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Differential modulation of extracellular levels of 5-hydroxytryptamine in the rat frontal cortex by (R)- and (S)-zacopride

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1 The ability of various anxiolytic and potential anxiolytic agents to modify 5-hydroxytryptamine (5-HT) release in the frontal cortex of the rat was assessed by the microdialysis technique.

2 The benzodiazepine receptor agonist, diazepam (2.5 mg kg^{-1}, i.p.), the 5-HT$_{1A}$ receptor agonist 8-hydroxy-2-(di-n-propylamino) tetralin (8-OH-DPAT, 0.32 mg kg^{-1}, s.c.) and the 5-HT$_{1A}$ receptor partial agonist buspirone (4.0 mg kg^{-1}, i.p.) maximally reduced extracellular levels of 5-HT in the rat frontal cortex by approximately 50–60%, 70–80% and 30–40%, respectively.

3 (R)-zacopride (1.0–100 μg kg^{-1}, i.p.) dose-dependently reduced extracellular levels of 5-HT in the rat frontal cortex (approximately 80% maximal reduction) whereas the other 5-HT$_3$ receptor antagonists ondansetron (10 μg kg^{-1}, i.p.) and (S)-zacopride (10–100 μg kg^{-1}, i.p.) were ineffective.

4 In contrast to (S)-zacopride (100 nM; administered via the microdialysis probe), (R)-zacopride (1.0–100 nM; administered via the microdialysis probe) induced a concentration-dependent reduction in extracellular levels of 5-HT in the rat frontal cortex (approximately 70% maximal reduction).

5 In contrast to ondansetron (100 μg kg^{-1}, i.p.), (S)-zacopride (10–100 μg kg^{-1}, i.p.) dose-dependently reversed the (R)-zacopride (10 μg kg^{-1}, i.p.) induced reduction in extracellular levels of 5-HT in the rat frontal cortex. The highest dose of (S)-zacopride (100 μg kg^{-1}, i.p.) completely prevented the (R)-zacopride response. In addition, (S)-zacopride (100 nM; administered via the microdialysis probe) attenuated the inhibitory action of (R)-zacopride (10 nM; administered via the microdialysis probe) on extracellular levels of 5-HT in the rat frontal cortex.

6 In conclusion, the present study provides further evidence of the ability of diazepam, 8-OH-DPAT and buspirone to reduce the activity of the central 5-hydroxytryptaminergic system *in vivo*. Furthermore, the results indicate that the ability of (R)-zacopride to reduce the *in vivo* release of 5-HT in the rat frontal cortex does not correlate with its 5-HT$_3$ receptor antagonism. However, the differential affinity of (R)- and (S)-zacopride for a (S)-zacopride-insensitive (R)-zacopride site in rat cerebral cortex mirrors the relative activity of the two zacopride stereoisomers to modify the *in vivo* release of 5-HT in the frontal cortex of the rat and their ability to release suppressed behaviour in animal models of anxiety.

Keywords: 5-Hydroxytryptamine; anxiety; *in vivo* microdialysis; frontal cortex; 5-HT$_3$ receptor; 5-HT$_4$ receptor; benzamides

Introduction

A characteristic feature of many compounds that reduce the functional activity of the central 5-hydroxytryptaminergic system is the manifestation of anxiolytic-like behaviour (for reviews see Iversen, 1984; Chopin & Briley, 1987). Thus the clinical efficacy of the benzodiazepine receptor agonists and the 5-HT$_{1A}$ receptor agonist/partial agonists as anxiolytic agents has been attributed to a reduction in the neuronal release of 5-hydroxytryptamine (5-HT; e.g. Stein *et al.*, 1977; Chopin & Briley, 1987). In contrast, the anxiolytic-like activity of 5-HT$_3$ receptor antagonists (for reviews see Costall *et al.*, 1990; Barnes *et al.*, 1992a) is probably a consequence of preventing the natural agonist interacting with postsynaptic receptors.

Whilst structurally dissimilar 5-HT$_3$ receptor antagonists display anxiolytic-like activity in pre-clinical models and in preliminary trials with patients with generalized anxiety disorder (for reviews see Costall *et al.*, 1990; Barnes *et al.*, 1992a), the report that the (R)- and (S)-isomers of the benzamide derivative 5-HT$_3$ receptor antagonist, zacopride, display differential activity in animal models of anxiety remains an unexplained phenomenon (Barnes *et al.*, 1990a; Young & Johnson, 1991). Thus the (R)-isomer of zacopride possesses anxiolytic-like activity at low microgram doses whereas effective doses of (S)-zacopride are at least 4 orders of magnitude higher (Barnes *et al.*, 1990a; Young & Johnson, 1991). This potency difference, however, is not consistent with their affinities for the 5-HT$_3$ receptor since both possess considerable antagonistic activity at nanomolar concentrations (Barnes *et al.*, 1990a). The pharmacological diversity of the isomers of zacopride is further emphasized by the ability of (S)-zacopride to attenuate the anxiolytic profiles of (R)-zacopride (Barnes *et al.*, 1992b) and other pharmacologically distinct agents (Barnes *et al.*, 1992c).

In an attempt to reveal a neurochemical correlate for the ability of the isomers of zacopride to modulate suppressed behaviour, in the present studies we assess the actions of various anxiolytic and putative anxiolytic agents and the stereoisomers of zacopride, on the *in vivo* extracellular levels of 5-HT in the frontal cortex of freely-moving rats using the microdialysis technique. A preliminary account of this work has been presented to the British Pharmacological Society (Cheng *et al.*, 1992).

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Methods

Animals

Male hooded-Lister rats (300–350 g) were housed in groups of 6 in a controlled environment (temperature 21 ± 1°C) under a 12 h light/dark cycle (lights on 07 h 00 min–19 h 00 min) and were given free access to food and water.

Stereotaxic implantation of chronic indwelling guide cannulae

Rats were anaesthetized with sodium pentobarbitone (60 mg kg^{-1}, i.p.) and chloral hydrate (150 mg kg^{-1}, s.c.) before the insertion of 5 mm chronically indwelling guide cannulae (19 guage stainless steel tubing; Coopers Needle Work Ltd) which were positioned above the frontal cortex (final probe tip location; right hemisphere, A + 0.7, V − 7.0, L − 1.5, relative to Bregma at an angle of 45°) and secured to the skull with screws and dental cement. The guide cannulae were kept patent with stylets.

