Br. J. Pharmacol. (1991), **103**, 1148–1152

Comparison of the effects of the K^+-channel openers cromakalim and minoxidil sulphate on vascular smooth muscle

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1 The actions of the potassium channel openers, cromakalim and minoxidil sulphate, were compared in a range of isolated blood vessel preparations.

2 Cromakalim and minoxidil sulphate inhibited spontaneous mechanical activity of the guinea-pig portal vein and relaxed the noradrenaline precontracted rat aorta with similar potency. In contrast, minoxidil sulphate was less potent than cromakalim in inhibiting spontaneous activity in the rat portal vein and was essentially inactive in the noradrenaline precontracted rat mesenteric artery and rabbit aorta.

3 Minoxidil sulphate did not antagonize the effects of cromakalim in the rabbit aorta indicating it was not acting as a partial 'agonist'.

4 Charybdotoxin, noxiustoxin and rubidium failed to discriminate between cromakalim and minoxidil sulphate indicating that the apparently selective effects of minoxidil sulphate were not mediated by either Ca^{2+}-activated potassium channels, delayed rectifiers or rubidium impermeable potassium channels.

5 Glibenclamide antagonized the effects of cromakalim in an apparently competitive manner whereas the effects of minoxidil sulphate were antagonized in a non-competitive manner. The involvement of subtypes of ATP-sensitive potassium channels is discussed.

Keywords: Cromakalim; minoxidil sulphate; blood vessels; K^+-channels

Introduction

Cromakalim and minoxidil sulphate are thought to belong to a class of vasodilators collectively known as potassium channel openers. Thus, cromakalim has been shown to relax a variety of vascular smooth muscle preparations and these effects are generally found to be associated with increases in the $^{86}Rb^+$ or $^{42}K^+$ efflux rate coefficient and membrane hyperpolarization to a value close to the calculated potassium equilibrium potential (Hamilton *et al.*, 1986). Studies with minoxidil sulphate however, are far less numerous. Meisheri *et al.* (1988) reported that minoxidil sulphate relaxed the rabbit isolated mesenteric artery by a mechanism that was associated with stimulation of $^{42}K^+$ efflux. The ability of minoxidil sulphate to increase $^{42}K^+$ efflux and hyperpolarize the vascular smooth muscle cell membrane has subsequently been demonstrated in rat portal vein and rat aorta (Newgreen *et al.*, 1990) and in rabbit portal vein (Leblanc *et al.*, 1989), confirming a role for potassium permeability in the mechanism of action of this compound. However, despite the obvious similarities in the mechanisms of these compounds, several subtle differences have emerged. For example, although the hypoglycaemic sulphonylurea glibenclamide has been found to inhibit the effects of cromakalim in an apparently competitive manner (Buckingham *et al.*, 1989; Winquist *et al.*, 1989), glibenclamide antagonizes the effects of minoxidil sulphate in a non-competitive manner (Winquist *et al.*, 1989; Newgreen *et al.*, 1990). Furthermore, minoxidil sulphate, unlike cromakalim, failed to increase $^{86}Rb^+$ efflux from rat aorta (Newgreen *et al.*, 1990).

In order to investigate further similarities and differences in the mechanism of action of cromakalim and minoxidil sulphate the present study compares the actions of these compounds in a range of isolated vascular preparations. In addition, the interaction of cromakalim and minoxidil sulphate with a range of potassium channel blockers was investigated in an attempt to elucidate the nature of the potassium channels opened by these vasodilators.

