

# Inhibition by glibenclamide of the vasorelaxant action of cromakalim in the rat

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1 In rat isolated thoracic aortic rings pre-contracted with noradrenaline ( $10^{-6}$  M), cromakalim ( $3 \times 10^{-7}$ – $3 \times 10^{-5}$  M) produced concentration-related relaxation. This effect was progressively inhibited by increasing concentrations of the anti-diabetic sulphonylurea drug, glibenclamide ( $10^{-6}$ – $10^{-5}$  M).

2 In rat isolated portal veins, cromakalim ( $3 \times 10^{-8}$ – $10^{-6}$  M) produced concentration-related inhibition of the spontaneous contractive activity and glibenclamide ( $3 \times 10^{-7}$ – $3 \times 10^{-6}$  M) prevented this inhibitory action in a concentration-dependent manner.

3 In both rat aortic rings and portal veins, cromakalim ( $10^{-5}$  M) stimulated  $^{86}\text{Rb}$  efflux. Prior exposure to glibenclamide ( $10^{-7}$ – $10^{-6}$  M) produced a concentration-related inhibition of this response.

4 In conscious rats, cromakalim,  $0.075 \text{ mg kg}^{-1}$  i.v., produced a rapid and sustained fall in arterial blood pressure which was not influenced by pretreatment (2 h) with a large oral dose of glibenclamide ( $100 \text{ mg kg}^{-1}$ ).

5 In conscious rats, the hypotensive action of cromakalim,  $0.075 \text{ mg kg}^{-1}$  i.v., was abolished by pretreatment (30 min) with glibenclamide,  $20 \text{ mg kg}^{-1}$ , given by the intravenous route.

6 The results suggest that the vasorelaxant and hypotensive actions of cromakalim involve a  $\text{K}^+$  channel which can be inhibited by glibenclamide, but which may be distinct from the ATP-sensitive  $\text{K}^+$  channel of the pancreatic  $\beta$ -cell.

## Introduction

Cromakalim (BRL 34915) is a potent blood pressure lowering agent in laboratory animals (Buckingham *et al.*, 1986) and man (VandenBurg *et al.*, 1986; 1987; Eckl & Greb, 1987). It is thought that the ability of cromakalim to open smooth muscle cell membrane  $\text{K}^+$  channels underlies its relaxant effect not only in rabbit, rat and guinea-pig isolated blood vessels (Coldwell & Howlett, 1986; Hamilton *et al.*, 1986; Weir & Weston, 1986; Quast, 1987), but also in rat isolated uterus (Hollingsworth *et al.*, 1987), guinea-pig trachealis (Allen *et al.*, 1986) and guinea-pig and pig urinary bladder (Foster & Brading, 1987).

In rat pancreatic islet cells the antidiabetic sulphonylurea, tolbutamide, reduces  $^{86}\text{Rb}$  efflux (used as a tracer for  $\text{K}^+$ ) (Henquin, 1980) and this action causes depolarization (Henquin & Meissner, 1982), a rise in

the cytosolic free  $\text{Ca}^{2+}$  concentration (Abrahamsson *et al.*, 1985) and insulin secretion (see review by Gylfe *et al.*, 1984). Glucose is thought to stimulate insulin release by a similar mechanism (Atwater *et al.*, 1978; Ashcroft *et al.*, 1984). The glucose-sensitive pancreatic  $\text{K}^+$  channel and the  $\text{K}^+$  channel blocked by glibenclamide, a more potent sulphonylurea than tolbutamide, have both been shown to be regulated by intracellular ATP (Cook & Hales, 1984; Rorsman & Trube, 1985; Schmid-Antomarchi *et al.*, 1987). These results suggest that glucose and the sulphonylureas may act on the same membrane  $\text{K}^+$  channel in pancreatic islet cells.

In the present paper we have tested the possibility that glibenclamide acts as an antagonist of the vasorelaxant action of cromakalim both *in vitro* and *in vivo*. A preliminary account of some of these findings has been communicated to the British Pharmacological Society (Wilson *et al.*, 1988).

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## Methods

Male Sprague Dawley rats were used in all the experiments described below.

### *Isolated tissue experiments: tension studies*

The thoracic aorta was removed rapidly from rats (200–300 g) and cut into 4 rings, each 3–4 mm in width. Two stainless steel wire hooks were passed through the lumen of each ring. One wire was attached to the base of a 10 ml tissue bath and the second to an isometric tension transducer (Dynamometer UFI). Tissues were bathed in Krebs-Henseleit solution of the following composition (mM): NaCl 118.4, KCl 4.7, CaCl<sub>2</sub> 2.5, MgSO<sub>4</sub> 1.2, KH<sub>2</sub>PO<sub>4</sub> 1.2, NaHCO<sub>3</sub> 25.0 and D-glucose 10.1. The bathing solution was warmed to 37°C and gassed with 5% CO<sub>2</sub> in O<sub>2</sub> (pH = 7.3).