Microdialysis probe construction, implantation and collection of dialysates

Microdialysis probes were constructed 'in house'. Briefly, stainless steel 'bodies' (11 mm, 23 guage, Coopers Needle Work Ltd) and 'collars' (5 mm, 26 guage, Coopers Needle Work Ltd) were fixed into a perspex holder. Dialysis membrane (external/internal diameter 310/220 μm, molecular weight cut off 40,000; AN69, Hospal Medical) was glued to the tip of the stainless steel 'body' and the end sealed with epoxy resin (Araldite) leaving 4 mm in total length exposed to the brain area. A silica glass fibre (external/internal diameter 140/74 μm, Scientific Glass Engineering PTY Ltd) guided artificial cerebrospinal fluid (aCSF; mM: NaCl 126, $NaHCO_3$ 27.4, KCl 2.4, KH_2PO_4 0.5, $CaCl_2$ 1.1, $MgCl_2$ 1.3, $NaHPO_4$ 0.49 and glucose 7.0; pH 7.4) to the tip of the microdialysis probe, which subsequently eluted from the probe (via coiled polypropylene tubing) following passage between the outer surface of the silica glass fibre and the inner surface of the dialysis membrane.

At least 14 days after stereotaxic location of the guide cannulae, rats were immobilized by a soft-cloth wrapping technique and the microdialysis probe was gently implanted into the frontal cortex and secured with cyanoacrylate adhesive (Permabond C2).The rat was placed in a single animal cage (with free access to food and water) for 12 h before being transferred to the test cage (42 × 24 × 26 cm; length, width, height) where the animal also had free access to food and water. Following a 30 min period for the rat to habituate to the test box, the microdialysis probe was connected, via polypropylene tubing, to a microdialysis pump (CMA 100, Carnegie) and was perfused at a constant rate of 2.0 μl min^{-1} with aCSF. The dialysate collected over the first 60 min was discarded and subsequent samples were collected every 20 min for a period of up to 6 h. All samples were analysed immediately for levels of 5-HT and 5-hydroxyindoleacetic acid (5-HIAA) by high performance liquid chromatography (h.p.l.c.) with electrochemical detection (e.c.d.). At the end of the 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 frontal cortex were not included in the present study.

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

For the simultaneous determination of 5-HT and 5-HIAA levels in dialysates, the h.p.l.c.-e.c.d. system comprised of an isocratic pump (Waters 510 Solvent Delivery System) which was connected to an analytical column (Hypersil 5 ODS; 150 × 4.6 mm; HPLC Technology) via an automatic injector (Waters Wisp). The analytical column was protected by a C_{18} guard column (Guard-Pak, Waters). The effluent from the analytical column was passed into an electrochemical detector (ESA Coulochem with model 5011 analytical cell, working electrode potential + 0.45 V versus a solid state reference electrode incorporated within the analytical cell). The output from the electrode was monitored with a recording/plotting integrator (Hewlett-Packard 3392A). A guard cell (ESA model 5020) was placed between the pump and injector and was set at + 0.50 V (relative to a solid state reference electrode incorporated within the cell) to reduce the background current originating from the mobile phase. 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 column to column variation) 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

Buspirone (Mead Johnson), 8-OH-DPAT (8-hydroxy-2-(di-n-propylamino) tetralin, Research Biochemicals Inc.), ondansetron (Glaxo), pargyline (Sigma), tetrodotoxin (Sigma),

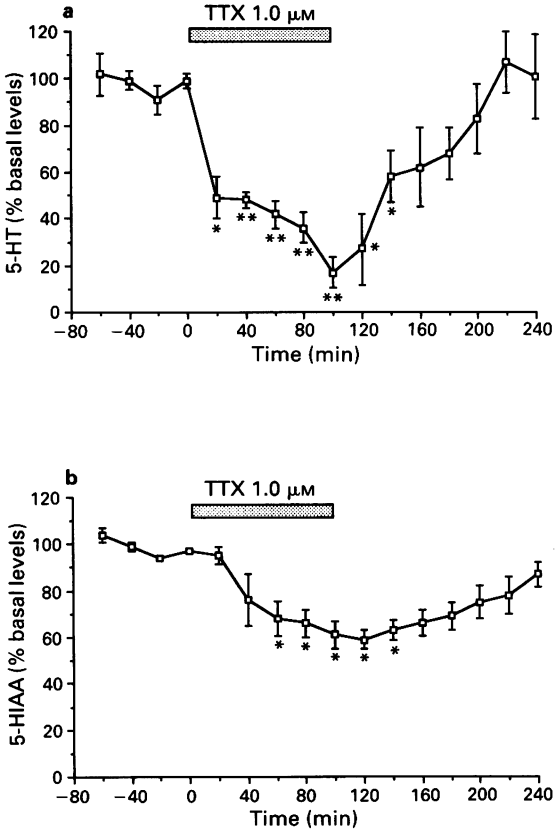

Figure 1 Effect of tetrodotoxin (1.0 μM; TTX) in the perfusing aCSF on 5-hydroxytryptamine (5-HT) (a) and 5-hydroxyindoleacetic acid (5-HIAA) (b) levels in rat frontal cortex dialysates. 5-HT and 5-HIAA levels in dialysates are expressed as the percentage of the meaned absolute amount in the 4 collections preceding the drug treatment. Data represent the mean ± s.e.mean (vertical bars) from 4 animals. ANOVA:$P<0.05$; *$P<0.05$; **$P<0.01$ (Dunnett's t test).

