ELSEVIER

European Journal of Pharmacology 297 (1996) 249–256

ejp

# Comparative effects of $K^+$ channel modulating agents on contractions of rat intestinal smooth muscle

Michael P. Davies *, Janice R. McCurrie, Diana Wood

*Postgraduate Studies in Pharmacology, The School of Pharmacy, University of Bradford, Bradford, West Yorkshire BD7 1DP, UK*

Received 29 June 1995; revised 24 October 1995; accepted 31 October 1995

**Abstract**

The effects of six $K^+$ channel openers were investigated on contractions of the rat ileum longitudinal muscle-myenteric plexus preparation elicited by electrical field stimulation and by $K^+$. Levcromakalim, pinacidil, RP 49356 (*N*-methyl-2-(3 pyridyl)-tetrahydrothiopyran-2-carbothioamide-1-oxide) and SDZ PCO 400 ((3*S*,4*R*)-3,4-dihydro-3-hydroxy-2,2-dimethyl-4-[(3-oxo-1-cyclopenten-1-yl)oxy]-2*H*-1-benzopyran-6-carbonitrile) completely abolished contractions elicited by electrical stimulation and caused complete relaxation of contractions elicited by $K^+$ with comparable $IC_{50}$ values. Minoxidil sulphate was much less potent and diazoxide was without effect in either protocol. The relaxant effects of these agents were antagonized by glibenclamide, tetraethylammonium and yohimbine in a manner which was not surmountable. The present study indicates that the relaxant effect of these compounds in intestinal smooth muscle is mediated through glibenclamide-sensitive ATP-dependent $K^+$ channels. These compounds did not preferentially inhibit either direct smooth muscle- or nerve-mediated responses. The present data may point to differences in the channels or their regulatory sites, in intestinal, compared with vascular, smooth muscle.

*Keywords:* $K^+$ channel, drug effect; Intestine, small; Glibenclamide

## 1. Introduction

$K^+$ channels comprise the most diverse group of ion channels so far investigated and a large number of agents have been shown to modulate their activities. Levcromakalim, pinacidil, diazoxide, minoxidil sulphate, RP 49356 (*N*-methyl-2-(3 pyridyl)-tetrahydrothiopyran-2-carbothioamide-1-oxide) and SDZ PCO 400 ((3*S*,4*R*)-3,4-dihydro-3-hydroxy-2,2-dimethyl-4-[(3-oxo-1-cyclopenten-1-yl)oxy]-2*H*-1-benzopyran-6-carbonitrile) are structurally diverse agents which have been reported to open membrane ATP-sensitive $K^+$ channels (Edwards and Weston, 1993). The pharmacology of these, and other $K^+$ channel modulating agents, has been studied on a wide variety of muscle preparations including cardiac (Escande et al., 1989), skeletal (Weik and Neumcke, 1990), vascular (Longmore et al., 1990), tracheal (Allen et al., 1986), uterine (Hollingsworth et al., 1987), bladder (Andersson et al., 1988) and also on pancreatic $\beta$-cells (Plant and Henquin, 1990) and neuronal preparations (Tremblay et al., 1991). The mechanism of action of the $K^+$ channel openers at the cellular level is complex and has been recently reviewed by Quast et al. (1994). Basically, these agents increase potassium conductance leading to a hyperpolarization of the cell membrane and subsequent relaxation or reduced membrane excitability. Consequently, $K^+$ channel openers relax smooth muscle preparations precontracted by various spasmogens. In addition, the $K^+$ channel opener, cromakalim, has been reported to inhibit smooth muscle contractions caused by endogenous acetylcholine release via electrical stimulation in the guinea pig ileum (Schwörer and Kilbinger, 1989). Inhibition of cholinergic transmission by cromakalim has been reported in airway smooth muscle preparations (McCaig and De Jonckheere, 1989) and it has been demonstrated that pinacidil can inhibit neurotransmitter release from the sympathetic innervation of the rat vas deferens (Soares-da-Silva and Fernandes, 1990).

Recently, some of these compounds have been shown to relax gastrointestinal smooth muscle such as the guinea-pig taenia caeci (Weir and Weston, 1986), rat gastric

* Corresponding author. Department of Physiology, University of Rochester School of Medicine, Rochester, NY 14642, USA. Tel.: (716) 275 4222; fax: (716) 461 3259; e-mail: davies@uhura.cc.rochester.edu.


*SSDI* 0014-2999(95)00740-7

fundus (Lefebvre and Horacek, 1992), cat gastric antrum (Kortezova et al., 1992) and canine colon (Richardson et al., 1992). Also, some of these agents are reported to inhibit electrically evoked contractions of guinea-pig ileum (Zini et al., 1991). Due to their relaxant effects on vascular smooth muscle, the development of the $K^+$ channel opening agents was initially directed towards potential treatment of cardiovascular disorders. In addition, they have also been considered for the treatment of asthma and urinary incontinence because of their actions on airway and bladder smooth muscle respectively. It is evident, because of their effects on gastrointestinal tissue, that such agents may offer considerable therapeutic potential in the treatment of gastrointestinal motility disorders or conditions such as irritable bowel syndrome and oesophageal spasm. In addition, consideration must be given to the possible effects on gastrointestinal function following administration for other therapeutic applications.

Numerous naturally occurring and synthetic substances have been shown to block ATP-sensitive $K^+$ channels and to antagonize the electrophysiological and relaxant effects of the $K^+$ channel openers. The majority of these blockers are very limited in their specificity for this channel; however, the sulphonylurea, glibenclamide, has shown specificity to block ATP-sensitive channels in a variety of preparations (Quast and Cook, 1989) and has therefore proved to be a useful tool in attempting to characterize the $K^+$ channel sub-types opened in various preparations by a range of agents.

