Br. J. Pharmacol. (1989), **98**, 851–864

Properties of the cromakalim-induced potassium conductance in smooth muscle cells isolated from the rabbit portal vein

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1 Single smooth muscle cells were isolated freshly from the rabbit portal vein and membrane currents were recorded by the whole-cell or excised patch configurations of the patch-clamp technique at room temperature.

2 Cromakalim (Ckm, 10 μM) induced a potassium current (I_{Ckm}) that showed no pronounced voltage-dependence and had low current noise.

3 This current, I_{Ckm}, was inhibited by (in order of potency): phencyclidine > quinidine > 4-aminopyridine > tetraethylammonium ions (TEA). These drugs inhibited the delayed rectifier current, I_{dK}, which is activated by depolarization of the cell, with the same order of potency.

4 Large conductance calcium-activated potassium channels (L_{KCa}) in isolated membrane patches were blocked by (in order of potency) quinidine > TEA $\approx$ phencyclidine. 4-Aminopyridine was ineffective. A similar order of potency was found for block of spontaneous transient outward currents thought to represent bursts of openings of L_{KCa} channels.

5 The low current noise of I_{Ckm} at positive potentials, and its susceptibility to inhibitors indicated that it was not carried by L_{KCa} channels, and that it may be carried by channels which underlie I_{dK}. It was observed that when I_{Ckm} was activated, I_{dK} was reduced. However, in two experiments, I_{Ckm} was much more susceptible to glibenclamide than I_{dK}; possible reasons for this are discussed.

Introduction

Cromakalim (Ckm; BRL 34915) belongs to a group of drugs that appear to have a novel mechanism of action and are presently termed K^+-channel openers (Weston & Abbott, 1987; Cook, 1988). It is active on cardiac muscle (Osterrieder, 1988) and on neurones (Alzheimer & ten Bruggencate, 1988) but appears to be most potent on smooth muscle. Although Ckm has a number of potential clinical applications, emphasis has been placed on its possible use as a peripherally acting anti-hypertensive (Vandenburg *et al.*, 1986), where it seems to act directly on the vascular muscle, independently of the endothelium (Southerton *et al.*, 1987). The decrease in muscle tone has been proposed to result from a hyperpolarization caused by an increase in the K^+-conductance of the smooth muscle cell membrane (Hamilton *et al.*, 1986). However, other mechanisms may contribute to the action of Ckm, because the spontaneous electrical and phasic mechanical activity of smooth muscle (e.g. Weir & Weston, 1986b; Quast, 1987) can be inhibited without hyperpolarization occurring (see also Cook, 1988).

This study has investigated the mechanism underlying the hyperpolarization by evaluating the effects of Ckm on the membrane currents of single rabbit portal vein smooth muscle cells. Ckm was found to increase the membrane K^+-conductance. The voltage-dependence of this conductance was examined and the hypothesis assessed that Ckm acts to increase the open-time of a population of K^+-channels identifiable in these cells by other means. Preliminary findings have been communicated (Beech & Bolton, 1987a,b).

Methods

Cell dispersion

Adult, New Zealand White rabbits (2.5–3 kg) were killed by injection of a lethal dose of sodium pentobarbitone. The main branch of the portal-mesenteric

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vein was removed and dissected free of fat and connective tissue. It was cut into small pieces ($\sim 2 \times 3$ mm) and six were incubated in low-Ca^{2+} (20–30 μM) physiological salt solution (PSS) for 10 min (36°C) and then re-suspended in a mixture of papain (5 mg ml), DL-dithiothreitol (DTT, 3–5 mM) and bovine serum albumin (BSA, 2–4 mg ml^{-1}) in the low-Ca^{2+} solution for 20–30 min at 36°C. Tissue segments were then removed from the mixture and mildly agitated in the low-Ca^{2+} PSS. The isolated cells were centrifuged (100 g for 1.5 min), the pellet re-suspended in PSS containing 0.8 mM $CaCl_2$ and the suspension stored on glass cover-slips at 4°C. Cells were used between 2 and 12 h after isolation.

Current recording and analysis

Recordings were made by using the whole-cell or excised-patch configurations of the patch-clamp technique (Hamill *et al.*, 1981). Patch pipettes were made of borosilicate glass (external diameter 1.4–1.6 mm, internal diameter 0.6–0.8 mm) and had resistances of 1–4 MΩ (whole-cell recording; access resistance = 5–10 MΩ) or 5–10 MΩ (excised-patch recording). A List EPC-7 current-voltage converter was used. Data were recorded on FM-tape (Racal) and were either displayed on a chart recorder (Gould 2400S) or digitized off-line by means of a CED 1401 analogue-to-digital interface (Cambridge Electronic Design), stored on floppy diskettes and then displayed by a graph plotter (7470A Hewlett Packard). Digital records were analysed by a BBC microcomputer in conjunction with the CED 1401. All currents were low-pass filtered at 1 kHz with an 8-pole Bessel filter (Barr & Stroud; attenuation rate = 48 dB/octave). Functions were fitted by the iterative algorithm of Marquardt (1963), with the criterion of minimizing the unweighted sum of the squares of the deviations. Leakage current (whole-cell) was subtracted by an analogue circuit. Twenty mV hyperpolarizing steps (each 0.5 s) were applied 1 s after each depolarizing step and the inward current required was inverted, multiplied by the appropriate factor and subtracted from the current elicited by the depolarizing step.

Solutions and reagents

The PSS used in the bath had the following composition (mM): Na^+ 126, K^+ 6, Ca^{2+} 1.7, Mg^{2+} 1.2, Cl^- 137.8, glucose 14, HEPES 10.5 and was titrated to pH 7.2 with NaOH. When the ionic composition of this solution was altered, the osmolarity was maintained by adjusting the NaCl concentration. Recording pipette (=intracellular) solution had the following composition (mM): K^+ 133.5, Mg^{2+} 1.2, Cl^- 136, EGTA 0.8, glucose 14, HEPES 10.5 and was titrated to pH 7.2 with NaOH. The Ca^{2+} concentration of this solution was estimated to be 1 nM (when 10 nM Ca^{2+} was required 0.07 mM $CaCl_2$ was added). For some experiments GTP ($\sim$0.2 mM) and/or ATP (1 mM) were included in the pipette solution in an attempt to improve the responsiveness of the cells to Ckm. PSS containing 4-aminopyridine (4-AP, >1 mM) was alkaline and was titrated to pH 7.2 with HCl. PSS containing phencyclidine (100 μM) was acidic and was titrated with NaOH to pH 7.2. Experiments were performed at room temperature (20–23°C).

Papain (type 4), DL-dithiothreitol (DTT), bovine serum albumin (BSA), tetraethylammonium chloride (TEA), 4-aminopyridine (4-AP), quinine, procaine, phencyclidine hydrochloride, glibenclamide, guanosine 5'-triphosphate (GTP), adenosine 5'-triphosphate (ATP), ethylene glycol *bis*-(b-aminoethyl ether) N,N,N',N'-tetraacetic acid (EGTA), N-2-hydroxyethylpiperazine-N'-2-ethanesulphonic acid (HEPES) were all obtained from Sigma. Quinidine hydrochloride (BDH) and (±)-6-cyano-3,4-dihydro-2,2 dimethyl-trans-4-(2-oxo-1-pyrollidyl)-2H-benzo[b]pyrano-3-ol (cromakalim, Beecham) were also used. Charybdotoxin from *Leiurus quinquestriatus* venom (for the separation procedure see Castle & Strong, 1986) and apamin were generous gifts from Dr P.N. Strong.

Drugs were bath applied by an infusion-withdrawal system. The bath volume was $\sim$0.5 ml and exchange was judged to be complete within 15–20 s. All responses were assessed after complete equilibration of drugs.

Results

Patch-clamp recording in the 'current-clamp' mode enabled an estimation of the resting membrane potential of single cells which had known ionic gradients, to be made. For the example shown (Figure 1A), the ionic gradients were physiological (see Methods) and the potential fluctuated around −35 mV. Application of Ckm (10 μM) increased the potential to about −70 mV and this was maintained in the presence of Ckm. The response reached a maximum slowly, after about 4–5 min, and a decrease in voltage noise occurred concomitant with the hyperpolarization. Increasing the concentration beyond 10 μM had very little further effect; 1 μM Ckm produced only a small hyperpolarization (2–5 mV).

Voltage-clamp of single cells using low resistance pipettes permitted the recording of current across the cell membrane when the ionic gradients were known. Figure 1B shows current, relative to zero current (i.e.

