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β-Adrenoceptor subtypes and the opening of plasmalemmal K^+-channels in trachealis muscle: electrophysiological and mechanical studies in guinea-pig tissue

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1 Mechanical and electrophysiological studies of guinea-pig isolated trachealis have been made with the objectives of (a) identifying which of the β-adrenoceptor subtypes mediates the opening of plasmalemmal K^+-channels, (b) gaining further insight into the properties of the novel, long-acting β-adrenoceptor agonist, salmeterol and (c) clarifying the role of K^+-channel opening in mediating the relaxant actions of agonists at β-adrenoceptors.

2 Noradrenaline (10 nM–100 μM) caused a concentration-dependent increase in the rate of beating of guinea-pig isolated atria. The selective β_1-adrenoceptor blocking drug, CGP 20712A (100 nM–10 μM) caused concentration-dependent antagonism of noradrenaline. The selective β_2-adrenoceptor blocking drug, ICI 118551, also produced concentration-dependent antagonism of noradrenaline, but only when used in concentrations greater than 300 nM.

3 Cromakalim (100 nM–10 μM), isoprenaline (1–100 nM), procaterol (0.1–30 nM), salbutamol (1 nM–1 μM), salmeterol (1–100 nM) and theophylline (1 μM–1 mM) each caused concentration-dependent suppression of the spontaneous tone of guinea-pig isolated trachealis.

4 ICI 118551 (10 nM–1 μM) antagonized isoprenaline, procaterol and salmeterol in suppressing the spontaneous tone of the isolated trachea. The antagonism was concentration-dependent. In contrast, ICI 118551 (1 μM) antagonized neither cromakalim nor theophylline. CGP 20712A (up to 1 μM) failed to antagonize cromakalim, isoprenaline, procaterol, salmeterol or theophylline. In trachea treated with indomethacin (2.8 μM) and carbachol (10 μM), salmeterol (1 μM) antagonized the effects of isoprenaline but not aminophylline.

5 Intracellular electrophysiological recording from guinea-pig isolated trachealis showed that the relaxant effects of cromakalim (10 μM), isoprenaline (100 nM), procaterol (10 nM) and salbutamol (10 nM–1 μM) were accompanied by the suppression of spontaneous electrical slow waves and by cellular hyperpolarization. In contrast, the relaxant effects of salmeterol (10 nM–1 μM) were not accompanied by significant cellular hyperpolarization.

6 CGP 20712A (1 μM) inhibited the hyperpolarization but not the relaxation induced by isoprenaline (100 nM). In contrast ICI 118551 (100 nM) inhibited both the hyperpolarization and the relaxation induced by isoprenaline (100 nM). Neither CGP 20712A (1 μM) nor ICI 118551 (100 nM) inhibited the hyperpolarization induced by cromakalim (10 μM). Salmeterol (1 μM) inhibited the hyperpolarization induced by isoprenaline (100 nM) but not that induced by cromakalim (10 μM).

7 It is concluded that activation of either β_1- or β_2-adrenoceptors can promote the opening of K^+-channels in the trachealis plasmalemma. The poor ability of salmeterol to hyperpolarize trachealis muscle reflects neither its selectivity in activating β_2-adrenoceptors as opposed to β_1-adrenoceptors nor a non-specific action in stabilizing the cell membrane. Instead, it may reflect low intrinsic efficacy of the drug at β_2-adrenoceptors. The opening of plasmalemmal K^+-channels plays a supportive rather than a crucial role in mediating the tracheal relaxant actions of agonists at β-adrenoceptors.

Keywords: Trachealis muscle; electrophysiology; β-adrenoceptor subtypes; K^+-channels; isoprenaline; procaterol; salbutamol; salmeterol; CGP 20712A; ICI 118551

Introduction

In bovine and canine trachealis the mechano-inhibitory effects of agonists at β-adrenoceptors (adrenaline, isoprenaline and procaterol) are associated with hyperpolarization of the muscle cells (Suzuki *et al.*, 1976; Kirkpatrick, 1981; Ito & Tajima, 1982; Cameron *et al.*, 1983; Fujiwara *et al.*, 1988). In human and guinea-pig trachealis, agonists at β-adrenoceptors, such as isoprenaline and terbutaline, suppress spontaneous mechanical tone, suppress electrical slow waves and evoke cellular hyperpolarization (Allen *et al.*, 1985; Honda *et al.*, 1986; Honda & Tomita, 1987). Since the slow wave suppression and the hyperpolarization are inhibited by propranolol (Kirkpatrick, 1981; Ito & Tajima, 1982; Allen *et al.*, 1985; Honda *et al.*, 1986) it seems clear that these electrophysiological changes are mediated by the activation of β-adrenoceptors. However, the adrenoceptor subtype (β_1- or β_2-) responsible for mediating these electrophysiological changes has yet to be determined.

Electrotonic potentials induced by transmembrane current pulses are reduced in amplitude during the hyperpolarization of airways smooth muscle induced by agonists at β-adrenoceptors (Ito & Tajima, 1982; Cameron *et al.*, 1983; Fujiwara *et al.*, 1988). This may suggest that the hyperpolarization is a consequence of an increase in membrane conductance. Furthermore, since isoprenaline-induced hyperpolarization approaches the predicted K^+ equilibrium potential for trachealis muscle (Kirkpatrick, 1981; Allen *et al.*, 1985) it is likely that this conductance change involves the opening of membrane K^+-channels. Direct support for this suggestion comes from the patch clamp studies of Kume *et al.* (1989).

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Using cells freshly dispersed from rabbit trachealis smooth muscle, and recording from cell-attached patches, these authors showed that the addition of isoprenaline (0.2 μM) to the bathing medium stimulated the opening of plasmalemmal K^+-channels of large conductance.

The ability of charybdotoxin, in guinea-pig trachealis and human bronchus, to antagonize agonists at β-adrenoceptors such as isoprenaline and salbutamol (Jones *et al.*, 1990; Murray *et al.*, 1991; Miura *et al.*, 1992) suggests that the opening of the large conductance, Ca^{2+}-dependent K^+-channel (BK_{Ca}) is involved in the relaxant activity of the agonists at β-adrenoceptors. However, whether BK_{Ca} channel opening plays a crucial or merely a supportive role in mediating the mechano-inhibitory effects of the agonists at β-adrenoceptors is currently a matter of active debate (Jones *et al.*, 1990; Murray *et al.*, 1991; Cook & Small, 1991; 1992).

