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Cromakalim-induced relaxation of guinea-pig isolated trachealis: antagonism by glibenclamide and by phentolamine

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1 Tested against the spontaneous tone of guinea-pig isolated trachealis, cromakalim (0.1–100 μM), isoprenaline (1 nM–1 μM) and theophylline (1 μM–1 mM) each produced concentration-dependent relaxation.

2 Glibenclamide (0.1–10 μM) did not itself alter the spontaneous tone of the trachea nor did it modify the relaxant actions of isoprenaline or theophylline. In contrast, glibenclamide (0.1 and 1 μM) caused a concentration-dependent rightward shift of the log concentration-effect curve of cromakalim. Glibenclamide (10 μM) reduced the slope of the log concentration-effect curve of cromakalim and moved the foot of the curve back towards the control position.

3 Phentolamine (1, 10 and 100 μM) did not itself alter the spontaneous tone of the trachea nor did it modify the relaxant actions of isoprenaline or theophylline. In contrast phentolamine caused concentration-dependent depression of the log concentration-effect curve of cromakalim.

4 Neither prazosin (1 μM) nor yohimbine (10 μM) modified the spontaneous tone of the trachea. Prazosin and yohimbine each failed to antagonise the effects of cromakalim, isoprenaline and theophylline.

5 Intracellular electrophysiological recording showed that glibenclamide (1 μM) and phentolamine (100 μM) caused minor change in the resting membrane potential of trachealis cells. Slow wave activity was slightly depressed by these agents. In contrast tetraethylammonium (TEA; 8 mM) caused marked depolarisation, and promoted the conversion of slow waves into regenerative action potentials. These electrical changes were accompanied by tonic tension development.

6 Phentolamine (100 μM) and glibenclamide (1 μM) reduced and reversed both the relaxation and the hyperpolarisation induced by cromakalim (10 μM).

7 It is concluded that glibenclamide and phentolamine each provide selective antagonism of the relaxant action of cromakalim in guinea-pig trachealis. These agents also inhibit the plasmalemmal hyperpolarisation induced by cromakalim. The effect of phentolamine is unrelated to the blockade of α_1- or α_2-adrenoceptors. If either glibenclamide or phentolamine act to block the K^+ channels opened by cromakalim, then such channels are not identical to those which endow the trachealis plasmalemma with its powerful rectifying behaviour.

Introduction

The K^+ channel opening drug, cromakalim, causes concentration-dependent suppression of the spontaneous tone of guinea-pig isolated trachealis (Allen *et al.*, 1986; Arch *et al.*, 1988). This effect is associated both with the stimulation of $^{86}Rb^+$ efflux and with hyperpolarisation of the trachealis plasmalemma to a value close to the K^+ equilibrium potential (Allen *et al.*, 1986). K^+ channel blocking agents such as procaine and tetraethylammonium (TEA) inhibit cromakalim-induced hyperpolarisation and relaxation (Allen *et al.*, 1986) but since these agents are known to block several types of K^+ channel (Yamanaka *et al.*, 1985; Cook, 1988), they do not serve to identify the K^+ channel opened by cromakalim. In guinea-pig trachealis this channel is not sensitive to apamin (Allen *et al.*, 1986).

It has recently been shown (Quast & Cook, 1988; Wilson *et al.*, 1988; Newgreen *et al.*, 1989) that glibenclamide can antagonise cromakalim-induced relaxation of vascular smooth muscle. Furthermore,

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McPherson & Angus (1989) have shown that phentolamine can antagonise the effects of cromakalim on vascular smooth muscle by an action unrelated to the blockade of α-adrenoceptors. Observations of this kind prompted the experiments of the present study, in which we have sought to determine whether glibenclamide and phentolamine can provide selective antagonism of the effects of cromakalim on trachealis muscle. It was reasoned that such experiments might shed more light on the nature of the K^+ channel opened by cromakalim in this tissue. A preliminary account of this work has been communicated to the British Pharmacological Society (Murray & Small, 1989).

Methods

Guinea-pigs (350–550 g) of either sex were killed by stunning and bleeding. Tracheae were excised, cleaned of adhering adipose and connective tissue and opened by cutting longitudinally through the cartilage rings diametrically opposite the trachealis.

Tissue bath studies of mechanical activity

Small segments of trachea were set-up for the isometric recording of tension changes essentially as described by Foster *et al.* (1983). At the outset of each experiment tissues were subjected to an imposed tension of 1.25 g. Approximately 20 min later, aminophylline (1 mM) was added in order to determine the recorder pen position at zero tone. The aminophylline was then washed from the tissues and when tone subsequently reached its peak value, study of relaxant drugs commenced.

