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Evaluation of ICI D7114, a putative stimulant of brown adipocytes, on histamine-contracted guinea-pig ileum

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1 Experiments were performed to characterize the effects of the novel brown adipocyte stimulant, ICI D7114, in the guinea-pig isolated ileum, right atrium and tracheal chain. In the ileum, agonist-induced inhibition of the contractile response to either histamine or prostaglandin E_2 (PGE_2) was assessed, along with effects on resting rate in the atrium and resting tone in the tracheal chain. In the latter two preparations, antagonism of isoprenaline-induced responses by ICI D7114 was also assessed.

2 Inhibitory responses were obtained in the ileum to ICI D7114, isoprenaline, BRL37344, and noradrenaline. The responses to ICI D7114, isoprenaline and BRL37344 were resistant to blockade with propranolol (5 μM), naloxone (1 μM), methysergide (0.1 μM), cimetidine, indomethacin and 8-phenyltheophylline (all 10 μM). These responses to isoprenaline, in the presence of propranolol (5 μM), were competitively antagonized by alprenolol (1–100 μM) with a pA_2 value of 6.44. The responses to ICI D7114 and BRL37344 were antagonised by single concentrations of alprenolol (1 μM) with apparent pK_B values of 6.53 and 6.57 respectively. These data indicate an effect of ICI D7114 at the atypical β-adrenoceptor in the guinea-pig ileum.

3 The order and relative potency of agonists at the atypical β-adrenoceptor was BRL37344 (4) > isoprenaline (1) = ICI D7114 (1.1) > noradrenaline (0.5).

4 ICI D7114 (1 nM–10 μM) caused no significant change in the rate of beating or the resting tone of the guinea-pig right atrium or tracheal chain respectively. It did, however, cause selective blockade of the responses to isoprenaline in these tissues (apparent pK_B values 7.63 and 5.85 in atrium and tracheal chain respectively). Responses to histamine (atrium) and aminophylline (tracheal chain) were not significantly affected by 10 μM ICI D7114.

5 These results demonstrate that ICI D7114 possesses selective agonist activity at atypical β-adrenoceptors in the guinea-pig ileum and its use as a tool may help to establish a role for the atypical β-adrenoceptor in the control of gastrointestinal motility.

Keywords: Guinea-pig ileum pre-contracted; inhibitory action of ICI D7114; atypical β-adrenoceptors; antagonist action of alprenolol

Introduction

It was suggested by Arch *et al.* (1984) that stimulation of lipolysis following activation of brown adipocytes was the result of an interaction of noradrenaline with an atypical β-adrenoceptor. The authors demonstrated that a series of compounds possessed high potency at this atypical β-adrenoceptor. In particular, BRL37344, (sodium-4-[2-[2-hydroxy-2-(3-chlorophenyl) ethylaminopropyl] phenoxyacetate sesquihydrate) was shown to stimulate lipolysis at much lower concentrations than those required to affect atrial rate or tracheal tone. Since these observations were made, there has been speculation as to the possible existence of a similar atypical β-adrenoceptor that might be involved in the control of gut motility. Bond & Clarke (1987, 1988) identified a propranolol-resistant component to the isoprenaline-induced inhibitory response in electrically stimulated or histamine pre-contracted guinea-pig ileum and further demonstrated that BRL37344 possessed similar potent inhibitory effects. In a more recent study (Blue *et al.*, 1990), the potent non-selective β-adrenoceptor antagonists (−)-alprenolol and (−)-dihydroalprenolol, were shown to produce competitive antagonism of the responses to isoprenaline and BRL37344. However, the affinity constants for these agents were significantly lower than would have been predicted for an interaction at either β_1- or β_2-adrenoceptors, hence it was suggested that an atypical β-adrenoceptor might be responsible for the effects observed. Similar observations have been made in a variety of tissues where responses to catecholamines appear to be resistant to α- or β-adrenoceptor blockade. These tissues include rat stomach fundus (Kelly & MacDonald, 1990), distal colon (McLaughlin & MacDonald, 1990), small intestine (van der Vliet *et al.*, 1990) and rabbit jejunum (Norman & Leathard, 1990). Moreover, Bianchetti & Manara (1990) have recently developed a series of phenylethanolaminotetralines that show a high degree of selectivity for the atypical β-adrenoceptor in rat colon.

It also now appears that atypical β-adrenoceptors may be associated with neuronally-released transmitter. Taneja & Clarke (1991) have recently demonstrated that in electrically stimulated guinea-pig ileum, the neuronally released natural transmitter of the sympathetic nerves, noradrenaline, may, in addition to its actions on classical adrenoceptors, also act at atypical β-adrenoceptors. There is a possibility, therefore, that atypical β-adrenoceptors participate in the physiological mechanisms associated with the control of gut motility.

Hence, there appears to be considerable evidence to support the existence of the atypical β-adrenoceptor in gastrointestinal tissue and that this site appears to bear some resemblance to that already identified in brown adipocytes. ICI D7114 ((S)-4-[2-hydroxy-3-phenoxy-propylamino-ethoxy]-*N*-(2-methoxyethyl) phenoxyacetamide) is a recently developed β-adrenoceptor agonist that stimulates brown adipocytes via an atypical β-adrenoceptor (Holloway *et al.*, 1991a,b). It was of interest, therefore, to evaluate the action of this agent in an isolated tissue system where atypical β-adrenoceptors have been postulated and compare this to any effect at β_1- or β_2-adrenoceptors. The current study describes the pharmacological action of ICI D7114 in the histamine pre-contracted guinea-pig ileum, right atrium and tracheal chain prepara-

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tions. A preliminary account of some of this work was presented to the British Pharmacological Society in Glasgow, July, 1991.