For other experiments, portal veins were removed and suspended, by means of cotton ties, in 10 ml tissue baths containing Krebs-Henseleit solution as described above.

An initial tension of 2 g was applied to aortic rings and 1 g to portal veins; these tensions were then reset after 1 h and kept constant thereafter. The tissues were allowed to equilibrate for 2 h with exchange of bathing fluid by overflow every 20 min. Isometric tension was recorded on a Lectromed 4-channel system. The spontaneous contractile activity of the portal veins was quantified using integrators (Beecham Pharmaceuticals Research Division Instrument Services) working on a 1 min time cycle.

At the end of the equilibration period in rat aorta, a known maximally effective concentration of noradrenaline (10<sup>-6</sup> M) was added to each bath and the tissue contractions allowed to reach a plateau. This was obtained within 10–15 min of the addition of noradrenaline. Cromakalim was then added, in volumes of 70–200 μl in a cumulative manner, in half log unit increments commencing at 10<sup>-7</sup> M. The relaxation response to each concentration was allowed to reach a plateau before the next addition was made. Relaxation responses were usually complete within 10 min. When consecutive additions of cromakalim produced no further relaxation, the tissues were washed every 5 min over a 45 min period, during which the baseline was always restored. Subsequently, the rings were contracted once more with noradrenaline and, when a plateau was reached, glibenclamide (10<sup>-6</sup>, 3 × 10<sup>-6</sup> or 10<sup>-5</sup> M) or an equivalent volume of vehicle (dimethylsulphoxide, DMSO) was added and allowed to equilibrate for 15 min before the cromakalim concentration-relaxation curve was repeated.

In rat portal vein the vasorelaxant action of cro-

makalim was studied in terms of its ability to inhibit spontaneous phasic activity. In these experiments, cumulative additions of cromakalim were made at 20 min intervals until a maximum inhibitory effect was produced. Washing the tissues every 5 min over a 45 min period then restored phasic contractile activity, and the cumulative concentration-inhibitory effect curve for cromakalim was repeated following 15 min incubation with glibenclamide (3 × 10<sup>-7</sup>, 10<sup>-6</sup> or 3 × 10<sup>-6</sup> M) or vehicle, as described above.

The effects of cromakalim on aortic rings have been expressed as a percentage of the maximum possible relaxation i.e. the return of tension to the pre-noradrenaline level. Effects on portal vein have been expressed as a percentage reduction in the mean integrated response (the mean of the last 6 integrals in the 20 min period following each drug addition). Arithmetic mean peak responses (± s.e.mean) and geometric mean IC<sub>40</sub> (aortic rings) or IC<sub>50</sub> values (portal veins), together with 95% confidence limits, were calculated from individual experiments. The effects of glibenclamide have been quantified by calculating concentration-ratios at the IC<sub>40</sub> or IC<sub>50</sub> level.

### *Isolated tissue experiments: <sup>86</sup>Rb efflux studies*

For <sup>86</sup>Rb efflux studies, hepatic portal veins and segments of thoracic aorta (each 10–20 mg wet weight) were removed rapidly from rats (200–350 g) and suspended in a tissue bath containing 200 ml of HEPES buffer which was gassed with 5% CO<sub>2</sub> in O<sub>2</sub> at 37°C (pH = 7.4). The composition of the HEPES medium was as follows (mM): NaCl 120, KCl 6.0, CaCl<sub>2</sub> 2.5, MgCl<sub>2</sub> 1.2, HEPES 5.0, and D-glucose 11.4. After 30 min, 100–200 μCi <sup>86</sup>Rb (1–6 mCi mg<sup>-1</sup>) was added to the bath and the tissues allowed to equilibrate for 90 min. Each tissue was then transferred, at 3 min intervals, through a series of plastic vials containing 3 ml of aerated buffer but no radioactivity. Cromakalim (10<sup>-5</sup> M) was present from the 30th to the 48th min of this efflux period. Experiments were conducted in the absence and in the continuous presence of three concentrations of glibenclamide (10<sup>-7</sup>, 3 × 10<sup>-7</sup> and 10<sup>-6</sup> M). The radioactive content of the vials was determined by liquid scintillation counting. Results have been expressed as rate coefficients which were calculated as the <sup>86</sup>Rb released during each 3 min period as a percentage of the mean tissue <sup>86</sup>Rb remaining during that period. The average efflux rate between the 21st and 30th min of the efflux period was taken as the basal rate. The maximum stimulation of <sup>86</sup>Rb efflux in the presence of cromakalim was expressed as a percentage of the basal efflux rate.