Cumulative concentration-effect curves were constructed for all relaxant drugs examined. Three fold, concentration increments were used for cromakalim, and ten fold increments for isoprenaline and theophylline. Each concentration increment was allowed 8 min, 4 min and 5 min tissue contact respectively for these drugs.

Pilot experiments revealed that some tissue desensitization to cromakalim occurred when cumulative concentration-effect curves for this agent were repeatedly constructed on the tissue. Accordingly, desensitization to cromakalim was minimised by interposing a concentration-effect curve for theophylline between successive cromakalim curves. Following construction of initial log concentration-effect curves for cromakalim and theophylline, test tissues were exposed to an antagonist (glibenclamide, phentolamine, prazosin or yohimbine). Forty min later, concentration-effect curves for cromakalim and theophylline were reconstructed in the presence of the antagonist. Isoprenaline was studied in a similar fashion except that concentration-effect curves for theophylline were not interposed between successive isoprenaline curves. In all experiments time-matched control tissues were treated identically to test tissues but were not exposed to the antagonist under investigation.

Intracellular electrophysiological recording

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). Accordingly, part of the trachealis was immobilized to permit long-term intracellular electrical recording, while mechanical activity of contiguous muscle bundles was measured under an initial imposed tension of 1.25 g. The recording microelectrodes were filled with 3 M KCl and were of resistance greater than 40 MΩ.

Three types of electrophysiological experiments were performed. In the first type of experiment, a trachealis cell was impaled and, after a lapse of several minutes to check the stability of the electrical record, glibenclamide (1 μM), phentolamine (100 μM) or tetraethylammonium (TEA, 8 mM) was added to the Krebs solution superfusing the tissue. The effects of each of these agents on electrical and mechanical activity were monitored for a minimum of 10 min.

The second type of experiment was conducted either in normal Krebs solution or in Krebs solution containing glibenclamide (1 μM) or phentolamine (100 μM). Following cellular impalement, cromakalim (10 μM) was added to the superfusing solution and the effects of cromakalim (in the absence and presence of glibenclamide or phentolamine) on electrical and mechanical activity were monitored for a minimum of 4 min.

The third type of electrophysiological experiment was conducted in normal Krebs solution. Following cellular impalement, cromakalim (10 μM) was added to the superfusing solution. Eight min later, glibenclamide (1 μM) or phentolamine (100 μM) was added to the superfusate. The effects of the drug combination on the electrical and mechanical activity were monitored for as long as the microelectrode tip remained within the impaled cell.

Drugs, solutions and statistical analysis of results

Drug concentrations are expressed in terms of the molar concentration of the active species. The following drugs were used: aminophylline (BDH), cromakalim (Beecham Research Laboratories), glibenclamide (Hoechst), (−)-isoprenaline hydro-

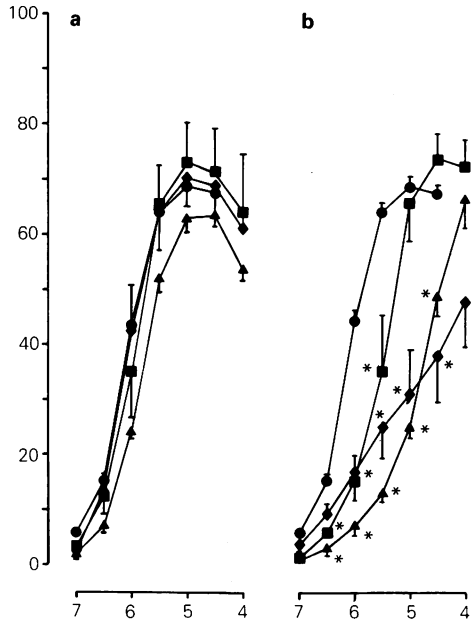

Figure 1 Cromakalim-induced relaxation of guinea-pig isolated trachealis: antagonism by glibenclamide. Abscissae: molar concentration of cromakalim on a log scale. Ordinate scale: relaxation expressed as a percentage of the maximal relaxation induced by aminophylline. (a) Time-matched control tissues. (b) Test tissues. In each case (●) indicates the pooled, initial log concentration-effect curve for cromakalim. Subsequent log concentration-effect curves were constructed in the absence (a, time-matched controls) or presence (b, test tissues) of glibenclamide 0.1 μM (■), 1 μM (▲) or 10 μM (◆). Data points are the means of values from at least six tissues. Vertical lines show s.e.mean. *indicates a significant ($P < 0.05$) difference from the corresponding point in the time-matched control tissues.

