The contribution of Rb-permeable potassium channels to the relaxant and membrane hyperpolarizing actions of cromakalim, RP49356 and diazoxide in bovine tracheal smooth muscle

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1 Cromakalim (1 and 10 μM), RP49356 (5 and 50 μM) and diazoxide (100 and 300 μM) produced full relaxation of smooth muscle strips pre-contracted with 25 mM KCl. These agents caused membrane hyperpolarization and increased ^{42}K and ^{86}Rb efflux. The time taken to achieve the maximum change in each of these parameters (t_{max}) was less for the higher concentration levels of cromakalim, RP49356 and diazoxide than for the lower concentration levels.

2 Calculation of permeability (P) changes showed that cromakalim (1 and 10 μM) produced a greater rise in PK than PRb, although the PRb:PK ratio was similar at both concentration levels. Similarly RP49356 produced a greater change in PK than PRb. However, in contrast to cromakalim, this difference was more marked at the higher concentration (50 μM) and was reflected by a differential effect of the two concentrations of RP49356 on the PRb:PK ratio. Diazoxide (100 and 300 μM) produced similar changes in PK and PRb.

3 For cromakalim (1 and 10 μM) the t_{max} for the electrical and mechanical effects and also the profile of change in these parameters corresponded to changes in both PK and PRb. For RP49356 (5 μM), changes in tension and membrane potential were related to both changes in PK and PRb, whereas at 50 μM these responses more closely corresponded to changes in PK. For diazoxide (100 and 300 μM) the electrical and mechanical effects corresponded to changes in both PK and PRb.

4 The results show that changes in ^{42}K and ^{86}Rb efflux induced by cromakalim, RP49356 and diazoxide are good indicators of changes in membrane PK and PRb evoked by these agents. Furthermore, it is concluded that the K channels involved in the mechanical and electrical effects of cromakalim are represented by the opening of a single population through which Rb can pass less easily than K, whilst the K channels associated with actions of diazoxide are equally permeable to both K and Rb. In contrast, the relaxant and membrane hyperpolarizing actions of RP49356 may involve the opening of more than one group of K channels which differ in their permeability to Rb.

Keywords: K-channels; RP49356; diazoxide; cromakalim; rubidium; hyperpolarization; ion flux; tracheal smooth muscle

Introduction

Changes in ^{42}K efflux from smooth muscle tissues evoked by potassium (K) channel opening drugs such as cromakalim are generally regarded as satisfactory indicators of changes in membrane K permeability (PK). A number of studies have measured the effects of K channel openers on K efflux using ^{86}Rb as a substitute for K, since this radionuclide has a more convenient half-life and is available with a higher specific activity (e.g. Hamilton *et al.*, 1986; Quast, 1987). Although Rb has similar chemical and physical properties to K (Ussing, 1960), patch-clamp studies have shown that some K channels can discriminate between these two ions (e.g. mouse exocrine acinar cells: large conductance Ca-activated K channel; Gallacher *et al.*, 1984; rat pancreas; ATP-sensitive K channels; Ashcroft *et al.*, 1989).

In smooth muscle tissues there are several reports that K channels opened by cromakalim-like drugs may also distinguish between K and Rb. In some vascular tissues, cromakalim and pinacidil evoke larger increases in K efflux than Rb efflux and the ratio of K:Rb flux varies with concentration of K channel opener used (Quast & Baumlim, 1988; Videbaek *et al.*, 1988; Bray & Weston, 1989). In guinea-pig bladder and rat aorta, cromakalim and minoxidil sulphate respectively produce marked increases in K efflux with no detectable effect

on the loss of Rb (Foster *et al.*, 1989; Newgreen *et al.*, 1990) and in rat bladder, cromakalim-stimulated increases in Rb efflux, but not K efflux, show 'desensitisation' on a second exposure to this drug (Edwards & Weston, 1989). In rabbit aorta, cromakalim (10 μM)-induced changes in Rb efflux are more sensitive to the effects of glibenclamide than K efflux (Bray & Weston, 1989) whilst in guinea-pig bladder replacement of extracellular KCl with RbCl abolishes the relaxant effect of cromakalim (Foster *et al.*, 1989).

