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Pharmacological properties of GR38032F, a novel antagonist at 5-HT_3 receptors

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1 This paper describes the pharmacology of the novel 5-hydroxytryptamine$_3$ (5-HT_3) receptor antagonist GR38032F.

2 On the isolated vagus nerve and superior cervical ganglion of the rat, **R,S**-GR38032F behaved as a reversible competitive antagonist of 5-HT-induced depolarization with pK_B values of 8.61 ± 0.08 ($n = 19$) and 8.13 ± 0.07 ($n = 16$), respectively. The resolved **R**- and **S**-isomers of GR38032F were approximately equipotent as 5-HT antagonists on the rat vagus nerve: the pK_B values were 8.95 ± 0.05 ($n = 16$) and 8.63 ± 0.08 ($n = 17$), respectively. **R,S**-GR38032F was also an effective antagonist of 5-HT on the rabbit isolated vagus nerve: in this case the pK_B value was 9.40 ± 0.14 ($n = 4$).

3 On the rabbit isolated heart, low concentrations of **R,S**-GR38032F (3×10^{-11}–1×10^{-9} M) antagonized the positive chronotropic effect of 5-HT and 2-methyl-5-hydroxytryptamine (2-methyl-5-HT). However, the effects of the compound did not appear consistent with simple reversible competition.

4 On the longitudinal smooth muscle of the guinea-pig ileum, **R,S**-GR38032F caused concentration-dependent parallel rightward displacement of the 2-methyl-5-HT concentration-contraction response curve; in contrast, a portion of the response to 5-HT appeared resistant to **R,S**-GR38032F. pK_B values estimated from the effects of the compound against 2-methyl-5-HT or the inhibitable portion of the response to 5-HT were 7.31 ± 0.06 ($n = 8$) and 7.33 ± 0.13 ($n = 8$), respectively. Against 2-methyl-5-HT, **R**-GR38032F seemed more potent (pK_B 7.20 ± 0.10; $n = 6$) than S-GR38032F (pK_B 6.30 ± 0.05; $n = 6$).

5 **R,S**-GR38032F is highly selective for 5-HT_3 receptors, and at concentrations of 3×10^{-6}–3×10^{-5} M, had negligible agonist or antagonist activity on other 5-HT or non-5-HT receptor-containing tissues on which it was tested.

6 The potency and duration of action of **R,S**-GR38032F in blocking 5-HT_3 receptors *in vivo* were assessed by measuring its ability to antagonize the bradycardic response to 5-HT or 2-methyl-5-HT administered intravenously (i.v.) to anaesthetized animals. For i.v. administration to the rat, the ED_{50} for **R,S**-GR38032F against 2-methyl-5-HT (100 $\mu g\,kg^{-1}$) was 0.4 (95% confidence limits 0.18–0.87) $\mu g\,kg^{-1}$ ($n = 10$); the corresponding value for oral administration to this species was 7.0 (3.0–22.0) $\mu g\,kg^{-1}$ ($n = 8$–10 per dose level). **R,S**-GR38032F was similarly effective in the anaesthetized cat.

7 The present results are discussed with reference to the postulated existence of subtypes of the 5-HT_3 receptor.

Introduction

The classification of 5-hydroxytryptamine (5-HT) receptors has become increasingly complex especially as attempts are made to reconcile classification systems based on functional and binding assays. However, with the recent introduction of highly selective compounds, it has been possible to propose a working classification of functional 5-HT receptors (Bradley *et al.*, 1986). In particular, notable advances have been made in characterizing the 5-HT 'M' receptor described by Gaddum & Picarelli (1957), now referred to as the 5-HT_3 receptor. Thus, MDL 72222 and ICS 205-930 are potent and selective antagonists of some of the effects of 5-HT on mammalian peripheral neurones (Fozard, 1984; Azami *et*

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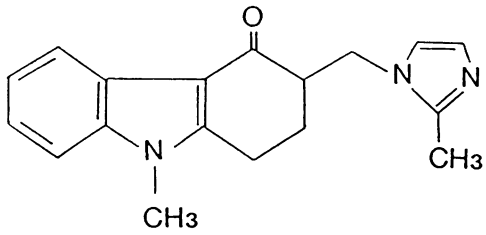

Figure 1 The structure of GR38032F.

al., 1985; Richardson *et al.*, 1985); sensitivity to blockade by these compounds enables these responses to be defined as mediated via 5-HT_3 receptors (see Bradley *et al.*, 1986). However, results in the literature indicate that for both MDL 72222 and ICS 205-930 there are marked discrepancies in their apparent affinities in different tissues. The magnitude of these discrepancies has prompted other authors to suggest that there may be subtypes of 5-HT_3 receptor (Fozard, 1984; Richardson *et al.*, 1985).

We describe here the actions of a novel, potent and highly selective 5-HT_3 antagonist, GR38032F (Figure 1), in a wide range of biological preparations *in vitro* and *in vivo*. In addition, we consider how the results obtained with GR38032F may contribute to the discussion of 5-HT_3 receptor subtypes. A preliminary account of some of the data has been presented to the British Pharmacological Society (Brittain *et al.*, 1987).

Methods

Vagus nerve and superior cervical ganglion

5-HT-induced depolarization of rat isolated superior cervical ganglia (SCG) and vagus nerves (VN) was recorded extracellularly using conventional techniques (Ireland *et al.*, 1987). In some experiments, 5-HT-induced depolarization of rabbit VN was studied. Male hooded rats (200–350 g; Glaxo) were stunned by a blow to the back of the head and killed by cardiac puncture; male Californian rabbits (2.5–3.0 kg; Froxfield) were killed by cervical dislocation. SCG or segments of cervical VN (approximately 10–20 mm long and minus the nodose ganglion) were excised as rapidly as possible, de-sheathed and mounted in two-compartment Perspex baths. Each VN was positioned so that approximately 50% lay in the first compartment while the remainder projected through a greased slot into the second. Each SCG was mounted with the ganglion lying in the first compartment, and the internal carotid nerve projecting through the greased slot into the second. The d.c. potential between the two compartments was measured via silver-silver chloride electrodes. Preparations were maintained at 27°C (see Ireland & Tyers, 1987). Both compartments of the recording bath were perfused continuously with Krebs-Henseleit medium dripped directly onto the tissue. Drugs were applied at known concentration via the superfusion stream into the first compartment only. The methods used for quantifying the effects of agonists on the VN and SCG are described in detail elsewhere (Ireland & Tyers, 1987). Experiments on 5-HT-induced depolarization of the SCG, but not the VN, were performed in the presence of the 5-HT uptake inhibitor paroxetine (1×10^{-6} M) to avoid complications arising from 5-HT uptake (Ireland *et al.*, 1987). In experiments to assess the selectivity of GR38032F, measurement was made of the effects of the compound against depolarization of the SCG induced by γ-aminobutyric acid (GABA) or the nicotinic agonist 1,1-dimethyl-4-phenylpiperazinium iodide (DMPP).

Rabbit isolated heart

Male Californian rabbits (2.5–3.0 kg Froxfield) were given heparin (500 u kg^{-1}) via an ear vein. After 3–4 min, the animals were killed by cervical dislocation. The hearts were excised as rapidly as possible and transferred to ice-cold, oxygenated Krebs-Henseleit medium (approximately 200 ml per heart). Perfusion of hearts using the Langendorff technique was commenced within 10 min of isolation. Preparations were perfused at a constant rate of approximately 12 ml min^{-1} with low-calcium Krebs-Henseleit medium at 35.5°C delivered from a peristaltic pump (Watson-Marlow 502S/301); the actual flow-rate was adjusted to optimize the basal performance of each heart. All drugs were applied at known concentrations via the perfusion stream. The mechanical activity of each heart was recorded via a Grass FT.03 isometric transducer attached to the right ventricle. The frequency of ventricular contraction was derived using a Lectromed 4520 rate meter. All experiments were performed in the presence of atropine (1.4×10^{-6} M) and desmethylimipramine (2×10^{-7} M) to block, respectively, the indirect negative chronotropic effect of 5-HT, and the uptake of 5-HT into noradrenergic terminals (see Fozard & Mwaluko, 1976). In some experiments, the cardio-accelerator nerve was stimulated electrically at 0.5–10 Hz with square-wave pulses of 3 ms duration delivered at supramaximal voltage via platinum-wire electrodes.

Guinea-pig ileum longitudinal muscle-myenteric plexus preparation (GPI)

Male Dunkin-Hartley guinea-pigs (Porcellus), weighing 250–300 g, were killed by cervical dislocation. A 3 cm portion of ileum was excised about 1 cm from

the ileo-caecal junction and the longitudinal muscle layer was removed using the method of Rang (1964). Strips of muscle were each placed in a 10 ml organ bath containing Krebs-Henseleit solution aerated with 95% O_2 and 5% CO_2 and maintained at 37 ± 1°C. Tissues were placed under an initial tension of 0.5–1 g. Agonist solutions (100 μl at up to 1×10^{-2} M) were injected rapidly into the bath and evoked contractions recorded isometrically using Grass FT.03 transducers.

Concentration-response curves for both 5-HT and 2-methyl-5-HT were constructed non-cumulatively using a 15 min dose-cycle to avoid desensitization. Measurements were made at the highest point of the response and agonists were washed out as soon as the peak response was reached.

In some experiments, measurement was made of the effects of GR38032F on contractile responses induced by histamine.

Measurement of the effects of antagonists against 5-HT$_3$-receptor mediated responses in vitro

Control agonist concentration-response curves were determined for each isolated tissue preparation using non-cumulative dosing schedules. The chosen concentration of antagonist was then applied and allowed to attain apparent equilibrium. On the rabbit heart, rat SCG and rat and rabbit VN, equilibrium was assumed to be complete when two successive responses to a given concentration of agonist (approximating to the EC_{50} in the presence of antagonist) were equal to within $\pm 10\%$. On the GPI, the effects of antagonists were measured after a pre-incubation period of 60 min. Each tissue preparation was exposed to only one concentration of antagonist. Lateral displacements of agonist concentration-response curves were measured at the control half-maximal response level. The negative logarithm of the apparent dissociation constant for an antagonist (pK_B) was estimated by calculation of the mean ($\pm$s.e.) of the individual results: pK_B = log(dose-ratio − 1) − log (antagonist concentration). The effect of each concentration of antagonist was measured on at least four preparations of a given tissue, each obtained from a different animal.

