Characterization of responses to cromakalim and pinacidil in smooth and cardiac muscle by use of selective antagonists

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1 In dog isolated coronary artery (precontracted with endothelin, 10 nM) cromakalim (0.1–30 μ M) and pinacidil (1–30 μ M) produced concentration-dependent vasorelaxant responses. The effects of these compounds could be blocked by glibenclamide (3 μ M), phentolamine (30 μ M) or alinidine (30 μ M) to a similar extent, indicating that both agents alter vascular tone through the same mechanism in this preparation.

2 The ability of the antagonists glibenclamide, phentolamine and alinidine to block the response to cromakalim in a number of smooth muscle types from the guinea-pig was determined. Cromakalim (0.1–30 μ M) produced concentration-dependent relaxant responses in thoracic aorta (precontracted with endothelin, 30 nM), ileum (precontracted with K^+ , 25 mM) and trachea (spontaneously contracted). Responses to cromakalim in all tissues could be blocked by the three antagonists. However, significantly higher concentrations of the antagonists were required to block responses in the thoracic aorta than in the ileum or trachea. Given that the rank order of potency of the antagonists was similar in all tissues (i.e. glibenclamide > phentolamine = alinidine), this result may suggest vascular K^+ channels opened by cromakalim are quantitatively but not qualitatively different in vascular compared with non-vascular smooth muscle. Glibenclamide was approximately 10 times more potent than phentolamine or alinidine.

3 Cromakalim had minimal functional effects on the rat spontaneously beating right atrial (rate) or electrically driven left ventricular strip (force) preparations. Similarly the three antagonists studied failed to alter force generation in the right ventricular strip. However alinidine and phentolamine did produce a dose-related bradycardia in the spontaneously beating right atria. This effect appears to be unrelated to blockade of the K^+ channel opened by cromakalim since glibenclamide, the most potent K^+ channel antagonist studied, failed to produce the same response.

4 It would appear that the K^+ channel opened by cromakalim is present in a number of vascular and non-vascular smooth muscle. Based on the potency of the three antagonists studied, there appears to be little heterogeneity in the process activated by cromakalim in vascular and non-vascular smooth muscle.

Introduction

Cromakalim (Hamilton *et al.*, 1986; Quast, 1987; Wilson *et al.*, 1988) and pinacidil (see Videbaek *et al.*, 1988) are two new antihypertensives which are thought to produce their vasodilator actions by opening K^+ channels in the plasma membrane of smooth muscle cells. However, the exact nature of the interaction between these compounds and the cell, the events leading to the observed efflux of K^+ and the mechanism resulting in relaxation are unclear. For example, detailed radioligand binding studies have failed to identify receptors for cromakalim in tissues where it causes relaxation (Coldwell & Howlett, 1987). In addition, some recent studies have suggested that while the hyperpolarization observed with these compounds is important in their mechanism of action, in some tissues at least it is not the subsequent closing of voltage-sensitive Ca^{2+} channels that produces the observed relaxation (Gillespie & Sheng, 1988).

Previous studies using vascular and non-vascular preparations have shown that phentolamine, alinidine (McPherson & Angus, 1989) and glibenclamide (Quast, 1988; Eltze, 1989; Buckingham *et al.*, 1989) can antagonize the response to cromakalim and pinacidil. The purpose of the present work was to determine whether the K^+ channels activated by cromakalim and pinacidil were functionally similar in terms of their sensitivity to the antagonists phentolamine, alinidine and glibenclamide. In addition we asked whether there were fundamental tissue differences in the K^+ channel activated by cromakalim in a number of vascular and non-vascular smooth muscle preparations, judged by their sensitivity to the three antagonists.

Methods

General methods

A number of vascular, non-vascular and cardiac preparations were studied *in vitro*. All experiments were carried out in 25 ml organ baths containing a modified Krebs buffer (composition in mM: NaCl 119, KCl 4.7, $MgSO_4 \cdot 7H_2O$ 1.17, $NaHCO_3$ 25, KH_2PO_4 1.18, $CaCl_2$ 2.5 and glucose 11) maintained at 37°C and gassed with 5% CO_2 in O_2 . Changes in isometric force development of the preparations were monitored with Grass (FT03) force displacement transducers coupled to a Grass polygraph (model 7). Each preparation was placed under an initial passive force and allowed to equilibrate for 30 min before the addition of any drugs.

