

Characterisation of the effects of potassium channel modulating agents on mouse intestinal smooth muscle

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Abstract

The actions of agents which modulate ATP-sensitive potassium (K_{ATP}) channels in excitable cells were investigated in an in-vitro preparation of mouse ileum from which the mucosa was removed. A range of potassium channel openers of diverse structure, cromakalim (0.1–100 μM), pinacidil (0.1–200 μM) and its analogue P1060 (0.1–200 μM), SDZ PCO400 ((-)-(3*S*,4*R*)-3,4-dihydro-3-hydroxy-2,2-dimethyl-4-(3-oxo-cyclopent-1-enyloxy)-2*H*-1-benzopyran-6-carbonitrile) (0.3–60 μM), caused concentration-related reduction in twitch height of electrical field stimulated ileum. P1060 and SDZ PCO400 were the most potent agents; diazoxide (0.1–100 μM) was without effect. The order of inhibitory potency, based on EC50 values (concentration of a relaxant producing 50% of the maximum inhibition of twitch) was: P1060 = SDZ PCO400 > cromakalim > pinacidil. The relaxant effect of the potassium channel openers was antagonised by the sulfonylureas glibenclamide (0.1–1.0 μM) and glipizide (3–30 μM) but the nature of the antagonism differed. Antagonism of P1060 and SDZ PCO400 by glibenclamide appeared to be competitive whereas the antagonism of relaxation induced by cromakalim and pinacidil was apparently not competitive. Both phentolamine (1–10 μM) and tolbutamide (100–300 μM) showed competitive antagonism of the actions of pinacidil while yohimbine (1–20 μM) did not antagonise relaxation and appeared to have actions at sites other than the K_{ATP} channel in this preparation. The relative effectiveness of the antagonists on pinacidil-induced relaxation was found to be: glibenclamide > phentolamine > tolbutamide > yohimbine, which is in agreement with studies in other tissues. The results show that many structurally diverse potassium channel openers are potent relaxants of mouse ileum. These observations are consistent with the existence of ATP-dependent K^+ channels in murine intestinal muscle which, however, differ somewhat in properties from those reported for vascular muscle and pancreatic β -cells.

Introduction

Potassium (K^+) channels regulate the excitability of smooth muscle cells and many agents of diverse chemical structure are known to open K^+ channels in smooth muscle. The best known of these agents, such as pinacidil and cromakalim, open adenosine 5'-triphosphate (ATP)- and glibenclamide-sensitive K^+ channels (K_{ATP} channels) causing hyperpolarization of the smooth muscle cell membrane, a decrease in cell excitability and relaxation. The K_{ATP} channels are composed of two proteins: a regulatory protein containing the sulfonylurea receptor together with an inward rectifier K^+ channel (Yokoshiki et al 1998). K_{ATP} channels are inhibited by a rise in cytoplasmic ATP and so provide a link between cellular metabolism and excitability.

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There are subtypes of the sulfonylurea receptor (SUR): SUR1 occurs in pancreatic β -cells, SUR2A and SUR2B in cardiac and smooth muscle, respectively, and the ATP-sensitive K^+ channels demonstrate a heterogeneous pharmacology. Not only are there several sulfonylurea subtypes (Inagaki et al 1996) but it appears that there are differences in localisation and properties of the channels between tissues (Bryan & Aguilar-Bryan 1999). However, the subtype(s) of sulfonylurea receptor present in intestinal muscle has not yet been described. The K^+ channel openers produce relaxant effects in most types of smooth muscle and, in view of the selectivity between tissues which has been demonstrated, these agents may offer considerable therapeutic potential in the treatment of asthma, cardiovascular disease, urinary incontinence and disorders of gastrointestinal motility such as oesophageal spasm and irritable bowel syndrome. There are, however, relatively few reports on the actions of K^+ channel modulators on gastrointestinal smooth muscle.

