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β_1- and β_2-adrenoceptor antagonist activities of ICI-215001, a putative β_3-adrenoceptor agonist

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1 The present study was undertaken to characterize the β_3-adrenoceptor agonist activity of ICI-215001 and to determine whether it exhibits additional activities on β_1- and β_2-adrenoceptors in isolated spontaneously beating atrium, trachea and ileum of guinea-pig.

2 In guinea-pig atrium, isoprenaline, a non-selective β-adrenoceptor agonist, caused concentration-dependent, positive chronotropic effects that were inhibited by atenolol, a selective β_1-antagonist. ICI-215001 also competitively antagonized the increase in heart rate caused by isoprenaline.

3 ICI-215001 exhibited low intrinsic activity at increasing the beating rate of atrium and no activity on resting or induced tone of tracheal strips.

4 In strips of guinea-pig trachea, contracted submaximally with carbachol, isoprenaline, caused concentration-dependent relaxations. Both ICI-118551, a selective β_2-adrenoceptor antagonist, and ICI-215001 competitively inhibited the relaxations caused by isoprenaline.

5 In isolated strips of guinea-pig ileum longitudinal smooth muscle contracted with histamine, isoprenaline and ICI-215001 caused relaxations which were inhibited by alprenolol, a β-adrenoceptor antagonist with modest affinity for β_3-adrenoceptors, but were resistant to ICI-118551 and atenolol.

6 These results indicate that ICI-215001 exhibits β_3-adrenoceptor agonist activity as demonstrated by relaxations mediated via atypical β-adrenoceptors in the longitudinal smooth muscle of guinea-pig ileum. Further, the studies demonstrate that ICI-215001 can act as an antagonist at β_1- and β_2-adrenoceptors in situations where its intrinsic agonist activity is low.

Keywords: Atypical β-adrenoceptors; guinea-pig atrium; guinea-pig ileum; guinea-pig trachea; ICI-215001; relaxation

Introduction

The subclassification of β-adrenoceptors into the two subgroups of β_1 and β_2 was based on the selectivity of action of different β-agonists as cardiac stimulants and bronchodilators, respectively (Lands *et al.*, 1967). Quantitative pharmacological experiments have shown the existence of adrenoceptors distinct from the defined α- and β-subtypes (Bond *et al.*, 1986; 1988). This 'atypical' β adrenoceptor is resistant to blockade by α-adrenoceptor antagonists and β-adrenoceptor antagonists (Bond *et al.*, 1988). Furthermore a human gene has been isolated from brown adipocytes which encodes for a β-adrenoceptor distinct from β_1- or β_2-receptors, and was referred to as 'the β_3-adrenergic receptor' (Emorine *et al.*, 1989; Krief *et al.*, 1993). The β_3-adrenoceptors have gained attention as potential therapeutic targets for specific agonists that might provide antiobesity, thermoregulatory or antidiabetic properties (Arch *et al.*, 1984).

Furthermore, others have reported a propranolol-resistant component to the relaxations of various sections of gastrointestinal tract induced by isoprenaline and other β-adrenoceptor agonists, which resembles the one described in brown adipocytes, implying that an 'atypical' β-adrenoceptor might be responsible for these effects (Bond *et al.*, 1988; Blue *et al.*, 1990; Taneja & Clarke, 1991). From the above account it is clear that the 'atypical' β-adrenoceptor is a pharmacologically defined entity and little information exists with regard to its functional characterization and the interaction of selective β_3-adrenoceptor agonists with other β-adrenoceptor types.

It has recently been reported that ICI-215001 and its prodrug form, ICI-D7114, {4-[2-[(2-hydroxy-3-phenoxypropyl) amino] ethoxy] phenoxyacetamides} have potent activity at 'atypical' β-adrenoceptors in brown adipocytes leading to an increased whole body temperature (Holloway *et al.*, 1991; Champigny *et al.*, 1992). It was of interest to characterize further β_3-adrenoceptor agonist activity of ICI-215001 and to evaluate whether it exhibits additional activities at β_1- and β_2-adrenoceptor subtypes. This task has been approached by examining its direct agonist activity in guinea-pig isolated ileum as well as by comparing its antagonist activity with competitive antagonists selective for β_1-adrenoceptors (atenolol) and β_2-adrenoceptors (ICI-118551) in isolated spontaneously beating atrium and tracheal preparations of guinea-pig (Lands *et al.*, 1986; Bilski *et al.*, 1983).

