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Effect of cromakalim and glibenclamide on spontaneous and evoked motility of the guinea-pig isolated renal pelvis and ureter

[1]Carlo Alberto Maggi, Sandro Giuliani & Paolo Santicioli

Pharmacology Department, A. Menarini Pharmaceuticals, Via Sette Santi 2, 50131, Florence, Italy

1 We have investigated the effect of the potassium (K) channel opener, cromakalim, on the spontaneous myogenic activity of the guinea-pig isolated renal pelvis and on myogenic contractions evoked by direct electrical stimulation of the guinea-pig isolated ureter.

2 In the presence of Bay K 8644 (1 μM), electrical stimulation of the guinea-pig ureter (10 Hz for 1 s, pulse width 5 ms, 60 V) produced regular tetrodotoxin-(1 μM) resistant phasic contractions which were suppressed by 3 μM cromakalim. Glibenclamide (0.1–3 μM), 4-aminopyridine (4-AP, 0.1–2 mM) and tetraethylammonium (TEA, 1–10 mM) produced a concentration-dependent inhibition of the effect of cromakalim with the rank order of potency (EC_{50} in parentheses): glibenclamide (0.64 μM)>>4-AP (1.11 mM)>TEA (6.6 mM). Apamin (0.1–0.3 μM) was without effect.

3 Cromakalim (0.1–10 μM) produced concentration-dependent inhibition and suppression of spontaneous contractions of the guinea-pig isolated renal pelvis and of evoked contractions of the ureter with EC_{50} values of 0.71 and 0.47 μM, respectively.

4 Glibenclamide (1 μM) produced a rightward shift of the concentration-response curve to cromakalim in both the renal pelvis and ureter, without producing depression of the maximal inhibitory effect. Glibenclamide did not affect the spontaneous activity of the renal pelvis while it produced a slight enhancement (10–15% increase) of evoked contractions of the ureter. Glibenclamide did not affect the inhibitory action of the adenylate cyclase activator, forskolin, in the renal pelvis or ureter.

5 In electrophysiological experiments (sucrose gap), cromakalim (0.3 and 1 μM) produced hyperpolarization of ureter smooth muscle. Cromakalim also produced a transient suppression of action potentials and accompanying phasic contractions evoked by electrical stimulation. Before suppression of evoked contractions, a shortening of action potential duration was observed concomitant with the developing hyperpolarization produced by cromakalim. A lower concentration (0.1 μM) of cromakalim did not affect membrane potential but shortened action potential duration and reduced the evoked contraction.

6 Glibenclamide (1 μM) inhibited the hyperpolarizing action of cromakalim and prevented its inhibitory action on evoked action potentials and contractions of the ureter. Glibenclamide also produced a slight prolongation of action potential duration and increased the amplitude and duration of the accompanying mechanical response.

7 These findings demonstrate that activation of cromakalim- and glibenclamide-sensitive K channels produces a powerful mechanism for regulation of pyeloureteral motility and suppression of latent pacemakers of the ureter in guinea-pig. Glibenclamide-sensitive K channels take part in determining action potential shape and duration in the guinea-pig ureter.

Keywords: Potassium channels; guinea-pig ureter; electromechanical coupling; guinea-pig renal pelvis; cromakalim; glibenclamide; adenylate cyclase; forskolin

Introduction

Potassium (K) ions play an important role in determining the excitability of smooth muscle cells. Various types of outward K currents occur in smooth muscle, which influence the shape of the action potential, generation of spontaneous activity and the mechanical responses brought about by different stimuli. In the past few years, a number of pharmacological tools has been developed which affect in a selective manner only certain types of K channels (Cook & Quast, 1990). Among these, the development of the benzopyran derivative, BRL 34915, or cromakalim, which acts as a K channel opener, has represented one of the most interesting advances (Hamilton *et al.*, 1986; Weir & Weston, 1986; Quast, 1987). As expected from its agonist action on certain K channels, cromakalim produces smooth muscle hyperpolarization and reduces the probability of the opening of voltage-sensitive calcium channels (Cook & Quast, 1990, for review). These electrophysiological effects determine a remarkable relaxant action on smooth muscles, especially evident when cromakalim is challenged against stimuli which use voltage-dependent calcium channels to produce smooth muscle contraction.

The nature of K channels opened by cromakalim in smooth muscle has been a matter of debate (see Cook & Quast, 1990). In various preparations, the hyperpolarizing action of cromakalim and its smooth muscle relaxant activity are selectively blocked, in a competitive manner, by antidiabetic agents of the sulphonylurea group, such as glibenclamide (Quast & Cook, 1988; Wilson, 1989; Standen *et al.*, 1989). These findings support the idea that the K channel activated by cromakalim in smooth muscle could be an ATP-sensitive K channel, similar to that regulating insulin secretion, although the pharmacology of the ATP-sensitive K channels in smooth muscle and the peripheral nervous system may not be identical to that of the pancreatic and cardiac ATP-sensitive K channels (e.g. Zini *et al.*, 1991; Takano & Noma, 1993).

[1] Author for correspondence.

The smooth muscle relaxant activity of cromakalim has been documented in the urinary bladder from various species: at this level, cromakalim stimulates K efflux, produces hyperpolarization and reduces the spontaneous and stimulated activity of urinary bladder smooth muscle (Foster *et al.*, 1989; Fujii *et al.*, 1990; Grant & Zuzack, 1991). ATP-sensitive K channels activated by cromakalim and blocked by glibenclamide have recently been described in the guinea-pig urinary bladder (Bonev & Nelson, 1993). On the basis of these results, a possible therapeutic use of K channel openers in the treatment of motility disorders of the urinary tract has been suggested. However, the possible action of cromakalim on motility of the upper urinary tract has not been investigated. Since the activation of voltage-dependent calcium channels is very important for regulation of pyeloureteral motility (Weiss, 1992 for review), a powerful action of K channel openers at this level may be expected.

The aim of the present study was to evaluate the effect of cromakalim and glibenclamide, on the motility of the guinea-pig upper urinary tract. In a first series of experiments, we evaluated the effect of cromakalim on evoked contractions of the guinea-pig ureter and spontaneous contractions of the guinea-pig isolated renal pelvis. In a second series, the electrophysiological effect of cromakalim on membrane potential and evoked action potentials of the guinea-pig ureter were investigated by a sucrose gap technique.