The type of antagonism which exists between K^+ channel openers and agents such as the sulfonylurea glibenclamide, which has shown specificity to block K_{ATP} channels (Quast & Cook 1989), is controversial. For example, McPherson & Angus (1990) reported competitive antagonism with pinacidil in the dog coronary artery whereas Steinberg et al (1991), using the cephalic vein of the same species, concluded that antagonism was not competitive. The majority of studies on the functional effects of K^+ channel modulators have been carried out on tissues other than smooth muscle from the gastrointestinal tract. In the few studies on gastrointestinal muscle published, some inconsistencies in the type of antagonism observed have also been reported. For example, Zini et al (1991) found that glibenclamide competitively antagonised the effects of cromakalim in the guinea-pig small intestine; however, when pinacidil was used as the K^+ channel activator in rat gastric fundus, the antagonism by glibenclamide was not surmountable (Lefebvre & Horacek 1992). Published reports on the actions of K^+ channel modulating agents on gastrointestinal smooth muscle include studies on guinea-pig intestine (Weir & Weston 1986; Schwörer & Kilbinger 1989; Zini et al 1991), rat stomach (Lefebvre & Horacek 1992) and rat ileum (Davies et al 1991, 1996). Only one report on the effects of these agents in murine gastrointestinal tissue is available (Lebrun & Fontaine 1990). Because of the increasing importance of mice in transgenic studies, the mouse small intestine was chosen for this study, in which the effects of a variety of K^+ channel modulators were characterised in-vitro.

In this study, the relaxant effects of the K^+ channel openers cromakalim, SDZ PCO400, pinacidil, P1060 (a pinacidil analogue; Edwards & Weston 1990) and diazoxide, were investigated in a preparation of mouse mucosa-free ileum. The aim was to characterise the actions of the K^+ channel openers and the nature of the antagonism of these actions by the K_{ATP} channel blocker glibenclamide and the structurally related compound, glipizide, together with other agents reported to possess K_{ATP} channel blocking activity, tolbutamide, phentolamine and yohimbine (Schwieter et al 1992). Contractions of the ileum were induced either by electrical stimulation or by potassium chloride (KCl) to determine whether the effects of K^+ channel openers are dependent upon the stimulus used to elicit contraction.

A preliminary account of these studies has been presented to the British Pharmacological Society (Yeung et al 1995a) and the British Pharmaceutical Conference (Yeung et al 1995b).

Materials and Methods

Drugs and solutions

The following agents used in this study were generous gifts: pinacidil and P1060 from Leo Pharmaceuticals; SDZ PCO400 from Sandoz Pharmaceuticals. Cromakalim, diazoxide, yohimbine, atropine, tetrodotoxin and tolbutamide were obtained from the Sigma-Aldrich Company. Glibenclamide and glipizide were obtained from Research Biochemicals International and phentolamine from Ciba.

The K^+ channel openers were dissolved in 40% alcohol to give 10 mM stock solutions; subsequent dilutions were carried out daily using Krebs' solution. Stock solutions of glibenclamide, glipizide and tolbutamide were prepared by dissolving 30 mg in 3 mL of a solution prepared by combining (in the following order) 1.0 mL ethanol, 1.0 mL polyethylene glycol, 0.6 mL 1 M NaOH and 0.4 mL distilled water, to make a 20 mM solution. Subsequent dilutions were made in distilled water.

Preparations

Mucosa-free ileum preparations were obtained from adult male Bantin and Kingman White mice. A length of ileum (1.0–1.2 cm, approximately 2 cm from the ileocaecal junction) was cut and a plastic tube of appropriate diameter passed through the lumen to remove the ileal mucosa. The mucosa-free segment was attached by cotton thread to a tissue holder electrode (stainless

steel electrodes 7 mm apart, 40 mm long, mounted on perspex) under 0.5 g tension in a 20-mL organ bath containing Krebs' solution at 37°C, gassed with 95% O₂-5% CO₂. The Krebs' solution was of the following composition (mM): NaCl 118, NaHCO₃ 25, glucose 11.1, KCl 4.75, MgSO₄ 1.2, KH₂PO₄ 1.2 and CaCl₂ 2.5. Each preparation was allowed to equilibrate for 60 min during which time it was washed twice. Isometric contractions were measured using a Washington Dynamometer UFI transducer and recorded on a Grass model 7D Polygraph.

