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Cromakalim- and RP 49356-induced $^{42}K^+$ and $^{86}Rb^+$ efflux in rat myometrium

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Abstract

Previous studies had failed to observe cromakalim-induced $^{42}K^+$ or $^{86}Rb^+$ efflux from the myometrium of the pregnant rat in contrast to positive findings in other smooth muscles. In the current study, in myometrium from the non-pregnant rat, cromakalim (10 μM) and RP 49356 ({(±)-*N*-methyl-2-(3-pyridyl)-tetrahydrothiopyran-2-carbothioamide-1-oxide}; 10 μM) induced small increases in $^{42}K^+$ or $^{86}Rb^+$ efflux but much less than did oxytocin (20 nM) or KCl (20 mM). The cromakalim-induced increase in $^{42}K^+$ efflux was enhanced 3.5-fold in the presence of KCl (20 mM) plus (+)-*cis*-diltiazem (3 μM), a property shared by RP 49356. Glibenclamide (10 μM) partially reduced the cromakalim-induced $^{42}K^+$ efflux, in the presence of KCl and (+)-*cis*-diltiazem, but did not affect the KCl-induced $^{42}K^+$ efflux. The data provides further support for the idea that cromakalim and RP 49356 are able to open potassium channels in rat myometrium. It would appear that their actions in this tissue are dependent on the extracellular K^+ concentration and/or membrane potential.

Keywords: Cromakalim; RP 49356; Myometrium; $^{42}K^+$ efflux; $^{86}Rb^+$ efflux; K^+ channel

1. Introduction

Cromakalim and RP 49356 are suggested to produce relaxation of smooth muscle by opening potassium (K^+) channels in the plasmalemma (Mondot et al., 1988; Edwards and Weston, 1990a,b; Cook and Quast, 1992). Crucial experimental evidence is their ability to increase the efflux of $^{42}K^+$, $^{43}K^+$ or $^{86}Rb^+$ (a marker for K^+) from pre-loaded tissues. Such findings have been made in many smooth muscles including rat aorta (Weir and Weston, 1986b), rat portal vein (Hamilton et al., 1986; Quast and Baumlin, 1988), guinea pig taenia coli (Weir and Weston, 1986a) and guinea pig trachealis (Allen et al., 1986).

Cromakalim is a relaxant of the isolated uterus of the pregnant rat with a mechanical profile similar to that reported in other smooth muscles in that it selectively inhibited spasm to low but not high concentrations of potassium chloride (KCl) and was antagonized by various potassium channel blockers (Hollingsworth et al., 1987, 1989, 1994; Piper et al., 1990). Such observations have been extended to mechanical studies in the uterus of the non-pregnant rat where both cromakalim and RP 49356 were relaxants and were selectively antagonized by glibenclamide (Piper et al., 1990, 1992). However, cromakalim failed to alter $^{86}Rb^+$ (Hollingsworth et al., 1987) or $^{42}K^+$ (Hollingsworth et al., 1989) efflux from the uterus of the pregnant rat casting doubt as to its mechanism of action in this tissue.

The aim of the current study was to explore further these unexplained findings by investigation of the effects of both cromakalim and RP 49356 on $^{42}K^+$ and $^{86}Rb^+$ efflux in the myometrium of the non-pregnant rat. Oxytocin and KCl were used as positive controls to show that drug-induced $^{42}K^+$ and $^{86}Rb^+$ fluxes could be detected in myometrium. Cox (1990) failed to observe cromakalim-induced $^{86}Rb^+$ efflux from the rat tail artery in a normal physiological salt solution (PSS) but measured a large cromakalim-induced efflux in a PSS with a raised K^+ concentration. Similar observations have been made with high concentrations of potassium channel openers in the rat ileum (Davies et

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al., 1993). To establish whether such a phenomenom exists in the uterus, similar experiments were conducted in the presence of KCl (20 mM), a concentration known to produce partial depolarisation of rat uterus (Mollard et al., 1986). The experiments were performed with a modification of the method of Cox (1990), the addition of (+)-*cis*-diltiazem, a blocker of L-type Ca^{2+} channels. Diltiazem would prevent K^+-induced Ca^{2+} entry and, therefore, any Ca^{2+}-activated K^+ efflux. Consequently cromakalim- and RP 49356-induced $^{42}K^+$ efflux could be studied under depolarising conditions without the complication of any reduction they might produce in Ca^{2+}-activated K^+ efflux.

2. Materials and methods

2.1. Myometrial strips

Uteri were obtained from 17β-oestradiol benzoate (100 μg/kg 20–24 h prior)-treated Sprague-Dawley rats. In preliminary experiments neither oxytocin (20 nM) nor cromakalim (10 μM) induced $^{42}K^+$ or $^{86}Rb^+$ efflux from whole uterus, so further experiments were conducted in myometrial strips. Myometrial strips of longitudinal muscle were produced by stripping away the endometrium and circular muscle using forceps.

