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TWO COMPONENTS OF POTASSIUM CURRENT ACTIVATED BY DEPOLARIZATION OF SINGLE SMOOTH MUSCLE CELLS FROM THE RABBIT PORTAL VEIN

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SUMMARY

1. Using the patch-clamp technique at 20–23 °C membrane currents were recorded from single smooth muscle cells enzymatically isolated from the rabbit portal vein. Single-channel currents were observed in outside-out patches excised from these.

2. Outward current elicited upon depolarization from −70 mV was not activated as a result of Ca^{2+} influx. It could be divided into two components: an inactivating, 4-aminopyridine- and phencyclidine-sensitive low-noise current (I_{dK}), and a non-inactivating, tetraethylammonium (TEA)- and charybdotoxin-sensitive high-noise current (I_{cK}).

3. I_{dK} activated with a threshold around −40 mV and was carried by K^+. It was substantially inhibited by 4-aminopyridine (5 mM) or phencyclidine (0·1 mM) but was insensitive to TEA^+ (4 mM), charybdotoxin (0·1 μM) or apamin (0·1 μM). Upon stepping to 0 mV it reached a maximum within about 0·2 s. The time course of its activation could be described by a fourth-order single exponential; the time constants of these exponentials changed e-fold every 56 mV. It inactivated in a time- and voltage-dependent manner with a fast and slow component, and was about 50% available at −30 mV. From single-channel recordings in isolated patches single channels underlying this current have a small unitary conductance (around 5 pS).

4. I_{cK} did not inactivate significantly over 6 s. It activated with a less negative threshold than I_{dK}, usually near 0 mV when the pipette solution contained 0·8 mM-EGTA with no added calcium. It was blocked by TEA (4 mM) or charybdotoxin (0·1 μM), but not by 4-aminopyridine (5 mM), phencyclidine (0·1 mM) or apamin (0·1 μM). Estimates of the single-channel conductance from the noise variance of the whole-cell current I_{cK} indicated a value at +80 mV of 115 pS, very similar to that of the large-conductance Ca^{2+}-activated K^+ channels studied in these cells using single-channel recording.

5. The results suggest that outward current evoked by depolarization from the resting potential can be carried by 100 pS Ca^{2+}-activated K^+ channels and by small-conductance delayed-rectifier K^+ channels. It is likely that opening of both types of channel contributes to the repolarization phase of the action potential in this smooth muscle.

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INTRODUCTION

The rabbit portal vein contracts phasically and exhibits spontaneous electrical activity characterized by multispike complexes on slow waves of depolarization (Holman, Kasby, Suthers & Wilson, 1968). The membrane properties are similar to those of the rabbit mesenteric vein (Somlyo, Vinall & Somlyo, 1969), and guinea-pig (Kuriyama, Ohshima & Sakamoto, 1971) and rat (Daemers-Lambert, 1976) portal veins. Phasic contraction is associated with spike generation and in smooth muscle the majority of the upstroke of this spike is due to an influx of calcium ions (for review, see Tomita, 1981), probably through voltage-activated Ca^{2+} channels, which have been described for rabbit portal vein (Ohya, Kitamura & Kuriyama, 1988). The repolarization may be due in part to the inactivation of these Ca^{2+} channels, but seems also to result from the activation of K^+ channels as tetraethylammonium has considerable effect on spike duration and frequency (Suzuki, Nishiyama & Inomata, 1963; Osa, 1974).

Studies of these K^+ currents in other smooth muscles have revealed some dependence on Ca^{2+} influx but also indicate a Ca^{2+}-insensitive, voltage-dependent current contribution; for example see Wiegel, Connor & Prosser (1979); Mironneau & Savineau (1980). However, interpretation is difficult because of the multicellular nature of the tissues which compromises the control of ionic gradients and of voltage (see Bolton, Tomita & Vassort, 1981). However, studies on single enzymatically isolated smooth muscle cells, where good ionic and voltage control can be obtained, have shown again that part of the outward current in some smooth muscles (Walsh & Singer, 1981; Mitra & Morad, 1985; Ohya, Kitamura & Kuriyama, 1987) but not others (Aaronson, Bolton & Lang, 1985) is linked to the influx of Ca^{2+}. The channels activated as a result of Ca^{2+} influx are probably large-conductance Ca^{2+}-activated K^+ channels, which have been well characterized in smooth muscle (for example Benham, Bolton, Lang & Takewaki, 1984, 1986; Inoue, Kitamura & Kuriyama, 1985). In guinea-pig bladder smooth muscle there is evidence that apamin-sensitive K^+ channels generate the after-hyperpolarization (Fujii, 1987), although this seems not to be the case in the guinea-pig ileum (Nakao, Inoue, Yamanaka & Kitamura, 1986). We find that the outward current is not activated by Ca^{2+} influx under the conditions of these experiments and separate the current carried by large-conductance Ca^{2+}-activated K^+ channels from other, voltage-activated outward current, which we postulate to be carried principally by voltage-dependent K^+ channels; this latter current shows the characteristics of a delayed rectifier. Preliminary findings have been communicated (Beech & Bolton, 1987).

METHODS

Cell dispersion

Adult, male, New Zealand White rabbits (2–2·5 kg) were killed by injection of a lethal dose of sodium pentobarbitone (May & Baker Ltd). The main branch of the portal–mesenteric vein was removed and placed in physiological saline solution (PSS). It was dissected free of fat and connective tissue, and cut into small pieces ($\sim 2 \times 3$ mm). Six pieces were incubated at 36 °C in low-Ca^{2+} salt solution for 10 min and then re-suspended in a mixture of papain (5 mg/ml), dithiothreitol (3–5 mM), and bovine serum albumin (2–4 mg/ml) in a low-Ca^{2+} salt solution (20–30 μM-Ca^{2+}), for 20–30 min at 36 °C. The pieces of tissue were then removed from the mixture and mildly agitated

in the low-Ca^{2+} salt solution. The isolated cells were then centrifuged (100 *g* for 1·5 min), the pellet re-suspended in PSS and the suspension stored on glass cover-slips at 4 °C. Cells were used between 2 and 12 h after isolation.

Solutions and reagents

Physiological salt solution (PSS) in the bath had the following composition (mM): Na^+, 126; K^+, 6; Ca^{2+}, 1·7; Mg^{2+}, 1·2; Cl^-, 138; glucose, 14; HEPES, 10·5 and was titrated to pH 7·2 with NaOH (~4 mM). Pipette (intracellular) solution had the following composition (mM): K^+, 134; Mg^{2+}, 1·2; Cl^-, 136; EGTA, 0·8; glucose, 14; HEPES, 10·5 and was titrated to pH 7·2 with NaOH. For Ca^{2+}-free solutions the $CaCl_2$ was replaced by an equimolar amount of NaCl, and a contamination of Ca^{2+} (10 μM) was assumed. The free Ca^{2+} concentration in the pipette solution was estimated to be 1 nM. Solutions containing 4-AP (> 1 mM) were slightly alkaline and were titrated to pH 7·2 with HCl.