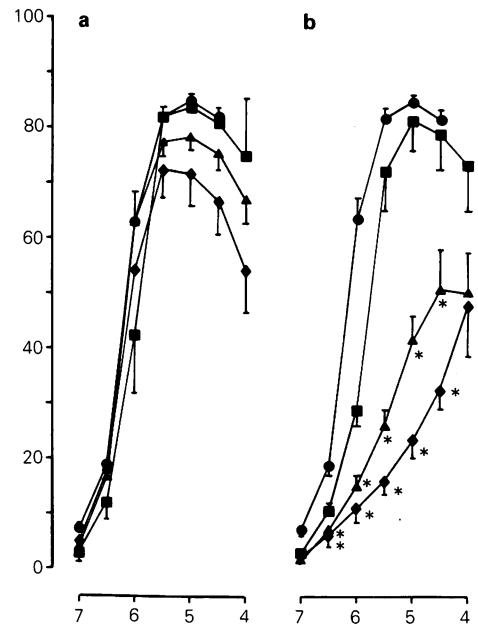

Figure 2 Cromakalim-induced relaxation of guinea-pig isolated trachealis: antagonism by phentolamine. Abscissae: molar concentration of cromakalim on a log scale. Ordinate scale: relaxation expressed as a percentage of the maximal relaxation induced by aminophylline. (a) Time-matched control tissues. (b) Test tissues. In each case (●) indicates the pooled, initial log concentration-effect curve for cromakalim. Subsequent log concentration-effect curves were constructed in the absence (a, time-matched controls) or presence (b, test tissues) of phentolamine 1 μM (■), 10 μM (▲) or 100 μM (◆). Data points are the means of values from at least six tissues. Vertical lines show s.e.mean. *indicates a significant ($P < 0.05$) difference from the corresponding point in the time-matched control tissues.

chloride (Sigma), phentolamine mesylate (Ciba), prazosin hydrochloride (Pfizer), tetraethylammonium bromide (Sigma), theophylline (Sigma), yohimbine hydrochloride (Sigma). While stock solutions of most drugs were prepared in distilled water, those of cromakalim were prepared in 70% v/v ethanol and those of glibenclamide and phentolamine in absolute ethanol. A stock solution of isoprenaline was prepared in 0.1 M HCl and dilutions of this agent were prepared in distilled water containing 0.57 mM ascorbic acid as an antioxidant.

The Krebs solution used in all the experiments 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.

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

Results

Tissue bath studies of mechanical activity

Cromakalim (0.1–100 μM; Figures 1, 2, 3), isoprenaline (1 nM–1 μM) and theophylline (1 μM–1 mM) each produced concentration-dependent suppression of the spontaneous tone of the trachea. As observed previously (Small *et al.*, 1989), the maximal relaxant effects of isoprenaline and theophylline were identical to that of aminophylline. In contrast the maximal relaxant effect of cromakalim was approximately 70–80% (Figures 1, 2, 3) of that of aminophylline.

Glibenclamide (0.1–10 μM) caused little, if any change in the tone of the trachea (Figure 4) and did not modify the relaxant actions of either isoprenaline or theophylline. Mean (±s.e.mean; $n > 6$) pD_2 values in the absence and in the presence of 10 μM

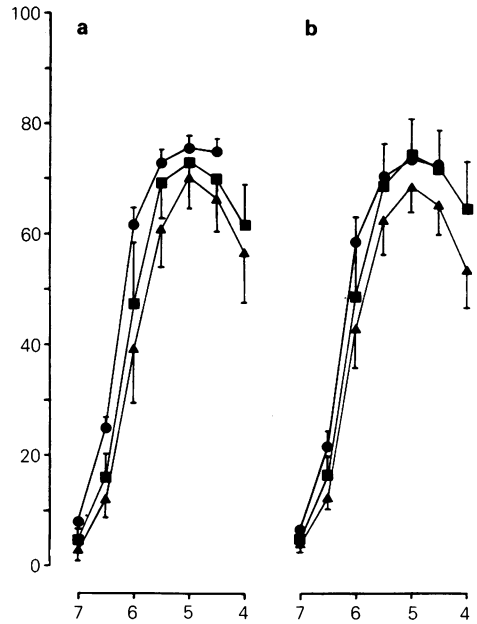

Figure 3 Cromakalim-induced relaxation of guinea-pig isolated trachealis: the failure of prazosin (1 μM) or yohimbine (10 μM) to provide antagonism. Abscissae: molar concentration of cromakalim on a log scale: Ordinate scale: relaxation expressed as a percentage of the maximal relaxation induced by aminophylline. (a) Effects of prazosin (1 μM). (b) Effects of yohimbine (10 μM). In each case (●) indicates the pooled, initial log concentration-effect curve for cromakalim. Subsequent log concentration-effect curves were constructed in the presence of the putative antagonist ((■); test tissues) or its vehicle ((▲); time-matched control tissues). Data represent the means of values from at least six tissues. Vertical lines show s.e.mean. Note that prazosin (1 μM) and yohimbine (10 μM) each failed to antagonise cromakalim.