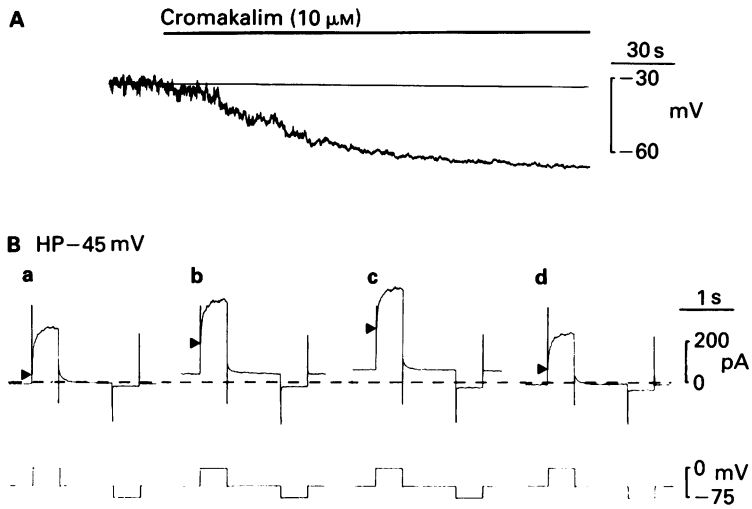

Figure 1 Effects of bath applied cromakalim (Ckm; 10 μM) on membrane potential and currents recorded from rabbit portal vein single smooth muscle cells bathed in physiological salt solution (PSS). (A) Pipette current was maintained constant and membrane potential recorded. Ckm increased the membrane potential by about 35 mV after 4 min. (B) Voltage-clamped cell, where the broken line represents zero holding current. The cell was held at −45 mV and stepped to 0 and −75 mV, each for 0.5 s every 5 s. Records show the control (a), 3 min (b) and 5 min (c) after the application of Ckm, and after repeated washing (d). The outward current induced by Ckm (I_{Ckm}) was associated with an increase in membrane conductance (g_{Ckm}). Quasi-instantaneous current is marked (▶).

when the holding potential (HP) was the resting membrane potential) recorded from a cell clamped at −45 mV and bathed in PSS. The step to 0 mV elicited a capacity current (outward), followed by a quasi-instantaneous current (the difference between the current at the holding potential and the current level to which the capacity current decays before voltage-gated currents develop) and then a time-dependent outward current. The step to −75 mV induced a capacity current (inward) followed by a time-independent inward current. Application of Ckm (10 μM) generated an outward current (I_{Ckm}) with respect to the control holding current which averaged 43.7 ± 5.3 pA (mean ± s.e.mean, $n = 12$; HP −40 mV, K^+-equilibrium potential (E_K) at −78 mV). I_{Ckm} was always associated with an increase in the quasi-instantaneous current, indicating an increased membrane conductance (g_{Ckm}). The fact that the current was outward and was carried by K^+ (see below) suggested that the effect was not due to a decrease in the seal resistance. Figure 1B shows that the current required to clamp the cell at −75 mV was similar for both the control and after equilibration with Ckm, suggesting that I_{Ckm} reversed near this potential, that is, near E_K (at −78 mV). The effect of Ckm on the quasi-instantaneous current reversed slowly after extensive washing (Figure 1Bd).

Reversal potential of I_{Ckm}

To determine the ion selectivity of g_{Ckm} the potential at which I_{Ckm} reversed was estimated. Cells were equilibrated for 10 min after establishing the whole-cell configuration in an attempt to minimize the effect of any slowly shifting junction potential. To find the reversal potential the currents required to step the cell for 100 ms to various potentials more negative inside than the holding potential were measured and plotted against voltage both in the absence of Ckm and after I_{Ckm} had developed fully (see Figure 2a). The voltage at which the two lines intersected gave the reversal potential for I_{Ckm}.

For the cell illustrated in Figure 2a, E_K was at −58 mV and I_{Ckm} reversed close to this at −59 mV (−55 ± 2 mV, $n = 3$). For the cell illustrated in Figure 2b, E_K was at −78 mV and for the plot the control current was subtracted so that only I_{Ckm} is shown. At a holding potential of −50 mV, I_{Ckm} reversed close to E_K at −77 mV (−73 ± 3 mV, $n = 5$), and at a holding potential of +30 mV (1 min was allowed for inactivation of voltage-gated channels: see below) the reversal potential was similar. These results suggested that g_{Ckm} was K^+-selective. The small discrepancy between the mean reversal potential for I_{Ckm} and E_K can probably be attributed to a junction potential, especially since spontaneous

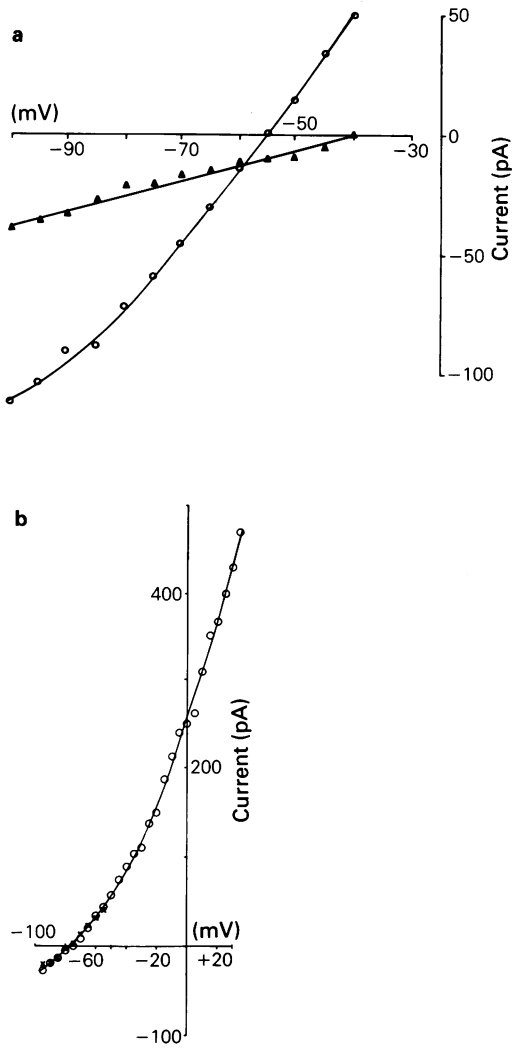

Figure 2 Measurement of the reversal potential for the cromakalim (Ckm)-induced potassium current (I_{Ckm}). Abscissae: transmembrane potential (mV). Ordinate scale: inward (−) and outward (+) transmembrane current. (a) The K^+ equilibrium potential (E_K) was at −58 mV (bath K^+ concentration = 13 mM) and the cell was held at −40 mV. The points represent the currents required to hyperpolarize the cell from the holding potential plotted relative to zero current; control (▲), 10 μM Ckm (○). (b) I_{Ckm} measured in two experiments on one cell which was bathed in PSS (E_K at −78 mV). Two holding potentials were used; −50 mV (×) and +30 mV (○) and each was established for 1 min before the hyperpolarizing steps were applied (method as for a). Current in the absence of Ckm was subtracted from current with 10 μM Ckm present and the line was drawn by eye. Positive current is outward, negative current is inward according to the usual convention.

transient outward currents (see below) were found to reverse at the same potential as I_{Ckm} (Beech, 1988).

The relationship between current and potential (Figure 2b) was represented by a shallow curve indicating that g_{Ckm} showed little voltage-dependence. The curved nature of the voltage/current relationship may reflect the equivalent relationship for the single channel currents underlying the response, as mild outward rectification is commonly observed for single K^+-channels recorded from the excised patch when exposed to an asymmetrical K^+-gradient (e.g. see Benham *et al.*, 1986).

Effects of K^+ channel inhibitors on I_{Ckm}

The effects of inhibitors on I_{Ckm} were determined by applying increasing concentrations of each inhibitor once I_{Ckm} had been established. Inhibition was measured as the change in holding current relative to the control level and to I_{Ckm} in the absence of inhibitor. For these experiments, cells were held at −40 mV to establish the largest driving force without activating I_{dK} (the delayed outward rectifier K^+-current of these cells: see below). Changes in the control holding current induced by the inhibitors were assessed in separate cells ($n = 3$–4): small inward shifts were found for quinidine, 4-AP and TEA and points shown in Figure 7a have been corrected for these effects.