Evaluation of possible activity of GR38032F at functional non-5-HT$_3$ receptors

5-HT$_1$-like and 5-HT$_2$ receptors GR38032F was examined for its ability to mimic or antagonize 5-HT-induced relaxation of the α-methyl-5-HT contracted cat isolated saphenous vein and 5-HT-induced contraction of the dog isolated saphenous vein. Both these effects of 5-HT are mediated via 5-HT$_1$-like receptors (Bradley *et al.*, 1986). GR38032F was tested for activity at 5-HT$_2$ receptors using the *in vitro* rabbit thoracic aorta; this preparation is contracted by 5-HT. Details of all three assays are given elsewhere (Apperley *et al.*, 1976; Apperley *et al.*, 1980; Feniuk *et al.*, 1983).

α-Adrenoceptors Potential effects of GR38032F at α_1- or α_2-adrenoceptors were assessed using isolated preparations of the rat anococcygeus muscle and the rat vas deferens, respectively. Tissues were excised from adult male animals (260–450 g; C.D. strain, Charles River) killed by a blow to the head followed by exsanguination. Anococcygeus muscles were prepared according to the method of Gillespie (1972). Agonist-induced contractions were recorded isometrically from preparations bathed with Krebs-Henseleit medium bubbled with 5% CO_2 and 95% O_2 and maintained at 37°C. GR38032F was examined for its ability to mimic or antagonize prazosin (6×10^{-8} M)-sensitive contractile responses to a submaximal concentration of phenylephrine (6×10^{-7} M). Phenylephrine was applied using a 30 s contact time and 3 min dose cycle.

The prostatic portions of horizontally-bisected vasa deferentia, prepared as described by Brown *et al.* (1979), were individually superfused at a rate of 3 ml min^{-1} with sulphate-free Krebs-Henseleit medium at 31°C. Field stimulation (monophasic, supramaximal pulses, 2 ms, 0.1 Hz, 20 V maximum) was applied to each vas via two platinum ring electrodes spaced 20 mm apart. Evoked responses were recorded isometrically. GR38032F was examined for ability to antagonize yohimbine (1×10^{-7} M)-sensitive inhibition by UK14304 of the electrically-induced contractile response.

β-Adrenoceptors Possible activity of GR38032F at β_1- or β_2-adrenoceptors was assessed using, respectively, the isolated electrically-stimulated left atrium and the isolated uterus of the rat.

Uterine horns were obtained from Sprague-Dawley rats (150–200 g; Glaxo) in natural oestrus; atria were excised from male rats (200–250 g) of the same strain. Experiments were performed as described by Apperley *et al.* (1982).

Dopamine receptors Possible effects at central dopamine receptors were examined in conscious Sprague-Dawley rats (130–180 g; Glaxo). GR38032F, alone and in combination with apomorphine (1 mg kg^{-1}, i.v.), was examined for effects on stereotyped behaviour (repetitive licking, sniffing, head movements and chewing) as described by Gower & Marriott (1982). Animals were observed at 5 min intervals for a total of one hour.

Muscarinic receptors GR38032F was examined for its ability to antagonize the effects of (±)-muscarine in the rat isolated atrium. Male Lister-hooded rats (200–250 g; Glaxo) were killed by a blow to the head. The atria were excised and prepared as described by the Edinburgh Staff (1970). Preparations were incubated at 37°C in sulphate-free Krebs-Henseleit medium bubbled with 5% CO_2 and 95% O_2. Mechanical activity was recorded isometrically and the rate of contraction derived using an Ormed 5420 or 5421 rate meter. (±)-Muscarine (approximate $EC_{50} = 7 \times 10^{-7}$ M) applied for periods of 20 s at intervals of 5 min caused concentration-dependent falls in rate. On each preparation, a concentration-response curve to (±)-muscarine was determined both in the absence and in the presence of GR38032F.

Histamine H_2-receptors GR38032F was examined for activity at histamine H_2-receptors on the guinea-pig isolated atrium preparation. Assays were performed as described by Humphray *et al.* (1982).

Effects on the bradycardic response to 5-HT or 2-methyl-5-HT

Falls in heart rate induced by 2-methyl-5-HT or 5-HT were recorded in male C.D. rats (250–350 g; Charles River, U.K.) and male cats (2.2–2.8 kg; Glaxo).

Rats were anaesthetized with pentobarbitone (60 mg kg^{-1} i.p., May and Baker); in cats, pentobarbitone (10 mg kg^{-1} i.p., May and Baker) plus chloralose (80 mg kg^{-1} i.p., Glaxo) were used. Supplementary doses of anaesthetic were administered as required. The trachea of each animal was cannulated; in some, respiratory tidal volume was monitored via a Fleisch tube. Heart rate was derived from the ECG recorded via subcutaneous electrodes. In cats, left common carotid blood pressure was measured using a Bell and Howell type 4-422 blood pressure transducer filled with heparinized saline (20 u ml^{-1}; Evans Medical). Body temperature was monitored via a rectal probe and maintained at 37 ± 1.0°C using a thermostatically-controlled blanket (Harvard Bioscience). A polythene cannula (800/100/160/100; Portex) was inserted into the left external jugular vein (rats) or the left femoral vein (cats) to allow intravenous administration of drugs; the dose-volumes were 1.0 ml kg^{-1} and 0.1 ml kg^{-1}, respectively. In some cats, a polythene catheter was inserted into the duodenum approximately 6 cm distal to the pyloric sphincter to allow intraduodenal (i.d.) administration; in this case the dose-volume was 1.0 ml kg^{-1}. Other routes of administration used were sublingual, subcutaneous, intramuscular and intrarectal. Dose-volumes were all 0.1 ml kg^{-1} except for sublingual, which was 0.01 ml kg^{-1}.

In the rat, the bradycardic effects of single doses (approximating to the ED_{80}) of 5-HT or 2-methyl-5-HT were measured. Intravenously administered antagonists were tested at several doses per animal: ED_{50} values were calculated as the amount of compound required to reduce the response to agonist to 50% of the pre-dose control in the same animal. Antagonists to be tested for efficacy when given orally (dose volume 10 ml kg^{-1}) were administered to conscious rats that had been deprived of food overnight. These animals were anaesthetized and prepared surgically as described above, 10 to 20 min before testing the effects of 5-HT or 2-methyl-5-HT. In this case, the ED_{50} value was calculated as the dose of antagonist required to reduce the response to 5-HT or 2-methyl-5-HT to 50% of that recorded in vehicle-treated control animals.

In the cat, the dose-response curve for 2-methyl-5-HT-induced bradycardia was usually much steeper than that observed in the rat (see Results). This necessitated the use of a modified procedure to quantify the effects of antagonists. Thus, at specified times after administration of the antagonist, measurement was made of the apparent rightward displacement of the control 2-methyl-5-HT dose-response curve. Generally, such estimates were based on the effect of a single dose of the agonist (not exceeding 100 μg kg^{-1}), chosen to produce a response approximately equal to half the control maximum. In these experiments, onset time for an antagonist was calculated as the delay before a dose-ratio of 5 was achieved; duration was the period over which a dose-ratio of at least 5 was maintained.

Drugs and solutions

The composition of the normal Krebs-Henseleit medium used in the present study was (in mM): NaCl 118, $NaHCO_3$ 25, KH_2PO_4 1.18, KCl 4.7, $MgSO_4.7H_2O$ 1.18, $CaCl_2$ 2.5 and glucose 11.0. It was gassed with 95% O_2 and 5% CO_2. For experiments on the rabbit isolated heart, the amount of $CaCl_2$ added was reduced to 1.0 mM since this stabilized the basal activity of the preparation. Sulphate-free medium was prepared by the substitution of $MgCl_2$ (1.18 mM) for $MgSO_4.7H_2O$ (1.18 mM). The media were prepared using glass-distilled water and reagents, which were all A.R. grade, were purchased from commercial sources.

The following drugs were used: R-, S-, and RS-GR38032F (1,2,3,9-tetrahydro-3-[(methylimidazol-1-yl)methyl]-9-methyl-4H-carbazol-4-one hydrochloride, $2H_2O$; Glaxo Group Research Ltd); acetylcholine bromide (Sigma); apomorphine hydrochloride (Macfarlan Smith); atropine sulphate

(Sigma); desmethylimipramine hydrochloride (Ciba); 1,1-dimethyl-4-phenylpiperazinium iodide (DMPP; Sigma); γ-aminobutyric acid (GABA; Sigma); histamine acid phosphate (Sigma); 5-hydroxytryptamine creatinine sulphate (Sigma); (±)-α-methyl-5-hydroxytryptamine maleate (Glaxo); ICS 205-930 ((3α-tropanyl)-1H-indole-3-carboxylic acid ester; Sandoz); MDL 72222 (1αH,3α,5αH-tropan-3yl-3,5-dichlorobenzoate; Glaxo Group Research Ltd); methysergide hydrogen maleate (Sandoz); metoclopramide hydrochloride (Beecham); (±)-muscarine chloride (Sigma); phenylephrine hydrochloride (Sigma); prazosin hydrochloride (Pfizer); UK14304 (5-bromo-6-[2-imidazolin-2-ylamino]-quinoxaline tartrate; Pfizer); and yohimbine hydrochloride (Sigma).