As a result of these findings the present study was designed to compare the effects of a range of $K^+$ channel opening agents on the contractile properties of intestinal smooth muscle using the rat ileum longitudinal muscle-myenteric plexus preparation. The longitudinal muscle layer is integral to intestinal motility and this preparation of ileum possesses advantages in terms of drug diffusion compared to the intact ileum (Kilbinger, 1982). Contractions were induced either by electrical field stimulation or by KCl in order to determine whether the effects of the $K^+$ channel openers were dependent upon the stimulus used to elicit contractions. In addition, we have used glibenclamide and other $K^+$ channel blockers to investigate the possible involvement of ATP-sensitive $K^+$ channels in the relaxation of this preparation.

Preliminary reports of these results have been presented to the British Pharmacological Society (Davies et al., 1991a, b).

## 2. Materials and methods

### 2.1. Preparations

Longitudinal muscle-myenteric plexus preparations were obtained from adult male Sprague-Dawley rats by the method of Paton and Vizi (1969). The preparations, 2–3 cm long, were derived from the distal ileum, 5–25 cm from the ileo-caecal junction. They were placed under 1 g tension in Krebs' solution at 37°C and gassed with 95% $O_2$/5% $CO_2$. The Krebs' solution was of the following composition (mM): NaCl, 118; $NaHCO_3$, 25; glucose, 11.1; KCl, 4.75; $MgSO_4$, 1.2; $KH_2PO_4$, 1.2; $CaCl_2$, 2.5. Each preparation was allowed to equilibrate for 60 min before commencement of experiments. Isometric contractions were measured using a Washington Dynamometer UF1 transducer and recorded on a Grass 7D Polygraph. Between two and four preparations were obtained from each animal: $n$ values quoted are numbers of preparations, each derived from a different animal.

### 2.2. Experiments on preparations contracted by potassium chloride (KCl)

After equilibration, an isometric contraction was elicited to a concentration of KCl (15 mM) which gave a response 60–75% of the maximum (determined from preliminary experiments). KCl remained in contact with the preparation until a plateau of contraction was reached (approximately 5 min) after which time the tissue was washed. After a further 5 min the process was repeated and at the plateau of contraction one $K^+$ channel opener or its vehicle was added cumulatively: levcromakalim (50 nM–1.6 $\mu$M), pinacidil (0.1–13 $\mu$M), RP 49356 (1–13 $\mu$M), SDZ PCO 400 (1–13 $\mu$M), minoxidil sulphate (1–64 $\mu$M), diazoxide (1–64 $\mu$M). Subsequent concentrations were administered only when the response to the previous concentration was stable (2–7 min). Relaxation was expressed as a percentage reversal of the initial contraction elicited by 15 mM KCl. Any relaxant effect of the vehicle was subtracted from the apparent effect of the $K^+$ channel opener. This was only necessary with high concentrations of minoxidil sulphate and diazoxide. In experiments using levcromakalim, pinacidil and RP 49356, the procedure was repeated following a 20 min incubation with a single concentration of glibenclamide (30 nM–3 $\mu$M) or its vehicle. The effects of other $K^+$ channel blockers, tetraethylammonium (TEA, 0.3–3 mM) and yohimbine (10–100 $\mu$M), on the relaxant actions of levcromakalim were also investigated, adopting the procedure used with glibenclamide. Only one opener and one blocker were used in each tissue. Vehicle- and time-matched control experiments were performed for each $K^+$ channel opener and blocker used.

The effect of glibenclamide on pinacidil-induced relaxations of KCl-induced responses were studied as described earlier but with the addition of tetrodotoxin (1 $\mu$M) to the Krebs' solution throughout the experiment.

### 2.3. Experiments on preparations contracted by electrical field stimulation

After equilibration, isometric contractions were elicited by electrical field stimulation (0.1 Hz, 40 V, 1–5 ms pulse

width) delivered through parallel stainless steel electrodes from a Bioscience 200 stimulator. When stable twitches were secured, one $K^+$ channel opener was added cumulatively in the concentration range described above: subsequent concentrations were administered only when the previous concentration had exerted its full effect (1–3 min). Responses were expressed as the percentage inhibition of initial twitch height. When a maximal inhibition was obtained, a single concentration of glibenclamide (0.6 $\mu$M) was added to the preparation. This concentration had been shown, in experiments outlined in the previous section, to antagonize significantly the ability of the $K^+$ channel openers to relax KCl-elicited contractions. Any reversal by glibenclamide was expressed as a percentage of the initial twitch height before the addition of one $K^+$ channel opener. Vehicle- and time-matched control experiments were performed throughout the duration of these experiments to assess the maintenance of twitch height in the absence of drugs.

Preliminary experiments revealed that both atropine (0.1 $\mu$M) and tetrodotoxin (1.0 $\mu$M) completely inhibited the contractile responses to electrical field stimulation, indicating that these responses were mediated via stimulation of cholinergic neurones (data not shown).

### 2.4. Drugs and solutions

The following substances used in this study were generous gifts: levcromakalim (SmithKline Beecham Pharmaceuticals), pinacidil monohydrate (Leo Pharmaceuticals), RP 49356 (Rhone-Poulenc Rorer), SDZ PCO 400 (Sandoz Pharma), minoxidil sulphate (The Upjohn Co.). Diazoxide, tetraethylammonium chloride, yohimbine, atropine and tetrodotoxin were obtained from the Sigma Chemical Company. Glibenclamide was obtained from Research Biochemicals International.