The inhibitory effects of phencyclidine, quinidine, 4-AP and TEA on I_{Ckm} are illustrated in Figures 3 and 7a. Phencyclidine ($n = 3$) blocked I_{Ckm} completely at 100 μM, 50% block occurred at about 4 μM and the effect was fully reversible after wash-out (Figure 3a). Quinidine ($n = 3$) induced complete block at 100 μM and 50% block at about 10 μM (Figure 3b). 4-AP ($n = 4$) at 0.2 mM caused about 50% block but higher concentrations produced further block in some cells, whilst increasing I_{Ckm} in others (Figure 3c). TEA ($n = 5$) produced complete block at 40 mM and 50% block at about 7 mM (Figure 3d). I_{Ckm} was inhibited by quinine (0.1–0.4 mM, $n = 1$) or procaine (5 mM, $n = 2$), but was unaffected by charybdotoxin (100 nM, $n = 3$). The experiments indicated that the inhibitors had the following order of potency against I_{Ckm}; phencyclidine > quinidine = (quinine) > 4-AP > (procaine) > TEA (parentheses indicate when the concentration for 50% block was not determined).

Effects of inhibitors on large conductance Ca^{2+}-activated K^+ (L_{KCa}) channels

L_{KCa} channels are of large unitary conductance and have been characterized for a number of smooth muscle types (Inoue *et al.*, 1985; Benham *et al.*, 1986). In this study, unitary currents were recorded

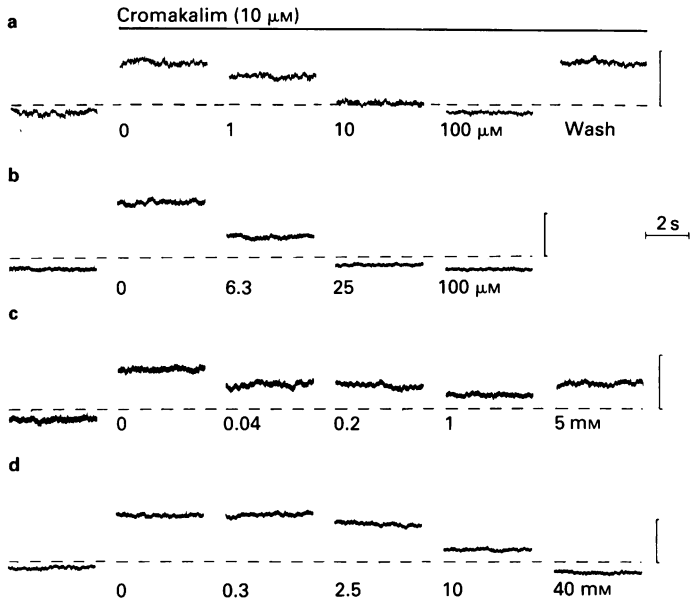

Figure 3 The effects of phencyclidine (a), quinidine (b), 4-aminopyridine (c) or tetraethylammonium (d) on holding current (HP −40 mV) in the presence of 10 μM cromakalim (Ckm). The broken lines represent zero holding current, the records on the left indicate the control current levels and all others are in the presence of 10 μM Ckm. The calibration bars represent 50 pA and upward deflection outward current.

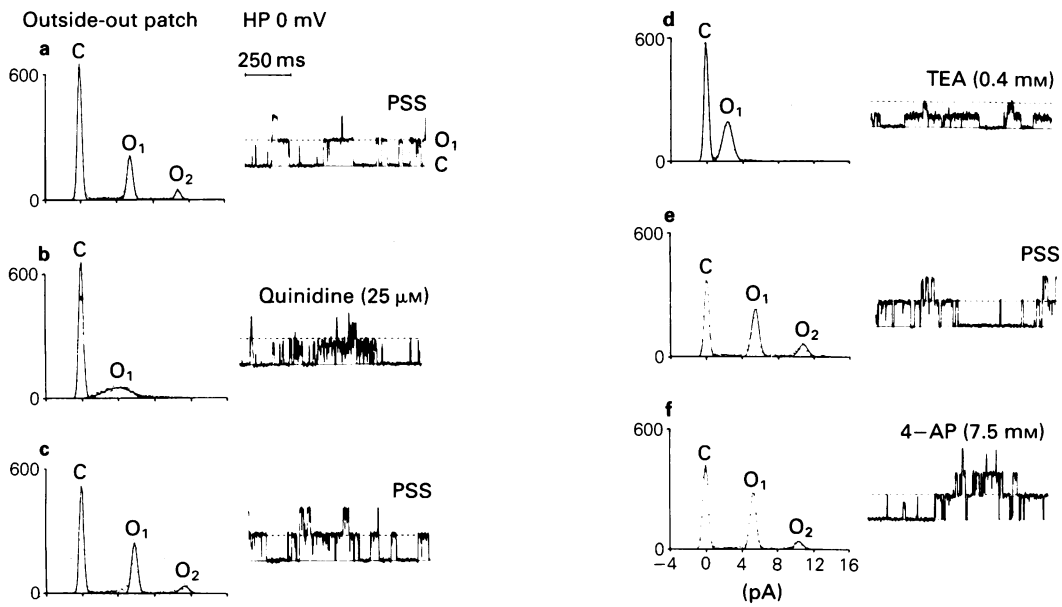

Figure 4 Effects of bath applied quinidine (b), tetraethylammonium (TEA, d) or 4-aminopyridine (4-AP, f) on large conductance Ca^{2+}-activated K^+ (L_{KCa}) channels in outside-out membrane patches exposed to a physiological K^+ gradient. The plots on the left show current amplitude histograms for 4 s of record (~4000 points) fitted by Gaussian functions. Signals were captured at 1 kHz and the bin size was 0.16 pA. Shown on the right are actual records (1 s) for the same experiments, where the continuous line indicates the current level when no channel openings were observed (C) and the broken line the first unitary current level (O_1) in the absence of any drug. PSS indicates the absence/wash-out of drug.