In the present study we have employed agonists (e.g. procaterol and salmeterol) and antagonists (e.g. CGP 20712A and ICI 118551) showing high selectivity for β_1- or β_2-adrenoceptors in experiments designed to determine which of the β-adrenoceptor subtypes mediates plasmalemmal K^+-channel opening in trachealis muscle. Other objectives of the present experiments were to shed more light on the role of K^+-channel opening in mediating the mechano-inhibitory effects of the agonists at β-adrenoceptors and to characterize further the pharmacological properties of the novel, long-acting β_2-adrenoceptor agonist, salmeterol (Ball *et al.*, 1991; Dougall *et al.*, 1991). The present electrophysiological and mechanical studies of guinea-pig trachealis were carried out in parallel with studies of mechanical activity and $^{86}Rb^+$ efflux in bovine trachealis (Chiu *et al.*, 1993). Some of our findings have been the subject of communications to the British Pharmacological Society (Chiu *et al.*, 1992; Cook *et al.*, 1992; Cook & Small, 1992).

Methods

Preparation of isolated tissues

Guinea-pigs of either sex (weight 300–800 g) were killed by stunning and bleeding. The heart was quickly excised from each animal and immersed in Krebs solution. Following removal of the pericardium, ventricular tissue was dissected from the atria taking care to preserve the integrity of the atrioventricular septum. Tracheae were excised from the animals, cleaned of adhering adipose and connective tissue and opened by cutting longitudinally, diametrically opposite the trachealis.

Tissue bath studies with isolated atria

Isolated, spontaneously-beating atria were set up in Krebs solution (37.5°C) under an initial resting tension of 1 g. Atrial tension changes were recorded with a force displacement transducer coupled to a Grass 7P1 preamplifier mounted in a Grass model 79D polygraph. The tension change signal was used to trigger a Grass model 7P44 tachograph so that recordings of atrial tension and rate of beating were made simultaneously. Once atrial rate had stabilized, test tissues were treated with CGP 20712A (100 nM, 1 μM or 10 μM) or ICI 118551 (100 nM, 300 nM, 1 μM or 10 μM) for 20 min prior to constructing a cumulative log concentration-chronotropic effect curve for noradrenaline (10 nM–100 μM) in the presence of one of the antagonists at β-adrenoceptors. Concentration increments for noradrenaline were made at 3 min intervals. Time-matched control tissues were treated identically to test tissues except that they were not exposed to CGP 20712A or ICI 118551.

Tissue bath studies with isolated trachea

Small segments of trachea were set up for the isometric recording of tension changes essentially as described by Foster *et al.* (1983). At the onset of each experiment, tissues were subjected to an imposed tension of 1.5 g. Approximately 20 min later, aminophylline (1 mM) was added in order to determine the recorder pen position at zero tissue tone. The aminophylline was removed from the tissue (initial wash followed by two further washes at 10 min intervals) and when spontaneous tone subsequently became maximal (40 min after initial wash), study of bronchodilator drugs commenced. A cumulative log concentration-effect curve against spontaneous tone was constructed for one of the following: cromakalim (100 nM–10 μM), isoprenaline (1–100 nM), procaterol (0.1–30 nM), salbutamol (1 nM–1 μM) or theophylline (1 μM–1 mM) in each test tissue. The concentration of isoprenaline was increased at 4 min intervals, that of procaterol, salbutamol or theophylline at 5 min intervals and that of cromakalim at 8 min intervals. Once the response to the highest concentration of the relaxant agonist had equilibrated, aminophylline was added to determine the recorder pen position at zero tissue tone. All relaxant responses were measured in terms of the maximal response to aminophylline. Following washout of the relaxant agonist, CGP 20712A (100 nM, 1 μM or 10 μM) or ICI 118551 (10 nM, 100 nM or 1 μM) was incubated with the tissue for a period of 40 min. The log concentration-effect curve for the relaxant agonist was then reconstructed in the presence of the antagonist. Time-matched control tissues were treated identically to test tissues but were not exposed to CGP 20712A or ICI 118551.

Responses of the isolated trachea to salmeterol required a protracted period (>30 min) to equilibrate and such responses proved very difficult to reverse by repeated changes of the bath fluid. Accordingly, salmeterol was studied by a modified protocol. Test tissues were preincubated (40 min) with CGP 20712A (100 nM, 1 μM or 10 μM) or ICI 118551 (10 nM, 100 nM or 1 μM) for 40 min prior to the application of a single concentration of salmeterol (1 nM, 10 nM, 100 nM, 1 μM, 10 μM or 100 μM). The relaxant response to salmeterol was allowed to develop for a period of 45 min before the addition of aminophylline (1 mM) in order to determine the pen position corresponding to zero tissue tone. Time-matched control tissues were treated similarly but were not exposed to CGP 20712A or ICI 118551.

A further group of experiments was performed in order to determine whether salmeterol might (as reported by Dougall *et al.*, 1991) act as a partial agonist at β_2-adrenoceptors. In these experiments, indomethacin (2.8 μM) was added to the Krebs solution 20 min after the initial setting up of the tracheal segments. Following indomethacin-induced suppression of spontaneous tissue tone (60 min), carbachol (10 μM) was added to the bath fluid. Once the spasmogenic response to carbachol had equilibrated (40 min), a cumulative log concentration-relaxation curve for isoprenaline (1 nM–10 μM) was constructed, essentially as described above. Following isoprenaline washout (40 min), test tissues were incubated with salmeterol (1 μM) for 45 min before reconstructing the log concentration-relaxation curve for isoprenaline in the presence of the salmeterol. Time-matched control tissues were treated similarly but were not exposed to salmeterol. Similar experiments were performed using aminophylline (1 μM–30 mM) instead of isoprenaline.

Intracellular electrophysiological recording from trachealis

Simultaneous recording of intracellular electrical activity and mechanical changes of a contiguous segment of trachea was performed with the technique and tissue holder described by Dixon & Small (1983). In brief, part of the trachealis was immobilized to permit long-term electrical recording while mechanical activity of contiguous muscle bundles was measured under an initial, imposed tension of 1.5 g. The recording microelectrodes were filled with 3 M KCl and were of resistance greater than 40MΩ.