Vascular and non-vascular smooth muscle

Dog isolated coronary artery Ring segments (4 mm long) of circumflex coronary arteries were obtained from greyhound dogs (20–30 kg) killed by injection of pentobarbitone (60 mg kg⁻¹, i.v.). Each tissue segment was mounted on wire hooks and placed under an initial passive force of 4 g. Active tone was induced by the addition of endothelin (ET-1) at a concentration (3–10 nM) that caused approximately 50–80% of the maximum response to this agent. Vasorelaxant responses to increasing concentrations of cromakalim (0.3–30 μ M), pinacidil (1–30 μ M) or isoprenaline (1–300 nM) were assessed in the absence and in the presence of the antagonists glibenclamide (3 μ M), phentolamine (30 μ M) or alinidine (30 μ M). The antagonists were added to the bath once the response to endothelin had reached a plateau. Ten minutes later one of the vasorelaxant drugs were tested. In this and other studies this period of exposure was found to cause maximal effects on the cromakalim response. The relaxant response to each agonist was

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expressed as a percentage of the maximum response obtained to isoprenaline ($10\text{ }\mu\text{M}$) which was added at the end of each experiment.

Smooth muscle preparations from the guinea-pig

Guinea-pigs were killed by a blow to the back of the head and the thoracic aorta, ileum or trachea removed. In each case the experimental protocol was the same. Relaxant responses to cromakalim were assessed in the absence and in the presence of increasing concentrations of glibenclamide ($0.1\text{--}10\text{ }\mu\text{M}$), phentolamine ($1\text{--}100\text{ }\mu\text{M}$) or alinidine ($1\text{--}100\text{ }\mu\text{M}$). Each preparation acted either as a control or was treated with one concentration of an antagonist. In this latter case the antagonist was added to the bath once the contractile response had reached a plateau. The preparation was allowed to equilibrate for 10 min before the concentration-effect curve to cromakalim was obtained. The relaxant responses to cromakalim were expressed as a percentage of the maximum relaxant response to papaverine (0.15 mM) which was added at the end of each experiment.

In the case of thoracic aorta, tone was induced in each vascular segment (see dog coronary artery) by the addition of endothelin ($3\text{--}10\text{ nM}$). Segments (2 cm long) of ileum were mounted longitudinally under 1 g force and precontracted with 25 mM KCl. The trachea single ring segments were suspended under 0.5 g force. Under these conditions the trachea exhibited spontaneous tone and thus the relaxant effect of cromakalim could be assessed directly.

Cardiac muscle

Rat spontaneously beating right atrium Rats ($250\text{--}300\text{ g}$) were killed by a blow to the head and their hearts removed. The right atria was dissected free and suspended under 0.5 g force. Isometric force (FT03 transducer) recorded from the spontaneously beating atrium was used to trigger a Grass (model 7P4) tachograph. Chronotropic responses to cromakalim, glibenclamide, phentolamine and alinidine were then assessed directly.

Rat electrically driven right ventricle strip Segments (1 cm long) of rat right ventricle were obtained and mounted under 1 g force. The strips were stimulated with a Grass (SD9) stimulator at 0.5 Hz , 5 ms duration and at a voltage sufficient to produce maximal contractile responses. Responses to increasing concentrations of cromakalim ($10\text{--}100\text{ }\mu\text{M}$) were assessed. Responses to glibenclamide ($30\text{ }\mu\text{M}$), phentolamine ($100\text{ }\mu\text{M}$) and alinidine ($100\text{ }\mu\text{M}$) were also assessed.