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Methods

Tissue bath experiments

Longitudinal sections of hepatic portal vein from male Dunkin Hartley guinea-pigs (200–350 g), longitudinal sections of portal vein and rings of thoracic aorta and superior mesenteric artery from female Alderley Park rats (200–300 g) or rings of thoracic aorta from male New Zealand White rabbits (2.5–3 kg) were suspended in 20 ml organ baths containing Krebs buffer of the following composition (mM): NaCl 120, $NaHCO_3$ 25, D-glucose 11.2, KH_2PO_4 1.2, $MgSO_4 7H_2O$ 1.2, KCl 4.7, $CaCl_2$ 2.5, ethylenediamine-tetra-acetic acid disodium salt (EDTA) 0.026, maintained at 37°C and bubbled with 95% O_2/5% CO_2. No attempt was made to remove the endothelium in any of the preparations studied. Isometric tension was recorded under a resting tension of 1 g. Tissues were allowed to equilibrate for a period of 1 h during which the Krebs buffer solution was changed every 20 min. Following the equilibration period, rings of aorta and mesenteric artery were challenged with a sub-maximal concentration of noradrenaline every 30 min until responses were consistent. At this point the spasmogen was allowed to remain in contact with the tissue and, once a stable plateau had developed (approximately 20 min), cumulative concentration-response curves were constructed to either cromakalim or minoxidil sulphate. Results were expressed as percentage relaxation of the response to the spasmogen. Additionally, cumulative concentration-response curves were constructed to cromakalim or minoxidil sulphate in guinea-pig or rat portal vein. A 30 min contact time was allowed for each concentration. The mean amplitude of the spontaneous changes in tension was calculated over the final 10 min of each contact period and expressed as a percentage inhibition of the mean control amplitude. Studies were also undertaken with potassium channel blockers. Cumulative concentration-response curves were constructed following 30 min incubation with potassium channel blocker or vehicle. Results are expressed as percentage inhibition of the mean amplitude of spontaneous activity

following addition of blocker or vehicle. In experiments involving replacement of potassium with rubidium, cumulative concentration-response curves were constructed using strips of rat portal vein following a 1 h equilibration in a modified Krebs solution (KH_2PO_4 replaced with an equimolar concentration of NaH_2PO_4, 2 mM KCl and 2 mM RbCl).

Schild analysis

Results showing evidence of competitive antagonism (parallel shifts without a reduction in maxima) were subjected to Schild analysis. Concentration ratios were calculated at the IC_{50} point from the mean control IC_{50} value determined in separate tissues in the presence of vehicle. Providing the slope of the Schild regression did not differ significantly from unity, pA_2 values were calculated from a least squares regression with slope corrected to unity.

Materials

Noradrenaline, glibenclamide and rubidium chloride were all obtained from Sigma. Cromakalim, minoxidil sulphate, noxiustoxin (NTX) and charybdotoxin (ChTX) were obtained from Chemistry Department II, ICI Pharmaceuticals. Cromakalim (1 mM), noxiustoxin (0.1 mM) and charybdotoxin (0.1 mM) were all dissolved in distilled water. Minoxidil sulphate (1 mM) was dissolved in 40% PEG200. Higher concentrations were obtained from a 10 mM stock dissolved in dimethylsulphoxide (DMSO). Noradrenaline (1 mM) was dissolved in water with an equal weight of ascorbic acid to prevent oxidation. All stocks were made up fresh every day.

Results

Guinea-pig portal vein

In control experiments cromakalim inhibited spontaneous mechanical activity in the guinea-pig portal vein with a mean pD_2 ($\pm$s.e.mean) of 7.4 $\pm$ 0.32 ($n = 4$, Figure 1a). Minoxidil sulphate also inhibited spontaneous mechanical activity with a mean pD_2 ($\pm$s.e.mean) of 7.5 $\pm$ 0.13 ($n = 4$, Figure 1b). These pD_2 values were not significantly different ($P > 0.05$, Student's t test).

Glibenclamide produced a marked, concentration-related rightward shift in the cromakalim concentration-response curve without altering the maximum inhibition of spontaneous activity (Figure 1a). Mean pD_2 values were 7.05 $\pm$ 0.14, 6.88 $\pm$ 0.1, 6.14 $\pm$ 0.12 and 5.16 $\pm$ 0.05 in the presence of vehicle, 0.1, 1 and 10 μM glibenclamide respectively. Schild analysis of concentration-ratios revealed a linear relationship with a slope of 1.07, which was not significantly different from unity and a pA_2 value of 6.8 $\pm$ 0.1. At the maximum concentration tested, glibenclamide was found to produce only a small increase in spontaneous activity (<20%).

Glibenclamide also produced a concentration-related rightward shift in the minoxidil sulphate concentration-response curve (Figure 1b). Mean minoxidil sulphate pD_2 values were 7.73 $\pm$ 0.25, 7.45 $\pm$ 0.35, 6.99 $\pm$ 0.39 and 6.1 $\pm$ 0.33 in the presence of vehicle, 0.1, 0.3, and 1 μM glibenclamide respectively. In the presence of 10 μM glibenclamide, minoxidil sulphate failed to inhibit spontaneous activity by 50% at concentrations up to and including 10 μM. Thus, unlike the antagonism of cromakalim the antagonism of minoxidil sulphate by glibenclamide was associated with a reduction in maximum inhibition of spontaneous activity. The maximum inhibition of spontaneous activity by minoxidil sulphate was 100 $\pm$ 0%, 100 $\pm$ 0%, 73 $\pm$ 12%, 59 $\pm$ 12% and 24 $\pm$ 8% in the presence of vehicle, 0.1, 0.3, 1 and 10 μM glibenclamide respectively.