Experiments on ileal preparations contracted by electrical field stimulation

After equilibration, preparations were stimulated for 20 min using the following stimulation parameters (EFS): 0.5 Hz, 30 V, using a double pulse with 40-ms delay between pulses, 1 ms pulse width. Contractile responses could be completely abolished following treatment with either atropine (0.1 μM) or tetrodotoxin (1.0 μM). Initial stimulation was followed by washing and a 3-min rest period before further procedures were started. The magnitude of the twitch contraction induced by EFS in the absence of drugs was considered to be 100%. When twitch height was constant, EFS was administered, using the parameters described above, in the presence of pinacidil (0.1–200 μM), cromakalim (0.1–100 μM), P1060 (0.1–200 μM), SDZ PCO400 (0.3–60 μM) or diazoxide (0.1–100 μM). These agents or the appropriate concentration of vehicle were administered cumulatively; subsequent concentrations were administered when the previous concentration had exerted a full effect (a minimum of 3 min). The final bath concentration of alcohol did not exceed 1.2% v/v. Only one of the agents was tested in each preparation.

To ascertain the effects of K⁺ channel blockers on the tissue, concentration–response curves to the K⁺ channel blockers glibenclamide (1–30 μM), glipizide (1 nM–60 μM), tolbutamide (1–100 μM), phentolamine (1–100 μM), yohimbine (1–100 μM) or appropriate vehicle were performed cumulatively on ileal segments contracted by EFS; subsequent concentrations were administered when the effects of the previous concentration reached a plateau. Time controls were performed in which the highest concentration of each K⁺ channel blocker alone remained in contact with the stimulated preparation for 40 min.

In further experiments, following an initial concentration–response curve to pinacidil, cromakalim, P1060 or SDZ PCO400, the preparation was washed 5 times at 5-

min intervals and re-tested with EFS to ensure absence of residual effects or changes in tissue sensitivity. A single concentration of one K⁺ channel blocker, glibenclamide (0.1–1 μM) or glipizide (3–60 μM), or vehicle was added to the bathing solution and incubated for 20 min, after which the concentration–response curve to the appropriate K⁺ channel opener was repeated in the continuing presence of the K⁺ channel blocker. Only one concentration of antagonist was added to each tissue in this group of experiments, to avoid effects of sensitisation or irreversible binding of the antagonist. The effects of other K⁺ channel blockers, phentolamine (1–10 μM) or tolbutamide (100–300 μM), were investigated on the relaxant effects of pinacidil only, using the protocol described above.

Comparison of the effects of K⁺ channel blockers in a single-point assay on field-stimulated ileum relaxed by pinacidil

Mucosa-free segments of mouse ileum were contracted by EFS as described above. When twitch height became constant, pinacidil was applied at a concentration which elicited 40–60% maximum relaxation, as determined above. At the plateau of relaxation produced by pinacidil, a single concentration of glibenclamide (1–10 μM), tolbutamide (10–100 μM), phentolamine (1–20 μM) or yohimbine (1–20 μM) was added. The results were expressed in terms of reversal by the K⁺ channel blockers of the pinacidil-induced relaxation (+) or production of further relaxation (–).

Experiments on mouse ileal segments contracted by potassium chloride

Following equilibration of the preparation, a single concentration of KCl (20 mM), which produced 60–70% maximum contraction of the ileum, was administered and remained in contact with the tissue until a plateau of contraction was reached. Pinacidil (0.5–100 μM) or the appropriate concentration of vehicle was added cumulatively and the relaxant effects expressed as a percentage inhibition of the contraction evoked by KCl. The procedure was repeated following 20 min incubation with a single concentration of glibenclamide (0.1–1.0 μM) or vehicle.

Analysis of data

Each point in figures represents the mean of *n* experiments, where *n* = the number of preparations, each

derived from a different mouse. Variation from the mean is represented by the standard error of the mean (\pm s.e.m.) calculated from original data. Differences between test and control values were assessed for significance using Student's paired *t*-test. A probability of $P < 0.05$ was considered to indicate a statistically significant difference between treatments.

EC50 values were used to compare the potency of relaxant agents in each experimental protocol. The EC50 value (geometric mean) with 95% confidence limits was calculated from original data in each experiment and represents the concentration of a relaxant which produced 50% of the maximum inhibition of twitch. EC50 values for the relaxant agents in the presence or absence of an antagonist drug were used to calculate a concentration ratio (EC50 of the relaxant in the presence of an antagonist divided by the EC50 for the relaxant alone). The concentration ratios are expressed as mean \pm s.e.m. Comparisons between EC50 values were performed using Student's unpaired *t*-test or paired *t*-test as appropriate. A probability of $P < 0.05$ was accepted as indicating a significant difference between values.