Assessment of the effects of K channel openers on K permeability (PK) using the data derived from $^{42}\text{K}/^{86}\text{Rb}$ efflux experiments is complicated by the ability of these agents to produce membrane hyperpolarization. Such a change in membrane potential increases the electrical force retaining K in the cell with a resultant underestimation of changes in membrane PK. Furthermore, if the channel is less permeable to Rb than K, then measurement of Rb flux will produce a greater error in the estimation of the effects of K channel openers on PK.

The aims of the present study were to determine firstly whether the K channels involved in the actions of cromakalim, RP49356 and diazoxide were permeable to Rb and/or K, and secondly whether there were any differences in the groups of K channels opened by the three K channel openers. Therefore, we examined the effects of cromakalim, RP49356 and diazoxide on K and Rb permeability (PK and PRb respectively) in bovine tracheal smooth muscle. We also investigated whether changes in PRb and/or PK corresponded to the changes in membrane potential and tension evoked by these K channel openers. Finally, we assessed whether measurement of changes in K and Rb efflux were good indicators of changes in PK and PRb respectively.

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A preliminary account of some of this work has been presented to the British Pharmacological Society (Longmore *et al.*, 1990a).

Methods

Fresh specimens of bovine trachea were obtained from Manchester Abattoir and were placed in cold (4°C) physiological salt solution (PSS) for transport to the laboratory (approximately 10 min). Each trachea was opened along its longitudinal axis and the smooth muscle was isolated and cleaned of any connective tissue. Strips of smooth muscle, each approximately 4 mm in width, were then prepared. In all experiments the appropriate time- and vehicle-matched controls were employed.

Tissue bath experiments

Strips of smooth muscle were mounted for isometric tension recording in a 20 ml organ bath containing PSS, pH 7.4, aerated with 95% O₂ and 5% CO₂. The tissues were suspended under 1 g tension and allowed to equilibrate for 60 min after which time tension was readjusted to 1 g. A further 30 min equilibration period was allowed before experiments started.

Calculation of IC₅₀ values

Tissues were pre-contracted with 25 mM KCl and when the contractile response had reached a plateau (approximately 10–20 min), the relaxant effects of cromakalim, RP49356 and diazoxide were assessed by cumulative addition to the tissue baths at 5 min intervals. The IC₅₀ values for the K channel openers were then calculated. In the case of cromakalim and RP49356 concentrations corresponding to approximately 5 and 50 times the IC₅₀ values were used in all subsequent experiments (i.e. 1 or 10 µM for cromakalim and 5 or 50 µM for RP49356). In the case of diazoxide, it was only possible to use concentrations corresponding to 5 and 15 times the IC₅₀ values (i.e. 100 and 300 µM) since at concentrations of diazoxide greater than 300 µM substantial effects of its vehicle (DMSO) were seen.

Time-course of mechano-inhibitory effects

Tissues were pre-contracted with 25 mM KCl and when the contractile response to this agent had reached a plateau each tissue was exposed to one of the following drugs for a period of 24 min: cromakalim (1 or 10 µM), RP49356 (5 or 50 µM) or diazoxide (100 or 300 µM). This contact time (24 min) was used in order to make the relaxant experiments comparable with the electrophysiological and ion flux studies. For the low concentrations of the K channel openers used it took up to 17 min for some responses to achieve their maximum (see Table 2). Therefore a contact time of 24 min was employed in all experiments in order to measure the responses at their plateau.

Electrophysiological measurements

A strip of smooth muscle was placed in a recording chamber (15 ml) through which PSS was flowing at the rate of 5 ml min⁻¹. The tissue was fixed to the Sylgard floor of the chamber with fine pins and allowed to equilibrate for 1 h. Glass microelectrodes (resistance 40–85 MΩ) were inserted into the smooth muscle cells and when a stable intracellular recording of membrane potential of at least 1 min duration had been obtained, cromakalim (1 and 10 µM), RP49356 (5 and 50 µM) or diazoxide (100 and 300 µM) were added to the PSS reservoir. Recordings were then made over a period of at least 10 min or until the electrode became dislodged. Results

were discarded if the resistance of electrode, when dislodged, markedly differed from that prior to impalement.