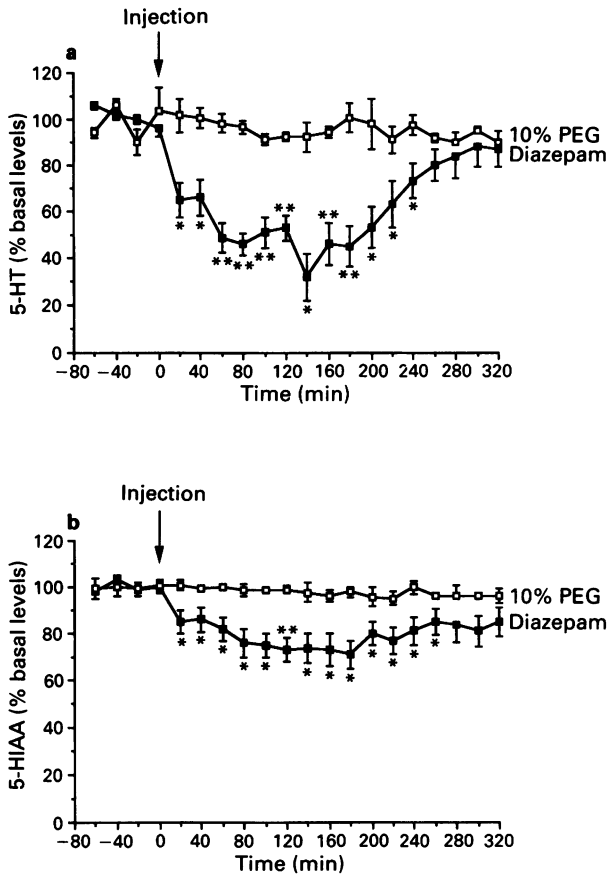

Figure 2 Effect of vehicle (10% v/v polyethylene glycol in saline, 1.0 ml kg^{-1}, i.p.; 10% PEG) and diazepam (2.5 mg kg^{-1}, i.p.) on 5-hydroxytryptamine (5-HT) (a) and 5-hydroxyindoleacetic acid (5-HIAA) (b) levels in rat frontal cortex dialysates. 5-HT and 5-HIAA levels in dialysates are expressed as the percentage of the meaned absolute amount in the 4 collections preceding the drug treatment. Data represent the mean ± s.e.mean (vertical bars) from 3–6 animals. ANOVA: $P<0.05$; $^{*}P<0.05$; $^{**}P<0.01$ (Dunnett's t test).

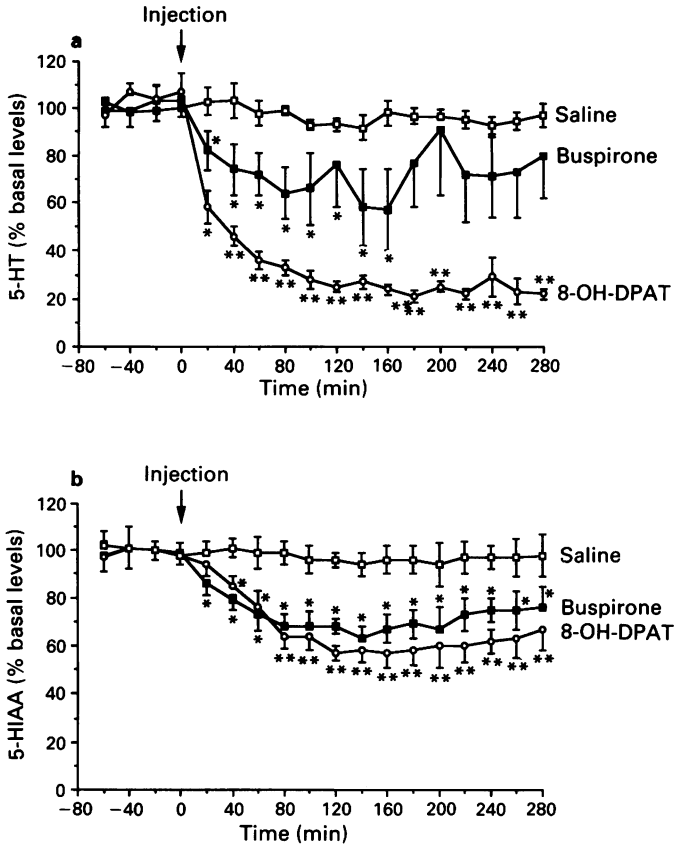

Figure 3 Effect of vehicle (saline, 1.0 ml kg^{-1}, i.p.), 8-hydroxy-2-(di-n-propylamino) tetralin (8-OH-DPAT), 0.32 mg kg^{-1}, s.c.) and buspirone (4 mg kg^{-1}, i.p.) on 5-hydroxytryptamine (5-HT) (a) and 5-hydroxyindoleacetic acid (5-HIAA) (b) levels in rat frontal cortex dialysates. 5-HT and 5-HIAA levels in dialysates are expressed as the percentage of the meaned absolute amount in the 4 collections preceding the drug treatment. Data represent the mean ± s.e.mean (vertical bars) from 6–8 animals. ANOVA: $P<0.05$; $^{*}P<0.05$; $^{**}P<0.01$ (Dunnett's t test).

(R)-zacopride (A.H. Robins) and (S)-zacopride (A.H. Robins) were dissolved in distilled water and diluted to volume in saline (0.9% w/v NaCl) or aCSF. Diazepam (Sigma) was dissolved in 10% v/v polyethylene glycol (in saline). All drugs were used as received and were freshly prepared immediately before use.

Results

Methodological results

The *in vitro* recoveries of 5-HT and 5-HIAA using the 'in-house' microdialysis probes were 24 ± 1 and 20 ± 1%, respectively (mean ± s.e.mean, 6 probes). The limits of detection for both 5-HT and 5-HIAA by the h.p.l.c.-e.c.d. assay was at least 1 pg (signal to noise ratio of 3:1; injection volume 40 µl).

Validation of the neuronal origin of the 5-HT in the dialysates

The basal level of 5-HT and 5-HIAA varied between 37–63 fmol/40 µl and 1.83–2.88 pmol/40 µl, respectively, in the dialysates obtained from the frontal cortex of freely-moving rats 12 h following the implantation of the dialysis probe.

Inclusion of tetrodotoxin (1.0 µM) in the perfusing aCSF reduced the levels of 5-HT and 5-HIAA in the frontal cortex dialysates by approximately 80 and 40%, respectively. These levels recovered to basal values following the removal of tetrodotoxin (Figure 1).

Removal of $CaCl_2$ from the aCSF (and inclusion of EGTA, 0.2 mM) maximally reduced the frontal cortex dialysate levels of 5-HT by approximately 50%. The 5-HT levels, however, began to return to basal values before the re-addition of $CaCl_2$ to the perfusing aCSF (data not shown).