2.2. $^{42}K^+$ and $^{86}Rb^+$ efflux

Ion efflux experiments broadly followed the method of Hollingsworth et al. (1987). Myometrial strips were loaded with either $^{42}K^+$ (1.35 μCi/ml) or $^{86}Rb^+$ (5 μCi/ml) plus $^{42}K^+$ (1.35 μCi/ml) for 180 min. Isotopes were allowed to efflux from the strips into 3 ml aliquots of a physiological salt solution (PSS) using 4 min collection periods. The media from the first 3 periods were discarded, since these resulted from washout of the isotope from the extracellular space, and then basal efflux collected for 3 periods. Tissues were subsequently exposed to PSS alone (control), PSS containing either oxytocin (20 nM), cromakalim (10 μM), RP 49356 (10 μM), KCl (20 mM, additional to that already present in the PSS) or PSS plus vehicle for cromakalim or RP 49356 (ethanol 0.07% v/v) for 6 periods. Strips were returned to PSS alone for 2 periods to assess reversibility. In some experiments tissues were exposed to (+)-*cis*-diltiazem (1 or 3 μM) or glibenclamide (10 μM) throughout the time of efflux. In other experiments strips were incubated in KCl (20 mM) plus (+)-*cis*-diltiazem (3 μM) for 8 min before exposure to cromakalim or RP 49356. (+)-*cis*-Diltiazem (1 μM) is able to produce near abolition of the mechanical response to KCl (20 mM; Granger et al., 1986). Six strips were obtained from each animal and the design balanced such that only 1 strip from the same animal received the same treatment. Efflux media and blotted strips were counted for $^{42}K^+$ or $^{86}Rb^+$ on a Packard Minaxi Auto-Gammma 5000 counter. The efflux data were expressed in terms of rate coefficient (fractional loss of $^{42}K^+$ or $^{86}Rb^+$ from the tissue per minute). In dual isotope studies the samples were counted again after a further 7 days, to allow for decay of $^{42}K^+$ (period equivalent to 14 half lives of $^{42}K^+$) and corrected for $^{86}Rb^+$ decay. This procedure allowed determination of the efflux of both $^{42}K^+$ and $^{86}Rb^+$. The data were also recalculated for each strip as the area under the rate coefficient versus time curve (AUC, units of %) to improve discrimination of

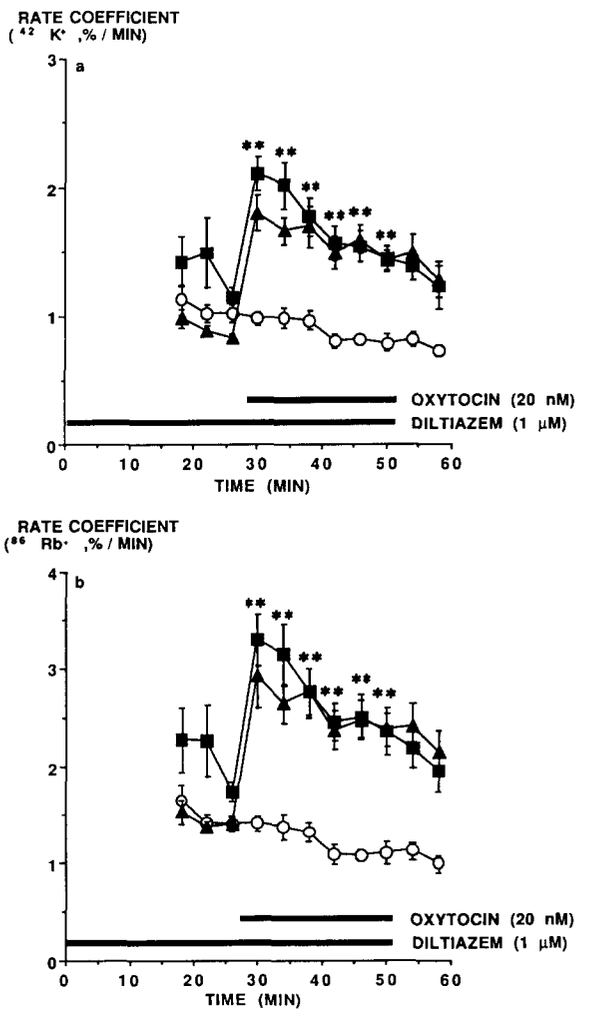

Fig. 1. Effect of PSS alone (○), oxytocin (20 nM, ■) alone and oxytocin (20 nM) in the presence of (+)-*cis*-diltiazem (1 μM) (▲) on the efflux of $^{42}K^+$ (a) and $^{86}Rb^+$ (b). Ordinate scales: $^{42}K^+$ or $^{86}Rb^+$ efflux rate coefficient expressed as percent loss of isotope from the tissue per minute. Abscissa scale: time (min) after start of efflux. The horizontal filled bars indicate the times of exposure to drugs. The points represent the means and the vertical lines the S.E.M. ($n = 6$–8). ** $P < 0.01$ = significant difference between efflux rate coefficient from tissues exposed to oxytocin alone and to those exposed to PSS alone.

the effects of inhibitors on drug-induced $^{42}K^+$ and $^{86}Rb^+$ efflux. Drug-induced AUC measurements were achieved by summing the differences between the rate coefficient during drug exposure and the rate coefficient during vehicle exposure in animal-matched tissues.