*Conscious rat studies*

Rats (315–385 g) were anaesthetized with pentobarbitone sodium,  $60 \text{ mg kg}^{-1}$  i.p., and polyethylene catheters filled with 5% w/v dextrose solution, were implanted in the aorta and vena cava (Buckingham, 1976). Following recovery, the animals were housed singly in a quiet room and used for experiment on the second post-operative day. Food was removed on the evening prior to the experiments. On each study day, polyethylene tube connectors containing 5% w/v dextrose solution were used to link the arterial catheter to a Bell and Howell physiological pressure transducer (type 4-422-0001) and the venous catheter to a 3-way tap. The arterial connector and catheter were heparinised ( $250 \text{ u ml}^{-1}$ ) to prevent blood clotting. Recordings of mean arterial blood pressure (MAP;  $1 \text{ mmHg} \approx 133 \text{ Pa}$ ) and heart rate from 8 rats were displayed simultaneously and continuously on Ormed multi-channel recorders, heart rate being triggered by the arterial pulse wave.

Rats were assigned to two matching groups on the basis of their baseline mean arterial pressures, estimated after an acclimatisation period of 2–3 h. When recordings of blood pressure and heart rate were stable, animals were treated with either glibenclamide,  $100 \text{ mg kg}^{-1}$  orally, or its vehicle (water),  $25 \text{ ml kg}^{-1}$  orally (time 0). Venous catheters were then connected to an infusion pump (Harvard Apparatus, Compact Infusion Pump, Type 975A) and a 5% w/v dextrose infusion was started ( $0.55 \text{ mg min}^{-1}$ ;  $0.65 \text{ ml h}^{-1}$ ). The infusion was interrupted briefly after 75 min when timolol,  $1 \text{ mg kg}^{-1}$  i.v., was injected, and again at 120 min when all rats also received cromakalim,  $0.075 \text{ mg kg}^{-1}$  i.v.

In a subsequent experiment, the effect of intravenously administered glibenclamide on the hypotensive response to cromakalim was studied. In this experiment timolol,  $2 \text{ mg kg}^{-1}$  i.v., was injected at time 0, immediately before starting the 5% w/v dextrose infusion. The intravenous infusion was stopped briefly at 30 min, to allow injection of glibenclamide,  $20 \text{ mg kg}^{-1}$ , or its vehicle,  $5 \text{ ml kg}^{-1}$  (see 'Drugs used' section), and again at 60 min when cromakalim,  $0.075 \text{ mg kg}^{-1}$  i.v., was given to all rats.

In both of these studies in conscious rats, blood pressure and heart rate were recorded for a further 2 h after the injection of cromakalim.

*Statistical evaluation of results*

For the tension experiments in isolated blood vessels the expression of results has been dealt with under an earlier heading in this section. Results in the  $^{86}\text{Rb}$  efflux experiments and in the conscious rat studies have been expressed as group mean data ( $\pm$ s.e.mean). Statistical evaluation of the results was

made using Student's *t* test for unpaired data. Probability values (*P*) of less than 0.05 were taken to indicate statistical significance.

*Drugs used*

Cromakalim was synthesized in these laboratories. Glibenclamide and timolol maleate were gifts from Roussel Laboratories and Merck, Sharp and Dohme, respectively. (–)-Noradrenaline bitartrate was purchased from Sigma.

For the tension experiments a cromakalim stock solution ( $10^{-2} \text{ M}$ ) was prepared in 50% v/v polyethylene glycol in water. (–)-Noradrenaline ( $10^{-2} \text{ M}$ ) was prepared in 0.1 M HCl to prevent oxidation. All dilutions were made in distilled water. Glibenclamide ( $10^{-3} \text{ M}$ ) was dissolved in and diluted with DMSO. All concentrations in the text refer to the concentration in the tissue bath.