Drugs

The following drugs were used: cromakalim (Beecham); pinacidil monohydrate (Leo Pharmaceuticals); endothelin (ET-1; porcine, human) (Auspep, Australia); (–)-isoprenaline bitartrate (Sigma); phentolamine mesylate (Ciba-Geigy); alinidine bromide (Eoehringer-Ingelheim); glibenclamide (Hoechst).

Stock solutions (10 mM) of phentolamine and alinidine were made in distilled water and diluted each day in the same vehicle. The poor solubility of cromakalim and pinacidil limited, in some tissues, the construction of full concentration-effect curves due to the adverse effects the organic solvents used. Glibenclamide (10 mM) was prepared in 100% methanol. Cromakalim (10 mM) and pinacidil (10 mM) stock was made in 50% (v/v) methanol; any precipitation could be reversed by gently warming the mixture. Isoprenaline (10 mM) stock was made in 0.01 M HCl. Dilutions of all stock solutions were made with distilled water.

Data analysis and statistics

pD_2 ($-\log \text{EC}_{50}$ value) and concentration-ratios were generally (see below) estimated by standard graphical procedures.

Statistical comparisons between two groups were made by Student's t test. Multiple comparisons of dependent samples were made by Bonferroni's t test. Multiple comparisons between independent samples were made by use of Scheffe's t test (see Wallenstein *et al.*, 1980). Results in the text are the mean $\pm$ s.e.mean for the specified number of experiments.

In some cases, concentration-effect curves were fitted by a non-linear curve fitting technique (McPherson *et al.*, 1983). The three parameter function gave estimates of the EC_{50} , maximum response and the slope factor (pseudo-Hill coefficient).

Results

Similarities in the actions of cromakalim and pinacidil on the dog coronary artery

Cromakalim ($0.3\text{--}30\text{ }\mu\text{M}$), pinacidil ($1\text{--}30\text{ }\mu\text{M}$) and isoprenaline ($1\text{--}300\text{ nM}$) caused concentration-dependent relaxation of the dog isolated coronary artery (Figure 1). In terms of the maximal relaxant response to isoprenaline ($= 100\%$) cromakalim ($30\text{ }\mu\text{M}$) and pinacidil ($30\text{ }\mu\text{M}$) caused a relaxation of

Figure 1 Mean concentration-effect curves for cromakalim (a), pinacidil (b) and isoprenaline (c) in dog coronary artery. Results are expressed as percentage of the contraction induced by endothelin (3 nM , 100%). The maximal possible tissue relaxant response was defined using isoprenaline ($10\text{ }\mu\text{M}$). Curves for each agonist were obtained in the absence ($\circ$) and in the presence of glibenclamide (Δ , $3\text{ }\mu\text{M}$), phentolamine ($\bullet$, $30\text{ }\mu\text{M}$) and alinidine ($\blacktriangle$, $30\text{ }\mu\text{M}$). Results are the mean from 3–4 separate experiments; s.e.mean shown by vertical bars.

85 ± 4% (*n* = 3) and 93 ± 5% (*n* = 3) respectively. Cromakalim (pD₂ ≈ 6.3) was approximately 50 and pinacidil (pD₂ ≈ 5.6) 250 times less potent than isoprenaline (pD₂ ≈ 8) (Figure 1.) The ability of glibenclamide (3 μM), phentolamine (30 μM) and alinidine (30 μM) to modify responses to the three agonists was tested. Isoprenaline vasorelaxant responses were unaffected by these compounds; however, those to cromakalim and pinacidil were markedly attenuated. Based on the concentration of agonist required to produce 50% relaxation (assuming that relaxation at 30 μM of each agonist = 100%) then the concentration-effect curves to cromakalim and pinacidil were both shifted 5–10 fold for each antagonist. The inability to construct full concentration-effect curves to the agonists (cromakalim and pinacidil) in the presence of higher concentrations of the antagonists precluded full quantitative analysis of the antagonism. However, the shift of the cromakalim concentration-effect curve by phentolamine and alinidine was accompanied by a significant reduction in the maximum response (*P* < 0.05, Scheffé's *t* test). In the case of pinacidil the shift produced by the three antagonists appeared more parallel (Figure 1) with little change in the maximal relaxation.