Methods

Male albino guinea-pigs weighing 250–300 g were stunned and bled. The renal pelvis and ureter (from the inferior renal pole to its entry into the bladder) were excised, cleaned of adhering fat and connective tissue and placed in oxygenated and warmed (95% O_2 and 5% CO_2, pH 7.4 at 37°C) Krebs solution which was used in all experiments. Krebs solution had the following composition (mmol l^{-1}): NaCl 119, $NaHCO_3$ 25, KH_2PO_4 1.2, $MgSO_4$ 1.5, KCl 4.7, $CaCl_2$ 2.5 and glucose 11. Since experiments performed in the ureter required the application of pulses of electrical stimulation to evoke action potentials (see sucrose gap experiments) and contractions, and because of the existence of a capsaicin-sensitive inhibitory innervation of the guinea-pig ureter (Maggi & Giuliani, 1991), all experiments were performed in capsaicin-pretreated ureters to eliminate this neural inhibitory influence on ureteral excitability. With this aim, the ureters were incubated for 15 min in Krebs solution containing 10 μM capsaicin: this *in vitro* capsaicin desensitization technique has been shown to produce blockade of neuropeptide release from capsaicin-sensitive primary afferents in the guinea-pig ureter (Maggi & Giuliani, 1991).

Organ bath experiments

The renal pelvis, cleaned of adhering tissue, was opened, connected to silk threads and mounted in a 5 ml organ bath for isotonic recording (load 2 mN) of mechanical activity along its circular axis, as described previously (Maggi & Giuliani, 1992). The ureter was placed in a Petri dish, a segment about 2 cm long was excised from its middle portion and the specimen was mounted in a 5 ml organ bath for isotonic recording (load 3 mN) of mechanical activity along its longitudinal axis, as described previously (Maggi & Giuliani, 1991).

In the ureter, the effect of cromakalim on phasic contractions produced by electrical depolarization of smooth muscle was investigated: with this aim, trains of pulses (10 Hz for 1 s, 60 V) of long width (5 ms) were delivered automatically at 1 min intervals by means of platinum wire electrodes placed at the top and bottom of the organ bath (field stimulation) by means of a GRASS S88 stimulator. We found that the mechanical response produced by electrical field stimulation in these conditions undergoes a progressive fading and becomes irregular during prolonged periods of stimulation; addition of the calcium channel agonist, Bay K 8644 (1 μM) produced a prompt enhancement of the evoked response which then remains regular for several hours, enabling pharmacological analysis of drug action.

In a first series of experiments a concentration of cromakalim (3 μM) which invariably produces total suppression of evoked contractions of the ureter, was used to analyse the effect of potassium channel blockers on the response to cromakalim. The K channel blockers, glibenclamide, 4-aminopyridine (4-AP), tetraethylammonium (TEA) or apamin were added to the bath when the amplitude of the evoked contractions had reached a steady state and their effect was observed for 15 min or until a steady state had been reached. At this time, cromakalim (3 μM) was added to the bath.

In a second series of experiments a cumulative concentration-response curve to cromakalim was produced, the next concentration being added when the effect of the preceding one had reached a steady state. Cumulative response-curves to the adenylate cyclase activator, forskolin, were produced in the same manner. The effect of glibenclamide on the curve to cromakalim was determined after 15 min contact time.

In the spontaneously active renal pelvis preparation, cumulative concentration-response curves to cromakalim and forskolin were produced, in the absence or presence of glibenclamide, as described above for the ureter. In the renal pelvis, the effect of cromakalim was evident either as a progressive reduction in the amplitude of contractions with minor effect on frequency until suppression of contractility or as a mixed inhibitory effect on frequency and amplitude of contraction until suppression. In the latter case, the inhibitory effect of cromakalim on the amplitude of contraction was relatively minor (< 50% inhibition) until suppression of spontaneous contractions occurred. For this reason a motility index (arbitrary units) was calculated in each preparation by multiplying the frequency of spontaneous contractions (contraction min^{-1}) and their amplitude (in mm of chart); the inhibitory effect of cromakalim was calculated as % inhibition of the basal motility index.

Sucrose gap experiments

Male albino guinea-pigs (250–300 g) were stunned and bled. The ureters were excised and placed in oxygenated (95% O_2 and 5% CO_2) Krebs solution. A single sucrose-gap, modified as described in details by Artemenko *et al.* (1982) and Hoyle (1987) was used to investigate changes in membrane potential and mechanical activity in response to electrical stimulation. The ureters were superfused with oxygenated Krebs solution at a rate of 1 ml min^{-1}. The temperature of the solution was kept constant at 35 ± 0.5°C.

Electrical field stimulation with single pulses using parameters sufficient to produce direct excitation of smooth muscle (50–60 V, 2–5 ms pulse width) evoked an action potential and accompanying contraction of the guinea-pig ureter, as described in a number of previous studies. We found that electrical and mechanical responses evoked in this way need quite a long interstimulus interval to be consistently reproduced over the long periods of time required for a pharmacological analysis of drug action. This phenomenon has been referred to as the 'refractory period' of the ureter (e.g. Cuthbert, 1965). We found that *in vitro* capsaicin pretreatment (10 μM for 20 min) to eliminate the CGRP-mediated inhibitory innervation (cf. Maggi & Giuliani, 1991) and addition of the calcium channel agonist, Bay K 8644 (1 μM) both exert a favourable effect on the reproducibility of the electrical and mechanical responses of the guinea-pig ureter (Maggi, Giuliani & Santicioli, unpublished observations).

Cromakalim was applied in the superfusion fluid for 4 min. Glibenclamide was superfused for 15 min before application

of cromakalim, this contact time being sufficient to produce maximal effects on the action potential of the guinea-pig ureter. In some experiments, glibenclamide was applied to preparations which had not been exposed to cromakalim: in these experiments glibenclamide produced effects on the action potential of the guinea-pig ureter which were similar to those observed in the remainders and the pooled data of the experiments are presented in the Results section. In a few experiments, the effect of cromakalim and glibenclamide were assessed on preparations not subjected to electrical stimulation.

Statistical analysis

Each value is mean ± s.e. of the mean. Statistical analysis was performed by means of Student's t test or by means of analysis of variance, when appropriate. Linear regression was performed by the least squares method: EC_{50} and 95% confidence limits were calculated accordingly.