Ion flux studies

Strips of smooth muscle were mounted on a syringe needle and each was suspended in a plastic vial containing 3 ml PSS. After 10 min the tissues were transferred to vials containing a modified PSS in which KCl had been replaced with ⁴²K₂CO₃ (1.57 µCi ml⁻¹) and/or to which ⁸⁶RbCl (5 µCi ml⁻¹; final concentration < 50 µM) had been added. After a 3 h loading period, ⁴²K and/or ⁸⁶Rb were allowed to efflux from the tissues by transferring them, at 4 min intervals, to a series of vials containing normal PSS (for further details see Hamilton *et al.*, 1986). Each tissue was exposed to one of the following drugs: cromakalim (1 or 10 µM), RP49356 (5 or 50 µM) or diazoxide (100 or 300 µM) with appropriate vehicle controls. The ⁴²K and ⁸⁶Rb content of the tissues and efflux samples were measured with a gamma counter. The samples were then stored at 20°C for 5–6 days and the remaining ⁸⁶Rb component was counted again, using a half-life correction factor. The ⁴²K counts were calculated by subtraction of the second counts (⁸⁶Rb only) from the first count (⁴²K and ⁸⁶Rb combined) and the data expressed in terms of the efflux rate coefficient (fractional loss of ⁴²K or ⁸⁶Rb from the tissue) over a 1 min period. In some experiments, for each individual time point, the fractional loss of ⁴²K or ⁸⁶Rb from strips of smooth muscle exposed to the drugs was expressed as a percentage of that from strips obtained from the same trachea but exposed to the vehicle control alone. Expression of the efflux data in this fashion eliminates differences in the basal loss of K and Rb and also reduces any decline in the rate of efflux throughout the course of the experiment which may occur as a result of depletion of the intracellular pool of K or Rb (Jones, 1980).

Drugs and solutions

The following substances were used: (±)-cromakalim (Beecham); (±)-N-methyl-2-(3-pyridinyl)-tetrahydrothiopyran-2-carbothioamide-1-oxide (RP49356) (Rhône-Poulenc); diazoxide (Glaxo); glibenclamide (Sigma); ⁴²K₂CO₃ (University of Manchester Reactor); ⁸⁶RbCl (Amersham). Stock solutions were prepared by dissolving cromakalim (10 mM) and RP49356 (10 mM) in 70% v/v ethanol:distilled water; diazoxide (10 mM) in DMSO (dimethyl sulphoxide; Sigma); glibenclamide (1 mM) in absolute ethanol. The PSS had the following composition (mM): NaCl 120, KCl 5.9, CaCl₂ 2.5, MgCl₂ 1.2, NaHCO₃ 25, KH₂PO₄ 1 and glucose 11. When KCl was used as the spasmogen the stated concentration excludes the KCl (5.9 mM) already present in the PSS.

Analysis of data

Data are expressed as mean ± s.e.mean and statistical comparisons were made with Student's *t* test (unpaired comparison). An *a priori* criterion of *P* < 0.05 was used to identify significant effects. The relationship between K efflux and PK is described by the following equation (adapted from Jones, 1980):

$$J_c = PK \cdot K_i \cdot \left(\frac{u}{1 - e^{-u}} \right) \quad (1)$$

where *J_c* = efflux (mol cm⁻¹ s⁻¹), *PK* = K permeability (cm s⁻¹), *K_i* = intracellular K concentration, *u* = *VF*/*RT* (*V* is membrane potential (mV); *F* is Faraday's constant (96500 C/mol); *R* is the gas constant (8.3 J/mol/°C); *T* is the absolute temperature (310°K at 37°C)).