The effect of cromakalim on vascular and non-vascular smooth muscle from the guinea-pig

The effect of cromakalim and its interaction with glibenclamide, phentolamine and alinidine was assessed in a number of preparations from the guinea-pig. Cromakalim caused concentration-dependent relaxation of the thoracic aorta (precontracted with endothelin), ileum (precontracted with potassium) and tracheal smooth muscle (spontaneously contracted). The potency (pD₂) of cromakalim ranged from ≈ 6.2 in the trachea to ≈ 5.6 in the thoracic aorta (see Table 1). The maximal relaxation produced by cromakalim was more variable (Table 1). Contractions in the thoracic aorta could be reversed an average of 50% while in the trachea the value was 80%. The concentration-effect curves for cromakalim were steep in all tissues (slope factors greater than unity in all cases; see Table 1).

Glibenclamide, phentolamine and alinidine were able to antagonize the actions of cromakalim in all preparations. Figures 2–4 show the mean data obtained in guinea-pig thoracic aorta, ileum and trachea respectively. In the ileal preparation phentolamine and alinidine (100 μM) caused a direct relaxation of the tone induced by K⁺. Results where this concentration of the antagonists was used could therefore not be calculated.

Due to the limited concentration-range over which the cromakalim curve could be constructed, it was not possible to quantitate fully the interaction between cromakalim and the antagonists. However, it was apparent from the studies that higher concentrations of antagonists were required to block the actions of cromakalim in the thoracic aorta than in the trachea or ileum. This is more clearly shown in Figure 5

Table 1 Effects of cromakalim on thoracic aorta, ileum and trachea from the guinea-pig

Tissue	n	pD ₂	Slope	% maximum relaxation
Thoracic aorta	11	5.58 ¹ * (0.07)	1.55 ¹ (0.14)	50 ¹ (3)
Ileum	12	5.78 ¹ (0.06)	1.44 ¹ (0.14)	68 ² (4)
Trachea	13	6.18 ² (0.07)	2.16 ¹ (0.21)	80 ³ (2)

* Values marked with different numeral were significantly different from others in the same group (*P* < 0.05, Scheffé's *t* test). pD₂ values are the -log EC₅₀ value. The slope is the Hill coefficient calculated by the non-linear curve fitting technique described in the methods. The values in parentheses are the s.e.mean from the specified number of experiments (*n*).

Figure 2 Mean concentration-effect curves for cromakalim obtained in guinea-pig thoracic aorta. Results are expressed as a percentage of the maximum contraction induced by endothelin (10 nM, 100%). The maximal possible tissue relaxant response was defined by use of papaverine (0.15 mM). Curves for cromakalim were constructed in the absence (○) and in the presence of increasing concentrations of: (a) glibenclamide 0.1 μM (●), 1 μM (△), 10 μM (▲) and 30 μM (□); (b) phentolamine 1 μM (●), 10 μM (△), 30 μM (▲) and 100 μM (□); (c) alinidine 1 μM (●), 10 μM (△), 30 μM (▲) and 100 μM (□). Results are the mean from 3 to 4 separate experiments; s.e.mean shown by vertical bars.

which shows the response to cromakalim (10 μM) in the absence and presence of increasing concentrations of antagonists (data extracted from Figures 2–4). In the trachea and ileum the response to cromakalim (10 μM) was significantly blocked by glibenclamide at concentrations in excess of 1 μM and phentolamine and alinidine in excess of 10 μM (Bonferroni's test, *P* < 0.05). However, in the thoracic aorta significant blockade of the response to this concentration of cromakalim required 10 μM glibenclamide and 30 μM phentolamine and alinidine. In all cases however glibenclamide was the most potent antagonist being approximately 3–10 times more active than phentolamine and alinidine.

Effects of cromakalim on the rat right atria and right ventricle strips

Cromakalim, in concentrations up to 30 μM, had no significant effect on the rat spontaneously beating right atrial preparation (Figure 6a). In addition, it did not affect the concentration-effect curve for isoprenaline-induced tachycardia (pD₂ ≈ 10 nM). The three antagonists studied had variable effects.