Methods

Tissue preparation

Guinea-pig ileum Guinea-pigs of either sex (Dunkin-Hartley strain, weighing 300–550 g) were killed by cervical dislocation and the distal ileum was removed. After washing out the luminal contents, segments 2–3 cm in length were set up in 10 ml organ baths under an initial resting tension of 500 mg in Krebs solution maintained at 37°C and gassed with 95% O_2, 5% CO_2. The composition (mM) of the Krebs solution was as follows: NaCl 118.9, $NaHCO_3$ 25.0, KCl 4.9, KH_2PO_4 111.2, $MgSO_4$ 1.2, glucose 11.1, $CaCl_2$ 2.5 and ascorbic acid 0.11. Atropine (0.1 μM), phentolamine (3 μM), cocaine (30 μM) and corticosterone (30 μM) were present throughout the experiments to inhibit muscarinic receptors, α-adrenoceptors, neuronal and extraneuronal uptake respectively. The concentrations of phentolamine and atropine were at least 100 times their equilibrium dissociation constants (Bond & Clarke, 1988). In experiments where the effects of compounds at the atypical β-adrenoceptor were assessed, propranolol (5 μM) was present. This concentration of propranolol was used as it had been previously demonstrated (a) to have no effect on smooth muscle tone (Bond & Clarke, 1988) and (b) to 'saturate' β_1- and β_2-adrenoceptors (Bond & Clarke, 1988). Hence, this concentration of propranolol should effectively block any activity of the agonists at classical β-adrenoceptors. In some experiments, the selective β_1- and β_2-adrenoceptor antagonists, CGP 20712A ((±)-1-[2 (3-carbomyl-4-hydroxyphenoxy)-ethylamino]-3-[4-(1-methyl-4-triflouromethyl-2-imidazolyl)-phenoxy]-2-propanol) and ICI 118551 (erythro- (±)-1-(7-methylindan-4-yloxy)-3-isopropylaminobutan-2-ol) were used (both 0.1 μM). The antagonism of isoprenaline with these two agents was compared to that obtained with propranolol (5 μM). Tissues were allowed to equilibrate for 30 min before starting experiments. Isotonic recording of tissue length changes were made (Mellor, 1984) and displayed on a Kipp and Zonen BD9 recorder.

Experiments using electrically stimulated guinea-pig ileum were also performed in order to determine the specificity of alprenolol. Ileum was obtained as outlined above and segments 2–3 cm in length were set up between stainless steel ring electrodes in 10 ml organ baths containing Mg^{2+}-free Krebs solution at 37°C and gassed with 95% O_2, 5% CO_2. Tissues were left to equilibrate under 500 mg tension for at least 30 min prior to field-stimulation (supramaximal square wave pulses, 1 ms in duration, delivered at a frequency of 0.1 Hz from a Grass S44 stimulator) to evoke acetylcholine release from enteric neurones. Isotonic recording of tissue length changes were measured as outlined above.

Guinea-pig right atrium Hearts were rapidly removed from the guinea-pigs (sex, strain, weight range as described above) and placed in oxygenated Krebs solution. The right atrium was quickly located, dissected free from the heart and mounted on to a pair of platinum electrodes. Once fixed to the electrodes, the tissue was immersed into a 20 ml organ bath containing Krebs solution (composition as outlined above) at 37°C and gassed with 95% O_2, 5% CO_2. Electrodes were connected to an ICI modular cardiotachometer which counted the number of spontaneous beats in 10 s epochs. A computer (IBM PS200 or equivalent) averaged the epochs in groups of 3 and displayed these on the computer screen at 1 min intervals. Tissues were allowed to equilibrate for 60 min with frequent washings with fresh Krebs solution. During this time, the spontaneous rate fell and became stable.

Guinea-pig tracheal chain Tracheae were removed from guinea-pigs (sex, strain, weight range as described above) and placed into oxygenated Krebs solution. The tissues were then cut into rings. Each preparation consisted of five rings sewn together. These were placed in 10 ml organ baths containing Krebs solution (composition as outlined above) at 37°C and gassed with 95% O_2, 5% CO_2. The tissues were left to equilibrate under 200 mg tension for 60 min during which time frequent rinses with fresh Krebs solution were made. Isotonic recording of tissue length changes were made as outlined above.

Experimental protocols

Guinea-pig ileum Inhibitory actions of the β-adrenoceptor agonists were determined by measuring the reduction in the contractile response to a submaximal concentration of histamine (0.5 μM, see Bond & Clarke, 1988). Thus, responses to histamine were obtained at 5 min intervals with 5 washouts between each application. When a suitable reproducible histamine response was obtained, this was set as 100% and all subsequent responses were compared as a percentage change. Increasing concentrations of β-adrenoceptor agonist were added in a sequential manner 3 min prior to the addition of histamine and retained in the organ bath during the response to histamine. In experiments where antagonist drugs were used, these were added to the reservoir of Krebs and allowed 30 min to equilibrate with the tissue prior to addition of histamine. It was found that when alprenolol was employed as an antagonist, responses to histamine were significantly inhibited by the presence of this agent. In this case, a submaximal concentration of PGE_2 (50 ng ml^{-1}, approx. 0.1 nM) was used instead of histamine.

Concentration-response curves were plotted for the β-adrenoceptor agonists and potency was expressed as an IC_{30} value. This represents the concentration of agonist causing 30% inhibition of the response to histamine. Values are expressed as the mean together with the respective 95% confidence limits. IC_{30} were used to allow direct comparison between this study and those previously performed by other workers using guinea-pig ileum (i.e. Bond & Clarke, 1988).

Some experiments using guinea-pig ileum were performed with electrically-stimulated preparations. These tissues were stimulated to cause release of acetylcholine from enteric neurones, resulting in a 'twitch' response. Following 30 min equilibration, during which time twitch responses became stable, morphine (1 nM–10 μM) was added cumulatively and the inhibition of the twitch response measured. Following a washout period of 30 min stimulation was continued and alprenolol (10 μM) was added to the bath for 10 min after which time, the inhibition-response to morphine was repeated.

Concentration-inhibition curves were plotted for the effects of morphine in the absence and presence of alprenolol. From these curves, IC_{50} values were derived, i.e. the concentration of morphine causing 50% inhibition, and expressed as mean values together with their respective 95% confidence limits.

Guinea-pig right atrium Spontaneous rate of beating of the right atrium was increased by adding either isoprenaline or histamine in a cumulative fashion until no further change in rate was observed. The tissues were then washed several times and left for 30 min for rates to return to normal. ICI D7114 (10 nM–10 μM) was then added to the bathing Krebs solution and left in contact with the tissues for 60 min after which time the concentration-responses to either isoprenaline or histamine were repeated. From the concentration-response curves, EC_{50} values, i.e. the concentration of agonist producing an increase in rate that was 50% of the maximum response, were derived.