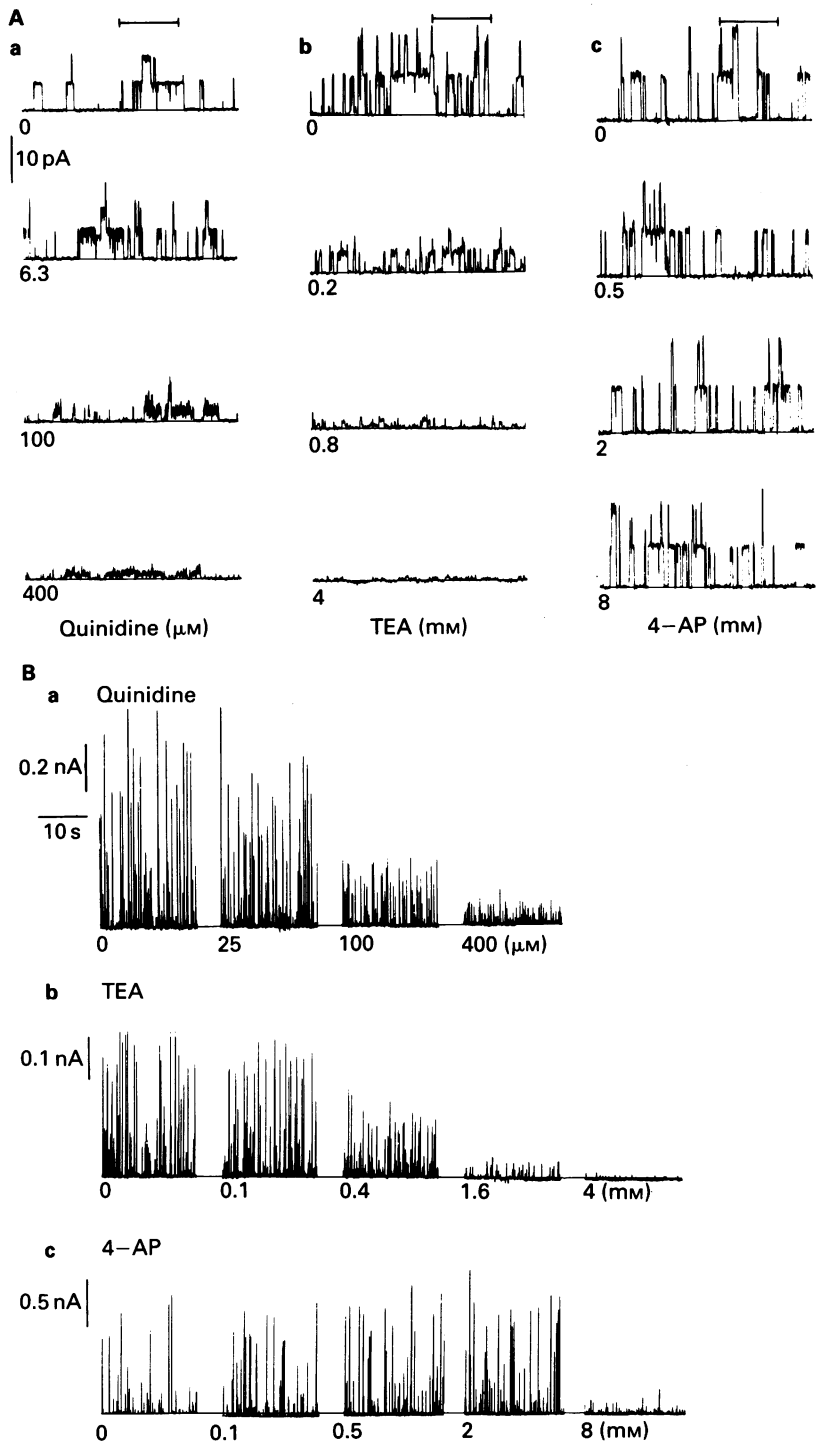

Figure 5 Effects of quinidine (a), tetraethylammonium (TEA, b) or 4-aminopyridine (4-AP, c) on large conductance Ca^{2+}-activated K^+ (L_{Kca}) channels (outside-out patch) and on spontaneous transient outward currents (STOCs) (whole-cell). (A) Unitary currents through L_{KCa} channels (physiological K^+-gradient). Upward deflection indicates outward current and continuous lines mark the current level when no channels were open. The Ca^{2+}-concentration in the pipette was 1 nM (a,c) or 10 nM (b), patches were held at 0 mV (a), +20 mV (b) and +35 mV (c), and the time calibration bars represent 500 ms (a,b) and 125 ms (c). (B) STOCs recorded from single cells bathed in PSS; holding potentials were −10 mV (a,c) and −35 mV (b). Quinidine (a) or TEA (b) each reduced STOCs in a concentration-dependent manner, whilst 4-AP (c) had no effect at concentrations up to 2 mM but caused a marked reduction in STOC amplitude at 8 mM.

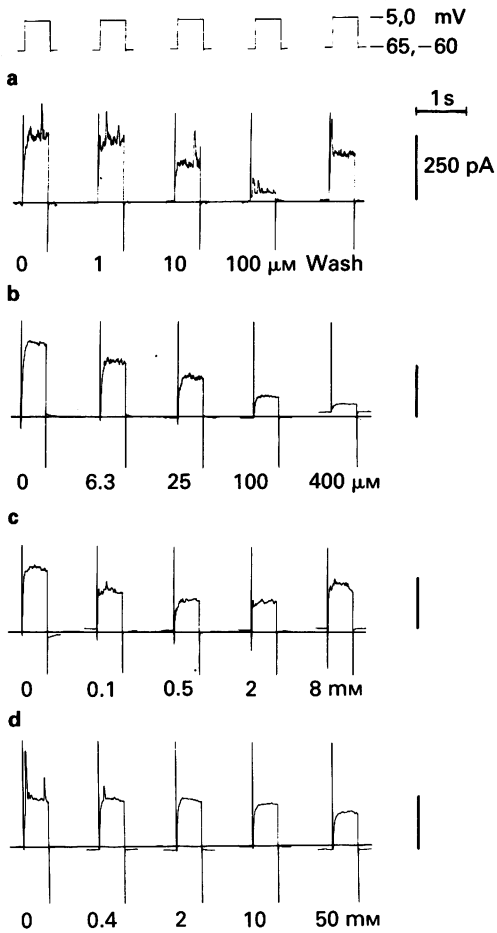

Figure 6 The effects of phencyclidine (a), quinidine (b), 4-aminopyridine (4-AP) (c) or tetraethylammonium (TEA) (d) on whole-cell current (I_{dK}) activated upon depolarization to 0 mV (a; HP −60 mV) or −5 mV (b,c, d; HP −65 mV) for cells bathed in PSS. The steps were of 0.5 s duration and all vertical calibration bars represent 250 pA. Scaled leakage current was subtracted. Control currents (0) are shown on the left, upward deflection indicates outward current and continuous lines mark the control current at the holding potential. The time-dependent outward current measured at the end of the depolarizing step was taken to represent I_{dK}.

through these channels in the excised outside-out patch. As described by Inoue *et al.* (1985), the channels have a conductance of about 100 pS at 0 mV when exposed to a physiological K^+-gradient, are activated upon depolarization and have an opening probability dependent on the internal Ca^{2+}-concentration. It was necessary to buffer Ca^{2+} to < 10 nM to ensure a low probability of opening, for it was common to observe many active L_{KCa} channels (> 10) in each patch. Patches were clamped between −20 and +35 mV so that only 2 to 3 unitary current levels were observed. The effects of K^+ channel inhibitors were assessed by measuring the decrease in unitary current with increasing concentration of inhibitor bath-applied to the outside of the membrane, on the assumption that open-channel block (see Hille, 1984) was the major effect.

Figure 4 shows an assessment of the effects of quinidine (b), TEA (d) or 4-AP (f) on L_{KCa} channels in the outside-out patch. Current amplitude histograms were constructed and each peak was fitted by a Gaussian (Normal) function of the form;

$$f = f_{max}\{\exp((i - a)^2/2\sigma^2)\},$$

where f is the number of points in each bin (the frequency), i the current amplitude, a the mean of the curve and σ^2 the variance about the mean. Quinidine or TEA, but not 4-AP produced a leftward shift of the peak(s) for the open state(s), indicating a decrease in unitary current, and increased σ^2, which describes the noise in the open state. No change in the opening probability of the channels in the presence of any of the inhibitors was indicated. This suggested that the block by TEA or quinidine could be described by a decrease in the mean unitary current and that 4-AP neither decreased the unitary current nor modified the gating of these channels.

Quinidine ($n = 3$) and TEA ($n = 3$–4) each reduced the unitary current in a concentration-dependent manner (Figure 5A), 50% reduction occurring at about 90 and 300 μM respectively (Figure 7b). 4-AP ($n = 3$) (up to 8 mM) bath-applied to the outside-out (Figures 4 and 5Ac) or inside-out patch had no effect when applied to either side of the membrane. Phencyclidine (≤ 10 μM) in one experiment had little effect on the channels but induced some open channel block at 100 μM.

Effects of inhibitors on spontaneous transient outward currents

Spontaneous transient outward currents (STOCs) are thought to result from the opening of K^+ channels activated by a spontaneous, cyclical release of Ca^{2+} from stores within the cell (Benham & Bolton, 1986). To test the postulate that the channels opened were L_{KCa} channels, the effects of K^+ channel inhibitors on STOCs were assessed to enable a comparison to be made with the single L_{KCa} channel data. Effects of STOCs were evaluated by measuring the sum of their amplitudes over a 1 min period. Quinidine ($n = 3$) and TEA ($n = 13$) caused a 50% reduction at about 80 μM and 0.5 mM respectively

(Figure 5Ba,b), but phencyclidine (up to 100 μM) had little effect in 3 experiments (see Figure 6a) suggesting that STOCs were carried by L_{KCa} channels. Complete block of STOCs by 4 mM TEA indicated that the small conductance Ca^{2+}-activated K^+ channels described by Inoue *et al.* (1985) play little role in the generation of STOCs. 4-AP ($n = 4$) at a high concentration (≥ 5 mM) tended to inhibit the STOCs of some cells (Figure 5Bc). However, this seemed to result from an effect on the Ca^{2+}-stores and not from a direct effect on the L_{KCa} channels (Beech, 1988).

Effects of inhibitors on current carried by delayed rectifier K^+ channels (I_{dK})

When the EGTA concentration in the pipette was ≥ 0.8 mM the time- and voltage-dependent outward current (I_{dK}) elicited upon stepping cells to potentials negative of 0 mV has been shown to be carried principally by delayed rectifier-like K^+-channels, which are of small conductance (Beech & Bolton, 1989b). Preliminary experiments (Beech & Bolton, 1987a) indicated that these channels have a pharmacological profile unlike that of the L_{KCa} channels. Experiments were performed in PSS, so both Ca^{2+}- and K^+-currents were expected to be activated upon depolarization. However, the net current was principally I_{dK} at the end of the 0.5 s test pulse, since the Ca^{2+}-current is almost completely inactivated at this time (Ohya *et al.*, 1988). ATP was not included in the pipette solution as it accentuates the slow Ca^{2+}-current component of smooth muscle cells (Ohya *et al.*, 1987). Cl^--current did not interfere since currents were assessed with a test potential near 0 mV, the Cl^--equilibrium potential. The current measured at the end of the 0.5 s pulse was, therefore, taken to represent I_{dK}.