glibenclamide were 7.90 ± 0.12 and 8.13 ± 0.08 respectively for isoprenaline and 4.08 ± 0.07 and 4.23 ± 0.12 respectively for theophylline. In contrast, glibenclamide antagonised the action of cromakalim. Glibenclamide (0.1 and 1 μM) caused a concentration-dependent rightward shift of the log concentration-effect curve for cromakalim without changing the slope or maximal effect. However, glibenclamide (10 μM) reduced the slope of the log concentration-effect curve of cromakalim and moved the curve leftwards back towards the pre-glibenclamide position (Figure 1).

Phentolamine (1–100 μM) also caused little, if any, change in the tone of the trachea (Figure 4) and did not modify the relaxant action of either isoprenaline or theophylline. Mean ($n > 6$) pD_2 values in the absence and in the presence of 100 μM phentolamine were 8.31 ± 0.06 and 8.37 ± 0.09 respectively for isoprenaline and 4.29 ± 0.05 and 4.27 ± 0.06 respectively for theophylline. In contrast, phentolamine antagonised the action of cromakalim. Phentolamine (1 μM) caused a small rightward shift in the log concentration-effect curve for cromakalim without depressing the maximal response. At a higher concentration (10 μM and 100 μM) phentolamine reduced the slope of the log concentration-effect curve for cromakalim and reduced the maximal response (Figure 2).

Neither prazosin (0.01–1.0 μM) nor yohimbine (1–10 μM) caused a change in the tone of the trachea, or modified the relaxant actions of isoprenaline or theophylline. Furthermore, neither prazosin (1 μM) nor yohimbine (10 μM) modified the relaxant action of cromakalim (Figure 3).

Intracellular electrophysiological recordings

The effects of glibenclamide, phentolamine and TEA on the spontaneous electrical and mechanical activity of the trachea were each examined on a minimum of six different tissues. Glibenclamide (1 μM) or phentolamine (100 μM) added to the Krebs solution superfusing the trachea produced little or no change in mechanical tone. Monitored over a 10 min period, these agents caused some suppression of spontaneous slow wave activity. Glibenclamide did not significantly alter the resting membrane potential but phentolamine always evoked a slight depolarisation (Table 1 and Figure 4). In contrast, TEA (8 mM) caused marked depolarisation of the trachealis cells, increased or initiated slow wave activity and promoted the conversion of slow waves into regenerative action potentials. These electrical changes were accompanied by tonic tension development (Table 1 and Figure 4).

As we have previously demonstrated (Allen *et al.*, 1986), treatment of the trachealis muscle with cromakalim (10 μM) suppressed spontaneous slow waves, hyperpolarised the trachealis cells and relaxed the tissue (Figure 5). When the same concentration of cromakalim was tested in tissues equilibrated with Krebs solution containing glibenclamide (1 μM) or phentolamine (100 μM), the hyperpolarisation (Figure 5 and Table 2) and the relaxation induced by cromakalim were both reduced. That glibenclamide and phentolamine could antagonise both the hyperpolarisation and the relaxation induced by cromakalim was also demonstrated in experiments where the tissues were first exposed to cromakalim and then glibenclamide, or phentolamine was added in an attempt to reverse the effects of the K^+ channel opener.