2.3. Drugs and solutions

The following drugs were used: cromakalim (SmithKline Beechams), RP 49356 {(±)-*N*-methyl-2-(3-pyridyl)-tetrahydrothiopyran-2-carbothioamide-1-oxide; Rhône Poulenc Rorer}, oxytocin acetate (grade X, Sigma), 17β-oestradiol benzoate (Sigma), (+)-*cis*-diltiazem (Synthelabo), glibenclamide (Hoechst). The stock solutions (10 mM) of cromakalim or RP 49356 were prepared in 70% (v/v) ethanol/isotonic saline, of glibenclamide (10 mM) in 95% ethanol and oxytocin in distilled water. 17β-Oestradiol benzoate was prepared as a 100 μg/ml solution in arachis oil. The composition of the PSS was (mM): Na^+ 143; K^+ 5.9; Ca^{2+} 2.55; Mg^{2+} 1.2; SO_4^{2-} 1.2; $H_2PO_4^-$ 1.2; Cl^- 128; HCO_3^- 25; glucose 11.

2.4. Analysis of results

Data are expressed as means ± S.E.M. The differences between means were tested for significance by ANOVA followed by the Studentised range test (Goldstein, 1968). Comparison of the mean values of AUC between different drug-treated groups was made by the non-paired Student's *t*-test. Comparison of the $^{42}K^+$ and $^{86}Rb^+$ efflux from the same tissues to the same treatment was carried out using the paired Student's *t*-test.

3. Results

3.1. Oxytocin-, cromakalim- and RP 49356-induced efflux of $^{42}K^+$ and $^{86}Rb^+$

Oxytocin (20 nM) caused a marked and sustained increase in the efflux rate of $^{42}K^+$ and $^{86}Rb^+$ over the entire drug exposure period (Fig. 1). The mean AUC for $^{86}Rb^+$ efflux was 56% of that to $^{42}K^+$ which was significantly less ($P < 0.05$, Table 1).

Cromakalim (10 μM) produced a very small and non-sustained rise in $^{42}K^+$ and $^{86}Rb^+$ efflux in normal PSS (Fig. 2a,b). The AUC for $^{86}Rb^+$ efflux stimulated by cromakalim was 18 ± 10% ($n = 6$) of the cromakalim-stimulated $^{42}K^+$ efflux which was significantly less ($P < 0.005$; Table 1). There was a significant increase in $^{42}K^+$ and $^{86}Rb^+$ efflux at one time period during exposure to RP 49356 (10 μM) (Fig. 2c,d). RP 49356 did not produce changes in the AUC for $^{42}K^+$ or $^{86}Rb^+$ efflux (Table 1).

3.2. Effect of raised KCl concentrations and (+)-cis-diltiazem on drug-induced $^{42}K^+$ efflux, blockade by glibenclamide

KCl (20 mM) produced a marked and sustained increase in the efflux of $^{42}K^+$ (Fig. 3). Diltazem (1 or 3 μM) significantly ($P < 0.001$) reduced KCl-induced efflux of $^{42}K^+$ by 55.4 ± 7.8% ($n = 7$) and 47.5 ± 4.4% ($n = 7$) respectively, values which were not significantly different from each other (Table 1). (+)-*cis*-Diltiazem (1 μM) did not modify the oxytocin-induced increase in efflux of $^{42}K^+$ or $^{86}Rb^+$ (Fig. 1, Table 1).

In a medium which contained (+)-*cis*-diltiazem (3 μM) throughout the time of efflux and KCl (20 mM) for 8 min before and during the exposure to cromakalim (10 μM) or RP 49356 (10 μM), cromakalim

Table 1
Effects of KCl, oxytocin, cromakalim and RP 49356 on the rate of efflux of $^{42}K^+$ and $^{86}Rb^+$ from rat myometrium