Unless stated otherwise, drugs were dissolved in the appropriate Krebs-Henseleit medium for experiments *in vitro*, 0.9% (w/v) saline for intravenous (i.v.), subcutaneous (s.c.) and intramuscular (i.m.) injection or distilled water for oral (p.o.), sublingual (s.l.) and intraduodenal (i.d.) administration. Stock solutions of desmethylimipramine, histamine, methysergide, GR38032F, MDL 72222, muscarine, ICS 205-930, prazosin or yohimbine were prepared in distilled water. These could be diluted with Krebs-Henseleit medium or 0.9% saline as appropriate, without causing visible precipitation. All solutions were prepared immediately before use. For *in vivo* experiments, doses refer to the free base. Unless stated otherwise, the results described for GR38032F were obtained using the racemate.

Results

Effects of antagonists on 5-HT-induced depolarization of the vagus nerve and superior cervical ganglion

On the rat VN, 5-HT (1×10^{-7}–3×10^{-5} M) induced rapid, concentration-related depolarization responses. Similar responses were obtained on the rat SCG in the presence of the 5-HT uptake inhibitor paroxetine, using 5-HT concentrations ranging from 1×10^{-6} to 1×10^{-4} M. 5-HT-induced depolarization of both the VN and SCG of the rat is stable and reproducible (Ireland *et al.*, 1987). GR38032F (1×10^{-8}–3×10^{-7} M) caused parallel rightward displacements of the 5-HT concentration-depolarization response curve on both preparations, with no significant reduction in the amplitude of the maximum response (Figure 2). Plots of log (DR − 1) against log concentration of antagonist (Arunlakshana & Schild, 1959) approximated to straight lines with gradients of 1.23 (95% confidence limits 0.93–1.53) and 1.16 (0.89–1.43) on the VN and SCG, respectively (Figure 2). The pK_B values calculated by constraining the gradients to unity were 8.61 ± 0.08 ($n = 19$) and 8.13 ± 0.07 ($n = 16$), respectively. On the VN, the R and S isomers of GR38032F caused effects similar to that of the racemate (Figure 2), the pK_B values and gradients (in parentheses) were 8.95 ± 0.05 (1.13 (0.94–1.31); $n = 16$) and 8.63 ± 0.08 (1.12 (0.83–1.41); $n = 17$), respectively. 5-HT-induced hyperpolarization of the rat SCG (Ireland, 1987) was not blocked by GR38032F (1×10^{-5} M).

On the rabbit isolated VN, 5-HT (1×10^{-6}–3×10^{-4} M) induced reproducible depolarizations similar to those observed on the rat preparation. Metoclopramide (1×10^{-6}–3×10^{-5} M) caused parallel rightward displacement of the 5-HT concentration-depolarization response curve on the rabbit VN with no reduction in the amplitude of the maximum response (Figure 3). A plot of the antagonism data according to the method of Arunlakshana & Schild (1959) had a slope of 0.62 (95% confidence limits 0.53–0.72; Figure 3); the pA_2 value calculated from the log (DR − 1) = 0 intercept was 7.7. GR38032F (3×10^{-8} M) caused a rightward displacement of the 5-HT concentration-depolarization curve on the rabbit VN, accompanied by a small reduction in the maximum response (Figure 3). The provisional pK_B value calculated from the effect of this single concentration of GR38032F was 9.40 ± 0.14 ($n = 4$).

In the experiments described here, metoclopramide and GR38032F achieved apparent equilibrium within 60 min and 90–120 min, respectively. Neither antagonist changed the extracellularly-recorded resting potential in any of the preparations examined.

Effects of agonists and antagonists on the rabbit isolated heart

In the rabbit isolated heart, in the presence of atropine and desmethylimipramine, application of known concentrations of 5-HT (2×10^{-6}–7×10^{-5} M) caused rapid, concentration-dependent positive chronotropic responses which reached a maximum within 1–2 min. 2-Methyl-5-HT (7×10^{-6}–1×10^{-4} M) mimicked the effect of 5-HT. Generally, the responses to both compounds were poorly maintained despite the continued presence of agonist. On discontinuing agonist application, basal heart rate (approximately 100 beats min^{-1}) was reattained within 5 to 10 min. Concentration-response curves were constructed non-cumulatively, using randomized concentrations. No tachyphylaxis was observed when an interval of approximately 20 min was allowed between applications. The amplitudes of the maximal response to 5-HT and 2-methyl-5-HT were similar, being typically a doubling of basal rate. 2-Methyl-5-HT was slightly less active than 5-HT, the mean (±s.e. mean) equipotent molar ratio being

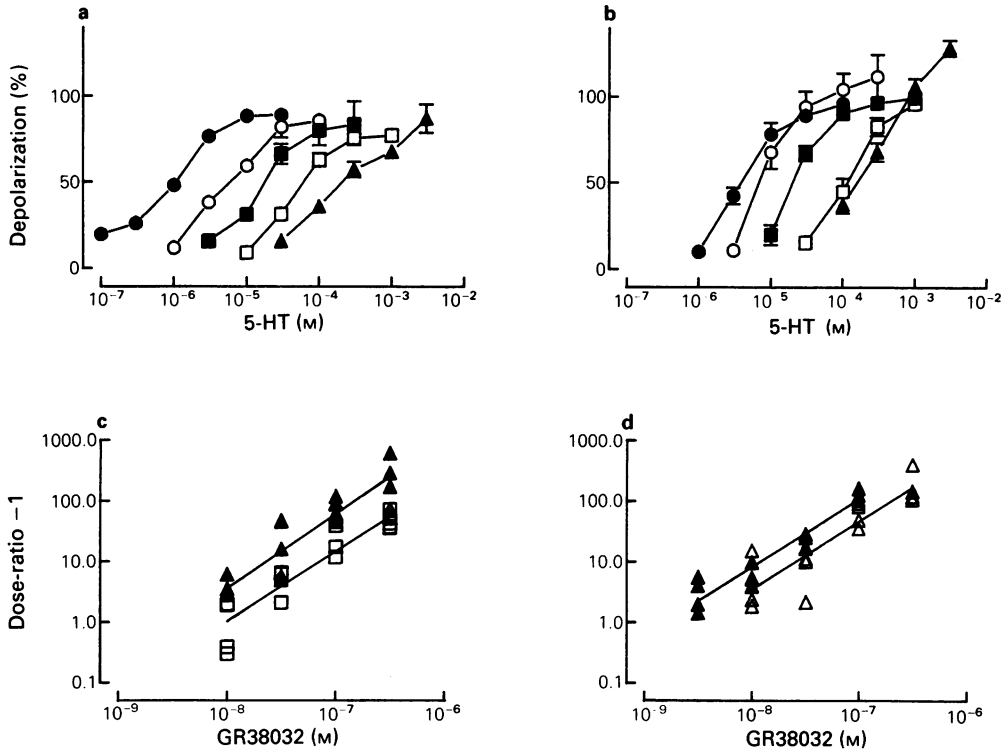

Figure 2 Antagonism by GR38032F of 5-hydroxytryptamine (5-HT)-induced depolarization of the rat isolated vagus nerve (a) and superior cervical ganglion (SCG) (b). In (a) and (b), results are expressed as a percentage of the estimated control maximum. Symbols indicate controls (●) or the presence of GR38032F at 1×10^{-8} (○), 3×10^{-8} (■), 1×10^{-7} (□) or 3×10^{-7} (▲) M. Concentration-response curves were constructed using non-cumulative additions of 5-HT; each preparation was exposed to only one concentration of GR38032F. Each point is the mean of single determinations in 4 preparations, with the vertical line indicating the s.e. mean. (c) Data from the experiments illustrated in (a) and (b) plotted according to Arunlakshana & Schild (1959). Symbols indicate results obtained from the vagus nerve (▲) and SCG (□). (d) Antagonism of 5-HT-induced depolarization of the rat vagus nerve by the R- (▲) and S- (△) isomers of GR38032F; data are plotted as in (c). In (c) and (d), each point represents the result from a separate tissue and straight lines were fitted by linear regression analysis. All results for the SCG were recorded in the presence of the 5-HT uptake inhibitor, paroxetine (1×10^{-6} M).

1.4 ± 0.15 ($n = 6$). Metoclopramide (7×10^{-7}–6×10^{-6} M) caused parallel rightward displacements of the 5-HT concentration-response curve on the rabbit heart. No significant change in the amplitude of the maximum response was observed, despite the need to use 5-HT concentrations as high as 1×10^{-3} M (Figure 4). Metoclopramide appeared to have equilibrated within one hour of commencing its application. A plot of the antagonism data according to the method of Arunlakshana & Schild (1959) approximated to a straight line with a slope of 0.79 (95% confidence limits 0.46–1.12) (Figure 4). The pK_B value calculated by constraining the gradient to unity was 6.81 ± 0.06 ($n = 18$). The effects of metoclopramide against 2-methyl-5-HT were not examined in the present study.