ChTX (0.1 μM) enhanced the spontaneous mechanical activity of the guinea-pig portal vein by 37 $\pm$ 8% ($n = 8$). However, ChTX (0.1 μM) failed to antagonize the effects of cromakalim.

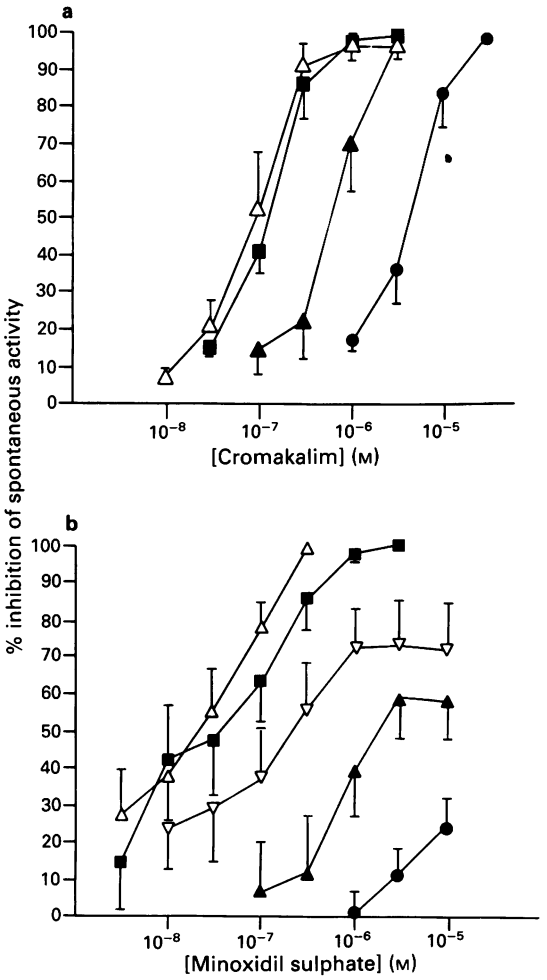

Figure 1 Effect of (a) cromakalim and (b) minoxidil sulphate on the spontaneous mechanical activity of the guinea-pig portal vein in the presence of vehicle (△) and glibenclamide, 0.1 μM (■), 0.3 μM (▽) 1 μM (▲) and 10 μM (●). Symbols represent the mean of 6 experiments. Bars represent the s.e.mean.

Mean cromakalim pD_2 values were 7.13 $\pm$ 0.31 and 7.53 $\pm$ 0.13 ($P > 0.05$) in the presence of vehicle and ChTX respectively. Similarly, ChTX (0.1 μM) failed to antagonize minoxidil sulphate induced inhibition of spontaneous activity. Thus, mean minoxidil sulphate pD_2 values were 7.30 $\pm$ 0.12 and 7.15 $\pm$ 0.12 ($P > 0.05$) in the presence of vehicle and ChTX respectively.

NTX (1 μM) enhanced the spontaneous mechanical activity of the guinea-pig portal vein by 29 $\pm$ 17% ($n = 8$). NTX (1 μM) was also without effect on the concentration-effect curves for cromakalim and minoxidil sulphate. Thus, mean cromakalim pD_2 values were 7.16 $\pm$ 0.11 and 7.57 $\pm$ 0.15 ($P > 0.05$) in the presence of vehicle and NTX respectively and mean minoxidil sulphate pD_2 values were 7.19 $\pm$ 0.17 and 7.28 $\pm$ 0.01 ($P > 0.05$) in the presence of vehicle and NTX respectively.