After impalement of a trachealis cell, several minutes were allowed to elapse to check the stability of the record of electrical activity. Tracheal relaxant drugs (cromakalim, 10 μM; isoprenaline, 100 nM; procaterol, 10 nM; salbutamol, 10 nM, 100 nM or 1 μM; salmeterol, 10 nM, 100 nM or 1 μM) were then studied by their addition to the Krebs solution superfusing the tissue. Whenever possible, the effects of relaxant drugs on the electrical activity of the impaled cell were monitored for periods of 4 (isoprenaline), 5 (salbutamol), 6 (procaterol), 8 (cromakalim) or 14 min (salmeterol). When the effects of the tracheal relaxant drugs were examined in the presence of a modifying agent (CGP 20712A, ICI 118551 or salmeterol), the modifying agent was allowed to pre-equilibrate with the tissue for at least 20 min.

Drugs and solutions/statistical analysis of results

Drug concentrations are expressed in terms of the molar concentration of the active species. The following substances were used: aminophylline (BDH), CGP 20712A (2-hydroxy-5-(2-((2-hydroxy-3-(4-((1-methyl-4-trifluoro-methyl)-1H-imidazole-2-yl)-phenoxy)propyl)amino)ethoxy)-benzamide monomethanesulphonate; Ciba-Geigy Pharmaceuticals), cromakalim (SmithKline Beecham Pharmaceuticals), ICI 118551 (erythro-DL-1-(7-methylindan-4-yloxyl)-3-(isopropylamino-butan-2-ol hydrochloride); ICI Pharmaceuticals), (−)-isoprenaline hydrochloride (Sigma), (−)-noradrenaline bitartrate (Sigma), procaterol (Sigma), salbutamol (Glaxo Group Research), salmeterol (Glaxo Group Research), theophylline (Sigma). Stock solutions of most drugs were prepared in twice-distilled water. That of cromakalim was prepared in 70% ethanol. Isoprenaline, noradrenaline, salbutamol and salmeterol stock solutions were prepared in 0.1 M HCl. Dilutions of isoprenaline and noradrenaline were made with distilled water containing 0.57 mM ascorbic acid.

The Krebs solution used in the tissue bath experiments and for the microelectrode recording of membrane potential changes had the following composition (mM): Na^+ 143.5, K^+ 5.9, Ca^{2+} 2.6, Mg^{2+} 1.2, Cl^- 127.6, HCO_3^- 25, SO_4^{2-} 1.2, $H_2PO_4^-$ 1.2 and glucose 11.1 This solution (pH 7.4) was maintained at 37°C and was gassed with a mixture of 95% O_2:5% CO_2.

The significance of differences between means was assessed by use of a two-tailed, unpaired t test. The null hypothesis was rejected when $P < 0.05$.

Results

Studies with isolated atria

Noradrenaline (10 nM−100 μM) caused a concentration-dependent increase in the rate of atrial beating. CGP 20712A (100 nM−10 μM) did not significantly alter the resting atrial rate but antagonized noradrenaline in a concentration-dependent fashion. At a concentration of 1 μM, CGP 20712A caused a rightward shift of the noradrenaline log concentration-effect curve which was greater than 100 fold (Figure 1a). ICI 118551 (100 nM−10 μM) caused no change in the resting rate of atrial beating. At concentrations in the range 100−300 nM, ICI 118551 failed to modify the chronotropic action of noradrenaline. However, at concentrations in the range 1−10 μM, ICI 118551 antagonized noradrenaline in a concentration-dependent manner (Figure 1b).

Studies of the mechanical activity of isolated trachealis

Isoprenaline (1−100 nM; Figure 2), procaterol (0.1−30 nM; Figure 2), salbutamol (1 nM−1 μM) and salmeterol (1−100 nM; Figure 3) each caused concentration-dependent suppression of the spontaneous tone of guinea-pig isolated trachealis. Each of these agents was able to cause full suppression of the spontaneous tone of the trachea. The rank

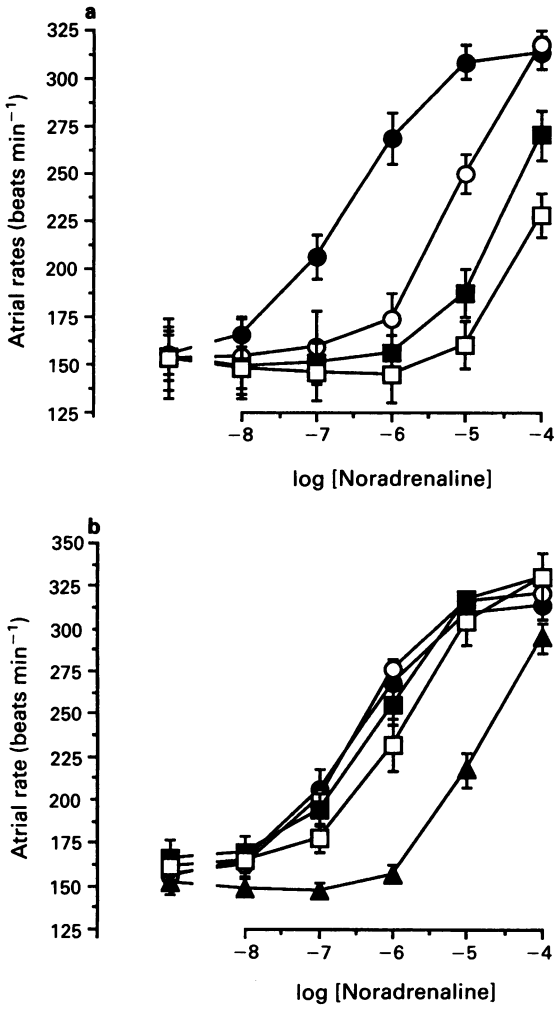

Figure 1 The effects of (a) CGP 20712A and (b) ICI 118551 against the chronotropic action of noradrenaline acting on guinea-pig isolated atria. In each panel the abscissa scale indicates the molar concentration of noradrenaline on a $\log_{10}$ scale while the ordinate scale indicates atrial rate in beats min^{-1}. In both panels (●) indicates the time-matched control log concentration-effect curve for noradrenaline. (a) Shows log concentration-effect curves for noradrenaline obtained in the presence of 100 nM (○), 1 μM (■) and 10 μM (□) CGP 20712A; (b) shows log concentration-effect curves for noradrenaline obtained in the presence of 100 nM (○), 300 nM (■), 1 μM (□) and 10 μM (▲) ICI 118551. All data points indicate the mean (±s.e.mean) of values obtained from at least 6 tissues. The data points shown on the extreme left in each panel indicate the resting atrial rate prior to the administration of noradrenaline.

order of relaxant potency for the agonists at β-adrenoceptors was procaterol > isoprenaline = salmeterol > salbutamol (Table 1). Cromakalim (100 nM−10 μM) and theophylline (1 μM−1 mM) also produced concentration-dependent suppression of tracheal tone. The maximal relaxant effect of theophylline was equivalent to that of aminophylline, whereas the maximal effect of cromakalim was 80−90% of that of aminophylline (data not shown).