Effect of monoamine oxidase inhibition on the dialysate levels of 5-HT and 5-HIAA

Peripheral administration of the non-selective monoamine oxidase inhibitor, pargyline (75 mg kg^{-1}, i.p.) increased the frontal cortex dialysate levels of 5-HT by up to 800% and decreased the levels of 5-HIAA (maximally to 30–40% of basal levels) over the 320 min period following the peripheral injection (data not shown).

Effect of systemic drug administration on the dialysate levels of 5-HT and 5-HIAA

Administration of the benzodiazepine receptor agonist, diazepam (2.5 mg kg^{-1}, i.p.), reduced the frontal cortex dialysate levels of 5-HT and 5-HIAA (Figure 2). Injection of diazepam's vehicle (10% v/v polyethylene glycol in saline, i.p.) failed to alter dialysate levels of either 5-HT or 5-HIAA (Figure 2).

The 5-HT_{1A} receptor agonist, 8-OH-DPAT (0.32 mg kg^{-1},

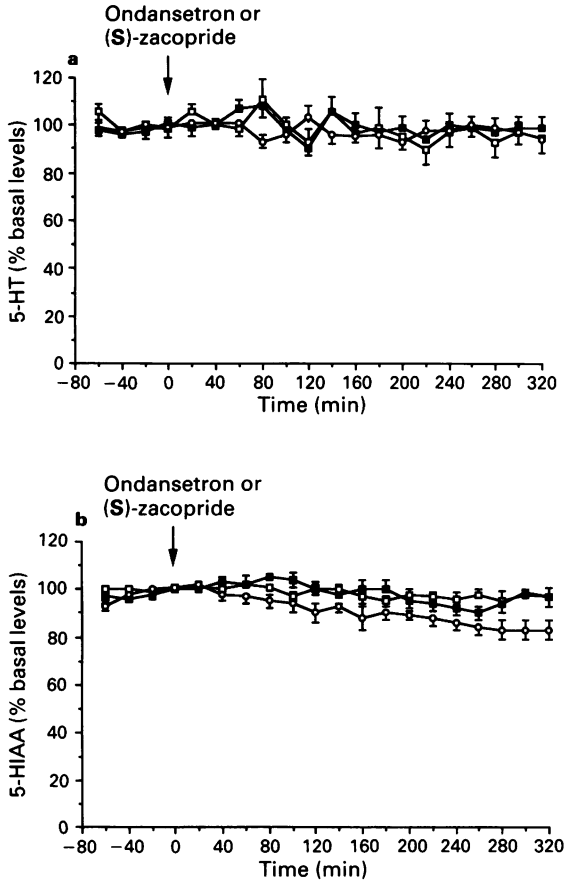

Figure 4 Effect of ondansetron (10 μg kg^{-1}, i.p. (○)), and (S)-zacopride (10 μg kg^{-1}, i.p. (□) and 100 μg kg^{-1}, i.p. (■)) on 5-hydroxytryptamine (5-HT) (a) and 5-hydroxyindoleacetic acid (5-HIAA) (b) levels in rat frontal cortex dialysates. 5-HT and 5-HIAA levels in dialysates are expressed as the percentage of the meaned absolute amount in the 4 collections preceding the drug treatment. Data represent the mean ± s.e.mean (vertical bars) from 8 animals. ANOVA: $P > 0.05$.

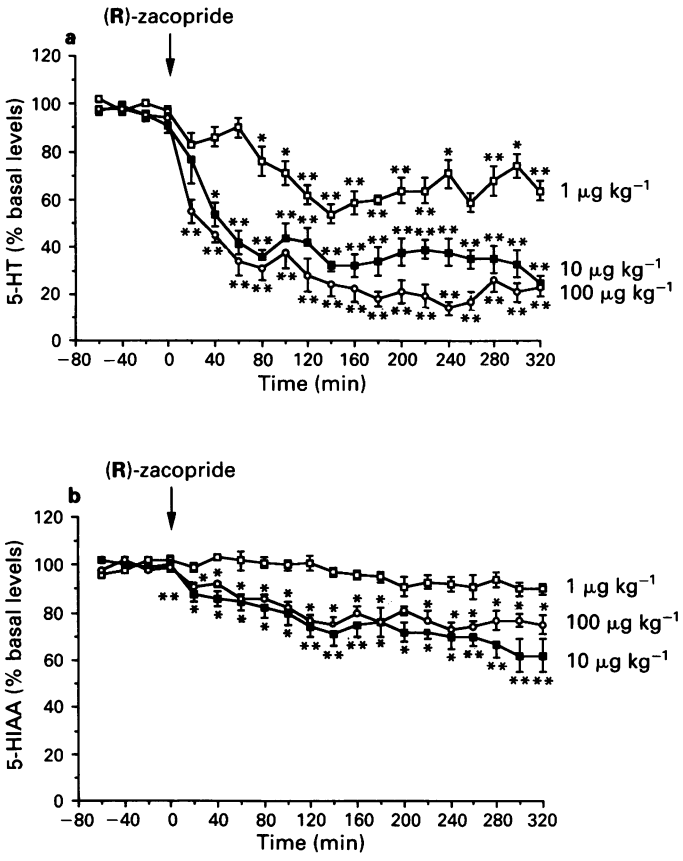

Figure 5 Effect of (R)-zacopride (1.0–100 μg kg^{-1}, i.p.) on 5-hydroxytryptamine (5-HT) (a) and 5-hydroxyindoleacetic acid (5-HIAA) (b) levels in rat frontal cortex dialysates. 5-HT and 5-HIAA levels in dialysates are expressed as the percentage of the meaned absolute amount in the 4 collections preceding the drug treatment. Data represent the mean ± s.e.mean (vertical bars) from 8 animals. ANOVA: $P < 0.05$; $^{*}P < 0.05$; $^{**}P < 0.01$ (Dunnett's t test).

s.c.) and partial agonist buspirone (4.0 mg kg^{-1}, i.p.) decreased the levels of 5-HT and 5-HIAA in the frontal cortex dialysates (Figure 3). Subcutaneous (data not shown) or intra-peritoneal injection of vehicle (saline) failed to alter dialysate levels of either 5-HT or 5-HIAA (Figure 3).