Drug	Concentration	Modifying drug	Concentration	Efflux	
				$^{42}K^+$	$^{86}Rb^+$
KCl	20 mM	–	–	11.85 ± 1.31 [a]	–
KCl	20 mM	(+)-*cis*-Diltiazem	1 μM	4.93 ± 0.74 [a]	–
KCl	20 mM	–	–	9.59 ± 0.54 [b]	–
KCl	20 mM	(+)-*cis*-Diltiazem	3 μM	5.13 ± 0.58 [b]	–
Oxytocin	20 nM	–	–	9.14 ± 1.62 [c]	5.09 ± 0.87 [c]
Oxytocin	20 nM	(+)-*cis*-Diltiazem	1 μM	7.29 ± 1.18	4.30 ± 0.61
(+)-*cis*-Diltiazem	1 μM	–	–	0.28 ± 0.66	−0.30 ± 0.32
Cromakalim	10 μM	–	–	3.75 ± 1.80 [d]	0.58 ± 0.27 [d]
RP 49356	10 μM	–	–	0.76 ± 0.30	0.10 ± 0.41

Data are expressed as the drug-induced rate coefficient as area under the efflux-time curve above values in vehicle controls (expressed as a percentage) and are given as means ± S.E.M. ($n = 6$–8). Statistical comparisons were made, where appropriate, using an unpaired Student's *t*-test ([c,d] $P < 0.05$; [a,b] $P < 0.01$).

and RP 49356 each produced a marked and sustained increase in $^{42}K^+$ efflux (Fig. 4). The mean AUC for the increases in efflux caused by cromakalim (10 μM) and RP 49356 (10 μM) were not significantly different from each other ($P > 0.05$, Table 2). The cromakalim-induced efflux in the presence of KCl (20 mM) and (+)-*cis*-diltiazem (3 μM) was approximately 3.5-fold greater than that in normal PSS in tissues matched from the same animals. However, it is difficult to make this comparison as basal $^{42}K^+$ efflux differed in the two groups due to the effect of KCl.

Exposure of strips to glibenclamide (10 μM) throughout the efflux time did not alter the increased $^{42}K^+$ efflux induced by KCl (20 mM) in the presence of (+)-*cis*-diltiazem (3 μM) but reduced by 40.2 ± 14.4% ($n = 6$, $P < 0.01$) the cromakalim (10 μM)-induced $^{42}K^+$ efflux in this medium (Fig. 5, Table 2).

4. Discussion

The ability of oxytocin and KCl to evoke marked increases in $^{42}K^+$ and $^{86}Rb^+$ efflux from the rat myometrium demonstrates that the method is capable of detecting such changes in this tissue. This is the first report of significant increases in $^{42}K^+$ and $^{86}Rb^+$ ef-