Methods

Guinea atrium

Male Hartley guinea-pigs (~300 g) were killed by exsanguination after asphyxia with carbon dioxide. The pericardium was carefully removed from the heart and the right atrium was dissected. A suture was tied to the upper and lower tip of the atrium. The spontaneously beating atrium was suspended between a fixed end and the distal end was connected to a force transducer for measurement of beating rate. Beating rates were determined by tachograph which integrated the beating rate to a linear scale on the recorder. The atria were placed in an organ bath filled with physiological salt solution containing (mM): NaCl 118.3, KCl 4.7, $MgSO_4$ 1.2, KH_2PO_4 1.2, $CaCl_2$ 2.5, $NaHCO_3$ 25.0, disodium EDTA 0.026 and glucose 5.5. The solutions were kept at 37°C and were continuously gassed with 95% O_2: 5% CO_2 to maintain the pH at 7.4. The resting tension was set at 1 g during a 1 h equilibration period. Cumulative concentration-response curves for the positive chronotropic effect of isoprenaline and ICI-215001 were determined. The beating rate was assessed 1 min after the addition of each successive concentration of β-adrenoceptor agonists. For assessment of antagonist activity, the responses of the atrium to isoprena-

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line were determined in the presence of ICI-215001 or atenolol (0.1–10 μM). Antagonists were added 30 min before addition of agonists.

Guinea-pig trachea

Tracheal strips (~5 mm long) were prepared and suspended between a fixed base and strain gauge for measurement of isometric circumferential force. The strips were placed in organ baths filled with physiological salt solution which were kept at 37°C and were continuously gassed with 95% O_2: 5% CO_2. The length of the smooth muscle was increased stepwise over 90 min to adjust basal tension to 2 g. This tension was found to be optimal for contractions of guinea-pig trachea by testing the contractions to potassium (80 mM). Once basal tension was established, the length of the strips was not altered thereafter. The tracheal strips were contracted with carbachol and then exposed to increasing half-log cumulative concentrations of agonists.

Guinea-pig ileum

After careful flushing of the luminal contents, the outer layer of ileum containing longitudinal smooth muscle was carefully removed with a cotton swab. Each strip (~3 cm long) was tied with a nylon suture at each end, and was mounted on a force transducer under an initial resting tension of 0.5 g, which was found to be optimal for contractions by testing repeated contractions to potassium (80 mM). To measure relaxations, strips were contracted with histamine and were then exposed to increasing cumulative concentration of agonists.

In guinea-pig trachea and ileum studies, phentolamine (10 μM) and indomethacin (3 μM) were added to block α-adrenoceptors and to inhibit rhythmic motility, respectively. In ileal preparations, atropine (0.1 μM) was added to block muscarinic receptors. The inhibitors had no effect on the resting or induced tone of tissues.

Drugs

The pharmacological agents used were the following: atenolol, (−)-alprenolol, atropine, carbachol, histamine, indomethacin, (−)-isoprenaline, phenylephrine, phentolamine, salbutamol, sodium nitroprusside (Sigma Chemical, St. Louis, MO, U.S.A.), ICI-215001 and ICI-118551 {erythro-1-(7-methylindan-4-yloxy)-3-isopropylamino-butan-2-ol} (imperial Chemical Industries, Macclesfield, England). Unless otherwise specified, drugs were dissolved in distilled water. ICI-215001 was solubilized in dimethyl sulphoxide and further dilutions were made in water. Indomethacin was prepared in 2% Na_2CO_3.