GR38032F (3×10^{-11}–1×10^{-9} M) antagonized the positive chronotropic effect of both 5-HT and 2-methyl-5-HT on the rabbit isolated heart. Apparent equilibrium was attained between one and two hours after commencing applications. However, both the quantitative and qualitative effects of low concentrations of GR38032F varied considerably between heart preparations. For example, in some preparations exposed to GR38032F, 3×10^{-11} M, a parallel rightward shift of the agonist concentration-response curve with little change in the amplitude of the maximum response was observed ($n = 2$ of 7 with 5-HT, 3 of 6 with 2-methyl-5-HT). In contrast, in other preparations in the presence of the same concentration of GR38032F, the maximum response that could be elicited by either agonist at concentra-

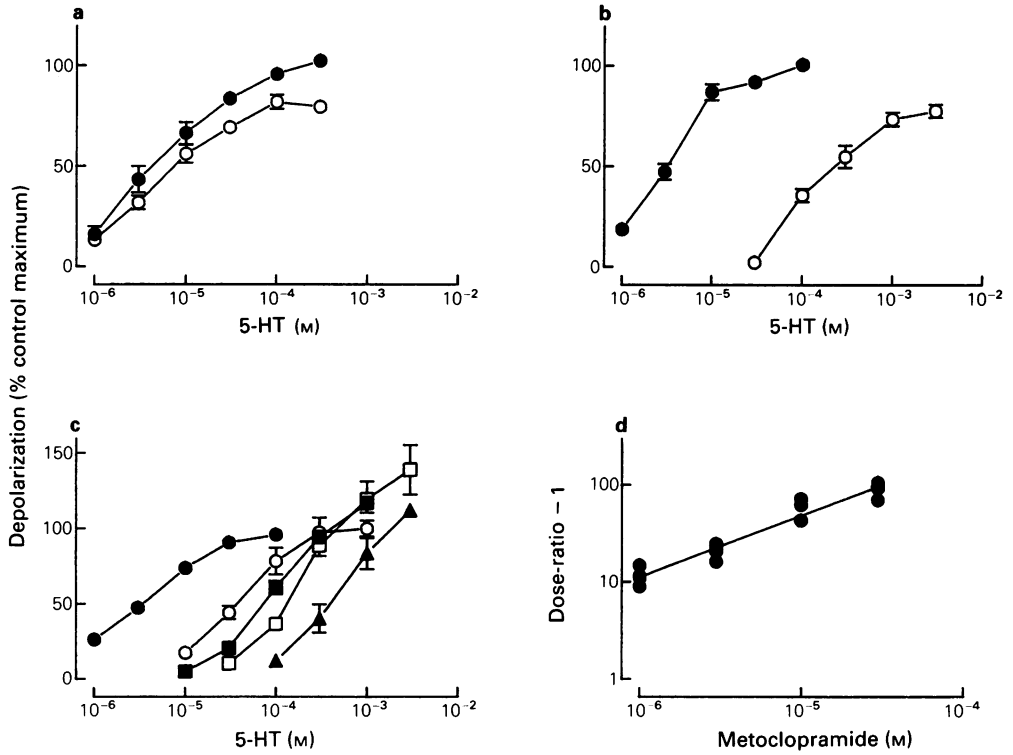

Figure 3 Antagonism by GR38032F and metoclopramide of 5-hydroxytryptamine (5-HT)-induced depolarization of the rabbit isolated vagus nerve. Concentration-response curves were constructed using non-cumulative additions of 5-HT. In (a), no antagonist was applied: symbols represent the first (●) and second (○) concentration-response curves obtained at an interval of 60 min on the same preparations. (b) The effect of GR38032F (3×10^{-8} M); symbols represent control responses (●) or the presence of antagonist (○). (c) The effects of metoclopramide: symbols indicate control responses (●) or the presence of metoclopramide at 1×10^{-6} (○), 3×10^{-6} (■), 1×10^{-5} (□) or 3×10^{-5} (▲) M. In (a–c), each preparation was exposed to only one concentration of antagonist and each point is the mean of single determinations in at least four separate preparations with the vertical lines indicating the s.e. mean. (d) 'Schild' plot of the data obtained in the experiments illustrated in (c); each point represents the result from a separate preparation.

tions up to 1×10^{-3} M, was less than 30% of the control maximum (Figure 4) ($n = 2$ of 7 with 5-HT, 2 of 6 with 2-methyl-5-HT). In the remaining preparations, the effects of GR38032F (3×10^{-11} M) appeared intermediate. A higher concentration of GR38032F (1×10^{-9} M) ($n = 4$) always caused marked depression of the amplitude of the maximum response. If these effects of GR38032F were due to an action at the 5-HT_3 receptor, the results shown in Figure 4 suggest that at this site the compound has a dissociation constant of less than 3×10^{-11} M.

Electrical stimulation of the cardio-accelerator nerve at 0.5 to 10.0 Hz, caused a frequency-dependent positive chronotropic response in the rabbit isolated heart. This was unaffected by GR38032F (1×10^{-6} M), although it was rapidly and completely abolished by tetrodotoxin (1×10^{-6} M) ($n = 3$).

Guinea-pig ileum longitudinal muscle-myenteric plexus preparation

On the GPI, both 5-HT (3×10^{-9}–3×10^{-5} M) and 2-methyl-5-HT (3×10^{-7}–1×10^{-4} M) caused concentration-related contractions. The responses consisted of a rapid initial contraction which quickly decayed to a lower but sustained level. In the present study, only the amplitude of the peak response was quantified. As demonstrated previously (Buchheit *et al.*, 1985), the concentration-contraction response curve to 5-HT consisted of two distinct phases. In contrast, that to 2-methyl-5-HT seemed monophasic. The amplitudes of the maximum response to each agonist were similar (Figure 5). Two concentration-contraction response curves to 5-HT or 2-methyl-5-HT constructed on the same GPI preparation at an interval of 60 min, were superimposable (result not

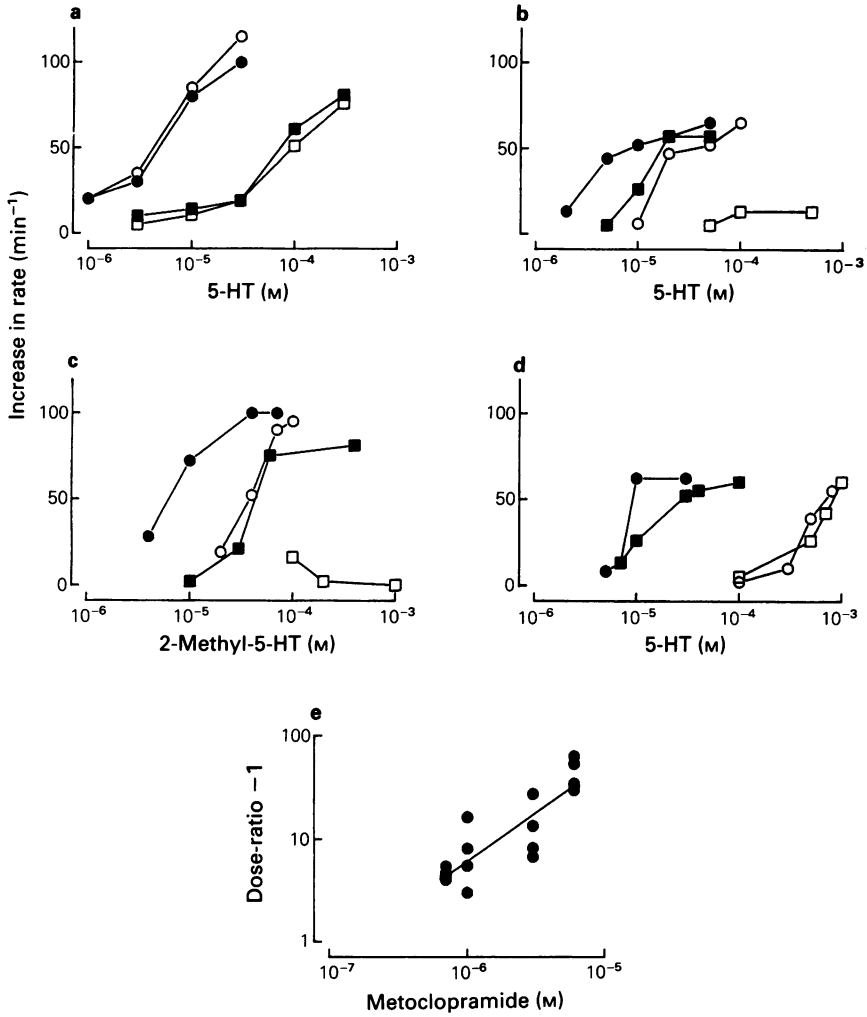

Figure 4 Effects of antagonists on the positive chronotropic effect of 5-hydroxytryptamine (5-HT) on the rabbit isolated heart. In (a–d), responses of one heart preparation that had high sensitivity to agonist (●, ○) and one that had low sensitivity (■, □) are shown; these are representative of the range of effects observed. In (a), no antagonist was applied: (●, ■) represent the first and (○, □) the second concentration-response curves determined on the same preparations at an interval of 60 min. (b–d) The effect of GR38032F (3×10^{-11} M) (b and c) or metoclopramide (6×10^{-6} M) (d) on responses induced by 5-HT or 2-methyl-5-hydroxytryptamine (2-methyl-5-HT). (●, ■) Show control responses, (○, □) the presence of antagonist. (e) Antagonism by metoclopramide of the positive chronotropic effect of 5-HT plotted according to Arunlakshana & Schild (1959). Each point represents the result obtained in a separate heart preparation, the straight line was fitted by linear regression.

shown). Responses to 5-HT or 2-methyl-5-HT were unaffected by methysergide (1×10^{-7} M).

