



# Comparison of the profiles of agonists as stimulants of the $\beta_3$ -adrenoceptor *in vitro* with their gastroprotective effects in the conscious rat

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**1** This paper compares the activity of a range of agonists as stimulants of the  $\beta_3$ -adrenoceptor in rat isolated oesophagus with their ability to afford protection against indomethacin-induced gastric damage in the conscious rat.

**2** The  $\beta_3$ -adrenoceptor agonists, CL 316243 and BRL 37344, the non-selective  $\beta$ -adrenoceptor agonist, isoprenaline and the selective  $\beta_2$ -adrenoceptor agonist, salmeterol, all evoked concentration-dependent relaxation of precontracted muscularis mucosa from rat oesophagus. The rank order of agonist potency was BRL 37344 > CL 316243 > isoprenaline > salmeterol. The selective  $\beta_1$ -adrenoceptor agonist, denopamine, did not relax the preparation.

**3** The relaxant responses to all agonists were resistant to blockade by atenolol (10  $\mu$ M), and ICI 118551 (1  $\mu$ M) thus suggesting that they were not mediated by either  $\beta_1$ - or  $\beta_2$ -adrenoceptor stimulation. In contrast, cyanopindolol and propranolol did inhibit responses to BRL 37344, CL 316243 and isoprenaline, giving  $pA_2$  values or  $pK_B$  estimates which were consistent with an interaction at  $\beta_3$ -adrenoceptors (i.e. approximately 8.0 and 6.5 respectively). However, responses to salmeterol were resistant to blockade by all the antagonists tested, which suggests that the high (> 1  $\mu$ M) concentrations of salmeterol used exerted non-specific relaxant effects.

**4** The agonist effects of CL 316243 and BRL 37344 on  $\beta_1$ - and  $\beta_2$ -adrenoceptors were assessed on guinea-pig right atrium and precontracted trachea respectively. Both agonists had minimal activity as stimulants of heart rate, but did relax trachea, being 380 (CL 316243) and 21 (BRL 37344) fold less potent than isoprenaline.

**5** CL 316243 and BRL 37344 were potent inhibitors of indomethacin-induced gastric antral ulceration in the conscious rat ( $ED_{50}$  values = 0.24 and 0.09  $\mu$ mol  $kg^{-1}$ , p.o.) Salmeterol was approximately 100 times less potent than BRL 37344 as a gastroprotective agent and denopamine was without effect.

**6** The gastroprotective effects of CL 316243 and BRL 37344 were resistant to blockade by ICI 118551 (10 mg  $kg^{-1}$ , p.o.) and propranolol (10 mg  $kg^{-1}$ , p.o.). In contrast, both antagonists caused dose-related inhibition of the protective action of salmeterol (10 mg  $kg^{-1}$ , p.o.). Cyanopindolol was not assessed as an antagonist *in vivo* because preliminary experiments revealed that it exacerbated indomethacin-induced gastric damage in its own right.

**7** In conclusion, the  $\beta_3$ -adrenoceptor agonists CL 316243 and BRL 37344 were potent inhibitors of indomethacin-induced gastric antral ulceration in the rat. These data suggest that an agonist which is potent and selective for the human  $\beta_3$ -adrenoceptor may confer mucosal protection in man.

**Keywords:** Gastroprotection;  $\beta_3$ -adrenoceptor; CL 316243; BRL 37344; ulceration

## Introduction

The existence of atypical  $\beta$ -adrenoceptors (also referred to as  $\beta_3$ -adrenoceptors) is now well established (Arch & Kaumann, 1993; Howe, 1993). There are several pharmacological properties which discriminate this form of  $\beta$ -adrenoceptor from others (Blin *et al.*, 1994), the major factors being resistance to blockade by a range of conventional  $\beta$ -adrenoceptor antagonists and high potency of a series of novel synthetic agonists (Arch *et al.*, 1984; Wilson *et al.*, 1984). The majority of evidence for a functional role of  $\beta_3$ -adrenoceptors has been derived from studies in which the effects of these agonists on lipid metabolism have been examined. In rodents, a range of  $\beta_3$ -adrenoceptor agonists are potent lipolytic and thermogenic agents (Wilson *et al.*, 1984; Holloway *et al.*, 1989; Bloom *et al.*, 1992) and as a result their therapeutic use as anti-obesity agents is now under evaluation. However, the corresponding

role of the  $\beta_3$ -adrenoceptor in man is still subject to controversy, although a recent study does suggest that it mediates a lipolytic response in human white fat (Lönnqvist *et al.*, 1993).