Stock solutions of levcromakalim, pinacidil, RP 49356, SDZ PCO 400 and minoxidil sulphate were prepared by dissolving the agents in ethanol 50%, 50%, 100%, 100% and 50% respectively to give 10 mM stock solutions. Subsequent dilutions were made in Krebs' solution, or distilled water for minoxidil sulphate. Diazoxide was dissolved in dimethylformamide (DMF). Subsequent dilutions were made in distilled water.

Stock solutions of glibenclamide were prepared by dissolving 30 mg of glibenclamide in 3 ml of 14% distilled water, 20% 1 M NaOH, 33% ethanol and 33% polyethylene glycol 400 (PEG 400) to make a 20 mM solution. Subsequent dilutions were made in distilled water.

### 2.5. Analysis of data

Results are expressed as the mean percentage value ± the standard error of the mean (S.E.M.). $IC_{50}$ value is defined as the concentration of drug required to produce 50% inhibition of KCl- or electrically elicited contractions. Statistical comparisons between $IC_{50}$ values or two groups of data, in the absence and presence of antagonists, were carried out on original data using Student's unpaired *t*-test, with Dunnett's correction when multiple comparisons were performed with the same control group. A probability of $P < 0.05$ was accepted as showing a statistically significant difference between values. Schild regression analysis was performed as described by Arunlakshana and Schild (1959) where the $\log_{10}$ (DR − 1) was plotted against the $\log_{10}$ molar concentration of $K^+$ channel blocker. DR is the dose ratio for each concentration of blocker. The slope of the plotted regression line was calculated and used to assess the nature of antagonism.

## 3. Results

### 3.1. Effects of $K^+$ channel openers on KCl-elicited contractions

Levcromakalim, pinacidil, RP 49356 and SDZ PCO 400 caused concentration-dependent and complete reversal of KCl-induced contractions (Fig. 1). The $IC_{50}$ values are presented in Table 1: the values for inhibition of KCl-induced contractions were of similar magnitude for all these agents. The $IC_{50}$ value for levcromakalim was significantly smaller than the values for SDZ PCO 400, RP 49356 and pinacidil ($P < 0.05$) which were not signifi-

Table 1
Comparative $IC_{50}$ values for inhibition of contractions elicited by 15 mM KCl and by electrical field stimulation

| | Levcromakalim | SDZ PCO 400 | RP 49356 | Pinacidil |
|---|---|---|---|---|
| *KCl* | | | | |
| $IC_{50}$ value ($\mu$M) | 0.16 ± 0.02 * | 0.33 ± 0.03 | 0.47 ± 0.06 | 0.65 ± 0.07 |
| *n* | 10 | 5 | 16 | 24 |
| *EFS* | | | | |
| $IC_{50}$ value ($\mu$M) | 0.10 ± 0.02 * | 0.18 ± 0.02 | 1.04 ± 0.28 * | 1.17 ± 0.16 * |
| *n* | 6 | 5 | 5 | 6 |

The $IC_{50}$ values for inhibition of KCl-induced (*KCl*), and electrically induced (*EFS*) contractions for each $K^+$ channel opener are shown. Each value represents the mean value ± S.E.M. from *n* experiments. $IC_{50}$ values for each agent in relaxing KCl- or EFS-induced contractions were compared statistically with the corresponding $IC_{50}$ value for SDZ PCO 400. Significant differences are indicated by * ($P < 0.05$).

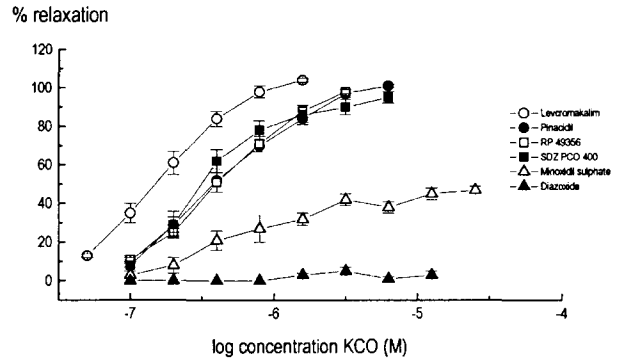


Fig. 1. Relaxant effects of $K^+$ channel openers (KCO) on contractions elicited by 15 mM KCl. Preparations of rat ileum longitudinal muscle-myenteric plexus were initially contracted by application of 15 mM KCl, and levcromakalim, pinacidil, RP 49356, SDZ PCO 400, minoxidil sulphate and diazoxide were added cumulatively ($n$ = 10, 24, 16, 5, 5, 7 respectively). The percentage relaxation of initial KCl-elicited contraction is plotted against the log concentration of drug. Data are expressed as the mean ± S.E.M. for each concentration of each drug.

cantly different from each other. The order of inhibitory potency based on their $IC_{50}$ values was: levcromakalim > SDZ PCO 400 = RP 49356 = pinacidil. Minoxidil sulphate had only limited effects and diazoxide was apparently ineffective in this protocol (Fig. 1).

### 3.2. Effects of $K^+$ channel openers on electrically induced contractions

Levcromakalim, pinacidil, RP 49356 and SDZ PCO 400 caused concentration-dependent and complete reversal of electrically induced contractions (Fig. 2). The $IC_{50}$ values for these agents are shown in Table 1: the value for levcromakalim was significantly smaller than that for SDZ PCO 400 ($P < 0.05$). The $IC_{50}$ for RP 49356 was significantly greater than that for SDZ PCO 400 ($P < 0.05$) but was not different from that for pinacidil. The order of inhibitory potency of these agents based on their $IC_{50}$ values was thus: levcromakalim > SDZ PCO 400 > RP 49356 = pinacidil. Minoxidil sulphate was only weakly effective using this protocol and diazoxide was without apparent effect.