Ondansetron (10 μg kg^{-1}, i.p.) and (S)-zacopride (10–100 μg kg^{-1}, i.p.) failed to alter the levels of 5-HT in the dialysates from rat frontal cortex (Figure 4). Furthermore, of these treatments, only (S)-zacopride (100 μg kg^{-1}, i.p.) induced a small reduction in the dialysate levels of 5-HIAA (by some 15–20%) which was maximal at the end of the experiment (320 min after the injection; Figure 4).

(R)-zacopride (1.0–100 μg kg^{-1}, i.p.) reduced the dialysate levels of 5-HT from rat frontal cortex in a dose-dependent manner; the highest dose decreasing the levels by some 70–80% (Figure 5). At all of the doses tested, the maximal decreases in dialysate 5-HT levels were detected approximately 140 min following the injection and the reduced levels remained near maximal for the remainder of the experiment (320 min after the injection). (R)-zacopride (10–100 μg kg^{-1}, i.p.) also reduced the dialysate levels of 5-HIAA (by upto 40%) whilst a lower dose (1.0 μg kg^{-1}, i.p.) was without effect. The (R)-zacopride-induced reduction in 5-HIAA levels reached a maximum at approximately 120 min after the injection of 100 μg kg^{-1} (and remained so for the duration of the experiment), whilst the 5-HIAA levels continued to decrease up to 320 min following the injection of 10 μg kg^{-1} (R)-zacopride (Figure 5).

Effect of central drug administration on the dialysate levels of 5-HT and 5-HIAA

(R)-zacopride (1–100 nM) administered via the perfusing aCSF induced a concentration-dependent decrease in the dialysate levels of 5-HT and 5-HIAA from the rat frontal cortex (Figure 6). The highest concentration maximally reduced 5-HT and 5-HIAA levels by approximately 70% (Figure 6); the reduction being maximal 60 min after the introduction of (R)-zacopride (Figure 6).

(S)-zacopride (100 nM) administered via the perfusing aCSF failed to modify the dialysate levels of either 5-HT or 5-HIAA from the rat frontal cortex (Figure 6).

Effect of (S)-zacopride and ondansetron on the (R)-zacopride-induced reduction in the dialysate levels of 5-HT and 5-HIAA

(S)-zacopride (10–100 μg kg^{-1}, i.p.) administered 5 min prior to the injection of (R)-zacopride (10 μg kg^{-1}, i.p.) dose-dependently prevented the reduction in extracellular levels of 5-HT and 5-HIAA (Figure 7).

In contrast, injection of saline (0.9% w/v NaCl i.p.) or ondansetron (100 μg kg^{-1}, i.p.) 5 min prior to the injection of (R)-zacopride (10 μg kg^{-1}, i.p.) failed to attenuate the (R)-zacopride-induced (10 μg kg^{-1}, i.p.) reduction in dialysate levels of either 5-HT or 5-HIAA (Figure 7).

Following a reduction in 5-HT dialysate levels induced by (R)-zacopride (10 nM; administered via the perfusing aCSF), the addition of (S)-zacopride (100 nM) to the aCSF

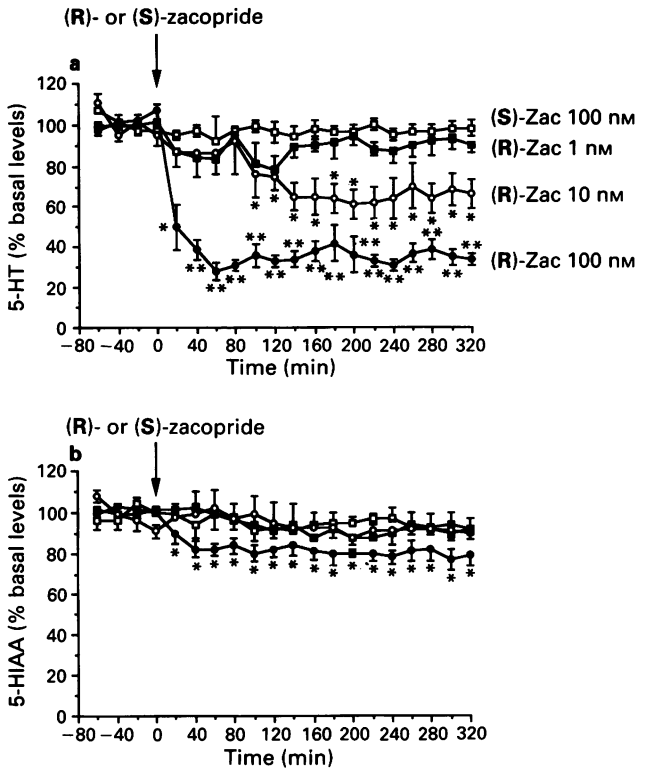

Figure 6 Effect of (R)-zacopride (1.0–100 nM; (R)-Zac) and (S)-zacopride (100 nM; (S)-Zac) administered via the perfusing aCSF on 5-hydroxytryptamine (5-HT) (a) and 5-hydroxyindoleacetic acid (5-HIAA) (b) levels in rat frontal cortex dialysates. 5-HT and 5-HIAA levels in dialysates are expressed as the percentage of the meaned absolute amount in the 4 collections preceding the drug treatment. Data represent the mean ± s.e.mean (vertical bars) from 8 animals. ANOVA: $P<0.05$; $^{*}P<0.05$; $^{**}P<0.01$. (Dunnett's t test).