Guinea-pig tracheal chain Spontaneous tone of the tracheal chain was reduced by cumulative addition of either iso-

prenaline or aminophylline until no further reduction could be obtained. The tissues were then washed several times until tone had returned; 60 min later, a second concentration response to either isoprenaline or aminophylline was repeated and the tissues were once again washed. When tone had returned, ICI D7114 (1 nM–10 μM) was added to the Krebs bathing medium and left to equilibrate for 60 min after which time, concentration-responses to isoprenaline or aminophylline were repeated. From the concentration-response curves, EC_{50} values were derived.

pA_2 and pK_B values

Agonist concentration-ratios obtained from individual experiments were plotted by the method of Arunlakshana & Schild (1959). Linear regression analysis was used to calculate the pA_2 value and slope of the line. Antagonism was considered to be competitive if the slope of the regression line did not differ significantly from unity. In cases where a single concentration of antagonist was used, 'apparent' pK_B values were derived by the concentration-ratio method of Furchgott (1972), where:

$$K_B = [\text{antagonist}]/\text{agonist concn. ratio} - 1$$

and following on from this:

$$-\log_{10} K_B = pK_B$$

Statistics

Significance levels, where quoted, were determined by Student's paired *t* test after checking the homogeneity of the variances. *P* values of less than 0.05 were considered to indicate a significant difference between the responses being compared.

Drugs

The following drugs were used: histamine dihydrochloride, (−)-isoprenaline hydrochloride, (−)-noradrenaline bitartarate, atropine sulphate, 8-phenyltheophylline, corticosterone-21-acetate, prostaglandin E_2 (all purchased from Sigma Chemical Co.). CGP 20712A, phentolamine mesylate, cocaine hydrochloride (purchased from CIBA-Geigy Pharmaceuticals), methysergide bimaleate (purchased from Sandoz Pharmaceuticals), (±)-propranolol, (±)-alprenolol, (Zeneca Pharmaceuticals), ICI D7114 and BRL37344 (synthesized by B. Rao, Research Function, Zeneca Pharmaceuticals, Alderley Park, Macclesfield). All other chemicals were of analytical grade from either BDH Ltd., Poole, Dorset or Fisons Ltd., Loughborough, Leicester. ICI D7114, BRL37344, methysergide, corticosterone, (±)-alprenolol and 8-phenytheophylline were initially dissolved in dimethyl sulphoxide (DMSO) and subsequently diluted in distilled water. All other agents were dissolved in distilled water.

Results

Guinea-pig ileum

One of the characteristics that helps identify the presence of atypical β-adrenoceptors is the low antagonist potency of propranolol against β-adrenoceptor agonists. In the current studies, the effects of propranolol (5 μM) on inhibitory responses to isoprenaline, BRL37344 and ICI D7114 were evaluated. In the case of isoprenaline, the effects of propranolol were compared with those obtained with CGP 20712A and ICI 118551 (both 0.1 μM), alone or in combination. The effects of propranolol are shown in Figure 1 (a–c). The mean concentration-ratios were 5.7 [2.2–9.0], 12.9 [1.1–32.7] and 1.6 [1.0–2.3] (all $n = 4$) against isoprenaline, BRL37344 and ICI D7114 respectively. These values equate

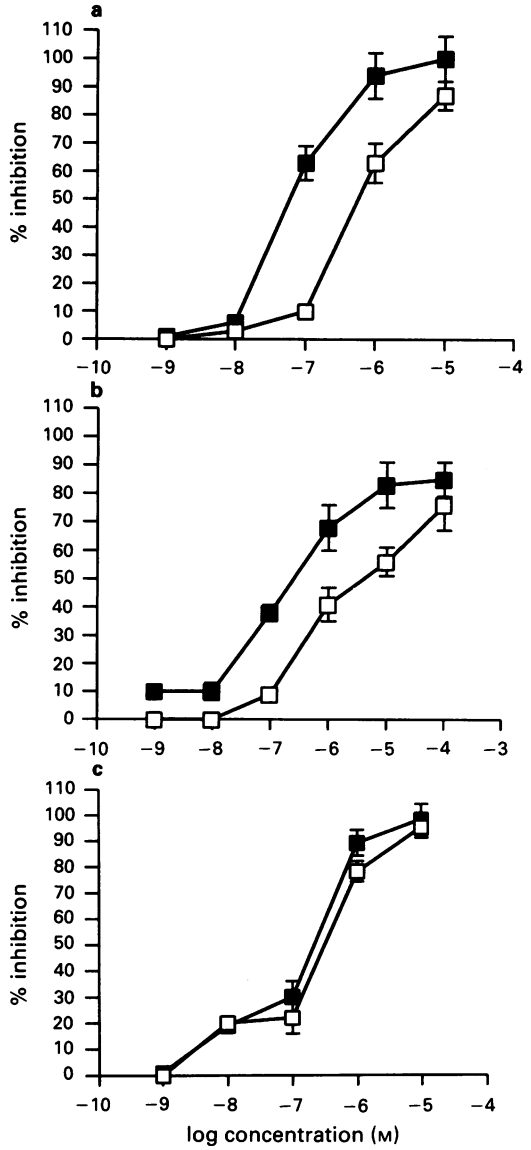

Figure 1 Inhibitory effects of (a) isoprenaline, (b) BRL37344 and (c) ICI D7114 on the contractile responses to histamine (0.5 μM) in guinea-pig isolated ileum: interaction with propranolol (5 μM). (■) Control responses; (□) responses in the presence of propranolol. Each point represents the mean ± s.e.mean percentage (where this exceeds the symbol size) inhibition obtained from tissues from 4 guinea-pigs.

to 'apparent' pK_B values of 5.97 [5.38–6.20], 6.37 [4.30–6.80] and 5.07 [4.3–5.4] respectively. In the presence of CGP 20712A or ICI 118551 alone, small rightward shifts of the isoprenaline concentration-responses were seen (concentration-ratios 2.2 [1.5–3.0] and 4.6 [1.8–11.4] respectively, 'apparent' pK_B values of 7.08 [6.69–7.30] and 7.56 [6.90–8.02], all $n = 4$). When these two antagonists were used in combination, again, small shifts in the concentration-response curves to isoprenaline were observed (concentration-ratio 8.1 [0.7–21.2], 'apparent' pK_B value of 7.85 [6.00–8.3], $n = 4$). The low values obtained with propranolol and either CGP 20712A or ICI 118551 indicate a lack of involvement of either β_1- and β_2-adrenoceptors. In the subsequent studies, propranolol (5 μM) was employed throughout.