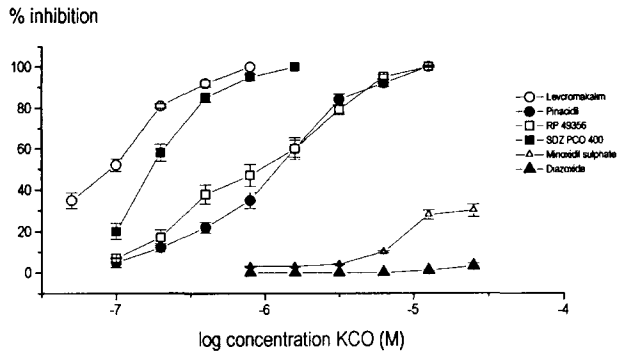


Fig. 2. Inhibitory effects of $K^+$ channel openers (KCO) on contractions elicited by electrical field stimulation. Preparations of rat ileum longitudinal muscle-myenteric plexus were repeatedly contracted by electrical stimulation (0.1 Hz, 1–5 ms pulse width, 40 V) and levcromakalim, pinacidil, RP 49356, SDZ PCO 400, minoxidil sulphate and diazoxide were added cumulatively. The percentage inhibition of the initial control contraction is plotted against the log concentration of drug. Data are expressed as the mean ± S.E.M. for each concentration of each drug ($n$ = 5–6).

### 3.3. Comparison of the actions of $K^+$ channel openers on KCl-elicited or electrically induced contractions

In general, the magnitude of electrically stimulated contractions was comparable with contractions elicited by 15 mM KCl. The onset of action of the effective opening agents was more rapid on electrically evoked responses (1–3 min to reach steady-state) than on responses elicited by KCl (2–7 min to reach steady-state). In both types of experiment the onset of action of levcromakalim and SDZ PCO 400 was more rapid than that of pinacidil and RP 49356. The ability of the most effective openers to inhibit electrically induced contractions was compared with their ability to relax KCl-stimulated contractions (Table 1). There were small but significant differences in the $IC_{50}$ values for pinacidil, RP 49356 and SDZ PCO 400 in reversing contractions elicited by electrical stimulation compared with the $IC_{50}$ values in inhibiting contractions elicited by KCl ($P < 0.05$). SDZ PCO 400 was more potent whereas RP 49356 and pinacidil were less potent. The values for levcromakalim were not different ($P > 0.05$) using the two protocols.

Although the maximum responses to minoxidil sulphate were very similar using the two protocols, this agent appeared to cause inhibition of KCl-induced contractions at lower concentrations than those required to inhibit contractions elicited by electrical stimulation. This phenomenon is illustrated by the different shapes of the concentration-response curves for minoxidil sulphate shown in Figs. 1 and 2. Diazoxide was without effect in either protocol.

The vehicles for levcromakalim, pinacidil, RP 49356 and SDZ PCO 400 had no significant effects on the contractions elicited either by KCl or by electrical stimulation at the concentrations used. At the higher concentrations of minoxidil sulphate (≥ 10 μM) and diazoxide (≥ 1 μM), vehicle effects were apparent. These have been accounted for by subtraction of time-matched vehicle effects from the apparent effect of the opener.

### 3.4. Effects of $K^+$ channel blocking agents

#### 3.4.1. Glibenclamide

In KCl-contracted preparations, glibenclamide (30 nM–3 μM) caused non-parallel rightward displacements of the concentration-response curves for levcromakalim, pinacidil and RP 49356. The vehicle for glibenclamide was

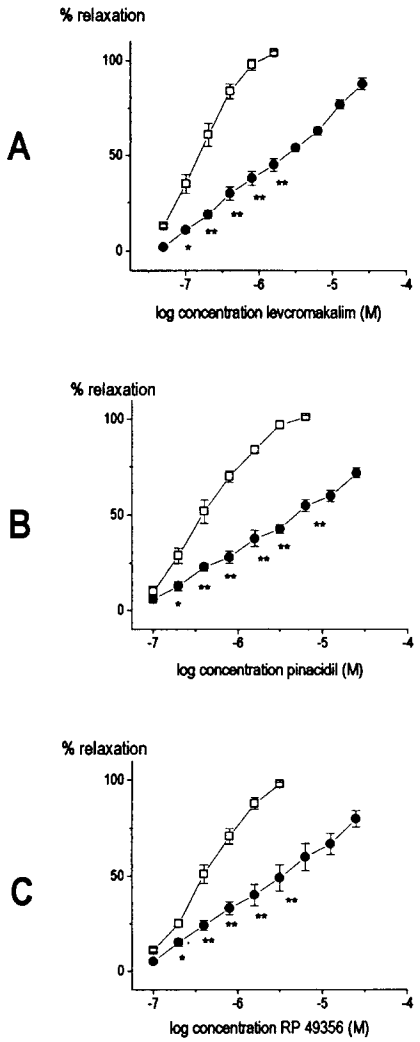


Fig. 3. The effect of glibenclamide on $K^+$ channel opener-induced relaxations of KCl-elicited contractions. The relaxant effects of (A) levcromakalim, (B) pinacidil and (C) RP 49356 were determined in the absence (□) and presence (●) of 3 $\mu$M glibenclamide ($n = 8$, 5, 5 for each drug respectively). The effects of glibenclamide were concentration-dependent but only the effect of the highest concentration used is shown here. Data are expressed as the mean ± S.E.M. for each point. Comparisons between means were made using Student's unpaired $t$-test. Significant differences are shown by * ($P < 0.05$) and * * ($P < 0.01$).

without effect. Fig. 3 shows the effect of the highest concentration of glibenclamide (3 $\mu$M) on the concentration-response relationship for these three agents. At the concentrations used, glibenclamide had no effect on the resting tone of the preparations or on contractions elicited by 15 mM KCl. Schild analysis, incorporating all concentrations of glibenclamide, revealed that the slopes of the regression lines were 0.48 for levcromakalim ($n = 19$), 0.42 for pinacidil ($n = 16$) and 0.70 for RP 49356 ($n = 13$). Since these values differed markedly from unity, the nature of the antagonism of openers by glibenclamide appeared not to be competitive. Therefore, $pA_2$ values were not calculated since this type of analysis is only applicable to competitive antagonists. In order to make comparisons of the glibenclamide effect on the openers used, the concentration of glibenclamide required to produce a 2-fold increase in the $IC_{50}$ value for each agonist was calculated. These values were found to be 26 nM with levcromakalim ($n = 19$), 78 nM with pinacidil ($n = 16$) and 141 nM with RP 49356 ($n = 13$).