Drugs

Drugs used were: cromakalim (Beecham), nifedipine, glibenclamide, forskolin, capsaicin and 4-aminopyridine (Sigma), tetrodotoxin (Sankyo), tetraethylammonium (Serva), apamin (Peninsula), Bay K 8644 (methyl-1,4-dihydro-2,6-dimethyl-3-nitro-4-(2-trifluoromethylphenyl)-pyridine-5-carboxylate, Calbiochem).

Results

Guinea-pig ureter

The unstimulated guinea-pig isolated ureter is mechanically quiescent. Phasic contractions were produced by electrical field stimulation with train of pulses (10 Hz, 60 V, 5 ms pulse width for 1 s every 60 s, Figure 1). The phasic contractions evoked under these experimental conditions (capsaicin pretreatment, Bay K 8644 1 μM in the bath) are tetrodotoxin-(1 μM) resistant indicating that they depend upon direct activation of smooth muscle cells and are abolished by 10–30 μM nifedipine ($n = 4$) showing the involvement of voltage-dependent calcium channels.

Cromakalim (3 μM) produced a prompt suppression of evoked contractions of the guinea-pig ureter. This effect was concentration-dependently prevented (Figure 2) by glibenclamide (EC_{50} 0.64 μM, 95% c.l. 0.50–0.72 μM), 4-AP (EC_{50} 1.11 mM, 1.05–1.45 mM) and TEA (EC_{50} 6.6 mM, 3.5–36 mM). Glibenclamide, 4-AP and TEA also enhanced the amplitude of evoked ureteral contractions but the degree of enhancement was barely correlated with the extent of inhibition of the suppressive action of 3 μM cromakalim: the amplitude of evoked ureteral contractions was enhanced by 8 ± 2, 14 ± 3 and 14 ± 4% following 0.3, 1 and 3 μM glibenclamide, respectively ($n = 4–7$ for each concentration tested); by 5 ± 1, 10 ± 2, 14 ± 4 and 36 ± 5% following 0.1, 0.5, 1 and 2 mM 4-AP, respectively ($n = 4–6$); by 11 ± 2, 39 ± 6, 62 ± 13 and 82 ± 7% following 1, 2, 5 and 10 mM TEA, respectively ($n = 4–6$). Apamin (0.1–0.3 μM, $n = 4$) had no consistent effect on the amplitude of evoked contractions nor on the inhibitory action of 3 μM cromakalim.

Cromakalim (0.1–3 μM) produced a concentration-dependent inhibition and eventually suppressed the evoked contractions of the guinea-pig ureter (Figures 1 and 3). The EC_{50} was 0.47 μM (0.18–0.90 μM). The inhibitory effect of cromakalim was promptly reversed by washing or by addition of 1 μM glibenclamide (Figure 1). Glibenclamide (1 μM) produced a slight and steady enhancement of evoked contractions to 109 ± 2% of control value (Figure 1): although quantitatively small, this effect was consistently observed in all preparations tested ($n = 8$) and was statistically significant ($P < 0.05$). In the presence of glibenclamide, the concentration-dependent inhibitory effect of cromakalim was shifted rightward without depression of the maximal inhibitory effect attainable, i.e. a pattern consistent with competitive antagonism (Figure 3, Table 1).

Forskolin produced a concentration-dependent inhibition of evoked contractions of the guinea-pig ureter (Figure 3, Table 1): the maximal inhibitory effect averaged 57 ± 6%

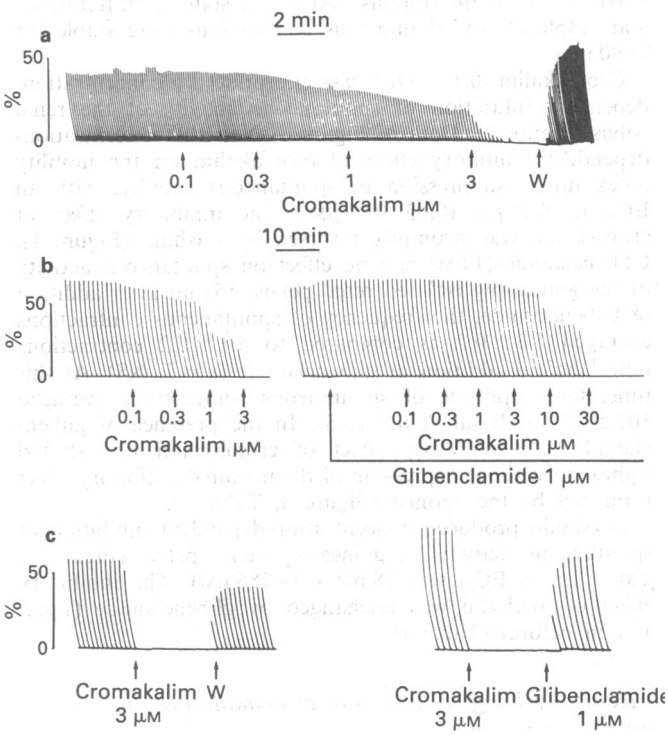

Figure 1 (a) Concentration-dependent inhibition by cromakalim of spontaneous activity of the guinea-pig isolated renal pelvis and reversal by washing (W). (b) Concentration-dependent inhibition by cromakalim of electrically-evoked contractions of the guinea-pig isolated ureter in the absence (left) and presence (right) of glibenclamide. Note that glibenclamide produced a small enhancement in amplitude of the evoked contractions. (c) Suppression by 3 μM cromakalim of electrically-evoked contractions of the guinea-pig isolated ureter and reversal of cromakalim inhibition by washing (W, left) and glibenclamide (right). In all panels, vertical bars indicate % of maximal contractile response to 80 mM KCl.