Isoprenaline (1 nM–1 mM), BRL37344 (1 nM–1 mM), ICI D7114 (1 nM–1 mM) and noradrenaline (1 nM–1 mM) all produced concentration-related inhibition of the histamine response in the guinea-pig ileum (Figure 2). Isoprenaline and ICI D7114 were approximately equipotent (IC_{30} values 0.20 μM [0.16–0.28 μM] and 0.22 μM [0.04–0.40 μM], $n = 30$

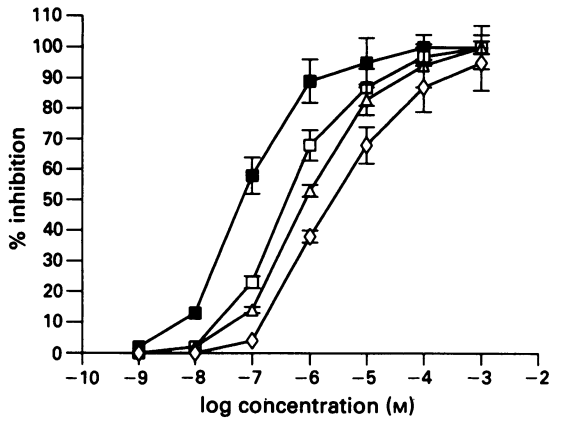

Figure 2 Inhibitory effects of BRL37344 (■, $n = 30$), isoprenaline (□, $n = 30$), ICI D7114 (△, $n = 30$) and noradrenaline (◇, $n = 8$) on the contractile responses to histamine (0.5 μM) in the guinea-pig isolated ileum. Each point represents the mean ± s.e.mean (where this exceeds the symbol size) percentage inhibition and the number of guinea-pigs is given as n.

and 8 respectively). BRL37344 was significantly ($P < 0.01$) more potent than either isoprenaline or ICI D7114 (IC_{30} value 0.052 μM [0.02–0.10 μM], $n = 30$). Noradrenaline (IC_{30} value 0.41 μM [0.16–0.50 μM], $n = 6$) was one-half as potent as isoprenaline. All agonists produced similar maximum responses.

Moreover, the inhibitory responses to isoprenaline, ICI D7114 and BRL37344 were insensitive to the antagonist effects of methysergide (0.1 μM), cimetidine (10 μM), naloxone (1 μM), indomethacin (10 μM) and 8-phenytheophylline (10 μM). However, the inhibitory responses to BRL37344 were significantly ($P < 0.05$) potentiated in the presence of this latter agent (concentration-ratio 0.2 [0.03–0.6]).

The non-selective β-adrenoceptor antagonist (±)-alprenolol, at concentrations above 1 μM, caused a significant inhibition of the histamine-induced contraction of the guinea-pig ileum (data not shown). When PGE_2 was used to contract the tissues, this inhibitory effect was not apparent. Under these latter conditions, alprenolol produced competitive antagonism of the inhibitory responses to isoprenaline (Figure 3a). The Schild plot (Figure 3b) of these data gave a pA_2 value of 6.44 [6.25–6.64, $n = 12$] with a slope (1.10 [0.73–1.45]) not significantly different from unity. Single concentrations of alprenolol (1 μM) also caused antagonism of the inhibitory responses to both BRL37344 (Figure 4a) and ICI D7114 (Figure 4b) in histamine contracted tissues ('apparent' pK_B values 6.57 [6.01–7.42, $n = 4$] and 6.53 [6.26–6.72, $n = 4$] respectively). These values were not significantly different ($P > 0.05$) from those obtained with alprenolol versus isoprenaline in tissues where PGE_2 instead of histamine was used as the contractile agent.

To check on the specificity of alprenolol as a β-adrenoceptor antagonist, its effects were also assessed in the field stimulated guinea-pig ileum against morphine-induced inhibition of twitch. Thus, 10 μM alprenolol caused some inhibition of the twitch response in its own right (40 ± 10%), but was without significant ($P > 0.05$) antagonist effect on the morphine-induced inhibitory effect (Figure 5). In fact, the concentration-response curve to morphine was shifted to the left, although this was not statistically significant ($P > 0.05$, $n = 4$).

Guinea-pig right atrium and tracheal chain

In the guinea-pig right atrium, ICI D7114 (1 nM–10 μM) produced no significant changes in the resting rates of the tissues (0, 13 and 11% increase at 0.001, 0.1 and 10 μM, $n = 4$ in all cases). At 1 nM, no significant antagonist activity was observed, whereas at 0.1 μM and 1 μM, ICI D7114 produced

