Regionally selective cholinergic stimulation by BRL 24924 in the human isolated gut

TRACEY A. BURKE & G. J. SANGER

Beecham Pharmaceuticals, Medicinal Research Centre, Coldharbour Road, The Pinnacles, Harlow, Essex CM19 5AD

1 The effects of BRL 24924 on cholinergic activity was studied in longitudinal muscle strips obtained from human stomach and colon (taenia). Contractions were evoked by exogenous acetylcholine (ACh) or by electrical field stimulation (EFS) of cholinergic neurones. Inhibitory nerve activity (predominantly non-adrenergic, non-cholinergic; NANC) was detected by measuring the relaxations evoked by EFS in the presence of atropine 1.4 μM .

2 In the stomach, BRL 24924 0.28–28 μM consistently increased the EFS-evoked contractions; lower concentrations (0.0028 and 0.028 μM) increased the contractions in most, but not all of the specimens studied. Since BRL 24924 28 μM did not affect NANC-mediated, EFS-evoked relaxations, and since high concentrations of BRL 24924 (28 and 282 μM) were required to increase ACh-evoked contractions, the increase in EFS-evoked contractions caused by BRL 24924 may be due to a facilitation of ACh release.

3 In the colon, BRL 24924 0.0028–282 μM did not affect EFS-evoked contractions.

4 BRL 24924 may therefore be regionally selective in its ability to stimulate human gastrointestinal cholinergic activity.

Keywords BRL 24924 gastrointestinal tract cholinergic mechanisms 5-hydroxytryptamine

Introduction

BRL 24924 is a novel substituted benzamide (Figure 1), which in animals, stimulates gastrointestinal motility and emptying, but has negligible affinity for dopamine receptors (Cooper *et al.*, 1986; McClelland, 1987). BRL 24924 has therefore entered clinical trials as a new gastric prokinetic agent, lacking the side-effects, sometimes detected with metoclopramide (Maxolon; Beecham Pharmaceuticals), which are associated with antagonism of dopamine receptors in the central nervous system. Preliminary results with BRL 24924 in volunteers support the animal findings (Rapeport & Thompson, 1987a,b; Staniforth & Corbett, 1987). In addition to acting as a stimulant of gut motility, BRL 24924 is also an antagonist of 5-hydroxytryptamine₃ (5-HT₃)

receptors (Sanger, 1987a), which explains the mechanism by which, in ferrets, BRL 24924 can inhibit the emesis evoked by cytotoxic drugs (Miner & Sanger, 1986; Miner *et al.*, 1987) or by total body X-irradiation (Andrews *et al.*, 1987; Andrews & Hawthorn, 1987; Miner *et al.*, 1987).

To characterise the mechanisms by which BRL 24924 stimulates gastric motility, the actions of the compound have now been investigated in

Figure 1 Structure of BRL 24924.

human isolated stomach and colonic muscle. For the stomach, similar studies previously demonstrated that metoclopramide may stimulate electrically-evoked, cholinergically-mediated contractions by acting pre-junctionally to increase acetylcholine (ACh) release (Sanger, 1985). Increased release of ACh from gut cholinergic neurones may play a major role in the mechanisms by which metoclopramide stimulates gut motility (Sanger, 1984), and it was considered important to look for similar actions with BRL 24924.

Methods

Macroscopically-normal specimens of human stomach and taenia coli were obtained from surgery for benign or malignant disease. Muscle was used after storage for one to two nights at 4° C in Krebs solution (NaCl 121.5, CaCl₂ 2.5, KH₂PO₄ 1.2, KCl 4.7, MgSO₄ 1.2, NaHCO₃ 25.0, dextrose 5.6 mM) equilibrated with 5% CO₂ in O₂. The muscle layers were removed from the mucosa and submucosa by dividing along the submucosal plexus. Muscle strips approximately 4 mm wide and 30 mm long, were cut parallel to the longitudinal muscle fibres. Each strip was suspended under a 1 g load in a 10 ml tissue bath containing Krebs solution maintained at 37° C and bubbled with 5% CO₂ in O₂. Responses were magnified 6–18 times and registered with isotonic transducers.

The intrinsic nerves of the muscle strips were activated by electrical field stimulation (EFS), as described previously (Sanger, 1985). For both stomach and colonic muscle strips, bipolar rectangular pulses of 1 ms duration were given every 10 min for 30 s, at 5 Hz frequency and at maximum-effective voltage (80–120 V cm⁻¹). After each period of stimulation, the muscle was washed by replacing the bathing solution. In all experiments with EFS, consistent contractions or relaxations were first obtained, and then increasing concentrations of BRL 24924 were added every 10 min.