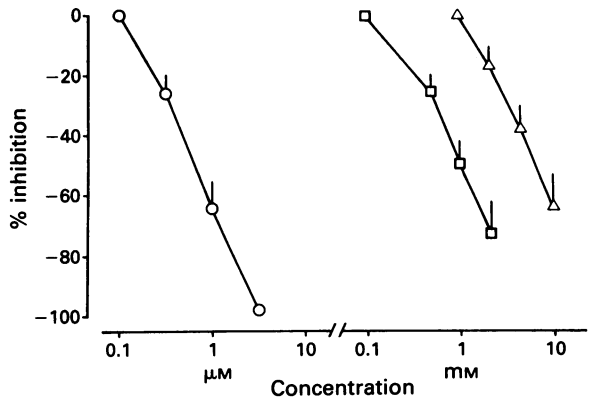

Figure 2 Concentration-dependent inhibition by glibenclamide (○), 4-aminopyridine (□) and tetraethylammonium (△) of the effect of cromakalim (3 μM) on electrically-evoked contractions of the guinea-pig isolated ureter. Each value is the mean ± s.e.mean of 4–7 experiments.

inhibition and its EC_{50} was 0.38 μM (0.11–3.2 μM). Glibenclamide (1 μM) did not significantly affect the inhibitory action of forskolin (Figure 3, Table 1).

Guinea-pig renal pelvis

The guinea-pig isolated renal pelvis showed spontaneous mechanical activity at a frequency of 4–7 contractions min^{-1} (Figure 1). At steady state (30–60 min from setup), the amplitude of spontaneous contractions averaged about 50% of maximal contractile response. At steady state, both frequency and amplitude of spontaneous contractions were stable for >90 min.

Cromakalim (0.1–10 μM, $n = 7$) produced a concentration-dependent inhibition of spontaneous activity of the renal pelvis (Figure 1). Data in Figure 3 show the concentration-dependent inhibitory effect of cromakalim on the motility index up to suppression of spontaneous activity, with an EC_{50} of 0.71 μM (0.65–0.75 μM). The inhibitory effect of cromakalim was promptly reversed by washing (Figure 1). Glibenclamide (1 μM) had no effect on spontaneous activity of the guinea-pig isolated renal pelvis: 15 min after addition of glibenclamide, the frequency of spontaneous contractions averaged 5.3 ± 0.2 as compared to 5.1 ± 0.2 contractions min^{-1} before addition of glibenclamide ($n = 7$, NS). At this time, the amplitude of spontaneous contractions averaged 101 ± 4% of basal values (NS). In the presence of glibenclamide, the inhibitory effect of cromakalim was shifted rightward without depression of the maximal inhibitory effect produced by the agonist (Figure 3, Table 1).

Forskolin produced concentration-dependent inhibition of spontaneous activity of guinea-pig renal pelvis (Figure 3, Table 1): its EC_{50} was 78 nM (41–186 nM). The inhibitory effect of forskolin was unchanged by glibenclamide (1 μM, 15 min before) (Table 1).

Electrophysiological action of cromakalim on the guinea-pig ureter

The effects of cromakalim and glibenclamide have been investigated in capsaicin-pretreated ureters and in the presence of 1 μM Bay K 8644: in these conditions, electrical stimulation with single shocks at 50–60 V and pulse width of 2–5 ms automatically delivered at 60 s intervals evoked reproducible electrical and mechanical responses (Figure 4a).

The action potential of the ureter elicited under these experimental conditions is characterized by a rapidly rising depolarization followed by a plateau with superimposed oscillations, repolarization and slight after-hyperpolarization (Figure 5). Quantitative data on action potential and accompanying contraction are given in Table 2.

Cromakalim (0.1 μM, applied by superfusion for 4 min, $n = 4$) did not affect membrane potential but produced a shortening (24% reduction) of action potential duration (from 1742 ± 220 to 1333 ± 210 ms, effect measured at 5 min from start of superfusion with cromakalim) without affecting the amplitude of the action potential (8.4 ± 1.3 and 8.8 ± 1.4 mV before and after cromakalim, respectively). The accompanying phasic contraction was slightly reduced (– 10%) in amplitude (from 3.9 ± 0.1 to 3.5 ± 0.09 mN) and shortened (– 22%) in duration (from 2071 ± 280 to 1612 ± 150 ms).

At higher concentrations (0.3–1 μM) cromakalim produced various effects (Figures 4, 5 and 6), summarized as follows: (a) hyperpolarization of the smooth muscle which averaged

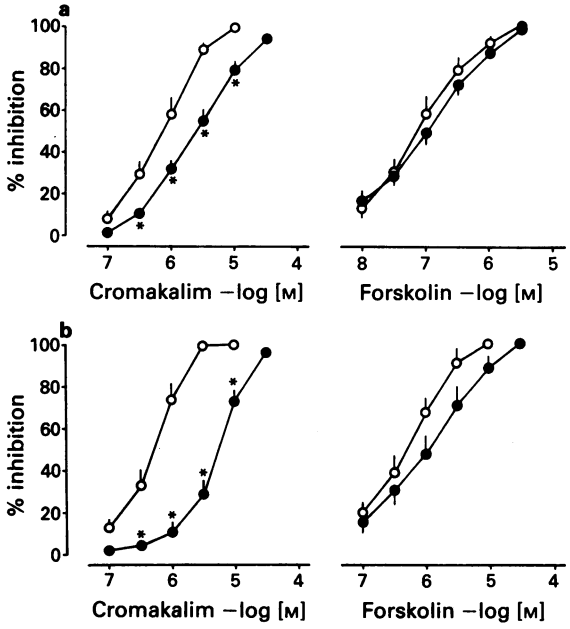

Figure 3 Concentration-dependent inhibition by cromakalim (left panels) and forskolin (right panels) of spontaneous activity of the guinea-pig isolated renal pelvis (a) and electrically-evoked contractions of the guinea-pig isolated ureter (b) in the absence (○) and presence (●) of glibenclamide. Each value is the mean ± s.e.mean of 6–8 experiments. *Significantly different from control, $P < 0.05$.

Table 1 EC_{50} (95% c.l. in brackets) of cromakalim and forskolin for inhibition of spontaneous (renal pelvis) and evoked (ureter) contractions of the guinea-pig pyeloureteral system in the absence and presence of glibenclamide (1 μM 15 min beforehand)

Guinea-pig renal pelvis	*Control*		*Glibenclamide* (1 μM)	
	EC_{50}	E_{max} (% inhibition)	EC_{50}	E_{max} (% inhibition)
Cromakalim	0.71 μM (0.65–0.75)	100	2.36 μM* (2.15–2.66)	100
Forskolin	78 nM (41–186)	100	93 nM (53–180)	100
Guinea-pig ureter	*Control*		*Glibenclamide* (1 μM)	
	EC_{50}	E_{max} (% inhibition)	EC_{50}	E_{max} (% inhibition)
Cromakalim	0.47 μM (0.18–0.90)	100	5.07 μM* (1.1–8.8)	100
Forskolin	0.38 μM (0.11–3.2)	57 ± 6	1.32 μM (1.2–1.4)	56 ± 6

*Significantly different from EC_{50} value recorded in the absence of glibenclamide. Each value is the mean of 6–8 experiments.