The  $\beta_3$ -adrenoceptor is also located within the gastrointestinal tract and isolated preparations taken from several species show that here, it mediates relaxation (reviewed by Arch & Kaumann, 1993). As a consequence, Bianchetti & Manara (1990) suggested that  $\beta_3$ -adrenoceptor agonists may alleviate intestinal hypermotility disorders. We now describe an additional property of  $\beta_3$ -adrenoceptor agonists which may be of potential therapeutic use, the ability to confer protection against gastric damage induced by a non-steroidal anti-inflammatory drug (NSAID). Ulceration was induced in the gastric antrum of the rat as described by Satoh *et al.* (1981). This model was chosen because the antrum is the primary site of NSAID-induced damage in man (Roth & Bennett, 1987). In the current study we have examined the ability of two  $\beta_3$ -adrenoceptor agonists, BRL 37344 (Arch *et al.*, 1984) and CL 316243 (Bloom *et al.*, 1992) to protect against indomethacin-induced ulceration and compared their profiles

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with that of the potent and selective  $\beta_2$ -adrenoceptor agonist, salmeterol (Ball *et al.*, 1991) and the  $\beta_1$ -adrenoceptor agonist, denopamine (Nagao *et al.*, 1984). Before undertaking the *in vivo* studies, an assessment of the agonists' abilities to stimulate  $\beta$ -adrenoceptors in isolated tissues was carried out. The following responses were examined: relaxations of rat oesophageal muscularis mucosa and guinea-pig trachea and elevation of guinea-pig atrial rate, which are mediated predominantly, although not exclusively, by  $\beta_3$ - (de Boer *et al.*, 1993),  $\beta_2$ - (Carswell & Nahorski, 1983) and  $\beta_1$ - (Hedberg *et al.*, 1980) adrenoceptor stimulation, respectively.

## Methods

### Isolated preparations

**Rat oesophageal muscularis mucosae** Female Wistar rats (Allen & Hanbury's strain) weighing 100–200 g were killed by stunning and cervical dislocation. The abdomen was opened and the distal 2 cm of oesophagus removed. The muscularis externa was split lengthways and cut away, leaving the inner smooth muscle tube. This was suspended vertically under an initial tension of 0.5 g in modified Krebs-Henseleit solution at 32°C and gassed with 95% O<sub>2</sub>/5% CO<sub>2</sub>. Changes in tension were monitored via an isometric force-displacement transducer (Dynamometer UF1) coupled to an Ormed recorder. The bathing solution was changed 3–4 times and the tissue was then allowed to equilibrate for 30 min before agonists were applied. Throughout the experiment, the Krebs-Henseleit solution contained indomethacin (2.8  $\mu$ M) to inhibit formation of cyclo-oxygenase products, ascorbate (0.11 mM) to prevent catecholamine oxidation and isobutylmethylxanthine (IBMX (3  $\mu$ M)) to prolong responses mediated by formation of adenosine 3':5'-cyclic monophosphate (cyclic AMP). In all except the preliminary series of experiments, atenolol (10  $\mu$ M) and ICI 118551 (1  $\mu$ M) were also included in the bathing solution to avoid  $\beta_1$ - and  $\beta_2$ -adrenoceptor activation respectively.

**Guinea-pig trachea** Male Dunkin-Hartley guinea-pigs (350–500 g) were killed by stunning and exsanguination for supply of trachea and right atrium (see below). The trachea was cut into transverse sections, 3–4 cartilage rings wide. Each tracheal ring was then opened by cutting the cartilage opposite the trachealis muscle (Coburn & Tomita, 1973). The resulting strip preparations were suspended in organ baths via cotton threads tied through the upper and lower cartilages under an initial tension of 1.5 g in bathing solution maintained at 37°C. Two preparations were obtained from each animal. The recording system and equilibration conditions were the same as those described above. In these experiments the Krebs-Henseleit solution contained indomethacin (2.8  $\mu$ M), ascorbate (0.11 mM), atenolol (10  $\mu$ M), and atropine (0.4  $\mu$ M, to prevent muscarinic cholinergic activation).

**Guinea-pig atrium** The right atrium was removed rapidly and mounted for recording of isometric tension in modified Krebs-Henseleit solution (containing ascorbate (0.11 mM) and ICI 118551 (1  $\mu$ M)) at 32°C. The initial resting tension on the preparation was adjusted to 1.0 g. The rate of spontaneous beats was measured by linking the transducer signal to a cardiometer and was allowed to stabilize before the experiment started (30–45 min after dissection).

**Experimental protocols** Tone was elevated in oesophageal preparations by inclusion of 1  $\mu$ M carbachol in the bathing solution. This concentration of spasmogen evoked a well-maintained contraction which was between 70–80% of the maximal response. A cumulative concentration-response curve for the relaxant effect of isoprenaline was constructed on each preparation. The tissues were then washed repeatedly and allowed to recover for 1 h, after which they were recontracted. A further concentration-response curve was then constructed to

either isoprenaline (in tissues which served as controls), to ensure that the EC<sub>50</sub> value (see below) was constant (within two fold), or to a test agonist. Isoprenaline (1–100  $\mu$ M) was also administered in the presence of a test agonist to determine the maximal response in each preparation. In some experiments an antagonist was added to the bath 30 min prior to construction of the second concentration-response curve.

A similar protocol was adopted for guinea-pig trachea. However, in this case, the spasmogen used was prostaglandin F<sub>2 $\alpha$</sub>  (0.3 or 1  $\mu$ M) and the recovery period between curves was 30 min.