The interaction between relaxants and antagonists was examined using Schild regression analysis, performed as described by Arunlakshana & Schild (1959), where the $\log_{10}(\text{concentration ratio} - 1)$ was plotted against the $\log_{10}(\text{M})$ concentration of K^+ channel blocker, with concentration ratios corresponding to the concentration ratio for each concentration of blocker. The slope of the plotted regression line was calculated and used in the assessment of the nature of antagonism observed.

Results

Effects of K^+ channel openers and blockers on field-stimulated mouse ileum

The K^+ channel openers elicited concentration-dependent reduction in twitch height in field-stimulated mouse ileum preparations. Complete relaxation of preparations could be obtained with the K^+ channel openers, with the exception of diazoxide. P1060 and SDZ PCO400 were the most potent agents (Figure 1). The EC50 values (95% confidence limits) obtained were as follows: pinacidil, $4.30 \mu\text{M}$ (3.80–5.88, $n = 19$); cromakalim, $3.41 \mu\text{M}$ (3.01–4.49, $n = 25$); P1060, $1.60 \mu\text{M}$ (1.48–1.98, $n = 30$) and SDZ PCO400, $1.96 \mu\text{M}$ (1.71–2.58, $n = 27$). Diazoxide (0.1–100 μM) did not relax this tissue (results not shown). The relaxant effect of the K^+

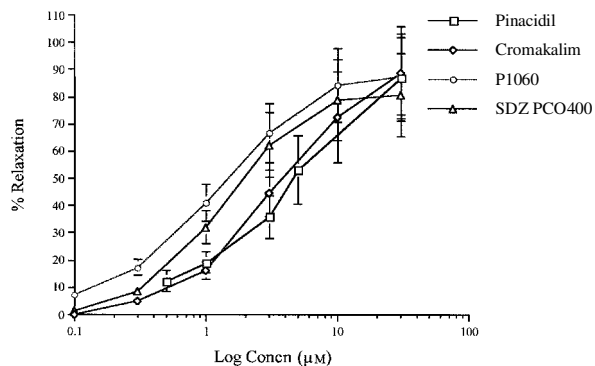


Figure 1 Concentration–response curves for the reduction in magnitude of twitches elicited by electrical field stimulation (0.5 Hz, supramaximal voltage) in the mouse mucosa-free ileum preparation by pinacidil, P1060, cromakalim and SDZ PCO400. Drugs were added cumulatively, $n = 25$ –30. Each point represents the mean and the vertical bars represent s.e.m.

channel openers was reversible and no change in tissue sensitivity was observed within the experimental period. No vehicle effects were observed.

Glibenclamide (0.1–1.0 μM) and glipizide (3–30 μM) antagonised the relaxant effects of all K^+ channel openers tested, causing concentration-related, parallel shifts in the concentration–response curves to pinacidil (0.1–200 μM), cromakalim (0.1–100 μM), P1060 (0.1–200 μM) and SDZ PCO400 (0.3–60 μM). Maximal responses were retained. Concentration ratios for each K^+ channel opener in the presence of glibenclamide and glipizide are shown in Table 1. Both antagonists effectively reversed the relaxant effects of the K^+ channel openers but had no effects on the resting tone of the preparation or on the maximum twitch height obtained. However, at concentrations higher than those used in this study, glibenclamide ($> 10 \mu\text{M}$) relaxed the preparation and slightly reduced twitch height (data not shown).

Slopes obtained from Schild plots for pinacidil, cromakalim, P1060 and SDZ PCO400 are compared in Table 2. The slopes for P1060 and SDZ PCO400, in the presence of either glibenclamide or glipizide, were not different from unity (Student's unpaired *t*-test), suggesting competitive antagonism between these agents. However, slopes for pinacidil and cromakalim were significantly different from unity, the Schild plot slopes for pinacidil with either glibenclamide or glipizide being less than unity. The pA_2 values thus obtained for P1060 with glibenclamide or glipizide were 7.3 and 5.8, respectively. The pA_2 values obtained for SDZ PCO400 with glibenclamide or glipizide, 7.3 and 5.7, respectively, were very similar.