Phencyclidine ($n = 3$) reduced I_{dK} with almost complete block at 100 μM, 50% block at about 30 μM and recovery on wash-out (Figure 6a and Figure 7c). Quinidine ($n = 3$) also reduced I_{dK} with almost complete block at 400 μM (Figure 6b) and 50% inhibition at about 30 μM. TEA ($n = 4$) at 50 mM produced only a 40% reduction of I_{dK} (Figure 6d). In three cells studied 4-AP reduced I_{dK} at concentrations up to 2 mM, inducing 50% block at about 0.2 mM (Figures 6c and 7c). Figure 6c shows an increase in transient net inward current in the presence of 4-AP, but this probably reflects a net effect due to reduced outward current. However, high concentrations of 4-AP (≥ 5 mM) tended to augment both outward and inward currents. Charybdotoxin (100 nM) (Beech & Bolton, 1989b) or apamin (100 nM) had no effect on I_{dK}. Thus, I_{dK} and I_{Ckm} showed the same rank order of susceptibility to the four inhibitors, phencyclidine

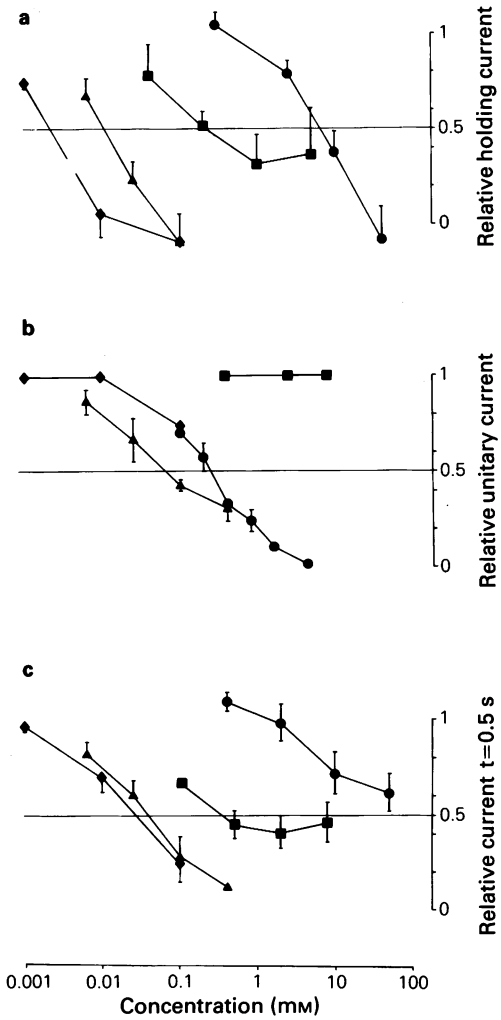

Figure 7 Pharmacological profiles for whole-cell cromakalim-induced potassium current (I_{Ckm}, a), the large conductance Ca^{2+}-activated K^+ (L_{KCa}) single channel current (b) and for whole-cell delayed rectifier current (I_{dK}, c). The points represent the means of 3 to 5 experiments where standard error bars are drawn. The inhibitors: phencyclidine (◆), quinidine (▲), 4-aminopyridine (■) or tetraethylammonium (●) were bath applied and their concentration plotted on a $\log_{10}$ scale. (a) I_{Ckm}, the ordinate scale represents the holding current, where 1 is I_{Ckm} and 0 the current level before the application of Ckm. (b) L_{KCa} single channel currents. The ordinate scale represents unitary current where 1 is the current through the fully open channel and 0 that through the fully closed channel without drug. (c) I_{dK}, the ordinate scale represents scaled leakage subtracted outward current elicited upon stepping to 0 mV, where 1 is the current before drug application and 0 the current at the holding potential (−60 or −65 mV).

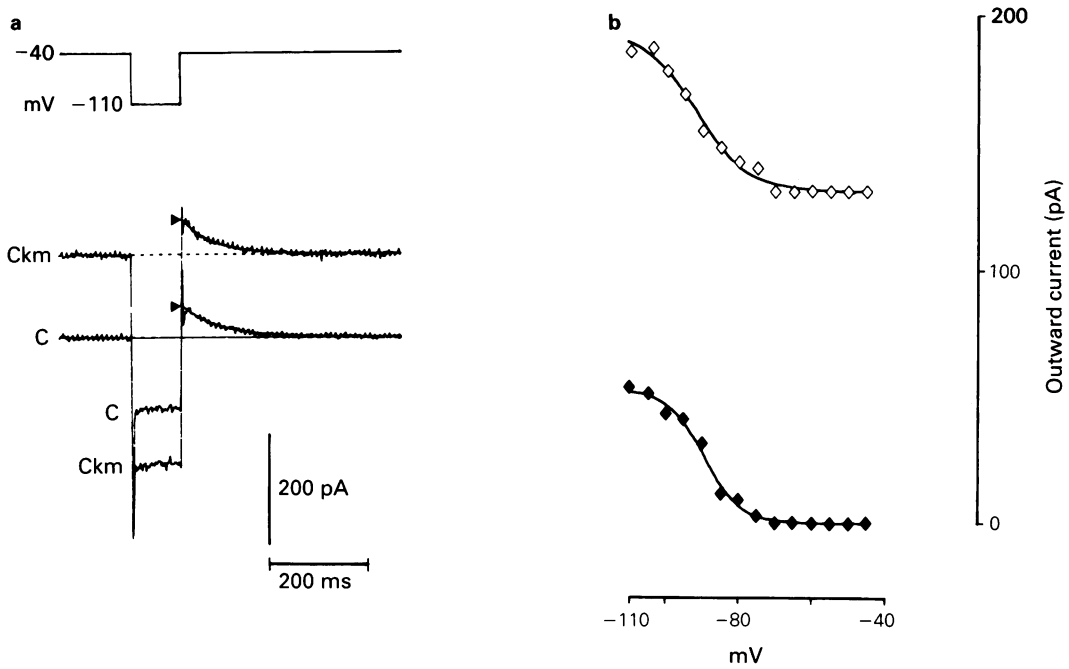

Figure 8 The lack of effect of 10 μM cromakalim (Ckm) on the transient voltage-dependent outward current (I_{fo}). (a) Current recorded from a cell bathed in PSS, showing the effect of a 100 ms step to −110 mV from the HP of −40 mV; for the control (C) and during the maximum response to Ckm. Records are digital (2 KHz). The horizontal lines (solid for C, broken for Ckm) mark the current level for the −40 mV HP before the step was applied (zero current in b is measured from the solid line). The distance between each of these lines and the appropriate, arrowed current level gives the amplitude of I_{fo} at time zero for the step back to −40 mV. The time-dependent decay of I_{fo} was fitted by a single exponential (shown). (b) The same cell, the inactivation curve for I_{fo} was plotted for 100 ms hyperpolarizing steps from −40 mV; for the control (C; ◆) and in the presence of Ckm (◇). Points were fitted by a Boltzmann equation (see text), indicating that I_{fo} was 50% inactivated at −87.3 mV for the control and at −92 mV in the presence of Ckm.

> quinidine > 4-AP > TEA, and neither were susceptible to block by apamin or charybdotoxin at concentrations which block other potassium currents.

Lack of effect of cromakalim on the transient, voltage-dependent outward current

The transient voltage-dependent outward current (I_{fo}; Beech, 1988; Beech & Bolton, 1988, 1989a) is an outward current, carried principally by K^+, that has fast kinetics and is activated upon depolarization from very negative HPs, usually negative to −50 mV. It closely resembles the A-current of neuronal cells (reviewed by Rogawski, 1985), which is thought to regulate spike frequency. The effect of Ckm on I_{fo} was investigated because preliminary experiments have suggested that I_{fo} shows a similar susceptibility to K^+-channel inhibitors as does I_{dK} (and I_{Ckm}). I_{fo} was blocked by bath applied phencyclidine or 4-AP but not by TEA, apamin (100 nM) or charybdotoxin (100 nM) (Beech & Bolton, 1989a). In addition, if Ckm changed the voltage-dependence of I_{fo} this might change spike frequency in the whole smooth muscle tissue, perhaps explaining why Ckm can sometimes inhibit spontaneous spike activity at concentrations that produce no appreciable hyperpolarization (see Cook, 1988).