In experiments on atria, cumulative concentration-response curves to isoprenaline were repeated at hourly intervals until constant (i.e. until the EC<sub>50</sub> values (see below) varied by less than two fold). This usually was achieved with the first two curves. A further curve was then constructed to either isoprenaline alone (in control preparations) or a test agonist plus subsequent addition of isoprenaline (1–100  $\mu$ M).

### Animal studies – assessment of the protective effect of $\beta$ -adrenoceptor agonists against gastric ulceration in rats

**Induction of gastric antral ulceration** The method used was largely that described by Satoh *et al.* (1981). Briefly, food (but not water) was withheld for 24 h from female, random bred, hooded rats (supplied by Glaxo UK) weighing 70–120 g. Access was then allowed to food (rat and mouse No 1 maintenance diet (SDS Ltd, Witham, Essex)) for 90 min. Unless stated otherwise, a single dose of  $\beta$ -adrenoceptor agonist was then administered p.o. (1 ml 100 g<sup>-1</sup>). Indomethacin (60 mg kg<sup>-1</sup>, 1 ml 100 g<sup>-1</sup> body weight) was then injected subcutaneously. Control rats received the subcutaneous injection of indomethacin and oral administration of vehicle (0.5% methyl cellulose in distilled water) for the  $\beta$ -adrenoceptor agonist. The animals were then allowed continued access to food but water was withdrawn. In some studies an antagonist was administered p.o. 30 min prior to the  $\beta$ -adrenoceptor stimulant.

**Assessment of gastric damage** The methods used were those described by Trevethick *et al.* (1993). The animals were killed by cervical dislocation 6 h after dosing with indomethacin. The stomachs were removed, opened along the greater curvature and washed in 0.9% saline. An assessment of gastric damage was carried out by an observer who was unaware of the dosing regimen. A transparent plastic grid divided into 1 mm<sup>2</sup> sections was placed over the antrum and the area of macroscopic damage assessed as the total area of visible lesions in mm<sup>2</sup>. This value was then expressed as a percentage of the total antral area. The stomachs were then fixed in 10% (v/v) buffered formalin and tissue sections taken for histological assessment (Trevethick *et al.*, 1993). Each section was examined for the presence of the following: (i) superficial erosion – damage to surface epithelium only; (ii) deep erosion – glandular epithelium still present; (iii) ulceration – complete loss of the epithelium to the level of the muscularis mucosae.

The length of antral mucosa showing each degree of damage was measured with a calibrated eyepiece scale and calculated as a percentage of the total antral mucosal area.

### Analysis of results

All results are expressed as either geometric means with 95% confidence limits or arithmetic means  $\pm$  s.e. mean of *n* observations. Differences in mean values from isolated tissue experiments were determined by Student's *t* test; *P* < 0.05 was considered significant. For oesophagus and trachea, responses were expressed as percentage reversal of spasmogen-induced tone. In atria, all responses were expressed as a proportion of the maximal tachycardia induced by isoprenaline. For all preparations the EC<sub>50</sub> value refers to the concentration of agonist required to produce half the maximum effect. The potency of an antagonist in the oesophagus was assessed by

calculating a concentration ratio (i.e.  $EC_{50}$  value obtained in the presence of antagonist divided by the corresponding control value from another tissue used in the same experiment). When three or more concentrations of antagonist were used, the concentration ratios were pooled to construct a Schild plot, from which a  $pA_2$  value was derived. The slope of each Schild plot was shown not to be significantly different from 1 using a  $t$  test based on the standard error of the slope estimated from the linear regression. When only a single concentration of antagonist was utilised, an estimate of its  $pK_B$  value was obtained from the Gaddum equation (Gaddum, 1957).

In order to compare data from *in vivo* experiments, an  $ED_{50}$  value (i.e. dose of agonist required to reduce the extent of antral macroscopic damage by 50%) was calculated for each  $\beta$ -adrenoceptor stimulant. Statistical comparisons were performed with the Mann-Whitney U test. A  $P$  value  $<0.05$  was considered statistically significant.

### Compounds

The following compounds were used: ascorbic acid, carbachol (BDH), atenolol, atropine hydrochloride, 5-hydroxytryptamine creatinine sulphate (5-HT), indomethacin, 3-isobutyl-1-methyl-xanthine, isoprenaline hemisulphate, propranolol hydrochloride (Sigma), ICI 118551 (( $\pm$ )-1-[2,3-(dihydro-7-methyl-1H-inden-4-yl)oxy]-3-[(1-methylethyl)amino]-2-butanolol hydrochloride) (Cambridge Research Biochemicals), prostaglandin  $F_{2\alpha}$  tromethamine salt (Lutalyse, Upjohn), BRL 37344, (( $R,R$ )-[ $\pm$ ]-[4-[2-[2-(3-chlorophenyl)-2-hydroxyethylamino]propyl]phenoxy]acetic acid), CL316243 (( $R,R$ )-5-[2-[2-(3-chlorophenyl)-2-hydroxyethylamino]propyl]-1,3-benzodioxole-2,2-dicarboxylic acid disodium salt), cyanopindolol, denopamine and salmeterol (Glaxo).