Table 1 The antagonistic effects of glibenclamide and glipizide on relaxant effects of pinacidil, cromakalim, P1060 and SDZ PCO400 in mouse ileum preparations contracted by electrical field stimulation.

K ⁺ channel opener	Glibenclamide (μM)	Concn ratio	n	Glipizide (μM)	Concn ratio	n
Pinacidil	0.1	2.8±0.6*	4	3.0	2.6±0.4*	4
	0.3	7.3±1.8***	4	10.0	4.8±0.8**	4
	1.0	9.2±3.3**	4	30.0	8.1±1.0**	6
Cromakalim	0.1	1.8±0.1*	4	10.0	4.5±0.4*	4
	0.3	3.9±0.4**	4	30.0	7.2±0.5**	4
	1.0	19.9±3.5***	5	60.0	9.4±0.9**	4
P1060	0.1	3.6±0.8***	6	3.0	3.6±0.8**	4
	0.3	9.4±3.3**	4	10.0	3.9±0.3**	4
	1.0	10.0±5.5***	7	30.0	27.5±6.3***	5
SDZ PCO400	0.1	2.9±0.6*	4	3.0	2.6±0.3**	4
	0.3	8.8±2.6**	4	10.0	7.0±0.6**	4
	1.0	15.6±2.9***	4	30.0	25.2±2.1**	4

The concentration ratios were calculated by dividing the EC₅₀ of the relaxant in the presence of antagonist by the EC₅₀ of the relaxant alone. The calculated concentration ratios are expressed as mean ± s.e.m. The extent of shift in the concentration–response curve to each K⁺ channel opener was analysed using Student's unpaired *t*-test (**P* < 0.05, ***P* < 0.01, ****P* < 0.001, EC₅₀ of relaxant alone vs EC₅₀ of the relaxant in the presence of antagonist).

Table 2 Schild plot slopes for antagonism by glibenclamide and glipizide of mouse ileum responses to pinacidil, cromakalim, P1060 and SDZ PCO400.

	Glibenclamide	n	Glipizide	n
Pinacidil	0.64 (0.30–0.93)*	12	0.68 (0.40–0.95)*	14
Cromakalim	1.39 (1.21–1.57)*	13	0.51 (0.31–0.71)*	12
P1060	1.24 (0.98–1.50)	17	1.04 (0.60–1.48)	13
SDZ PCO400	0.92 (0.53–1.32)	12	1.19 (1.00–1.37)	12

Schild plot slopes (from a graph of log (concentration ratio – 1) against log antagonist concentration) were examined using simple regression analysis. The calculated Schild plot slopes are expressed as mean slope (95% confidence limits). **P* < 0.05 indicates significant difference from unity.

Effects of phentolamine and tolbutamide on relaxant responses to pinacidil in field-stimulated ileum

Both phentolamine (1–10 μM) and tolbutamide (100–300 μM) caused concentration-dependent, rightward shifts of the pinacidil concentration–response curves, which remained parallel. The Schild plot slopes (95% confidence limits) for pinacidil in the presence of phentolamine or tolbutamide were 0.82 (0.25–1.39, *n* = 12) and 1.16 (0.29–2.04, *n* = 12), respectively. These values were not different from unity. The pA₂ values thus obtained were 6.3 and 4.0 for phentolamine and tolbutamide, respectively.

Comparison of the actions of K⁺ channel blockers in a single-point assay in preparations contracted by EFS

To compare the actions of various K⁺ channel blockers with those of glibenclamide, a modified protocol was adopted in which these agents were applied to field-stimulated preparations relaxed by pinacidil (5 μM, 40–60% of maximal relaxation). The effects of glibenclamide (1–10 μM), tolbutamide (10–100 μM), phentolamine (1–20 μM) and yohimbine (1–20 μM) on the relaxant actions of pinacidil (3 μM) are shown in Figure 2. Glibenclamide, tolbutamide and phentolamine reversed the pinacidil-induced relaxation of the tissue in a concentration-dependent manner, glibenclamide being the most effective agent. The relative effectiveness of the agents in reversing pinacidil-induced relaxation (% recovery ± s.e.m.) was: glibenclamide (+50.25 ± 4.17%) > phentolamine (+34.83 ± 3.46%) > tolbutamide (+15.26 ± 0.92%). Yohimbine caused little reversal of the effects of pinacidil and high yohimbine concentrations (> 20 μM) increased relaxation of the tissue (Figure 2).