In the present experiments the effects of cromakalim, RP49356 and diazoxide on K and Rb efflux and membrane potential were used to determine the effects on PK and PRb respectively. The calculation of PK and PRb (expressed as arbitrary units; *U*) was based on a modification of Equation

(1):

$$\text{PK (or PRb)} = \frac{\text{efflux (\% control) at time } t \text{ min}}{K_i} \times \frac{1 - e^{-u}}{u} \text{ at time } t \text{ min} \quad (2)$$

Firstly, for each concentration of each drug, the mean membrane potential change obtained from $n = 2$ or 3 cells was measured at time points corresponding to those used in the efflux studies (i.e. 0, 4, 8, 12, 16, 20 and 24 min). The difficulty in maintaining impalement of smooth muscle cells with micro-electrodes limited the number of cells which could be successfully studied over the full 24 min period. These data were used to calculate the $(1 - e^{-u})$ divided by u term and the efflux data obtained from each individual tissue were multiplied by this factor and divided by K_i (105 mM from Kirkpatrick, 1981) to give PK or PRb (measured in arbitrary units U). Finally, for each individual tissue the ratio of the maximum changes in PRb and PK evoked by each drug was calculated (i.e. $\text{PRb}_{\text{max}}/\text{PK}_{\text{max}}$).

Results

Tissue bath studies

Figure 1 shows the relaxant concentration-effect curves for cromakalim, RP49356 and diazoxide in bovine tracheal smooth muscle pre-contracted with 25 mM KCl. The IC_{50} values for cromakalim, RP49356 and diazoxide were $0.19 \pm 0.02 \mu\text{M}$, $1.65 \pm 0.30 \mu\text{M}$ and $21.3 \pm 3.6 \mu\text{M}$ respectively ($n = 4-6$). The higher concentrations of each K channel opener (cromakalim, $10 \mu\text{M}$; RP49356, $50 \mu\text{M}$; diazoxide, $300 \mu\text{M}$) produced complete relaxation of the 25 mM KCl-induced contractions. The time taken to achieve a maximal effect (t_{max}) ranged between 7 and 9 min. The lower concentra-

Figure 1 The effects of cromakalim (▲), RP49356 (●), diazoxide (■) and vehicle control (○, ethanol; □, dimethyl sulphoxide) on 25 mM KCl-induced contractions in bovine tracheal smooth muscle. Ordinate scale: % initial 25 mM KCl-induced contraction; abscissa scale: concentration of K channel opener (μM) on a logarithmic scale. Points show mean values ($n = 4-6$). Vertical bars signify $\pm$ s.e.mean.

Figure 2 Typical examples of the effect of (a) cromakalim $10 \mu\text{M}$, (b) RP49356 $50 \mu\text{M}$ and (c) diazoxide $100 \mu\text{M}$ on membrane potential in bovine tracheal smooth muscle. The (●) indicates the point at which the drugs were added to the PSS reservoir and the values refer to the resting membrane potential (mV).

tions of each K channel opener (cromakalim, $1 \mu\text{M}$; RP49356, $5 \mu\text{M}$; diazoxide, $100 \mu\text{M}$) also caused complete relaxation, although it took longer for this effect to be achieved (11–18 min). For details see Tables 1 and 2. DMSO (the vehicle for diazoxide) itself produced a marked relaxation although this effect occurred over a longer time-course than the effects of diazoxide itself.

Electrophysiological measurements

The mean resting membrane potential of bovine tracheal smooth muscles cells was $-61.6 \text{ mV} \pm 1.5 \text{ mV}$ ($n = 28$ cells obtained with 18 different trachea) and showed little spontaneous fluctuation during an experiment. The effects of cromakalim, RP49356 and diazoxide on membrane potential and the time taken to attain the maximum effect (t_{max}) are summarised in Tables 1 and 2. Each agent produced membrane hyperpolarization which was relatively well maintained throughout the period of impalement (10–24 min). Figure 2 shows typical examples of the effects of cromakalim ($10 \mu\text{M}$), RP49356 ($50 \mu\text{M}$) and diazoxide ($100 \mu\text{M}$) on membrane potential. The solvents used (ethanol or DMSO) had no apparent effect on the level of membrane potential, but membrane noise was increased in the presence of DMSO.