GR38032F (1×10^{-7} and 1×10^{-6} M) antagonized contractions of the GPI induced by either 5-HT or 2-methyl-5-HT. Against 5-HT, GR38032F caused parallel rightward displacements of the upper phase of the concentration-response curve, with little effect on the lower phase. In contrast, against 2-methyl-5-HT, GR38032F caused parallel rightward displacements of the entire curve (Figure 5). Unfortunately, a wider range of concentrations of antagonist could not be tested since the limited solubility of both 5-HT and 2-methyl-5-HT restricted the extent to which rightward displacement of the concentration-response curves for these agonists could be quantified (see Methods). Therefore, pK_B

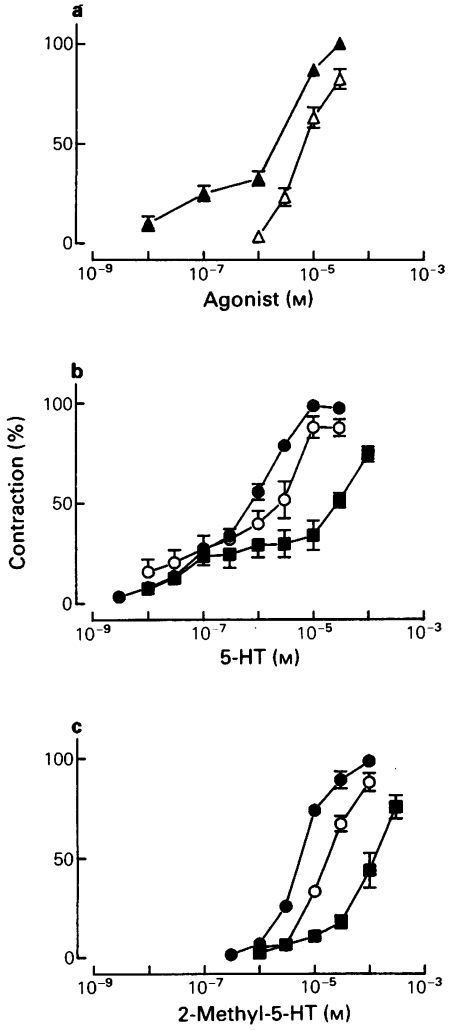

Figure 5 Effects of agonists and antagonists on the guinea-pig isolated ileum longitudinal muscle-myenteric plexus preparation. (a) Responses to 5-hydroxytryptamine (5-HT) (▲) and 2-methyl-5-hydroxytryptamine (2-methyl-5-HT) (△). Results which are expressed as a percentage of the maximum response to 5-HT, were obtained from four preparations, each exposed to both agonists. (b and c) Effects of GR38032F on contractions induced by 5-HT (b) or 2-methyl-5-HT (c). Symbols indicate control responses (●) or the presence of GR38032F at 1×10^{-7} M (○) or 1×10^{-6} M (■); results are expressed as a percentage of the control maximum. In (a–c), each point is the mean of single determinations in at least four preparations, with the vertical lines indicating the s.e. mean.

values were calculated from the effects of GR38032F, 1×10^{-7} and 1×10^{-6} M. Against 5-HT these were 7.12 ± 0.17 ($n = 4$) and 7.55 ± 0.07 ($n = 4$), respectively, (mean 7.33 ± 0.13, $n = 8$); against 2-methyl-5-HT, the corresponding values were 7.31 ± 0.07 ($n = 4$) and 7.31 ± 0.12 ($n = 4$), respectively, (mean 7.31 ± 0.06, $n = 8$). Doubling the pre-incubation period with GR38032F (5×10^{-7} M) to two hours did not change its apparent potency: in this case the pK_B value (against 2-methyl-5-HT) was 7.10 ± 0.10 ($n = 4$).

Both isomers of GR38032F were found to be effective antagonists of 5-HT and 2-methyl-5-HT-induced contraction of the GPI. Like the racemate, S-GR38032F (3×10^{-6} M) and R-GR38032F (3×10^{-7} M) caused parallel rightward displacements of the entire concentration-response curve to 2-methyl-5-HT and of the upper phase only of the concentration-response curve to 5-HT (results not shown). pK_B values calculated from the effects of S-GR38032F (3×10^{-6} M) against 5-HT and 2-methyl-5-HT were 6.42 ± 0.10 ($n = 4$) and 6.30 ± 0.05 ($n = 6$), respectively. The corresponding values for R-GR38032F (3×10^{-7} M) were 7.14 ± 0.13 ($n = 4$) and 7.20 ± 0.10 ($n = 6$), respectively; each was significantly higher than the corresponding value for the S-isomer ($P < 0.01$ and $P < 0.001$, respectively).

Metoclopramide (1×10^{-5} M) caused parallel rightward displacement of the 2-methyl-5-HT concentration-contraction response curve; the mean pK_B calculated from the effect of this single concentration was 5.47 ± 0.09 ($n = 4$).

Selectivity of action in vitro

In the spontaneously-beating isolated atrium of the rat, a high concentration of GR38032F (1×10^{-5} M) caused a mean reduction in rate of 15% ($n = 2$), although the compound had no significant effect at 1×10^{-6} M. Furthermore, GR38032F (1×10^{-5} M) did not antagonize falls in rate induced by (±)-muscarine. GR38032F at concentrations up to 2×10^{-5} M, did not exert significant agonist effects on any other isolated preparation examined.

GR38032F (3×10^{-6}–3×10^{-5} M) was either inactive or only weakly active as an antagonist at all the functional non-5-HT_3 receptors examined (Table 1). Since high concentrations of the 5-HT_3 antagonists MDL 72222 and metoclopramide, inhibit depolarization of the rat SCG induced by the nicotinic agonist DMPP (Fortune & Ireland, 1984; Ireland *et al.*, 1982), it was of interest to see whether GR38032F shared this property. High concentrations of GR38032F (3×10^{-5} M) reduced (by 53.7 and 30%, respectively, in two preparations) the maximum response to DMPP, although it caused little rightward shift of the concentration-depolarization response curve. In contrast, a lower concentration of the antagonist (3×10^{-6} M) had no significant effect on DMPP-induced depolarization.

Table 1 Antagonist effects of GR38032F at non-5-HT_3 receptors *in vitro*

Preparation	*Receptor*	*Agonist*	*Potency estimate* (pA_2)
Cat saphenous vein	5-HT_1-like	5-HT	<5.0
Dog saphenous vein	5-HT_1-like	5-HT	<4.6
Rabbit aorta	5-HT_2	5-HT	4.6
Rat anococcygeus muscle	α_1-Adrenoceptor	Phenylephrine	<5.2
Rat vas deferens	α_2-Adrenoceptor	UK14304	<5.2
Rat atrium (paced)	β_1-Adrenoceptor	Isoprenaline	<4.5
Rat atrium (unpaced)	Muscarinic	Muscarine	<5.0
Rat SCG	Nicotinic	DMPP	<5.5
Rat SCG	$GABA_A$	GABA	<4.5
Guinea-pig ileum	H_1-histamine	Histamine	<5.0
Guinea-pig atrium	H_2-histamine	Histamine	5.5

pA_2 values were determined according to the method of Schild (1947) from the effects of GR38032F (3×10^{-6}–3×10^{-5} M) applied to at least two examples of each preparation. Abbreviations: SCG, superior cervical ganglion; 5-HT, 5-hydroxytryptamine; DMPP, 1,1-dimethyl-4-phenylpiperazinium iodide; GABA, γ-aminobutyric acid.

Activity in vivo

In the anaesthetized rat, rapid intravenous injection of 2-methyl-5-HT (30–300 $\mu g\,kg^{-1}$) or 5-HT (10–100 $\mu g\,kg^{-1}$) produced dose-related transient falls in heart rate (Figure 6). Such effects have been described previously (see Fozard & Host, 1982; Richardson *et al.*, 1985). In the present experiments using the rat, antagonists were tested for ability to block the response to a standard dose of 2-methyl-5-HT (100 $\mu g\,kg^{-1}$) or 5-HT (30 $\mu g\,kg^{-1}$); these doses approximated to the ED_{80} for the agonist. Each dose of antagonist was tested on 8–10 animals.

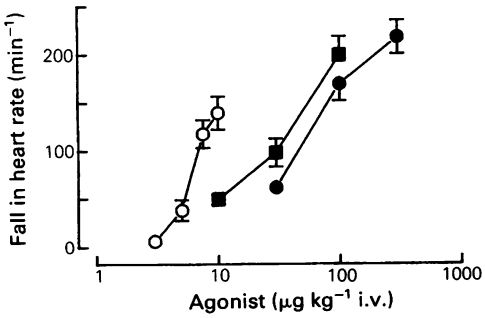

Figure 6 Comparison of the falls in heart rate produced by 2-methyl-5-hydroxytryptamine (2-methyl-5-HT) in the anaesthetized cat (○) and the anaesthetized rat (●). In the rat, the effects of 5-hydroxytryptamine (5-HT) (■) were also measured. Each point is the mean of single determinations in 8–10 animals, with the full range of doses shown being tested in each animal. The vertical lines indicate the s.e. mean. Note that in the 9 cats tested, the mean basal heart rate was $172 \pm 12\,min^{-1}$ and that in the rat, doses of 5-HT greater than 100 $\mu g\,kg^{-1}$ or of 2-methyl-5-HT greater than 300 $\mu g\,kg^{-1}$ were usually fatal.

Metoclopramide (1–30 $mg\,kg^{-1}$ p.o.; 60 min pretreatment) caused dose-dependent inhibition of the falls in heart rate produced by 2-methyl-5-HT or 5-HT (Figure 7). The mean ED_{50} for metoclopramide was 4.2 (95% confidence limits 2.4–7.1) $mg\,kg^{-1}$ p.o. The corresponding value against 5-HT was 5.3 (1.5–17.6) $mg\,kg^{-1}$ p.o. Intravenously administered metoclopramide (3–300 $\mu g\,kg^{-1}$; 5 min pretreatment) also inhibited the falls in heart rate induced by 2-methyl-5-HT (Figure 7). In this case, the mean ED_{50} against 2-methyl-5-HT was 24.0 (6.1–150.8) $\mu g\,kg^{-1}$. Approximately 50% of rats did not survive repeated administration of 5-HT (30 $\mu g\,kg^{-1}$ i.v.). Therefore, the effects of intravenously-administered antagonists were not tested against 5-HT.

In the rat, GR38032F was a potent antagonist of both 2-methyl-5-HT and 5-HT-induced falls in heart rate with good duration of action (Figure 7). Following oral administration (60 min pretreatment), the ED_{50} values (with 95% confidence limits) for GR38032F against 2-methyl-5-HT and 5-HT were 7.0 (3.0–22.0) $\mu g\,kg^{-1}$ and 8.0 (4.0–34.0) $\mu g\,kg^{-1}$, respectively. The effects of intravenously administered GR38032F were measured against 2-methyl-5-HT only: with a pretreatment time of 5 min, the mean ED_{50} was 0.42 (0.18–0.87) $\mu g\,kg^{-1}$.

In the anaesthetized cat, rapid intravenous injection of 2-methyl-5-HT (3–20 $\mu g\,kg^{-1}$) caused transient but reproducible falls in heart rate and blood pressure; for a given animal, the ED_{50} values for these two effects of 2-methyl-5-HT were similar. High doses of 2-methyl-5-HT (10–20 $\mu g\,kg^{-1}$) generally also caused apnoea of short duration (less than one min). Typically, the dose-response curve for the falls in heart rate was much steeper in the cat than in the rat (Figure 6).