Papain (type 4), dithiothreitol, bovine serum albumin, tetraethylammonium chloride (TEA), 4-aminopyridine (4-AP), phencyclidine (PCP), ethylene glycol bis-(β-aminoethyl ether) *N,N,N',N'*-tetraacetic acid (EGTA) and *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulphonic acid (HEPES) were obtained from Sigma, and the charybdotoxin from the venom of the Israeli scorpion, *Leiurus quinquestriatus* (for the separation procedure see Castle & Strong, 1986) and the apamin were generous donations from Dr P. N. Strong.

Current recording and analysis

Recordings were made by using both the whole-cell and excised-patch configurations of the patch-clamp technique (Hamill, Marty, Neher, Sakmann & Sigworth, 1981) at room temperature (20–23 °C). Patch pipettes had a resistance of 1–4 MΩ. In the most extreme case of a 150 mV step the outward current was about 1 nA corresponding to less than 6% error in the voltage. In most cases the error was much less than this. For excised patch recording pipettes had a higher resistance of 8–10 MΩ and were fire-polished and coated in Sylgard (Dow Corning, USA). The glass was borosilicate and had an external diameter 1·4–1·6 mm and an internal diameter 0·6–0·8 mm. The current amplifier was a List EPC-7 (List-Electronic) and data was recorded on FM tape (Racal) at tape speeds of 3·75 or 7·5 in/s.

Data were digitized off-line by using a CED 1401 analog-to-digital interface (Cambridge Electronic Design), stored on floppy diskettes and then analysed using a BBC microcomputer in conjunction with the CED 1401. Single-channel currents were displayed by using a chart recorder (Gould 2400S) and were low-pass filtered (8-pole Bessel: attenuation rate, 48 dB/octave). Whole-cell currents were digitized at a frequency of 0·5 kHz except where indicated. Functions were fitted by using the iterative algorithm of Marquardt & Levenberg with the criterion of minimizing the unweighted sum of the squares of the deviations. The goodness of fit was evaluated by eye, except for the straight line where the correlation coefficient was used.

When using the whole-cell configuration leakage current was estimated for each bathing solution and then subtracted. It was evaluated by measuring the mean inward current (over 100 ms) required to hyperpolarize the cell from the holding potential to various test potentials (rectification was not observed upon hyperpolarization). The points were plotted, a straight line fitted to data points (the correlation coefficient was between 0·95 and 0·99) and the gradient and Y-intercept used to estimate the leakage component. This was then subtracted from the current activated upon depolarization, assuming the leakage did not rectify over the potential range studied. When establishing the inactivation curve for I_{dK}, the leakage was assessed by measuring current elicited by depolarization to potentials negative of threshold as currents were unstable negative of the holding potential. None of the cells described in this paper displayed the transient, voltage-dependent outward current which has a very negative threshold (Beech & Bolton, 1989).

RESULTS

Outward current characteristics

Depolarization of single cells from holding potentials between −50 and −70 mV in physiological salt solution (PSS) resulted in the development of a time- and

voltage-dependent outward current which decayed slowly (Fig. 1). In some cells this was preceded by a small transient net inward current. At very positive potentials the peak-to-peak current noise increased considerably. In some cells spontaneous

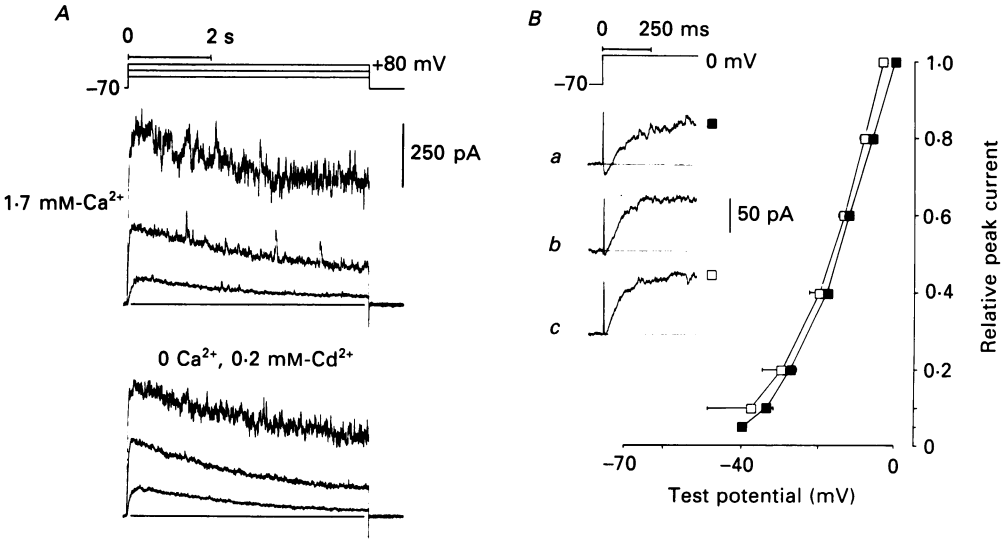

Fig. 1. Dependence of outward current on Ca^{2+} influx. *A*, currents were elicited by 6 s steps to 0, +40, and +80 mV from a holding potential (HP) of −70 mV. After control responses were obtained in normal solution (1·7 mM-Ca^{2+}, upper records) the cell was bathed in PSS with 0·2 mM-Cd^{2+} and no added Ca^{2+}. *B*, for the same cell, current elicited by depolarizing to negative potentials. The cell was bathed in PSS (*a*; ■, $n = 9$–10), Ca^{2+}-free solution (*b*) and Ca^{2+}-free solution containing 0·2 mM-Cd^{2+} (*c*; □, $n = 3$–4). The inset currents were elicited by stepping to 0 mV from a HP of −70 mV. The analog signal was digitized at 5 kHz and every two points were averaged. The graph shows peak outward current plotted against test potential. Currents were normalized to peak current ($\approx$ 200 pA) elicited by the test pulse to 0 mV when in PSS. The points are interpolated and represent the mean potential (±S.E.M.) to evoke a given current. Threshold for outward current was at about −40 mV and the Ca^{2+}-free solution containing 0·2 mM-Cd^{2+} had little effect on this. Leakage currents were estimated for each solution and subtracted.

transient outward currents (STOCs; Benham & Bolton, 1986) were observed, but on depolarization no initial rapid peak to the outward current was seen such as occurs in rabbit intestinal smooth muscle (Benham & Bolton, 1986; Ohya *et al.* 1987).