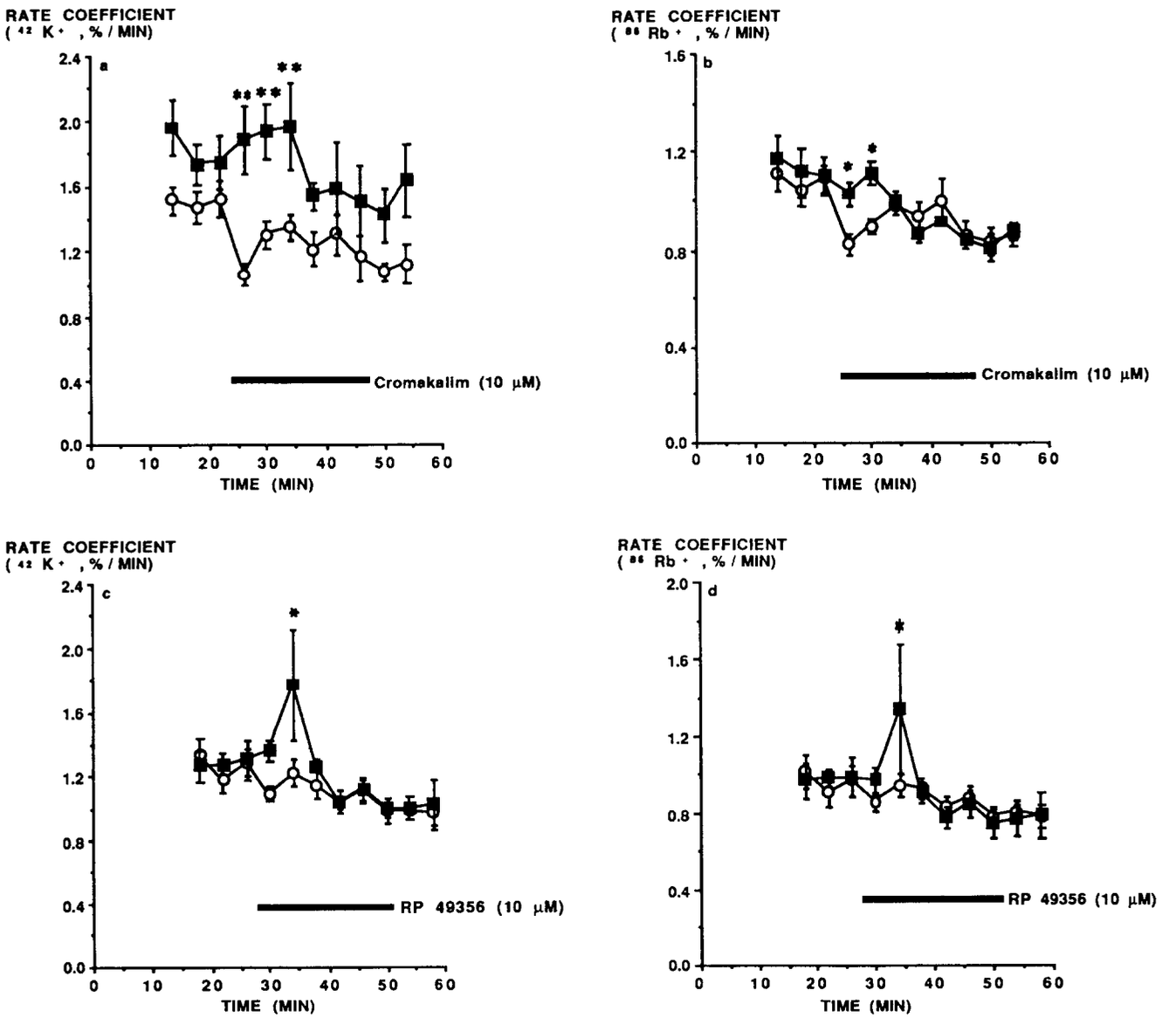

Fig. 2. Effect of cromakalim (10 μM; a,b; ■), RP 49356 (10 μM; c,d; ■) and vehicle (ethanol 0.07% v/v, ○) on $^{42}K^+$ (a,c) and $^{86}Rb^+$ (b,d) efflux. Ordinate scales: $^{42}K^+$ or $^{86}Rb^+$ efflux rate coefficient expressed as percent loss of isotope from the tissue per minute. Abscissa scale: time (min) after start of efflux. The horizontal filled bars indicate the times of exposure to drugs. The points represent the means and the vertical lines the S.E.M. ($n = 6$–8). * $P < 0.05$, ** $P < 0.01$ = significant difference between efflux rate coefficient from tissues exposed to cromakalim or RP 49356 and to those exposed to vehicle.

fluxes to cromakalim and RP 49356 in rat myometrium. These data support the concept (Hollingsworth et al., 1987,1989,1994; Piper et al., 1990,1992) that potassium channel opening plays a role in their relaxant actions in rat myometrium as in other smooth muscles.

4.1. Oxytocin-induced $^{42}K^+$ and $^{86}Rb^+$ efflux

We have shown that oxytocin can augment the efflux of $^{42}K^+$ and $^{86}Rb^+$ from rat myometrium. Presumably the enhanced $^{42}K^+$ and $^{86}Rb^+$ efflux in the presence of the spasmogen oxytocin is due to greater opening of Ca^{2+}-activated K^+ channels. Previous studies have indicated that the mechanical response to oxytocin involves both increased Ca^{2+} entry through L-type Ca^{2+} channels and the release of intracellular Ca^{2+} from stores mediated by enhanced formation of inositol trisphosphate (Edwards et al., 1986; Marc et al., 1986; Anwer et al., 1989). The predominant role of intracellular Ca^{2+} release in the action of oxytocin at higher oxytocin concentrations, such as at 20 nM (Edwards et al., 1986), may explain the insensitivity of the oxytocin-induced $^{42}K^+$ efflux to (+)-*cis*-diltiazem.