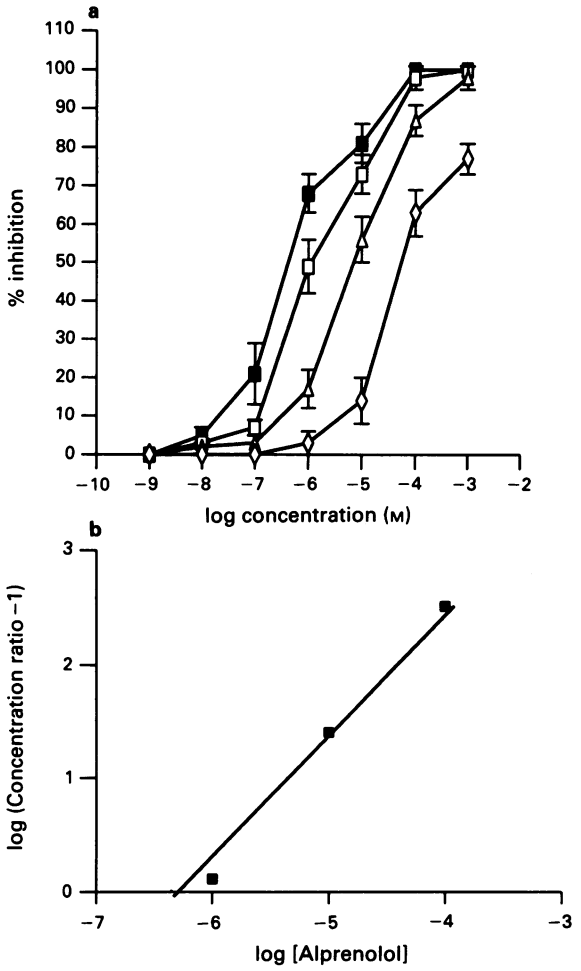

Figure 3 (a) Inhibitory effects of isoprenaline on the contractile response to prostaglandin E_2 (50 ng ml^{-1}) in the guinea-pig isolated ileum: interaction with alprenolol 1 μM–100 μM. (■) Control responses ($n = 12$); responses in the presence of 1 μM (□, $n = 4$), 10 μM (△, $n = 4$) and 100 μM (◇, $n = 4$) alprenolol. Each point represents the mean ± s.e.mean (where this exceeds the symbol size) percentage inhibition and the number of guinea-pigs is given as n. (b) Arunlakshana & Schild (1959) plot for alprenolol versus isoprenaline. Concentration-ratios were measured from the IC_{30} values obtained from the curve shown in (a).

some degree of β_1-adrenoceptor antagonism (concentration-ratios 5.8 [4.0–7.3] and 397.3 [132.0–875.0] respectively). These values equate to 'apparent' pK_B values of 7.68 [7.48–7.79] and 7.59 [7.12–7.94] respectively. Some depression in the maximum response obtained to isprenaline was also seen at these higher concentrations of ICI D7114 (Figure 6a). The effect of ICI D7114 in the guinea-pig right atrium appeared to be specific for β_1-adrenoceptors as high concentrations of the compound (10 μM) failed to antagonize significantly the effects of histamine in this preparation (concentration-ratio 2.28 [1.52–3.75], $n = 4$). In the tracheal chain preparation (Figure 6b), no reduction in resting tone was seen with ICI D7114 at either 1 nM or 0.1 μM. At 10 μM, however, ICI D7114 produced a small and gradual reduction in tone (15% of the isoprenaline maximum response). Moreover, at this high concentration, ICI D7114 produced weak antagonism of the isoprenaline-induced relaxation (concentration ratio 8.07 [6.92–9.29]. This value equates to an 'apparent' pK_B of 5.85 [5.77–5.92]. The weak effect of ICI D7114 in the trachea appeared to be specific for the β-adrenoceptor as the compound (10 μM) failed to antagonize significantly the relaxant responses to aminophylline in this preparation. There was, however, a small but statistically insignificant leftward shift in the aminophylline concentration-response curve (concentration-ratio 0.43 [0.33–0.56]).

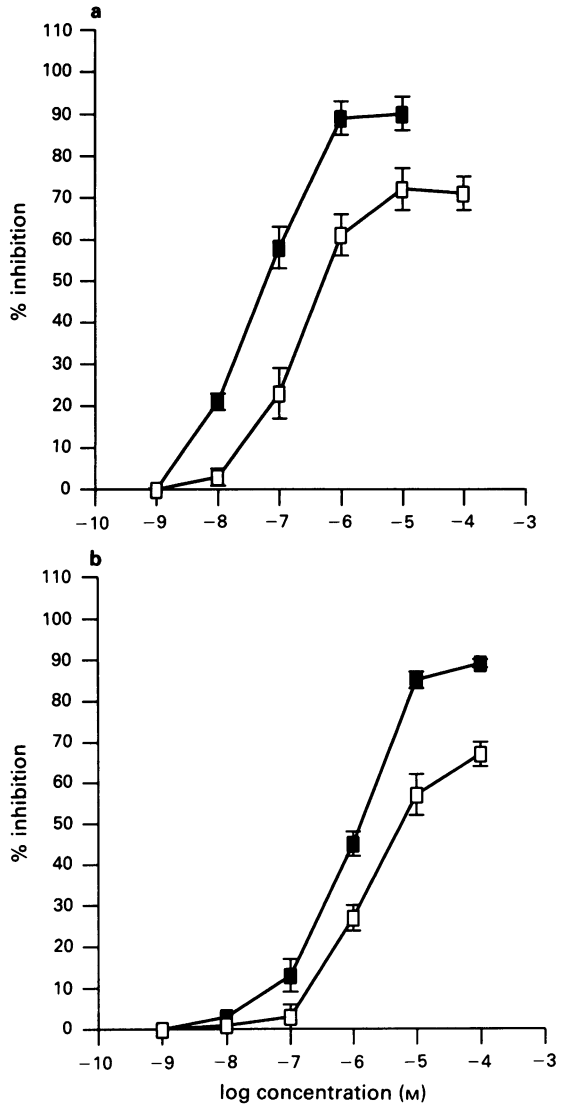

Figure 4 Inhibitory effects of (a) BRL37344 and (b) ICI D7114 on the contractile response to histamine (0.5 μM) in the guinea-pig isolated ileum: interaction with alprenolol (1 μM). (■) Control responses: (□) responses in the presence of alprenolol. Each point represents the mean ± s.e.mean (where this exceeds symbol size) percentage inhibition obtained from tissues from 4 guinea-pigs.

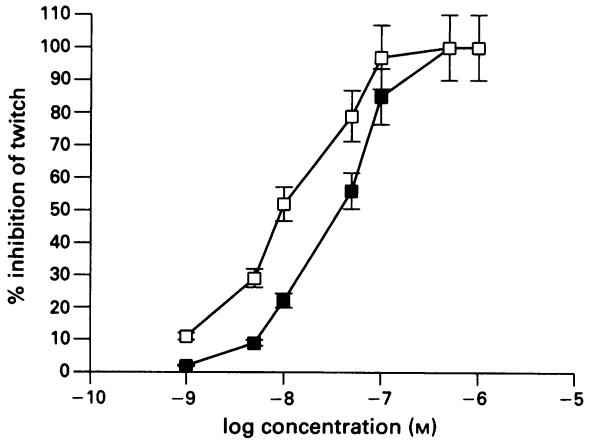

Figure 5 Concentration-responses to morphine in the guinea-pig field-stimulated isolated ileum: interaction with alprenolol. (■) Control concentration-response curve to morphine and (□) concentration-response curve to morphine in the presence of alprenolol (10 μM). Each point represents the mean ± s.e.mean (where this exceeds the symbol size) percentage inhibition obtained in ileum from 4 guinea-pigs.