Ion flux experiments

Figure 3 shows the effects of cromakalim ($10 \mu\text{M}$) on ^{42}K efflux from tissues which were loaded in a solution containing either ^{42}K alone or ^{42}K and ^{86}Rb . The loss of ^{42}K was similar in both experimental groups indicating that at the concentrations used, Rb itself did not influence the loss of K from the

Table 1 Maximum responses in bovine isolated tracheal muscle observed in presence of K channel openers

		Tension (%)	Membrane potential (mV)	K efflux (% control)	Rb efflux (% control)	PK ($\text{U} \times 10^2$)	PRb ($\text{U} \times 10^2$)	PRb/PK
Cromakalim	$1 \mu\text{M}$	97.0 ± 6.9	-74.4 ± 1.6	233 ± 32	156 ± 13	120 ± 77	79 ± 4	0.61 ± 0.07
Cromakalim	$10 \mu\text{M}$	97.0 ± 4.0	-79.2 ± 1.3	337 ± 43	235 ± 18	168 ± 21	111 ± 7	0.66 ± 0.08
RP49356	$5 \mu\text{M}$	95.6 ± 3.5	-76.6 ± 1.3	203 ± 28	185 ± 13	108 ± 7	88 ± 8	0.81 ± 0.05
RP49356	$50 \mu\text{M}$	105.3 ± 7.4	-82.5 ± 1.1	419 ± 56	249 ± 13	251 ± 24	136 ± 7	0.57 ± 0.06
Diazoxide	$100 \mu\text{M}$	97.9 ± 12.5	-71.9 ± 2.2	173 ± 24	154 ± 20	75 ± 11	65 ± 7	0.98 ± 0.18
Diazoxide	$300 \mu\text{M}$	100.7 ± 5.2	-80.9 ± 1.0	212 ± 17	190 ± 28	107 ± 10	91 ± 14	0.84 ± 0.16

Values are mean $\pm$ s.e.mean; $n = 3-7$.

Table 2 $t_{\max}$ (min) values i.e. time taken to achieve maximal effect in bovine isolated tracheal muscle

		Tension	Membrane potential	K efflux	Rb efflux	PK	PRb
Cromakalim	1 μM	11.2 $\pm$ 3.1	17.0 $\pm$ 2.7	16–20	>24	16–20	20–24
Cromakalim	10 μM	8.7 $\pm$ 1.9	6.2 $\pm$ 0.5	8–12	>24	8–12	8–12
RP49356	5 μM	12.2 $\pm$ 3.9	12.3 $\pm$ 0.7	20–24	20–24	20–24	20–24
RP49356	50 μM	8.3 $\pm$ 3.1	5.5 $\pm$ 0.5	8–12	20–24	8–12	20–24
Diazoxide	100 μM	12.7 $\pm$ 0.8	14.5 $\pm$ 2.8	>24	16–20	20–24	16–20
Diazoxide	300 μM	7.4 $\pm$ 1.1	7.0 $\pm$ 0.8	>24	16–20	16–20	16–20

Values are mean $\pm$ s.e.mean; $n = 3-7$.

Figure 3 Effect of cromakalim on ^{42}K efflux from bovine tracheal smooth muscle. Square symbols show the loss of ^{42}K from tissues loaded with ^{42}K alone and round symbols show ^{42}K efflux from tissues loaded with ^{42}K and ^{36}Rb . The filled symbols show the effects of cromakalim (10 μM) and the open symbols show the effects of vehicle (ethanol) control. Ordinate scale: K efflux rate coefficient (% per min); abscissa scale: time into efflux (min). Points show mean values ($n = 6$) and vertical bars signify $\pm$ s.e.mean. The horizontal bar indicates the period of time over which the tissues were exposed to the modifying agents.

Figure 4 The relationship between the effects of RP49356 (50 μM)-induced changes in (a) K efflux and K permeability and (b) Rb efflux and Rb permeability: (○) show effects on ion flux and (●) show effects on permeability. Points show mean values ($n = 4-5$) and vertical bars signify $\pm$ s.e.mean. The horizontal bar indicates the period of time over which the tissues were exposed to RP49356 (50 μM).

tissues. For both concentrations of cromakalim, RP49356 and diazoxide the profile of changes in K and Rb efflux paralleled the profile of change in PK and PRb respectively. This is illustrated in Figure 4 which shows the relationship between RP49356 (50 μM)-evoked changes in K and Rb effluxes and permeabilities.