Ckm (10 μM) in 3 experiments had little effect on the amplitude, kinetics or inactivation curve of I_{fo} (Figure 8). In addition, I_{Ckm} was 2.5 × larger than I_{fo} at −40 mV. Thus, although I_{fo} was probably not maximally activated upon stepping to −40 mV it was reasonable to presume that if I_{Ckm} was carried by the same channels then a reduction in the amplitude of I_{fo} would occur, but in fact it was slightly increased. It should be noted that the inactivation

curve for I_{f_0} is not in its steady-state position on the voltage axis as the hyperpolarizing steps used were short (100 ms). When the channels were given sufficient time for steady-state to be achieved at each potential the current was 50% available at about −78 mV (Beech & Bolton, 1989a).

Effect of cromakalim on I_{dK}

Because I_{dK} and I_{Ckm} had a similar susceptibility to a number of blocking agents, it seemed possible that Ckm may act on I_{dK} or a component of it. A depolarizing pulse from a negative holding potential caused activation of I_{dK} which increased to a peak and then declined; if the pulse was sufficiently long (several seconds) I_{dK} was almost completely inactivated. Holding for 20 s at 0 mV for example inactivated I_{dK} almost completely leaving only 150 pA of non-inactivating current as shown in Figure 9b (●) where the points were fitted by a curve described by a Boltzmann equation.

$$I = (I_{max} - I_{min})/\{1 + \exp((V - V_h)/V_s)\} + I_{min},$$

where V_h is the potential at which half of the channels were available for activation, V_s the slope factor, I the current available and V the conditioning voltage. Examples of actual records from two conditioning potentials are shown in Figure 9a. Ckm did not induce any appreciable shift in the threshold for activation of I_{dK}.

The application of cromakalim produced outward current at the conditioning potential if this was positive to E_K (Figure 9a, the −90 mV conditioning potential is close to E_K and little I_{Ckm} is apparent). Stepping to the test potential (+40 mV) in the presence of cromakalim produced a peak current which was a little larger than in its absence if the conditioning potential was −90 mV (Figure 9a and b), but if the conditioning potential was 0 mV then peak outward current was substantially increased (by about 150 pA in Figure 9a,b). Measurements were made at the peak of the outward current shortly after the beginning of the test pulse (actually the outward current record is almost flat during the test pulse upon stepping from 0 mV in Figure 9a). At the end of the test pulse (4 s) cromakalim also increased outward current more if the conditioning potential was 0 mV rather than −90 mV (Figure 9a), although the effect of varying the conditioning potential was less (Figure 9b). The increase in quasi-instantaneous current produced by Ckm was similar in size to the effect of Ckm on outward current, measured either at peak or the end of the pulse, if I_{dK} was inactivated by holding for 20 s at 0 mV before the test pulse (Figure 9). These results show that when Ckm activated outward current, I_{dK} was reduced and that when I_{dK}

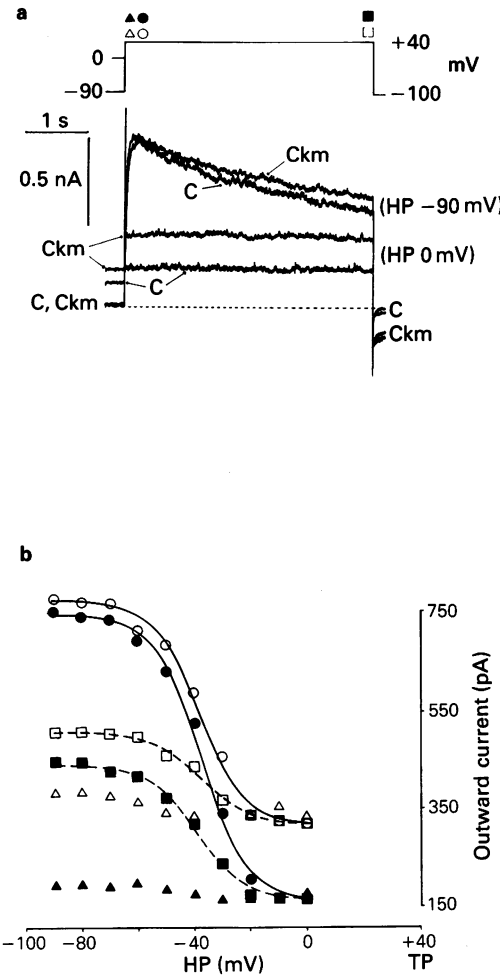

Figure 9 The effect of cromakalim (Ckm, 10 μM) on the whole cell delayed rectifier current (I_{dK}). Currents were recorded from cells bathed in PSS. (a) Effects of two different 20 s conditioning voltage steps (from −100 mV to −90 mV and from −100 mV to 0 mV) on current elicited upon stepping to +40 mV for 4 s, for the control (C) and in the presence of Ckm. The illustrated traces commence shortly before the step to +40 mV. The broken line represents the current level at the −100 mV HP (current above this line being referred to as outward, see b). Leakage and capacity currents were not subtracted. Records are digital (0.7 kHz). (b) The same experiment (HP −100 mV), outward current at +40 mV (see above) plotted against conditioning voltage. Quasi-instantaneous current at time ≈ 3 ms (control, ▲; Ckm, △), peak current (control, ●; Ckm, ○) and that after 4 s (control, ■; Ckm, □) are shown (see a). Points were fitted by the Boltzmann equation (see text). V_h values were −37 and −38 mV, and V_s 8.1 and 8.3 mV for peak current, before and in the presence of Ckm, respectively.

was inactivated by holding at 0 mV, Ckm had a larger effect. This type of result would be expected if Ckm and depolarization acted on the same population of K-channels. Thus, if Ckm has already opened a proportion of these channels, a smaller proportion will be available to be opened by a depolarizing step.

Figure 9a is of additional interest as steps to +40 mV have been shown to activate L_{KCa} channels (Beech & Bolton, 1989b). These channels do not inactivate and they contribute the major noise component because their unitary conductance is large. The unitary current amplitude (i) and probability of opening (p) of a channel population are related to the mean macroscopic current (I) and its variance (σ^2) by the relationship, $\sigma^2 = i(1 - p)I$ (see Hille, 1984). Thus if the probability of opening is low the effect of increasing i is to increase noise (σ^2). However, for the experiment shown (Figure 9), Ckm decreased the variance about a single exponential fitted to the current decay at +40 mV from about 4.5× that about the mean at −90 mV to about 4×. This suggested that Ckm had not increased the opening probability of L_{KCa} channels in this experiment. It is unlikely that Ckm blocks the channels underlying I_{dK} because the total outward current elicited upon depolarization (i.e. including leakage current) was never observed to be less in the presence of Ckm (10–100 μM) than it was in the control.

Discussion

These results suggest that cromakalim induced opening of a population of channels, which allowed a potassium current to flow in these smooth muscle cells; normally this would cause hyperpolarization of the cells. The current, I_{Ckm}, showed no pronounced voltage-dependence and was highly selective for K^+. The sensitivity of this current to various inhibitors was very similar to that of the Ckm-induced $^{86}Rb^+$/$^{42}K^+$-efflux (Quast, 1987; Coldwell & Howlett, 1987; Weir & Strong, 1988; Quast & Cook, 1988) or relaxation (Allen *et al.*, 1986; Wilson, 1987) demonstrated in smooth muscle tissues, suggesting that I_{Ckm} and Ckm-induced $^{86}Rb/^{42}K$ efflux and relaxation have a common underlying mechanism.

Several types of K^+-channel have been proposed to exist in smooth muscle but certain of these channels do not appear to be involved in the generation of I_{Ckm}. Firstly, it seems reasonable to exclude an apamin-sensitive K^+-channel in the action of Ckm (Weir & Weston, 1986a; Allen *et al.*, 1986; Coldwell & Howlett, 1987). Secondly, as phencyclidine did not affect the resting K^+-conductance in the present experiments, the K^+-channel type which underlies this would seem not to generate I_{Ckm}. The channels underlying the M-current (Sims *et al.*, 1985) are unlikely to be involved as they are not inhibited by 4-AP (Brown & Adams, 1980). The single voltage-independent K^+-channel (K_M) described by Inoue *et al.* (1986) was shown to close if extracellular Ca^{2+} was reduced to <10 μM, an effect opposite to that obtained for the Ckm-induced $^{86}Rb^+$-efflux (Howlett & Coldwell, 1987). Although there are insufficient data available to assess the possible role of the slow, potential sensitive K^+-channels (Benham & Bolton, 1983) or the small conductance Ca^{2+}-activated K^+ channels (Inoue *et al.*, 1985) of smooth muscle cells, preliminary experiments with excised inside- or outside-out patches have not revealed such channels to be opened by Ckm (unpublished observation, Beech & Bolton). Ckm was found not to affect I_{fo}, which is a transient and rapidly inactivating potassium current available only at strongly negative potentials (Figure 8).