Figure 3 Mean concentration-effect curves for cromakalim obtained in guinea-pig ileum. Results are expressed as a percentage of the maximum contraction induced by K^+ (25 mM, 100%). The maximal possible tissue relaxant response was defined by use of papaverine (0.15 mM). Curves for cromakalim were constructed in the absence ($\circ$) and in the presence of increasing concentrations of: (a) glibenclamide; (b) phentolamine; (c) alinidine. The concentrations used are identical to those described in Figure 2. The results are the mean from 4 separate experiments; s.e.mean shown by vertical bars.

Alinidine and phentolamine produced a concentration-dependent bradycardia (Figure 6b). In this series of experiments alinidine was approximately 10 times more potent than phentolamine. Glibenclamide did not effect the rate of beating of the right atria at concentrations up to $3 \mu\text{M}$ (Figure 6b).

In the driven right ventricle strip preparation of the rat cromakalim was generally inactive at concentrations up to $30 \mu\text{M}$. At $100 \mu\text{M}$, the highest concentration tested, a small negative inotropic effect ($<20\%$) was observed in 2 out of 4 preparations studied. Due to the variability in the response to cromakalim, and the high concentrations required, it was not possible to study the interaction with the K^+ channel blockers. Glibenclamide ($30 \mu\text{M}$), phentolamine and alinidine (both $100 \mu\text{M}$) did not affect the force of contraction in this preparation.

Discussion

The major finding from this study was that cromakalim does not appear to show selectivity in its potency in relaxing vascu-

Figure 4 Mean concentration-effect curves for cromakalim obtained in guinea-pig tracheal rings. Results are expressed as a percentage of the maximum tone which developed spontaneously (100%). The maximal possible tissue relaxant response was defined by use of papaverine (0.15 mM). Curves for cromakalim were constructed in the absence ($\circ$) and in the presence of increasing concentrations of: (a) glibenclamide; (b) phentolamine; (c) alinidine. The concentrations used are identical to those described in Figure 2. The results are the mean from 4–5 separate experiments; s.e.mean shown by vertical bars.

lar and non-vascular smooth muscle. In addition, three structurally different antagonists displayed the same relative ability to antagonize the actions of cromakalim in all tissues. Thus the mechanism underlying the activity of cromakalim (i.e. K^+ channel opening) is apparently ubiquitous in smooth muscle.

On the basis of the similar degree of antagonism by glibenclamide, phentolamine and alinidine of the relaxation response to pinacidil and cromakalim, it would appear that both compounds are acting through a similar mechanism to cause relaxation in dog isolated coronary artery. However, given that the antagonism produced by the three inhibitors is non-competitive in nature with cromakalim as the agonist (i.e. the maximum vasorelaxant response was diminished) but apparently competitive with pinacidil (i.e. the curves were shifted in a parallel fashion) it is possible that some qualitative differences exist. In the guinea-pig pulmonary artery, Eltze (1988) obtained identical pA_2 values for glibenclamide, independent of whether pinacidil or cromakalim were used as the agonist. The results indicate that the interaction between K^+ channel openers and the antagonists are complex, given that in the dog coronary artery the interaction is non-competitive (present study, McPherson & Angus, 1989) but competitive in

Figure 5 Mean concentration-effect curves of increasing concentrations of glibenclamide (a), phentolamine (b) and alinidine (c) in reducing the relaxation response to cromakalim ($10 \mu\text{M}$) in preparations from the guinea-pig (Δ) thoracic aorta, ($\circ$) ileum and ($\bullet$) trachea. Results (derived from Figures 2–4) are expressed as a percentage of the maximum tone present in each preparation before the addition of cromakalim. The maximal possible tissue relaxant response was defined by use of papaverine (0.15 mM). In each panel (C) represents the amount of tone in the presence of cromakalim ($10 \mu\text{M}$) but in the absence of antagonist. Results are mean from 3–5 separate experiments; s.e.mean shown by vertical bars.

the guinea-pig pulmonary artery (Eltze, 1988). Clearly much more work is required in this area.