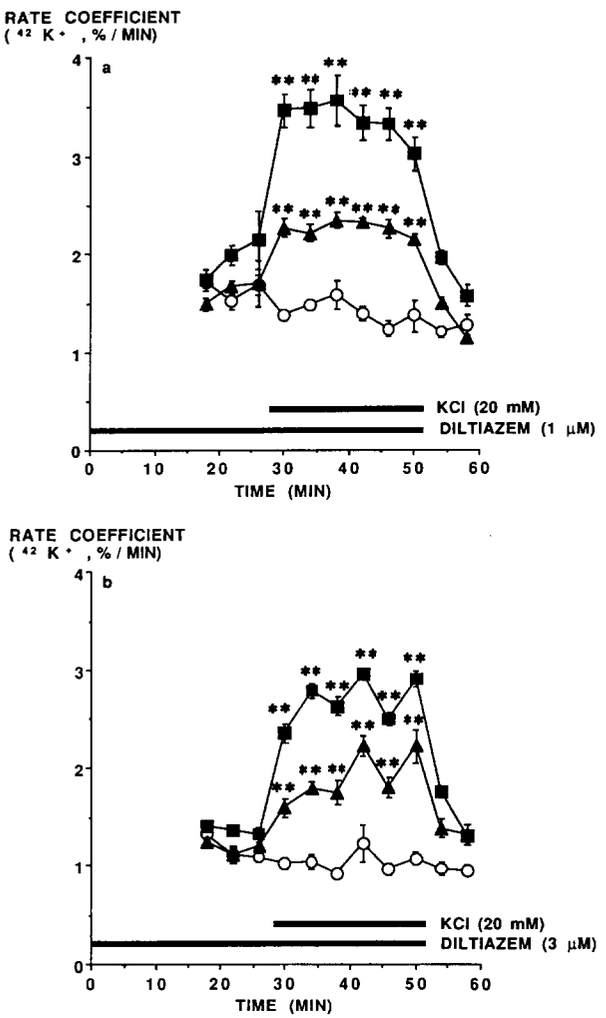

Fig. 3. Effect of KCl (20 mM) in the absence (■) or presence of (+)-*cis*-diltiazem (1 μM, a, ▲; 3 μM, b, ▲) or PSS alone (○) on $^{42}K^+$ efflux. Ordinate scales: $^{42}K^+$ efflux rate coefficient expressed as percent loss of isotope from the tissue per minute. Abscissa scale: time (min) after start of efflux. The horizontal filled bars indicate the times of exposure to drugs. The points represent the means and the vertical lines the S.E.M. ($n = 6–8$). ** $P < 0.01$ = significant difference between efflux rate coefficient from tissues exposed to KCl and those exposed to PSS alone, or from tissues exposed to KCl plus (+)-*cis*-diltiazem compared to those exposed only to KCl.

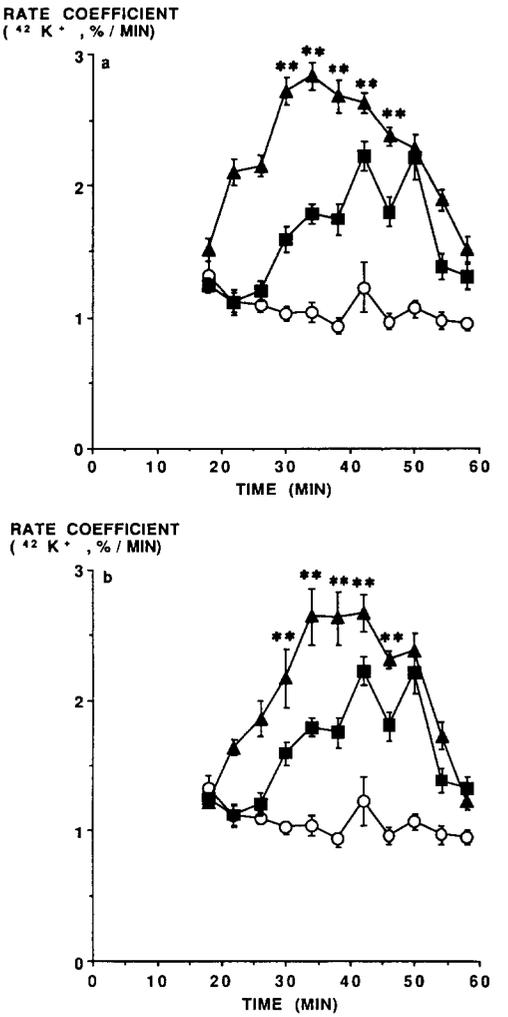

Fig. 4. $^{42}K^+$ efflux from rat myometrial strips exposed to PSS alone (○); exposed to (+)-*cis*-diltiazem (3 μM) from 0 to 52 min and to KCl (20 mM) from 28 to 52 min (■); exposed to (+)-*cis*-diltiazem (3 μM) from 0 to 52 min, to KCl (20 mM) from 20 to 52 min and to cromakalim (10 μM, a, ▲) or RP 49356 (10 μM, b, ▲) from 28 to 52 min. Ordinate scales: $^{42}K^+$ efflux rate coefficient expressed as percent loss of isotope from the tissue per minute. Abscissa scale: time (min) after start of efflux. The points represent the means and the vertical lines the S.E.M. ($n = 6–8$). ** $P < 0.01$ = significant difference between efflux rate coefficient from tissues exposed to cromakalim or RP49356 plus KCl and (+)-*cis*-diltiazem compared to those exposed just to KCl plus (+)-*cis*-diltiazem.