At the concentrations used above, these antagonists did not reduce the magnitude of the twitch elicited by field stimulation of the ileum.

Effects of pinacidil and glibenclamide on ileum contracted by KCl

KCl (20 μM) caused concentration-related contractile responses which were relaxed by pinacidil (0.5–100 μM)

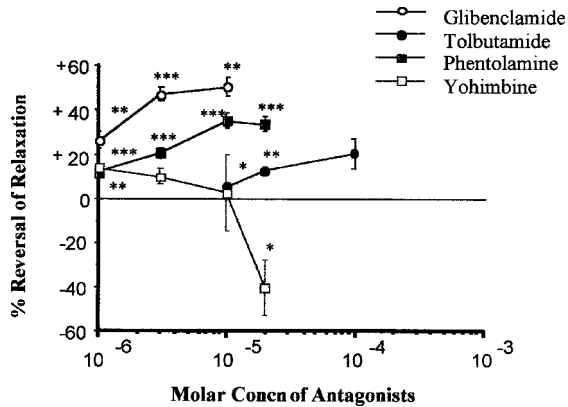


Figure 2 Antagonism of the relaxant effects of a single concentration of pinacidil ($5 \mu\text{M}$) on mouse ileum contracted by electrical field stimulation (0.5 Hz, supramaximal voltage) by addition of glibenclamide ($1\text{--}10 \mu\text{M}$), tolbutamide ($10\text{--}100 \mu\text{M}$), phentolamine ($1\text{--}20 \mu\text{M}$) or yohimbine ($1\text{--}20 \mu\text{M}$). The abscissa shows the molar concentration of the antagonists; the ordinate shows the percentage recovery (+) or further reduction in tone (-) of pinacidil-induced relaxation observed in the presence of the K^+ channel blocking agents. Values are means (\pm s.e.m.) of 4–8 experiments. Comparisons between means were made using Student's paired *t*-test (* $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$, vs effect of pinacidil alone).

(Figure 3). The EC_{50} value (95% confidence limits) for pinacidil was found to be $3.02 \mu\text{M}$ ($2.68\text{--}3.85$, $n = 24$) which was not significantly different from that obtained in field-stimulated preparations, $4.30 \mu\text{M}$ ($3.80\text{--}5.88$, $n = 19$). Thus, pinacidil possessed a similar potency against both contractile stimuli.

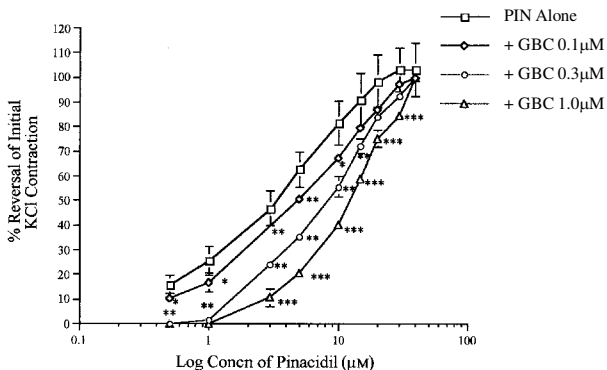


Figure 3 Effect of glibenclamide (GBC) on pinacidil (PIN)-induced relaxation of the mouse ileum preparation contracted by KCl (20 mM). The relaxant effects of pinacidil were obtained in the absence or presence of glibenclamide (0.1 , 0.3 or $1.0 \mu\text{M}$, $n = 5\text{--}8$). Each point represents the mean and the vertical bars represent the s.e.m. Comparisons between means were made using Student's paired *t*-test (* $P < 0.05$; ** $P < 0.01$ and *** $P < 0.001$, vs effect of pinacidil alone).

