Br. J. Pharmac. (1987), **91**, 77–87

Increased gut cholinergic activity and antagonism of 5-hydroxytryptamine M-receptors by BRL 24924: potential clinical importance of BRL 24924

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1 The mechanisms by which BRL 24924 ([(±)-(endo)]-4-amino-5-chloro-2-methoxy-N-(1-azabicyclo-[3.3.1]-non-4-yl) benzamide hydrochloride stimulates gut motility and the relationships between BRL 24924 and 5-hydroxytryptamine (5-HT) receptors have been studied.

2 In guinea-pig isolated ileum, BRL 24924 (10^{-14}–10^{-6} M) increased electrically-evoked, cholinergically-mediated contractions, probably by increasing acetylcholine (ACh) release. This action of BRL 24924 was prevented by the presence of high concentrations of 5-HT, but not by hexamethonium, phentolamine and propranolol, methysergide or ICS 205–930.

3 The mechanism by which BRL 24924 can increase gut ACh release is not certain, but most likely involves activation of an enteric 5-HT receptor which differs from those 5-HT M-receptors antagonized by ICS 205-930 or by higher concentrations of BRL 24924 in other test systems.

4 BRL 24924 antagonized 5-HT-evoked, cholinergically-mediated contractions of guinea-pig isolated ileum ($pA_2 = 7.56 \pm 0.12$). Similar and higher concentrations of BRL 24924 did not antagonize contractions evoked by nicotinic receptor stimulation. In rabbit isolated heart, BRL 24924 1–10 nM reduced the tachycardia evoked by 5-HT.

5 In anaesthetized rats, BRL 24924 0.3–83 nmol kg^{-1} i.v. antagonized the Bezold-Jarisch reflex evoked by 5-HT; the ID_{50} for BRL 24924 was 10.2 ± 3.0 nmol kg^{-1} (3.7 ± 1.1 μg kg^{-1}). A direct action of BRL 24924 on nerve function was excluded.

6 In rat cortex, BRL 24924 10^{-6} M did not displace [^{3}H]-5-HT or [^{3}H]-ketanserin binding to 5-HT_1 and 5-HT_2 receptors.

7 The actions of BRL 24924 are discussed in terms of its potential clinical use as a stimulant of gastric motility and as a 5-HT M-receptor antagonist.

Introduction

BRL 24924 is a novel substituted benzamide which potently increases rat gastric motility and has a low affinity for dopamine receptors (Cooper *et al.*, 1986). In this respect, BRL 24924 is unlike metoclopramide (a gut stimulant and a dopamine antagonist; McRitchie *et al.*, 1984) and similar but more potent than either BRL 20627 (McClelland *et al.*, 1983) or cisapride (Schuurkes *et al.*, 1985).

Metoclopramide, BRL 20627 and cisapride are thought to stimulate gut motility mostly by increasing acetylcholine (ACh) release from the cholinergic nerves of the gut (McClelland & Sanger, 1983; Sanger, 1984; Schuurkes *et al.*, 1985). For metoclopramide, this increase in ACh release may be due to antagonism of 5-hydroxytryptamine (5-HT) M-receptors (5-HT_3 receptors; Bradley *et al.*, 1986) modulating ACh release in the gut (Bianchi *et al.*, 1970; Buchheit *et al.*, 1985a). However, the evidence is not conclusive and an increase in ACh release due to stimulation of enteric 5-HT receptors has been proposed (Kilbinger *et al.*, 1982; Sanger, 1985a).

The ability of BRL 24924 to increase ACh release in guinea-pig isolated ileum has now been studied and the relationships between BRL 24924 and 5-HT receptors investigated in the gut and in other, non-gastrointestinal tissues. Some of these results were previously communicated to the British Pharmacological Society (Dunbar *et al.*, 1986).

Methods

Guinea-pig ileum longitudinal muscle-myenteric plexus

Male guinea-pigs, 300–400 g in weight were used. Distal ileum was removed at least 10 cm proximal to the caecum and preparations of longitudinal muscle-myenteric plexus were cut 2–3 cm long (Ambache & Freeman, 1968). The muscle-nerve strips were suspended under a 0.5 g load in 10 ml tissue baths containing Krebs solution (NaCl 121.5, $CaCl_2$ 2.5, KH_2PO_4 1.2, KCl 4.7, $MgSO_4$ 1.2, $NaHCO_3$ 25.0, dextrose 5.6 mM) bubbled with 5% CO_2 in O_2 and maintained at 37°C. Responses were registered and magnified 6–18 times with isotonic transducers.

For electrical field stimulation (EFS) of the myenteric neurones, bipolar rectangular pulses were passed between 2 platinum wire electrodes 25 mm long and 5 mm apart, suspended either side of the muscle strip and insulated on entry to the bathing solution. EFS was given as 0.5 ms pulses at 0.1 Hz frequency and at the minimum voltage which evoked maximum muscle contractions (25–40 V). After obtaining consistent contractions, 10 min after washout and replacement of the bathing solution, cumulative concentration-response curves were constructed for BRL 24924 by adding increasing concentrations at 5 min intervals. The effects of BRL 24924 on the EFS-evoked contractions were calculated as a % of the contraction heights measured before addition of BRL 24924. In studying the effects of other drugs on the responses evoked by BRL 24924, a single concentration of BRL 24924 was tested before and then again after recovery and 30 min tissue preincubation with the potentially modifying drug; contact time for BRL 24924 was 5 min. In experiments with 5-HT as the potentially modifying substance, BRL 24924 was added 10 min after addition of 5-HT and the effects of 5-HT were compared on separate tissues in which saline and BRL 24924 were added.