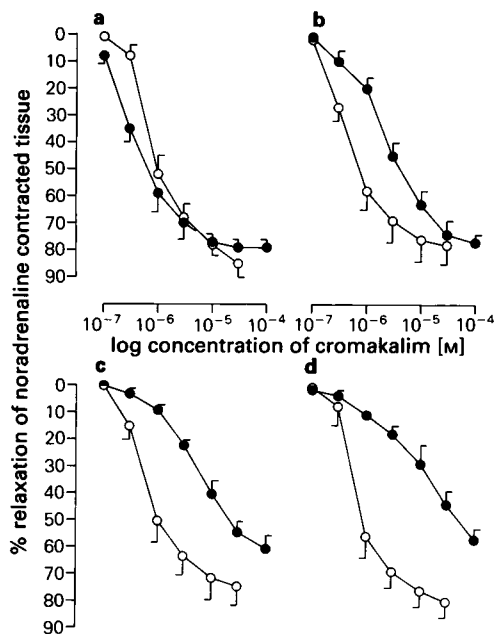
For  $^{86}\text{Rb}$  efflux studies, stock solutions of cromakalim and glibenclamide were prepared in ethanol ( $5 \times 10^{-3} \text{ M}$ ). Subsequent dilutions of both solutions were in HEPES buffer.

For conscious rat studies, both timolol maleate and cromakalim were dissolved in sterile 0.9% w/v saline. The doses of timolol used in these experiments ( $1$  or  $2 \text{ mg kg}^{-1}$ ) are expressed as free base. Glibenclamide was suspended in water for oral administration. For intravenous administration, glibenclamide was sonicated in 1 M NaOH then diluted 1 in 40 with 5% dextrose.

**Results***Isolated tissue experiments: tension studies*

**Aortic rings** Cromakalim ( $3 \times 10^{-7}$ – $3 \times 10^{-5} \text{ M}$ ) produced concentration-related relaxation of rat isolated aortic rings pre-contracted with noradrenaline ( $10^{-6} \text{ M}$ ) (Figure 1), with a mean ( $\pm$ s.e.mean) maximum response from the four experiments (i.e.  $n = 20$ ) of  $79.0 \pm 2.3\%$ . In the control experiment (Figure 1a), the tissues exhibited an increased sensitivity to cromakalim in the second concentration-response curve ( $\text{IC}_{40} = 4.4 [2.4\text{--}7.9] \times 10^{-7} \text{ M}$  compared with  $7.5 [4.9\text{--}11.0] \times 10^{-7} \text{ M}$ ) to yield a concentration ratio of 0.62. This value, therefore, was used in the calculation of concentration-ratios in the experiments with glibenclamide.

Addition of glibenclamide ( $10^{-6}$ – $10^{-5} \text{ M}$ ) to noradrenaline-contracted aortic rings produced no change in tension. However, glibenclamide produced concentration-related displacements to the right of the cromakalim concentration-response curve (Figure 1b, c, d). The  $\text{IC}_{40}$  values for cromakalim were  $2.7 [1.8\text{--}4.0] \times 10^{-6} \text{ M}$ ,  $1.1 [0.7\text{--}1.7] \times 10^{-5} \text{ M}$

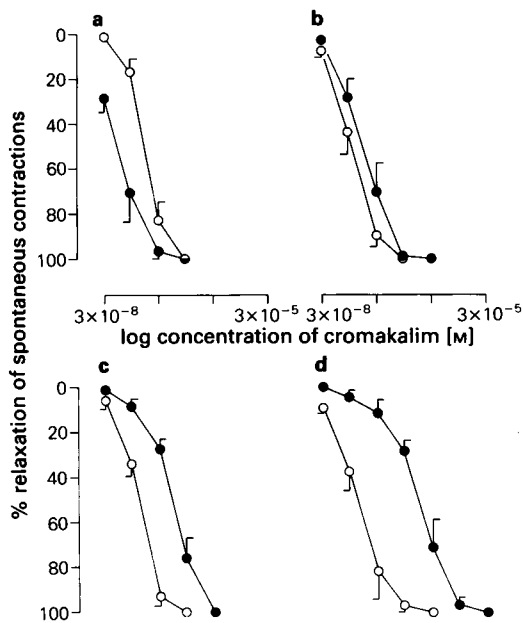


**Figure 1** Demonstration of the antagonism of the vasorelaxant effect of cromakalim by glibenclamide in rat isolated aorta. In each of the four panels (○) depict the % relaxation of tissues pre-contracted with noradrenaline ( $10^{-6}$  M) produced by increasing concentrations of cromakalim; (●) depict a second cromakalim concentration-relaxation response in the presence of (a) glibenclamide vehicle, and in (b), (c) and (d), increasing concentrations of glibenclamide ( $10^{-6}$ ,  $3 \times 10^{-6}$  and  $10^{-5}$  M, respectively;  $n = 5$ ).

and  $2.2 [1.0-4.7] \times 10^{-5}$  M in the presence of  $10^{-6}$ ,  $3 \times 10^{-6}$  and  $10^{-5}$  M glibenclamide, respectively. The calculated concentration ratios were 10.1, 26.1 and 103.2, respectively.