Both the relaxant effects of pinacidil on KCl-stimulated preparations and the antagonism of these effects by glibenclamide (30 nM–3 $\mu$M) were unaffected by the presence of tetrodotoxin (1 $\mu$M). This confirmed that the actions of these agents on KCl-elicited responses were via mechanisms directly associated with the ileal smooth muscle and not via neuronally mediated effects.

Glibenclamide, at a concentration previously shown to antagonize significantly the ability of the $K^+$ channel openers to relax KCl-elicited contractions (0.6 $\mu$M), was added to preparations in which twitch responses elicited by electrical stimulation had been inhibited by one of the effective openers. Glibenclamide caused a relatively fast reversal of the inhibition and, thus, a restoration of the twitch response. Glibenclamide (0.6 $\mu$M) reversed the inhibition of twitch response caused by levcromakalim, pinacidil, RP 49356 and SDZ PCO 400 to 89.6 ± 3.5%, 78.1 ± 6.5%, 76.6 ± 8.3% and 88.0 ± 9.2% of control levels respectively ($n = 4$ for each agent). These values were not significantly different from each other.

### 3.4.2. TEA

The non-selective $K^+$ channel blocker TEA (0.3–3 mM) caused non-parallel rightward shifts of concentration-response curves for levcromakalim in preparations contracted by KCl but was much less potent than glibenclamide (Fig. 4A). Using Schild analysis the slope of the regression lines was calculated to be 1.32 ($n = 11$) indicating that the antagonism was not competitive. The concentration required to produce a 2-fold increase in $IC_{50}$ was 1.7 mM.

### 3.4.3. Yohimbine

Yohimbine, in a concentration range (10–100 $\mu$M) found to block $K^+$ channels in pancreatic $\beta$-cells by Plant and Henquin (1990), was used to antagonize the inhibitory effects of levcromakalim on KCl-induced contractions. Yohimbine displaced the levcromakalim concentration response curve in a non-parallel manner (Fig. 4B) and Schild analysis yielded a regression line with a slope of 1.4. These results are not consistent with competitive antagonism. The concentration of yohimbine required to produce a 2-fold increase in the $IC_{50}$ value for levcromakalim against KCl was 41.7 $\mu$M ($n = 13$).

From these experiments it is clear that, in terms of potency in antagonizing the relaxant effects of levcromakalim on contractions elicited by 15 mM KCl on the rat ileum longitudinal muscle-myenteric plexus preparation, glibenclamide was the most potent antagonist. Gliben-

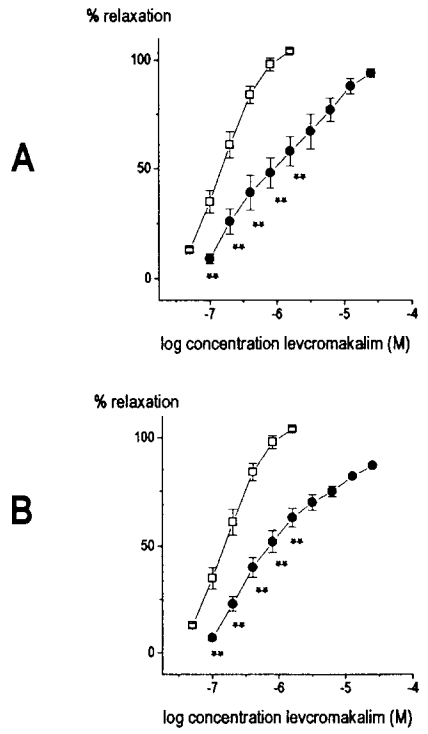


Fig. 4. The effect of TEA and yohimbine on levcromakalim-induced relaxations of KCl-elicited contractions. (A) The effects of levcromakalim were determined in the absence (□) and presence (●) of 3 mM TEA ($n = 5$). (B) The effects of levcromakalim were determined in the absence (□) and presence (●) of 100 μM yohimbine ($n = 5$). The effects of TEA and yohimbine were concentration-dependent but only the effects of the highest concentrations used are shown here. Data are expressed as the mean ± S.E.M. for each point. Comparisons between means were made using Student's unpaired $t$-test. Significant differences are shown by * ($P < 0.05$) and ** ($P < 0.01$).

clamide was approximately 1600 times more potent than yohimbine which in turn was about 40 times more potent than TEA. However, none of the antagonists used appeared to act in a competitive manner.

## 4. Discussion

The present results provide the first direct comparison of the effects of several $K^+$ channel openers on intestinal smooth muscle, using two experimental protocols. Levcromakalim, pinacidil, RP 49356 and SDZ PCO 400 inhibited contractions of the rat ileum longitudinal muscle-myenteric plexus preparation evoked by electrical field stimulation and by KCl. The $IC_{50}$ values for inhibition of KCl-elicited contractions reported here are similar to those reported for other smooth muscle preparations (Longmore et al., 1990; McPherson and Angus, 1990). In the present study, levcromakalim was the agent most potent in its ability to inhibit KCl-elicited contractions and pinacidil, RP 49356 and SDZ PCO 400 were very similar in their potencies. Comparative data for RP 49356 and SDZ PCO 400 are rare; however, it is widely reported that levcromakalim is more potent than pinacidil in most tissues, although in the rat detrusor muscle pinacidil has been shown to be somewhat more potent than levcromakalim (Edwards et al., 1991).