Effect of Ca^{2+} influx on outward current

When cells were bathed in PSS a small, transient net inward current was sometimes observed upon application of a depolarizing voltage step (Fig. 1*Ba*). This appeared after the capacity current and before the outward current. In cells perfused internally with 10 mM-TEA and NaCl to replace KCl, K^+ currents were blocked and net inward currents were observed throughout the voltage step in a number of cells. Studies of other smooth muscles have shown that this Ca^{2+} influx can contribute to the generation of the outward current (e.g. Ohya *et al.* 1987). However, in portal vein

cells that exhibited a net inward current, both with or without leakage current subtraction (EGTA concentration in the pipette, 0·8 mM), abolition of Ca^{2+} influx sometimes caused a small increase in peak outward current elicited by test potentials negative to 0 mV but a decrease in current at very positive potentials.

Close examination of the current elicited by depolarizing to 0 mV revealed that the peak outward current was increased slightly in both Ca^{2+}-free PSS and Ca^{2+}-free PSS containing cadmium ions (Fig. 1*Ba–c*) when compared with the control, and that the rate of development of the outward current was accelerated. This may be due to loss of voltage-activated inward Ca^{2+} current and also to a surface potential effect (see Beech & Bolton, 1989). Outward current elicited by very positive test potentials decreased when Ca^{2+} inward current was reduced or abolished by adding cadmium to this solution (Fig. 1*A*). STOCs (evident in Fig. 1*A*) which are Ca^{2+}-activated K^+ currents (Benham & Bolton, 1986), were abolished in Ca^{2+}-free solution, implying that intracellular Ca^{2+} stores are rather rapidly depleted in this cell type.

Two components of outward current

Two components of outward current could be distinguished. At potentials negative to 0 mV, TEA (4 mM) had very little effect on the size of the outward current. Current noise was also little if at all changed (Fig. 2*A* and *C*). However, this current was inhibited substantially by 4-AP (5 mM, six experiments) or phencyclidine (0·1 mM, six experiments) (Fig. 3*A* and *B*). Stepping to positive potentials evoked a much noisier outward current (Fig. 2*A* and *B*) upon which spontaneous transient outward currents (Benham & Bolton, 1986) were often superimposed. A large component of the outward current evoked at positive potentials was blocked by TEA (4 mM, seven experiments; Fig. 2*A* and *C*), which also markedly reduced the current noise and abolished STOCs. This suggested that this outward current component was carried by the large-conductance Ca^{2+}-activated K^+ channels, which are prevalent in these cells and are blocked by external TEA (Beech, 1988).

Further evidence that this component of outward current (I_{cK}) was carried by large Ca^{2+}-activated K^+ channels came from the action of charybdotoxin (ChTX, 0·1 μM), which also blocks these channels in smooth (Beech, Bolton, Castle & Strong, 1987) and skeletal muscles (Miller, Moczydlowski, Lattore & Phillips, 1985). This blocked both STOCs and a component of outward current evoked by stepping to positive potentials which was similar in size to the component blocked by 4 mM-TEA. ChTX also reduced the noise (Fig. 2*B*). However, after block by TEA or ChTX the remaining and less noisy component of outward current decayed at a virtually unchanged rate when compared to the outward current before drug application (Fig. 2*A* and *B*) suggesting that the component which was blocked was time invariant. Ca^{2+}-activated K^+ channels do not inactivate significantly in smooth muscle (Benham *et al.* 1986). The results showed that the decay of outward current was largely due to the TEA-insensitive, 4-AP-sensitive component, which will be termed I_{dK} because of its similarity to delayed rectifier current described in other excitable cells (for review see Thompson & Aldrich, 1980). 4-AP blocks the delayed rectifier K^+ current in squid axon (Yeh, Oxford, Wu & Narahashi, 1976) and phencyclidine blocks voltage-gated K^+ channels in hippocampal neurones (Ffrench-Mullen, Suzuki, Barker & Rogawski, 1988).