**Portal veins** Cromakalim ( $3 \times 10^{-8}$ – $10^{-6}$  M) produced concentration-related inhibition of the spontaneous contractile activity of rat isolated portal veins (Figure 2). Complete abolition of activity was always achieved. In the control experiment (Figure 2a), a considerable increase in sensitivity to cromakalim was observed in the second concentration-response curve ( $IC_{50} = 6.3 [4.8-8.3] \times 10^{-8}$  M compared with  $1.8 [1.6-2.0] \times 10^{-7}$  M). This concentration-ratio (0.40), therefore, was used in the calculation of concentration-ratios in the experiments with glibenclamide.

When glibenclamide ( $3 \times 10^{-7}$ – $3 \times 10^{-6}$  M) was added to portal veins there was no effect on the integrated activity of the preparations. However, glibenclamide produced concentration-related, parallel



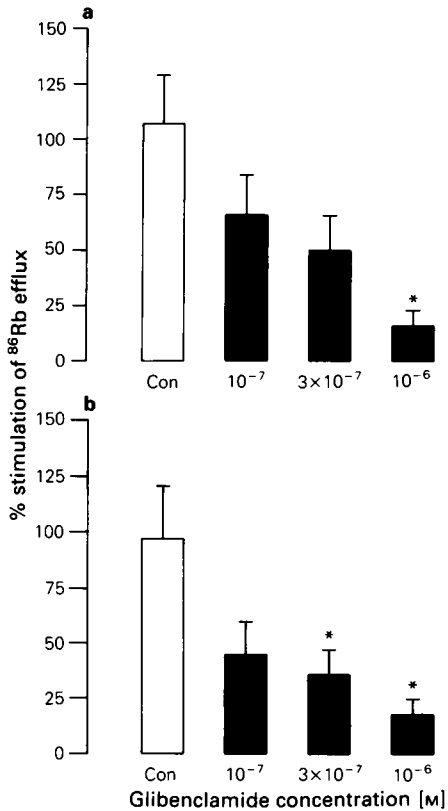
**Figure 2** Demonstration of the antagonism of the vasorelaxant effect of cromakalim by glibenclamide in rat isolated portal vein. In each panel (○) depict the % relaxation of spontaneous contractions produced by increasing concentrations by cromakalim; (●) represent a second cromakalim concentration-relaxation response in the presence of (a) glibenclamide vehicle, and in (b), (c) and (d), increasing concentrations of glibenclamide ( $3 \times 10^{-7}$ ,  $10^{-6}$  and  $3 \times 10^{-6}$  M, respectively;  $n = 5$ ).

displacements to the right of the cromakalim concentration-response curve (Figure 2b, c, d).  $IC_{50}$  values for cromakalim were  $2.0 [1.6-2.4] \times 10^{-7}$  M,  $5.6 [4.6-6.7] \times 10^{-7}$  M and  $1.9 [1.5-2.4] \times 10^{-6}$  M in the presence of  $3 \times 10^{-7}$ ,  $10^{-6}$  and  $3 \times 10^{-6}$  M glibenclamide, respectively. The calculated concentration-ratios were 4.6, 10.4 and 36.1, respectively.

#### Isolated tissue experiments: $^{86}\text{Rb}$ efflux studies

**Aortic rings** At peak effect, cromakalim ( $10^{-5}$  M) caused a  $107 \pm 22\%$  stimulation of  $^{86}\text{Rb}$  efflux in control tissues and increasing concentrations of glibenclamide reduced progressively the degree of stimulation (Figure 3a). However, this inhibitory effect was only statistically significant at the highest concentration of the sulphonylurea ( $10^{-6}$  M), when  $^{86}\text{Rb}$  efflux was raised only  $16 \pm 7\%$  by cromakalim.

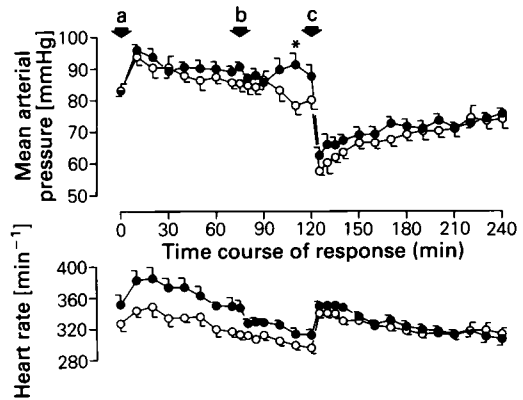
The basal  $^{86}\text{Rb}$  efflux rate coefficient ( $1.9 \pm 0.5\%$   $3 \text{ min}^{-1}$ ) was not significantly changed by the presence of different concentrations of glibenclamide.