Levcromakalim was also more potent than pinacidil in inhibiting contractions elicited by electrical field stimulation. The order of potency of the $K^+$ channel openers for inhibition of electrically elicited contractions was very similar compared with the order for KCl-induced contractions. The slight differences, however, were statistically significant and may suggest differences in the mechanism of action of pinacidil, RP 49356 and SDZ PCO in their effect on contractions evoked by electrical stimulation compared with contractions elicited by KCl. However, the differences in potency were small, approximately 2-fold, suggesting that none of these compounds preferentially inhibited either direct smooth muscle- or nerve-mediated responses, and that these compounds may share a common mechanism of action.

The mechanism by which $K^+$ channel openers relax KCl-induced smooth muscle contractions is believed to involve hyperpolarization of the cell membrane (Quast et al., 1994). However, the way in which the $K^+$ channel openers exert their inhibitory effect on electrically stimulated contractions is controversial and may include a prejunctional action in addition to postjunctional membrane hyperpolarization. It was reported that in guinea-pig and rabbit mesenteric arteries pinacidil and cromakalim had no effect on presynaptic neurotransmitter release (Nakashima et al., 1990; McHarg et al., 1990). However, in airway smooth muscle preparations inhibition of cholinergic transmission has been found (McCaig and De Jonckheere, 1989). Similarly, in the guinea-pig ileum, Schwörer and Kilbinger (1989) reported that cromakalim reduced cholinergic transmission: pinacidil has been shown to inhibit release of dopamine and noradrenaline in the rat vas deferens (Soares-da-Silva and Fernandes, 1990) and Tremblay et al. (1991) have identified prejunctional glibenclamide binding sites in central neurones. Thus, the possibility that at least part of the mechanism of action of the $K^+$ channel openers in electrically stimulated rat ileum may be prejunctional cannot be discounted.

One of the major findings of the present study was the low potency of minoxidil sulphate and the apparent absence of effect of diazoxide in both experimental protocols. This contrasts with the reported effectiveness of these agents on other, particularly vascular, preparations (Newgreen et al., 1990; Winquist et al., 1989). Piper et al. (1990) reported that minoxidil sulphate was much less potent than cromakalim, pinacidil and RP 49356 in relaxing isolated uterine tissue contracted by oxytocin. The results of the present study and that of Piper et al. (1990) suggest that minoxidil sulphate may be more specific for vascular compared with intestinal and uterine smooth muscle, possibly due to differences in the channels or their

regulatory sites. The observation that there were marked differences in the potency of the $K^+$ channel openers in vascular smooth muscle compared with pancreatic $\beta$-cells supports the hypothesis that differences may exist between the $K^+$ channels in various tissues (Edwards and Weston, 1993).

Although it is generally accepted that diazoxide is much less potent than other $K^+$ channel openers in smooth muscle preparations, little evidence has been reported supporting the possibility that diazoxide may possess selectivity for vascular smooth muscle. In fact, it is well documented that diazoxide discriminates poorly between $K^+$ channels in vascular smooth muscle and pancreatic $\beta$-cells (Quast and Cook, 1989; Edwards and Weston, 1993). However, the lack of effect of diazoxide in the present study contrasts with the findings of Zini et al. (1991), who reported that diazoxide, albeit in higher concentrations than levcromakalim, cromakalim and RP 49356, produced inhibition of contractions of the guinea-pig ileum longitudinal muscle-myenteric plexus preparation induced by electrical field stimulation. The $IC_{50}$ values for inhibition by levcromakalim and RP 49356 were similar to those reported here using rat ileum. In support of the results presented here, no effect of diazoxide was observed on rubidium efflux from rat ileum longitudinal muscle-myenteric plexus preparations even after stimulation with KCl (Davies et al., 1993), indicating that $K^+$ efflux was not promoted.

Glibenclamide is a well documented and potent blocker of the ATP-sensitive $K^+$ channel and a large number of pharmacological studies have involved the use of glibenclamide to antagonize the relaxant effects of the $K^+$ channel openers (Longmore et al., 1990; Piper et al., 1990; McPherson and Angus, 1990). In the present study, glibenclamide reversed the maximal inhibition of electrically induced twitch responses exerted by levcromakalim, pinacidil, RP 49356 and SDZ PCO 400, which is consistent with the results reported by Zini et al. (1991) using guinea-pig ileum. Also, in the present study glibenclamide antagonized the relaxant responses of levcromakalim, pinacidil and RP 49356 on KCl-induced contractions in a concentration-dependent manner and at concentrations similar to those described in other smooth muscles (Cavero et al., 1989; Piper et al., 1990; McPherson and Angus, 1990). Although glibenclamide was a relatively potent antagonist of these $K^+$ channel openers, the nature of the antagonism was found to be unsurmountable. Similarly, non-surmountable antagonism has been reported in a variety of smooth muscle preparations (Lefebvre and Horacek, 1992; Piper et al., 1990; Masuzawa et al., 1990).