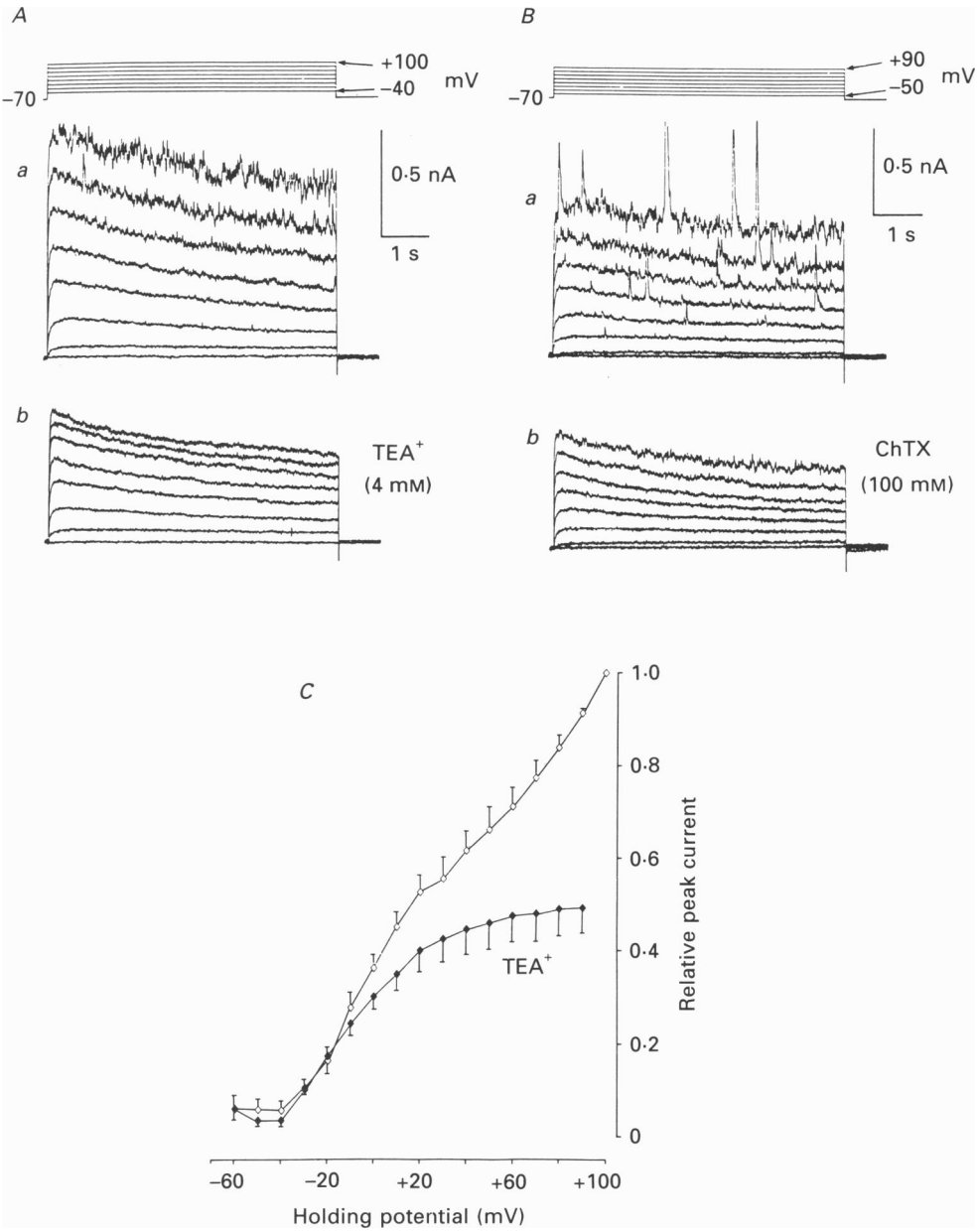

Fig. 2. The effects of TEA or charybdotoxin (ChTX) on outward currents elicited from a HP of −70 mV when in PSS. *A*, TEA. The test potentials were −40 to +100 mV in 20 mV increments, each for 6 s. The control (*a*) and after the equilibration with 4 mM-TEA (*b*) are shown. Note the low current noise at +80 and +100 mV in *b*. *B*, ChTX. The test potentials were −50 to +90 mV in 20 mV increments, each for 6 s. The control (*a*) and after equilibration with 0·1 μM-ChTX (*b*) are shown. Note the low current noise at +90 mV and the absence of STOCs (see text) in *b*. *C*, the mean conductance (mean ± S.E.M., $n = 4$) at peak outward current was plotted against test potential on a relative scale for the control in PSS (◇) and after equilibration with 4 mM-TEA (◆). Conductance was estimated assuming the charge carrier was principally K^+, which had an equilibrium potential at about −77 mV (see Fig. 6). The TEA-resistant K^+ conductance activated with a threshold around −40 mV. Leakage currents were subtracted.

The results suggest that a current, I_{dK}, was evoked by steps to potentials negative to 0 mV and that this current contributed almost all the outward current observed since TEA or ChTX had little effect (Fig. 2) and phencyclidine induced complete, and 4-AP substantial, block (Fig. 3). When outward current was evoked by stepping

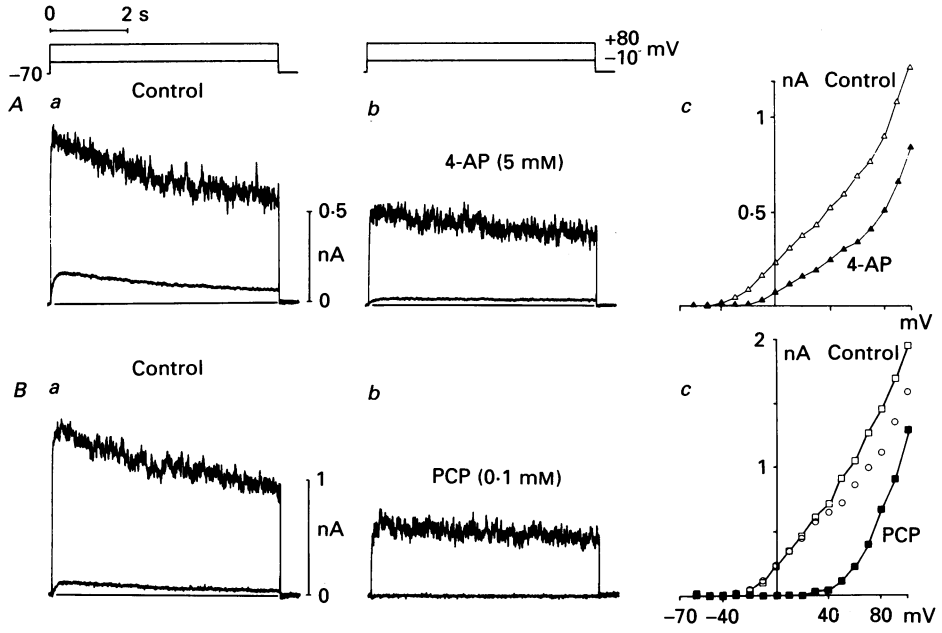

Fig. 3. The effect of 4-aminopyridine (4-AP) or phencyclidine (PCP) on outward current elicited from a HP −70 mV when in PSS. *A*, 4-AP (5 mM). The test potentials were −10 and +80 mV, each for 6 s. The control (*a*) and after equilibration with 4-AP for 2 min (*b*) are shown (marked). *c*, complete current–voltage relationship for the same experiment: △, control; ▲, 4-AP. *B*, PCP (0·1 mM), using the same protocol as for *A*. For the current–voltage relationship (*c*): □, control; ■, PCP; ○, wash-out. Both 4-AP and PCP produced substantial block of current at −10 mV but had little effect on the noise of the current at +80 mV. Leakage currents were subtracted.

to positive potentials a second component, I_{cK}, became apparent and its contribution was as much as 40% at +80 mV (Fig. 2). Phencyclidine or 4-AP produced 50% block of I_{dK} at about 0·1 and 0·2 mM respectively and substantially reduced outward current at +80 mV by blocking the contribution of I_{dK} (Fig. 3*Ac* and *Bc*). At positive potentials a major contribution to outward current was made by I_{cK}, which seemed to be carried by large-conductance Ca^{2+}-activated K^+ channels as it was blocked by TEA or ChTX. Negative to 0 mV, however, I_{cK} was small. This was consistent with previous experiments on Ca^{2+}-activated K^+ channels in smooth muscle (Benham *et al.* 1986) which showed that with an intracellular solution containing no added calcium and 0·8 mM-EGTA (as used here in the pipette solution) the opening of these channels in isolated inside-out patches was detected only at very positive potentials. The number of these channels in a single cell probably approaches 10000 (between one and ten are observed in an isolated membrane patch; Benham *et al.* 1986). If the

probability of the open state was only 0·005 at +80 mV, current through these channels would be about 900 pA (=50 × 18 pA) not dissimilar from the size of the TEA-sensitive, 4-AP-resistant component observed (Figs 2 and 3).