CGP 20712A (in concentration up to 1 μM) did not alter the spontaneous tone of the trachea, nor did it antagonize isoprenaline, procaterol, salbutamol or salmeterol. However, at a concentration of 10 μM, CGP 20712A caused minor (< four fold) antagonism of procaterol and salbutamol (Figures 2 and 3; Table 1). CGP 20712A (1 μM) did not modify the relaxant actions of either cromakalim or theophylline (Table 1). Like CGP 20712A, ICI 118551 (10 nM−1 μM) did not alter the spontaneous tone of the trachea, nor did it (at a concentration of 100 nM) antagonize either cromakalim or theophylline (Table 1). However, ICI

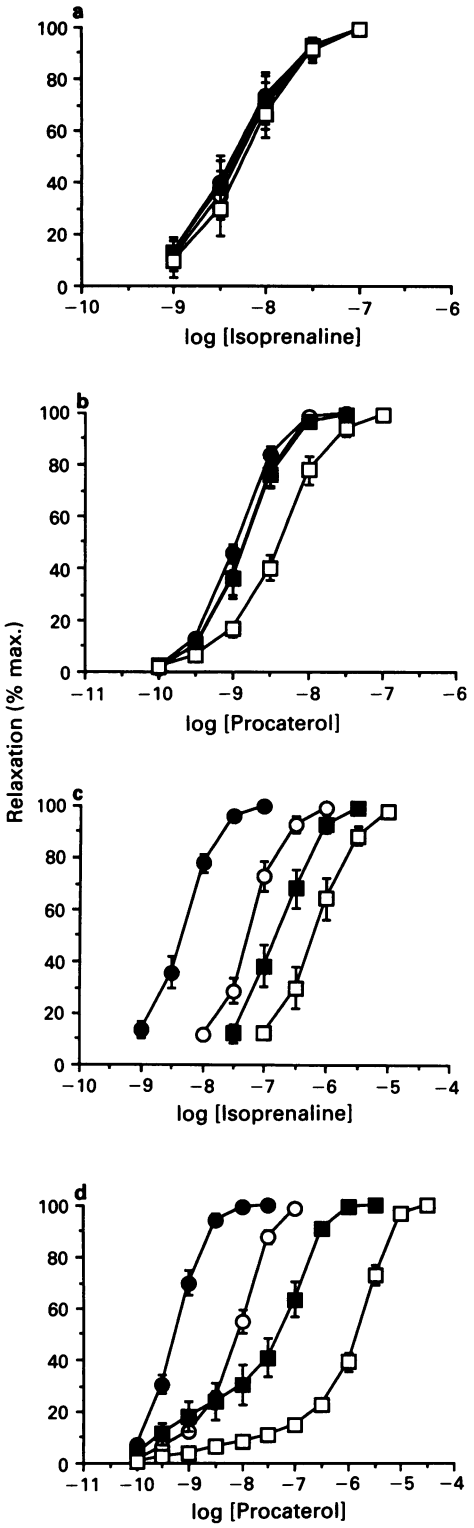

Figure 2 The effects of CGP 20712A and ICI 118551 against the actions of isoprenaline and procaterol in suppressing the spontaneous tone of guinea-pig isolated trachealis muscle. In each case the abscissa scale indicates the $\log_{10}$ molar concentration of isoprenaline or procaterol while the ordinate scale indicates relaxation expressed as a percentage of the maximal relaxation induced by aminophylline. In all panels (●) indicates the log concentration-effect curve of the agonist at β-adrenoceptors as observed in the absence of any antagonist. (a) and (b) show log concentration-effect curves for the agonists at β-adrenoceptors as observed in the presence of 100 nM (○), 1 μM (■) or 10 μM (□) CGP 20712A; (c) and (d) show log concentration-effect curves for the agonist at β-adrenoceptors as observed in the presence of 10 nM (○), 100 nM (■) or 1 μM (□) ICI 118551. In each panel all data points indicate the means of values from at least 6 issues ± s.e.mean.

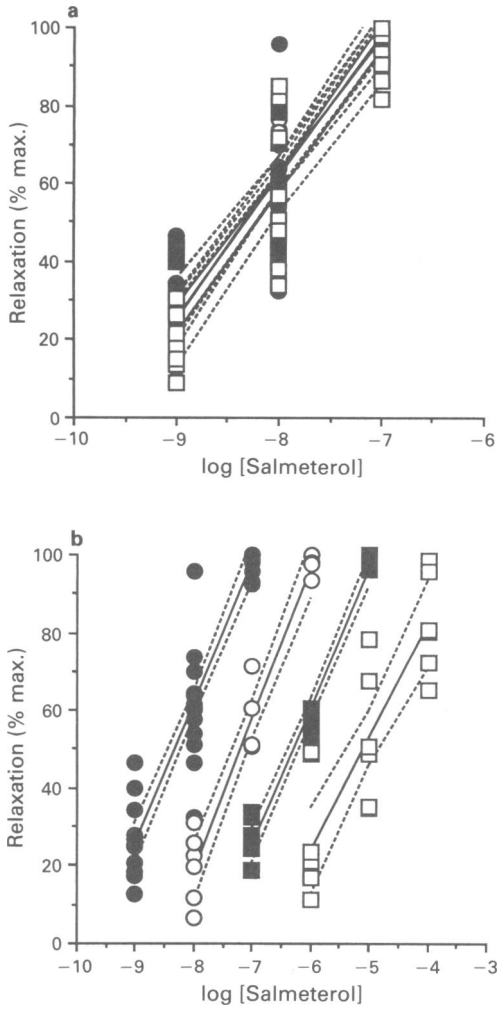

Figure 3 The effects of (a) CGP 20712A and (b) ICI 118551 against the action of salmeterol in suppressing the spontaneous tone of guinea-pig isolated trachealis muscle. In each case the abscissa scale indicates the $\log_{10}$ molar concentration of salmeterol and the ordinate scale indicates relaxation expressed as a percentage of the maximal relaxation induced by aminophylline. In both panels the solid lines indicate the linear regression of response versus the log molar concentration of salmeterol while the broken lines indicate the upper and lower 95% confidence limits of the regression lines. In (a) responses to salmeterol were obtained in the absence (●) and presence of 100 nM (○), 1 μM (■) and 10 μM (□) CGP 20712A. In (b) responses to salmeterol were obtained in the absence (●) and presence of 10 nM (○), 100 nM (■) and 1 μM (□) ICI 118551.