In experiments not primarily concerned with EFS, submaximally-effective concentrations of exogenous ACh (0.04–0.41 μM; 20 s contact, 10 min cycle) were used to evoke contractions approximately equal in height to the EFS-evoked contractions. The ACh-evoked contractions were 52 ± 7% (mean ± s.e.mean) of the maximum contraction evoked by higher concentrations of ACh ($n = 8$). After obtaining consistent contractions, BRL 24924 was added in increasing concentrations after washout of the bathing solution following each addition of ACh. The effects of BRL 24924 on the ACh-evoked contractions were calculated as a % of the contraction heights obtained prior to addition of BRL 24924.

Concentration-response curves for 5-HT were constructed in the presence of methysergide 0.2 μM (to block non-neuronally-mediated contractions which may be evoked with 5-HT), using a modified technique of Buchheit *et al.* (1985b). The heights of the 5-HT-evoked contractions were calculated as a % of a previously obtained maximum ACh-evoked contraction. Increasing concentrations of 5-HT were added every 15 min, with 60 s contact times for low concentrations (0.003–1.3 μM) which were slow to evoke a contraction, and 30 s contact times for higher concentrations (3 μM–1.3 mM) which evoked a contraction and subsequent fading of the contraction within 30 s. In separate experiments in which single concentrations of 5-HT were repeatedly administered (not shown), these procedures caused no apparent tachyphylaxis. However, to avoid extensive tissue fatigue, only one concentration-response curve was constructed in each tissue, following 30 min preincubation with saline or with a single concentration of BRL 24924.

Concentration-response curves for the nicotinic agonist, dimethylphenylpiperazinium iodide (DMPP), were constructed by adding increasing concentrations to the bathing solution for 30 s and at 10 min intervals. After obtaining a control concentration-response curve, a single concentration of BRL 24924 was added to the bathing solution for 30 min before retesting the effects of DMPP. This procedure was repeated twice more with higher concentrations of BRL 24924. The results were compared with control experiments in which the effects of saline were tested against DMPP in a separate tissue from the same ileum. By this comparison the results were corrected for slight tissue fatigue (Furchgott, 1968). The contractions evoked by each concentration of DMPP were calculated as a % of the maximum contraction evoked by DMPP in the control concentration-response curve, before BRL 24924 or saline was added.

Rabbit isolated heart

Rabbit hearts were prepared according to the Langendorff technique, and the ability of 5-HT to stimulate noradrenergic neurones was evaluated by measuring the resultant tachycardia. The coronary arteries were perfused with Krebs solution containing atropine 1.4 μM, maintained at 37°C and at approximately 60 cmH_2O perfusion pressure (see Fozard, 1984a). Right ventricular isometric tension and rate of contraction were recorded. Dose-response curves to 5-HT were constructed using bolus injections of increasing doses (0.3 nmol–3 μmol) into the perfusate, every 5 min and in 0.1 ml volumes delivered within 1 s; for the higher doses of 5-HT (0.1–3 μmol), longer time intervals between doses (6–10 min) were sometimes required in order to allow for recovery of the response. Changes in rate of ventricular contraction evoked by 5-HT were then measured. For each experiment, up to 4 dose-response curves were constructed. Between each curve, the tissue was equilibrated for 30 min with

fresh perfusate containing a single concentration of BRL 24924; the concentration of BRL 24924 was increased after each dose-response curve. The tachycardia evoked by 5-HT was calculated as a % of the maximum tachycardia evoked by 5-HT during the first, control dose-response curve. In control experiments where BRL 24924 was not added, there were no statistically significant ($P > 0.05$) differences between 4 consecutive 5-HT dose-response curves ($n = 4$).

Rat forestomach longitudinal muscle

Strips of rat forestomach longitudinal muscle were prepared (Sanger, 1985a), with atropine 1.4 μM present in the Krebs solution. Isotonic concentration-response curves were constructed for 5-HT and then concentrations of 5-HT were chosen to give contractions approximately 50% of maximum (1.3–2.6 nM; 90 s contact, 15 min cycle). After obtaining consistent contractions with 5-HT, BRL 24924 was added in increasing concentrations following washout of each dose of 5-HT. The effects of BRL 24924 on the 5-HT-evoked contractions were calculated as a % of the contractions obtained before addition of BRL 24924.

Bezold-Jarisch effect in anaesthetized rats

Male rats 260–290 g were anaesthetized with urethane 1.25 g kg^{-1} i.p., and the trachea cannulated. The jugular vein was cannulated for intravenous (i.v.) injection of drugs. Blood pressure was recorded from a cannula in the left carotid artery and connected to a heparin/saline-filled pressure transducer. Continuous heart rate measurements were taken from the blood pressure recordings. The Bezold-Jarisch effect was evoked by rapid, bolus i.v. injections of 5-HT (see Fozard, 1984a) and measurements were made of the fall in heart rate. In each rat, consistent responses were first established with the minimum dose of 5-HT that evoked a clear fall in heart rate. Injections of 5-HT were given every 12 min and a dose-response curve for BRL 24924 was established by injecting increasing doses of compound 5 min before each injection of 5-HT. The effect of BRL 24924 on the 5-HT-evoked bradycardia was calculated as a % of the bradycardia evoked by 5-HT before injection of BRL 24924. In separate experiments to measure the duration of 5-HT antagonism caused by BRL 24924, a single dose of compound was injected 5 min before 5-HT, and the effects of 7 repeated challenges with 5-HT were then monitored. The effects of BRL 24924 on the efferent vagal limb of the Bezold-Jarisch reflex were checked by electrically stimulating the peripheral end of a cut vagus nerve. Unipolar electrical stimulation was applied every 5 min via a pair of silver electrodes, using 1 ms rectangular pulses in 5 s trains with a maximally-effective voltage (20 V at 10 Hz). Pulse frequency varied from 5–30 Hz and frequency-response curves were constructed before and 10 min after i.v. injection of a single dose of BRL 24924.