1.36 ± 0.16 and 1.85 ± 0.15 mV at 0.3 and 1 μM, respectively ($n = 8$); hyperpolarization produced by cromakalim developed fully in 4–6 min and recovered following washout; (b) the hyperpolarization was followed by progressive reduction in the duration of the action potential plateau and decrease in contraction amplitude (Figure 5); this inhibitory effect led to suppression of the action potential and accompanying contraction; both electrical and mechanical responses recovered following washout of cromakalim; after application of 0.3 and 1 μM cromakalim the evoked action potentials and contractions were suppressed for 2 ± 0.6 and 8 ± 2 min, respectively ($n = 8$).

Glibenclamide (1 μM, superfused for 15 min, $n = 10$) did not produce consistent changes of membrane potential: a slight depolarization was observed in 5 cases, a slight hyperpolarization in 2 cases and no change in 3 cases. Glibenclamide produced a slight but significant ($P < 0.05$) prolongation of action potential duration (20% increase), increased the number of spikes, amplitude (9% increase) and duration (15% increase) of accompanying contraction (Table 2, Figure 7). A shortening in the delay between stimulus application and onset of the action potential and an increased afterhyperpolarization were also observed in some preparations after glibenclamide (Figure 7), but these effects were not consistently observed in all preparations tested and did not reach statistical significance (Table 2).

In the presence of glibenclamide (1 μM, 15 min before), the hyperpolarizing action of cromakalim (0.3–1 μM) on membrane potential and the inhibitory effects on evoked action potential and contraction were significantly reduced; (Table 3, Figure 6). The inhibition by glibenclamide was overcome when a larger concentration of cromakalim (10 μM) was tested (Figure 6 and Table 3).

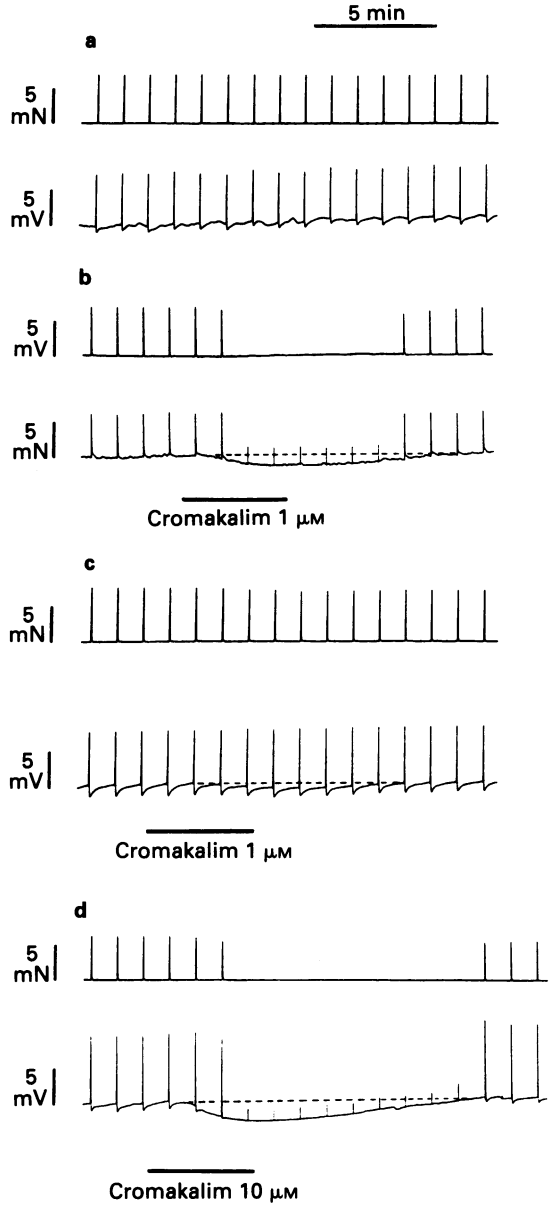

Figure 4 Electrical and mechanical activity of the guinea-pig isolated ureter evoked by electrical stimulation (sucrose gap recording). In capsaicin pretreated ureters and in the presence of Bay K 8644, electrical stimuli applied at intervals of 60 s produced regular action potentials (lower tracing in each panel) and accompanying phasic contractions of the ureter (upper tracing in each panel). (a) Shows reproducibility of the evoked responses. Cromakalim (b) produced hyperpolarization and suppression of evoked action potentials and contractions (the small upward deflections remaining during cromakalim action are artifacts of the stimulus). In the presence of glibenclamide (c and d) the hyperpolarization produced by 1 μM cromakalim and its inhibitory action on evoked action potentials and contractions were inhibited (c) but the inhibitory effect of glibenclamide was overcome by using a larger concentration of cromakalim (10 μM, d).

Discussion

The present findings demonstrate that the K channel opener, cromakalim, inhibits the contractile activity of the guinea-pig isolated renal pelvis and ureter at concentrations comparable to those found effective in other studies on vascular and nonvascular smooth muscles (e.g. Buckingham *et al.*, 1989;

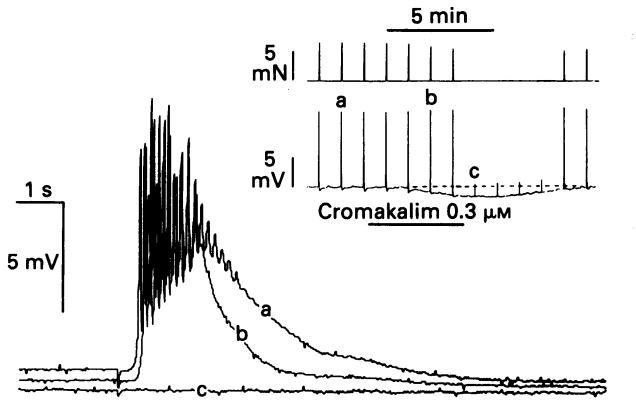

Figure 5 Superimposed tracings showing the reduction of action potential duration during the developing hyperpolarization produced by cromakalim in the guinea-pig ureter. Tracing (a) is control action potential; tracing (b) was obtained at 3 min after start of superfusion with 0.3 μM cromakalim; tracing (c) was obtained at 5 min from start of superfusion with cromakalim. The superimposed action potentials recorded in (a), (b) and (c) are shown in the inset at a low chart speed along with the accompanying mechanical response of the ureter.