Data analysis

Changes in sinus rate are expressed as a percentage of the maximum increase in beating rate caused by isoprenaline (10 μM). Relaxations are expressed as percentage decrease in tension from the level of induced tone. The half-maximal inhibitory concentration (IC_{50}) was determined graphically as the concentration causing 50% of the maximal relaxation. Antagonist potencies were evaluated by calculating their pA_2 values. Concentration-response curves for agonists were made in the presence of three different concentrations of antagonist. Schild plots were constructed from the individual experiments and the pA_2 values were calculated. (Arunlakshana & Schild, 1959). Antagonism was considered to be competitive if the slope of the regression line did not significantly differ from unity. The values are expressed as means ± s.e. Statistical evaluation of the data was made using repeated measures of analysis of variance or Student's *t* test for paired comparisons of mean values. Values with *P* less than 0.05 were regarded as significant. In all experiments, *n* equals the number of guinea-pigs from which the tissues were taken.

Results

Guinea-pig atrium

Isoprenaline (0.1 nM–10 μM), a non-selective β-adrenoceptor agonist, had a concentration-dependent positive chronotropic effect on the atrium (EC_{50}: 7.1 ± 1.2 nM, $n = 12$). Atenolol (0.1–10 μM) shifted the isoprenaline concentration-response curves, resulting in Schild plots with slopes not significantly different from unity (pA_2: 6.1 ± 0.2; with isoprenaline as the agonist). ICI-215001 (0.1, 1 and 10 μM) also competitively antagonized the increase in beating rate caused by isoprenaline, yielding a Schild plot with a slope not significantly different from unity and a pA_2 value of 6.7 ± 0.2, $n = 7$ (Figure 1). In the guinea-pig atrium, ICI-215001 (0.1, 1, 10 μM) had weak agonist activity (6.1 ± 2.6, 11 ± 2.6 and 11 ± 6.4% maximal isoprenaline, respectively, $n = 7$).

Guinea-pig trachea

In strips of guinea-pig trachea contracted submaximally with carbachol (1 μM), isoprenaline (0.1 nM–1 μM) caused concentration-dependent relaxations (IC_{50}: 6.1 ± 2.5 nM, $n = 10$). These relaxations were competitively antagonized by ICI-118551 (pA_2: 7.4 ± 0.8). In the guinea-pig trachea, ICI-215001 (0.1, 1, 10 μM) produced competitive antagonism of isoprenaline-induced relaxations yielding a Schild plot with a slope not significantly different from unity and a pA_2 value of 7.3 ± 0.4, $n = 7$ (Figure 2). ICI-215001 (0.1–10 μM) had no effect on the resting or induced tone of tracheal strips.

Guinea-pig ileum

In strips of guinea-pig ileum longitudinal smooth muscle contracted with histamine (10 μM), isoprenaline (0.01–100 μM), caused concentration-dependent relaxations (IC_{50}: 31 ± 0.8 nM, $n = 7$). These relaxations were significantly inhibited by treatment of strips with (−)-alprenolol (1, 10 μM) (Figure 3). The inhibition was more marked with the higher concentration of (−)-alprenolol (10 μM). In contrast, isoprenaline-induced relaxations were resistant to treatment of strips with atenolol (10 μM) or ICI-118551 (10 μM). In guinea-pig ileum longitudinal muscle, ICI-215001 (0.01–10 μM) caused concentration-dependent relaxations (IC_{50}: 316 ± 0.6 nM, $n = 8$) (Figure 4). These relaxations were markedly inhibited by (−)-alprenolol (1, 10 μM) (Figure 4). By contrast, (−)-alprenolol (10 μM) did not have any significant effect on the

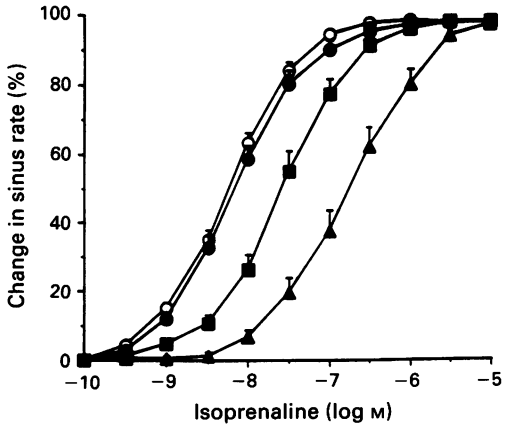

Figure 1 In control (○) spontaneously beating guinea-pig atrium, isoprenaline caused a concentration-dependent positive chronotropic effect. ICI-215001 in concentrations 0.1 μM (●), 1 μM (■) and 10 μM (▲) competitively antagonized the increase in sinus rate caused by isoprenaline, resulting in a parallel right-ward shift of the concentration-response curves. Values are expressed as maximal response to isoprenaline (10 μM), means ± s.e., $n = 12$.