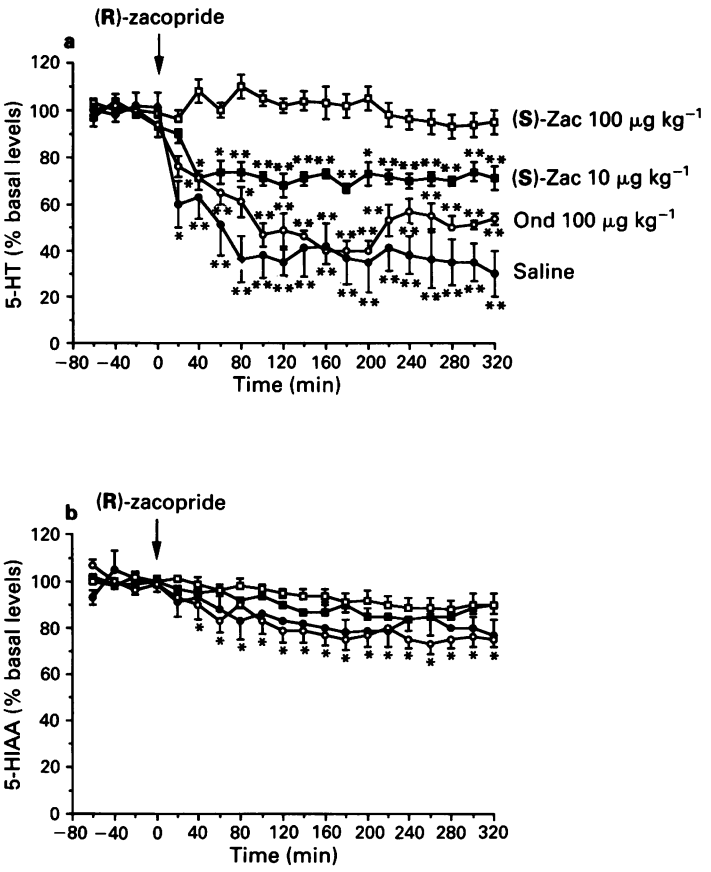

Figure 7 Ability of saline vehicle (0.9% w/v NaCl i.p.; Saline), ondansetron (100 μg kg^{-1}, i.p.; Ond) or (S)-zacopride (10–100 μg kg^{-1}, i.p.; (S)-Zac) to modify the (R)-zacopride-induced reduction in dialysate levels of 5-hydroxytryptamine (5-HT) (a) and 5-hydroxyindoleacetic acid (5-HIAA) (b) from rat frontal cortex. Saline vehicle, ondansetron or (S)-zacopride were administered 5 min prior to the injection of (R)-zacopride (10 μg kg^{-1}, i.p.). 5-HT and 5-HIAA 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 (vertical bars) from 8 animals. ANOVA: $P<0.05$; $^{*}P<0.05$; $^{**}P<0.01$ (Dunnett's t test).

attenuated the (R)-zacopride-induced reduction in dialysate 5-HT levels (Figure 8).

Discussion

In the present studies, the effect of anxiolytic and putative anxiolytic agents on 5-HT release from the frontal cortex was assessed by the microdialysis technique in freely moving rats. The finding that the dialysate 5-HT levels were sensitive to the addition of the sodium channel blocker, tetrodotoxin, to the perfusing aCSF and to the removal of $CaCl_2$ from the aCSF, indicates that a relatively large component (at least 70–80%) of the quantified 5-HT was neuronal in origin, this being consistent with previous reports (e.g. Auerbach *et al.*, 1989; Carboni & DiChiara, 1989). Likewise the response following inhibition of monoamine oxidase subsequent to peripheral administration of pargyline (e.g. Carboni & DiChiara, 1989), where a gradual increase in the levels of 5-HT and a concomitant decrease in the levels of the metabolite of 5-HT, 5-HIAA, presumably reflected a decrease in the metabolism of this monoamine.

As has been previously demonstrated in the rat frontal cortex and/or ventral hippocampus (e.g. Maidment *et al.*, 1986; Pei *et al.*, 1989; Sharp *et al.*, 1989), 5-HT release in the frontal cortex, measured by *in vivo* microdialysis, was decreased following administration of the benzodiazepine receptor agonist, diazepam, and the 5-HT_{1A} receptor agonist 8-OH-DPAT and partial agonist buspirone. Such findings are consistent with the action of benzodiazepine receptor agonists to potentiate the inhibitory action of γ-aminobutyric acid (GABA) (e.g. Enna & Mohler, 1987) and the actions of 5-HT_{1A} receptor ligands with intrinsic activity to reduce the release of 5-HT in terminal regions following interaction with somato-dendritic 5-HT_{1A} autoreceptors (Maidment *et al.*, 1986; Auerbach *et al.*, 1989; Hutson *et al.*, 1989; Sharp *et al.*, 1989). The maintenance of a maximal reduction in dialysate 5-HT levels following 8-OH-DPAT administration at the termination of the experiment (over 4 h after the subcutaneous injection) may at first appear unexpected since this compound is rapidly metabolized (half life < 30 min; Perry & Fuller, 1989). It should be noted, however, that previous studies have demonstrated that comparable doses of 8-OH-DPAT induce a maximal response for at least as long as in the present study (e.g. Maidment *et al.*, 1986).

The 5-HT_3 receptor antagonist, ondansetron (Butler *et al.*, 1988) when used at a dose known to reduce the behavioural response to an aversive situation (Costall *et al.*, 1990) failed to alter the release of 5-HT in the frontal cortex. This was not entirely unexpected since 5-HT_3 receptor recognition sites do not appear to be predominantly presynaptic in their location since their density is not reduced following selective lesion of the central 5-hydroxytryptaminergic system (Barnes *et al.*, unpublished observation). The absence of any detectable change in frontal cortex dialysate 5-HT levels by the acute administration of ondansetron also indicates that modulation by 5-HT_3 receptors of release via postsynaptic