Further support for the suggestion that I_{cK} was carried by the large-conductance (100 pS) Ca^{2+}-activated K^+ channels came from measurements of the root mean square (r.m.s.) of the current noise, σ (pA). The r.m.s. noise of I_{cK} was estimated at +80 mV after holding at −20 mV for 2 min, which completely inactivated I_{dK} channels (Fig. 8). If the unitary channel current underlying this is $i = \sigma^2/[I(1-p)]$ where I is the mean current (pA) and p the probability of channel opening (assumed to be very low such that $(1-p) \approx 1$) then the unitary current was 18 pA, representing a K^+ channel conductance of 115 pS, very close to the single-channel measurement under these ionic conditions (Benham *et al.* 1986). In 4 mM-TEA, after completely inactivating I_{dK} channels by holding at −20 mV, the noise was very substantially reduced (its variance declined from $\sigma^2 = 7094$ to $\sigma^2 = 56$ pA^2, Fig. 4*B*) suggesting that very small-conductance channels were responsible for the remaining leak current (Fig. 4*B*). TEA at 4 mM is about ten times the IC_{50} for Ca^{2+}-activated K^+ channels (Beech, 1988) and completely and reversibly blocked single-channel current through these in isolated patches (Fig. 4*C*), even at strongly positive potentials (not shown).

During a step to a positive potential from a −70 mV holding potential, I_{dK} channels were activated (in contrast to stepping from a −20 mV holding potential where they were inactivated) and so they contributed to total outward current and to current noise. Assuming 4 mM-TEA completely blocked I_{cK} channels (Fig. 4*C*) the relative contribution of I_{dK} to r.m.s. of current noise was about 20% at +100 mV (Fig. 4*A*) i.e. the contribution of I_{dK} channel openings to total noise variance was about 4%. This suggests the conductance of an I_{dK} channel is about 5 pS since leakage channels contributed less than 1% of total noise variance (Fig. 4*B*). No great reliance can be placed on this estimate even though it is coincidentally close to the conductance seen with single-channel studies (see below) because of uncertainty about whether the probability of I_{dK} channels being open is very low. Nevertheless, it is clear that I_{dK} channels have a much smaller conductance than I_{cK} channels and the result makes it plausible that the small-conductance channels seen in isolated patches (see below) underlie I_{dK}.

The channels underlying I_{dK} were searched for in outside-out patches held at −70 mV and stepped to 0 mV, a potential where the contribution from I_{cK} was very small. Unitary current levels of 0·4 and 0·7 pA were observed in nine experiments, corresponding to single K^+ channel (chord) conductances of 5 and 8 pS. Most patches exhibited only one or two active channels and this activity was particularly evident at early times during the step to 0 mV. Occasionally, many small channels were observed to open (Fig. 5) and this activity showed a clear decline during the 6 s test step. Averaging the currents of eight records gave a time-course of inactivation not dissimilar from whole-cell outward current at this potential (cf. Fig. 5*A* with Figs 1, 2 and 3). Interspersed with the small unitary currents of 0·4 and 0·7 pA were brief openings of channels, presumably large-conductance Ca^{2+}-activated K^+ channels. These were blocked by ChTX (0·1 μM), which did not affect the smaller unitary currents. The currents observed could not be carried by chloride as 0 mV was the

chloride equilibrium potential. Holding at −20 mV for 2 min, which inactivated whole-cell I_{dK}, abolished the small unitary currents but not those through the large-conductance Ca^{2+}-activated K^+ channels (Fig. 5*B*).

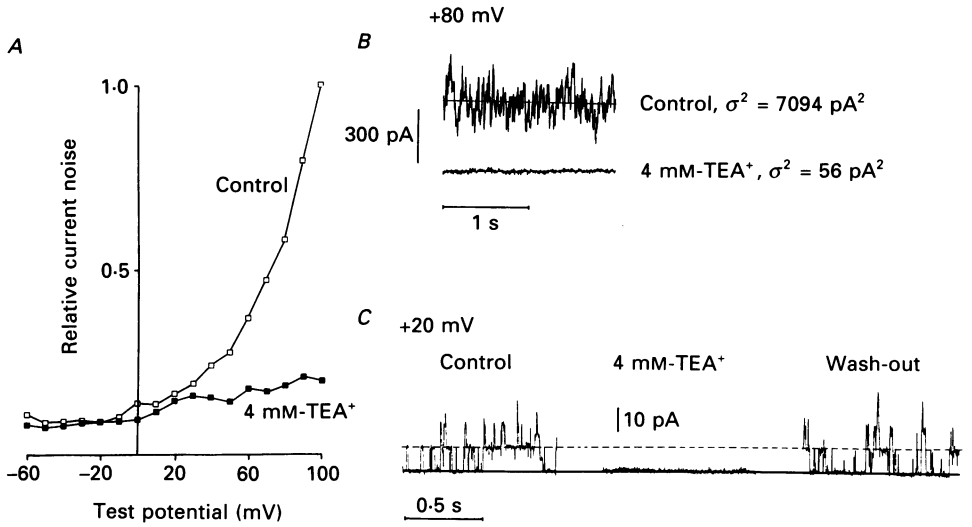

Fig. 4. Large-conductance Ca^{2+}-activated K^+ channels carry the noisy, non-inactivating component of outward current (I_{cK}). *A*, relative r.m.s. noise of outward current (σ), elicited from a HP of −70 mV, about a fitted single exponential (between 1 and 5 s after the start of the voltage step) plotted against step potential. The control (□) and in the presence of 4 mM-TEA (■) are shown (marked). *B*, variance of the whole-cell current at +80 mV elicited from a HP of −20 mV, which inactivates I_{dK}, in the absence and presence of 4 mM-TEA (bath applied). Records were not low-pass filtered and were digitized at 2 kHz. The horizontal lines are fitted straight lines. The calculated variances (σ^2) are shown. For a K^+-selective channel this indicated a unitary conductance of 115 pS for the control current. *C*, complete block of large-conductance Ca^{2+}-activated K^+ channels by 4 mM-external TEA. Unitary K^+ currents were recorded through these channels (~100 pS) in an outside-out patch held at +20 mV and bathed in PSS. Upward deflection indicates channel opening and outward current. Dashed line indicates current level when one channel is open, continous line when all channels are closed. Records were digitized at 1 kHz. Channel openings were not observed when in TEA but were similar to the control after wash-out (marked).