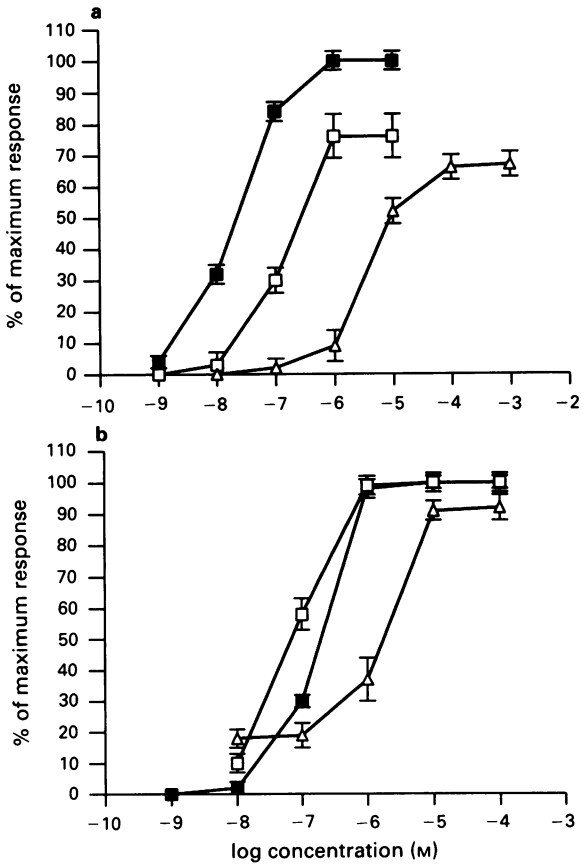

Figure 6 Concentration-responses to isoprenaline in (a) guinea-pig isolated right atria and (b) tracheal chain preparations: interaction with ICI D7114, 0.1 μM and 10 μM. (■) Control responses; responses in the presence of 0.1 μM (□) and 10 μM (△) ICI D7114. Each point represents the mean ± s.e.mean (where this exceeds the symbol size) percentage increase in rate (atria) or decrease in tone (trachea) obtained from 4 guinea-pigs. Effects of ICI D7114 at the lowest concentration used, i.e. 1 nM, have been omitted from the figures for clarity.

Discussion

ICI D7114 has previously been shown to stimulate oxygen consumption potently in conscious rats, cats and dogs and in rat isolated brown adipocytes (Holloway *et al.*, 1991a,b). We have now demonstrated that, in addition to its effect on metabolic parameters, ICI D7114 also causes an inhibition of the response to histamine in the guinea-pig ileum pretreated with a variety of pharmacological agents, including β_1- and β_2-adrenoceptor antagonists. This model has previously been reported to reveal the atypical β-adrenoceptor (Bond & Clarke, 1988). The effect of ICI D7114 in the guinea-pig ileum was similar to that observed with BRL37344, a compound recognised as a potent and selective atypical β-adrenoceptor agonist (Arch *et al.*, 1984), and was only weakly antagonized by (±)-propranolol at concentrations as high as 5 μM. Furthermore, both the isoprenaline and BRL37344-induced inhibition of the histamine responses were also only weakly antagonized by this high concentration of (±)-propranolol. These findings and those that the selective β_1- and β_2-adrenoceptor antagonists (CGP 20712A and ICI 118551) also produced only weak antagonism of the responses to isoprenaline, suggest that conventional β_1- and β_2-adrenoceptors play a minor role in the inhibitory responses produced by isoprenaline, ICI D7114 and BRL37344. These observations are in agreement with the findings of other workers employing the guinea-pig ileum (Bond & Clarke, 1988), rat gastric fundus (McLaughlin & MacDonald, 1991) distal colon (McLaughlin & MacDonald,

1990) and small intestine (van der Vliet *et al.*, 1990), although in this latter preparation, BRL37344 expresses itself as a partial agonist producing a relaxation that was only 60% of that achieved with a maximal concentration of isoprenaline.

In the current study, it was demonstrated that no significant effect of either ICI D7114 or BRL37344 was mediated via α-adrenoceptors, 5-HT_1, 5-HT_2 histamine H_2, opioid, adenosine receptors or via an interaction with prostanoid biosynthesis. It was interesting, however, that the inhibitory responses to BRL37344 were significantly enhanced in the presence of 8-phenyltheophylline. Although the exact reason(s) for this effect are not known, it could be that some degree of phosphodiesterase inhibition was induced by the presence of 8-phenyltheophylline. This, could in turn, lead to an enhancement of relaxation/inhibition. It appears that in the current study BRL37344 was more sensitive to this effect than any of the other β-adrenoceptor agonists employed. In tissues containing β_1- and β_2-adrenoceptors (guinea-pig right atrium and tracheal chain, respectively), ICI D7114 produced only weak agonist activity. BRL37344, on the other hand, has been shown to possess significant agonist activity at both β_1- and β_2-adrenoceptors. Concentrations of 0.68 μM and 0.04 μM caused half-maximal responses in guinea-pig right atrium and trachea respectively (Arch *et al.*, 1984). As ICI D7114 possessed little intrinsic activity in either guinea-pig right atrium (a tissue containing predominantly β-adrenoceptors) or the tracheal chain (a tissue containing predominantly β_2-adrenoceptors), it was checked for its ability to produce antagonism of isoprenaline at these sites. The data revealed β_1- and weak β_2-adrenoceptor antagonist effects to be present and this appeared to be specific for the β-adrenoceptors as no effects were observed with ICI D7114 against either histamine-induced chronotropism or aminophylline-induced relaxation in the atrium and trachea, respectively. Moreover, the effects of ICI D7114 at β_1- and β_2-adrenoceptors have been shown not to be responsible for the action of the compound on brown adipocytes (Holloway *et al.*, 1991a) or its inhibitory effect on the contractile response to histamine in the guinea-pig ileum (current study).