In experiments with exogenous ACh, concentration-response curves were first obtained using 30 s contact times and a 10 min cycle time. Concentrations were then chosen (4–400 µM for stomach, 2–200 µM for colon) to give muscle contractions which were approximately 50% of the maximum contractions which could be evoked using higher concentrations of ACh. After obtaining consistent contractions, increasing concentrations of BRL 24924 were added every 10 min.

Results are expressed as medians with semi-quartile ranges in parentheses, and analysed using the Wilcoxon matched pairs test.

Drugs used

The following drugs were dissolved in 154 mM saline: acetylcholine perchlorate, atropine sulphate (BDH), tetrodotoxin (Sigma) and BRL 24924 ([(+)-(endo)]-4-amino-5-chloro-2-methoxy-N-(1-azabicyclo-[3.3.1]-non-4-yl) benzamide hydrochloride; Beecham). Barium chloride (BaCl₂; BDH) was dissolved in distilled water.

Results

Stomach specimens were obtained from the gastric body, antrum or pylorus from eight male and six female patients (median age 63 years; range 52–84 years). Colonic specimens were obtained from the ascending, transverse or sigmoid colon of seven male and seven female patients (median age 73 years; range 55–83 years). The numbers of specimens examined were too small to determine how stomach or colonic region, disease, age or sex may affect the responses to BRL 24924.

Stomach

EFS-evoked muscle contractions in the stomach have previously been shown to be prevented by atropine 1.4 µM or by tetrodotoxin 0.1 µM, indicating predominant cholinergic activation (Sanger, 1985). In tissues incubated with atropine 1.4 µM (to block the cholinergically-mediated contractions) and where necessary, barium chloride 0.48 mM (to raise muscle tone), EFS caused relaxations which may be due predominantly to activation of non-cholinergic, non-adrenergic inhibitory neurones (Bennett & Stockley, 1975; Sanger, 1985).

BRL 24924 0.28–28 µM concentration-dependently and consistently increased the contractions evoked by EFS; BRL 24924 282 µM caused inhibition. Lower concentrations of BRL 24924 (0.0028 and 0.028 µM) also increased the contractions in most, but not all of the specimens studied (Figure 2). In these experiments, BRL 24924 0.0028–28 µM had no consistent effects on resting muscle tone or spontaneous contractions. However, at the highest concentration of BRL 24924 used (282 µM), muscle tone was reduced in four of the 10 specimens studied. BRL 24924 28 µM had no effect on the muscle relaxations evoked by EFS in the presence of atropine 1.4 µM. Relaxations were 100 (100–113)% of the relaxations obtained before adding BRL 24924 to the bathing solution ($n = 6$; $P > 0.05$).

In muscle strips not stimulated electrically and in which no atropine was added, BRL 24924

Figure 2 Effects of BRL 24924 on (a) EFS-evoked contractions and on (b) ACh-evoked contractions in human isolated stomach. After obtaining consistent contractions in response to EFS (5Hz frequency, 1 ms pulse width, 30 s train, maximum-effective voltage, 10 min intervals) or to ACh (approximately 50% maximum ACh contraction, 30 s contact, 10 min intervals), BRL 24924 was added in increasing concentrations every 10 min. The effects of BRL 24924 on the contractions were calculated as a % of the pre-BRL 24924 contractions. These are shown here as medians (columns) with semiquartile ranges (bars), and were analysed using the Wilcoxon matched pairs test; * $P < 0.05$, ** $P < 0.01$; $n = 10$ for EFS, $n = 8$ for ACh.

0.0028–2.8 μM had no effects on submaximal ACh-evoked contractions. Higher concentrations of BRL 24924 (28 and 282 μM) increased the contractions (Figure 2).

Colon

In the taenia coli, EFS-evoked contractions were prevented by atropine 1.4 μM ($n = 7$) or by tetrodotoxin 0.05–2.5 μM ($n = 5$). In the presence of atropine 1.4 μM , EFS evoked muscle relaxations which were prevented by tetrodotoxin 0.05–2.5 μM ($n = 4$), and which may be due predominantly to activation of non-cholinergic, nonadrenergic inhibitory neurones (Bennett & Stockley, 1975; Stockley & Bennett, 1977).

BRL 24924 0.0028–282 μM had no consistent effects on resting muscle tone or spontaneous contractions, and did not affect the contractions evoked by EFS (Figure 3).