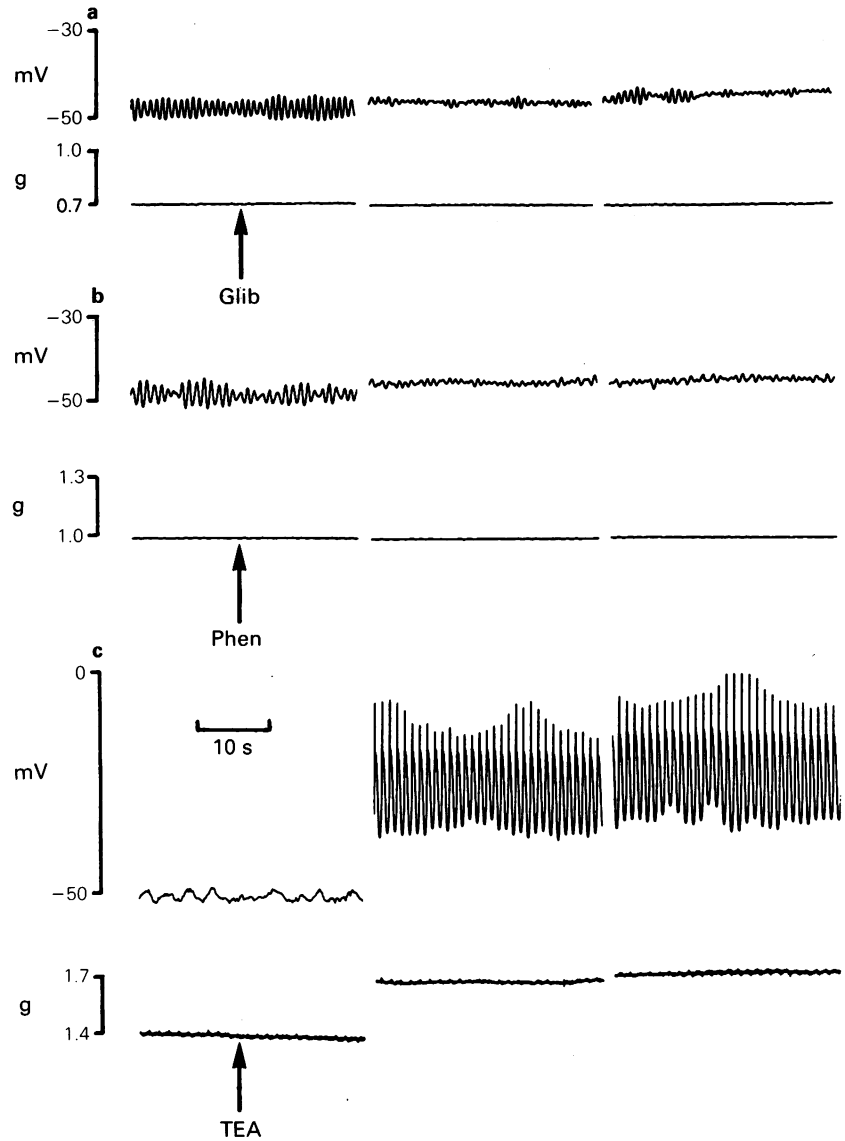

Figure 4 Guinea-pig isolated trachealis: the effects of some antagonists of cromakalim on electrical and mechanical activity. In each row of recordings the upper trace represents membrane potential changes recorded from a single cell, while the lower trace represents the mechanical activity of a contiguous segment of trachea. In each row the left hand panel indicates control activity and tissue exposure to the indicated drug commences at the arrow. The centre and right hand panels show activity recorded 5 and 10 min (respectively) after tissue exposure to the relevant drug. Note (in a and b, respectively) that glibenclamide (Glib) (1 μM) and phentolamine (Phen) (100 μM) caused some suppression of spontaneous slow wave activity and very minor depolarisation. These electrical changes were accompanied by little or no change in mechanical tone. In contrast (c) tetraethylammonium (TEA) (8 mM) evoked marked depolarisation, promoted the discharge of regenerative action potentials and caused tonic tension development.

The electrophysiological experiments involving tracheal exposure to cromakalim (10 μM) and the subsequent addition either of glibenclamide (1 μM) or of phentolamine (100 μM) involved maintaining a recording of membrane potential for a total of 30–40 min. During this period marked relaxation (induced by cromakalim) and subsequent recovery occurred. Accordingly such experiments proved

Table 1 The effects of glibenclamide (1 μM), phentolamine (100 μM) and tetraethylammonium (TEA, 8 mM) on the resting membrane potential and mechanical tone of guinea-pig isolated trachea

Treatment	*Membrane potential change* (mV)	*Tension change* (mg)	*Number of tissues examined*
Glibenclamide 1 μM	−1.0 ± 1.4	0.0 ± 13.0	11
Phentolamine 100 μM	−4.3 ± 0.5*	0.0 ± 31.0	11
TEA 8 mM	−25 ± 3.8*	+750 ± 130*	6

Data represent mean ± s.e.mean changes observed 10 min after tissue exposure to the drug. − indicates depolarisation or relaxation. + indicates hyperpolarisation or contraction. * indicates a change significantly ($P < 0.05$) different from zero.

extremely difficult to complete. The microelectrode often became dislodged from the impaled cell before the effects of glibenclamide or phentolamine had fully developed. Nevertheless, on all occasions where this experiment was attempted, it was clear that glibenclamide (1 μM) or phentolamine (100 μM) reversed both the electrical and mechanical effects of cromakalim (10 μM). The records presented in Figure 6 clearly illustrate this and are representative of the few occasions when the microelectrode impalement of a single cell was maintained for the full duration of the experiment.