A number of studies have indicated that Ckm increases the open-time of the large conductance of Ca^{2+}-activated K^+-channels (L_{KCa}): Kusano *et al.* (1987) observed an increase in open-time for on-cell patch recordings; Gelband *et al.* (1988) a decrease in the time constant of the long closed state for Ckm (0.05 μM) applied to the outside of the membrane; and Trieschmann *et al.* (1988) an increase in opening probability resulting from a decrease in the closed time for Ckm (1 μM) applied to the inside of the membrane. The present experiments do not support a significant action on L_{KCa} channels, principally on the basis of the susceptibility of I_{Ckm} to inhibitors and the low noise of I_{Ckm} which positively excludes these large conductance (about 100 pS) channels. Large conductance Ca^{2+}-activated K^+-channels, and spontaneous transient outward currents (STOCs) showed a different inhibitor sensitivity to I_{Ckm}, in particular, they were insensitive to 4-AP, much more sensitive to TEA and blocked by charybdotoxin (Beech *et al.*, 1987; Kusano *et al.*, 1987). Ckm-induced $^{86}Rb^+$-efflux (Weir & Strong, 1988) and I_{Ckm} were not.

A possible explanation for the current evoked by Ckm is that this drug short-circuits the voltage-gate of delayed rectifier K^+-channels which carry I_{dK}, so that an increased open-time is maintained independent of the membrane potential or the state of the channels. Thus, Ckm may open the delayed rectifier K^+-channels from either their resting or inactivated state, as I_{Ckm} could be induced at all potentials examined (−100 to +40 mV). The evidence for this came from experiments which showed that I_{dK} was reduced when I_{Ckm} was activated. I_{Ckm} also showed a similar sensitivity to that of I_{dK} to several inhibitors. If I_{Ckm} has some explanation other than the above, then the time-dependent change in the magnitude of

I_{Ckm} when I_{dK} is evoked by a depolarizing pulse needs to be explained. Other substances have been described which act in a way analogous to that suggested for Ckm: maitotoxin induces a sustained inward Ca^{2+}-current and a concomitant decrease in the normal voltage-activated Ca^{2+}-current (Kobayashi *et al.*, 1987), and veratridine holds Na^+-channels in an open-state (transition only from the normal open-state) such that the voltage-activated Na^+-current is reduced (Sutro, 1986).

Although the rank order of potencies of various inhibitors were the same for I_{Ckm} and I_{dK}, there were minor differences in potency of individual inhibitors on I_{Ckm} and on I_{dK} which could be attributed to the method of analysis. For example, tachyphylaxis of the Ckm-induced response, a decrease in the seal resistance (not compensated for by the control experiments) or a voltage-dependence of the block could have contributed to making I_{Ckm} appear more sensitive to inhibition than I_{dK}. There was, however, a marked discrepancy when comparing the sensitivities of the currents to glibenclamide in two experiments, I_{Ckm} being much more strongly affected (Figure 10). This observation requires further investigation. The effects of glibenclamide may indicate that Ckm channels are similar to the ATP-regulated K^+-channels of cardiac cells (Noma, 1983) or pancreatic β-cells (Cook & Hales, 1984), which are also inhibited by this substance (Sturgess *et al.*, 1985), albeit at a lower concentration (e.g. see Fosset *et al.*, 1988).

However, we and others have not identified ATP-regulated K^+-channels in smooth muscle cells from portal vein or small intestine (Ohya *et al.*, 1987; Beech, 1988), although they have been claimed to be present in mesenteric artery (Davies *et al.*, 1989). The current-voltage relationship of I_{Ckm} was unaffected by 1 mM ATP intracellularly (Beech & Bolton, unpublished) and, moreover, ATP regulated K^+-channels rectify inwardly whereas I_{Ckm} rectifies outwardly. Several other observations are difficult to reconcile with the idea that ATP-regulated K^+-channels underlie I_{Ckm}. The channels described by Davies *et al.* (1989) had a conductance of 135 pS which is the same as that of L_{KCa} channels and 2–3 times larger than that of any other ATP-sensitive channel (Ashcroft, 1988). Any current through these 135 pS channels is likely to be extremely noisy; for example, in Figure 10 the extra 500 pA of current produced by Ckm would represent the opening (on average) of 37 channels each carrying 13.5 pA. Random fluctuations in the number of open channels would produce a conspicuously noisy current. However, we found that Ckm reduced current noise (Figure 9) rather than increased it, which suggests that the unitary current carried by channels opened by Ckm might be small. I_{dK} channels have a conductance of about 5 pS (Beech & Bolton, 1989b). In contrast, the current carried by L_{KCa} channels in single portal vein cells is extremely noisy at positive potentials and this current, and the current noise, is drastically reduced by TEA or charybdotoxin (Beech & Bolton, 1989b). However, glibenclamide had no appreciable effect on current noise (Figure 10) which argues strongly against the idea that a large conductance channel similar in size to the L_{KCa} channel underlies the Ckm-induced current. Also, Ashford *et al.* (1988) have shown that Ckm has no effect on

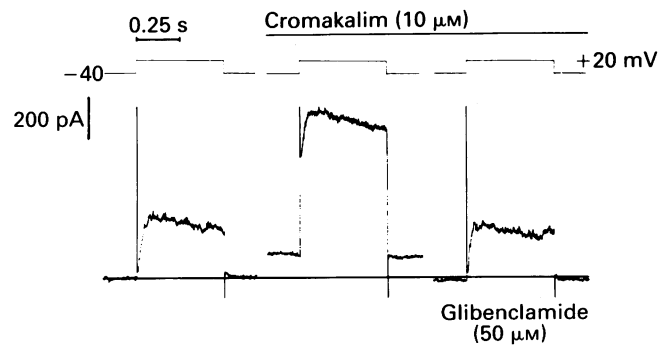

Figure 10 Effect of glibenclamide (50 μM) on the outward current to cromakalim (10 μM). Potential dependent outward current was elicited by stepping from the holding potential of −40 mV to +20 mV. Cromakalim was applied and evoked an outward current (upward displacement of holding current) and outward current was much larger upon stepping to +20 mV. Glibenclamide reversed the effects of cromakalim with only a small effect on the potential-dependent component of current evoked by the +20 mV test step; this current is largely the delayed rectifier current (I_{dK}).

single, diazoxide-activatable, ATP-regulated K^+-channels from an insulin-secreting cell-line. Thus it is more likely that block of I_{Ckm} by glibenclamide results from competition at the Ckm-binding site rather than the blockade of the channel carrying the current.

D.J.B. was supported by the Wellcome Trust and the work by the MRC. We thank Dr J.S. Denbigh for writing the data analysis programmes, Dr P.N. Strong for the generous gifts of the toxins and Beecham Pharmaceuticals for the cromakalim.

References

ALLEN, S.L., BOYLE, J.P., CORTICO, J., FOSTER, R.W., MORGAN, G.P. & SMALL, R.C. (1986). Electrical and mechanical effects of BRL 34915 in guinea-pig isolated trachealis. *Br. J. Pharmacol.*, **89**, 395–405.

ALZHEIMER, C. & TEN BRUGGENCATE, G. (1988). Actions of BRL 34915 (cromakalim) upon convulsive discharges in the guinea-pig hippocampal slices. *Naunyn-Schmiedebergs Arch. Pharmacol.*, **337**, 429–434.

ASHCROFT, F.M. (1988). Adenosine 5'-triphosphate-sensitive potassium channels. *Ann. Rev. Neurosci.*, **11**, 97–118.

ASHFORD, M.L.J., HALES, C. & KOZLOWSKI, R.Z. (1988). Diazoxide, but not BRL 34915, activates ATP-sensitive potassium channels in a rat insulinoma cell line. *J. Physiol.*, **409**, 53P.

BEECH, D.J. (1988). Pharmacology of voltage-dependent ion channels in rabbit vascular smooth muscle. *Ph.D. Thesis, University of London.*

BEECH, D.J. & BOLTON, T.B. (1987a). The effects of tetraethylammonium ions, 4-aminopyridine or quinidine on K^+-currents in single smooth muscle cells of the rabbit portal vein. *Biomed. Biochim. Acta.*, **46**, S673–676.

BEECH, D.J. & BOLTON, T.B. (1987b). Effects of BRL 34915 on membrane currents recorded from single smooth muscle cells from the rabbit portal vein. *Br. J. Pharmacol.*, **92**, 550P.

BEECH, D.J. & BOLTON, T.B. (1988). A transient, voltage-dependent potassium current recorded from single smooth muscle cells of the rabbit portal vein. *Pflügers Archiv.*, **411**, R200.