The results also highlight the differences in the mechanism of action of cromakalim and pinacidil compared with nicorandil. Previous studies (Eltze, 1988; McPherson & Angus, 1989) have shown that the effects of another purported K⁺ channel opener, nicorandil, were insensitive to glibenclamide, phentolamine and alinidine. However, it is known that nicorandil can cause smooth muscle relaxation by a mechanism other than K⁺ channel opening (Wier & Weston, 1986; Coldwell & Howlett, 1987).

The effects of cromakalim are not restricted to vascular smooth muscle since relaxant responses to this compound have also been reported in a number of non-vascular smooth muscle preparations (present study; see Allen *et al.*, 1986; Weir & Weston, 1986; Arch *et al.*, 1988; Buchheit & Bertholet, 1988; Eltze, 1989). The results from this study indicate that the effect of cromakalim on smooth muscle (vascular and non-vascular) is very similar. Thus cromakalim produced relaxant responses over a similar concentration-range in a number of guinea-pig smooth muscle preparations. The response to cromakalim in the thoracic aorta however

Figure 6 (a) Mean concentration-effect curves for cromakalim ($\bullet$) and isoprenaline in the rat spontaneously beating right atrial preparation. Curves for isoprenaline were constructed in the absence ($\circ$) and in the presence (Δ) of cromakalim ($30 \mu\text{M}$). Results are expressed in absolute heart rate responses (HR) and are the mean from 4–5 separate experiments. (b) Mean concentration-effect curves for glibenclamide ($\circ$), phentolamine (Δ) and alinidine ($\bullet$) obtained in the rat right atria. Results are expressed in absolute heart rate responses (HR) and are the mean from 3–4 separate experiments. Vertical bars show s.e.mean.

appeared to be less sensitive to the effects of the three antagonists than those in the trachea or ileum. This result may indicate some subtle differences in the process activated by cromakalim in vascular compared with non-vascular smooth muscle. However, the exact mechanism underlying the interaction between the antagonists and cromakalim is unknown at present. It is possible for example that the potency of the antagonists is partially dependent on the nature of the agent used to produce tone. We are currently investigating this possibility. Despite these reservations, the finding that the response to cromakalim can be blocked by all three antagonists with the same rank order of potency (i.e. glibenclamide > phentolamine = alinidine) would suggest that the process activated by cromakalim is similar in all preparations studied. However, it is possible that the agents that are currently available to block the action of cromakalim are non-selective. Consequently any conclusion regarding the homogeneity of the K⁺ channel opened by cromakalim should be viewed with some caution.

We also examined the cardiac actions of cromakalim. Previous studies have shown that cromakalim reduces action potential duration in guinea-pig papillary muscle (Scholtysik, 1987) and myocytes (Osterrieder, 1988). However, these cardiac effects of cromakalim occur at concentrations (10 – $300 \mu\text{M}$) 10–100 fold greater than those causing vascular and non-vascular smooth muscle relaxation (0.1 – $1 \mu\text{M}$). We found the cardiac effects of cromakalim to be very poor. It had no effect on the spontaneously beating right atria in concentrations up to $30 \mu\text{M}$. In the electrically driven left ventricle strip, cromakalim caused a small and inconsistent negative inotropic action. On the basis of these results it would appear that there is minimal functional importance for the K⁺ channel

opened by cromakalim in these preparations or, alternatively, that the channel functions but is poorly coupled to any cromakalim-modulated mechanism.