Control experiments revealed that tissue exposure to cromakalim (10 μM) for periods in excess of 8 min led to some spontaneous reversal of electrical and mechanical responses. However, this proceeded more slowly than that induced by either glibenclamide or phentolamine. In view of the difficulties of maintaining long-term recordings of membrane potential, quantitative comparison of spontaneous and drug-induced reversal of responses to cromakalim was made in experiments where mechanical activity alone was measured. At the time (20 min) when glibenclamide (1 μM) and phentolamine (100 μM) had caused reversal of 79.9 ± 5.7% and 116.1 ± 3.7% respectively, a spontaneous reversal of 19.1 ± 3.9% was observed.

Discussion

The antagonism of cromakalim by glibenclamide

There have been several studies (Quast & Cook, 1988; Wilson *et al.*, 1988; Newgreen *et al.*, 1989) showing that glibenclamide is able to antagonise cromakalim acting on vascular smooth muscle. That the action of glibenclamide exhibits some selectivity is suggested by its ability, in rat thoracic aorta, to antagonise the relaxant action of cromakalim but not that of sodium nitroprusside (Newgreen *et al.*, 1989). The results of the present study show that glibenclamide can antagonise both the relaxant action of cromakalim in trachealis muscle and also its ability to hyperpolarise the trachealis cells. Furthermore, the selectivity of the action of glibenclamide is confirmed by its failure to antagonise the tracheal relaxant actions of isoprenaline or theophylline.

Glibenclamide (0.1–20 nM) increases insulin secretion by blocking ATP-dependent K^+ channels in

Table 2 The effects of pretreatment with glibenclamide (1 μM) or phentolamine (100 μM) on the tracheal hyperpolarisation induced by cromakalim (10 μM)

Treatment	*Hyperpolarisation* (mV) *induced at the stated time after the administration of cromakalim* 4 min	8 min	*Number of tissues examined*
Cromakalim 10 μM	19.3 ± 2.2	20.0 ± 2.1	8
Glibenclamide 1 μM + cromakalim 10 μM	7.3 ± 2.0*	11.0 ± 2.6*	6
Phentolamine 100 μM + cromakalim 10 μM	3.3 ± 1.3*	1.0 ± 2.3*	4

Data represent mean ± s.e.mean of values from at least 4 tissues. * indicates a significant ($P < 0.05$) difference from the corresponding value for cromakalim alone.