Rat portal vein

Cromakalim completely inhibited the spontaneous mechanical activity of the rat portal vein with a mean pD_2 value of 6.9 $\pm$ 0.08 ($n = 8$; Figure 2a). Minoxidil sulphate also inhibited the spontaneous activity of the rat portal vein. However, minoxidil sulphate was both less potent (mean $pD_2 = 6.2 \pm 0.12$) and less efficacious (mean max. inhibition = 70 $\pm$ 4%) than cromakalim ($P < 0.05$, Student's t test, Figure 2b). Following incubation in modified Krebs solution (Rb Krebs) the concentration-response curve to cromakalim was shifted significantly ($P < 0.05$) to the right by a factor of 8.3 $\pm$ 3.5 (Figure 2a). Cromakalim mean pD_2 values were 6.9 $\pm$ 0.08

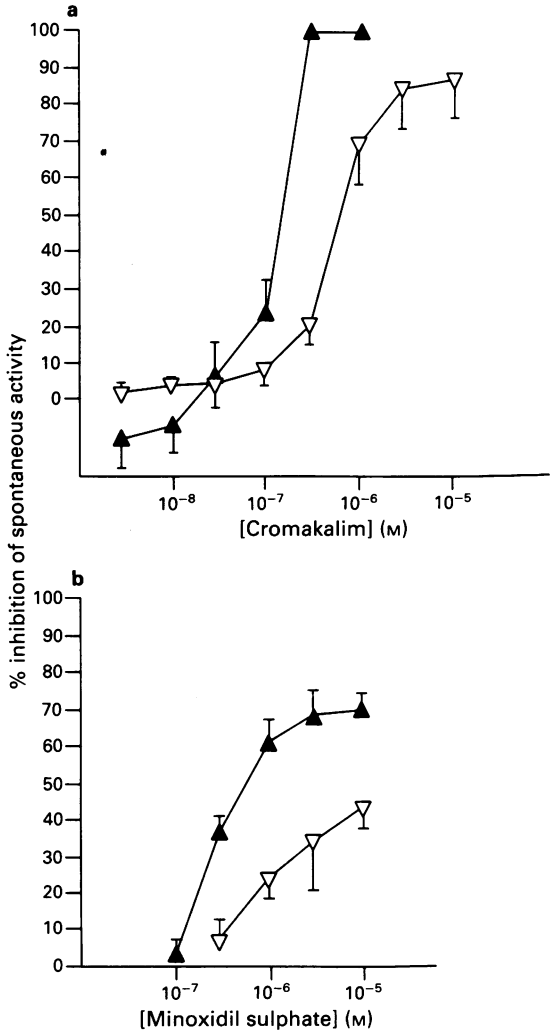

Figure 2 The effect of (a) cromakalim and (b) minoxidil sulphate on the spontaneous activity of the rat portal vein. Tissues were incubated in normal (▲) or rubidium (▽) Krebs solution. Symbols represent the mean of 8 experiments. Bars represent s.e.mean.

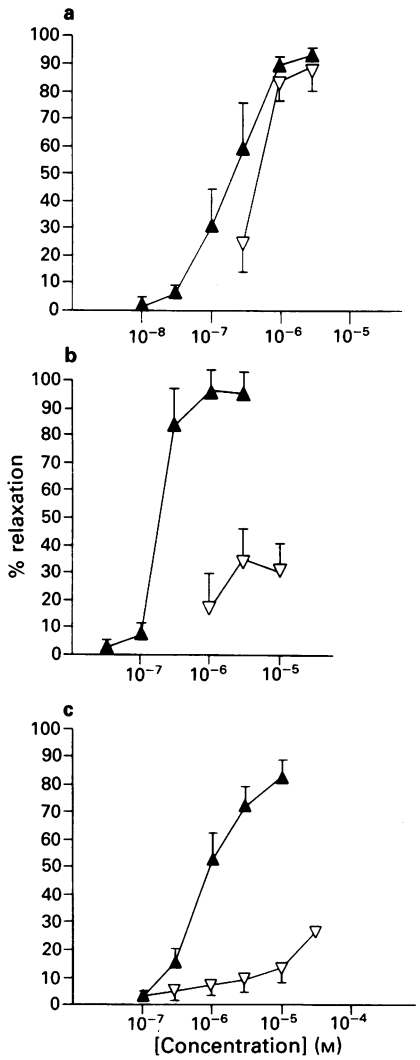

Figure 3 Comparison of the effects of cromakalim (▲) and minoxidil sulphate (▽) on (a) the noradrenaline (0.03 μM) precontracted rat thoracic aorta, (b) the noradrenaline (0.3 μM) precontracted rat mesenteric artery and (c) the noradrenaline (1 μM) precontracted rabbit thoracic aorta. Symbols represent the mean of 4 experiments. Bars represent the s.e.mean.