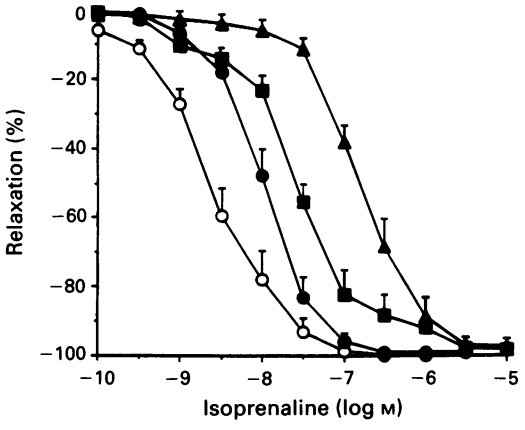

Figure 2 In control (○) guinea-pig tracheal strips contracted with carbachol (1 μM), isoprenaline caused concentration-dependent relaxations. These relaxations were competitively antagonized by ICI-215001 in concentrations 0.1 μM (●), 1 μM (■) and 10 μM (▲). $n = 7$.

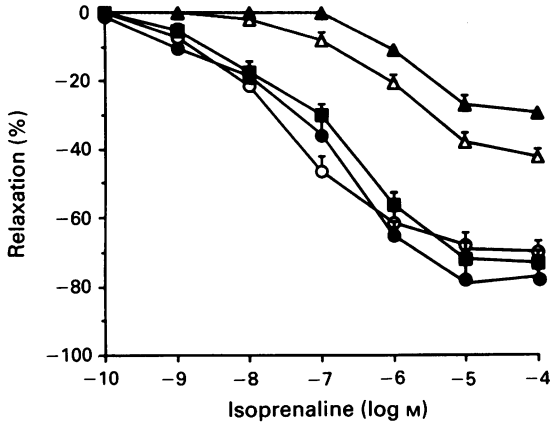

Figure 3 In control (○) isolated longitudinal muscle of guinea-pig ileum contracted with histamine (10 μM), isoprenaline caused concentration-dependent relaxations. These relaxations were significantly ($P < 0.05$) inhibited by alprenolol in concentrations 1 μM (▲) and 10 μM (△) but not by atenolol 10 μM (■) or ICI-118551 10 μM (●). $n = 7$.

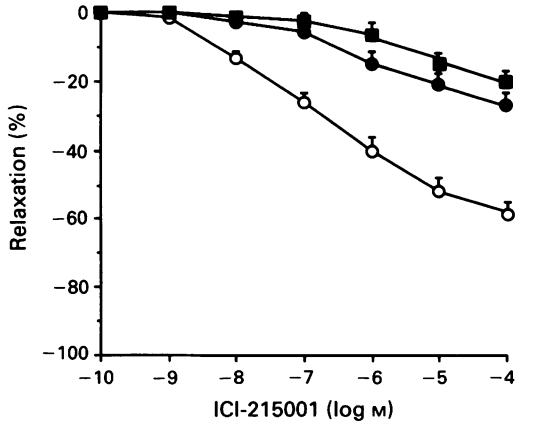

Figure 4 In control (○) isolated longitudinal muscle of guinea-pig ileum contracted with histamine (10 μM), ICI-215001 caused concentration-dependent relaxations, that were significantly ($P < 0.05$) inhibited by alprenolol at concentrations of 1 μM (●) and 10 μM (■). $n = 8$.

relaxations caused by sodium nitroprusside (1 μM, in absence and presence of alprenolol: 99 ± 0.9 vs 97 ± 2.1%, respectively, $n = 6$). In guinea-pig ileum longitudinal smooth muscle, salbutamol, a selective β_2-adrenoceptor agonist (0.1 nM–100 μM), did not cause any relaxations (data not shown, $n = 4$).