The rank order of agonist potency of isoprenaline, BRL37344 and noradrenaline obtained in the current study is in good agreement with that obtained by other workers (e.g. Bond & Clarke, 1988), from which it may be inferred that similar mechanisms/receptor sites were involved in these determinations in both studies. However, the usefulness of relative agonist potency has it limitations when attempting to classify/categorize the receptor(s) involved. For example, similar (or even identical) receptors can behave quite differently depending on the stimulus-response relationship (Kenakin & Beek, 1980) and if agonists fail to achieve full efficacy, then any comparison is negated. Hence, in tissues where agents behave as partial agonists (BRL37344, rat jejunum, van der Vliet *et al.*, 1990), or have no efficacy whatsoever, (ICI D7114, rat ileum, Growcott *et al.*, 1992; rat colon, MacDonald & Lamont, 1992), then rank orders agonist potency cannot be utilised in the discussion of receptor homogeneity/heterogeneity.

Hence, it is pertinent, whenever possible, to use selective and competitive antagonists to classify receptor sub-types. In an attempt to characterize further the atypical β-adrenoceptor, Blue *et al.* (1990) employed alprenolol or dihydroalprenolol as antagonists. In the current study, alprenolol was used to characterize effects of agonists at the atypical β-adrenoceptor. This agent possessed some degree of 'partial agonist' activity in the guinea-pig ileum, such that at concentrations above 1 μM responses to histamine were almost abolished and the twitch responses in the electrically-stimulated preparation were markedly attenuated. This 'partial agonist' effect has been previously observed in the electrically-stimulated guinea-pig ileum (Blue *et al.*, 1990) and these authors suggest that this latter action of alprenolol might be due to an effect at atypical β-adrenoceptors. These observations are not totally surprising as other pindolol analogues have been demonstrated to possess partial agonist activity in rat adipocytes (Engel *et al.*, 1981). Thus, in the current study, alprenolol produced competitive antagonism of the inhibitory responses to isoprenaline, the Schild analysis revealing a pA_2 value of 6.44. This value is not dissimilar to that reported by Blue *et al.* (1990) where a pA_2 of 6.47 was obtained for (−)-alprenolol against the inhibitory effects of isoprenaline in the electrically stimulated guinea-pig ileum or that obtained by Nials *et al.* (1992) in the guinea-pig gastric fundus ($pA_2 = 6.0$ versus isoprenaline). In the current study, when a single concentration (1 μM) of alprenolol was used to antagonize the inhibitory effects of either ICI D7114 or BRL37344, the calculated 'apparent' pK_B values were 6.53 and 6.57 respectively, which were not significantly ($P > 0.05$) different from the value obtained against isoprenaline. These values are somewhat lower than those obtained for alprenolol at β_1- and β_2-adrenoceptors (pA_2 values = 8.1 and 8.0 respectively, Hoefle *et al.*, 1975) lending support to the notion that a single population of atypical β-adrenoceptors are involved in the inhibitory responses to agents such as ICI D7114. However, there is a possibility that atypical β-adrenoceptors may not be homogeneous. In the rat colon, Bianchetti & Manara (1991) found that alprenolol caused competitive antagonism of the inhibitory responses to isoprenaline (pA_2 7.6) and a novel phenylethanolaminotetraline, SR 5875A (pA_2 7.5). These values are substantially higher than those previously reported for the effect of alprenolol (e.g. Blue *et al.*, 1988) and are closer to the affinity of alprenolol for classical β-adrenoceptors (i.e. pA_2 approx. 8.0). Thus, it could be argued that SR 58375A is acting at both atypical and classical β-adrenoceptors in the study of Bianchetti & Manara (1990) as it is not clear from their findings whether β_1- and β_2-adrenoceptors were blocked when assessment of the antagonist potency of alprenolol was made.

Now that the human atypical β-adrenoceptor has been cloned (Emorine *et al.*, 1989), it was interesting to check for homology between atypical sites identified in animal tissue and those found in human tissue. Already, there appears to be some variance in data. In the human cloned atypical β-adrenoceptor, it has been shown that cyclic AMP production is potently stimulated by BRL37344 (Emorine *et al.*, 1989). However, this same agent has been shown to lack intrinsic activity on human colon (McLaughlin *et al.*, 1991) and adipocytes (Hollenga *et al.*, 1990). It is possible that BRL37344 could therefore act as an antagonist, in the situation where agonist efficacy is low; however, neither McLaughlin *et al.* (1991) nor Hollenga *et al.* (1990) checked for this possibility.

In conclusion, therefore, the present study has indicated that a potent and selective lipolytic agent, ICI D7114, possesses properties similar to BRL37344. The inhibitory effects in the guinea-pig ileum appear to be mediated via an atypical β-adrenoceptor; whether this site is identical to that identified on adipose tissue remains to be resolved. ICI D7114 should provide a valuable tool to probe these sites and help reveal the true nature of the atypical β-adrenoceptor.

References

ARCH, J.R.S., AINSWORTH, A.T., CAWTHORNE, M.A., PIERCEY, V., SENNITT, M.V., THODY, V.E., WILSON, C. & WILSON, S. (1984). Atypical β-adrenoceptors on brown adipocytes as a target for anti-obesity drugs. *Nature*, **309**, 163–165.

ARUNLAKSHANA, O. & SCHILD, H.O. (1959). Some quantitative uses of drug antagonists. *Br. J. Pharmacol. Chemother.*, **14**, 48–57.

BIANCHETTI, A. & MANARA, L. (1990). In vitro inhibition of intestinal motility by phenylethanolaminotetralines: evidence of atypical β-adrenoceptors in rat colon. *Br. J. Pharmacol.*, **100**, 831–839.