Table 2 Membrane hyperpolarization produced by cromakalim in the guinea-pig ureter in the absence and presence of glibenclamide (1 μM, 15 min beforehand)

		Membrane hyperpolarization (mV)	
		Control	*After glibenclamide*
Cromakalim	0.3 μM	1.36 ± 0.16	0.40 ± 0.13*
	1.0 μM	1.85 ± 0.15	0.92 ± 0.13*
	10 μM	not tested	2.18 ± 0.34

Each value is mean ± s.e.mean of 4–8 experiments.
*Significantly different from control values, $P < 0.05$.

Foster et al., 1989; Fujii et al., 1990). Since the spontaneous activity of the renal pelvis and the electrically-evoked contractions of the ureter are highly if not exclusively dependent on activation of voltage-sensitive calcium channels (Shuba, 1977; Brading et al., 1983; Weiss, 1992 for review), we reasoned that cromakalim should exert a powerful suppressant activity on smooth muscle of the pyeloureteral complex. In both preparations, the contractile activity is abolished by nifedipine indicating the absolute requirement for activation of L-type voltage-sensitive calcium channels. In the ureter, relatively high (10–30 μM) concentrations of nifedipine were required to abolish contractility because of the presence of Bay K 8644 which was added to provide the regular and reproducible responses necessary for pharmacological analysis of the inhibitory action of cromakalim. Since cromakalim produces hyperpolarization of smooth muscles, including that of the urinary bladder (Foster et al., 1989; Fujii et al., 1990), its inhibitory activity in the pyeloureteral smooth muscle may be explained through a similar mechanism. This conclusion is supported by electrophysiological analysis of cromakalim action in the guinea-pig ureter, where developing hyperpolarization by cromakalim first produced a shortening of action potential duration and eventually suppressed the evoked electrical and mechanical activity. At 0.1 μM, cromakalim produced a small shortening of action potential duration and decreased the evoked contraction without producing overt changes of resting membrane potential. This effect is quite similar to that produced by nifedipine at low concentrations which do not abolish the action potential of the ureter (unpublished observations). Although cromakalim at high concentrations may possess calcium channel blocking properties (for e.g. 3–30 μM, Okabe et al., 1990), the present experiments have been performed in the presence of Bay K 8644 and it appears unlikely that direct blockade of calcium channels accounts for the reduced duration of action potential duration produced by 0.1 μM cromakalim. We speculate that the K channel agonist action of cromakalim may have been insufficient to produce membrane hyperpolarization at resting membrane potential but could have been efficient at the level of membrane potential attained during the plateau of the action potential.

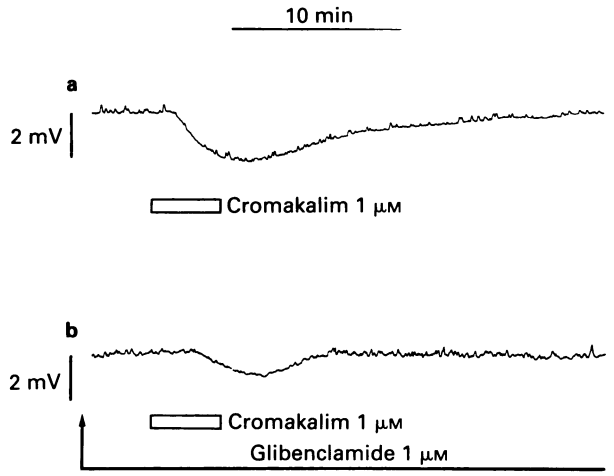

Figure 6 Membrane hyperpolarization produced by 1 μM cromakalim in the guinea-pig ureter (a) and its antagonism by 1 μM glibenclamide (b).

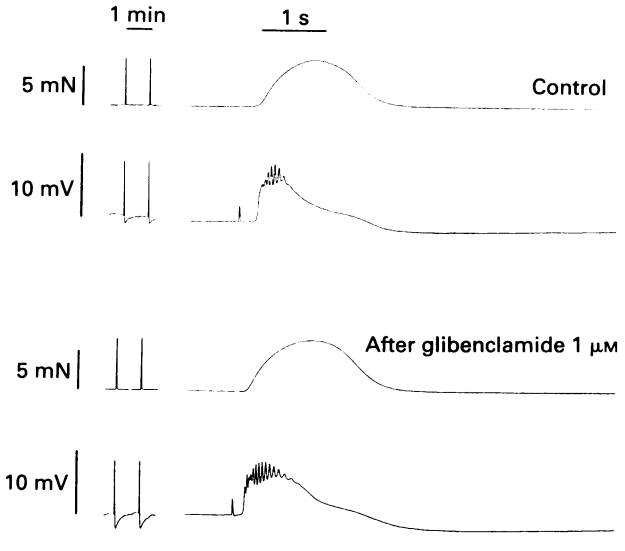

Figure 7 Effect of 1 μM glibenclamide on action potential and accompanying contraction produced by electrical stimulation of the guinea-pig ureter. The effect of glibenclamide is shown after 15 min superfusion with the drug. See text for details.

Although the nature of K channels activated by cromakalim in smooth muscle may not be identical to the ATP-dependent K channels regulating insulin secretion in the pancreas, the hyperpolarizing action and smooth muscle relaxant properties of cromakalim are sensitive to the inhibitory action of the sulphonylurea-type antidiabetics like glibenclamide (e.g. Buckingham et al., 1989; Cavero et al., 1989; Quast & Cook, 1989; Standen et al., 1989), including its actions on the guinea-pig urinary bladder smooth muscle (Fujii et al., 1990; Grant & Zuzack, 1991).

