Comparison of the effects of several potassium-channel openers on rat bladder and rat portal vein *in vitro*

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1 The ability of several K-channel openers to inhibit KCl-induced contractions of rat bladder detrusor and spontaneous mechanical activity in rat portal vein was examined.

2 Lemakalim, pinacidil, Ro 31-6930, RP 49356, P1060 and S 0121 dose-dependently relaxed rat detrusor, precontracted with 20 mM KCl. With the exception of pinacidil, concentrations of these agents below 30 μ M did not inhibit 80 mM KCl-induced contractions. Pinacidil (10 μ M) produced a small, but significant ($P < 0.05$) relaxation of 80 mM KCl-induced mechanical activity. Minoxidil sulphate and BRL 38226 produced some relaxation of 20 mM but not 80 mM KCl-induced contractions.

3 Glibenclamide (0.3–3 μ M) antagonized the relaxant effects of lemakalim, pinacidil, Ro 31-6930, RP 49356, P1060 and S 0121 in a competitive manner (pA_2 values 6.3–6.6). The effects of minoxidil sulphate and BRL 38226 were fully antagonized by 3 μ M glibenclamide.

4 Lemakalim, pinacidil, S 0121, BRL 38226 and minoxidil sulphate were each approximately 8 times more potent as inhibitors of the spontaneous contractions of rat portal vein than KCl-induced contractions of the rat detrusor. Minoxidil sulphate was approximately 30 times more potent in the rat portal vein than in the bladder. This may indicate that either minoxidil sulphate is acting at different recognition sites in these two tissues, or that this compound has an additional mechanism of action in the portal vein.

5 With the exception of minoxidil sulphate, all the compounds tested stimulated ^{86}Rb efflux and ^{42}K efflux from preloaded rat detrusor strips. The stimulated ^{86}Rb efflux was qualitatively but not quantitatively similar to the stimulated ^{42}K efflux. Minoxidil sulphate stimulated ^{42}K efflux from rat portal vein but not from rat bladder.

6 It is concluded that all the compounds tested cause relaxation of rat detrusor predominantly by K-channel opening. Selectivity for bladder rather than vascular smooth muscle was not shown by any compound.

Introduction

K-channel opening drugs such as cromakalim and pinacidil are currently being evaluated for the treatment of hypertension (Singer *et al.*, 1989) and asthma (Williams *et al.*, 1990) and they may also prove to be beneficial in the control of other conditions including bladder instability. Typically, the unstable bladder is treated with antimuscarinic drugs (e.g. oxybutinine, terodiline or propantheline) or with tranquilliser-sedative-hypnotic drugs (e.g. imipramine) although these agents are not ideal (Mundy & Stephenson, 1984; Andersson & Mattiasson, 1988). Pinacidil and cromakalim are capable of reducing spontaneous mechanical activity in rat, pig or human detrusor (Foster *et al.*, 1989a,b; Fovaeus *et al.*, 1989; Malmgren *et al.*, 1990) and *in vivo* experiments have demonstrated that cromakalim and pinacidil abolish the contractions associated with bladder outflow obstruction in pig and rat without affecting the ability to void urine (Foster *et al.*, 1989a; Malmgren *et al.*, 1989).

Such data suggest that K-channel openers could be clinically useful in the treatment of the irritable bladder syndrome, a condition which is very prevalent, especially in males with benign prostatic hyperplasia (McGuire, 1984). However, because of their relaxant effect on the vasculature, these agents may also reduce blood pressure in normotensive patients, an effect which has already been demonstrated for cromakalim (Eckl & Greb, 1987; Fox *et al.*, 1987) and for pinacidil (see review by Friedel & Brogden, 1990). To be clinically useful in the irritable bladder syndrome, the K-channel opener should ideally be a selective inhibitor of detrusor muscle contractions in comparison to effects on vascular smooth muscle.