Glibenclamide, 0.1 , 0.3 and $1.0 \mu\text{M}$, antagonised the relaxant response to pinacidil with concentration ratios 2.0 ± 0.2 ($n = 8$), 3.0 ± 0.7 ($n = 6$) and 5.0 ± 0.4 ($n = 5$), respectively (Figure 3). Schild-plot analysis showed that glibenclamide antagonised actions of pinacidil competitively in KCl-contracted preparations; the slope (95% confidence limits) was not significantly different from unity (0.70 ($0.3\text{--}1.10$), $n = 19$). The pA_2 of 6.8 thus calculated was not significantly different from that obtained with P1060 and SDZ PCO400 in preparations stimulated by EFS.

Discussion

This study, on the mucosa-free preparation of mouse ileum, provides a direct comparison of the ability of several K^+ channel opening agents to reduce the magnitude of twitch responses elicited by EFS. The order of inhibitory potency of the K^+ channel openers, based on their EC_{50} values, was $\text{P1060} = \text{SDZ PCO400} > \text{cromakalim} > \text{pinacidil}$. A similar relative rank order of potency has been reported in other studies in-vitro: $\text{P1060} > \text{pinacidil}$ in rat portal vein (Weston et al 1988); $\text{SDZ PCO400} > \text{pinacidil}$ in rat ileum (Davies et al 1996); $\text{cromakalim} > \text{pinacidil}$ in rabbit aorta (Cook et al 1988), dog arterial strips (Masuzawa et al 1990) and guinea-pig pulmonary artery (Eltze 1989); $\text{P1060} > \text{cromakalim}$ in rat portal vein (Hu et al 1990). Diazoxide failed to relax the mouse ileum and has also been reported to be ineffective in relaxing electrically stimulated rat longitudinal muscle–myenteric plexus preparations (Davies et al 1996). However, Zini et al (1991) reported that diazoxide inhibited contraction of guinea-pig longitudinal muscle–myenteric plexus preparations induced by EFS. In contrast, diazoxide has been shown to relax vascular muscle preparations (Newgreen et al 1990). Our results are consistent with the suggestion that there are differences in the K^+ channels, their distribution or their regulatory sites in vascular muscles, compared with intestinal and other smooth muscle types (Edwards & Weston 1993).

The sulfonylurea glibenclamide is a potent blocker of K_{ATP} channels (Quast & Cook 1989) in pancreatic β -cells and vascular smooth muscle. In this study, glibenclamide antagonised the relaxant effects of a range of K^+ channel opening agents in intestinal muscle. Glipizide showed similar but less potent antagonism. The concentration ratios obtained (Table 1) showed that both glibenclamide and glipizide caused a concentration-dependent rightward shift of the concentration–response curves to all K^+ channel openers,

glibenclamide being over 40 times more potent than glipizide in the mouse intestine.

The type of antagonism existing between K^+ channel openers and blockers remains unclear. In this study, antagonism of the actions of pinacidil and cromakalim by glibenclamide and glipizide appeared to be non-competitive since the Schild plot slopes differed from unity (Table 2). Antagonism between the pinacidil analogue, P1060, or SDZ PCO400 and the sulfonylureas was apparently competitive. The variability in the results of Schild slope determination observed with structurally different K^+ channel modulators in the ileum is consistent with the presence of more than one isoform of the K_{ATP} channel (Inagaki et al 1996). Selectivity for K^+ channel openers between different smooth muscle types has previously been reported (Wellman & Quayle 1997) and two patterns of behaviour of K_{ATP} channels have also been observed in rat portal vein smooth muscle (Zhang & Bolton 1996). Previous studies on the type of antagonism occurring between glibenclamide and K^+ channel openers in smooth muscle preparations have provided conflicting evidence. In some studies, particularly in vascular muscle, antagonism between pinacidil and glibenclamide was reported to be competitive (Wilson et al 1988; Quast & Cook 1989; Newgreen et al 1990). However, other authors doubt that the antagonism is of the simple competitive type (McPherson & Angus 1989, 1990; Masuzawa et al 1990; Lefebvre & Horacek 1992). In addition, Schild analysis in our study showed some differences between the antagonism of cromakalim by the sulfonylureas glibenclamide (slope greater than unity) and glipizide (slope less than unity, Table 2). Zini et al (1991) reported that binding of glibenclamide in the guinea-pig ileum was not inhibited by cromakalim, although it was displaced by the more potent agent, RP 49356. It is possible that K^+ channel openers such as pinacidil and cromakalim and the K^+ channel blocker glibenclamide bind to different sites which are allosterically coupled, as suggested by Bray & Quast (1992) or that cromakalim and pinacidil possess a K^+ channel opening action plus an additional effect; consequently the functional antagonism between these K^+ channel openers and glibenclamide would be unlikely to be competitive. Both pinacidil (Anabuki et al 1990; Yanagisawa et al 1990) and cromakalim (Joseph et al 1996) have been reported to affect calcium movement in smooth muscle and may have influenced calcium movement in our study. Changes in internal calcium concentration can both inactivate K_{ATP} channels and modify the activity of the active channels in adult mouse skeletal muscle (Hehl et al 1994); similar mechanisms may operate in murine smooth muscle.