Radioligand binding studies

Binding to 5-HT$_1$ and 5-HT$_2$ receptors was estimated according to Bennett & Snyder (1976) and Leysen *et al.* (1982), using rat cortical membranes, [^{3}H]-5-HT 4 nM and [^{3}H]-ketanserin 0.5 nM. BRL 24924 10 μM was tested in triplicate for displacement of either radioligand. Specific binding was defined as the difference between total counts obtained in the presence and absence of, respectively, an excess of unlabelled 5-HT (10 μM) and methysergide (2 μM).

Statistical analysis

Results are expressed as means ± standard error of the means and were analysed by Student's paired or unpaired t test. pA_2 and pD'_2 values were determined by the methods of Arunlakshana & Schild (1959) and Van Rossum (1963) respectively.

Drugs used

The following were dissolved in 154 mM saline: BRL 24924 ([(±)-(endo)]-4-amino-5-chloro-2-methoxy-N-(1-azabicyclo-[3.3.1]-non-4-yl) benzamide hydrochloride; Beecham Pharmaceuticals), acetylcholine perchlorate, atropine sulphate, nicotine hydrogen tartrate (BDH), DMPP, 5-hydroxytryptamine creatinine sulphate (protected from light), tetrodotoxin (Sigma), hexamethonium bromide (May and Baker), methysergide hydrogen maleate (Sandoz), phentolamine mesylate (Ciba), and propranolol hydrochloride (ICI). ICS 205-930 (indole-3-carboxylic acid-tropine-ester hydrochloride) was synthesized in house, dissolved (20 mM) in distilled water adjusted to pH 4 with tartaric acid and then further diluted with water. Noradrenaline bitartrate (Koch and Light) was freshly dissolved in 0.57 μM ascorbic acid solution. 5-Hydroxy[G-^{3}H]-tryptamine creatinine sulphate (specific activity 10–20 Ci mmol^{-1}; Amersham) and [ethylene-^{3}H]-ketanserin hydrochloride (60–95 Ci mmol^{-1}; New England Nuclear) were dissolved in the appropriate buffer solutions for radioligand binding.

Results

Effects of BRL 24924 on cholinergic activity in guinea-pig isolated ileum

Maximum contractions evoked by electrical field

stimulation (EFS) were prevented by atropine 1 μM ($n = 7$) or by tetrodotoxin 0.5 μM ($n = 4$). Hexamethonium 28 μM reduced the contractions by 10 ± 4% ($P < 0.05$, $n = 6$), suggesting that the contractions evoked by EFS were predominantly due to activation of post-ganglionic cholinergic neurones.

BRL 24924 0.00001 nM–1.0 μM dose-dependently increased the maximum contractions evoked by EFS; lower and higher concentrations of BRL 24924 had no significant effects (Figure 1). There were no effects of BRL 24924 on muscle tone. In separate experiments in which single concentrations of BRL 24924 were added to the bath, the increase in EFS-evoked contractions recorded 5 min after addition of BRL 24924, 0.1 nM or 10 nM, to the bathing solution was the same as that recorded 30 min after addition of BRL 24924 ($P > 0.1$, $n = 6$ each). The increase in EFS-evoked contractions 5 min after addition of BRL 24924 1 μM (34 ± 12%) was less after 30 min (29 ± 12%; $P < 0.01$, $n = 6$). In these experiments, muscle tone was unaffected by 0.1 nM or 10 nM BRL 24924, but there was a small, short-lived increase in tone with 1 μM BRL 24924. The increase in EFS-evoked contractions caused by BRL 24924 10 nM was not blocked by hexamethonium 28 μM, phentolamine 1.3 μM and propranolol 0.9 μM, methysergide 0.2 μM or by ICS 205-930 0.1 μM (Table 1). These antagonists respectively prevented submaximal contractions evoked by DMPP 3 μM ($n = 6$), prevented the inhibition of EFS-evoked contractions caused by noradrenaline 3 nM (which reduced EFS-evoked contractions by 42 ± 6%), antagonized 5-HT D-receptor-mediated contractions (see Costa & Furness, 1979) and reduced contractions evoked by 5-HT acting on cholinergic neurones (unpublished observations; see also Buchheit *et al.*, 1985b). The presence of low concentrations of 5-HT (0.3–30 nM) had no effects or caused a sustained increase in the EFS-evoked contractions, but did not prevent the actions of BRL 24924. The maximum increase in contraction