**Figure 3** Demonstration of the antagonism by glibenclamide of the stimulation of <sup>86</sup>Rb efflux produced by cromakalim (10<sup>-5</sup> M) in (a) rat isolated aorta, and (b) rat isolated portal vein. In each panel the open columns depict the group mean % stimulation of <sup>86</sup>Rb efflux evoked by cromakalim alone; s.e.mean shown by vertical bar. The consecutive solid columns depict responses to cromakalim in the presence of increasing concentrations of glibenclamide (10<sup>-7</sup>, 3 × 10<sup>-7</sup>, 10<sup>-6</sup> M, respectively; n = 7 or 8). Statistically significant differences between the control and glibenclamide treatments: \*P < 0.05.

**Portal veins** In control tissues, <sup>86</sup>Rb efflux was increased by a maximum of 97 ± 24% in the presence of cromakalim, 10<sup>-5</sup> M (Figure 3b). As in rat aorta, this stimulatory effect was reduced by increasing concentrations of glibenclamide. At a glibenclamide concentration of 10<sup>-6</sup> M, <sup>86</sup>Rb efflux was elevated only 18 ± 7% by cromakalim.

Again the basal <sup>86</sup>Rb efflux rate coefficient (1.8 ± 0.5% 3 min<sup>-1</sup>) was not significantly changed in the presence of the different concentrations of glibenclamide.

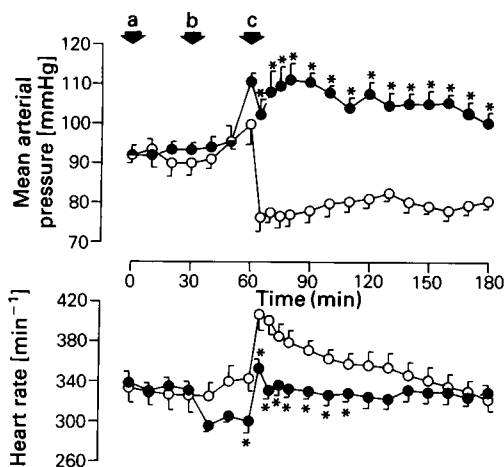


**Figure 4** Demonstration of the lack of an interaction between orally administered glibenclamide and cromakalim on blood pressure and heart rate in conscious rats. In both panels (○) depict group mean responses (s.e.mean shown by vertical bars) in the control group (n = 8) which received a 25 ml kg<sup>-1</sup> water load orally at time 0 (marked 'a' on the figure); (●) depict the group given glibenclamide, 100 mg kg<sup>-1</sup> orally, at time 0 (n = 7). Subsequently, both groups received an intravenous injection of timolol, 1 mg kg<sup>-1</sup>, at 75 min (marked 'b') and cromakalim, 0.075 mg kg<sup>-1</sup> at 120 min (marked 'c'). Statistically significant differences between the groups: \*P < 0.05.

#### Conscious rat studies

Compared with vehicle-treated control rats, the oral administration of glibenclamide, 100 mg kg<sup>-1</sup>, produced no significant change in either mean arterial blood pressure or heart rate in the 75 min before the intravenous administration of the β-adrenoceptor antagonist timolol, 1 mg kg<sup>-1</sup>, to both experimental groups (Figure 4). In the period between 90 min and 120 min the group mean blood pressures did exhibit some divergence, though a significantly higher pressure in glibenclamide-treated rats occurred only at 110 min. However, group mean heart rates in the two groups were very similar following the administration of timolol. Intravenous injection of cromakalim, 0.075 mg kg<sup>-1</sup>, at 120 min caused an immediate fall in blood pressure which was of a similar magnitude in the two groups. Despite the presence of timolol, cromakalim also increased heart rate slightly and to the same extent in both groups. For both parameters the peak responses were seen within 5 min, thereafter gradually returning towards their pre-cromakalim values. These results demonstrate a lack of effect of oral pretreatment with glibenclamide on the cardiovascular response to i.v. cromakalim.