BLUE, D.R., BOND, R.A., ADHAM, N., DELMONDO, R., MICHEL, A.D., EGLEN, R., WHITING, R.L. & CLARKE, D.E. (1990). Interaction of dihydroalprenolol and cyanopindiolol with atypical β-adrenoceptors in guinea-pig ileum. *J. Pharmacol. Exp. Ther.*, **252**, 1034–1042.

BLUE, D.R., BOND, R.A. & CLARKE, D.E. (1988). Blockade of putative adrenoceptor in guinea-pig ileum by alprenolol. *Br. J. Pharmacol.*, **95**, 541P.

BOND, R.A. & CLARKE, D.E. (1987). A response to isoprenaline unrelated to α- or β-adrenoceptor agonism. *Br. J. Pharmacol.*, **91**, 683–686.

BOND, R.A. & CLARKE, D.E. (1988). Agonist and antagonist characterization of a putative adrenoceptor with distinct pharmacological properties from the α- and β-subtypes. *Br. J. Pharmacol.*, **95**, 723–734.

EMORINE, L.J., MARULLO, S., BRIEND-SUTREN, M.N., PATEY, G., TATE, K., DELAVIER-KLUTCHKO, C. & STROSBERG, D.A. (1989). Molecular characterization of the human β_3-adrenergic receptor. *Science*, **245**, 1118–1121.

ENGEL, G., HOYER, D., BERTHOLD, R. & WAGNER, H. (1981). (±)[^{125}Iodo]cyano-pindolol, a new ligand for β-adrenoceptors: Identification and quantitation of sub-classes of β-adrenoceptor in guinea-pig. *Naunyn-Schmied. Arch. Pharmacol.*, **317**, 277–285.

FURCHGOTT, R.F. (1972). The classification of adrenoceptors (adrenergic receptors). An evaluation from the standpoint of receptor theory. In *Handbook of Experimental Pharmacology*, Vol. 33, pp. 283–335. Berlin: Springer Verlag.

GROWCOTT, J.W., HOLLOWAY, B., WILSON, C., MAINWARING, S. & GREEN, M. (1992). The novel brown adipocyte stimulant, ICI D7114, reveals possible differences in atypical β-adrenoceptor reserve. *Br. J. Pharmacol.*, **105**, 232P.

HOEFLE, M.L., HASTINGS, S.G., MEYER, R.F., COREY, R.M., HOLMES, A. & STRATTON, C.D. (1975). Cardioselective β-adrenergic bloking agents1. 1[(3,4-dimethoxyphenethyl)amino]-3-aryloxy-2-propanols. *J. Med. Chem.*, **18**, 148–152.

HOLLENGA, C., HAAS, M., DEINUM, J.T. & ZAAGSMA, J. (1990). Discrepancies in lipolytic activities induced by β-adrenoceptor agonists in human and rat adipocytes. *Horm. Metab. Res.*, **22**, 17–21.

HOLLOWAY, B.R., BRISCOE, M., GROWCOTT, J.W., WILSON, C. & STOCK, M.J. (1991a). In vitro and in vivo selectivity of ICI D7114 for brown fat and thermogenesis. In *Adrenoceptors: Structures, Mechanisms, Function. Advances in Pharmacological Sciences*, pp. 313–314. Basel: Birkhauser Verlag.

HOLLOWAY, B.R., HOWE, R., RAO, B.S., STRIBLING, D., MAYERS, R.M., BRISCOE, M. & JACKSON, J. (1991b). ICI D7114 a novel selective β-adrenoceptor agonist selectively stimulates brown fat and increases whole body oxygen consumption. *Br. J. Pharmacol.*, **103**, 97–104.

KELLY, J. & MACDONALD, A. (1990). Relaxant effects of α-adrenoceptor agonists in the isolated rat gastric fundus. *J. Pharm. Pharmacol.*, **42**, 30–34.

KENAKIN, T.P. & BEEK, D. (1980). Is prenalterol (H133/80) really a selective β_1-adrenoceptor agonist? Tissue selectivity resulting from differences in stimulus-response relationships. *J. Pharmacol. Exp. Ther.*, **213**, 406–413.

MACDONALD, A. & LAMONT, M. (1992). Relative contributions of β-adrenoceptor subtypes to isoprenaline-induced relaxation of rat distal colon. *Br. J. Pharmacol.*, **107**, 24P.

MCLAUGHLIN, D.P. & MACDONALD, A. (1990). Evidence for the existence of atypical β-adrenoceptors (β_3-adrenoceptors) in the rat distal colon *in vitro*. *Br. J. Pharmacol.*, **101**, 569–574.

MCLAUGHLIN, D.P. & MACDONALD, A. (1991). Characterization of catecholamine-mediated relaxations in rat isolated gastric fundus: evidence for an atypical β-adrenoceptor. *Br. J. Pharmacol.*, **103**, 1351–1356.

MCLAUGHLIN, D.P., FULTON, J.D., MACDONALD, E., SCOTT, P.J.W. & MACDONALD, A. (1991). β-adrenoceptors mediating relaxation in human colon *in vitro*. *Br. J. Pharmacol.*, **104**, 152P.

MELLOR, P.M. (1984). A precision isotonic measuring system for isolated tissues. *J. Pharmacol. Method.* **12**, 259–260.

NIALS, A.T., BARKER, R.C., COLEMAN, R.A. & VARDEEY, C.J. (1992). Further characterization of the atypical β-adrenoceptors in guinea-pig gastic fundus. *Br. J. Pharmacol.*, **106**, 66P.

NORMAN, B.J. & LEATHARD, H.L. (1990). Evidence that an atypical β-adrenoceptor mediates the inhibition of spontaneous rhythmical contractions of rabbit isolated jejunum induced by ritodrine and salbutamol. *Br. J. Pharmacol.*, **101**, 27–30.

TANEJA, D.T. & CLARKE, D.E. (1991). Evidence for a noradrenergic innervation to 'atypical' β-adrenoceptors (or putative β_3-adrenoceptors) in the ileum of the guinea-pig. *J. Pharmacol. Exp. Ther.*, **260**, 192–200.

VAN DER VLIET, A., RADEMAKER, B. & BAST, A. (1990). A β-adrenoceptor with atypical characteristics is involved in the relaxation of the rat small intestine. *J. Pharmacol. Exp. Ther.*, **255**, 218–226.

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