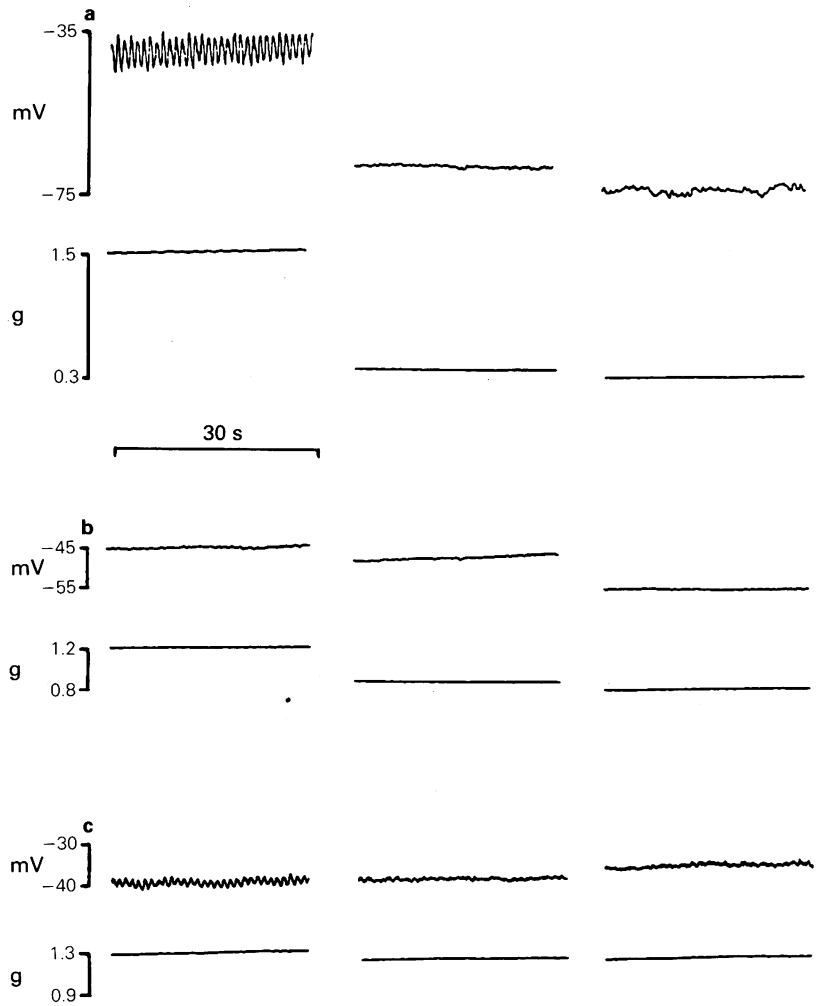

Figure 5 Guinea-pig isolated trachealis: the electrical and mechanical effects of cromakalim (10 μM) and their antagonism by tissue pretreatment with glibenclamide or phentolamine. In each row of recordings the upper trace represents membrane potential changes recorded from a single cell, while the lower trace represents the mechanical activity of a contiguous segment of trachea. In each row the left hand panel indicates control activity. The centre and right hand panels indicate activity recorded 4 and 8 min respectively after tissue exposure to cromakalim (10 μM). (a) No antagonist present. (b and c) Glibenclamide (1 μM) and phentolamine (100 μM), respectively, present throughout. Note that glibenclamide and phentolamine each reduced the ability of cromakalim to hyperpolarise and relax the trachealis muscle.

RINm5F insulinoma cells (Schmid-Antomarchi *et al.*, 1987). This has prompted the suggestion (Newgreen *et al.*, 1989) that glibenclamide may block the K^+ channel opened by cromakalim in smooth muscle. However, the high concentrations (0.1–10 μM) of glibenclamide required for antagonism of cromakalim in smooth muscle indicate that the cromakalim-opened K^+ channel in smooth muscle may not be identical to the ATP-dependent K^+ channel of pancreatic β-cells.

If glibenclamide indeed acts to block the K^+ channel opened by cromakalim in guinea-pig trachealis, then its failure to cause significant membrane potential change (Figure 4 and Table 1) suggests that the cromakalim-sensitive K^+ channel is not open under normal circumstances and does not play an important role in determining the resting membrane potential. A similar suggestion has been made with regard to the action of glibenclamide in vascular smooth muscle (Quast & Cook, 1988).

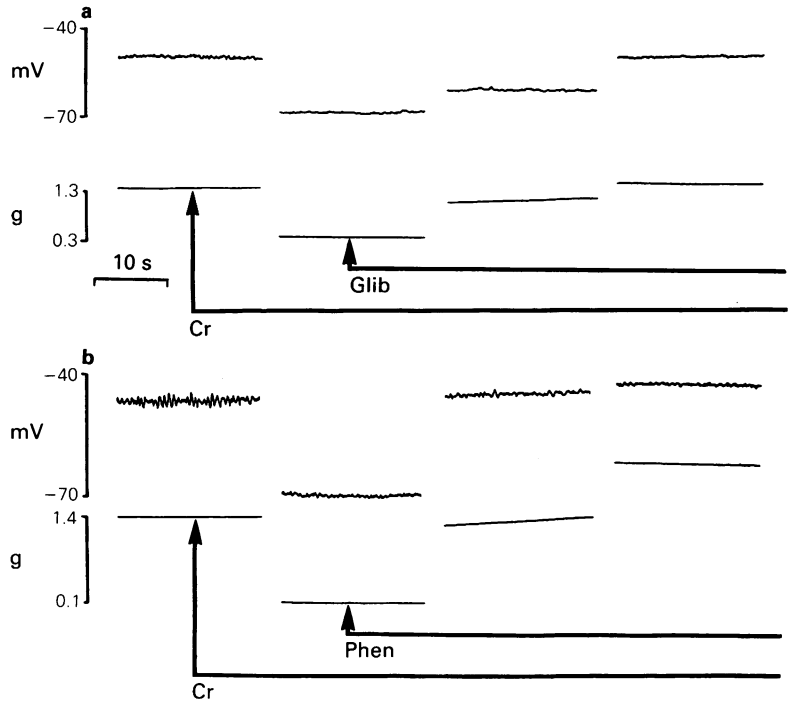

Figure 6 The electrical and mechanical effects of cromakalim (10 μM) in guinea-pig isolated trachealis: their reversal by glibenclamide or phentolamine. In each row of recordings the upper trace represents membrane potential changes recorded from a single cell, while the lower trace represents the mechanical activity of a contiguous segment of trachea. In each row the left hand panel indicates control activity and tissue exposure to cromakalim (Cr) (10 μM) commences at the arrow. Subsequent panels show activity recorded 8 min after tissue exposure to cromakalim (when glibenclamide (Glib, 1 μM; a) or phentolamine (Phen, 100 μM; b) was added at the arrow) and 18 and 28 min after tissue exposure to cromakalim. Note that, 20 min after the addition of glibenclamide or phentolamine, the membrane potential changes and tension changes evoked by cromakalim were effectively reversed towards the pre-cromakalim levels.