and 6.15 ± 0.13 in normal and Rb Krebs respectively. This shift was associated with a reduction in the maximum inhibition of spontaneous activity achieved by cromakalim (100 ± 0% and 87 ± 9% in normal and Rb Krebs respectively). Similar effects were observed with minoxidil sulphate. Thus following incubation with Rb Krebs the concentration-response curve to minoxidil sulphate was shifted to the right and the maximum inhibition reduced to the extent that an IC_{50} was achieved in only two out of four experiments (Figure 2b). Mean maximum % inhibition was 70 ± 4% and 44 ± 6% in normal and Rb Krebs respectively. Comparison of concentration-response curves at the IC_{30} level revealed a mean concentration ratio (from mean IC_{30} in normal Krebs) of 14.2 ± 8.0 (mean −log IC_{30} values = 6.6 ± 0.06 and 5.6 ± 0.2 in normal and Rb Krebs respectively; $P < 0.05$). Concentration-ratios for cromakalim and minoxidil sulphate were not significantly different ($P > 0.05$).

Rat thoracic aorta

Noradrenaline (0.03 μM) contracted rat thoracic aorta by 95 ± 1% of the noradrenaline maximum response (data not shown). Cromakalim and minoxidil sulphate relaxed the noradrenaline (0.03 μM) precontracted rat aorta with mean (± s.e.mean) pD_2 values of 6.7 ± 0.21 ($n = 4$) and 6.29 ± 0.07 ($n = 4$) respectively. These pD_2 values were not significantly different ($P > 0.05$; Figure 3a).

Rat mesenteric artery

Noradrenaline (0.3 μM) contracted the rat mesenteric artery by 97 ± 3% of the noradrenaline maximum response (data not shown). Cromakalim relaxed the rat mesenteric artery precontracted with noradrenaline (0.3 μM) with a mean pD_2 of 6.71 ± 0.09 ($n = 4$, Figure 3b). Minoxidil sulphate however, failed to relax the noradrenaline precontracted rat mesenteric artery by 50% at concentrations up to 10 μM (Figure 3b). The mean maximum % relaxation produced by minoxidil sulphate was 36 ± 11%.

Rabbit thoracic aorta

Noradrenaline (1 μM) contracted the rabbit thoracic aorta by 83 ± 1.3% of the noradrenaline maximum response (data not shown). Cromakalim relaxed the noradrenaline (1 μM) precontracted rabbit aorta with a mean pD_2 of 5.97 ± 0.15 ($n = 4$, Figure 3c). As found in the rat mesenteric artery, (Figure 3b), minoxidil sulphate failed to relax the rabbit aorta by 50% at concentrations up to 30 μM. The mean maximum % relaxation produced by minoxidil sulphate was 27 ± 1.0%. In additional experiments the relaxant effect of a single concentration of minoxidil sulphate (10 μM) was assessed. Minoxidil sulphate (10 μM) relaxed the NA (1 μM) precontracted rabbit aorta by

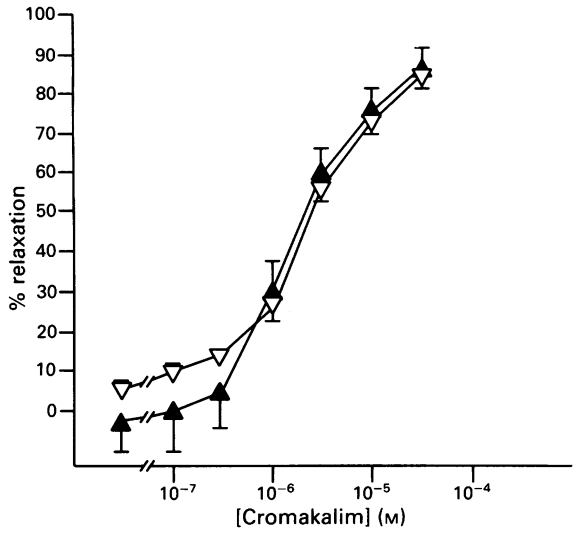

Figure 4 The effect of cromakalim on the noradrenaline (1 μM) precontracted rabbit thoracic aorta in the presence of vehicle (▲) or minoxidil sulphate (▽). Symbols represent the mean of 4 experiments. Bars represent the s.e.mean.