The aim of the present study was to compare the *in vitro* effects of several K-channel openers on bladder and vascular

smooth muscle to determine any degree of tissue specificity. Two of the benzopyrans used in this study were the single enantiomeric components of the racemate cromakalim, the relatively inactive BRL 38226 and the biologically active lemakalim (BRL 38227) (Buckingham *et al.*, 1986; Bray *et al.*, 1987; Hof *et al.*, 1988). The two other benzopyrans were S 0121, which differs from BRL 38226 only by the presence of a methyl substituent on the lactam ring, and Ro 31-6930 which is a non-chiral molecule (lacking the hydroxyl group at the 3 position) with a six-membered ring replacing the lactam ring in the 4 position. We also used pinacidil and the related compound P 1060, both K-channel openers of the guanidine group (Edwards & Weston, 1990). In addition, the effects of RP 49356, a substituted thioformamide which opens ATP-sensitive K-channels in isolated cardiac myocytes (Escande *et al.*, 1988), and minoxidil sulphate which opens K-channels in isolated smooth muscle (Meisheri *et al.*, 1988; Leblanc *et al.*, 1989; Newgreen *et al.*, 1990) were studied. Their activity and therapeutic potential have been recently reviewed (Edwards & Weston, 1989; 1990).

Methods

Experiments were performed on bladders or portal veins isolated from male Sprague-Dawley rats (280–350 g) which were killed by stunning and bleeding.

Tissue bath experiments

Bladder detrusor Four longitudinal strips of detrusor were dissected from each bladder and each was mounted for isometric tension recording under a resting tension of 1 g. Tissues were allowed to equilibrate for 1 h in Krebs solution at 37°C, aerated with 95% O₂:5% CO₂. During this period the tissues

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were frequently washed and their resting tension continuously adjusted until it was maintained in the range 0.75–1 g.

To evaluate the spasmolytic effects of the K-channel openers, the detrusor strips were first exposed to 20 mM or 80 mM KCl. Maximal mechanical effects were produced within 15–20 min of exposure to KCl, after which time the tissues were exposed to a single K-channel opener using a cumulative protocol and with concentration increments at 5 min intervals. After a maximum relaxant response to the K-channel opener had been achieved, 1 mM aminophylline was added to produce full relaxation. For each tissue, the mechanical activity in the presence of each concentration of K-channel opener applied was integrated and expressed as a percentage of the response to 20 mM KCl (=100%) with the response to aminophylline defining the mechanical zero point (=0%). Following initial exposure to a K-channel opener, tissues were washed frequently for 15 min and then incubated with glibenclamide for 30 min. During the last 15 min of a glibenclamide incubation, 20 mM KCl was also present. The responses to the K-channel openers were reassessed in the continued presence of glibenclamide. In this manner, each tissue was sequentially exposed to a total of 2 or 3 increasing concentrations of glibenclamide (typically 0.1 μ M, 1 μ M and 3 μ M). Concentration-effect curves for the K-channel openers were also repeated in tissues which were not exposed to glibenclamide to detect any time-dependent changes in tissue sensitivity.

Portal vein Whole portal veins were mounted under 1 g tension for isometric recording. The tissues were allowed to equilibrate in Krebs solution for 1 h before they were exposed at 5 min intervals to increasing concentrations of a single K-channel opener using a cumulative protocol.

Mechanical responses of both the portal vein and bladder were recorded and the activity integrated with respect to time by use of an Apple Macintosh Plus computer in conjunction with a MacLab system (World Precision Instruments) and software (MacLab system chart recorder software programme version 2.5) supplied by Analog Digital Instruments.

Efflux studies

Each rat detrusor was cut into 4 equal pieces as already described and each was allocated to a separate treatment group as follows: (1) vehicle control, (2) K-channel opener, (3) K-channel opener + 1 μ M glibenclamide, (4) K-channel opener + 3 μ M glibenclamide. Each group contained tissues from 5 different animals. Whole portal veins were similarly grouped ($n = 5$). Pieces of detrusor or whole portal veins were then impaled on syringe needles and attached to a gassing manifold. The tissues were allowed to equilibrate for about 1 h in normal Krebs at 37°C and then incubated for 3 h in Krebs solution in which the potassium was derived from $^{42}\text{K}_2\text{CO}_3$ and to which ^{86}Rb had been added to give final activities of 1.57 $\mu\text{Ci ml}^{-1}$ ^{42}K and 5 $\mu\text{Ci ml}^{-1}$ ^{86}Rb . The final concentration of Rb in the Krebs solution did not exceed 60 μM . Following this loading period, each tissue was passed at 4 min intervals through a series of tubes containing 3 ml normal Krebs together with K-channel opener or glibenclamide as appropriate for the treatment group. The tissues were then blotted and the total (^{42}K and ^{86}Rb) activity of the tissue and of the efflux samples corresponding to each collection period was determined in a γ -counter. After approximately 10 half-lives of ^{42}K (5 days), the samples and tissues were recounted to determine the activity associated with the ^{86}Rb (using a correction factor to compensate for ^{86}Rb decay). The values obtained were subtracted from the total activity counts to yield the counts due to ^{42}K . The fractional loss of ^{42}K and ^{86}Rb from each tissue was standardized for a 1 min period and expressed as a rate coefficient (% per min).