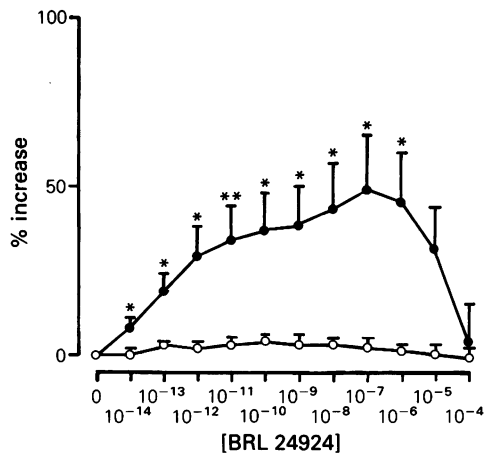

Figure 1 Increase in electrically-evoked contractions by BRL 24924 in guinea-pig isolated ileum. Contractions were evoked by electrical field stimulation at 0.1 Hz frequency, using unipolar rectangular pulses of 0.5 ms duration and at maximally-effective voltage. After obtaining consistent contractions 10 min after changing the bathing solution, increasing concentrations of BRL 24924 (●) or equivalent volumes of saline (○) were added to the Krebs solution at 5 min intervals. Results are given as the % increase in height of the electrically-evoked contractions; $^*P < 0.05$; $^{**}P < 0.01$ comparison with saline, $n = 8$ for BRL 24924 and saline.

Table 1 Effects of drug antagonists on the increase in EFS-evoked contractions caused by BRL 24924 in guinea-pig isolated ileum

Antagonist and concentration used (μM)		*Effects on EFS-evoked contractions* (% control)	*Effects on response to* BRL 24924 (ratio)
Saline	0.1 ml	88 ± 3	1.0 ± 0.1
Hexamethonium	28	70 ± 7	1.6 ± 0.2
Phentolamine and Propranolol	1.3 + 0.9	64 ± 6**	1.2 ± 0.2
Methysergide	0.2	116 ± 6**	0.9 ± 0.3
ICS 205-930	0.1	79 ± 2*	1.0 ± 0.3

The effects of BRL 24924 10 nM were tested before and then after 30 min incubation of the tissue with an antagonist. The results are expressed as a ratio of the % increase in contractions to EFS caused by BRL 24924 in the presence of antagonist, divided by the increase caused by BRL 24924 in the absence of antagonist (Sanger, 1985a). The effects of the antagonists on the contractions to EFS in the absence of BRL 24924 were calculated as a % of the pre-antagonist control contractions. Resting muscle tone was not affected by any of the antagonists used. Compared with the effects of saline, $^*P < 0.05$; $^{**}P < 0.01$; $n = 6$ each.

heights caused by these concentrations of 5-HT was similar to the increase caused by BRL 24924 10 nM. In contrast, higher concentrations of 5-HT (0.3 and 3.0 μM) had no effects or reduced the EFS-evoked contractions and prevented the increase in contractions caused by BRL 24924 10 nM (Figure 2). Similar results were obtained with 5-HT and BRL 24924 1 μM (not shown).

BRL 24924 0.00001 nM–100 μM had no effects ($P > 0.05$, $n = 8$ each) on contractions evoked by exogenous ACh.

Effects of BRL 24924 on contractions evoked by 5-HT or by DMPP in guinea-pig isolated ileum

Contractions evoked by low concentrations of 5-HT (0.003–1.3 μM) were slow to develop and were maintained once the contraction had been fully developed. In contrast, contractions evoked by higher concentrations of 5-HT quickly reached their maximum and then rapidly relaxed back towards the resting tension (see Buchheit *et al.*, 1985b). BRL 24924 10 nM had no effects on the 5-HT concentration-response curve, but higher concentrations of BRL 24924 (0.1 and 1.0 μM) dose-dependently antagonized the submaximal contractions evoked by low and by high concentrations of 5-HT ($pA_2 = 7.56 \pm 0.12$; Figure 3). Contractions evoked by DMPP were unaffected or increased by BRL 24924 0.1, 1.0 and 10 μM (Figure 3).

Inhibition by BRL 24924 of 5-HT-evoked tachycardia in rabbit isolated heart

BRL 24924 1–10 nM concentration-dependently reduced the tachycardia evoked by 5-HT (Figure 4). The negative logarithm of the molar concentration of BRL 24924 which reduced the 5-HT-evoked tachycardia by a factor of two was 8.94 ± 0.07 ($n = 7$). However, because of the substantial antagonism caused by BRL 24924 5 and 10 nM, it was not always possible to re-obtain a maximum effect of 5-HT in the presence of BRL 24924 and the slope of an Arunlakshana & Schild (1959) plot was significantly different from unity (1.9 ± 0.1; $P < 0.001$).

Effects of BRL 24924 on the Bezold-Jarisch reflex

BRL 24924 0.3–83 nmol kg^{-1} i.v. dose-dependently reduced the reflex bradycardia evoked by 5-HT; the ID_{50} was 10.2 ± 3.0 nmol kg^{-1} (3.7 ± 1.1 μg kg^{-1}) i.v. ($n = 8$). These doses of BRL 24924 did not by themselves affect resting blood pressure or heart rate. A single dose of BRL 24924 27.7 nmol kg^{-1} (10 μg kg^{-1}) i.v. caused a short-lived reduction in the bradycardia evoked by 5-HT, whereas a higher dose 277 nmol kg^{-1} (100 μg kg^{-1}) almost abolished the Bezold-Jarisch reflex and this was followed by a slight recovery over an 89 min observation period (Figure 5). BRL 24924 277 nmol kg^{-1} i.v. did not affect the