The present findings demonstrate that a similar mechanism occurs in the guinea-pig pyeloureteral system, based on the following results: (a) the inhibitory effect of cromakalim on motility of the renal pelvis and ureter was antagonized by glibenclamide in a manner consistent with competitive antagonism at a common target of action; (b) TEA and 4-AP were much weaker antagonists of the action of cromakalim as compared to glibenclamide (cf. Wilson et al., 1988; Winquist et al., 1989); (c) the antagonism by glibenclamide is selective because the depression of contractility produced by forskolin, a well known activator of adenylate cyclase

Table 3 Effect of glibenclamide (1 μM for 15 min) on action potential and contraction of the guinea-pig ureter

Action potential		*Control*	*After glibenclamide*
Delay	(ms)	172 ± 34	140 ± 30
Amplitude	(mV)	9.61 ± 0.7	9.05 ± 0.7
Duration	(90%, ms)	1431 ± 118	1821 ± 135 *
Afterhyperpolarization	(mV)	0.92 ± 0.14	1.19 ± 0.20
Contraction			
Amplitude	(mN)	4.52 ± 0.53	4.92 ± 0.61*
Duration	(90%, ms)	1953 ± 114	2240 ± 1.92*

Each value is mean ± s.e.mean of 8 determinations.
*Significantly different from control, $P<0.05$.

(Seamon *et al.*, 1981), was unaffected by glibenclamide; (d) the antagonism by glibenclamide of hyperpolarization of the ureter produced by cromakalim was overcome by increasing the concentration of cromakalim.

Interestingly, forskolin was unable to suppress totally the evoked contractions of the ureter, while it effectively abolished spontaneous mechanical activity of the renal pelvis. In contrast, cromakalim abolished spontaneous and evoked activity in the two preparations. Since forskolin reportedly blocks various types of K channels (Cook & Quast, 1990 for review), and K channel blockers (glibenclamide, TEA and 4-AP) enhance evoked ureteral contractions, it may be argued that the relaxant action of forskolin in the ureter could be counteracted by its K channel blocking activity, leading to an incomplete suppression of evoked motility. However, isoprenaline was similarly unable to totally suppress evoked contractions of the ureter under comparable experimental conditions (unpublished observations).

A somewhat unexpected finding of this study was the prolongation of action potential and enhancement of evoked contractility produced by glibenclamide in the guinea-pig ureter. Although quantitatively small, these effects were consistenly observed in all preparations tested. According to previous studies on mechanisms regulating the shape and duration of the action potential of the guinea-pig ureter (Shuba, 1977) the effect of glibenclamide would be consistent with a blockade of K channels. Although glibenclamide, even at high concentrations, does not affect electrical and mechanical activity of smooth muscles in general (e.g. Buckingham *et al.*, 1989, rat aorta and portal vein; Katayama *et al.*, 1993, guinea-pig stomach), previous studies have reported an increase in spontaneous mechanical activity (Fujii *et al.*, 1990) and membrane depolarization (Seki *et al.*, 1992) by 0.5–1 μM glibenclamide in the smooth muscle of the guinea-pig urinary bladder. These findings raise the possibility that glibenclamide-sensitive K channels play a role in determining action potential shape and duration in the guinea-pig ureter.

Blockade of calcium- and voltage-dependent K currents is likely to explain the enhancement of ureteral contractions produced by TEA and 4-AP, respectively. In fact, two major outward currents have been detected in ureter smooth muscle, a calcium-dependent K current which is blocked by TEA and a voltage-dependent A-current which is blocked by 4-AP (Imaizumi *et al.*, 1989; 1990; Lang, 1989). Although glibenclamide is remarkably selective in blocking the ATP-sensitive K channels (Cook & Quast, 1990), a few reports have described a blocking action on certain voltage-gated K currents in neuronal cells (Reeve *et al.*,1992; Crepel *et al.*, 1993) and this may explain the effect of glibenclamide on the action potential and contractility of the guinea-pig ureter.

In conclusion, the present findings demonstrate that cromakalim produces a powerful suppression of spontaneous and evoked contractility of the guinea-pig isolated renal pelvis and ureter, which is sensitive to blockade by glibenclamide. Since the motility of the pyeloureteral system is highly dependent upon activation of voltage-dependent calcium channels, the hyperpolarizing action produced by activation of cromakalim- and glibenclamide-sensitive K channels provides a powerful mechanism for regulating ureteral peristalsis. It is an important topic for future studies to assess whether mediators or transmitters present in the ureter may use this mechanism for producing inhibition of ureteral motility and suppression of latent pacemakers.

References

ARTEMENKO, D.P., BURY, V.A., VLADIMIROVA, I.A. & SHUBA, M.F. (1982). Modification of the single sucrose-gap method. *Physiol. Zhurn.*, **28,** 374–380.

BONEV, A.D. & NELSON, M.T. (1993). ATP-sensitive potassium channels in smooth muscle cells from guinea-pig urinary bladder. *Am. J. Physiol.*, **264,** C1190–C1200.

BRADING, A.F., BURDYGA, TH.V. & SCRIPNYUK, Z.D. (1983). The effects of papaverine on the electrical and mechanical activity of the guinea-pig ureter. *J. Physiol.*, **334,** 79–89.

BUCKINGHAM, R.E., HAMILTON, T.C., HOWLETT, D.R., MOOTOO, S. & WILSON, C. (1989). Inhibition by glibenclamide of the vasorelaxant action of cromakalim in the rat. *Br. J. Pharmacol.*, **97,** 57–64.

CAVERO, I., MONDOT, S. & MESTRE, M. (1989). Vasorelaxant effects of cromakalim in rats are mediated by glibenclamide-sensitive potassium channels. *J. Pharmacol. Exp. Ther.*, **248,** 1261–1268.

COOK, N.S. & QUAST, U. (1990). Potassium channel pharmacology. In *Potassium Channels.* ed. Cook, N.S. pp. 181–258. Chichester, England: Ellis Horwood Series in Pharmaceutical Technology.

CREPEL, V.,, KRNJEVIC, K. & BEN-ARI, Y. (1993). Sulphonylureas reduce the slowly inactivating D-type outward current in rat hippocampal neurons. *J Physiol.*, **466,** 39–54.

CUTHBERT, A.W. (1965). The relation between response and the interval between stimuli of the isolated guinea-pig ureter. *J. Physiol.*, **180,** 225–238.

FOSTER, C.D., FUJII, K., KINGDON, J. & BRADING, A.F. (1989). The effect of cromakalim on the smooth muscle of the guinea-pig urinary bladder. *Br. J. Pharmacol.*, **97,** 281–291.