118551 antagonized isoprenaline, procaterol, salbutamol and salmeterol in a concentration-dependent manner (Figures 2 and 3, Table 1).

In tracheal segments treated with indomethacin (2.8 μM) and carbachol (10 μM), isoprenaline (1 nM–10 μM) and aminophylline (1 μM–30 mM) each evoked concentration-dependent relaxation. The incubation of test tissues with salmeterol (1 μM) caused some reduction (approximately 20%) in the carbachol-induced tone. This reduction in tone did not render the tissues more sensitive to isoprenaline. On the contrary, the log concentration-effect curve for isoprenaline seen in the salmeterol-treated tissues was shifted to the right ($-\log EC_{50}$ values for control and test tissues were 6.62 ± 0.06 and 5.59 ± 0.11 respectively; mean ± s.e.mean, $n = 6$) compared with that seen in the time-matched control tissues. Furthermore, the maximal response to isoprenaline was slightly reduced in the test tissues (data not shown). Salmeterol (1 μM) did not, however, antagonize aminophylline ($-\log EC_{50}$ values for control and test tissues 3.33 ± 0.15 and 3.64 ± 0.10 respectively) in suppressing carbachol-induced contraction of the trachea.

Table 1 The effects of CGP 20712A and ICI 118551 against the actions of cromakalim, theophylline and some agonists at β-adrenoceptors in suppressing the spontaneous tone of guinea-pig isolated trachealis

	Time-matched control	*CGP 20712A*		
Agonist	(no antagonist)	100 nM	1 μM	10 μM
Cromakalim	6.50 ± 0.10	†	6.53 ± 0.10	†
Theophylline	4.32 ± 0.06	†	4.29 ± 0.05	†
Isoprenaline	8.35 ± 0.10	8.27 ± 0.15	8.32 ± 0.15	8.23 ± 0.15
Procaterol	8.93 ± 0.05	8.85 ± 0.09	8.87 ± 0.08	8.36 ± 0.08*
Salbutamol	7.70 ± 0.10	7.63 ± 0.11	7.59 ± 0.11	7.26 ± 0.09*
Salmeterol	8.34 (8.21–8.50)	8.33 (8.21–8.45)	8.36 (8.22–8.52)	8.20 (8.06–8.36)
	Time-matched control	*ICI 118551*		
Agonist	(no antagonist)	10 nM	100 nM	1 μM
Cromakalim	6.09 ± 0.10	†	5.79 ± 0.10	†
Theophylline	4.13 ± 0.05	†	3.97 ± 0.04	†
Isoprenaline	8.39 ± 0.05	7.27 ± 0.07*	6.78 ± 0.12*	6.18 ± 0.12*
Procaterol	9.23 ± 0.06	8.07 ± 0.07*	7.47 ± 0.25*	5.84 ± 0.05*
Salbutamol	7.86 ± 0.11	6.54 ± 0.22*	5.50 ± 0.32*	4.36 ± 0.15*
Salmeterol	8.29 (8.14–8.45)	7.18* (7.06–7.32)	6.29* (6.21–6.37)	5.09* (4.84–5.36)

For most agents the data indicate mean (± s.e.mean) values of $-\log_{10} EC_{50}$. For salmeterol the mean $-\log_{10} EC_{50}$ and its lower and upper 95% confidence limits are shown. For all agents, data from at least 6 tissues contribute to the mean.
†indicates a parameter not measured.
*indicates a value significantly ($P < 0.05$) different from that observed in the absence of the relevant antagonist.

Intracellular electrophysiological recording from trachealis

The addition of either cromakalim (10 μM) or isoprenaline (100 nM; Figures 4 and 6) to the Krebs solution superfusing the trachealis muscle reduced the mechanical tone of the tissue, suppressed spontaneous electrical slow waves and caused hyperpolarization of the impaled cell. In this respect we have been able to confirm the results of our earlier studies (Allen *et al.*, 1986). Procaterol (10 nM) and salbutamol (10 nM–1 μM) also produced relaxation accompanied by slow wave suppression and by cellular hyperpolarization (Figure 4 and Table 2).

As anticipated from the results of the tissue bath studies, salmeterol (10 nM–1 μM) produced relaxant effects, but these were very slow to develop compared with those of the other agonists at β-adrenoceptors. Salmeterol (10 nM–1 μM) failed to evoke significant hyperpolarization of the trachealis cells (Figure 5 and Table 2). At concentrations of 100 nM and 1 μM this agent did, however cause some reduction in slow wave amplitude or frequency (Figure 5b,c). Pretreatment of the trachealis with salmeterol (1 μM) abolished the mechanical tone of the tissue, failed to modify the hyperpolarization induced by cromakalim (10 μM) but reduced the ability of isoprenaline (100 nM) to cause hyperpolarization of the impaled cell (Table 2).

The addition of CGP 20712A (1 μM) to the Krebs solution superfusing the trachealis muscle modified neither the mechanical tone of the tissue nor the electrical behaviour of the impaled cell. CGP 20712A (1 μM) did not modify the relaxant action of cromakalim (10 μM) nor the ability of cromakalim to hyperpolarize the trachealis cells (Table 2). CGP 20712A (1 μM) did not reduce the relaxant action of isoprenaline (100 nM) but significantly reduced the hyperpolarization induced by isoprenaline (Figure 6 and Table 2).

ICI 118551 (100 nM) did not itself modify either the mechanical tone of the tissue or the ongoing electrical behaviour of the impaled cell. ICI 118551 (100 nM) also failed to modify electrical and mechanical responses of the trachea to cromakalim (Table 2). In contrast ICI 118551 (100 nM) significantly reduced both the relaxation induced by isoprenaline and the ability of isoprenaline to hyperpolarize the trachealis cells (Figure 6 and Table 2).