Drugs and solutions

The following substances were used: BRL 38226 ((+)-3R,4S-6-cyano-3,4-dihydro-2,2-dimethyl-*trans*-4-(2-oxo-pyrrolidin-1'-

yl)-2H-1-benzopyran-3-ol) and lemakalim ((-)-3S,4R-6-cyano-3,4-dihydro-2,2-dimethyl-*trans*-4-(2'-oxopyrrolidin-1'-yl)-2H-1-benzopyran-3-ol; SmithKline Beecham), pinacidil (($\pm$)-N'-cyano-N-4-pyridyl-N'-1,2,2-trimethylpropyl-guanidine mono-hydrate; Leo), P1060 (N'-cyano-N-(3-pyridyl)-N'-(*t*-butyl) guanidine; Pfizer), Ro 31-6930 (2-(6-cyano-2,2-dimethyl-2H-1benzopyran-4-yl)pyridine 1-oxide; Roche), RP 49356 (($\pm$)-N-methyl-2(3-pyridinyl)-tetrahydrothiopyran-2-carbothioamide-1-oxide; Rhône-Poulenc), S 0121 ((-)-3R,4S,5'R-6-cyano-3,4-dihydro-2,2-dimethyl-*trans*-4-(2'-oxo-5'methylpyrrolidin-1'-yl)-2H-1-benzopyran-3-ol; Hoechst), minoxidil sulphate (2,4-diamino-6-piperidinyl-pyrimidine-3-oxide sulphate; ICI), glibenclamide and aminophylline (Sigma), $^{42}\text{K}_2\text{CO}_3$ (University of Manchester Reactor Facilities, Risley). Glibenclamide was made as a 1 mM stock solution in absolute ethanol and aminophylline as a 100 mM stock solution in distilled water. All other compounds were prepared as stock solutions in 70% or 100% ethanol and diluted with twice distilled water. Minoxidil sulphate was dissolved immediately before use.

The Krebs solution had the following composition (mM): Na^+ 142.7, K^+ 5.9, Ca^{2+} 2.6, Mg^{2+} 1.2, Cl^- 127.6, H_2PO_4^- 1.2, HCO_3^- 25, SO_4^{2-} 1.2 and glucose 11.1.

Statistical analysis

The significance of differences between two means was assessed by Student's *t* test.

Results

Effects of K-channel openers on mechanical activity

In vitro, strips of detrusor muscle showed spontaneous contractions, although the tension generated during each contraction was small. The addition of 20 mM KCl increased the frequency and amplitude of the phasic tension waves. Figure 1a shows that lemakalim produced a concentration-dependent reduction in the amplitude of contractions generated by 20 mM KCl, an inhibitory effect that was antagonized by glibenclamide in a manner suggestive of competitive antagonism. Time-matched concentration-effect curves to lemakalim showed that there was no alteration in the tissue response with time or previous exposure to lemakalim (data not shown). Pinacidil, Ro 31-6930, RP 49356, P1060 and S 0121 each inhibited contractions produced by 20 mM KCl, effects which were antagonized by glibenclamide in a competitive-like manner (Figure 1b–f). Minoxidil sulphate was relatively impotent, and was inhibited by glibenclamide in a non-competitive manner (Figure 1g). The highest concentration of BRL 38226 tested in detrusor, 30 μM , produced only a slight inhibition in the response to 20 mM KCl, an effect that was antagonized by glibenclamide (Figure 1h). Table 1 summarises some of the characteristics of the K-channel openers in relaxing a 20 mM KCl-induced contraction of rat detrusor. The rank order of potency was Ro 31-6930 > P1060 = lemakalim $\geq$ pinacidil > RP 49356 > S 0121 > minoxidil sulphate > BRL 38226.