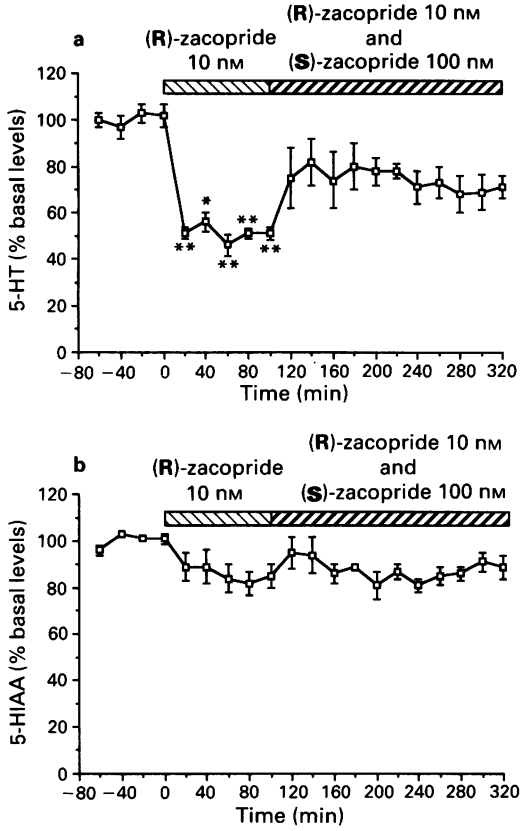

Figure 8 Ability of (S)-zacopride (100 nM) administered via the perfusing aCSF to modify the (R)-zacopride-induced reduction in dialysate levels of 5-hydroxytryptamine (5-HT) (a) and 5-hydroxyindoleacetic acid (5-HIAA) (b) from rat frontal cortex. (R)-zacopride was added to the aCSF at $t = 0$ and (S)-zacopride was added to the aCSF after an additional 100 min. 5-HT and 5-HIAA levels in dialysates are expressed as the percentage of the meaned absolute amount in the 4 collections preceding the drug treatment. Data represent the mean ± s.e.mean (vertical bars) from 8 animals. ANOVA: $P<0.05$; $*P<0.05$; $**P<0.01$ (Dunnett's t test).

feedback systems is not involved. In accordance with the results obtained with ondansetron, the structurally dissimilar 5-HT_3 receptor antagonist, (S)-zacopride (Barnes *et al.*, 1990a,b), also failed to modify the *in vivo* release of 5-HT from the rat frontal cortex. These findings therefore imply that the dose-dependent ability of (R)-zacopride to reduce extracellular levels of 5-HT in the frontal cortex was not a consequence of antagonism of 5-HT_3 receptors. However, this neurochemical response may provide the basis for the anxiolytic-like action of (R)-zacopride (see Introduction).

The ability of nanomolar concentrations of (R)-zacopride to reduce the apparent terminal release of 5-HT in the rat frontal cortex was detected when (R)-zacopride was administered via the microdialysis probe. It would therefore be predicted that the response was a probable consequence of recognition site interaction within the frontal cortex. However, the potential involvement of additional areas following systemic administration of (R)-zacopride cannot be excluded and warrants further investigation.

At present we have no evidence as to the nature of the (R)-zacopride recognition site. Whilst this site may represent a receptor, the potential for the involvement of an uptake system or an enzyme in the (R)-zacopride-induced reduction in extracellular levels of 5-HT should not be ruled out. It may be relevant, however, that (R)-zacopride displays low nanomolar affinity for a recognition site within the brain for which (S)-zacopride displays at least 3 orders of magnitude lower affinity (Barnes *et al.*, 1990a; Kidd *et al.*, 1992). Such an affinity difference is more consistent with the differential potency of these two isomers in pre-clinical models designed to assess potential anxiolytic activity (Barnes *et al.*, 1990a; Young & Johnson, 1991) and their ability to reduce extracellular levels of 5-HT within the rat frontal cortex (present studies) compared with their ability to interact with 5-HT_3 receptors (Barnes *et al.*, 1990a,b) or stimulate 5-HT_4 receptors (Eglen *et al.*, 1990; Baxter *et al.*, 1991). It must be emphasized, however, that the pharmacological knowledge relating to this (S)-zacopride-insensitive (R)-zacopride recognition site is limited (Barnes *et al.*, 1990a; Kidd *et al.*, 1992).

Since behavioural experiments in the mouse have identified that the anxiolytic-like actions of (R)-zacopride are reversed by (S)-zacopride (Barnes *et al.*, 1992b), it is of considerable interest that in the present studies, administration of (S)-zacopride prevented the (R)-zacopride-induced reduction in extracellular 5-HT levels in the rat frontal cortex. The ability of (S)-zacopride to evoke this response when administered via the microdialysis probe indicates that this pharmacological interaction probably occurs within the frontal cortex. It is unlikely, however, that (S)-zacopride is competing with (R)-zacopride for the (R)-zacopride site which may mediate the reduction in 5-HT release since radioligand binding studies have demonstrated that (S)-zacopride displays relatively low affinity for this site (Barnes *et al.*, 1990a; Kidd *et al.*, 1992). With respect to the ability of (S)-zacopride to antagonize the (R)-zacopride-induced reduction in extracellular levels of 5-HT, it is interesting that (S)-zacopride in its own right failed to enhance extracellular 5-HT levels—such a finding may be explained by the requirement to reduce 5-hydroxytryptaminergic tone before functional expression of this (S)-zacopride-induced response. Such a hypothesis remains to be explored as does the pharmacological characterization of the 'receptor' responsible for reversing the (R)-zacopride-induced reduction in extracellular levels of 5-HT.

Regardless of the precise mechanism of action, the ability of (R)-zacopride to reduce extracellular levels of 5-HT within the rat frontal cortex and the apparent failure of (S)-zacopride to elicit a similar response in the same model provides a neurochemical response which mirrors the differential activities of the zacopride isomers in animal models of anxiety (Barnes *et al.*, 1990a; Young & Johnson, 1991). Furthermore, the capacity of (S)-zacopride to prevent the (R)-zacopride-induced reduction in 5-HT release may be relevant to the ability of (S)-zacopride to antagonize the anxiolytic profile of action of (R)-zacopride (Barnes *et al.*, 1992b).

In summary, the present studies have demonstrated that extracellular levels of 5-HT in the frontal cortex of freely moving rats (the majority of which is apparently neuronal in origin) are reduced by drugs with agonistic activity at the benzodiazepine and 5-HT_{1A} receptor but not by the 5-HT_3 receptor antagonists, ondansetron and (S)-zacopride. The ability of (R)-zacopride, therefore, to reduce the extracellular levels of 5-HT, following peripheral or central administration, is unlikely to result solely from an antagonistic interaction with 5-HT_3 receptors and may be a consequence of interaction with a (S)-zacopride insensitive 'receptor'. Such an interaction may provide a neurochemical basis for the anxiolytic-like actions of (R)-zacopride.