Figure 3 Effects of BRL 24924 on EFS-evoked contractions in human isolated taenia coli. The experimental procedure for EFS was as described in Figure 2 for the stomach. $P > 0.05$ for each concentration of BRL 24924, $n = 8$.

Discussion

In human isolated stomach, low concentrations of BRL 24924 increased the electrically-evoked, cholinergically-mediated contractions. Since BRL 24924 28 μM did not affect the muscle relaxations obtained in response to EFS after cholinergic antagonism by atropine, BRL 24924 may increase the EFS-evoked contractions by increasing cholinergic activity and not by reducing non-adrenergic, non-cholinergic inhibitory nerve activity.

BRL 24924 did not increase the contractions evoked by exogenous ACh, except when high concentrations were added to the bathing solution. Low concentrations of BRL 24924 may therefore increase EFS-evoked contractions by acting pre-junctionally, to increase ACh release. A predominant post-junctional action of BRL 24924 seems unlikely, in view of the failure of similarly low concentrations of BRL 24924 to increase the contractions evoked by ACh. Nevertheless, these experiments provide only an indirect assessment of ACh release and confirmation of the conclusions must now be obtained by measuring ACh release, using techniques such as gas chromatography-mass spectrometry.

Similar conclusions were reached in studies with metoclopramide (Sanger, 1985), but in comparison, BRL 24924 is a more potent stimulant of cholinergic activity in the human stomach. Furthermore, BRL 24924 did not increase the EFS-evoked, cholinergically-mediated contractions of human isolated taenia coli and may therefore show regional selectivity in its ability to facilitate gastrointestinal cholinergic activity.

The mechanisms by which BRL 24924 may increase neuronal ACh release in the gut have been investigated in animals (Sanger, 1987a), but not in man. Dopamine antagonism is not likely to be involved since BRL 24924 has a poor affinity for dopamine receptors (Cooper *et al.*, 1986). Furthermore, the dopamine D_2 -receptor antagonist domperidone, does not increase cholinergic activity in human isolated stomach (Sanger, 1985). In guinea-pig isolated ileum, it has been suggested that the increased cholinergic activity caused by metoclopramide can be explained by an activation of enteric receptors for 5-hydroxytryptamine (5-HT) which modulate ACh release in the gut (Kilbinger *et al.*, 1982; Sanger, 1987b). This action may also be true for BRL 24924 (Sanger, 1987a). These 5-HT receptors are probably located within the myenteric plexus, since in both the present study with

human stomach and in guinea-pig ileum (Sanger, 1987a,b), the mucosa and submucosa had been removed from the muscle strips studied. The possibility that metoclopramide and related compounds increase gut ACh release by affecting receptors located within the mucosa (Costall *et al.*, 1986) is therefore not supported by our experiments.

If BRL 24924 activates enteric 5-HT receptors which modulate ACh release and gut motility, then these receptors may be different to the 5-HT₃ receptors antagonised elsewhere by BRL 24924 (Sanger, 1987a). In this respect, there is no correlation between the ability of different compounds to stimulate gut motility and their potency as 5-HT₃ receptor antagonists (Dunbar *et al.*, 1986). Furthermore, in human isolated stomach, the selective 5-HT₃ receptor antagonist BRL 43694 does not increase cholinergic activity (unpublished) and, in guinea-pig isolated ileum, the 5-HT₃ receptor antagonist ICS 205-930 does not increase EFS-evoked cholinergic activity and does not prevent the stimulatory actions of BRL 24924 or metoclopramide (Sanger, 1987a,b). ICS 205-930 has, however, been shown to increase cholinergic activity in guinea-pig isolated stomach, but the effect is not clearly shared by another 5-HT₃ receptor antagonist, MDL 72222 (Buchheit *et al.*, 1985).

There is evidence to suggest that the 5-HT₃ receptor antagonists ICS 205-930, MDL 72222 and GR 38032F may increase gastric emptying in conscious guinea-pigs, possibly by acting within the central nervous system (Buchheit *et al.*, 1985; Costall *et al.*, 1987). However, since ICS 205-930 does not increase small intestinal motility in conscious fed or fasted dogs (Davidson & Pilot, 1986) and since BRL 43694 did not increase conscious rat interdigestive gastric motility (Fake *et al.*, 1987), there may be species or methodological differences in the actions of these compounds. It is therefore conceivable that BRL 24924 could increase gastric motility by acting within the central nervous system in some, but not all, species or conditions of study. However, the clear ability of BRL 24924 to increase gut cholinergic activity in human isolated stomach, suggests a predominant action within the myenteric nerves of the gut, and this is a property not obviously shared by other 5-HT₃ receptor antagonists.

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