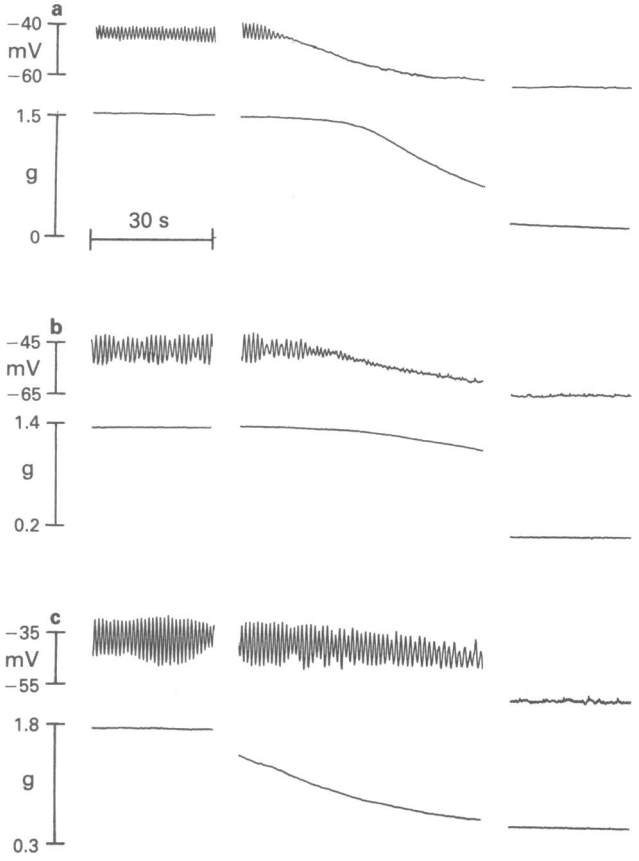

Figure 4 The effects of (a) isoprenaline (100 nM) (b) salbutamol (1 μM) and (c) procaterol (10 nM) on the electrical and mechanical activity of guinea-pig isolated trachealis. In each row of recordings the upper trace represents membrane potential changes recorded from a single cell while the lower trace represents tension changes recorded from a contiguous segment of trachea. In each row the left hand panel represents control activity recorded immediately prior to the administration of the agonist at β-adrenoceptors. The centre panels show activity recorded 45 s after the administration of the β-adrenoceptor agonist. The right hand panels indicate activity recorded 4 (isoprenaline), 5 (salbutamol) or 6 min (procaterol) after the administration of the β-adrenoceptor agonist.

Table 2 Some drug effects on the electrical and mechanical activity of guinea-pig isolated trachealis muscle

Agent (s)	*Hyperpolarization* (mV)	*Relaxation* (g)	(*n*)
Isoprenaline (100 nM)	13.6 ± 1.3*	1.20 ± 0.10	9
Isoprenaline (100 nM) + CGP 20712A (1 μM)	5.8 ± 1.4*†	1.10 ± 0.20	11
Isoprenaline (100 nM) + ICI 118551 (100 nM)	−0.4 ± 0.8†	0.14 ± 0.06	7
Isoprenaline (100 nM) + salmeterol (1 μM)	−4.1 ± 1.4*†	–	8
Procaterol (10 nM)	6.8 ± 2.4*	1.30 ± 0.10	9
Salbutamol (10 nM)	−0.3 ± 1.7	0.50 ± 0.10	8
Salbutamol (100 nM)	7.7 ± 1.5*	1.50 ± 0.20	9
Salbutamol (1 μM)	9.7 ± 1.6*	1.10 ± 0.10	6
Salmeterol (10 nM)	0.3 ± 1.4	0.38 ± 0.10	4
Salmeterol (100 nM)	3.4 ± 2.1	0.76 ± 0.11	12
Salmeterol (1 μM)	2.2 ± 1.1	0.89 ± 0.10	12
Cromakalim (10 μM)	23.7 ± 3.7*	0.90 ± 0.20	4
Cromakalim (10 μM) + CGP 20712A (1 μM)	21.0 ± 2.6*	0.70 ± 0.10	4
Cromakalim (10 μM) + ICI 118551 (100 nM)	22.3 ± 0.9*	0.91 ± 0.18	4
Cromakalim (10 μM) + salmeterol (1 μM)	23.0 ± 2.7*	–	4

Data indicate the means (± s.e.mean) of values from the indicated (*n*) number of tissues.
Measurements of membrane potential and tension changes were made 4, 5, 8, 6 and 14 min respectively after the administration of isoprenaline, salbutamol, cromakalim, procaterol and salmeterol.
*indicates a mean value of membrane potential change significantly ($P < 0.05$) different from zero.
†indicates a significant ($P < 0.05$) difference from the corresponding value for the agonist measured in the absence of any antagonist.

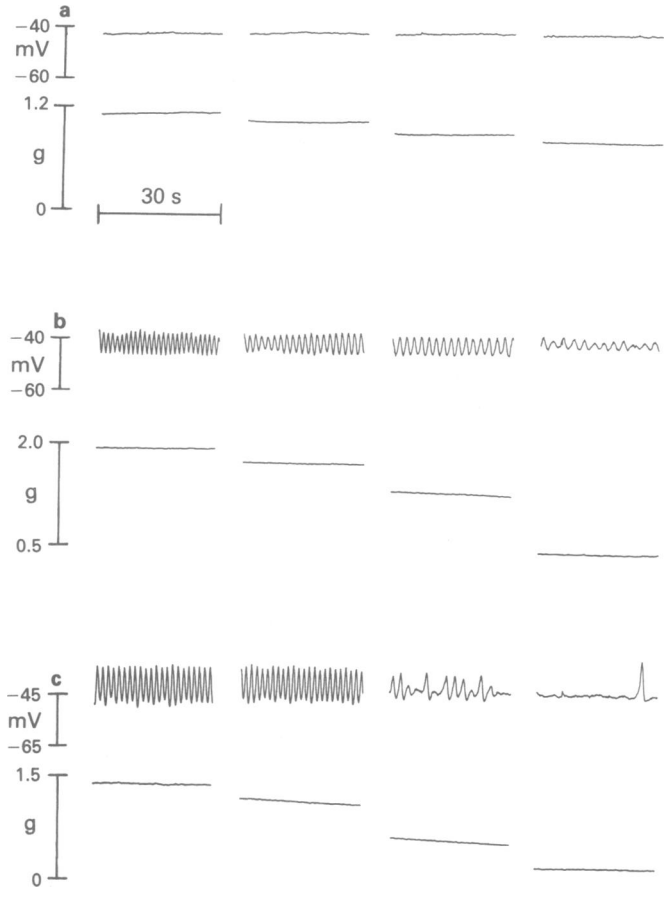

Figure 5 The effects of salmeterol 10 nM (a), 100 nM (b) and 1 μM (c) on the electrical and mechanical activity of guinea-pig isolated trachealis. In each row of recordings the upper trace represents membrane potential changes recorded from a single cell while the lower trace represents tension changes recorded from a contiguous segment of trachea. In each row the left hand panel represents control activity recorded immediately prior to the administration of salmeterol. The subsequent panels show activity recorded 1, 4 and 14 min respectively after the administration of salmeterol. Note that, at the outset, the cell illustrated in (a) lacked spontaneous electrical slow wave activity. Note also the failure of salmeterol (10 nM–1 μM) to cause cellular hyperpolarization.