In the anaesthetized cat, intravenous application

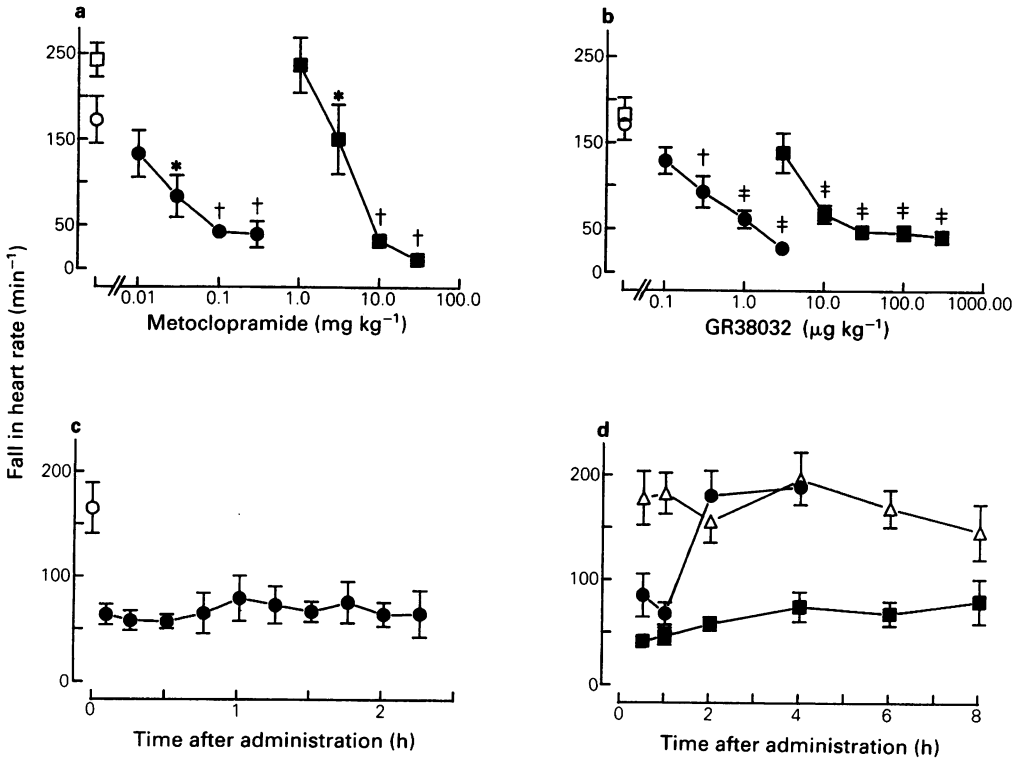

Figure 7 Effects of antagonists on the fall in heart rate produced by 2-methyl-5-hydroxytryptamine (2-methyl-5-HT; 100 $\mu g\,kg^{-1}$) administered intravenously (i.v.) to the anaesthetized rat. (a and b) Dose-inhibition curves for metoclopramide (a) and GR38032F (b) given i.v.; (5 min pretreatment) (●) or orally (p.o.; 60 min pretreatment) (■). (○, □) Indicate the appropriate control responses to 2-methyl-5-HT; significance of differences from control as indicated (*$P < 0.05$; †$P < 0.01$; ‡$P < 0.001$, t test). (c and d) Duration of action of GR38032F administered i.v. (c) at 0.42 $\mu g\,kg^{-1}$ or p.o. (d) at 10 (●) or 100 (■) $\mu g\,kg^{-1}$. Control responses are indicated by (○) and (△). The inhibitory effects of GR38032F, 0.42 $\mu g\,kg^{-1}$ i.v. or 100 $\mu g\,kg^{-1}$ p.o. were significant ($P < 0.05$) at all time-points shown; GR38032F, 10 $\mu g\,kg^{-1}$ p.o. was effective ($P < 0.05$) at 30 and 60 min only. In (a–d), each point is the mean of single determinations in 8–10 animals, with the vertical lines indicating the s.e. mean. Injection of the vehicle for 2-methyl-5-HT (1 ml kg^{-1}) caused a fall in heart rate of 20–30 min^{-1}.

of MDL 72222 (30–300 $\mu g\,kg^{-1}$), ICS 205-930 (1–10 $\mu g\,kg^{-1}$) or GR38032F (1–30 $\mu g\,kg^{-1}$) caused marked inhibition of the effects of 2-methyl-5-HT (Figure 8). GR38032F (10–100 $\mu g\,kg^{-1}$) was also highly effective following sublingual administration (Table 2) or when given via the subcutaneous, intramuscular, intrarectal or intraduodenal routes (results not shown).

Table 2 Time-course of the inhibition by GR38032F of the bradycardic response to 2-methyl-5-HT in the anaesthetized cat

Dose ($\mu g\,kg^{-1}$)	*Route*	n	*Onset to DR = 5* (min)	*Duration of DR ⩾ 5* (min)
3	i.v.	3	<2	4.7 ± 0.3
10	i.v.	4	<2	24.5 ± 2.2
30	i.v.	3	<2	103.0 ± 18.6
30	s.l.	4	20.8 ± 4.7	167.3 ± 49.1

Values are the mean ± s.e. mean of single determinations made in the number of animals indicated. In some experiments, onset of blockade was too rapid to measure precisely—the value given is the maximum time to the first observation. Abbreviations: i.v. = intravenous; s.l. = sublingual; DR = dose-ratio.

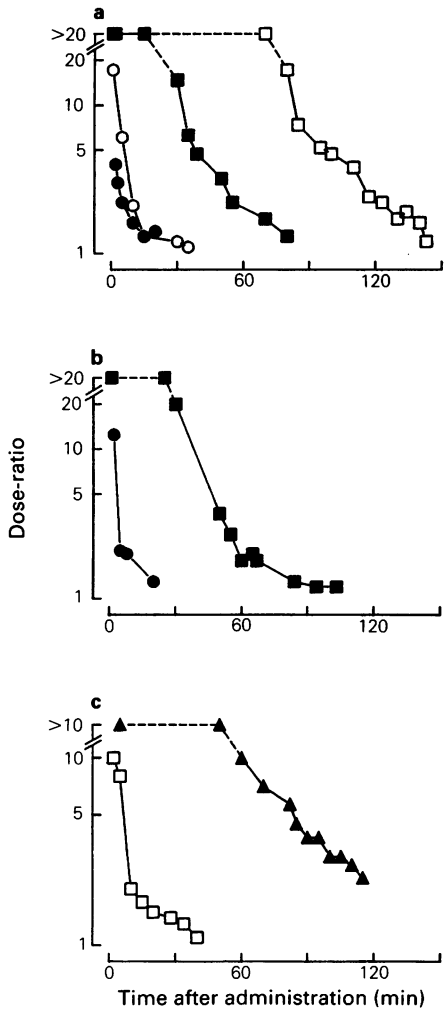

Figure 8 Antagonism of 2-methyl-5-hydroxytryptamine-induced falls in heart rate in the anaesthetized cat. Symbols indicate the time course of the antagonism following intravenous administration (at 0 min) of GR38032F (a), ICS 205-930 (b) or MDL 72222 (c) at 1 (●), 3 (○), 10 (■), 30 (□) or 300 (▲) $\mu g\,kg^{-1}$. Each set of points represents data for a single animal; effects similar to those shown were obtained with each dose of GR38032F in a further 2–5 animals. Note that dose-ratios greater than 20 could not be quantified; in (c) the limit was 10.

Selectivity of action in vivo

GR38032F (1 $mg\,kg^{-1}$, i.v.) produced no change in the stereotypy score in the rat. In the same test, apomorphine (1 $mg\,kg^{-1}$, i.v.) produced a marked increase in stereotypy (1 $mg\,kg^{-1}$ i.v. mean score 12.0 per 30 min), but GR38032F (1 and 3 $mg\,kg^{-1}$ i.p.) administered 1 h before failed ($P < 0.05$; $n = 6$) to modify this response (mean score 12.5 per 30 min).

Discussion

GR38032F is a potent and highly selective antagonist at 5-HT_3 receptors. In the two preparations where it was possible to test a wide range of concentrations, the rat VN and SCG, GR38032F appeared to be a simple, reversible competitive antagonist with pK_B values of 8.61 and 8.13. In this respect, GR38032F behaved in a similar fashion to metoclopramide which causes concentration-dependent parallel displacement of concentration-response curves in both preparations, when tested under the conditions used in the present study (Ireland *et al.*, 1987; Ireland & Tyers, 1987). However, metoclopramide is about 100 times less potent than GR38032F (pK_B values of 6.60 and 6.25 on rat VN and rat SCG, respectively). The other recently described compounds, MDL 72222 and ICS 205–930, are highly potent 5-HT_3 antagonists in the rat VN but analysis of their actions is complicated by the fact that they cause a depression of the maximum response to 5-HT in this preparation (Ireland & Tyers, 1987). A similar non-parallel rightward displacement of concentration-response curves has been described for MDL 72222 in the rabbit heart, rabbit SCG and rabbit nodose ganglion (NG) (Fozard, 1984; Azami *et al.*, 1985; Fozard *et al.*, 1985). In contrast, ICS 205-930 has been reported to cause simple competitive antagonism in the rabbit heart and VN (Richardson *et al.*, 1985), but it causes pronounced reduction in maximum response in the rabbit NG and SCG (Round & Wallis, 1986).

GR38032F also blocked 5-HT-induced depolarization in the rabbit VN. In this preparation, GR38032F appeared to be about ten times more potent than in the rat VN but some depression of the maximum response was observed. In the rabbit VN, metoclopramide caused parallel righward shifts of the 5-HT concentration-response curves, but the shallow Schild plot (slope = 0.62) is probably responsible for the high apparent pA_2 value for this compound.