Unless stated otherwise, drugs were dissolved and diluted in distilled water (for *in vitro* experiments) or saline (for administration to animals). All solutions of  $\beta$ -adrenoceptor agonists contained ascorbic acid (110  $\mu$ M). Indomethacin was dissolved in 1% (w/v)  $NaHCO_3$ . BRL 37344, salmeterol and cyanopindolol were dissolved in 5–10% dimethylsulphoxide (DMSO) for *in vitro* use, and in the case of cyanopindolol, for p.o. dosing. All  $\beta$ -adrenoceptor agonists were suspended in 0.5% w/v methylcellulose for p.o. dosing.

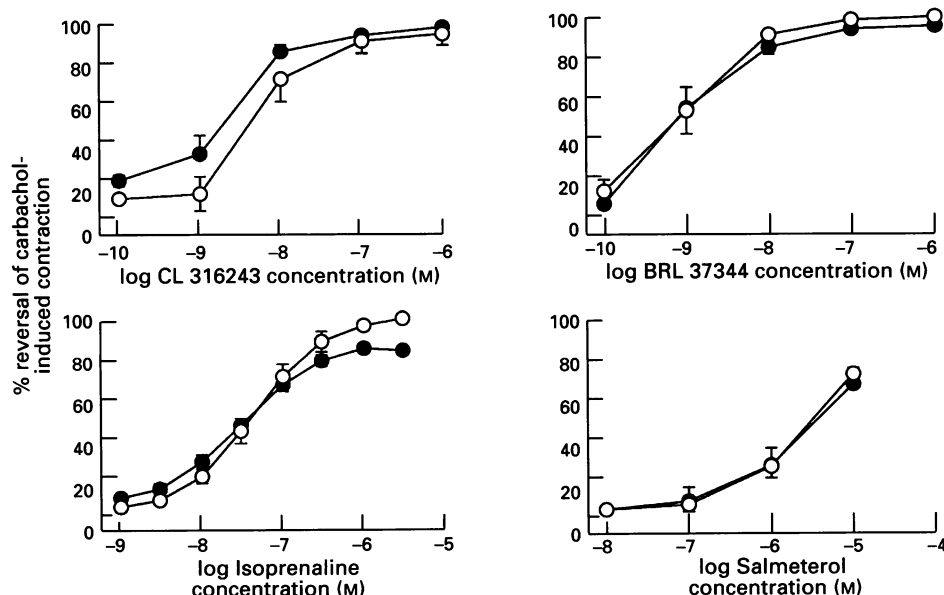
## Results

### Isolated preparations

**Rat oesophagus** Preliminary experiments showed that the definition of responses of the rat oesophagus to low concentrations ( $\leq 0.1 \mu$ M) of isoprenaline was increased by inclusion of IBMX in the bathing solution. A concentration of 3  $\mu$ M IBMX prolonged the plateau phase of the relaxations evoked by isoprenaline, and elevated the maximal response (from  $72 \pm 3$  to  $87 \pm 3\%$  reversal of tone ( $n \geq 7$ )) although it did not enhance sensitivity ( $EC_{50}$  values were 146 (71.6–296) nM and 43.2 (20.2–92.4) nM respectively in the absence and presence of IBMX). Treatment with 10  $\mu$ M IBMX led to a 20–30% depression of the contractile response to carbachol. Consequently, 3  $\mu$ M IBMX was used routinely in all subsequent experiments. Under these conditions, the contractile responses to carbachol (1  $\mu$ M) were similar on first ( $1.25 \pm 0.04$  g) and second ( $1.46 \pm 0.04$  g) challenge ( $n = 30$ ).

CL 316243, BRL 37344 and isoprenaline produced concentration-dependent relaxations in precontracted oesophageal preparations. The inhibitory responses to all three agonists were resistant to blockade by a combination of atenolol (10  $\mu$ M) and ICI 118551 (1  $\mu$ M) (Figure 1). The relative potencies of the agonists obtained in the presence of the  $\beta_1$ - and  $\beta_2$ -adrenoceptor antagonists are shown in Table 1. CL 316243 and BRL 37344 were approximately 27 and 41 fold more potent than isoprenaline. Salmeterol was included for comparison and was 78 fold less potent than isoprenaline. All agonists evoked a similar maximum response (i.e. 80–90% reversal of tone).

The relaxant effects of isoprenaline, BRL 37344, and CL 316243 on oesophagus were antagonized to a similar extent by cyanopindolol (0.1–1.0  $\mu$ M), giving  $pA_2/pK_B$  values of approximately 8.0 (Table 2). High concentrations of propranolol (3–30  $\mu$ M) also caused concentration-related inhibition of the relaxations evoked by these  $\beta$ -adrenoceptor stimulants ( $pA_2/pK_B$  values approximately 6.5; Table 2). However, the corresponding values when salmeterol was the relaxant were  $<6.5$  for cyanopindolol and  $<5.5$  for propranolol, which suggests that this response was not mediated via  $\beta$ -adreno-



**Figure 1** Concentration-effect curves for the relaxant response of rat precontracted oesophagus to  $\beta$ -adrenoceptor stimulants. Curves were obtained in the absence (●) or presence (○) of a combination of atenolol (10  $\mu$ M) and ICI 118551 (1  $\mu$ M). Each point is the mean ( $\pm$  s.e. mean) of  $n \geq 5$  observations.