Reversal potential for I_{dK}

To assess whether or not I_{dK} was carried principally by K^+ the tail current reversal potential was estimated for current activated by a depolarizing step to 0 mV where the K^+ equilibrium potential (E_K) was −78 mV, assuming the K^+ concentration in the cell to be the same as that in the pipette solution. Experiments were carried out in cells which did not display a net inward current after leakage subtraction on the basis that in these cells the contribution of Ca^{2+} current to the net current was minimal. Tail currents elicited were measured without leakage correction and expressed relative to the outward current flowing after 0·5 s at 0 mV, as some random

variation was observed in this value. The intersection point of the line fitted to the leakage current points and the curves drawn through the values of tail currents gave the reversal potential. It was at −76·5 mV for the experiment shown (Fig. 6) and for three cells the mean was −76·9±2·3 mV (mean±S.E.M.). The close proximity of the

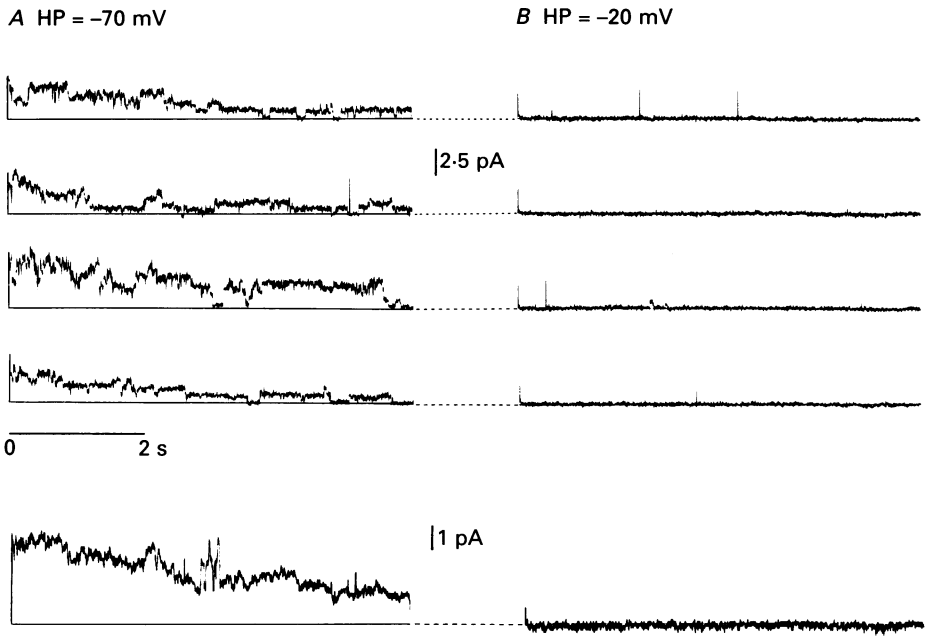

Fig. 5. Single-channel recordings from an outside-out patch. *A*, channel openings were elicited by 6 s steps to 0 mV from a holding potential of −70 mV and the interpulse interval was 20 s. *B*, in the same patch, stepping to 0 mV after holding at −20 mV for 2 min reveals loss of small unit current but larger unit current remains. The seal resistance was 3 GΩ and records were low-pass filtered at 300 Hz. The continuous lines represent zero current and zero time is at the beginning of the command voltage step. The lower traces are each averages of eight digital records.

reversal potential and E_K (−78·3 mV) suggested that the current was carried by K^+. Any voltage-activated Ca^{2+}-channel tail current was small and deactivated too rapidly to affect tail currents for I_{dK} which deactivated more slowly.

Activation kinetics of I_{dK}

The activation rate of outward current elicited by a depolarizing step was voltage-dependent, increasing for larger voltage steps. In addition, an initial lag phase was observed before the development of current, especially for test steps negative of 0 mV. To be sure that these properties were not influenced by any Ca^{2+} influx the experiments were carried out in Ca^{2+}-free PSS containing 0·2 mM-cadmium. The membrane voltage settled within 2–3 ms (reflected by the decay of the capacity current) and so would have little influence on the measured parameters. Current activated negative of 0 mV (mostly I_{dK}), was described well by a fourth-order

exponential (Fig. 7*A*), which took into consideration the initial lag phase. The function had the form (from Hodgkin & Huxley, 1952):

$$I = [(I_p)^{\frac{1}{4}} - \{(I_p)^{\frac{1}{4}} - (I_0)^{\frac{1}{4}}\} \exp(-t/q)]^4,$$

where I and t are current (A) and time (s) respectively, q is the time constant of the exponential, I_p the peak current elicited by that voltage step and I_0 the current at

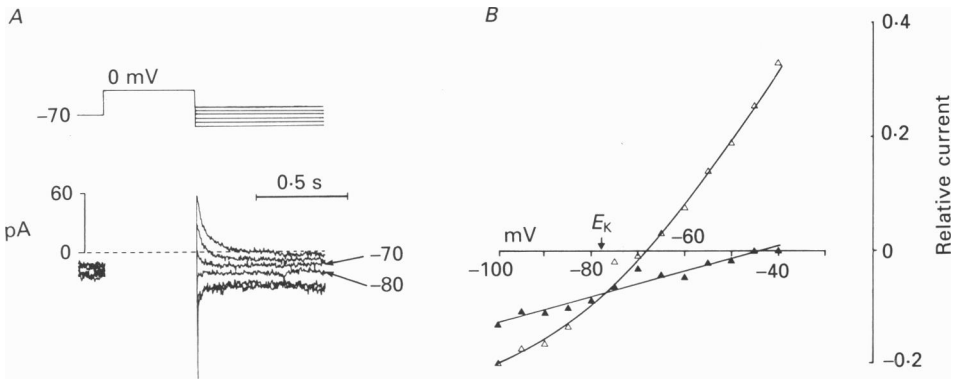

Fig. 6. Tail current reversal potential for outward current elicited by depolarizing to 0 mV from a HP of −70 mV when in PSS. *A*, the test step to 0 mV for 0·5 s elicited outward current (off-scale) which deactivated upon returning to various after-pulse potentials (−50 to −100 mV in 10 mV increments; shown). The dashed horizontal line marks zero current. *B*, for the same experiment, tail current size (△) (leak current not subtracted), and leak current measured 5 s after the end of the test pulse (▲) were plotted against after-pulse potential. Points were normalized with respect to current at the end of the test step (0 mV) and zero holding current occurred at the resting membrane potential of the cell. The straight line was fitted and the curve drawn by eye. The intersection point gave the reversal potential (−76·5 mV). The calculated K^+ equilibrium potential was −78·3 mV at 20 °C.

time zero (i.e. any instantaneous current not subtracted by the leakage correction procedure). The time constants for fitted fourth-order exponentials were related directly to the natural logarithm of the test potential such that they changed e-fold every 56 mV (Fig. 7*B*). This presumably reflected principally the behaviour of I_{dK} as I_{cK} was not a major current component at these potentials.

Figure 7*A* shows the current elicited at +50 mV fitted by a fourth-order exponential. In this case it was a reasonable fit, but some currents elicited by steps to positive potentials were not fitted well by this function and second-order exponentials provided a better description. This may have been due to the fact that I_{cK} was beginning to make a significant contribution. The activation kinetics of I_{cK} were not assessed as a reasonable definition of this current could only be observed at very positive potentials (e.g. +80 mV), presumably because so few channels were generating the current.