GR38032F is highly selective for 5-HT_3 receptors, having negligible agonist or antagonist activity at 5-HT_1-like receptors in the cat or dog saphenous vein preparations or at 5-HT_2 receptors in the rabbit aorta at concentrations up to 5×10^{-5} M. Thus, the compound has a selectivity ratio of more than one thousand for 5-HT_3 over 5-HT_1-like and 5-HT_2 receptors. Its selectivity with respect to non-5-HT receptors is of a similar magnitude. GR38032F had negligible agonist or antagonist activity at α_1-, α_2-, β_1- or β_2- adrenoceptors, was inactive at H_1-recep-

tors and weak antagonism of H_2-receptor-mediated responses was seen only at a high concentration (3×10^{-5} M). The lack of antagonist effect of GR38032F at muscarinic receptors in spontaneously beating atria suggests that the compound does not interfere with the vagal neuroeffector limb of the reflex arc involved in the bradycardic response to exogenous 5-HT or 2-methyl-5-HT *in vivo*. Nash & Wallis (1980) have suggested that there is a close relationship between nicotinic receptors and 5-HT receptors which mediate depolarization responses in the rabbit SCG. In the present experiments, GR38032F (3×10^{-6}–3×10^{-5} M) produced a negligible rightward shift in the concentration-response curve to DMPP but there was some reduction in the maximum response at 3×10^{-5} M. Again, there was a wide separation between this concentration and that required to produce antagonism of the effects of 5-HT in the same preparation. GR38032F (3×10^{-5} M) did not inhibit GABA-induced depolarization of the SCG. Finally, GR38032F (up to 1 mg kg^{-1}) did not block a dopamine receptor-mediated response measured *in vivo*. The possible interaction of GR38032F with 5-HT_1 or benzodiazepine binding sites has been examined also. No significant interaction was observed and this is reported elsewhere (Jones *et al.*, 1988).

The activity of GR38032F on 5-HT_3 receptors *in vivo* was assessed by measuring its ability to block the bradycardic response to 5-HT or 2-methyl 5-HT (see Fozard & Host, 1982; Richardson *et al.*, 1985). GR38032F was a potent antagonist in this model, in both species examined, with an ED_{50} value of 0.4 μg kg^{-1} intravenously in the rat. GR38032F was also well absorbed from the oral route in the rat. That the 2-methyl-5-HT-induced bradycardic response in the cat involves a 5-HT_3 receptor-mediated mechanism, as defined by Bradley *et al.* (1986), was confirmed by the activity of MDL 72222 and ICS 205-930. In the cat, GR38032F had a good duration of action following intravenous or sublingual administration: in either case, significant antagonism of 2-methyl-5-HT-induced bradycardic responses was maintained for > 100 min following a dose of 30 μg kg^{-1}.

The apparent affinity of GR38032F for 5-HT_3 receptors *in vitro* was tissue-dependent. A similar result has been obtained for MDL 72222, ICS 205-930, BRL 24924 and metoclopramide (see Table 3 and Donatsch *et al.*, 1984a; Fozard, 1984; Richardson *et al.*, 1985; Bradley *et al.*, 1986; Sanger, 1987). However, before concluding that this confirms the existence of subtypes of the 5-HT_3 receptor, alternative explanations should be considered. Factors which may influence estimates of antagonist potency include metabolism of the agonist, interaction of the antagonist with metabolic processes, tissue uptake of the agonist or antagonist, multiple actions of the agonist and failure of the antagonist to reach equilibrium (Kenakin, 1982).

In the rat SCG, Ireland *et al.* (1987) have shown that use of the 5-HT uptake inhibitor, paroxetine, leads to an increase in the apparent pK_B value obtained for metoclopramide. For GR38032F, some discrepancy remains between potency value obtained on the rat SCG in the presence of paroxetine and that obtained on the rat VN. This may be due to an inability to prevent completely the uptake of 5-HT at concentrations of paroxetine which do not interfere with activation of 5-HT receptors. There appears to be no saturable uptake system for 5-HT in the rat VN (Ireland *et al.*, 1987).

5-HT uptake systems are present in the guinea-pig ileum (Gershon & Altman, 1971). Therefore the possibility exists that saturable 5-HT uptake could contribute to the lower pK_B values observed for GR38032F in the GPI. However, other factors should be considered. For example, on this prep-

Table 3 Potencies of 5-HT_3 antagonists in isolated tissues

Compound	*Rabbit heart* (pA_2)	*Rabbit VN* (pA_2)	*Rat VN* (pK_B)	*Rat SCG* (pK_B)	*GPI* (pK_B)
GR38032F	(< 10.5)§	9.40	8.61	8.13	7.31
(S)-GR38032F	ND	ND	8.95	ND	6.30
(R)-GR38032F	ND	ND	8.63	ND	7.20
MDL 72222	9.27, 8.9	7.9	7.9*	ND	6.2¶
ICS 205-930	10.6	10.2	11.0*	ND	8.0
BRL 24924	8.9	ND	ND	ND	7.6
Metoclopramide	6.81†, 7.1, 7.2	7.7†, 7.3	6.60	6.25	5.47†

Abbreviations: VN = vagus nerve, SCG = superior cervical ganglion; GPI = guinea-pig ileum longitudinal muscle. Data for MDL 72222, ICS 205-930, BRL 24924 and metoclopramide are from published studies (Buchheit *et al.*, 1985; Donatsch *et al.*, 1984b, Fozard, 1984; Fozard & Mobarok Ali, 1978; Ireland *et al.*, 1987; Ireland & Tyers, 1987; Round & Wallis, 1985; Sanger, 1987), except those marked (†) which were determined in the present experiments. *pA_2 value calculated according to the method of Schild (1947). §See text. ¶pD_2 value from Donatsch *et al.* (1984b). ND = not determined.

aration, concentration-response curves for 5-HT are biphasic even in the presence of methysergide (Figure 5 and Buchheit *et al.*, 1985) and it is the second (higher concentrations) component which is sensitive to the effects of 5-HT_3 antagonists. However, it is unlikely that the low-concentration contractile component compromised estimation of the antagonist potency of GR38032F when using 5-HT as agonist. Thus, although this component was lacking from the concentration-response curve to 2-methyl-5-HT, similar pK_B values were obtained for GR38032F using either 5-HT or 2-methyl-5-HT. Interestingly, on the GPI, the antagonist potency of the **R**- and **S**-isomers of GR38032F differed significantly—the **R**-isomer was approximately 0.9 log units more potent. In contrast, on the VN, the two isomers were approximately equipotent. These results suggest that the resolved isomers of compounds such as GR38032F could provide a key to the definition of subtypes of the 5-HT_3 receptor.

In the present experiments, careful attention was paid to establishing experimental conditions which would ensure that the antagonist was tested as close to equilibrium as possible. This was achieved by including a lengthy pre-incubation period in the experimental protocol and, in most cases, by repeated application of a single concentration of agonist to test for temporal changes in the degree of antagonism. However, we cannot exclude the possibility that diffusion barriers or other tissue factors prevented equilibrium of biophase concentrations of agonist or antagonist with those in the bathing medium.

Attempts were made to reduce the influence of other tissue factors on the apparent potency of antagonists. For example, in studies using the rabbit heart, atropine and a monoamine uptake inhibitor (desmethylimipramine) were used to reduce possible complications arising from activation of muscarinic receptors and entry of 5-HT into noradrenergic neurones. Previous authors have employed bolus doses of agonist in this tissue. In the present experiments, rabbit heart preparations were perfused with known concentrations of agonist and antagonist with the aim of approaching equilibrium conditions more nearly. Nevertheless, although as in a previous study (Fozard & Mobarok Ali, 1978), metoclopramide behaved as a reversible competitive antagonist of 5-HT, GR38032F did not. Until it has been established how GR38032F antagonizes 5-HT on this preparation, caution should be exercised before using the present data to support the existence of subtypes of the 5-HT_3 receptor.

In summary, GR38032F is a potent and highly selective 5-HT_3 antagonist both *in vitro* and *in vivo*. When considered in the context of studies of its acute toxicological profile (Jones *et al.*, 1988), these results confirm that GR38032F appears likely to have a very favourable therapeutic ratio (see Brittain *et al.*, 1987). Experiments with GR38032F provide some evidence for different subtypes of 5-HT_3 receptor occurring in rat vagus nerve and guinea-pig ileum but such a subdivision remains to be confirmed. Tests in various animal models of central nervous system (Costall *et al.*, 1987a; Hagan *et al.*, 1987; Jones *et al.*, 1988) and gastrointestinal disorders (Costall *et al.*, 1987b, c; Andrews *et al.*, 1987) suggest that the compound may offer important advances in the therapy of schizophrenia, anxiety and emesis resulting from cancer chemotherapy and these possibilities are now being examined in patients.

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References

ANDREWS, P.L.R., BAILEY, H.E., HAWTHORN, J., STABLES, R. & TYERS, M.B. (1987). GR38032F, a novel 5-HT_3 receptor antagonist, can abolish emesis induced by cyclophosphamide or radiation in the ferret. *Br. J. Pharmacol.*, **91**, 417P.

APPERLEY, E., FENIUK, W., HUMPHREY, P.P.A. & LEVY, G.P. (1980). Evidence for two types of excitatory receptor for 5-hydroxytryptamine in dog isolated vasculature. *Br. J. Pharmacol.*, **68**, 215–224.

APPERLEY, E., HUMPHREY, P.P.A. & LEVY, G.P. (1976). Receptors for 5-hydroxytryptamine and noradrenaline in rabbit isolated ear artery and aorta. *Br. J. Pharmacol.*, **58**, 211–221.

APPERLEY, G.H., DREW, G.M. & SULLIVAN, A.T. (1982). Prenalterol is an agonist at β_2 as well as at β_1-adrenoceptors. *Eur. J. Pharmacol.*, **81**, 659–663.

ARUNLAKSHANA, O. & SCHILD, H.O. (1959). Some quantitative uses of drug antagonists. *Br. J. Pharmacol. Chemother.*, **14**, 48–58.