FUJII, K., FOSTER, C.D., BRADING, A.F. & PAREKH, A.B. (1990). Potassium channel blockers and the effects of cromakalim on the smooth muscle of the guinea-pig bladder. *Br. J. Pharmacol.*, **99,** 779–785.

GRANT, T.L. & ZUZACK, J.S. (1991). Effects of K channel blockers and cromakalim (BRL 34915) on the mechanical activity of guinea-pig detrusor smooth muscle. *J. Pharmacol. Exp. Ther.*, **259,** 1158–1164.

HAMILTON, T.C., WEIR, S.W. & WESTON, A.H. (1986). Comparison of the effects of BRL 34915 and verapamil on electrical and mechanical activity in rat portal vein. *Br. J. Pharmacol.*, **88,** 103–111.

HOYLE, C.H.V. (1987). A modified single sucrose gap – junction potentials and electrotonic potentials in gastrointestinal smooth muscle. *J. Pharmacol. Methods*, **18,** 219–226.

IMAIZUMI, Y., MURAKI, K. & WATANABE, M. (1989). Ionic currents in single smooth muscle cells from the ureter of the guinea-pig. *J. Physiol.*, **411,** 131–159.

IMAIZUMI, Y., MURAKI, K. & WATANABE, M. (1990). Characteristics of transient outward currents in single smooth muscle cells from the ureter of the guinea-pig. *J. Physiol.*, **427,** 301–324.

KATAYAMA, N., HUANG, S.M., TOMITA, T. & BRADING, A.F. (1993). Effects of cromakalim on the electrical slow wave in the circular muscle of the guinea-pig gastric antrum. *Br. J. Pharmacol.*, **109,** 1097–1100.

LANG, R.J. (1989). Identification of the major membrane currents in freshly dispersed single smooth muscle cells of guinea-pig ureter. *J. Physiol.*, **412,** 375–395.

MAGGI, C.A. & GIULIANI, S. (1991). The neurotransmitter role of CGRP in the rat and guinea-pig ureter: effect of a CGRP antagonist and species-related differences in the action of omega conotoxin on CGRP release from primary afferents. *Neuroscience*, **43,** 261–271.

MAGGI, C.A. & GIULIANI, S. (1992). Nonadrenergic noncholinergic excitatory innervation of the guinea-pig isolated renal pelvis: involvement of capsaicin-sensitive primary afferent neurons. *J. Urol.*, **147,** 1394–1397.

OKABE, K., KAJIOKA, S., NAKAO, K., KITAMURA, K., KURIYAMA, H. & WESTON, A.H. (1990). Actions of cromakalim on ionic currents recorded from single smooth muscle cells of the rat portal vein. *J. Pharmacol. Exp. Ther.*, **252,** 832–839.

QUAST, U. (1987). Effect of the K efflux stimulating vasodilator BRL 34915 on ^{86}Rb efflux and spontaneous activity in guinea-pig portal vein. *Br. J. Pharmacol.*, **91,** 569–578.

QUAST, U. & COOK, N.S. (1988). Potent inhibitors of the effects of the K channel opener, BRL 34915 in vascular smooth muscle. *Br. J. Pharmacol.*, **93,** 204P.

QUAST, U. & COOK, N.S. (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,** 261–271.

REEVE, H.L., VAUGHAN, P.F.T. & PEERS, C. (1992). Glibenclamide inhibits a voltage-gated K current in the human neuroblastoma cell line SH-SY5Y. *Neurosci. Lett.*, **135**, 37–40.

SEAMON, K.B., PADGETT, W. & DALY, J.W. (1981). Forskolin: unique diterpene activator of adenylate cyclase in membranes and intact cells. *Proc. Natl. Acad. Sci. U.S.A.*, **78**, 3363–3367.

SEKI, N., KARIM, O.M.A. & MOSTWIN, J.L. (1992). Effect of pinacidil on the membrane electrical activity of guinea-pig detrusor muscle. *J. Pharmacol. Exp. Ther.*, **263**, 816–822.

SHUBA, M.F. (1977). The effect of sodium-free and potassium-free solutions ionic current inhibitors and ouabain on electrophysiological properties of smooth muscle of the guinea-pig ureter. *J. Physiol.*, **264**, 837–851.

STANDEN, N.B., QUAYLE, J.M., DAVIES, N.W, BRAYDEN, J.E., HUANG, Y. & NELSON, M.T. (1989). Hyperpolarizing vasodilators activate ATP-sensitive K channels in arterial smooth muscle. *Science*, **245**, 177–180.

TAKANO, M. & NOMA, A. (1993). The ATP-sensitive K channel. *Prog. Neurobiol.*, **41**, 21–30.

WEIR, S.W. & WESTON, A.H. (1986). The effects of BRL 34915 and nicorandil on electrical and mechanical activity and on 86Rb efflux in rat blood vessels. *Br. J. Pharmacol.*, **88**, 121–128.

WEISS, R.M. (1992). Physiology and pharmacology of renal pelvis and ureter. In *Campbell's Urology*, ed. Walsh, P.C., Retik, A.B., Stamey, T.A. & Vaughan, E.D. Volume I, pp. 113–144. Philadelphia PA: W.B. Saunders Co.

WILSON, C. (1989). Inhibition by sulphonylureas of vasorelaxation induced by K channels activators in vitro. *J. Autonom. Pharmacol.*, **9**, 71–78.

WILSON, C., COLDWELL, M.C., HOWLETT, D.R., COOPER, S.M. & HAMILTON, T.C. (1988). Comparative effects of K channel blockade on the vasorelaxant activity of cromakalim, pinacidil and nicorandil. *Eur. J. Pharmacol.*, **152**, 331–339.

WINQUIST, R.J., HEANEY, L.A., WALLACE, W.A., BASKIN, E.P., STEIN, R.B., GARCIA, M.L. & KACZOROWSKI, G.J. (1989) Glyburide blocks the relaxation response to BRL 34915 (cromakalim) minoxidil sulfate and diazoxide in vascular smooth muscle. *J. Pharmacol. Exp. Ther.*, **248**, 149–156.

ZINI, S., BEN-ARI, Y. & ASHFORD, M.L.J. (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**, 566–573.

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