The effects of lemakalim, pinacidil, S 0121, BRL 38226 and minoxidil sulphate were also examined in portal veins (Figure 2), providing some comparison of the relative potency of these compounds in the two smooth muscle types. A comparison of the IC_{50} values (Table 1) shows that, with the exception of minoxidil sulphate, all the compounds tested were approximately 8 times more potent in the rat portal vein than in rat detrusor. Schild analysis of the inhibition of the relaxant responses of detrusor to the K-channel openers by glibenclamide yielded similar pA_2 values and, with the exception of pinacidil, slopes greater than 1 (Table 1). The pA_2 value for the antagonism by glibenclamide was derived from dose ratios of the mean responses of 4 tissues in the absence and in the presence of two (S 0121 – portal vein) or three concentrations

Figure 1 Suppression of 20 mM KCl-induced mechanical activity in rat detrusor by K-channel openers. Symbols represent the drug alone (○) or in the presence of 0.3 μM (●), 1 μM (▲) or 3 μM (■) glibenclamide. Data points represent the mean from at least 4 animals. Vertical bars indicate $\pm$ s.e.mean.

of glibenclamide. The pA_2 for glibenclamide against lemakalim in the bladder (6.61 ± 0.11) was similar to that in the portal vein (6.79 ± 0.11). In contrast the pA_2 for glibenclamide against pinacidil in the bladder (6.51 ± 0.11) was lower than that in the portal vein (7.21 ± 0.17 ; $P < 0.01$). The low potencies of BRL 38226 and S 0121 in the detrusor, and of BRL 38226 in portal vein, prevented determination of pA_2 values. The pA_2 for glibenclamide against S 0121 in the portal vein (7.01 ± 0.28) did not differ from that against pinacidil in this tissue, but was significantly higher than the value against lemakalim.

At concentrations below 30 μM neither RP 49356, lemakalim, P1060, S 0121 nor Ro 31-6930 had an effect on the response to 80 mM KCl (Figure 3). Each of these agents produced a small, but significant ($P < 0.05$; Student's *t* test),

reduction in the response to 80 mM KCl at the highest tested concentration (100 μM). Figure 3 shows that minoxidil sulphate caused no reduction in the magnitude of the contractions induced by 80 mM KCl. Concentrations of pinacidil above 10 μM however, produced a small but significant concentration-dependent decrease in the 80 mM KCl-induced contractions ($P < 0.05$).

Effects of K-channel openers on ^{42}K and ^{86}Rb efflux

Figure 4 shows stimulation of ^{42}K and ^{86}Rb efflux from bladder detrusor strips by lemakalim and antagonism of this effect by glibenclamide. Such a result is typical of K-channel openers in a variety of tissues. With the exception of minoxidil sulphate, all the agents tested produced similar effects on the

Figure 2 Suppression of spontaneous mechanical activity in rat portal vein by K-channel openers. Symbols represent the drug alone (○) or in the presence of 0.3 μM (●), 1 μM (▲) or 3 μM (■) glibenclamide. Data points represent the mean from at least 4 animals. Vertical bars indicate $\pm$ s.e.mean.

Table 1 A comparison of the IC_{50} values of some K-channel openers, pA_2 values and slopes for antagonism by glibenclamide in rat bladder strips and rat portal vein

	IC_{50} (μM)	pA_2	Slope
<i>Rat bladder</i>			
Ro 31-6930	0.12 (0.09–0.16)	6.54 ± 0.21	1.69
Lemakalim	0.86 (0.44–1.68)	6.61 ± 0.11	1.55
P1060	0.82 (0.52–1.29)	6.33 ± 0.15	1.36
*Pinacidil	1.59 (1.21–2.09)	6.51 ± 0.11	1.04
*RP 49356	2.69 (1.99–3.62)	6.30 ± 0.17	1.49
S 0121	5.84 (4.95–6.89)	ND	—
MxSO_4	23.51 (16.84–32.82)	ND	—
BRL 38226	> 20.00*	ND	—
<i>Rat portal vein</i>			
Lemakalim	0.08 (0.07–0.10)	6.79 ± 0.11	1.01
*Pinacidil	0.21 (0.15–0.30)	7.21 ± 0.17	0.75
S 0121	0.75 (0.64–0.89)	7.01 ± 0.28	0.71
MxSO_4 ($n = 2$)	1.02 (0.34–3.08)	ND	—
BRL 38226	5.93 (5.27–6.67)	ND	—