In the second conscious rat experiment, both groups were treated with timolol, 2 mg kg<sup>-1</sup> i.v., at



**Figure 5** Interaction between intravenously administered glibenclamide and cromakalim on blood pressure and heart rate in conscious rats. In both panels, group mean ( $n = 8$ ) responses (s.e.mean shown by vertical bars) of the control group are represented by (○) and the glibenclamide-treated group by (●). Both groups received an intravenous injection of timolol,  $2 \text{ mg kg}^{-1}$ , at time 0 (marked 'a' on figure). At 30 min (marked 'b'), the treated group received glibenclamide,  $20 \text{ mg kg}^{-1}$  i.v., and the control group received vehicle  $5 \text{ ml kg}^{-1}$ . At 60 min (marked 'c'), both groups received cromakalim,  $0.075 \text{ mg kg}^{-1}$  i.v. Statistically significant differences between the groups: \* $P < 0.05$ .

time 0 (Figure 5). Administration of glibenclamide,  $20 \text{ mg kg}^{-1}$  i.v., or its vehicle at time 30 min resulted in increases in blood pressure, though there was no statistically significant difference between the groups for the ensuing 30 min. Heart rate fell following glibenclamide injection though the difference between the groups was only significant at the 60 min time point. Administration of cromakalim,  $0.075 \text{ mg kg}^{-1}$  i.v., to control rats produced an immediate fall in blood pressure and, despite  $\beta$ -blockade with timolol, an increase in heart rate. In glibenclamide-treated rats, however, cromakalim caused only a small, transient fall in blood pressure showing that the sulphonylurea drug had prevented the hypotensive response. Despite  $\beta$ -blockade, and the absence of a hypotensive response in rats given the sulphonylurea, heart rate increased following cromakalim, suggesting a marginal fall in vascular resistance. However, following the peak response heart rate then fell to a plateau level similar to that seen just prior to glibenclamide injection.

## Discussion

In the present paper we have demonstrated that the sulphonylurea, glibenclamide, is an inhibitor of the ability of cromakalim to (i) relax rat isolated aortic rings pre-contracted with noradrenaline, (ii) suppress spontaneous contractile activity of rat isolated portal veins and (iii) stimulate  $^{86}\text{Rb}$  efflux from both of these tissues. Quast & Cook (1988) and Wilson (1989) have also recently demonstrated an interaction between glibenclamide and cromakalim in isolated blood vessels of the rat and rabbit. In view of the fact that vascular smooth muscle relaxant effects of cromakalim *in vitro* are thought to be mediated via an opening of cell membrane  $\text{K}^+$  channels (see Introduction), it can be inferred from the present results that glibenclamide blocks these same channels. There are already several publications which associate sulphonylurea-mediated stimulation of insulin secretion from pancreatic islet cells with blockade of ATP-sensitive  $\text{K}^+$  channels (see Introduction). Thus the present observations raise the question of the nature of the  $\text{K}^+$  channel(s) opened by cromakalim in vascular smooth muscle. If pancreatic islet cells and vascular smooth muscle cells possess similar ATP-sensitive  $\text{K}^+$  channels, two things might reasonably be expected: (i) the concentrations of glibenclamide required to block these channels would be of a similar order in the different tissues and (ii) cromakalim would stimulate insulin release from islet cells at concentrations similar to those causing vascular smooth muscle relaxation.

In the present study,  $10^{-7} \text{ M}$  glibenclamide appeared to be close to the threshold concentration required to reduce the  $^{86}\text{Rb}$  efflux stimulated by cromakalim ( $10^{-5} \text{ M}$ ) in both aorta and portal vein. Similarly, Garrino *et al.* (1985) found that  $10^{-7} \text{ M}$  glibenclamide reduced  $^{86}\text{Rb}$  efflux and increased insulin release from mouse islets perfused with a medium containing 3 mM glucose, although no attempt was made in that study to establish a threshold concentration for the sulphonylurea. Commonly, authors have tended to use higher concentrations of glibenclamide in their studies. For example, Matthews & Shotton (1984) employed  $10^{-6}$ – $10^{-4} \text{ M}$  glibenclamide to inhibit  $^{86}\text{Rb}$  efflux from rat isolated pancreatic islets. However, in a study by Schmid-Antomarchi *et al.* (1987), the  $^{86}\text{Rb}$  efflux stimulated by lowering the intracellular ATP content of rat RIN M 5F insulinoma cells, was half-maximally inhibited by  $6 \times 10^{-11} \text{ M}$  glibenclamide. Thus the issue of the glibenclamide concentrations required to block ATP-sensitive  $\text{K}^+$  channels in pancreatic islet  $\beta$ -cells compared with the  $\text{K}^+$  channels activated by cromakalim in isolated vascular smooth muscle is, at present, not completely resolved. Furthermore, although in the present studies cromakalim exerted

its maximum vascular relaxant effect at around  $10^{-6}$  M (portal vein) or  $10^{-5}$  M (aorta), Wilson *et al.* (1988) reported that in batch incubated rat pancreatic islets, cromakalim,  $10^{-5}$  M, neither reduced insulin secretion nor inhibited the increase produced by glibenclamide,  $5 \times 10^{-5}$  M. In view of these observations we feel that at present there is insufficient evidence from *in vitro* studies to conclude that glibenclamide might be blocking a cromakalim-activated ATP-sensitive  $K^+$  channel in vascular smooth muscle, though this possibility needs to be studied further.