**Table 1** Relative potencies of  $\beta$ -adrenoceptor stimulants in rat isolated oesophagus

Agonist	EC <sub>50</sub> value (nm)	n	Potency ratio
Isoprenaline	38 (24–60)	10	1
BRL37344	0.92 (0.25–3.35)	5	0.024
CL316243	1.41 (0.72–2.77)	6	0.037
Salmeterol	2982 (1662–5350)	8	78

The EC<sub>50</sub> values are geometric means with 95% confidence limits of *n* observations. The potency ratio is derived from the mean EC<sub>50</sub> value for each compound.

**Table 2** Antagonist effects of cyanopindolol and propranolol in rat precontracted oesophagus

Agonist	pA <sub>2</sub> (slope)/pK <sub>B</sub> value for Cyanopindolol	pA <sub>2</sub> (slope)/pK <sub>B</sub> value for Propranolol
Isoprenaline	8.19 ± 0.27 (0.84 ± 0.13)	6.50 ± 0.34 (0.88 ± 0.19)
BRL 37344	7.92 ± 0.16	NT
CL 316243	8.20 ± 0.39 (1.06 ± 0.24)	6.38
Salmeterol	< 6.5	< 5.5

Results are presented as mean ± s.e.mean of either pA<sub>2</sub> values (slopes shown in parentheses) or apparent pK<sub>B</sub> estimates (obtained with a single concentration of antagonist); *n* ≥ 4 except for propranolol against CL 316243, where *n* = 3. (NT denotes not tested).

ceptors. Likewise, 5-HT-mediated relaxations of the oesophagus were unaffected by cyanopindolol (1  $\mu$ M) or propranolol (30  $\mu$ M) (data not shown).

**Guinea-pig atrium and trachea** The agonist profiles of CL 316243, BRL 37344 and salmeterol relative to isoprenaline on guinea-pig atrium and trachea are shown in Table 3. The three agonists did not evoke tachycardia at concentrations up to 10  $\mu$ M. On trachea, salmeterol was 3 fold less potent than isoprenaline in evoking relaxation, whilst the corresponding potency ratios for BRL 37344 and CL 316243 were 21 and 380.

#### Animal studies

**Effects of  $\beta$ -adrenoceptor agonists on indomethacin-induced ulceration** Previous studies (Trevethick *et al.*, 1993) have shown that indomethacin (60 mg kg<sup>-1</sup> s.c.) evoked a reproducible

degree of gastric ulceration in the refed rat which is confined to the antrum. In the current study, the area of macroscopic damage induced by this dose of indomethacin was between 20–35% of the total antral area (mean value 23.8 ± 1.4%, *n* = 59). CL 316243, BRL 37344 and salmeterol caused dose-related inhibition (up to 90–100%) of indomethacin-induced antral damage (Figure 2). The ED<sub>50</sub> values (in  $\mu$ mol kg<sup>-1</sup> with 95% confidence limits) were: BRL 37344 0.09 (0.03–0.21), CL 316243 0.24 (0.12–0.40), salmeterol 7.9 (5.8–11.1). These values gave a relative order of potency of 1:2.7:87.8. In contrast, the  $\beta_1$ -adrenoceptor agonist, denopamine (up to 31  $\mu$ mol kg<sup>-1</sup> (10 mg kg<sup>-1</sup>), p.o.) did not afford protection against indomethacin-induced damage.

Histological analysis confirmed the protective effects of the  $\beta_3$ -adrenoceptor agonists and salmeterol (Figure 3). The development of frank ulceration was inhibited to a similar extent (i.e. by 80–90%) by CL 316243, BRL 37344 (0.5 and 0.3 mg kg<sup>-1</sup>, p.o. respectively) and by salmeterol (10 mg kg<sup>-1</sup>, p.o.).

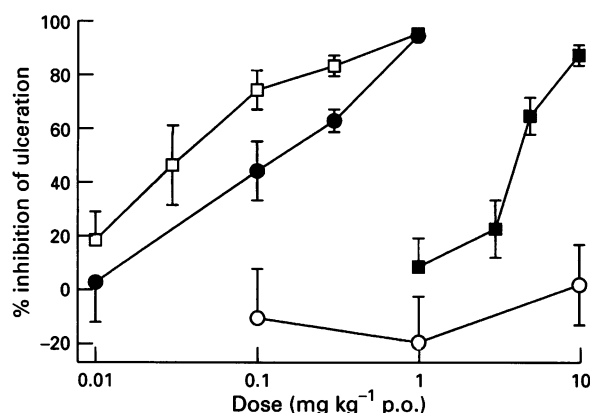
**Effects of antagonists on the gastroprotective effects of  $\beta$ -adrenoceptor stimulants** Administration of propranolol (0.1–10 mg kg<sup>-1</sup>, p.o.) or ICI 118551 (1.0–10 mg kg<sup>-1</sup>, p.o.) did not affect the level of ulceration in their own right, but did cause dose-related inhibition of the gastroprotective effect of salmeterol (10 mg kg<sup>-1</sup>, p.o.) (Figure 4). In contrast, the highest dose of propranolol or ICI 118551 (both of which caused 90–100% inhibition of the salmeterol response) did not alter the degree of protection afforded by either BRL 37344 (0.1 mg kg<sup>-1</sup>, p.o.) or CL 316243 (1 mg kg<sup>-1</sup>, p.o.) (Figure 4).