16.1 ± 3.1% when allowed to remain in contact with the tissues for a period of 60 min. Minoxidil sulphate was tested as an antagonist of the effects of cromakalim (Figure 4). Minoxidil sulphate (10 μM, 30 min incubation) failed to inhibit cromakalim-induced relaxation in the rabbit aorta (mean ± cromakalim pD_2 values were 5.68 ± 0.12 and 5.61 ± 0.03 in the presence of vehicle and minoxidil sulphate respectively).

Discussion

The present study compares the activity of cromakalim and minoxidil sulphate in a range of vascular preparations. Minoxidil sulphate was equipotent with cromakalim at inhibiting the spontaneous mechanical activity of the guinea-pig portal vein and at relaxing the noradrenaline precontracted rat aorta, was less potent and was less efficacious than cromakalim at inhibiting spontaneous activity in the rat portal vein and was essentially inactive against noradrenaline-induced tone in the rat superior mesenteric artery and the rabbit thoracic aorta. Thus, from the present study, it appears that striking differences exist in the profile of activity of the potassium channel openers cromakalim and minoxidil sulphate. Interestingly literature reports support this conclusion. For example, Winquist *et al.* (1989) and Newgreen *et al.* (1990) have reported that both cromakalim and minoxidil sulphate potently inhibited spontaneous activity in the rat portal vein and relaxed potassium-induced tone in the rat aorta. Piper & Hollingsworth (1989) however, have reported that minoxidil sulphate is essentially inactive compared to cromakalim in the rat uterus.

There are several possible explanations for the reported inactivity of minoxidil sulphate. Firstly, minoxidil sulphate is an unstable substance which readily breaks down to its parent compound minoxidil at room temperature. Minoxidil itself is a poor dilator of vascular smooth muscle (Towart, 1982; Kauffman *et al.*, 1986). However, such an explanation can be discounted since the sample used in the present study was stored at −20°C, the activity confirmed at regular intervals on the guinea-pig portal vein and the content of the sample verified by thin layer chromatography (results not shown).

Secondly, Newgreen *et al.* (1990) reported that the effects of minoxidil sulphate were somewhat slower than those of cromakalim in the rat portal vein. Thus, cromakalim was found to induce a maximum hyperpolarization over a period of 6 min whereas minoxidil sulphate produced a maximum hyperpolarization over a period of 12 min. Such a slowed or delayed response is unlikely to explain the findings of the present study since minoxidil sulphate failed to relax the precontracted rabbit aorta when allowed to remain in contact with the tissue for a period of 1 h.

A decrease in the number of spare receptors typically reduces the potency of full agonists whilst reducing both the potency and the maximum effect of partial agonists. Comparison of the concentration-response curves for cromakalim and minoxidil sulphate in the preparations used in the present study reveals such a trend raising the possibility that minoxidil sulphate may possess partial 'agonist' activity. Thus, minoxidil sulphate was maximally active in the guinea-pig portal vein, the tissue in which cromakalim was most potent, showed intermediate activity with a reduction in its maximum achievable effect in the rat portal vein and was only weakly active/inactive in the rat mesenteric artery and rabbit aorta, tissues in which cromakalim was least potent. However, minoxidil sulphate failed to antagonize the effect of cromakalim in tissues in which it is incapable itself of inducing a response suggesting that partial agonism is not an adequate explanation for the findings of this study.