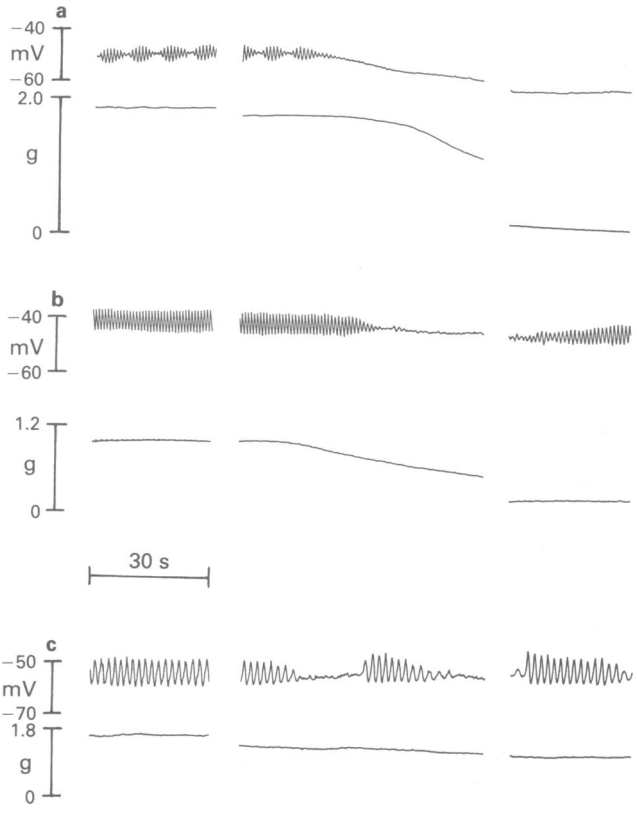

Figure 6 The effects of CGP 20712A and ICI 118551 on the electrical and mechanical responses of guinea-pig isolated trachealis to isoprenaline (100 nM). In each row of recordings the upper trace represents membrane potential changes recorded from a single cell while the lower trace represents tension changes recorded from a contiguous segment of trachea. In each row the left hand panel represents control activity recorded immediately prior to the addition of isoprenaline (100 nM). The subsequent panels show activity recorded 45 s and 4 min, respectively, after the addition of isoprenaline. (a) Shows the response to isoprenaline recorded in the absence of any modifying agent; (b) and (c) show responses to isoprenaline (100 nM) recorded from tissues equilibrated with CGP 20712A (1 μM) and ICI 118551 (100 nM) respectively. Note that CGP 20712A inhibited the hyperpolarization but not the relaxation induced by isoprenaline. Note also that ICI 118551 attenuated both the hyperpolarization and the relaxation induced by isoprenaline.

Discussion

Properties of CGP 20712A and ICI 118551

In the present study, CGP 20712A (100 nM–10 μM) failed to modify the spontaneous tone or spontaneous electrical activity of guinea-pig trachealis muscle. CGP 20712A (1 μM) failed to inhibit the tracheal relaxant action of theophylline (Table 1). Furthermore, the same concentration of CGP 20712A failed to inhibit the tracheal relaxation or the cellular hyperpolarization induced by the potassium channel opener, cromakalim (Tables 1 and 2). Collectively, these findings suggest that CGP 20712A (100 nM–10 μM) does not directly inhibit the plasmalemmal K^+-channels controlling the excitability of the trachealis cells nor the K^+-channel (K_{KCO}) involved in the action of cromakalim. The fact that CGP 20712A (100 nM–1 μM) antagonized noradrenaline in increasing atrial rate (Figure 1) without antagonizing isoprenaline, procaterol, salbutamol or salmeterol in suppressing tracheal tone (Figures 2 and 3; Table 1) is entirely consistent with reports (Dooley & Bittinger, 1984; Lemoine *et al.*, 1985) that CGP 20712A is an antagonist having very much higher (at least 10,000 times) affinity for β_1- than for β_2-adrenoceptors.

ICI 118551 (10 nM–100 nM) failed to modify the spontaneous tone or the spontaneous electrical activity of guinea-pig trachealis muscle. ICI 118551 (100 nM) failed to inhibit the tracheal relaxant action of theophylline (Table 1). Furthermore, the same concentration of ICI 118551 failed to inhibit the tracheal relaxation or the cellular hyperpolarization induced by cromakalim (Tables 1 and 2). Collectively, these observations suggest that, like CGP 20712A, ICI 118551 does not directly inhibit the plasmalemmal K^+-channels controlling the excitability of the trachealis cells nor does it directly inhibit K_{KCO}. Our observation that ICI 118551 (10–100 nM) antagonized isoprenaline, procaterol, salbutamol and salmeterol in suppressing tracheal tone without antagonizing noradrenaline in increasing atrial rate (Figures 1, 2 and 3; Table 1) is entirely consistent with reports (O'Donnell & Wanstall, 1980; Bilski *et al.*, 1983; Rimele *et al.*, 1988) that ICI 118551 is an antagonist having much higher (50–125 times) affinity for β_2-adrenoceptors than for β_1-adrenoceptors.

The results of our mechanical studies in the atria and trachea suggest that CGP 20712A (1 μM) produces marked antagonism at β_1-adrenoceptors with negligible concurrent antagonism at β_2-adrenoceptors. Similarly, it may be suggested that ICI 118551 (100 nM) produces marked antagonism at β_2-adrenoceptors with negligible concurrent antagonism at β_1-adrenoceptors.

Effects of salmeterol on the electrical and mechanical activity of trachealis muscle

Earlier reports (Ball *et al.*, 1991; Dougall *et al.*, 1991) that salmeterol exerts relaxant effects in trachealis muscle, that such effects require a protracted (>20 min) period to reach equilibrium and are poorly reversible by removal of the drug from the bathing medium have been confirmed in the present study. The claim (Ball *et al.*, 1991) that salmeterol is an agonist that is highly selective for β_2- as opposed to β_1-adrenoceptors is supported by the present findings that the tracheal relaxant action of salmeterol was antagonized by ICI 118551 but not by CGP 20712A (Figure 3 and Table 1).