The resting PK ($\text{U} \times 10^2$) and PRb ($\text{U} \times 10^2$) were 36.6 ± 1.4 and 32.1 ± 1.9 respectively ($n = 31-37$). Cromakalim (1 and 10 μM) produced larger increases in PK than PRb and the PRb/PK ratio did not differ significantly at the two concentration levels. RP49356 (5 and 50 μM), similarly to cromakalim, produced larger increments in PK than PRb; however, in contrast to cromakalim, there was a significant difference in the PRb/PK ratio for each concentration used. Diazoxide (100 and 300 μM) produced similar changes in PK and PRb. For details see Table 1.

At the lower concentration levels all three K channel openers produced similar changes in PRb (range 109% to 148%). Cromakalim (1 μM) and RP49356 (5 μM) produced comparable rises in PK whereas diazoxide (100 μM), which represents a similar effective relaxant concentration (i.e. 5 times the IC_{50} value), produced a significantly smaller change in PK. Comparison of the effects of the higher concentrations showed that RP49356 (50 μM) produced a significantly greater increase in PK than cromakalim (10 μM), which in turn was more effective than diazoxide (300 μM). The smaller effect of diazoxide (300 μM) on PK is likely to reflect the lower effective relaxant concentration of this K channel opener.

The effects of cromakalim, RP49356 and diazoxide on PK and PRb, tension and membrane potential are summarised in Figures 5, 6 and 7 respectively.

Figure 5 The effect of (a) cromakalim 1 μM and (b) cromakalim 10 μM on K permeability (○), Rb permeability (●), membrane potential (▲) and tension (■). Points show mean values ($n = 3-7$) and the vertical bars signify $\pm$ s.e.mean.

Figure 6 The effect of (a) RP49356 $5 \mu M$ and (b) RP49356 $50 \mu M$ on K permeability ($\circ$), Rb permeability ($\bullet$), membrane potential ($\blacktriangle$) and tension ($\blacksquare$). Points show mean values ($n = 3-7$) and the vertical bars signify $\pm$ s.e.mean.

Discussion

Bovine tracheal smooth muscle has little if any spontaneous tone and therefore, the relaxant effects of cromakalim, RP49356 and diazoxide were studied in strips of smooth muscle pre-contracted with 25 mM KCl. All three K channel openers produced full relaxation and the rank order of potency was cromakalim > RP49356 > diazoxide with potency ratios of 1:9:112 when IC_{50} values were compared. These values are comparable to those reported for cromakalim and RP49356 in guinea-pig pulmonary artery (Eltze, 1989)

Figure 7 The effect of (a) diazoxide $100 \mu M$ and (b) diazoxide $300 \mu M$ on K permeability ($\circ$), Rb permeability ($\bullet$), membrane potential ($\blacktriangle$) and tension ($\blacksquare$). Points show mean values ($n = 3-7$) and the vertical bars signify $\pm$ s.e.mean.

and in rat portal vein (Longmore *et al.*, 1990b) and for cromakalim and diazoxide in rat aorta (Quast & Cook, 1989; Newgreen *et al.*, 1990) and in portal vein (Quast & Cook, 1989; Longmore *et al.*, 1990b).

Cromakalim, RP49356 and diazoxide each produced marked membrane hyperpolarization towards the calculated K equilibrium potential (E_K) in this tissue (-77 mV, assuming an intracellular K concentration of 105 mM, Kirkpatrick, 1981). Although the electrophysiological studies were carried out in the absence of 25 mM KCl, there was a good relationship between the mechanical and electrical effects observed in the different experimental series. There were considerable similarities in the profile of changes in tension and changes in membrane potential (see Figures 5-7) and the t_{max} values for the mechanical and electrical effects were similar for all drugs except in the case of cromakalim ($1 \mu M$) where the t_{max} value for the relaxant effect was less than that for the membrane potential effect. This may reflect the possibility that low concentrations of cromakalim produce smooth muscle relaxation via a mechanism which is independent of membrane hyperpolarization (Bray *et al.*, 1991).