The effect of cyanopindolol on the gastroprotective response was not evaluated in these studies because preliminary experiments showed that it markedly exacerbated the degree of antral damage produced by indomethacin.

**Table 3** Relative potencies of  $\beta$ -adrenoceptor stimulants in guinea-pig right atrium and trachea

Preparation	EC <sub>50</sub> for isoprenaline (nm)	EMCR BRL 37344	EMCR CL316243	EMCR salmeterol
Atrium	3.6 (1.6–8.4)	> 1315	≥ 5163	≥ 2834
Trachea	46.4 (19–116)	21 (17–25)	380 (34–4180)	3

Equipotent molar concentration ratios (EMCR), together with the EC<sub>50</sub> value for isoprenaline are presented as geometric mean values (with 95% confidence limits where possible; *n* ≥ 3 for all values except salmeterol on trachea, where (*n* = 2).



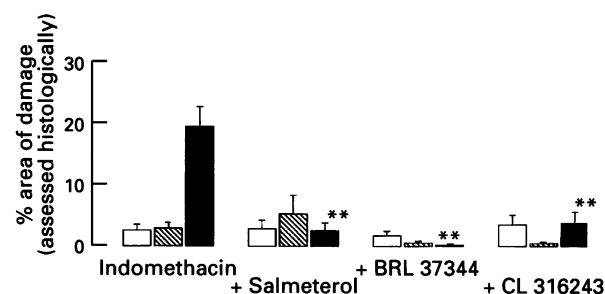
**Figure 2** Dose-response curves for the protective effect of  $\beta$ -adrenoceptor stimulants against indomethacin-induced gastric antral ulceration in the rat: ( $\square$ ) BRL 37344 ( $n=6$ ); ( $\bullet$ ) CL 316243 ( $n=6-12$ ); ( $\blacksquare$ ) salmeterol ( $n=13$ ); ( $\circ$ ) denopamine ( $n=11$ ). All values are arithmetic mean ( $\pm$  s.e.mean) of  $n$  observations.

## Discussion

The results of this study show that  $\beta$ -adrenoceptor agonists inhibit indomethacin-induced gastric antral ulceration in rats. The use of subtype-selective agonists suggests that both  $\beta_2$ - and  $\beta_3$ -adrenoceptors are involved in this gastroprotective response. In contrast, we have no evidence to suggest an involvement of  $\beta_1$ -adrenoceptors, as denopamine, a selective  $\beta_1$ -adrenoceptor agonist, did not inhibit ulceration at doses up to  $10 \text{ mg kg}^{-1}$ , p.o. which is a 13 fold higher dose than that required to produce a 50% increase in cardiac contractility in conscious rats (Yabana *et al.*, 1986).

The finding that the  $\beta_2$ -adrenoceptor selective agonist salmeterol, is gastroprotective is not surprising, as similar results have been obtained with salbutamol (Fielding *et al.*, 1975; Esplugues *et al.*, 1982). Isoprenaline has also been shown to afford protection against ethanol-induced gastric damage via stimulation of  $\beta_2$ -adrenoceptors (Howard *et al.*, 1993). The mechanisms by which this protective effect may be mediated are discussed in detail elsewhere (Esplugues *et al.*, 1982), and will not be reviewed here, as a  $\beta_2$ -adrenoceptor agonist was included in the current study for comparative purposes only.

The key result from the present series of experiments was the demonstration that CL 316243 and BRL 37344 inhibited indomethacin-induced gastric ulceration in the rat. The results from the pharmacological characterization carried out on isolated tissues show that both are potent  $\beta_3$ -adrenoceptor agonists, as judged by their ability to relax rat oesophagus muscularis mucosae, a preparation in which this response is mediated predominantly, if not exclusively, via  $\beta_3$ -adrenoceptors (De Boer *et al.*, 1993). Furthermore, the relaxation was resistant to blockade by a combination of atenolol and ICI 118551 at concentrations at least 100 fold higher than those required to block  $\beta_1$ - and  $\beta_2$ -adrenoceptors respectively. In contrast, the relaxant responses to isoprenaline and CL 316243 were blocked in a competitive manner by cyanopindolol and were also antagonized by high concentrations of propranolol. Overall, this pharmacological profile is consistent with that of the  $\beta_3$ -adrenoceptor. However, our  $\text{pA}_2$  values for cyanopindolol and propranolol are both higher (by 0.5–1.0 log unit) than those obtained on oesophagus by Ford *et al.* (1992). The reason for this is unknown, but the values that we quote are consistent with those which we obtain on other gastrointestinal preparations containing  $\beta_3$ -adrenoceptors (unpublished observations). Overall, in keeping with previous literature reports, (Arch *et al.*, 1984; Bloom *et al.*, 1992) both CL 316243 and BRL 37344 are potent and (as judged by their low degree of agonist activity on guinea-pig trachea and atria) selective,  $\beta_3$ -adrenoceptor stimulants. Salmeterol also relaxed