Values are geometric means (95% confidence limits) or arithmetic means $\pm$ s.e.mean ($n = 4-8$). In some instances the low potency of the K-channel opener prevented determination of the pA_2 value for antagonism by glibenclamide (ND). *Racemic mixtures; if the active enantiomeric component had been available, the IC_{50} value would theoretically have been halved.

detrusor strips. Glibenclamide alone was without effect on either ^{42}K or ^{86}Rb basal efflux but produced a concentration-dependent reduction in the efflux stimulated by lemakalim, P1060, Ro 31-6930, RP 49356, S 0121 and pinacidil. Figure 5 provides a comparison of the maximum increases in the ^{42}K and ^{86}Rb efflux rate coefficients (as a percentage of the efflux rate coefficient for each tissue at the time point immediately before addition of the K-channel opener) produced by approximately three times the maximum relaxant concentration of each K-channel opener. The corresponding drug-stimulated increases in the presence of 1 μM and 3 μM glibenclamide are also indicated. As the efflux rate coefficient falls during the efflux collection period (see Figure 5), the efflux rate coefficient for the control tissues in the time period which corresponded to the peak efflux collection period for drug-treated tissues was less than that which corresponded to the last collection period before addition of the drug. This is expressed as a fall in the 'max % increase in efflux rate coefficient' in Figure 5.

Unfortunately, insufficient minoxidil sulphate was available to allow investigation of the effect of three times the maximum relaxant concentration on efflux rates. The concentration used (30 μM) was less than the maximum relaxant concentration (100 μM). Compared with the vehicle control, this concentration of minoxidil sulphate had no effect on either ^{86}Rb efflux (data not shown) or ^{42}K efflux from detrusor strips (Figure 6a), but, in the same experiment, significantly increased ^{42}K

Figure 3 Effects of K-channel openers on mechanical activity of rat detrusor pre-contracted with 80 mM KCl. In (a), control (○); lemakalim (■); pinacidil (△) and S 0121 (▲). In (b), control (○); Ro 31-6930 (■); RP 49356 (□); minoxidil sulphate (▲) and P1060 (△). Data points represent the mean from 4 animals. Vertical bars indicate $\pm$ s.e.mean.

efflux from rat portal vein, an effect which was inhibited by 1 μ M glibenclamide (Figure 6b). The low potency of BRL 38226 also prevented the effects of an equipotent concentration of this K-channel opener from being tested. This concentration of the compound had no effect on ^{86}Rb efflux. Nevertheless, it was possible to show that BRL 38226 (100 μ M) increased ^{42}K efflux (Figure 5).

Discussion

With the exception of minoxidil sulphate and high concentrations of pinacidil and lemakalim, the compounds tested in the rat bladder in this study acted in a manner typical of potassium channel openers. They relaxed 20 mM but not 80 mM KCl-induced contractions and stimulated ^{42}K and ^{86}Rb efflux from preloaded tissues (Hamilton *et al.*, 1986; Weir & Weston, 1986). Although the K-channel openers were less potent in the bladder than in other tissues, their relative potencies were, in general, similar. Thus, the compound Ro 31-6930, which in this study was about 5 times more potent than lemakalim (biologically the more active enantiomer of the racemate cromakalim; Bray *et al.*, 1987; Hof *et al.*, 1988) in relaxing rat bladder, is approximately 6 times more potent than cromakalim in relaxing rat portal vein and 8–10 times more potent *in vivo* in reducing blood pressure in rats or cats (Paciorek *et al.*, 1990a,b). Similarly, the potency of RP 49356 in relation to lemakalim in rat bladder was comparable to the relative potencies of RP 49356 and cromakalim in guinea-pig pulmonary artery (Eltze, 1989a) or rat portal vein (Longmore *et al.*, 1990). Pinacidil, which is approximately 3 times less potent than lemakalim in rat aorta (Bray *et al.*, 1987) was also 3 times less potent in the bladder.

Figure 4 Rat detrusor: effect of 1 μ M (▲) or 3 μ M (■) glibenclamide on (a) ^{86}Rb or (b) ^{42}K efflux stimulated by 5 μ M lemakalim (●); (○) indicates vehicle controls. The horizontal bar indicates the time during which the tissues were exposed to lemakalim or vehicle. Glibenclamide was present for 28 min before and during the exposure to lemakalim. Each point represents the mean from 5 animals. Vertical bars indicate $\pm$ s.e.mean.