The antagonism of cromakalim by phentolamine

In vascular smooth muscle from the dog and other species, McPherson & Angus (1989) showed that phentolamine antagonised both the relaxant activity of cromakalim and its ability to hyperpolarise the tissue. The action of phentolamine was selective in that other vasorelaxants were not antagonised. Furthermore, since cromakalim was not antagonised by prazosin, rauwolscine or phenoxybenzamine, the action of phentolamine seemed unrelated to α-adrenoceptor blockade. Results of the present study suggest that phentolamine provides similar antagonism of cromakalim acting on trachealis muscle. The antagonism was selective in that phentolamine antagonised neither isoprenaline nor theophylline. The antagonism also seemed unrelated to the blockade of either α_1- or α_2-adrenoceptors since neither prazosin nor yohimbine antagonised cromakalim.

Tested on the trachealis, phentolamine (1 μM) caused some rightward shift in the cromakalim log concentration-effect curve. Higher concentrations of phentolamine depressed the curve and reduced the maximal effect of cromakalim (Figure 2). Similar changes in the cromakalim log concentration-effect curve were obtained in experiments with vascular smooth muscle (McPherson & Angus, 1989).

McPherson & Angus (1989) suggested that phentolamine might antagonise cromakalim by blocking the K^+ channel opened by cromakalim in smooth muscle. If this suggestion is correct, then the failure of phentolamine markedly to depolarise the trachealis (Figure 4 and Table 1) reinforces the evidence provided by experiments with glibenclamide, that the K^+ channel opened by cromakalim is not open under normal circumstances and does not play an important role in determining the resting membrane potential.

Does cromakalim open the K^+ channel responsible for the strong rectifying behaviour of the trachealis plasmalemma?

It has been known for almost two decades that the plasmalemma of trachealis cells is endowed with such strong rectifying properties that regenerative action potentials are not discharged spontaneously and cannot be evoked in response to the passage of cathodal current. Early evidence that this rectifying behaviour was linked to the activity of plasmalemmal K^+ channels included the observation that TEA (a K^+ channel inhibitor) could induce action potential discharge in trachealis muscle (Small & Foster, 1988). Measurement of electrotonic potentials evoked by anodal and cathodal current pulses in canine trachealis (Kannan *et al.*, 1983) has directly revealed the ability of TEA to reduce tracheal rectification. Furthermore, patch clamp recording from canine airways smooth muscle has revealed the existence of Ca^{2+}- and voltage-dependent K^+ channels in the plasmalemma. The high unitary conductance (270 pS), voltage-dependency and susceptibility of these channels to blockade by TEA, all suggest their involvement in the rectifying behaviour of the plasmalemma (McCann & Welsh, 1986).

Since TEA can, to some extent, inhibit the hyperpolarisation and relaxation of guinea-pig trachealis induced by cromakalim (Allen *et al.*, 1986), it might be argued that cromakalim opens the K^+ channels which endow the plasmalemma with its rectifying properties. However, TEA can block several different types of K^+ channel (Cook, 1988) and cannot therefore serve to identify the K^+ channel opened by cromakalim.

In the present study, glibenclamide and phentolamine each inhibited the mechanical and electrical effects of cromakalim. However, neither of these agents was able to mimic the effects of TEA in inducing the discharge of regenerative action potentials. This strongly indicates that glibenclamide and phentolamine do not reduce the rectifying behaviour of the trachealis plasmalemma.

As discussed above, the way in which glibenclamide and phentolamine interfere with the action of cromakalim in smooth muscle is currently poorly understood. These agents could prevent cromakalim binding to its site of action. They could interfere with biochemical processes linking the cromakalim site of action to the K^+ channel opened by cromakalim. Alternatively, as suggested by Newgreen *et al.* (1989) and McPherson & Angus (1989), they could act simply to block the relevant K^+ channel.

If it can be assumed that either of these agents block the K^+ channel opened by cromakalim, then the present results suggest that cromakalim does not act to open the large Ca^{2+}- and voltage-dependent K^+ channels which endow the trachealis plasmalemma with its strong rectifying properties.

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