor mediated tone on the dopaminergic neurones since the concentration of GR113808 used in the present study, especially when a 'recovery' factor is taken into account, would not be expected to antagonize 5-HT$_3$ receptor-mediated responses (Gale *et al.*, 1994). This may indicate, therefore, that there is an endogenous tone on the 5-HT$_4$ re-

ceptor in this preparation. This action of GR113808 was, however, not as evident with the *in vitro* static release preparation; presumably this reflects a reduced activity of the 5-hydroxytryptaminergic terminals in this preparation relative to the *in vivo* preparation.

As a note of caution, however, one of the agonists used in the present study, 5-MeOT, also displays affinity for some of the recently defined 5-HT receptor subtypes (e.g. 5-HT$_6$, 5-HT$_7$; for review see Hoyer *et al.*, 1994), whereas information concerning the interaction of 5-MeOT with 5-HT$_{5A}$ and 5-HT$_{5B}$ receptor subtypes has yet to be published. Furthermore, since the affinity of GR113808 for 5-HT$_{5A}$, 5-HT$_{5B}$, 5-HT$_6$ or 5-HT$_7$ receptor subtypes has yet to be documented, it remains a possibility that these receptors may contribute to some of the responses detected in the present study. It is of relevance, however, to our proposed classification of the receptor stimulating striatal dopamine release that the concentrations of methysergide, selected in the present study primarily to antagonize the previously described actions of 5-HT$_1$ and 5-HT$_2$ receptor subtypes, are sufficient to occupy in addition 5-HT$_{5A}$, 5-HT$_{5B}$, 5-HT$_6$ and 5-HT$_7$ receptor subtypes (for reviews see Boess & Martin, 1994; Hoyer *et al.*, 1994).

In addition to pharmacological characterization of the 5-HT$_4$ receptor mediated stimulation of striatal dopamine release, attempts were made to investigate the transduction system associated with the receptor. To date, the available molecular evidence and the majority of the functional data, indicate that the 5-HT$_4$ receptor is positively coupled to adenylate cyclase and therefore responses are a consequence of elevated cyclic AMP levels (see Introduction). In the present studies, we demonstrated that the membrane permeable phospholipase C/cyclic AMP-dependent protein kinase A inhibitor, H7 (e.g. Hidaka *et al.*, 1984) was able to prevent the stimulation of dopamine release induced by renzapride assessed in either the *in vitro* static release preparation or the *in vivo* release preparation. The nature of the release preparations prevented the use of the more selective peptide cyclic AMP-dependent protein kinase A inhibitors since these compounds display poor membrane penetration. Although the non-selective nature of H7 precludes any firm conclusions to be drawn with respect to the precise transduction system associated with the response, the data are consistent with a previous study (Fagni *et al.*, 1992), and at least demonstrate that the response is mediated via a metabotropic-like, rather than an ionotropic-like, receptor.

Whilst the present release data indicate a functional interaction between the 5-HT$_4$ receptor and dopaminergic neurone terminals in the rat striatum, this does not necessarily indicate that the 5-HT$_4$ receptors mediating the response are located on dopaminergic neurone terminals since an indirect interaction via another neurotransmitter system cannot be excluded. Indeed, our finding that the agonist-induced increase in dopamine release *in vitro* was prevented by tetrodotoxin may support the indirect action of the 5-HT$_4$ receptor in modulating the release of dopamine. It is therefore of interest that radioligand binding data have recently demonstrated that 5-HT$_4$ receptor levels are not altered in the striatum of rat brain following 6-hydroxydopamine lesion of the nigral-striatal dopaminergic system, whereas a reduction in radiolabelled striatal 5-HT$_4$ receptors was detected following kainic acid lesion of the striatum (Patel *et al.*, 1994). The lesion data indicate that a major population of 5-HT$_4$ receptors in the striatum is not located on dopaminergic neurone terminals but is located on inter-neurones. Interestingly, comparable findings have been demonstrated with human brain tissue from patients with Parkinson's disease and Huntington's disease (Reynolds *et al.*, 1995). These binding studies, however, do not exclude the possibility that a minor population of 5-HT$_4$ receptors are located on dopaminergic terminals in the striatum. The poor cellular resolution associated with autoradiographic radioligand binding would make it difficult to study the cellular distribution of the 5-HT$_4$ receptor in the striatum by this technique and therefore the results from *in situ* hybridisation studies are eagerly awaited to identify, with cellular resolution, the distribution in the brain of mRNA encoding the 5-HT$_4$ receptor. It may also be relevant that the 5-HT$_4$ receptor appears to exhibit two alternatively spliced variants (5-HT$_{4S}$ and 5-HT$_{4L}$), which, although pharmacologically similar, have a differential pattern of mRNA expression throughout the brain although both are present within the striatum (Gerald *et al.*, 1995), which is consistent with the lesion data discussed above.

The *in vitro* striatal slice studies demonstrated that the ability of 5-HT$_4$ receptor agonists to enhance dopamine release was transient, which may indicate that the receptor was prone to desensitization, consistent with previous *in vitro* responses mediated via the 5-HT$_4$ receptor (e.g. Ansanay *et al.*, 1992; for reviews see Bockaert *et al.*, 1992; Ford & Clarke, 1993). The initial detection of this phenomenon, using the perfused *in vitro* release preparation, provided the rationale for the subsequent use of the static *in vitro* release preparation since this allows faster drug application. With this latter preparation, however, renzapride evoked a comparable percentage increase in the dopamine release (which also desensitized). It was of interest that the desensitization of the response was less evident with the *in vivo* release preparation. The reason for this difference is not clear, but in addition to the possibility that receptor recruitment may be more efficient *in vivo*, if the 5-HT$_4$ receptor-mediated response was mediated via another neurotransmitter system (i.e. the 5-HT$_4$ receptor-mediated stimulation of the dopaminergic neurone was indirect), depletion of the intermediate neurotransmitter may contribute to the reduction of the response in the continued presence of the agonist. Such a depletion is likely to be more apparent in the *in vitro* preparations with the removal of neurochemical precursors (in the present *in vitro* studies, only tyrosine was added to the Krebs buffer to prevent depletion of dopamine).

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

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