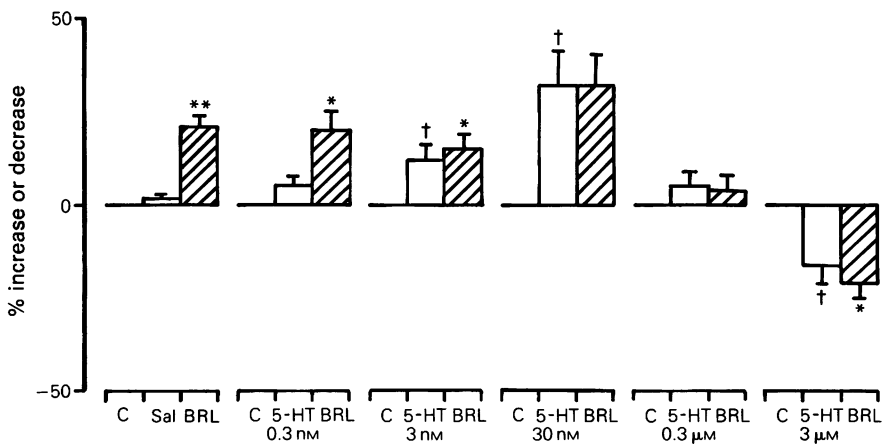

Figure 2 Effects of 5-hydroxytryptamine (5-HT) on the increase in electrically-evoked contractions caused by BRL 24924 10 nM in guinea-pig isolated ileum. Contractions were evoked by electrical field stimulation as described in Figure 1. After obtaining consistent contractions, saline or 5-HT was added to the bathing solution and after 10 min, BRL 24924 10 nM was added for 5 min. In each preparation, only a single concentration of 5-HT was used. Results are calculated as a % change in the height of the pre-drug control contractions (C) evoked by saline (Sal), 5-HT or by BRL 24924 (BRL); †$P < 0.05$, for the effect of 5-HT, compared with saline; *$P < 0.05$, **$P < 0.01$ for the effect of BRL 24924, compared with saline or 5-HT; $n = 30$ for saline, $n = 6$ for each concentration of 5-HT.

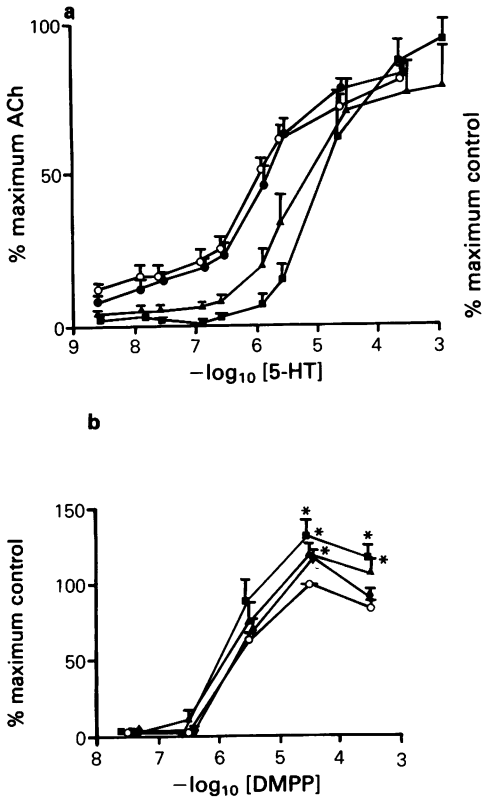

Figure 3 Comparison between the effects of BRL 24924 on contractions evoked by 5-hydroxytryptamine (5-HT) or by dimethylphenyl piperazinium (DMPP) in guinea-pig isolated ileum. (a) Concentration-response curves for 5-HT were constructed in the presence of methysergide 0.2 μM, by adding single concentrations to the bathing solution every 15 min. Contact times were 60 s for 0.003–1.3 μM 5-HT and 30 s for 3 μM–1.3 mM 5-HT. One concentration-response curve was constructed for each of 4 preparations from the same ileum, following 30 min preincubation with saline (○), BRL 24924 0.01 μM (●), 0.1 μM (▲) or 1.0 μM (■); $n = 6$ in each case. The results were calculated as a % of the maximum contraction previously obtained using acetylcholine (ACh). The pA_2 was determined according to Arunlakshana & Schild (1959) and was 7.56 ± 0.12; slope $= 0.91 \pm 0.10$ ($P > 0.05$). (b) Concentration-response curves for DMPP were constructed by adding single concentrations to the bathing solution for 30 s every 10 min. After establishing a control curve (○), 3 other curves were constructed following 30 min tissue preincubation with BRL 24924 0.1 μM (▲), 1.0 μM (■) and then 10.0 μM (◆). The results were calculated as a % of the maximum contraction evoked by DMPP in the control curve and were corrected for slight tissue fatigue by comparison with similar experiments in which saline, instead of BRL 24924, was added to a preparation from the same ileum (Furchgott, 1968). $^*P < 0.05$ comparison between control and BRL 24924; $n = 6$ each.