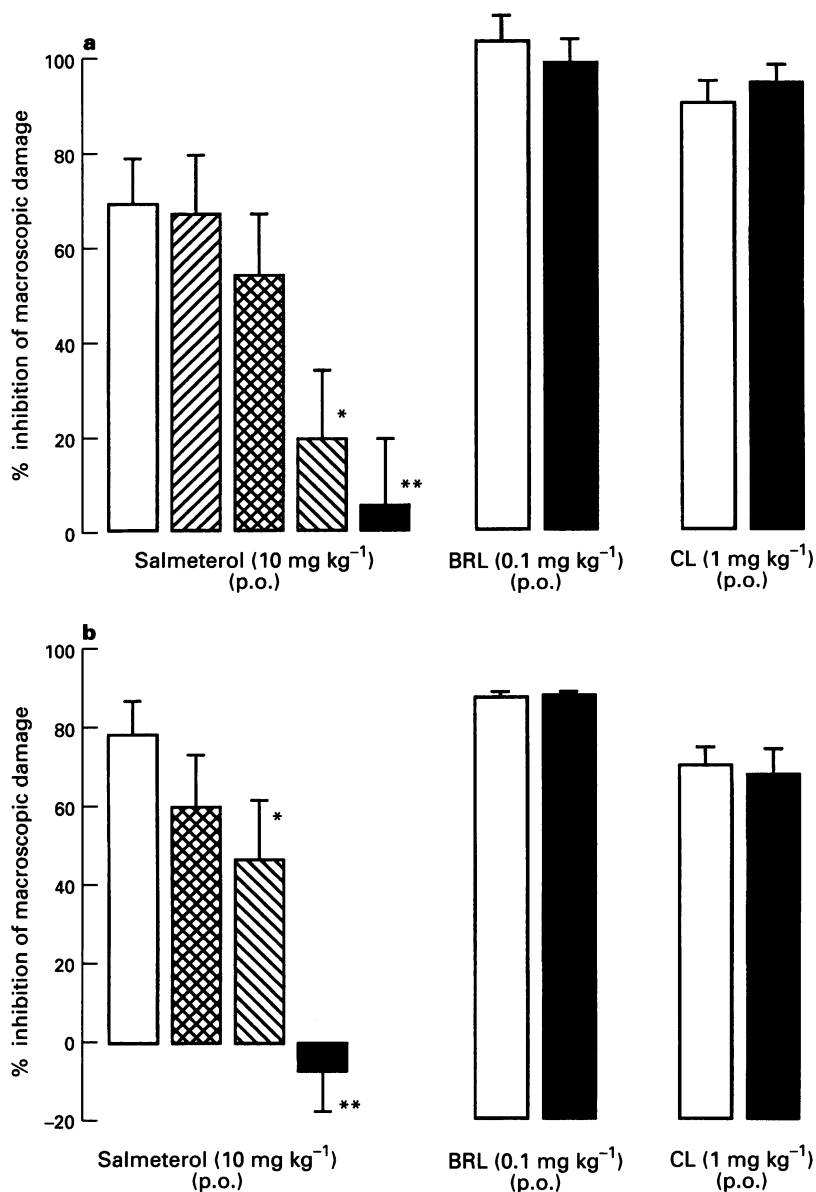
**Figure 3** Histological assessment of the protective effects of  $\beta$ -adrenoceptor stimulants against indomethacin-induced gastric antral damage in the rat. The effects of indomethacin ( $60 \text{ mg kg}^{-1}$ , s.c.) alone or in combination with salmeterol ( $10 \text{ mg kg}^{-1}$ , p.o.), BRL 37344 ( $0.3 \text{ mg kg}^{-1}$ , p.o.) or CL 316243 ( $0.5 \text{ mg kg}^{-1}$ , p.o.) on surface erosions (open columns), deep erosions (hatched columns) and frank ulceration (solid columns) are shown. \*\*Significant difference ( $P < 0.01$ , Mann-Whitney U test) between the group treated with  $\beta$ -adrenoceptor agonist and the corresponding group receiving indomethacin alone ( $n \geq 10$  in all cases).

rat oesophagus, but this response was not blocked by any of the antagonists used in this study, which suggests that it was not mediated via  $\beta$ -adrenoceptors. In view of the very high concentrations of salmeterol used, it is likely that this form of response is similar to that described previously by Barker *et al.* (1992) on guinea-pig gastric fundus.