Phentolamine and tolbutamide were reported to antagonise the actions of K^+ channel opening agents in vascular smooth muscle (McPherson & Angus 1990; Schwietert et al 1992) and yohimbine was shown to act as an antagonist in pancreatic β -cells (Plant & Henquin 1990). In this study, pinacidil, phentolamine and tolbutamide caused competitive antagonism. To compare their effectiveness as antagonists with glibenclamide, a simple, single-point assay was devised, and the ability of these agents to reverse the relaxation by pinacidil ($5 \mu M$) of EFS-induced twitches of the ileum was studied. The relative effectiveness of the antagonists was found to be: glibenclamide > phentolamine > tolbutamide by approximately 1.5 and 10 fold, respectively and is in good agreement with other studies (McPherson & Angus 1990; Schweitert et al 1992). Tolbutamide, at a low concentration ($10 \mu M$), partially reversed the pinacidil-induced relaxation (Figure 2). The effects of yohimbine, in a concentration range (1 – $20 \mu M$) found to block K^+ channels in pancreatic β -cells by Plant & Henquin (1990), differed from those of phentolamine and tolbutamide and increased pinacidil-induced relaxation (Figure 2). From these results it can be concluded that, in terms of antagonising the relaxant effects of pinacidil on contraction elicited by EFS, glibenclamide was the most potent of the antagonists tested.

Pinacidil also caused concentration-dependent relaxation of mouse ileum in preparations stimulated by KCl (20 mM). Similar relaxant potencies were observed in preparations contracted by both KCl and EFS, EC_{50} values being $3.02 \mu M$ (2.68 – 3.85) and $4.30 \mu M$ (3.80 – 5.88), respectively. However, the degree and type of antagonism observed between pinacidil and glibenclamide differed depending on the contractile agent used. In tissues contracted by KCl the antagonism between glibenclamide and pinacidil appeared competitive (the Schild plot slope was not different from unity). However, in field-stimulated preparations antagonism was apparently not competitive. In addition, the concentration ratio obtained in the presence of a specific concentration of glibenclamide (0.1 – $1 \mu M$) was consistently approximately 1.5- to 2.5-fold higher for electrically stimulated preparations than for those contracted by KCl. It is difficult to account for such differences, but possibly pinacidil acts on nerve in addition to smooth muscle and may have affected acetylcholine release during EFS (Soares-da-Silva & Fernandes 1990).

We conclude from these results that glibenclamide-sensitive, ATP-dependent K^+ channels are present in the mouse ileum. The K^+ channel openers used showed a potent inhibitory action on twitch heights of field-stimulated ileum which is in agreement with results

observed in the rat ileum longitudinal muscle–myenteric plexus (Davies et al 1996) and many other smooth muscle preparations. This action was antagonised by glibenclamide and glipizide and differences in the type and extent of antagonism observed in this tissue, compared with other types of smooth muscle and pancreatic β -cells, could be due to the presence of different sulfonylurea receptor subtypes associated with the K_{ATP} channel or to some other effect of pinacidil and cromakalim additional to actions at K_{ATP} channels. We also observed that agents other than sulfonylureas apparently cause competitive antagonism of K^+ channel opener-induced relaxation in this tissue. These experiments have characterised the responses of mouse field-stimulated ileum, a simple in-vitro model of intestinal motility, to known K^+ channel opening and blocking agents. With the further development of transgenic mouse models this mucosa-free ileum preparation may provide a convenient model for the evaluation of K^+ channel openers with tissue selectivity which are likely to be of therapeutic use in disorders of gastrointestinal motility.

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