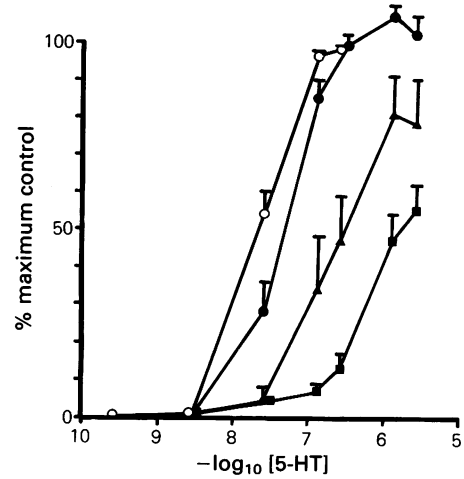

Figure 4 Antagonism by BRL 24924 of 5-hydroxytryptamine (5-HT)-evoked tachycardia in rabbit isolated heart. Atropine 1.4 μM was continually present in the perfusate. Dose-response curves for 5-HT were constructed by injection of increasing doses of 5-HT every 5 min (or longer if the resulting tachycardia was slow to recover). Up to 4 dose-response curves were constructed for each preparation, beginning with a control curve (○; $n = 8$) and then with the perfusate containing BRL 24924 1 nM (●; $n = 7$), 5 nM (▲; $n = 4$) and 10 nM (■; $n = 8$). Construction of each curve followed a 30 min perfusion with Krebs solution containing BRL 24924. In separate experiments, there were no significant differences ($P > 0.05$) between each of 4 consecutive dose-response curves constructed in the absence of BRL 24924 ($n = 4$). The results were calculated as a % of the maximum tachycardia evoked by 5-HT in the control curve. An apparent pA_2 ($= 8.94 \pm 0.07$) was estimated according to Arunlakshana & Schild (1959); slope $= 1.90 \pm 0.14$, $P < 0.001$.

bradycardia evoked by electrical stimulation of the vagus ($P > 0.1$ for the 5, 10, 20 and 30 Hz frequencies tested; $n = 4$).

Effects of BRL 24924 on other 5-HT-evoked responses

In rat isolated forestomach, BRL 24924 1 nM–0.1 μM had no effects on submaximal contractions evoked by 5-HT, whereas higher concentrations reduced the contractions; the concentration of BRL 24924 which reduced the 5-HT-evoked contractions by 50% was 1.2 ± 0.3 μM ($n = 6$).

In radioligand binding studies, BRL 24924 10 μM did not displace [^{3}H]-5-HT or [^{3}H]-ketanserin binding.

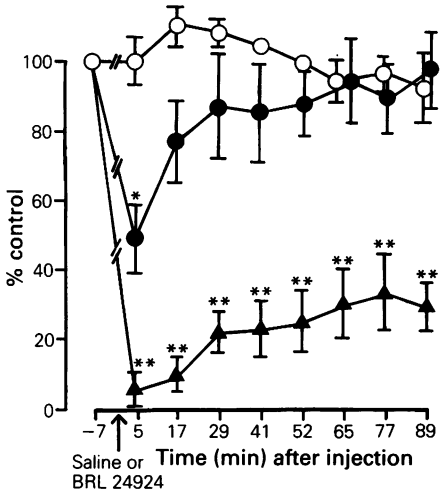

Figure 5 Inhibition by BRL 24924 of 5-hydroxytryptamine (5-HT)-evoked bradycardia (Bezold-Jarisch reflex) in anaesthetized rats. Bolus i.v.injections of 5-HT were given every 12 min until consistent responses were obtained. A single i.v. dose of saline (○; $n = 4$), BRL 24924 10 $\mu g\,kg^{-1}$ (●; $n = 12$) or 100 $\mu g\,kg^{-1}$ (▲; $n = 10$) was then given 5 min before a subsequent challenge with 5-HT and the effects on the 5-HT-evoked reflex bradycardia were monitored for up to to 89 min. The results were calculated as a % of the bradycardia evoked by 5-HT before injection of saline or BRL 24924. $*P < 0.05$; $**P < 0.01$, compared with the effects of saline.

Discussion

Stimulation of gut cholinergic activity

In guinea-pig isolated ileum, BRL 24924 potently increased the height of the EFS-evoked, cholinergically-mediated contractions. This action of BRL 24924 was maintained during the continued presence of the compound, and was therefore not clearly subject to tachyphylaxis. BRL 24924 had no effects on contractions evoked by exogenous ACh and may therefore increase EFS-evoked contractions by acting prejunctionally to increase the release of ACh from the cholinergic nerves. A similar action has previously been reported for metoclopramide (see Sanger, 1984), BRL 20627 (McClelland & Sanger, 1983) and for cisapride (Schuurkes *et al.*, 1985).

The ability of BRL 24924 to increase cholinergic activity was not prevented by hexamethonium and may therefore be due to a predominant action on postganglionic neurones. An action of BRL 24924 on α- or β-adrenoceptors was unlikely because of the failure of phentolamine and propranolol to antagonize the increase in EFS-evoked contractions caused by BRL 24924. Since this combination of adrenoceptor antagonists can also antagonize dopamine-evoked inhibition of cholinergic activity (Wikberg, 1978; Gorich *et al.*, 1982), an involvement of dopamine receptors in the cholinergic activity of BRL 24924 may also be excluded. This is supported by the low affinity of BRL 24924 for dopamine binding sites in rat cortex (Cooper *et al.*, 1986). Similar conclusions have previously been reached from experiments using metoclopramide (Kilbinger *et al.*, 1982; Zar *et al.*, 1982; Costall *et al.*, 1984; Sanger, 1985a).