Other agonists (i.e. salbutamol and procaterol) with reported selectivity for β_2- as opposed to β_1-adrenoceptors had relaxant effects in guinea-pig trachealis that were associated with hyperpolarization of the trachealis cells (Figure 4 and Table 2). In view of this, we were surprised to observe (Figure 5 and Table 2) that relaxant concentrations of salmeterol, while causing some inhibition of slow wave activity, failed significantly to modify the membrane potential of the trachealis cells.

We considered the possibility that salmeterol might, in addition to acting as an agonist at β_2-adrenoceptors, stabilize the trachealis cell membrane in a non-specific manner. A secondary action of this kind might prevent expression of any membrane hyperpolarization induced by the activation of β_2-adrenoceptors. However, salmeterol failed to reduce cromakalim-induced hyperpolarization (Table 2) suggesting that, at concentrations causing suppression of the spontaneous tone of guinea-pig trachea, salmeterol does not stabilize the plasmalemma by a non-specific mechanism.

Several groups of investigators (e.g. Ball *et al.*, 1991; Dougall *et al.*, 1991; Waldeck & Kallstrom, 1991) have reported that salmeterol is a partial rather than a full agonist at β_2-adrenoceptors. Our finding that salmeterol antagonized the relaxant action of isoprenaline (but not that of aminophylline) in carbachol-treated trachea supports this idea. It may also explain our observation that salmeterol could inhibit the hyperpolarization of trachealis cells induced by isoprenaline (Table 2). We therefore propose that the failure of salmeterol to induce significant hyperpolarization of the trachealis cells is a reflection of its low intrinsic efficacy (Dougall *et al.*, 1991) at β_2-adrenoceptors.

Subtypes of β-adrenoceptor and the activation of plasmalemmal K^+-channels

Electrophysiological studies of guinea-pig trachealis have shown that the relaxant action of isoprenaline is accompanied by cellular hyperpolarization (Allen *et al.*, 1985; Honda *et al.*, 1986) whereas no such membrane potential change accompanies the relaxant action of salmeterol (Cook & Small, 1992; Figure 5). In view of the high selectivity of salmeterol as an agonist at β_2- rather than β_1-adrenoceptors (Ball *et al.*, 1991) and the failure of salmeterol to evoke hyperpolarization of trachealis muscle, Cook & Small (1992) proposed that activation of β_1- but not β_2-adrenoceptors might promote the opening of plasmalemmal K^+-channels. However, several observations now suggest that this hypothesis is untenable.

Procaterol, salbutamol and terbutaline are all agonists selective for β_2- as opposed to β_1-adrenoceptors. Procaterol causes hyperpolarization of trachealis muscle from the guinea-pig (Figure 4) and the dog (Fujiwara *et al.*, 1988). Terbutaline (Honda *et al.*, 1986) and salbutamol (Figure 4) both induce hyperpolarization of guinea-pig trachealis muscle. Furthermore, as discussed above, the failure of salmeterol significantly to hyperpolarize guinea-pig trachealis may be attributed not to its great selectivity in activating β_2- as opposed to β_1-adrenoceptors, but instead to its low intrinsic efficacy at β_2-adrenoceptors. Therefore, although salmeterol fails to hyperpolarize trachealis cells, the ability of procaterol, salbutamol and terbutaline to induce cellular hyperpolarization suggests that the activation of β_2-adrenoceptors can promote plasmalemmal K^+-channel opening. This suggestion receives strong support from our findings (Figure 6, Table 2) that ICI 118551 (100 nM) ablated the tracheal hyperpolarization induced by isoprenaline.

Isoprenaline-induced hyperpolarization of guinea-pig trachealis was also significantly attenuated by CGP 20712A (1 μM; Figure 6; Table 2) suggesting that the activation of β_1-adrenoceptors can also promote K^+-channel opening in the plasmalemma. We thus conclude that the activation of either β_1- or β_2-adrenoceptors can promote K^+-channel opening in trachealis muscle. This conclusion receives strong support from the results of our studies of $^{86}Rb^+$ efflux from bovine trachealis (Chiu *et al.*, 1993).

Role of plasmalemmal K^+-channel opening in mediating the tracheal relaxation induced by agonists at β-adrenoceptors

In guinea-pig trachealis (Jones *et al.*, 1990; Murray *et al.*, 1991) and human bronchus (Miura *et al.*, 1992) charybdotoxin inhibits the relaxant effects of agonists at β-adreno-

ceptors such as isoprenaline and salbutamol. Since charybdotoxin has been reported to inhibit BK_{Ca} channels in airways smooth muscle (Green *et al.*, 1991; Murray *et al.*, 1991), these findings suggest that the opening of BK_{Ca} channels contributes to the relaxant activity of the agonists at β-adrenoceptors. It is commonly assumed that BK_{Ca} channel opening causes plasmalemmal hyperpolarization and therefore causes relaxation by inhibiting Ca^{2+} influx through voltage-operated channels. However, a number of findings now suggest that such a mechanism cannot play a crucial role in mediating the mechano-inhibition induced by the agonists at β-adrenoceptors.

In guinea-pig trachealis, for example, low concentrations of isoprenaline evoke relaxation without causing hyperpolarization (Allen *et al.*, 1985). In the same tissue, salmeterol is capable of fully suppressing spontaneous tone without causing significant change in membrane potential (Cook & Small, 1992; Figure 5 and Table 2). The K^+-channel inhibitor, procaine, can abolish the trachealis muscle hyperpolarization induced by isoprenaline without markedly affecting its relaxant activity (Allen *et al.*, 1985). Furthermore, CGP 20712A, an antagonist selective for β_1-adrenoceptors, can markedly attenuate the hyperpolarization but not the relaxation of guinea-pig trachealis induced by isoprenaline (Cook *et al.*, 1992; Figures 2 and 6 and Table 2). The relaxant activity of isoprenaline and salbutamol in trachealis is also largely retained when the tissue is bathed by a K^+-rich (80–120 mM) medium of osmolality similar to that of Krebs solution (Kumar, 1978; Allen *et al.*, 1985; Small *et al.*, 1989; Cook & Small, 1991). In this situation the K^+ equilibrium potential and the resting membrane potential are so closely apposed that K^+-channel opening can cause very little hyperpolarization. Accordingly, agonists at β-adrenoceptors cannot be expected to cause hyperpolarization sufficient to ensure the closure of voltage-operated Ca^{2+}-channels and hence the inhibition of Ca^{2+} influx. Unless K^+-channel opening can evoke relaxation by mechanisms not depending on hyperpolarization of the plasmalemma, we can conclude that K^+-channel opening induced by the agonists at β-adrenoceptors plays merely a supportive and not a crucial role in mediating their mechano-inhibitory effects.

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