BEECH, D.J. & BOLTON, T.B. (1989a). A voltage-dependent outward current with fast kinetics in single smooth cells isolated from rabbit portal vein. *J. Physiol.*, **412**, 397–414.

BEECH, D.J. & BOLTON, T.B. (1989b). Two components of potassium current activated by depolarization of single smooth muscle cells from the rabbit portal vein. *J. Physiol.*, (in press).

BEECH, D.J., BOLTON, T.B., CASTLE, N.A. & STRONG, P.N. (1987). Characterization of a toxin from scorpion (Leiurus quinquestriatus) venom that blocks in vitro both large (BK) K^+-channels in rabbit vascular smooth muscle and intermediate (IK) conductance Ca^{2+} channels in human red cells. *J. Physiol.*, **387**, 32P.

BENHAM, C.D. & BOLTON, T.B. (1983). Patch-clamp studies of slow potential-sensitive potassium channels in longitudinal smooth muscle cells of rabbit jejunum. *J. Physiol.*, **340**, 469–486.

BENHAM, C.D. & BOLTON, T.B. (1986). Spontaneous transient outward currents in single visceral and vascular smooth muscle cells of rabbit. *J. Physiol.*, **381**, 385–406.

BENHAM, C.D., BOLTON, T.B., LANG, R.J. & TAKEWAKI, T. (1986). Calcium-activated K-channels in single dispersed smooth muscle cells of rabbit jejunum and guinea-pig mesenteric artery. *J. Physiol.*, **371**, 45–67.

BROWN, D.A. & ADAMS, P.R. (1980). Muscarinic suppression of a novel voltage-sensitive K^+ current in a vertebrate neurone. *Nature*, **283**, 673–676.

CASTLE, N.A. & STRONG, P. (1986). Identification of two toxins from scorpion (Leiurus quinquestriatus) venom which block distinct classes of calcium-activated potassium channel. *FEBS*, **209**, 117–121.

COLDWELL, M.C. & HOWLETT, D.R. (1987). Specificity of action of the novel antihypertensive agent, BRL 34915, as a potassium channel activator: comparison with nicorandil. *Biochem. Pharmacol.*, **36**, 3663–3669.

COOK, D.L. & HALES, N. (1984). Intracellular ATP directly blocks K^+ channels in pancreatic B-cells. *Nature*, **311**, 271–273.

COOK, N.S. (1988). The pharmacology of potassium channels and their therapeutic potential. *Trends Pharmacol. Sci.*, **9**, 21–28.

DAVIES, N.W., NELSON, M., QUAYLE, J.M., STANDEN, N.B. & YU, H. (1989). ATP-sensitive potassium channels recorded from isolated arterial smooth muscle cells of rabbit and rat. *J. Physiol. Commun.* (in press).

FOSSET, M., DE WEILLE, J.R., GREEN, R.D., SCHMID-ANTOMARCHI, H. & LAZDUNSKI, M. (1988). Antidiabetic sulfonylureas control action potential properties in heart cells via high affinity receptors that are linked to ATP-dependent K^+ channels. *J. Biol. Chem.*, **263**, 7933–7936.

GELBAND, C.D., LODGE, N.J., TALVENHEIMO, J.A. & VAN BREEMEN, C. (1988). BRL 34915 increases P_{open} of the large conductance Ca^{2+} activated K^+ channel isolated from rabbit aorta in planar lipid bilayers. *Biophys. J.*, **53**, 149a.

HAMILL, O.P., MARTY, A., NEHER, E., SAKMANN, B. & SIGWORTH, F.J. (1981). Improved patch-clamp techniques for high-resolution current recording from cells and cell-free membrane patches. *Pflügers Archiv.*, **391**, 85–100.

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.

HILLE, B. (1984). *Ionic Channels of Excitable Membranes.* Sunderland, Massachusetts: Sinauer Associates Inc.

HOWLETT, D.R. & COLDWELL, M.C. (1987). BRL 34915 induced potassium channel activation: dependence on calcium ions for closing. *Br. J. Pharmacol.*, **91**, 400P.

INOUE, R., KITAMURA, K. & KURIYAMA, H. (1985). Two Ca-dependent K-channels classified by the application of tetraethylammonium distribute to smooth muscle membranes of the rabbit portal vein. *Pflügers Archiv.*, **405**, 173–179.

INOUE, R., OKABE, K., KITAMURA, K. & KURIYAMA, H. (1986). A newly identified Ca^{2+} dependent K^+ channel in the smooth muscle membrane of single cells dispersed from the rabbit portal vein. *Pflügers Archiv.*, **406**, 138–143.

KOBAYASHI, M., OCHI, R. & OHIZUMI, Y. (1987). Maitotoxin-activated single calcium channels in guinea-pig cardiac cells. *Br. J. Pharmacol.*, **92**, 665–671.

KUSANO, K., BARROS, F., KATZ, M., GARCIA, G., KACZOROWSKI, J.P. & REUBEN, J.P. (1987). Modulation of K channel activity in aortic smooth muscle by BRL 34915 and a scorpion toxin. *Biophys. J.*, **51**, 54a.

MARQUARDT, D.W. (1963). An algorithm for least-squares estimation of non-linear parameters. *J. Soc. Industrial Applied Math.*, **11**, 431–441.

NOMA, A. (1983). ATP-regulated K^+ channels in cardiac muscle. *Nature*, **305**, 147–148.

OHYA, Y., KITAMURA, K. & KURIYAMA, H. (1987). Modulation of ionic currents in smooth muscle balls of the rabbit intestine by intracellularly perfused ATP and cyclic AMP. *Pflügers Archiv.*, **408**, 465–473.

OHYA, Y., KITAMURA, K., KURIYAMA, H. (1988). Regulation of calcium current by intracellular calcium in smooth muscle cells of rabbit portal vein. *Circ. Res.*, **62**, 375–383.

OSTERRIEDER, W. (1988). Modification of K^+ conductance of heart cell membrane by BRL 34915. *Naunyn-Schmiedebergs Arch. Pharmacol.*, **337**, 93–97.

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.

ROGAWSKI, M.A. (1985). The A-current: how ubiquitous a feature of excitable cells is it? *Trends Neurosci.*, **8**, 214–219.

SIMS, S.M., SINGER, J.J. & WALSH, J.V. (1985). Cholinergic agonists suppress a potassium current in freshly dissociated smooth muscle cells of the toad. *J. Physiol.*, **367**, 503–529.

SOUTHERTON, J.S., TAYLOR, S.G. & WESTON, A.H. (1987). Comparison of the effects of BRL 34915 and of acetylcholine-liberated EDRF on rat aorta. *J. Physiol.*, **382**, 50P.

STURGESS, N.C., ASHFORD, M., COOK, D.L. & HALES, C.N. (1985). The sulphonylurea receptor may be an ATP-sensitive potassium channel. *Lancet*, 474–475.

SUTRO, J.B. (1986). Kinetics of veratridine action on Na channels of skeletal muscle. *J. Gen. Physiol.*, **87**, 1–24.

TRIESCHMANN, U., PICHLMAIER, M., KLÖCKNER, U. & ISENBERG, G. (1988). Vasorelaxation due to K-agonists. Single channel recordings from isolated human vascular myocytes. *Pflügers Archiv.*, **411**, R199.

VANDENBURG, M.J., WOODWARD, S.A., HOSSEIN, M., STEWART-LONG, P. & TASKER, T.C.G. (1986). Potassium channel activators lower blood pressure – an initial study of BRL 34915 in hypertensive patients. *J. Hypertension*, **4**, (*Suppl 6*), S166–S167.

WEIR, S.W. & STRONG, P.N. (1988). Inhibition of BRL 34915-stimulated $^{86}Rb^+$ efflux in rabbit aorta by fractionated *Leiurus quinquestriatus hebraeus* scorpion venom. *Br. J. Pharmacol.*, **93**, 202P.

WEIR, S.W. & WESTON, A.H. (1986a). Effect of apamin on responses to BRL 34915, nicorandil and other relaxants in the guinea-pig taenia caeci. *Br. J. Pharmacol.*, **88**, 113–120.

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

WESTON, A.H. & ABBOTT, A. (1987). New class of antihypertensive acts by opening K^+ channels. *Trends Pharmacol. Sci.*, **8**, 283–284.

WILSON, C. (1987). Antagonism of the vasorelaxant activity of BRL 34915 by K^+ channel blockers. *Br. J. Pharmacol.*, **91**, 401P.

(Received May 17, 1988
Revised May 11, 1989
Accepted June 5, 1989)