The increase in EFS-evoked contractions caused by BRL 24924 was not selectively affected by methysergide 0.2 μM, which itself increased the EFS-evoked contractions. This excitatory effect of methysergide may reflect the increased myoelectric activity which can be recorded from the ileum of conscious guinea-pigs after subcutaneous injection of methysergide (C.M. McClelland, personal communication). Relatively high concentrations of methysergide increase cholinergic activity in muscle strips taken from human isolated ileum and rectum (Turner, 1973). The mechanism of this action is obscure, but in certain vascular tissues, methysergide may act either as an antagonist or as a partial agonist at 5-HT receptors (Fenuik, 1984). Perhaps, in some gut tissues, methysergide activates an enteric 5-HT receptor to increase cholinergic activity. In contrast, ICS 205-930, 0.1 μM, did not increase the EFS-evoked contractions and did not prevent the increase in contractions (see Table 1) caused by BRL 24924. This concentration of ICS 205-930 selectively antagonizes 5-HT-evoked contractions of guinea-pig isolated ileum (pA_2 8.1; Buchheit *et al.*, 1985b). The 5-HT M-receptors antagonized by ICS 205-930 are therefore not likely to be involved in the mechanism by which BRL 24924 increases EFS-evoked contractions.

Increased cholinergic activity evoked by BRL 24924 could be prevented by the presence of high concentrations of 5-HT. These concentrations of 5-HT had no effect or caused a long-lasting reduction of EFS-evoked contractions in the absence of BRL 24924 (see also Kilbinger & Pfeuffer-Freiderich, 1985) and can also prevent the cholinergically-mediated actions of low (1 μM), but not of high (100 μM) concentrations of metoclopramide (unpublished). Lower concentrations of 5-HT caused a sustained increase in EFS-evoked contractions (confirming earlier observations; Sanger, 1985b), but did not prevent the increase caused by BRL 24924. Since both the excitatory and inhibitory actions of 5-HT or BRL 24924 are long-lasting, tachyphylaxis or depletion of a common second messenger are unlikely mechanisms by which 5-HT prevents the increase in cholinergic activity evoked by BRL 24924.

It has been suggested that metoclopramide might increase cholinergic activity in guinea-pig isolated

stomach by antagonizing an inhibitory action of endogenous 5-HT (Buchheit *et al.*, 1985a). However, in the present experiments with guinea-pig isolated ileum, inhibition of EFS-evoked contractions can only be detected with high concentrations of 5-HT. This action of 5-HT may be due to activation of 5-HT_1-like receptors, which are not antagonized by metoclopramide or by the 5-HT M-receptor antagonist MDL 72222 (Kilbinger & Pfeuffer-Friederich, 1985). Since BRL 24924 has a low affinity for 5-HT_1 binding sites in rat brain cortex, it is again unlikely that the increase in EFS-evoked contractions caused by BRL 24924 is due to antagonism of an inhibitory action of endogenous 5-HT, mediated by 5-HT_1 receptors. High concentrations of 5-HT may therefore prevent the actions of BRL 24924 by blocking receptors occupied by BRL 24924. This may be a direct effect of 5-HT on the receptors or may be a non-selective antagonism caused by 5-HT_1-like receptor activation, which also blocks the increase in EFS-evoked contractions seen with lower concentrations of 5-HT. BRL 24924 may therefore increase cholinergic activity in guinea-pig isolated ileum by affecting a receptor which modulates ACh release. This receptor cannot be defined with certainty, but is not a 5-HT_1-like or a 5-HT_2-receptor and cannot be activated or blocked by nicotinic receptor and adrenoceptor antagonists, or by the class of 5-HT M-receptor antagonists represented by ICS 205-930. However, it is suggested that BRL 24924 may activate a particular 5-HT receptor in the enteric nervous system. Activation by BRL 24924 of the enteric 5-HT receptor can therefore be mimicked by low concentrations of 5-HT and blocked by higher concentrations of 5-HT.

Other actions of BRL 24924 on 5-HT receptors

BRL 24924 may increase ACh release by activating enteric 5-HT receptors, but the compound can also antagonize other actions of 5-HT, mediated by 5-HT M-receptors in both gastrointestinal and non-gastrointestinal tissues. It is unlikely that BRL 24924 is a partial agonist and antagonist at the same 5-HT receptor, since the compound did not first mimic the actions of 5-HT in any of the models used to study 5-HT M-receptor antagonism. Instead, BRL 24924 may have different actions on the 5-HT receptors located on the different nerves studied. These different actions of BRL 24924 do not necessarily mean that there are subtypes of 5-HT receptors within the enteric and peripheral nervous systems, but the probability that this is so is suggested by the work of others (Wallis, 1981; Fozard, 1984b; Richardson *et al.*, 1985; Round & Wallis, 1987). More important, at present, is the concept that 5-HT M-receptor antagonism by a compound does not necessarily imply that this compound will also activate enteric 5-HT receptors which modulate ACh release. Thus, ICS 205-930 did not mimic or antagonize the increase in cholinergically-mediated contractions caused by BRL 24924 in guinea-pig ileum, whereas both compounds can antagonize 5-HT M-receptors in other test systems. Furthermore, other substituted benzamides which increase cholinergically-mediated contractions in isolated gut tissues (metoclopramide, BRL 20627, cisapride; see Introduction for references), may be considerably less effective 5-HT M-receptor antagonists, as judged by their ability to antagonize the Bezold-Jarisch reflex in anaesthetized rats (Dunbar *et al.*, 1986).