Competitive antagonism requires competition between an agonist and an antagonist for a common receptor site: Our results using glibenclamide suggest that this is not the underlying mechanism of antagonism in the rat ileum, and are supported by the findings of Zini et al. (1991) who reported that glibenclamide binding in the guinea-pig longitudinal muscle-myenteric plexus preparation was not inhibited by levcromakalim, cromakalim and diazoxide. However, these workers did observe displacement of glibenclamide binding in the presence of RP 49356. It is more likely that the $K^+$ channel openers and glibenclamide bind to different sites which are negatively allosterically coupled, as proposed by Bray and Quast (1992). This view is supported by the recent cloning of the cardiac ATP-sensitive $K^+$ channel (Ashford et al., 1994). When the gene encoding this channel was expressed in a mammalian cell line, the resulting channel activity possessed all of the essential features of the native channel. However, although the cloned channels were activated by pinacidil, they were not inhibited by glibenclamide, which strongly suggests two separate molecular entities. Consequently, functional antagonism between the $K^+$ channel openers and glibenclamide is not likely to be competitive.

A further possibility is that the non-surmountable antagonism by glibenclamide observed in the present study may result from an action of the openers not directly associated with the ileal smooth muscle, perhaps eliciting a neuronally mediated effect. However, the antagonism of pinacidil effects by glibenclamide was unchanged by tetrodotoxin. This finding appears to exclude the contribution of a tetrodotoxin-sensitive neuronal component in the actions of pinacidil and glibenclamide in KCl-stimulated preparations.

TEA, a non-selective $K^+$ channel blocker (Drukarch et al., 1989), was found to be a weak, non-surmountable antagonist of the relaxant effects of levcromakalim in preparations contracted by KCl (Fig. 4A). The millimolar concentrations required to shift the concentration-response curve for levcromakalim are similar to those required in other preparations (Allen et al., 1986; Wilson et al., 1988). Yohimbine, although not widely reported as a $K^+$ channel blocking agent, was found by Plant and Henquin (1990) to block the effects of diazoxide in pancreatic $\beta$-cells and in the present study was found to antagonize the relaxant effects of levcromakalim in a manner which was not surmountable (Fig. 4B). It was less potent than glibenclamide but more potent than TEA.

The results of the present study are consistent with the existence of a glibenclamide-sensitive, ATP-dependent $K^+$ channel in the rat ileum longitudinal muscle-myenteric plexus preparation. Our data suggest the involvement of this channel in the relaxant effects of levcromakalim, pinacidil, RP 49356 and SDZ PCO 400 on stimulated rat intestinal smooth muscle, which is less sensitive to minoxidil sulphate and insensitive to diazoxide. This finding may point to possible differences in the channel isoform found in intestinal, compared with vascular, smooth muscle. The relaxation of stimulated intestinal smooth muscle by these agents needs to be considered in the clinical evaluation of the $K^+$ channel openers. Indeed, our results confirm the possibility that these agents may possess therapeutic potential in the treatment of intestinal motility disorders.

## References


Allen, S.L., J.P. Boyle, J. Cortijo, R.W. Foster, G.P. Morgan and R.C. Small, 1986, Electrical and mechanical effects of BRL 34195 in guinea pig isolated trachealis, Br. J. Pharmacol. 89, 395.

Andersson, K.E., P.O. Andersson, M. Fovaeus, H. Hedlund, A. Malmgren and C. Sjogren, 1988, Effect of pinacidil on bladder muscle, Drugs 36 (Suppl. 7), 41.

Arunlakshana, O. and H.O. Schild, 1959, Some quantitative uses of drug antagonists, Br. J. Pharmacol. 14, 48.

Ashford, M.L., C.T. Bond, T.A. Blair and J.P. Adelman, 1994, Cloning and functional expression of a rat heart $K_{ATP}$ channel, Nature 370, 456.

Bray, K.M. and U. Quast, 1992, A specific binding site for $K^+$ channel openers in rat aorta, J. Biol. Chem. 267, 11689.

Cavero, I., S. Mondot and M. Mestre, 1989, Vasorelaxant effects of cromakalim in rats are mediated by glibenclamide-sensitive potassium channels, J. Pharmacol. Exp. Ther. 248, 1261.

Davies, M.P., J.R. McCurrie and D. Wood, 1991a, Comparative effects of four potassium channel openers on the rat ileum, Br. J. Pharmacol. 104, 163P.

Davies, M.P., J.R. McCurrie and D. Wood, 1991b, Antagonism of the relaxant effects of pinacidil and BRL 38227 on the rat ileum, J. Pharm. Pharmacol. 43, 127P.

Davies, M.P., J.R. McCurrie and D. Wood, 1993, Comparative effects of potassium channel openers on $^{86}$rubidium efflux from rat ileum, Br. J. Pharmacol. 110, 129P.

Drukarch, B., K.S. Kits, J.E. Leysen, E. Schepens and J.C. Stoof, 1989, Restricted usefulness of tetraethylammonium and 4-aminopyridine for the characterization of receptor-operated $K^+$-channels, Br. J. Pharmacol. 98, 113.

Edwards, G., M. Henshaw, M. Miller and A.H. Weston, 1991, Comparison of the effects of several potassium channel openers on rat bladder and rat portal vein in vitro, Br. J. Pharmacol. 102, 679.

Edwards, G. and A.H. Weston, 1993, The pharmacology of ATP-sensitive potassium channels, Annu. Rev. Pharmacol. Toxicol. 33, 597.

Escande, D., D. Thuringer, S. Le Guern, J. Courteix, M. Lavelle and I. Cavero, 1989, Potassium channel openers act through an activation of ATP-sensitive potassium channels in guinea pig cardiac myocytes, Pflüg. Arch. 414, 669.

Hollingsworth, M., T. Amedee, D. Edwards, J. Mironneau, J.P. Savineau, R.C. Small and A.H. Weston, 1987, The relaxant action of BRL 34915 in rat uterus, Br. J. Pharmacol. 91, 803.