Although we have been able to demonstrate an interaction between glibenclamide and cromakalim in rat isolated vascular smooth muscle, it was not possible to demonstrate a functional interaction *in vivo* when glibenclamide was administered by the oral route. Thus in conscious rats, a large oral dose of glibenclamide ( $100 \text{ mg kg}^{-1}$ ) did not antagonize the blood pressure lowering effect of what we would consider to be a modest intravenous dose of cromakalim ( $0.075 \text{ mg kg}^{-1}$ ) (Buckingham, 1988). Taking into account the necessity to minimize the dose of cromakalim, animals received the  $\beta$ -adrenoceptor antagonist, timolol, to prevent an anticipated sympathetically driven reflex increase in heart rate in order to amplify the hypotensive response. The fact that heart rate did increase slightly following cromakalim injection suggests that either the chosen dose of timolol ( $1 \text{ mg kg}^{-1}$ ) was insufficient for complete cardiac  $\beta$ -adrenoceptor blockade or that a pre-existing vagal tonic component was withdrawn as blood pressure fell. Grosset & Hicks (1986) detected no effect of cromakalim on the rate of beating of guinea-pig right atria *in vitro*, suggesting that this compound has no direct stimulatory effect on the cardiac pacemaker.

In a further (unpublished) experiment in conscious normotensive rats we found that a lower oral dose of glibenclamide ( $10 \text{ mg kg}^{-1}$ ) also did not antagonize the hypotensive action of cromakalim. Interestingly, Lorch *et al.* (1972) demonstrated that glibenclamide  $1\text{--}30 \mu\text{mol kg}^{-1}$  orally (approximately  $0.5\text{--}15 \text{ mg kg}^{-1}$ ) caused a dose-dependent hypoglycaemia in rats. In those experiments doses of 3 and  $30 \mu\text{mol kg}^{-1}$  orally were maximally effective within

1–3 h, suggesting that in our own studies glibenclamide would already have been maximally effective in stimulating insulin secretion at the time cromakalim was injected (2 h). Again in our studies, a continuous infusion of 5% w/v dextrose solution ( $0.55 \text{ mg dextrose min}^{-1}$ ) was given since in a preliminary experiment this protocol reduced, but did not prevent, the hypoglycaemia caused by glibenclamide,  $100 \text{ mg kg}^{-1}$  orally (results not shown).

Following the disclosure by Cavero *et al.* (1988) that, in anaesthetized rats, glibenclamide,  $20 \text{ mg kg}^{-1}$  i.v., prevented the hypotensive response to i.v. infusion of cromakalim,  $5 \mu\text{g kg}^{-1} \text{ min}^{-1}$  for 20 min, we used this dose of the sulphonylurea for a study in conscious rats. Indeed, glibenclamide,  $20 \text{ mg kg}^{-1}$  i.v., almost completely blocked the hypotensive response to cromakalim,  $0.075 \text{ mg kg}^{-1}$  i.v. In a recent study, Cavero *et al.* (1989) found that the threshold i.v. dose of glibenclamide for inhibition of cromakalim responses in the rat was between 5 and  $10 \text{ mg kg}^{-1}$ . Such doses, however, are far in excess of those reported by Lorch *et al.* (1972) to cause hypoglycaemia in rats ( $0.1\text{--}3.0 \mu\text{mol kg}^{-1}$ ,  $0.05\text{--}1.5 \text{ mg kg}^{-1}$  i.v.).

Nonetheless, the present evidence supports the view that cromakalim causes vascular relaxation both *in vitro* and *in vivo* by virtue of its ability to activate a glibenclamide-sensitive  $K^+$  channel in the smooth muscle cell membrane. However, the fact that there is such a large difference between the dose of glibenclamide required to block the hypotensive effect of cromakalim and that required to cause hypoglycaemia in the rat, suggests that the vascular  $K^+$  channel is much less sensitive to the sulphonylurea than the ATP-sensitive  $K^+$  channel in the pancreatic islet cell. Although this differential sensitivity of the two tissues to glibenclamide does not rule out the possibility that the cromakalim-activated  $K^+$  channel in vascular smooth muscle is also regulated by ATP, it is difficult to use the present evidence in favour of such a proposition.

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