Inactivation of I_{dK}

Outward current evoked at positive potentials (e.g. +80 mV) showed considerable inactivation during a 6 s pulse. The size of the peak outward current was reduced by

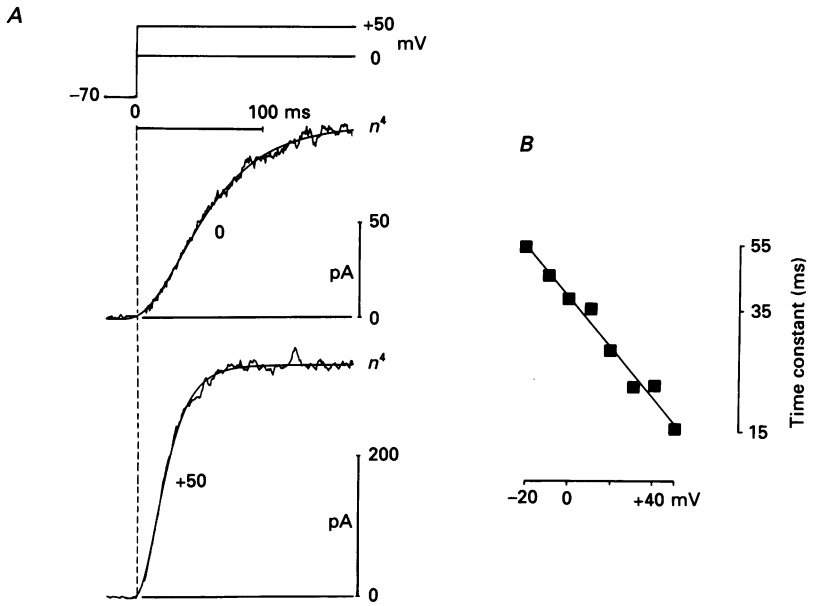

Fig. 7. Activation kinetics of outward current elicited by stepping from an HP of −70 mV when in Ca^{2+}-free PSS containing 0·2 mM-Cd^{2+}. *A*, currents are shown for the 0 and +50 mV test potentials (marked). The analog signal was digitized at 5 kHz and a four-point digital filter applied. The capacity current decayed completely within 3 ms and the digital points for this current were eliminated for the curve fitting and presentation. Fourth-order single exponentials (n^4, shown) were fitted to the currents between time zero (start of the voltage step; vertical dashed line) and time = 175 ms. *B*, the time constants (ms) of n^4 exponentials plotted on a natural logarithmic scale against test potential. The fitted straight line had a correlation coefficient of −0·98. The time constant changed e-fold every 56 mV.

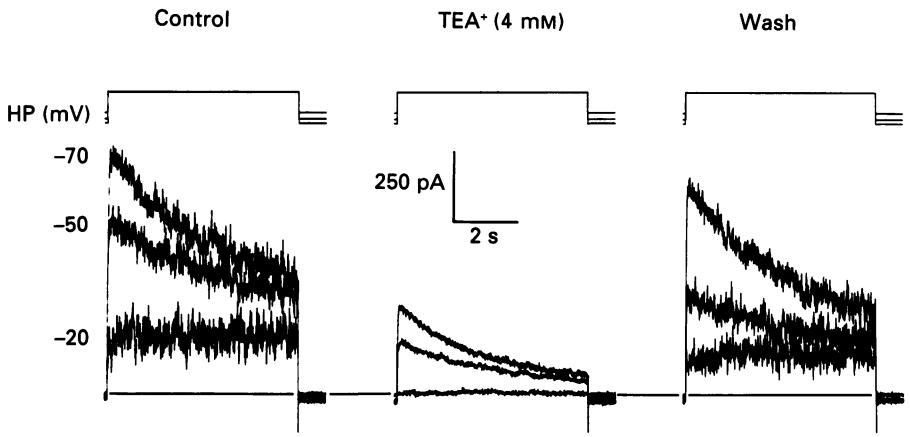

Fig. 8. The effect of holding potential (HP) and TEA on the outward current. The cell was held at each indicated HP for 2 min before stepping to +80 mV. Note that after holding at −20 mV current is much less and does not inactivate during the 6 s pulse. This current is completely blocked by 4 mm-TEA.

holding at less negative potentials than about −60 mV, and after holding at −20 mV for 20 s, current did not inactivate during a test pulse. This residual current was noisy and almost completely blocked by TEA (4 mM) suggesting that it was carried by Ca^{2+}-activated K^+ channels and that these do not inactivate (Fig. 8). TEA (4 mM) also reduced current evoked from holding potentials of −50 and −70 mV and the reduction was greater than from a holding potential of −20 mV probably indicating that this concentration also reduced I_{dK} to some extent.

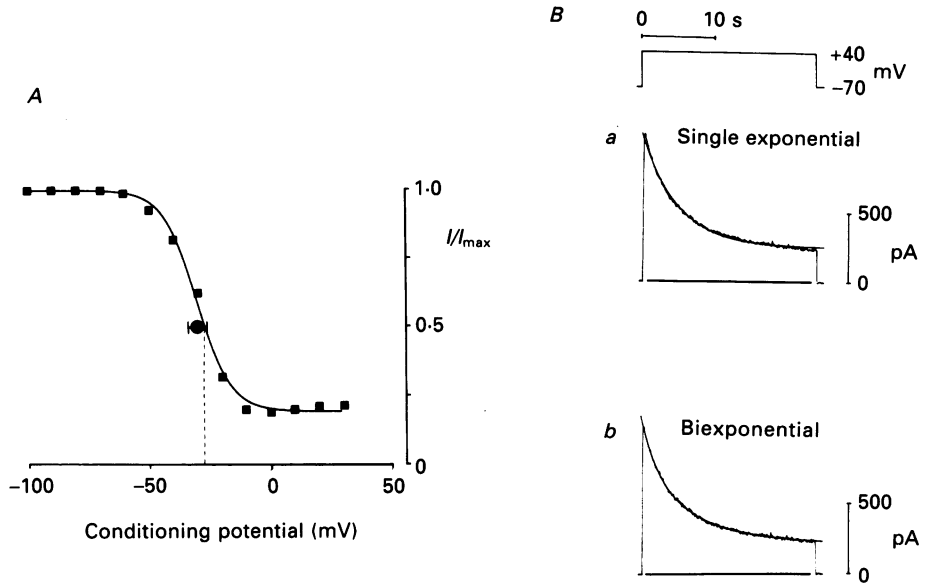

Fig. 9. Inactivation of outward current when in PSS. *A*, inactivation curve. Relative peak current (I/I_{max}) plotted against conditioning potential for one experiment. The holding potential was −100 mV, the conditioning potential step length was 20 s and the test potential +40 mV. The fitted curve described the Boltzmann equation (see text); V_h was −30 (at dashed vertical line) and V_s 7 mV. For six experiments the mean V_h value (±S.E.M.) was −30·2±3·6 mV (●). *B*, rate of inactivation of I_{dK} for a 24 s step to +40 mV from a holding potential of −70 mV. *a*, a fitted single exponential; *b* a fitted biexponential. Leakage current was subtracted.