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References

AUERBACH, S.B., MINZENBERG, M.J. & WILKINSON, L.O. (1989). Extracellular serotonin and 5-hydroxyindoleacetic acid in hypothalamus of the unanesthetized rat measured by in vivo dialysis coupled to high-performance liquid chromatography with electrochemical detection: dialysate serotonin reflects neuronal release. *Brain Res.*, **499,** 281–290.

BARNES, J.M., BARNES, N.M., CHAMPANERIA, S., COSTALL, B. & NAYLOR, R.J. (1990b). Characterisation and autoradiographic localisation of 5-HT_3 receptor recognition sites identified with [^{3}H]-(S)-zacopride in the forebrain of the rat. *Neuropharmacology*, **29,** 1037–1045.

BARNES, J.M., BARNES, N.M. & COOPER, S.J. (1992a). Behavioural pharmacology of 5-HT_3 receptor ligands. *Neurosci. Biobehav. Rev.*, **16,** 107–113.

BARNES, J.M., BARNES, N.M., COSTALL, B., DOMENEY, A.M., JOHNSON, D.N., KELLY, M.E., MUNSON, H.R., NAYLOR, R.J. & YOUNG, R. (1990a). The differential activities of R(+)- and S(−)-zacopride as 5-HT_3 receptor antagonists. *Pharmacol. Biochem. Behav.*, **37,** 717–727.

BARNES, N.M, CHENG, C.H.K., COSTALL, B., GE, J., KELLY, M.E. & NAYLOR, R.J. (1992c). Profiles of interaction of R(+)/S(−)-zacopride and anxiolytic agents in a mouse model. *Eur. J. Pharmacol.*, (in press).

BARNES, N.M, COSTALL, B., GE, J., KELLY, M.E. & NAYLOR, R.J. (1992b). The interaction of R(+) and S(−) zacopride with PCPA to modify rodent aversive behaviour. *Eur. J. Pharmacol.*, (in press).

BAXTER, G.S., CRAIG, D.A. & CLARKE, D.E. (1991). 5-Hydroxytryptamine$_4$ receptors mediate relaxation of the rat oesophageal tunica muscularis mucosae. *Naunyn Schmiedebergs Arch. Pharmacol.*, **343,** 439–446.

BUTLER, A., HILL, J.M., IRELAND, S.J., JORDAN, C.C. & TYERS, M.B. (1988). Pharmacological properties of GR38032F, a novel antagonist at 5-HT_3 receptors. *Br. J. Pharmacol.*, **94,** 397–412.

CARBONI, E. & DiCHIARA, G. (1989). Serotonin release estimated by transcortical dialysis in freely-moving rats. *Neuroscience*, **32,** 637–645.

CHENG, C.H.K., BARNES, N.M, COSTALL, B., GE, J. & NAYLOR, R.J. (1992). Differential activities of (**R**)- and (**S**)-zacopride to modify extracellular levels of 5-HT in the rat frontal cortex. *Br. J. Pharmacol.*, **105,** (Suppl.), 36P.

CHOPIN, P. & BRILEY, M. (1987). Animal models of anxiety: the effect of components that modify 5-HT neurotransmission. *Trends Pharmacol. Sci.*, **8,** 383–388.

COSTALL, B., NAYLOR, R.J. & TYERS, M.B. (1990). The psychopharmacology of 5-HT_3 receptors. *Pharmacol. Ther.*, **47,** 181–202.

EGLEN, R.M., SWANK, S.R., WALSH, L.K. & WHITING, R.L. (1990). Characterisation of 5-HT_3 and atypical 5-HT receptors mediating guinea-pig ileal contractions *in vitro*. *Br. J. Pharmacol.*, **101,** 513–520.

ENNA, S.J & MOHLER, H. (1987). Gamma-aminobutyric acid (GABA) receptors and their association with benzodiazepine recognition sites. In *Psychopharmacology: The Third Generation of Progress*. ed. Meltzer, H.Y. pp. 265–272. New York: Raven Press.

HUTSTON, P.H., SARNA, G.S., O'CONNELL, M.T. & CURZON, G. (1989). Hippocampal 5-HT synthesis and release in vivo is decreased by infusion of 8-OH-DPAT into the nucleus raphe dorsalis. *Neurosci. Letts.*, **100,** 276–280.

IVERSEN, S.D. (1984). 5-HT and anxiety. *Neuropharmacology*, **23,** 1553–1560.

KIDD, E., BOUCHELET DE VENDEGIES, I., LEVY, J.-C., HAMON, M. & GOZLAN, H. (1992). The potent 5-HT_3 receptor antagonist (R)-zacopride labels an additional high affinity site in the central nervous system. *Eur. J. Pharmacol.*, **211,** 133–136.

MAIDMENT, N.T., ROUTLEDGE, C., MARTIN, K.F., BRAZELL, M.P. & MARSDEN, C.A. (1986). Identification of neurotransmitter autoreceptors using measurement of release in vivo. In *Monitoring Neurotransmitter Release during Behaviour*. ed. Joseph, M.H., Fillenz, M., MacDonald, I.A. & Marsden, C.A. pp. 73–93. Chichester: Ellis Horwood Ltd.

PEI, Q., ZETTERSTROM, T. & FILLENZ, M. (1989). Both systemic and local administration of benzodiazepine agonists inhibit the in vivo release of 5-HT from ventral hippocampus. *Neuropharmacology*, **28,** 1061–1066.

PERRY, K.W. & FULLER, R.W. (1989). Determination of brain concentrations of 8-hydroxy-2-(di-n-propylamino)tetralin by liquid chromatography with electrochemical detection. *Biochem. Pharmacol.*, **38,** 3169–3173.

SHARP, T., BRAMWELL, S.R. & GRAHAME-SMITH, D.G. (1989). 5-HT_1 agonists reduce 5-hydroxytryptamine release in rat hippocampus *in vivo* as determined by brain microdialysis. *Br. J. Pharmacol.*, **96,** 283–290.

STEIN, L., BELLUZI, J.D. & WISE, C.D. (1977). Benzodiazepines: behavioural and neurochemical mechanisms. *Am. J. Psychiat.*, **134,** 665–669.

YOUNG, R. & JOHNSON, D.N. (1991). Anxiolytic-like activity of R(+)- and S(−)-zacopride in mice. *Eur. J. Pharmacol.*, **201,** 151–155.

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