It has been reported that although cromakalim and minoxidil sulphate are both capable of increasing $^{42}K^+$ efflux, only cromakalim was capable of increasing $^{86}Rb^+$ efflux from rat aorta (Newgreen *et al.*, 1990). Similar results have been reported in the rat portal vein (Newgreen *et al.*, 1990). On the basis of these results it is possible to speculate that minoxidil sulphate opens a different potassium channel from that modulated by cromakalim and that this channel is relatively impermeable to $^{86}Rb^+$. Previous electrophysiological studies have demonstrated the ability of cromakalim to open calcium-activated potassium channels (Kusano *et al.*, 1987; Trieschmann *et al.*, 1988; Gelband *et al.*, 1989) and the delayed rectifier (Beech & Bolton, 1989) in vascular smooth muscle. In addition, minoxidil sulphate has been reported to increase a potassium current which was sensitive to removal of extracellular calcium in coronary smooth muscle (Wilde & Lee, 1988). The possibility that cromakalim and minoxidil sulphate act via different potassium channels was therefore investigated in the present study by comparing the interactions of these compounds with charybdotoxin, a potent inhibitor of large conductance, calcium-activated potassium channels in a variety of tissues including vascular smooth muscle (Talvenheimo *et al.*, 1988) and noxiustoxin, a blocker of the delayed rectifier (Carbone *et al.*, 1982). The findings that neither charybdotoxin or noxiustoxin differentiate between the actions of cromakalim and minoxidil sulphate suggests that neither agent functionally interacts with large conductance calcium-activated potassium channels or delayed rectifiers and rules these channels out as mediators of the apparently selective effects of minoxidil sulphate. The lack of effect of these peptide channel blockers is unlikely to be due to their breakdown in the tissue bath since charybdotoxin induced an increase in the amplitude of the spontaneous activity of the guinea-pig portal vein which was sustained for several hours (data not shown). In addition, the effect of replacing potassium with rubidium was investigated. Rubidium has previously been shown to inhibit the actions of cromakalim in tracheal smooth muscle and in bladder smooth muscle and would be expected to block effects mediated via rubidium impermeable channels to a greater extent than those mediated via rubidium permeable channels. The finding that rubidium failed to block the effects of minoxidil sulphate to a greater extent than those of cromakalim in the rat portal vein is somewhat surprising and suggests that the channels mediating the effects of both cromakalim and minoxidil sulphate show a similar permeability to rubidium. The reason for the apparent discrepancy between the present functional studies and previously reported efflux studies is unclear (Foster *et al.*, 1989; Morris & Taylor, 1989).

The only other agent to antagonize effectively the actions of cromakalim and minoxidil sulphate was glibenclamide, which

antagonized the effects of both cromakalim and minoxidil sulphate over a similar concentration-range. However, whereas the antagonism of cromakalim by glibenclamide was apparently competitive, the antagonism of minoxidil sulphate by glibenclamide appeared non-competitive, findings consistent with previous reports (Buckingham et al., 1989; Winquist et al., 1989; Newgreen et al., 1990). If glibenclamide were to interact with more than one potassium channel with similar affinity then such observations could still be explained in terms of cromakalim and minoxidil sulphate interacting with different potassium channels. The channels activated by cromakalim and minoxidil sulphate may therefore represent subtypes of glibenclamide-sensitive potassium channels. In the absence of any compelling evidence to support an interaction between glibenclamide and any class of potassium channel other than the ATP-sensitive potassium channel, it remains a strong possibility that such sub-types may represent sub-types of ATP-sensitive channels. Sub-types of ATP-sensitive potassium channels have previously been suggested by Quast & Cook (1989) to account for differences in the properties of ATP-sensitive potassium channels in pancreatic, cardiac and neuronal tissues and arterial smooth muscle. Furthermore, ATP-sensitive potassium channels from different sites in the vasculature appear to show different properties. For example, ATP-sensitive potassium channels in cells dissociated from rat and rabbit mesenteric artery are reported to have a single channel conductance of 135 pS (Standen et al., 1989) whereas cells from rat portal vein are reported to express ATP-sensitive potassium channels with a single channel conductance of 10 pS (Kajioka et al., 1990).

Another possible explanation for the observed results is that cromakalim and minoxidil sulphate may modulate the same channel by different mechanisms. Indirect opening of potassium channels by minoxidil sulphate could explain the non-competitivity of the interaction between this agent and glibenclamide.

It has recently been reported that [^{35}S]-minoxidil sulphate labelled a 116 kD protein in rabbit mesenteric artery (Meisheri et al., 1990). Whether this site forms part of a particular subtype of ATP sensitive potassium channel or part of a cascade of events initiated by minoxidil sulphate which lead to potassium channel activation is unclear and is worthy of further study.

In summary, the results of the present study indicate that whereas cromakalim uniformly relaxes a variety of different blood vessel preparations, the actions of minoxidil sulphate appear more selective. The reasons for this are unclear. Minoxidil sulphate may interact with a different potassium channel from cromakalim although this channel is not charybdotoxin- or noxiustoxin-sensitive. The involvement of sub-types of the ATP sensitive potassium channel in the actions of cromakalim and minoxidil sulphate remains an attractive hypothesis and studies are planned to follow up this possibility.

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(Received November 6, 1990
Revised December 19, 1990
Accepted January 3, 1991)