One reason for examining cardiac preparations was that previous studies have shown that phentolamine (Angus, unpublished observations) and particularly alinidine (see Heinzow *et al.*, 1982) cause a marked bradycardia in spontaneously beating isolated atria and in the conscious rabbit. Given that both compounds interacted with the K^+ channel activated by cromakalim, we suggested (McPherson & Angus, 1989) that the cardiac effects of these compounds may, in some way, be related to this channel. From the present studies however, this would appear to be incorrect. We showed that glibenclamide was approximately 10 times more potent than alinidine and phentolamine in antagonizing the effects of cromakalim and pinacidil. However, in the spontaneously beating right atrial preparation, glibenclamide was inactive while alinidine was approximately 10 times more potent than phentolamine. In addition, in the right ventricle strip all antagonists were inactive despite cromakalim displaying some negative inotropic effects. Both these findings would indicate lack of involvement of the K^+ channel activated by cromakalim in regulating cardiac function in the rat.

The K^+ channel opened by compounds like cromakalim and pinacidil are widespread and involved in a number of processes. They regulate tone in vascular and non-vascular

smooth muscle (see above). In addition, recent studies (Ferrier *et al.*, 1989) have shown that cromakalim can directly release renin from rat isolated juxtaglomerular cells in addition to raising renin levels in man. Cromakalim has also been shown to modulate behavioural responses (Tricklebank *et al.*, 1988). This lack of specificity may limit usefulness of these compounds in conditions requiring chronic administration. However, recent studies have shown that diazoxide, a drug used for acute hypertensive crisis, may act through the same mechanism as cromakalim (Winquist *et al.*, 1989). Given that cromakalim is 100 times more potent than diazoxide in relaxing smooth muscle, it is possible that cromakalim would prove a better alternative to diazoxide in this acute condition.

In conclusion, the results from this study indicate that the mechanism by which K^+ channels are opened by cromakalim in both vascular and non-vascular smooth muscle is effectively homogeneous with respect to the actions of the three antagonists, glibenclamide, phentolamine and alinidine. There appears to be no importance of this potassium channel in regulating cardiac function in the rat under the conditions studied.