Discussion

In agreement with previous reports, isoprenaline-induced positive chronotropic effects were competitively antagonized by the selective β_1-adrenoceptor antagonist, atenolol, indicating that they are mediated by stimulation of β_1-adrenoceptors in the atrium (Lands *et al.*, 1967). An interesting finding in the present study is that ICI-215001 competitively antagonized the increase in beating rate caused by isoprenaline suggesting that ICI-215001 possesses β_1-adrenoceptor antagonist activity. Similarly, others have also shown that ICI-D7114, the prodrug form of ICI-215001, caused inhibition of responses to isoprenaline in guinea-pig atrium (Growcott *et al.*, 1993). In this regard, the affinity of ICI-215001 for β_1-adrenoceptors was compaable to that of atenolol. ICI-215001 exhibited low intrinsic activity on β_1-adrenoceptors as demonstrated by its weak effect on the beating rate of the atrium. In contrast to ICI-215001, another β_3-agonist, BRL-37344, has been reported to cause β_1-adrenoceptor-mediated positive chronotropic and inotropic responses in dog atria (Takayama *et al.*, 1993).

The inhibition by the selective β_2-antagonist, ICI-118551, of the relaxations of trachea caused by isoprenaline demonstrates that the relaxations were mediated by stimulation of β_2-adrenoceptors. The competitive antagonism by ICI-215001 of the relaxations caused by isoprenaline in trachea indicates that ICI-215001 exhibits affinity for β_2-adrenoceptors. In this regard, ICI-215001 and ICI-118551 showed similar affinity for β_2-adrenoceptors. Further, the absence of any effect of ICI-215001 in resting or contracted tracheal strips indicates its lack of direct intrinsic activity on β_2-adrenoceptors.

In an attempt to characterize further the 'atypical' β-adrenoceptors, we used the guinea-pig ileum which has been previously described as revealing the 'atypical' β-adrenoceptor (Bond *et al.*, 1988). Prior studies have suggested that β_2-adrenoceptors are located on circular smooth muscle or epithelial cells, whereas 'atypical' β-adrenoceptors are probably distributed on the longitudinal smooth muscle of the ileum (Van Der Vliet *et al.*, 1990). In the present study, efforts were made to confirm whether 'atypical' β-adrenoceptors mediate relaxations of the longitudinal muscle of the ileum. Indeed isoprenaline-induced relaxations were resistant to the selective β_1- and β_2-antagonists, implying that the conventional β_1- and β_2-adrenoceptors play no role in the inhibitory response produced by isoprenaline in this preparation. In addition, the lack of effect of salbutamol rules out β_2-adrenoceptor-mediated responses. Like isoprenaline, ICI-215001 also caused relaxation of the ileum suggesting that the relaxations are probably mediated by activation of 'atypical' β-adrenoceptors. This suggestion is strengthened by the blockade of isoprenaline- and ICI-215001-induced relaxations by (−)-alprenolol, a compound which exhibits moderate affinity for 'atypical' β-adrenoceptors (Blue *et al.*, 1990). The lack of effect of (−)-alprenolol on the relaxations caused by nitroprusside excludes a non-specific effect. These findings are in agreement with previous reports which have shown that relaxations induced by electrical stimulation of sympathetic neurones were blocked by (−)-alprenolol, indicating that the responses may be mediated via activation of β_3-adrenoceptors (Blue *et al.*, 1990; Taneja & Clarke, 1991). Furthermore, the current study has demonstrated that the 'atypical' β-adrenoceptors are distributed predominantly on the longitudinal smooth muscle cells which lie adjacent to the myenteric plexus. Whether the atypical β-adrenoceptors in the longitudinal muscle are the same as those β_3-receptors described in brown adipocytes is unknown. Because the 'atypical' β-

adrenoceptors have been shown to receive adrenergic innervation, this raises the possibility that these receptors may be a target site for modulating gut motility (Taneja & Clarke, 1991).

In summary, the present study has further characterized atypical β-adrenoceptors on the longitudinal smooth muscle of guinea-pig ileum in terms of the lack of affinity of β_1- and β_2-adrenoceptor antagonists. In addition to its agonist activity at atypical β-adrenoceptors, ICI-215001 also exhibits antagonist activity at β_1- and β_2-adrenoceptors.

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