4.2. KCl-induced $^{42}K^+$ and $^{86}Rb^+$ efflux

In contrast to oxytocin, the $^{42}K^+$ efflux induced by KCl was reduced by (+)-*cis*-diltiazem suggesting that

part of this effect of KCl was a consequence of Ca^{2+} entry through L-type Ca^{2+} channels inducing the opening of Ca^{2+}-activated K^+ channels. This conclusion is in line with previous mechanical, Ca^{2+} influx and electrophysiological data (Granger et al., 1986; Edwards et al., 1986; Mollard et al., 1986). (+)-*cis*-Diltiazem only inhibited partially the KCl-evoked increase in $^{42}K^+$ efflux with the inhibition by (+)-*cis*-diltiazem (3 μM) not greater than that by (+)-*cis*-diltiazem (1 μM), suggesting the effect of diltiazem was maximal. (+)-*cis*-Diltiazem possesses actions other than blockade of L-type Ca^{2+} channels at higher concentrations (Edwards et al., 1986) preventing the use of such concentrations. At least two possibilities exist which alone or together could explain a (+)-*cis*-diltiazem-resistant fraction of KCl-induced $^{42}K^+$ efflux. First, an increased extracellular K^+ concentration might augment $^{42}K^+$ exchange as reported in rabbit aorta (Aaronson and Jones, 1985). Second, the raised extracellular K^+ concentration would cause depolarisation (Mollard et al., 1986) and may cause the opening of voltage-dependent K^+ channels. Glibenclamide did not modify the (+)-*cis*-diltiazem-resistant KCl-induced $^{42}K^+$ efflux demonstrating that glibenclamide-sensitive K^+ channels are not involved.

4.3. Cromakalim- and RP 49356-induced $^{42}K^+$ and $^{86}Rb^+$ efflux

The cromakalim-and RP 49356-induced $^{42}K^+$ and $^{86}Rb^+$ effluxes were small in comparison to both oxytocin- and KCl-induced effluxes in rat myometrium (present study) and potassium channel opener-evoked effluxes in most other smooth muscles (see Introduction). One explanation for the small efflux could be that in a tissue like rat myometrium, which exhibits spontaneous mechanical activity, cromakalim or RP 49356 could decrease $^{42}K^+$ or $^{86}Rb^+$ efflux through Ca^{2+}-activated K^+ channels due to their indirect ability to reduce Ca^{2+} influx, as well as increasing $^{42}K^+$ or $^{86}Rb^+$ efflux via the K^+ channel opened by these drugs. These two phenomena would tend to cancel each other. An alternative suggestion, made previously (Hollingsworth et al., 1987,1989), is that in rat myometrium cromakalim and RP 49356 predominantly act upon K^+ channels involved in pacemaker currents. Increases in $^{42}K^+$ or $^{86}Rb^+$ efflux from the small proportion of pacemaker cells would not be detected.

This study has reported small effects of cromakalim and RP 49356 on $^{42}K^+$ and $^{86}Rb^+$ effluxes from uteri of non-pregnant rats in contrast to the absence of effects with uteri from late pregnant rats (Hollingsworth et al., 1987,1989). The above two explanations may be even more apposite for uteri from pregnant than non-pregnant rats.

As in other smooth muscles (Quast and Baumlin, 1988), the cromakalim-induced efflux of $^{86}Rb^+$ was less than that to $^{42}K^+$. Also, the $^{86}Rb^+/^{42}K^+$ efflux ratio was less for cromakalim than for oxytocin in myometrium. These data indicate that the K^+ channel involved in cromakalim's action may be selective for $^{42}K^+$ relative to $^{86}Rb^+$.

A single concentration (10 μM) of each of cromakalim and RP 49356 was used in the present study which is approximately 25-and 11-fold respectively the IC_{50} for relaxation (Piper et al., 1992). However, in most smooth muscles higher concentrations of potassium channel openers are necessary to increase $^{42}K^+$ or $^{86}Rb^+$ effluxes than to induce relaxation (Quast and Baumlin, 1988; Edwards and Weston, 1990a).

4.4. Cromakalim-and RP 49356-induced $^{42}K^+$ and $^{86}Rb^+$ efflux in the presence of KCl and (+)-cis-diltiazem

Cromakalim and RP 49356 produced a large and sustained increase in $^{42}K^+$ efflux in the presence of KCl (20 mM) and (+)-*cis*-diltiazem (3 μM). Previously

Table 2
Effects of cromakalim and RP 49356 on the rate of efflux of $^{42}K^+$ from rat myometrium and the modification by KCl, (+)-*cis*-diltiazem and glibenclamide

Drug	Concentration	Modifying drug	Concentration	Efflux of $^{42}K^+$
Cromakalim	10 μM	–	–	0.88 ± 0.67 [a]
RP 49356	10 μM	–	–	0.76 ± 0.30 [b]
Cromakalim	10 μM	KCl	20 mM	4.02 ± 0.74 [a]
		(+)-*cis*-Diltiazem	3 μM	
RP 49356	10 μM	KCl	20 mM	3.11 ± 0.97 [b]
		(+)-*cis*-Diltiazem	3 μM	
Cromakalim	10 μM	KCl	20 mM	2.20 ± 0.49
		(+)-*cis*-Diltiazem	3 μM	
		Glibenclamide	10 μM	
KCl	20 mM	(+)-*cis*-Diltiazem	3 μM	4.54 ± 0.46
		Glibenclamide	10 μM	

Data are expressed as the drug-induced rate coefficient as area under the efflux-time curve above values in vehicle controls (expressed as a percentage) and are given as means ± S.E.M. (n = 6–8). Statistical comparisons were made, where appropriate, using an unpaired Student's t-test ([a,b] $P < 0.01$).