AZAMI, J., FOZARD, J.R., ROUND, A.A. & WALLIS, D.I. (1985). The depolarising action of 5-hydroxytryptamine on rabbit vagal primary afferent and sympathetic neurones and its selective blockade by MDL 72222. *Naunyn-Schmiedebergs Arch. Pharmacol.*, **328**, 423–429.

BRADLEY, P.B., ENGEL, G., FENIUK, W., FOZARD, J.R., HUMPHREY, P.P.A., MIDDLEMISS, D.N., MYLECHARANE, E.J., RICHARDSON, B.P. & SAXENA, P.R. (1986). Proposals for the classification and nomencla-

ture of functional receptors for 5-hydroxytryptamine. *Neuropharmacology*, **25**, 563–576.

BRITTAIN, R.T., BUTLER, A., COATES, I.H., FORTUNE, D.H., HAGAN, R., HILL, J.M., HUMBER, D.C., HUMPHREY, P.P.A., IRELAND, S.J., JACK, D., JORDAN, C.C., OXFORD, A., STRAUGHAN, D.W. & TYERS, M.B. (1987). GR38032F, a novel selective 5-HT_3 receptor antagonist. *Br. J. Pharmacol. (Proc. Suppl.)*, **90**, 87P.

BROWN, C.M., McGRATH, J.C. & SUMMERS, R.J. (1979). The effects of α-adrenoceptor agonists and antagonists on responses of transmurally-stimulated prostatic and epididymal portions of the isolated vas deferens of the rat. *Br. J. Pharmacol.*, **66**, 553–564.

BUCHHEIT, K-H., ENGEL, G., MUTSCHLER, E. & RICHARDSON, B. (1985). Study of the contractile effect of 5-hydroxytryptamine (5-HT) in the isolated longitudinal muscle strip from guinea-pig ileum. *Naunyn-Schmiedebergs Arch. Pharmacol.*, **329**, 36–41.

COSTALL, B., DOMENEY, A.M., KELLY, M.E., NAYLOR, R.J. & TYERS, M.B. (1987a). The antipsychotic potential of GR38032F, a selective antagonist of 5-HT_3 receptors in the central nervous system. *Br. J. Pharmacol. (Proc. Suppl.)*, **90**, 89P.

COSTALL, B., GUNNING, S.J., NAYLOR, R.J. & TYERS, M.B. (1987b). The effect of GR38032F, a novel 5-HT_3-receptor antagonist on gastric emptying in the guinea-pig. *Br. J. Pharmacol.*, **91**, 263–264.

COSTALL, B., DOMENEY, A.M., GUNNING, S.J., NAYLOR, R.J., TATTERSHALL, F.D. & TYERS, M.B. (1987c). GR38032F: a potent and novel inhibitor of cisplatin-induced emesis in the ferret. *Br. J. Pharmacol. (Proc. Suppl.)*, **90**, 90P.

DONATSCH, P., ENGEL, G., RICHARDSON, B.P. & STADLER, P.A. (1984a). Subtypes of neuronal 5-hydroxytryptamine (5-HT) receptors as identified by competitive antagonists. *Br. J. Pharmacol. (Proc. Suppl.)*, **81**, 33P.

DONATSCH, P., ENGEL, G., RICHARDSON, B.P. & STADLER, P.A. (1984b). ICS 205-930: a highly selective and potent antagonist at peripheral neuronal 5-hydroxytryptamine (5-HT) receptors. *Br. J. Pharmacol. (Proc. Suppl.)*, **81**, 34P.

EDINBURGH STAFF (1970). *Pharmacological Experiments on Isolated Preparations*. Edinburgh, London and New York: Churchill Livingstone.

FENIUK, W., HUMPHREY, P.P.A. & WATTS, A.D. (1983). 5-Hydroxytryptamine-induced relaxation of isolated mammalian smooth muscle. *Eur. J. Pharmacol.*, **96**, 71–78.

FORTUNE, D.H. & IRELAND, S.J. (1984). Antagonism by MDL 72222 of 5-HT-induced depolarisations of the rat isolated vagus nerve and superior cervical ganglion. *Br. J. Pharmacol. (Proc. Suppl.)*, **77**, 520P.

FOZARD, J.R. (1984). MDL 72222: a potent and highly selective antagonist at neuronal 5-hydroxytryptamine receptors. *Naunyn-Schmiedebergs Arch. Pharmacol.*, **326**, 36–44.

FOZARD, J.R. & HOST, M. (1982). Selective inhibition of the Bezold-Jarisch effect of 5-HT in the rat by antagonists at neuronal 5-HT receptors. *Br. J. Pharmacol. (Proc. Suppl.)*, **77**, 520P.

FOZARD, J.R., HUMPHREYS, A., ROUND, A. & WALLIS, D.I. (1985). Further studies on the actions of MDL 72222 on responses to 5-HT in rabbit nodose (NG) and superior cervical (SCG) ganglia. *Br. J. Pharmacol. (Proc. Suppl.)*, **85**, 309P.

FOZARD, J.R. & MOBAROK ALI, A.T.M. (1978). Blockade of neuronal tryptamine receptors by metoclopramide. *Eur. J. Pharmacol.*, **49**, 109–112.

FOZARD, J.R. & MWALUKO, G.M.P. (1976). Mechanism of the indirect sympathomimetic effect of 5-hydroxytryptamine on the isolated heart of the rabbit. *Br. J. Pharmacol.*, **57**, 115–125.

GADDUM, J.H. & PICARELLI, Z.P. (1957). Two kinds of tryptamine receptor. *Br. J. Pharmacol. Chemother.*, **12**, 323–328.

GERSHON, M.D. & ALTMAN, R.F. (1971). An analysis of the uptake of 5-hydroxytryptamine by the myenteric plexus of the small intestine of the guinea-pig. *J. Pharmacol. Exp. Ther.*, **179**, 29–41.

GILLESPIE, J.S. (1972). The rat anococcygeus muscle and its response to nerve stimulation and to some drugs. *Br. J. Pharmacol.*, **45**, 404–416.

GOWER, A.J. & MARRIOTT, A.S. (1982). Pharmacological evidence for the sub-classification of central dopamine receptors in the rat. *Br. J. Pharmacol.*, **77**, 185–194.

HAGAN, R.M., BUTLER, A., HILL, J.M., JORDAN, C.C., IRELAND, S.J. & TYERS, M.B. (1987). Effect of the 5-HT_3 receptor antagonist, GR38032F, on responses to a neurokinin agonist into the ventral tegmental area of the rat brain. *Eur. J. Pharmacol.*, **138**, 303–305.

HUMPHRAY, J.M., DALY, M.J. & STABLES, R. (1982). Pharmacological interactions between ranitidine, cimetidine, metiamide and tiotidine at histamine H_2-receptor sites in guinea-pig atria. *Agents & Actions*, **12**, 138–141.

IRELAND, S.J. (1987). Origin of 5-hydroxytryptamine induced hyperpolarization of the rat isolated superior cervical ganglion and vagus nerve. *Br. J. Pharmacol.*, **92**, 407–416.

IRELAND, S.J., STRAUGHAN, D.W. & TYERS, M.B. (1982). Antagonism by metoclopramide and quipazine of 5-hydroxytryptamine-induced depolarisations of the rat isolated vagus nerve. *Br. J. Pharmacol. (Proc. Suppl.)*, **75**, 16P.

IRELAND, S.J., STRAUGHAN, D.W. & TYERS, M.B. (1987). Influence of 5-hydroxytryptamine uptake on the apparent 5-hydroxytryptamine antagonist potency of metoclopramide in the rat isolated superior cervical ganglion. *Br. J. Pharmacol.*, **90**, 151–160.

IRELAND, S.J. & TYERS, M.B. (1987). Pharmacological characterization of 5-hydroxytryptamine-induced depolarization of the rat isolated vagus nerve. *Br. J. Pharmacol.*, **90**, 229–238.

JONES, B.J., COSTALL, B., DOMENEY, A.M., KELLY, M.E., NAYLOR, R.J., OAKLEY, N.R. & TYERS, M.B. (1988). The potential anxiolytic activity of GR 38032F, a 5-HT_3-receptor antagonist. *Br. J. Pharmacol.*, (in press).

KENAKIN, T.P. (1982). Organ selectivity of drugs: Alternatives to receptor selectivity. *Trends Pharmacol. Sci.*, **3**, 153–156.

NASH, H.L. & WALLIS, D.I. (1980). Evidence suggesting that ganglion cell nicotinic and 5-hydroxytryptamine receptors may occupy adjacent membrane sites. *J. Physiol.*, **306**, 31P.

RANG, H.P. (1964). Stimulant actions of volatile anaesthetics on smooth muscle. *Br. J. Pharmacol. Chemother.*, **22**, 356–365.

RICHARDSON, B.P., ENGEL, G., DONATSCH, P. &

STADLER, P.A. (1985). Identification of serotonin M-receptor subtypes and their specific blockade by a new class of drugs. *Nature*, **316**, 126–131.

ROUND, A. & WALLIS, D.I. (1985). Selective blockade by ICS 205-930 of 5-HT depolarisations of rabbit vagal afferent and sympathetic ganglion cells. *Br. J. Pharmacol. (Proc. Suppl.)*, **86**, 734P.

ROUND, A. & WALLIS, D.I. (1986). The depolarising action of 5-hydroxytryptamine on rabbit vagal afferent and sympathetic neurones *in vitro* and its selective blockade by ICS 205-930. *Br. J. Pharmacol.*, **88**, 485–494.

SANGER, G.J. (1987). Increased gut cholinergic activity and antagonism of 5-hydroxytryptamine M-receptors by BRL 24924: potential clinical importance of BRL 24924. *Br. J. Pharmacol.*, **91**, 77–87.

SCHILD, H.O. (1947). pA, A new scale for the measurement of drug antagonism. *Br. J. Pharmacol. Chemother.*, **2**, 189–206.

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