The close correspondence between the electrical and mechanical effects, despite the mechanical studies being carried out in tissues pre-contracted with 25 mM KCl, most likely reflects the concentration of KCl used. Under these conditions the contractile effects of 25 mM KCl in bovine tracheal smooth muscle are associated with membrane depolarization to approximately -33 mV (Longmore *et al.*, 1991). Recent work in our laboratory (unpublished observations) suggests that within this tissue voltage-operated calcium channels are opened within the range -33 to -41 mV. Therefore full relaxation, mediated via closure of voltage-operated calcium channels, would not be predicted to occur until a K channel opener hyperpolarized the membrane potential to a value approximately equal to or greater than -41 mV. The calculated new E_K with an extracellular K concentration of 31 mM is -39 mV and this value would be predicted to be the maximum membrane potential observed in the presence of a K channel opener. Indeed, it has been shown that in the presence of 25 mM KCl, lemakalim (BRL 38227, the active enantiomer of cromakalim) does hyperpolarize the membrane to a value close to that of the calculated new E_K (Longmore *et al.*, 1991). Thus maximum relaxation would coincide with maximum changes in membrane potential.

In the present study cromakalim, RP49356 and diazoxide increased ^{42}K and ^{86}Rb efflux. It has been reported that for some K channels, Rb ions move through the channel more slowly than K and effectively block the channel or impair the movement of K (Gallacher *et al.*, 1984; Ashcroft *et al.*, 1989). Indeed, it has been shown that replacement of extracellular KCl with RbCl in guinea-pig bladder abolishes the relaxant effect of cromakalim (Foster *et al.*, 1989) and in guinea-pig tracheal smooth muscle a transient rather than sustained mechano-inhibitory action of cromakalim is observed (Morris & Taylor, 1989). However, in the present ion flux experiments it is unlikely that the presence of Rb (maximum concentration $< 50 \mu M$) caused channel blockade since cromakalim ($10 \mu M$)-stimulated ^{42}K efflux was similar from tissues loaded with solutions containing ^{42}K alone or ^{42}K and ^{86}Rb .

The three K channel openers produced substantial membrane hyperpolarization and thus measurement of changes in ^{42}K (or ^{86}Rb) efflux would be predicted to underestimate the effects of these agents on K permeability. The relationship between changes in K efflux and changes in PK is defined by Equation (2) shown above. The correction factor by which efflux was multiplied to derive permeability ranged between 37.4 and 63.0 at -61.6 mV (resting membrane potential) and -82.5 mV (the highest membrane potential seen in the presence of the potassium channel openers), respectively. Thus measurement of changes in K efflux induced by the K channel openers would underestimate the change in PK by a maximum of 1.74 fold. Nevertheless, it can be seen from Figure 3 that in the case of RP49356 ($50 \mu M$), the profile of

changes in ^{42}K or ^{86}Rb efflux closely corresponded to the profile of changes in K or Rb permeability. This was also the case for cromakalim (1 and $10\ \mu\text{M}$), RP49356 ($5\ \mu\text{M}$) and diazoxide ($100\ \mu\text{M}$ and $300\ \mu\text{M}$). Thus changes in K efflux appear to give a good indication of changes in PK.

It is well established that the basal loss of Rb from smooth muscle tissues is less than that of K (for example see Smith *et al.*, 1986; Videbaek *et al.*, 1988; Quast & Baumlim, 1988) and that K channels involved in the action of K channel openers may also differ in their permeability to Rb (see Introduction). In the present experiments, the flux data were expressed as a percentage of control, effectively eliminating differences in the basal loss of K and Rb. All three K channel openers tested produced increases in both PK and PRb. However, there were some differences. Cromakalim (1 and $10\ \mu\text{M}$) caused greater increases in PK than PRb and the PK:PRb ratio was similar at both concentrations tested (0.61 and 0.66 respectively). These results are similar to, although not entirely consistent with, the previously reported effects of cromakalim in rat and rabbit aorta and in guinea-pig portal vein (Quast & Baumlim, 1988; Bray & Weston, 1989) and those of pinacidil in rat mesenteric artery (Videbaek *et al.*, 1988). These previous studies showed that the magnitude of the PRb/PK ratio was dependent on the concentration of K channel opener used and it was suggested that different concentrations of either cromakalim or pinacidil open groups of K channels which differ in their permeability to Rb. However, the present results suggest that in bovine tracheal smooth muscle cromakalim (1 and $10\ \mu\text{M}$) opens either a single group of K channels through which Rb can pass less easily than K or that cromakalim opens two groups of channels one of which is permeable to both K and Rb and one which is relatively Rb-impermeable. At present it is not possible to distinguish between these two possibilities.