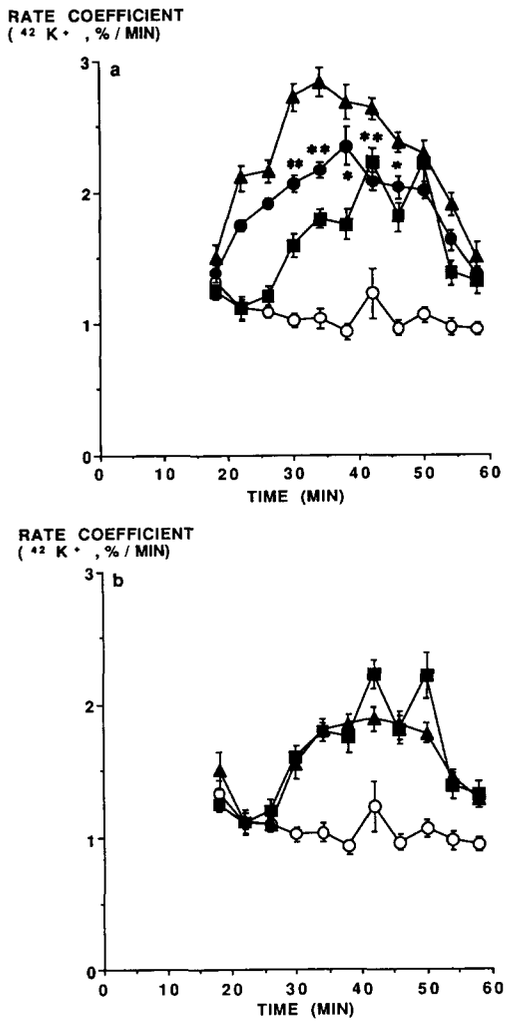

Fig. 5. $^{42}K^+$ efflux from rat myometrium. In (a) efflux from strips: exposed to PSS alone (○); exposed to (+)-*cis*-diltiazem (3 μM) and to KCl (20 mM) from 28 to 52 min (■); exposed to (+)-*cis*-diltiazem (3 μM) from 0 to 52 min, to KCl (20 mM) from 20 to 52 min and to cromakalim (10 μM) from 28 to 52 min (▲); exposed to (+)-*cis*-diltiazem (3 μM) plus glibenclamide (10 μM) from 0 to 52 min, to KCl (20 mM) from 20 to 52 min and to cromakalim (10 μM) from 28 to 52 min (●). In (b) efflux from strips: exposed to PSS alone (○); exposed to (+)-*cis*-diltiazem (3 μM) from 0 to 52 min and to KCl (20 mM) from 28 to 52 min (■); and exposed to (+)-*cis*-diltiazem (3 μM) and glibenclamide (10 μM) from 0 to 50 min and KCl (20 mM) from 28 to 52 min (▲). The data for the group exposed to diltiazem plus KCl are the same data as shown in Fig. 4. Ordinate scales: $^{42}K^+$ efflux rate coefficient expressed as percent loss of isotope from the tissue per minute. Abscissa scale: time (min) after start of efflux. The points represent the means and the vertical lines the S.E.M. ($n = 6$–8). $^*P < 0.05$, $^{**}P < 0.01$ = significant difference between efflux rate coefficient from tissues exposed to cromakalim in the presence of KCl, (+)-*cis*-diltiazem and glibenclamide (●) compared to those exposed to cromakalim in the presence of KCl plus (+)-*cis*-diltiazem (▲).

Cox (1990) had shown that cromakalim-induced $^{86}Rb^+$ efflux was absent in rat tail artery in a normal PSS but present in a medium containing KCl (15 mM). Similar data were obtained in rat ileum (Davies et al., 1993). The present studies suggest similarities in the mechanisms of cromakalim's action in the three tissues but extend the pharmacological analysis. The current data suggest that the actions of cromakalim and RP 49356 are dependent on the K^+ gradient across the membrane and/or the membrane potential. It is possible that cromakalim and RP 49356, in a KCl-enriched medium, recruit additional numbers of channels similar in nature to those they open in an unmodified PSS or they might cause the opening of channels other than those open in normal PSS or in either situation increase the frequency and/or duration of opening of these channels. It is clear that the K^+ channels involved in the cromakalim-induced $^{42}K^+$ efflux are only partially glibenclamide-sensitive as the response to cromakalim was reduced but not abolished as might be expected with this concentration of glibenclamide (10 μM).

In conclusion, cromakalim and RP 49356 appear to be able to open potassium channels as in other smooth muscles. It would appear that their action in myometrium is dependent on the extracellular K^+ concentration and/or the membrane potential.

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