In contrast to blood vessels, in which minoxidil sulphate is approximately 3 times less potent than cromakalim (Winquist *et al.*, 1989; Newgreen *et al.*, 1990), this agent was approximately 50 times less potent than lemakalim in relaxing the rat bladder when IC_{50} values were compared. The low potency of minoxidil sulphate in the bladder prevented us from examining the effects of maximal relaxant concentration of this agent on ^{42}K efflux from strips of detrusor, and the concentration used did not produce a detectable increase in ^{42}K efflux (although in the same experiment it did stimulate ^{42}K efflux from the rat portal vein). The reason for the low potency of minoxidil sulphate in the bladder is not immediately obvious, but could be due to a relatively low density of the K-channels through which this agent exerts its effects. A low K^+ conductance has previously been proposed by Brading (1987) to explain the low resting membrane potential in bladder (based on published values in rabbit and guinea-pig bladders of approx. -37 mV; Callahan & Creed, 1981; 1985). However, the low potency of minoxidil sulphate in rat bladder is consistent with a similar low potency in opening K-channels in brain slices (Schmid-Antomarchi *et al.*, 1990). It is possible either that the type of K^+ channel opened by minoxidil sulphate in blood vessels is different from that in bladder or brain, or that the channel in portal vein possesses a different recognition site for minoxidil sulphate. In the rat bladder, the relaxant effects of minoxidil sulphate may be solely due to K-channel opening, since this compound relaxed contractions induced by 20 mM but not 80 mM KCl. However, in the rat portal vein, Newgreen *et al.* (1990) found that minoxidil sulphate produced relatively little membrane hyperpolarization

Figure 5 Rat detrusor: maximum increases in (a) ^{42}K or (b) ^{86}Rb efflux rate coefficients stimulated by K-channel openers (open columns) and antagonism by $1\mu\text{M}$ (stippled columns) or $3\mu\text{M}$ (dark stippled columns) glibenclamide. The concentration of each K-channel opener used was approximately 3 times that which produced a maximal relaxation of rat detrusor precontracted with 20mM KCl. The concentrations used were: $3\mu\text{M}$ Ro 31-6930, $30\mu\text{M}$ RP 49356, $15\mu\text{M}$ lemakalim, $15\mu\text{M}$ P 1060, $30\mu\text{M}$ pinacidil, $30\mu\text{M}$ minoxidil sulphate (MxSO_4), $90\mu\text{M}$ S 0121 and $100\mu\text{M}$ BRL 38226. Each column represents the mean from 4–5 animals. Vertical bars indicate s.e.mean.

and proposed that the relaxant effects of this agent in this tissue might not be entirely due to the opening of potassium channels.

Pinacidil, in an equiactive mechano-inhibitory concentration to the other K-channel openers, stimulated efflux to a lesser extent. Moreover, in concentrations above $10\mu\text{M}$, pinacidil reduced the magnitude of contractions induced by 80mM KCl. This is consistent with an earlier report of the effects of pinacidil on human detrusor (Fovaeus *et al.*, 1989) and strongly suggests that part of the relaxant effect is due to a mechanism other than K-channel opening. In the human bladder, the contractile effect of KCl can be explained solely by entry of Ca^{2+} through voltage-sensitive Ca^{2+} channels (Maggi *et al.*, 1989). Assuming an intracellular K^+ concentration for bladder muscle in the range $125\text{--}182\text{mM}$ (Brading, 1981), the equilibrium potential for K^+ in depolarizing Krebs solution ($[\text{K}^+] = 86\text{mM}$) would be within the range -10mV to -20mV which should be sufficient to ensure that voltage-sensitive Ca^{2+} channels are in an open condition (Bolton *et al.*, 1984). The inhibitory effect of pinacidil, and possibly high concentrations of other K-channel openers under such conditions could therefore be due to inhibition of Ca^{2+} entry. This is supported by the recent finding that pinacidil inhibits Ca^{2+} influx into canine mesenteric arteries (Masuzawa *et al.*, 1990).

Figure 6 Minoxidil sulphate ($30\mu\text{M}$, $\square$) had no effect on the ^{42}K efflux rate coefficient in rat detrusor (a) but increased the efflux rate in rat portal vein (b). The effect of minoxidil sulphate on rat portal vein was fully antagonized by $1\mu\text{M}$ ($\blacktriangle$) or $3\mu\text{M}$ ($\square$) glibenclamide; ($\circ$) indicates vehicle controls. The horizontal bar indicates the time during which the tissues were exposed to minoxidil sulphate or vehicle. Glibenclamide was present for 28 min before and during the exposure to minoxidil sulphate. Each point represents the mean from 5 animals. Vertical bars indicate $\pm$ s.e.mean.