Kilbinger, H., 1982, The myenteric plexus-longitudinal muscle preparation, in: Progress in Cholinergic Biology: Model Cholinergic Synapses. eds. I. Hanin and A.M. Goldberg (Raven Press, New York) p. 137.

Kortezova, N., O. Bayguinov, K. Boev and M. Papasova, 1992, Effect of cromakalim on the smooth muscle of the cat gastric antrum, J. Pharm. Pharmacol. 44, 875.

Lefebvre, R.A. and J. Horacek, 1992, Relaxant effects of BRL 38227 and pinacidil on the rat gastric fundus, Eur. J. Pharmacol. 214, 1.

Longmore, J., D.T. Newgreen and A.H. Weston, 1990, Effects of cromakalim, RP49356, diazoxide, glibenclamide and galanin in rat portal vein, Eur. J. Pharmacol. 190, 75.

Masuzawa, K., T. Matsuda and M. Asano, 1990, Evidence that pinacidil may promote the opening of ATP-sensitive $K^+$ channels yet inhibit the opening of $Ca^{2+}$-activated $K^+$ channels in $K^+$-contracted canine mesenteric artery, Br. J. Pharmacol. 100, 143.

McCaig, D.J. and B. De Jonckheere, 1989, Effect of cromakalim on bronchoconstriction evoked by cholinergic nerve stimulation in guinea-pig isolated trachea, Br. J. Pharmacol. 98, 662.

McHarg, A.D., J.S. Southerton and A.H. Weston, 1990, A comparison of the actions of cromakalim and nifedipine on rabbit isolated mesenteric artery, Eur. J. Pharmacol. 185, 137.

McPherson, G.A. and J.A. Angus, 1990, Characterization of responses to cromakalim and pinacidil in smooth and cardiac muscle by use of selective antagonists, Br. J. Pharmacol. 100, 201.

Nakashima, M., Y. Li, N. Seki and H. Kuriyama, 1990, Pinacidil inhibits neuromuscular transmission indirectly in the guinea-pig and rabbit mesenteric arteries, Br. J. Pharmacol. 101, 581.

Newgreen, D.T., K.M. Bray, A.D. McHarg, A.H. Weston, S. Duty, B.S. Brown, P.B. Kay, G. Edwards, J. Longmore and J.S. Southerton, 1990, The action of diazoxide and minoxidil sulphate on rat blood vessels: a comparison with cromakalim, Br. J. Pharmacol. 100, 605.

Paton, W.D.M. and E.S. Vizi, 1969, The inhibitory action of noradrenaline and adrenaline on acetylcholine output in guinea pig ileum longitudinal muscle strip, Br. J. Pharmacol. 35, 10.

Piper, I., E. Minshall, S.J. Downing, M. Hollingsworth and H. Sadraei, 1990, Effects of several potassium channel openers and glibenclamide on the uterus of the rat, Br. J. Pharmacol. 101, 901.

Plant, T.D. and J.C. Henquin, 1990, Phentolamine and yohimbine inhibit ATP-sensitive $K^+$ channels in mouse pancreatic beta-cells, Br. J. Pharmacol. 101, 115.

Quast, U. and N.S. Cook, 1989, In vitro and in vivo comparison of two $K^+$ channel openers, diazoxide and cromakalim, and their inhibition by glibenclamide, J. Pharmacol. Exp. Ther. 250, 261.

Quast, U., J.M. Guillon and I. Cavero, 1994, Cellular pharmacology of potassium channel openers in vascular smooth muscle, Cardiovasc. Res. 28, 805.

Richardson, D., K.N. Alibhai and J.D. Huizinga, 1992, On the pharmacological and physiological role of glibenclamide-sensitive potassium channels in colonic smooth muscle, Pharmacol. Toxicol. 71, 365.

Schwörer, H. and H. Kilbinger, 1989, Effects of cromakalim on acetylcholine release and smooth muscle contraction in guinea-pig small intestine, Naunyn-Schmied. Arch. Pharmacol. 339, 706.

Soares-da-Silva, P. and M.H. Fernandes, 1990, Inhibition by the putative potassium channel opener pinacidil of the electrically-evoked release of endogenous dopamine and noradrenaline in the rat vas deferens, Naunyn-Schmied. Arch. Pharmacol. 342, 415.

Tremblay, E., S. Zini and Y. Ben-Ari, 1991, Autoradiographic study of the cellular localization of [$^3$H]glibenclamide binding sites in the rat hippocampus, Neurosci. Lett. 127, 21.

Weik, R. and B. Neumcke, 1990, Effects of potassium channel openers on single $K^+$ channels in mouse skeletal muscle, Naunyn-Schmied. Arch. Pharmacol. 342, 258.

Weir, S.W. and A.H. Weston, 1986, Effects of apamin on responses to BRL 34915, nicorandil and other relaxants in the guinea pig taenia caeci, Br. J. Pharmacol. 88, 113.

Wilson, C., M.C. Coldwell, D.R. Howlett, S.M. Cooper and T.C. Hamilton, 1988, Comparative effects of $K^+$ channel blockade on the vasorelaxant activity of cromakalim, pinacidil and nicorandil, Eur. J. Pharmacol. 152, 331.

Winquist, R.J., L.A. Heaney, A.A. Wallace, E.P. Baskin, R.B. Stein and M.L. Garcia, 1989, Glyburide blocks the relaxation response to BRL 34915 (cromakalim), minoxidil sulfate and diazoxide in vascular smooth muscle, J. Pharmacol. Exp. Ther. 248, 149.

Zini, S., Y. Ben-Ari and M.L. Ashford, 1991, Characterization of sulfonylurea receptors and the action of potassium channel openers on cholinergic neurotransmission in guinea pig isolated small intestine, J. Pharmacol. Exp. Ther. 259, 566.