Both CL 316243 and BRL 37344 caused dose-dependent inhibition of indomethacin-induced antral ulceration in the conscious rat. Both agonists were approximately 100 fold more potent than salmeterol as gastroprotective agents, but unlike salmeterol, their effects were not blocked by co-administration of propranolol or of ICI 118551 (up to  $10 \text{ mg kg}^{-1}$ , p.o.). The inability of propranolol to block the gastroprotective effects of the  $\beta_3$ -adrenoceptor agonists contrasts with its weak antagonist action on rat oesophagus. The most likely explanation of this discrepancy is that the plasma concentrations of propranolol attained were not sufficiently high to block  $\beta_3$ -adrenoceptors *in vivo*. Higher doses of propranolol were not tested because initial studies showed that they caused cardiovascular depression. Likewise, cyanopindolol could not be used as an antagonist *in vivo*, because it exacerbated gastric damage in its own right. Definitive proof that the gastroprotective effects of CL 316243 and BRL 37344 are mediated via  $\beta_3$ -adrenoceptors therefore awaits the development of a potent, selective,  $\beta_3$ -adrenoceptor antagonist.

The mechanism by which  $\beta_3$ -adrenoceptor agonists protect against NSAID-induced ulceration has not been investigated in the present study. However, whilst the current study was in progress, Kuratani *et al.* (1994) showed that a range of  $\beta_3$ -adrenoceptor agonists can enhance gastric antral mucosal blood flow in the halothane-anaesthetized rat. They speculated that this vasodilator action accounts for the gastroprotective response in the conscious rat. However, further experimentation is required to validate this hypothesis, as other studies have shown that NSAIDs in their own right can increase mucosal blood flow at the ulcer site (McGeavy & Moody, 1981; Lau *et al.*, 1992). Furthermore, a review of the literature by Jacobsen (1992) revealed that there is poor correlation between the abilities of a range of compounds to increase mucosal blood flow and afford gastroprotection.

A possible alternative, or additional site of action by which  $\beta_3$ -adrenoceptor agonists may act as gastroprotective agents is by virtue of their profound inhibitory effects on gastrointestinal motility. Takeuchi *et al.* (1986) demonstrated that indomethacin increased gastric motility in the conscious rat and the time course for the changes in motility was paralleled by that for lesion formation. They also noted that the doses of indomethacin required to affect motility and induce lesions were higher than that required to inhibit prostaglandin for-



**Figure 4** Effect of propranolol or ICI 118551 on the gastroprotective effect of  $\beta$ -adrenoceptor stimulants. The agonists (salmeterol, BRL 37344 (BRL) or CL 316243 (CL)) were administered either alone (open columns) or 30 min after antagonist. The upper figure (a) shows the effects of propranolol 0.1 (hatched column) 1.0 (cross hatched columns) 3.0 (reverse hatched column) and 10 (solid column)  $\text{mg kg}^{-1}$  (p.o.). The lower figure (b) shows the corresponding experiment using ICI 118551 1.0 (cross hatched columns) 3.0 (reverse hatched columns) and 10 (solid columns)  $\text{mg kg}^{-1}$  (p.o.). Significant differences (\* $P < 0.05$  and \*\* $P < 0.01$ ; Mann-Whitney U test) between the group treated with agonist alone and that receiving agonist plus antagonist ( $n = 5-10$  rats for each group).

mation in the gastric antrum, which suggests that it is not the absence of protective prostalands *per se* which is responsible for the ulcerogenic stimulus. Mersereau & Hinchey (1988) demonstrated that indomethacin markedly enhanced basal myoelectrical activity of rat gastric smooth muscle. They proposed that this altered smooth muscle state, in combination with the inhibition of prostaglandin synthesis, renders the mucosa vulnerable to injury by increased peristaltic action.

The  $\beta_3$ -adrenoceptor agonists have relaxant effects in a range of isolated preparations taken from the GI tract of various species (reviewed by Arch & Kaumann, 1993). Furthermore, studies using  $\beta_3$ -adrenoceptor agonists developed by Sanofi have shown that these compounds inhibit colonic myoelectric activity in both the anaesthetized (Giudice *et al.*, 1989) and conscious (Crocì *et al.*, 1991) rat. Thus it is likely that this inhibitory effect contributes in some way to the gastro-

protective effect of  $\beta_3$ -adrenoceptor agonists. However, exactly how this occurs is at present unclear, although it is of interest that Livingston *et al.* (1993) have shown that an increase in peristaltic activity (by field stimulation) of rat stomach leads to a reduction in blood flow which is particularly profound in the antral area. Thus it is possible that the  $\beta_3$ -adrenoceptor agonists confer protection in two ways: (i) by directly inhibiting the elevated level of contractile activity of gastric smooth muscle induced by indomethacin; (ii) by reversing the reduction in blood flow caused by this hypermotile state.

In conclusion, we have demonstrated that both  $\beta_2$ - and  $\beta_3$ -adrenoceptor agonists are capable of preventing gastric damage induced by indomethacin in the rat. Assessment of the therapeutic potential of this class of compounds as gastro-protective agents in man awaits the development of an agonist that is potent at the human  $\beta_3$ -adrenoceptor.

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