In the present study, consistent with the findings of Bray *et al.* (1987) and Hof *et al.* (1988), BRL 38226 abolished spontaneous activity in the rat portal vein but was far less potent than its enantiomer lemakalim (BRL 38227). Only the highest concentration of BRL 38226 used in this study ($30\mu\text{M}$) reduced 20mM KCl-induced activity of rat detrusor. However, since the K-channel openers were approximately 8 times more potent in reducing spontaneous activity in the rat portal vein than in reducing 20mM KCl-induced contractions in rat detrusor, concentrations of BRL 38226 less than $30\mu\text{M}$ were not expected to be effective. The highest concentration of BRL 38226 which we were able to use ($100\mu\text{M}$) had no effect on ^{86}Rb efflux, but produced a small increase in ^{42}K efflux. The relaxant effect of BRL 38226 is, therefore, likely to result from K-channel opening. In contrast, S 0121, which differs from BRL 38226 only by the addition of a methyl substituent at C5 on the lactam ring (see Edwards & Weston, 1990), fully relaxed 20mM KCl-induced activity but had no effect on 80mM KCl-contracted bladder strips. This, together with the ability of S 0121 to stimulate both ^{42}K and ^{86}Rb efflux, suggests that the relaxant effects of this compound are exerted via opening of K-channels. The greater potency of S 0121 in comparison to BRL 38226 was surprising and not predictable from current views on structure-activity relationships in the benzopyran K-channel openers (Edwards & Weston, 1990).

In the bladder, similar mean pA_2 values (range $6.3\text{--}6.6$) were obtained for the antagonism by glibenclamide of responses to lemakalim, pinacidil, Ro 31-6930, P1060 and RP 49356, sug-

gesting that these K-channel openers may be acting at the same site. In this tissue these values were low in comparison to those derived from other tissues, for example guinea-pig pulmonary artery (7.17–7.22; Eltze, 1989a), rabbit vas deferens (7.17; Eltze, 1989b), canine mesenteric artery (7.12–7.16; Masuzawa *et al.*, 1990), rat aorta and portal vein (7.2; Newgreen *et al.*, 1990), and rat portal vein (6.8–7.2; this study). Furthermore, for the bladder, the slopes of the Schild plots were greater than unity, in contrast to the situation in these other tissues. This might suggest that the K-channel openers are acting on channels in bladder which differ in their recognition sites from those in other types of smooth muscle. However, the relative potencies of the compounds tested in this study were similar to those reported in other tissues. Such data suggest that the channel types involved in the actions of the K-channel openers may be identical but that it is inappropriate to assume that the interaction between glibenclamide and the K-channel openers is competitive (for which there is no definitive evidence).

The antihypertensive effects of K-channel openers in animal models is now established (e.g. Arrigoni-Martelli *et al.*, 1980; Buckingham *et al.*, 1986; Paciorek *et al.*, 1990a) and cromakalim has also been demonstrated to be effective in man (Singer *et al.*, 1989). However, these agents may also have potential in the treatment of other disorders such as bladder instability;

cromakalim abolishes the spontaneous contractions of human unstable bladder (Foster *et al.*, 1989b). In the present study, a K-channel opener which was more selective for non-vascular than for vascular tissue was not identified. Thus, a possible disadvantage of using currently available K-channel openers to reduce smooth muscle activity in tissues other than the vasculature is that the higher doses of these agents which would be necessary could also reduce blood pressure in normotensive patients. Nevertheless, pinacidil and cromakalim are more potent *in vitro* in hypertrophied than in normal rat or pig bladder (Foster *et al.*, 1989b; Malmgren *et al.*, 1990). Furthermore, *in vivo*, both agents are effective inhibitors of the spontaneous contractions associated with bladder hypertrophy in rats and pigs (Foster *et al.*, 1989b; Malmgren *et al.*, 1990). In addition, Williams *et al.* (1990) have shown cromakalim to be effective in reducing nocturnal asthma using doses which have no effect on blood pressure. Further studies, together with the development of bladder-selective agents are clearly indicated.

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