The inactivation of I_{dK} was studied using 20 s holding periods at various potentials and measuring peak current evoked by stepping to +40 mV. I_{dK} was 50% available at −30·2±3·6 mV (mean±S.E.M., $n = 6$). A few experiments using a 40 s holding period were also done and the potential at which channels were 50% available was about 5 mV more negative. The voltage dependence of inactivation of I_{dK} could be fitted by a Boltzmann equation of the form:

$$I = (C_1 - C_2)/[1 + \exp((V - V_h)/V_s)] + C_2,$$

where C_1 and C_2 are the maximum and the minimum of the curve, V_h the holding potential for 50% inactivation and V_s the slope factor (Fig. 9*A*). I_{dK} slowly inactivated and its inactivation at +40 mV was better fitted by a biexponential than a single exponential. For three cells the time constants of the biexponential were 1·14±0·46 and 6·50±0·61 s at +40 mV (Fig. 9*B*).

DISCUSSION

Potassium current activated upon depolarization of the cell is known to be important for the repolarization of the spike (Suzuki & Inomata, 1981). In single cells from the rabbit portal vein we have shown that this current can be divided into two components; one carried by large-conductance Ca^{2+}-activated K^+ channels and the other by channels whose characteristics resemble those of delayed rectifier channels seen in other excitable tissues. These components were termed I_{cK} and I_{dK} respectively. Current activated negative of 0 mV was principally I_{dK} and was blocked by 4-AP but not by TEA; that activated at positive potentials consisted of both currents and was thus only partially blocked by 4-AP. I_{cK} was blocked completely by TEA (4 mM). The combination of 4-AP (5 mM) and TEA (4 mM) blocked almost all the outward current leaving only a small residual component. This remaining current was not studied as the extent and selectivity of the block of I_{dK} by 4-AP was not known.

Although outward current at very positive potentials was reduced in Ca^{2+}-free solution this need not necessarily indicate that outward current depends directly on Ca^{2+} influx. Inward Ca^{2+} current in these experiments was small, and it is more likely that the reduction of outward current in Ca^{2+}-free solution arises in some other way as the Ca^{2+}-activated current, I_{cK}, did not decline over a 6 s period despite the presence of 0·8 mM-EGTA in the pipette solution (Fig. 8). With this concentration of EGTA, the free ionized calcium in a steady state will be about 10^{-9} M. Strongly positive potentials still cause appreciable opening of Ca^{2+}-activated K^+ channels in isolated patches (Benham *et al.* 1986). In other smooth muscles, components of K^+ current have been ascribed to Ca^{2+} influx (Walsh & Singer, 1981; Klöckner & Isenberg, 1985; Mitra & Morad, 1985; Benham & Bolton, 1986; Ohya *et al.* 1987).

I_{dK} was a 4-AP-sensitive voltage-activated K^+ current similar to the current identified in rabbit pulmonary artery smooth muscle (Okabe, Kitamura & Kuriyama, 1987) and in rat uterine smooth muscle (Mironneau & Savineau, 1980). 4-AP causes an increase in spike frequency in guinea-pig pulmonary artery and rat portal vein muscles (Hara, Kitamura & Kuriyama, 1980) and block of an I_{dK}-like current may explain this effect. However, an effect of 4-AP on the A-current of portal vein smooth muscle (Beech & Bolton, 1989) must also be considered. Smooth muscle cells from the rabbit ileum (Ohya, Terada, Kitamura & Kuriyama, 1986) and guinea-pig urinary bladder (Klöckner & Isenberg, 1985) also exhibit a Ca^{2+} influx-independent voltage-activated K^+ current but these currents seem not to be blocked by 4−AP. In these cells this K^+ current may result from the voltage-activation of large Ca^{2+}-activated K^+ channels as has been proposed for human aorta smooth muscle (Bregestovski, Zamoyshi, Serebryakov, Toptygin & Antonov, 1985).

The inactivation of I_{dK} was slow and appeared to have two components. This has also been observed for delayed rectifiers of nerve (Schwarz & Vogel, 1971) and may be due to the existence of more than one type of delayed rectifier channel in a cell type (see Dubois, 1981). Slowly inactivating K^+ currents similar to I_{dK} have been reported for single smooth muscle cells from the rabbit small intestine (Terada, Kitamura & Kuriyama, 1987) and pulmonary artery (Okabe *et al.* 1987), and are also observed in other cell types such as superior cervical ganglia cells (Galvan &

Sedlmeir, 1984). The delayed rectifier K^+ current of skeletal muscle also inactivates (Adrian, Chandler & Hodgkin, 1970), although more rapidly so than the current in smooth muscle.

I_{cK} did not inactivate and any apparent current decay observed after block of I_{dK} by 4-AP or phenycyclidine almost certainly resulted from residual I_{dK} as stepping from a holding potential of −20 mV revealed a non-inactivating noisy outward current that was blocked completely by 4 mM-TEA. In addition, single large Ca^{2+}-activated K^+ channels in excised patches of membrane show no sign of inactivation (Benham *et al.* 1986; Beech, 1988) unlike Ca^{2+}-activated K^+ channels of rat skeletal muscle (Pallotta, 1985). I_{dK} was further distinguished from I_{cK} in that it showed no Ca^{2+} dependence. Delayed rectifier K^+ channels were not activated during the STOC and I_{dK} was not reduced when Ca^{2+} influx was abolished or the intracellular calcium was buffered strongly with 10 mM-EGTA. The slight N shape of the current–voltage relationships for outward current (see Fig. 2*C*) seemed not to reflect a dependence on voltage-activated Ca^{2+} inward current but to be associated with maximal activation of I_{dK}.

A further difference between I_{cK} and I_{dK} was the conductance of the ion channels underlying them. The channels carrying I_{cK} had a large unitary conductance (about 100 pS) and seemed to be large Ca^{2+}-activated K^+ channels, whereas the channels underlying I_{dK} had a low conductance of about 5 pS at 0 mV (both with a physiological K^+ gradient). A similar low conductance for delayed rectifier K^+ channels has been reported for avian hepatocytes (Marchetti, Premont & Brown, 1988), although other reports for delayed rectifiers indicate a slightly higher value between 10 and 20 pS (e.g. Conti & Neher, 1980; Shibasaki, 1987; Clapham & Logothetis, 1988).

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