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References

- ALLEN, S.L., BOYLE, J.P., CORTIJO, J., FOSTER, R.W., MORGAN, G.P. & SMALL, R.C. (1986). Electrical and mechanical effects of BRL 34915 in guinea-pig isolated trachealis. *Br. J. Pharmacol.*, **89**, 395–405.
- ARCH, J.R.S., BUCKLE, D.R., BUMSTEAD, J., CLARKE, G.D., TAYLOR, J.F. & TAYLOR, S.G. (1988). Evaluation of the potassium channel activator cromakalim (BRL 34915) as a bronchodilator in the guinea-pig: comparison with nifedipine. *Br. J. Pharmacol.*, **95**, 763–770.
- BUCHHEIT, K.H. & BERTHOLET, A. (1988). Inhibition of small intestinal motility by cromakalim (BRL 34915). *Eur. J. Pharmacol.*, **154**, 335–337.
- BUCKINGHAM, R.E., HAMILTON, T.C., HOWLETT, D.R., MOOTO, S. & WILSON, C. (1989). Inhibition by glibenclamide of the vasorelaxant action of cromakalim in the rat. *Br. J. Pharmacol.*, **97**, 57–64.
- COLDWELL, M.C. & HOWLETT, D.R. (1987). Specificity of action of the novel antihypertensive agent BRL 34915, as a potassium channel activator: Comparison with nicorandil. *Biochem. J.*, **36**, 3663–3669.
- ELTZE, M. (1988). Cromakalim, pinacidil and 49356 RP but not nicorandil relax guinea pig pulmonary artery via opening the same glibenclamide-sensitive K^+ channel. *Naunyn-Schmiedeberg Arch. Pharmacol.*, **339**, Suppl. R64.
- ELTZE, M. (1989). Competitive antagonism by glibenclamide of cromakalim inhibition of twitch contractions in rabbit vas deferens. *Eur. J. Pharmacol.*, **161**, 103–106.
- FERRIER, C.P., KURTZ, A., LEHNER, P., SHAW, S.G., PUSTERLA, C., SAXENHOFER, H. & WEIDMANN, P. (1989). Stimulation of renin secretion by potassium-channel activation with cromakalim. *Eur. J. Clin. Pharmacol.*, **36**, 443–447.
- GILLESPIE, J.S. & SHENG, H. (1988). The lack of involvement of cyclic nucleotides in the smooth muscle relaxant action of BRL 34915. *Br. J. Pharmacol.*, **94**, 1189–1197.
- HAMILTON, T.C., WEIR, S.W. & WESTON, A.H. (1986). Comparison of the effects of BRL 34915 and verapamil on electrical and mechanical activity in rat portal vein. *Br. J. Pharmacol.*, **88**, 103–111.
- HEINZOW, B.G.J., ANGUS, J.A. & KORNER, P.L. (1982). Effects of alinidine (ST567) on baroreceptor-heart rate reflexes and its interactions with clonidine on the baroreflex and on the sympathetic terminals of the isolated atrium. *Eur. J. Pharmacol.*, **84**, 177–187.
- McPHERSON, G.A. & ANGUS, J.A. (1989). Phentolamine and structurally related compounds selectively antagonise the vascular actions of the K^+ channel opener cromakalim. *Br. J. Pharmacol.*, **97**, 941–949.
- McPHERSON, G.A., MOLENAAR, P., RAPER, C. & MALTA, E. (1983). Analysis of dose-response curves and calculation of agonists dissociation constants using a weighted non-linear curve fitting technique. *J. Pharmacol. Methods*, **10**, 231–241.
- OSTERRIEDER, W. (1988). Modification of K^+ conductance of heart cell membrane by BRL 34915. *Naunyn-Schmiedeberg Arch. Pharmacol.*, **337**, 93–97.
- QUAST, U. (1987). Effect of the K^+ efflux stimulating dilator BRL 34915 on $86Rb^+$ efflux and spontaneous activity in the guinea-pig portal vein. *Br. J. Pharmacol.*, **91**, 569–578.
- QUAST, U. (1988). Inhibition of the effects of the K^+ channel stimulator cromakalim (BRL 34915) in vascular smooth muscle by glibenclamide and forskolin. *Naunyn-Schmiedeberg Arch. Pharmacol.*, **337**, Suppl. R72.
- SCHOLTYSIK, G. (1987). Evidence for inhibition by ICS 205–930 and stimulation by BRL 34915 of K^+ conductance in cardiac muscle. *Naunyn-Schmiedeberg Arch. Pharmacol.*, **335**, 692–696.
- TRICKLEBANK, M.D., FLOCKHART, G. & FREEDMAN, S.B. (1988). The potassium channel activator, BRL 34915, antagonises a behavioral response to the muscarinic agonist, pilocarpine. *Eur. J. Pharmacol.*, **151**, 349–350.
- VIDEBAEK, L.M., AALKJAER, C. & MULVANY, M. (1988). Pinacidil opens K^+ -selective channels causing hyperpolarization and relaxation of noradrenaline contractions in rat mesenteric resistance vessels. *Br. J. Pharmacol.*, **95**, 103–108.
- WALLENSTEIN, S., ZUCKER, C.L. & FLEISS, J.L. (1980). Some statistical method useful in circulation research. *Circ. Res.*, **47**, 1–9.
- WEIR, S.W. & WESTON, A.H. (1986). Effect of apamin on responses to BRL 34915, nicorandil and other vasorelaxants in the guinea-pig taenia caeci. *Br. J. Pharmacol.*, **88**, 113–120.
- WINQUIST, R.J., HEANEY, L.A., WALLACE, A.A., BASKIN, E.P., STEIN, R.B., GARCIA, M.L. & KACZOROWSKI, G.J. (1989). Glyburide blocks the relaxation response to BRL 34915 (cromakalim), minoxidil sulphate and diazoxide in vascular smooth muscle. *J. Pharmacol. Exp. Ther.*, **248**, 149–156.
- WILSON, C., COLDWELL, M.C., HOWLETT, D.R., COOPER, S.M. & HAMILTON, T.C. (1988). Comparative effects of K^+ channel blockade on the vasorelaxant activity of cromakalim, pinacidil and nicorandil. *Eur. J. Pharmacol.*, **152**, 331–339.

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