If BRL 24924 increases gut ACh release by activating enteric 5-HT receptors, then it may initially appear paradoxical that the compound can also antagonize, in an apparently competitive manner, contractions of guinea-pig ileum evoked by both low and high concentrations of 5-HT. ICS 205-930 can also antagonize 5-HT-evoked contractions, except that this compound was only effective against the contractions evoked by high, but not by low concentrations of 5-HT (Buchheit *et al.*, 1985b). These results may be explicable if the different actions of 5-HT and BRL 24924 were indicative of their effects on different nerve populations within the gut. Thus EFS-evoked cholinergically-mediated contractions may be increased by activating enteric 5-HT receptors using 5-HT (Sanger, 1985b), the 5-HT precursor 5-hydroxytryptophan (Sanger & McClelland, 1986) or BRL 24924; these actions may be related to an increase in co-ordinated and propulsive gut motility. In contrast, contractions of guinea-pig ileum evoked by 5-HT in the absence of electrical stimulation may be due to activation of gut afferent neurones, rather than those neurones which stimulate co-ordinated, propulsive gut motility; 5-HT-evoked contractions can be antagonized by capsaicin, which first stimulates and then depletes unmyelinated afferent nerve fibres of their neurotransmitters (Chahl, 1983; Buchheit *et al.*, 1985b). Stimulation by 5-HT of gut afferent neurones would evoke both an orthodromic and antidromic release of peptide neurotransmitter (including substance P) from afferent nerve fibres, which activate cholinergic neurones and cause muscle contraction. The concentrations of 5-HT required to evoke these contractions are higher than those required to increase electrically-evoked contractions (Sanger, 1985b), so that these different functions of 5-HT can be separately studied in guinea-pig isolated ileum. Clearly, further work with selective receptor antagonists is required to substantiate these suggestions.

BRL 24924 is also an antagonist of 5-HT M-receptors in non-gastrointestinal models of peripheral nerve activity, and these include the receptors located on noradrenergic efferent neurones in rabbit isolated heart and on the afferent neurones arising from the

heart, subserving the Bezold-Jarisch reflex in anaesthetized rats. This ability of BRL 24924 to antagonize 5-HT M-receptors seems selective, because of the low affinity of BRL 24924 for 5-HT_1 and 5-HT_2 binding sites in rat cortex. Furthermore the concentrations of BRL 24924 required to antagonize 5-HT-evoked contractions of rat isolated forestomach were higher than those required to antagonize 5-HT M-receptors in guinea-pig isolated ileum or in rabbit isolated heart. The receptors which mediate 5-HT-evoked contractions of rat forestomach are still poorly defined and are known as 5-HT D-receptors (Gaddum & Picarelli, 1957), but have more recently been characterized as 5-HT_2 (Gregg & Osborne, 1985), 5-HT_1-like (Clineschmidt *et al.*, 1985) or possibly a 5-HT_{1c} receptor subtype (Buchheit *et al.*, 1986).

Potential clinical significance of BRL 24924

Increased gut cholinergic activity due to activation by BRL 24924 of enteric 5-HT receptors may play a major role in the mechanism by which the compound stimulates gastric motility *in vivo* (see Cooper *et al.*, 1986). Previous studies with metoclopramide and related benzamides have shown a positive correlation between the ability to stimulate gastric motility and the ability to increase EFS-evoked, cholinergically-mediated contractions in isolated gut tissues (Sanger, 1984). In human isolated stomach, metoclopramide increases cholinergically-mediated contractions (Sanger, 1985c) and BRL 24924 can act in a similar manner (unpublished). BRL 24924 may therefore have clinical application in treating the kinds of disordered gut motility which are already successfully treated with metoclopramide (see Harrington *et al.*, 1983). The potential advantage of BRL 24924 over metoclopramide lies not just in its increased potency, but also in its poor affinity for dopamine receptors (Cooper *et al.*, 1986). Antagonism by metoclopramide of dopamine receptors can be associated with undesirable side-effects such as akathisia and hyperprolactinaemia, excluding some patients from potential treatment and precluding long-term treatment therapies (McRitchie *et al.*, 1984).

BRL 24924 is a potent 5-HT M-receptor antagonist and this property is unrelated to its more potent ability to increase gut cholinergic activity by activating enteric 5-HT receptors. Nevertheless, 5-HT M-receptor antagonism by BRL 24924 could increase gut motility by an action within the central nervous system. This was suggested by Buchheit *et al.* (1985a), to explain the actions of ICS 205-930 in conscious guinea-pigs. However, the ability of 5-HT M-receptor antagonists to increase gut motility may be species- or gut region-dependent, since ICS 205-930 did not increase the myoelectric activity recorded from the small intestine of conscious dogs (Davidson & Pilot, 1986).No information is yet available for man.

Antagonists of 5-HT M-receptors may be clinically useful for the intervention treatment of pain in migraine attacks (Loisy *et al.*, 1985), particularly if gastric motility can also be increased (Paratainen *et al.*, 1980). Cardiac arrhythmias associated with myocardial ischaemia may be reduced by 5-HT M-receptor antagonism (Williams *et al.*, 1985), and emesis evoked by cytotoxic drugs such as cisplatin may be prevented by 5-HT M-receptor antagonists (Miner & Sanger, 1986), such as BRL 24924 (Miner *et al.*, 1986; Andrews *et al.*, 1987).

I thank Miss J. Burridge, Mr A. Dunbar and Mr G. Pratt, for their expertise in carrying out the Bezold-Jarisch studies and the radioligand binding assays. Methysergide was a generous gift from Sandoz. My thanks also to Mrs A. Skinner for her patience and expertise in the preparation of this manuscript.

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(Received September 19, 1986.
Revised November 18, 1986.
Accepted November 25, 1986.)