RP49356, like cromakalim, also caused a greater increase in PK than PRb. However RP49356 differed from cromakalim since the two concentrations of RP49356 (5 and $50\ \mu\text{M}$) had differential effects on the PRb/PK ratio (see Table 1). At the lower concentration (RP49356 $5\ \mu\text{M}$) similar increases in both PK and PRb were observed whereas at the higher concentration (RP49356 $50\ \mu\text{M}$) a great stimulatory effect on PK than PRb was seen. These observations suggest that RP49356 can open at least two groups of K channels, the channel open at the $5\ \mu\text{M}$ level being permeable to both K and Rb whereas at the $50\ \mu\text{M}$ level there is an increased proportion of 'K-selective' channels open (i.e. channels which allow K to pass through more readily than Rb). This effect of RP49356 contrasts with the previously reported effects of pinacidil which at low concentrations opens 'K'-selective channels and at higher concentrations opens 'Rb-permeable' K channels (Videbaek *et al.*, 1988). Diazoxide ($100\ \mu\text{M}$ and $300\ \mu\text{M}$) produced similar changes in both PK and PRb, indicating that K channels involved in the action of this drug are equally permeable to both K and Rb. This effect of diazoxide is similar to that

found by Newgreen (personal communication) in rat aorta. Thus in the present experiments the effects of the K channel openers on Rb:K permeability ratio show some qualitative similarities to those found previously in other tissue types particularly vascular smooth muscle, although some discrepancies exist. These may reflect differences between the sensitivity or density of 'K-selective' and 'Rb-permeable' K channels in bovine tracheal smooth muscle and vascular tissues.

The present study also examined the relationship between the effects of cromakalim, RP49356 and diazoxide on tension, membrane potential, PK and PRb. In the case of cromakalim (1 and $10\ \mu\text{M}$) the mechanical and electrical effects appear to be related to changes in both PK and PRb, as all four curves rise together and reach their peak at approximately the same time point (see Figure 4 and Table 2). In the case of RP49356 ($50\ \mu\text{M}$) tension and membrane potential responses appear to correspond more closely to increases in PK since the maximum changes in these parameters temporally coincide whereas PRb continues to rise throughout the period of exposure to RP49356 ($50\ \mu\text{M}$; see Figure 4). For RP49356 ($5\ \mu\text{M}$) the mechanical and electrical effects appear to be related both to changes in PK and PRb since there was little difference in the profile of K and Rb permeability changes. Finally in the case of diazoxide (100 and $300\ \mu\text{M}$) there was little difference in the profile of change in PK and PRb. However, in contrast to the effects of cromakalim and RP49356 where t_{max} values for the mechanical, electrical and PK effects closely corresponded, the t_{max} values for diazoxide-induced changes in tension and membrane potential were less than those for PK and PRb. This separation of the mechanical and electrical effects from the effects on ion flux was more marked at the higher concentration of diazoxide ($300\ \mu\text{M}$). It is possible that at least part of the actions of diazoxide may be mediated by an additional action other than K channel opening. Indeed, Thorens & Haeusler (1979) showed that diazoxide (at concentrations $> 300\ \mu\text{M}$) exerted effects consistent with calcium channel blockade. Such an action may contribute to the relaxant effects seen in the present study but it would not account for the effects of this drug on membrane potential. Thus a further mechanism (e.g. decrease in chloride permeability) may also be involved.

In conclusion, the present results show that in bovine tracheal smooth muscle there are differences in the permeability of the channels involved in the actions of cromakalim, RP49356 and diazoxide to K and Rb. Furthermore, under the experimental conditions employed in this study, measurement of K efflux is a satisfactory indicator of changes in membrane K permeability evoked by K channel opening drugs. However, measurement changes in PRb may not always reflect changes in PK.

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