British Journal of Pharmacology (1995) 116, 3139-3148

The pharmacological properties of K^+ currents from rabbit isolated aortic smooth muscle cells

[1]Fiona C. Halliday, Philip I. Aaronson, A. Mark Evans & Alison M. Gurney

Department of Pharmacology, UMDS, St Thomas's Hospital, Lambeth Palace Road, London SE1 7EH

1 Using the whole-cell patch-clamp technique, the effects of several K^+ channel blocking drugs on K^+ current recorded from rabbit isolated aortic smooth muscle cells were investigated.

2 Upon depolarization from −80 mV, outward K^+ current composed of several distinct components were observed: a transient, 4-aminopyridine (4-AP)-sensitive component (I_t) and a sustained component (I_{sus}), comprising a 4-AP-sensitive delayed rectifier current ($I_{K(V)}$), and a noisy current which was sensitive to tetraethylammonium (TEA), and probably due to Ca^{2+}-activated K^+ current ($I_{K(Ca)}$).

3 Several drugs in clinical or experimental use have as part of their action an inhibitory effect on specific K^+ channels. Because of their differential K^+ channel blocking effects, these drugs were used in an attempt to characterize further the K^+ channels in rabbit aortic smooth muscle cells. Imipramine, phencyclidine, sotalol and amitriptyline failed to block selectively any of the components of K^+ current, and were thus of little value in isolating individual channel contributions. Clofilium showed selective block of $I_{K(V)}$ in the presence of TEA, but only at low stimulation frequencies (0.07 Hz). At higher frequencies (1 Hz) of depolarization, both I_t and $I_{K(V)}$ were suppressed to a similar extent. Thus, the blocking action of clofilium was use-dependent.

4 The voltage-dependent inactivation of I_t and the delayed rectifier were very similar although a brief (100 ms) pre-pulse to −30 mV could preferentially inactivate I_t. Together with the non-selective blocking effects of the K^+ channel blockers, similarities in the activation and inactivation of these two components suggest that they may not exist as separate ionic channels, but as distinct kinetic states within the same K^+ channel population.

5 The effects of all of these drugs on tension were examined in strips of rabbit aorta. The non-specific K^+ channel blockers caused only minor increases in basal tension. TEA and 4-AP by themselves caused significant increases in tension and were even more effective when applied together. There appeared to be no correlation between the effects of the drugs tested on tension and their actions on currents recorded from isolated myocytes. Thus tension studies are an inappropriate means of investigating the mechanism of action of these drugs, and studies on ionic currents in isolated myocytes cannot easily predict drug actions on intact tissues.

Keywords: Rabbit aorta; smooth muscle; K^+ channels; K^+ channel blockers

Introduction

Rabbit aorta belongs to the class of smooth muscle tissue which exhibits electrical quiescence, such that under normal physiological conditions, action potentials are rarely observed. That K^+ channels are responsible for minimizing the excitability of such tissue can be seen from experiments with K^+ channel blockers. For example, TEA (tetraethylammonium) has been shown to induce action potentials and phasic tension waves in rabbit pulmonary artery (Casteels *et al.*, 1977); the drug also induces pronounced depolarization and the appearance of action potentials in the rabbit ear artery (Droogmans *et al.*, 1977), and in the rabbit aorta TEA has been shown to induce fluctuations of the membrane potential (Mekata, 1974). Whether they exist in spontaneously active or quiescent tissue, the role of K^+ channels is to stabilize the membrane by drawing the membrane potential closer to the K^+ equilibrium potential and further from firing threshold. K^+ currents have been characterized in isolated cells from a variety of vascular smooth muscles, including rabbit pulmonary artery (Clapp & Gurney, 1991), mesenteric artery (Smirnov & Aaronson, 1992), portal vein (Beech & Bolton, 1989; Hume & Leblanc, 1989), and guinea-pig mesenteric artery (Bolton *et al.*, 1985). From these and other studies, it is apparent that several different types of voltage-sensitive K^+ channel exist in smooth muscle. Three groups, distinguished by their sensitivities to membrane potential and pharmacological agents, have been extensively characterized.

A voltage-gated K^+ current with rapid activation and inactivation kinetics similar to the neuronal fast 'A' current is found in pulmonary artery (Clapp & Gurney, 1991) and portal vein (Beech & Bolton, 1989; Hume & Leblanc, 1989). In large, quiescent arteries such as the aorta, this current may act as a 'brake' to counteract quickly any depolarizing influence. Most smooth muscle has been found to contain delayed rectifier channels which are also time- and voltage-dependent. Currents through these channels show slower activation and inactivation kinetics than the A-like current. Their activation requires membrane depolarization but not calcium. In excitable cells, currents through these channels help to keep action potentials short, whilst their role in quiescent tissue is probably to cause a general lowering of excitability (Longmore & Weston, 1990). A third type of voltage-sensitive channel is the Ca^{2+}-activated K^+ channel, the opening probability of which increases with rises in $[Ca^{2+}]_i$ and depolarization above the resting potential. These channels serve to terminate periods of Ca^{2+} entry by repolarizing or hyperpolarizing the cell. They appear to be the most abundant K^+ channel in several vascular smooth muscles (Beech & Bolton, 1989; Clapp & Gurney, 1991; Neylon *et al.*, 1994; Zhang & Cook, 1994).

Although the rabbit aorta is a widely used model for studying the regulation of vascular tone in normal and pathological conditions, there is little information available on the K^+ channels that are primarily responsible for the elec-

[1] Author for correspondence.

trophysiological properties of its smooth muscle. This paper describes the effects of a number of drugs on the K^+ currents recorded from these cells. These include tetraethylammonium (TEA) and 4-aminopyridine (4-AP), as well as other drugs which have a broader spectrum of activities on various ion channels, but also block specific K^+ channels in some tissues. In addition, the effects of all of these drugs on tension regulation is investigated. A preliminary account of this work has appeared in abstract form (Halliday *et al.*, 1994).

Methods

Male New Zealand rabbits (2–2.5 kg) were killed by a lethal i.v. dose of sodium pentobarbitone (60 mg kg^{-1}; C-Vet Ltd, Bury St Edmunds) and exsanguinated. A segment of thoracic aorta was excised and placed in physiological solution and connective tissue removed.

Cell isolation

Cells were enzymatically isolated by the method of Clapp and Gurney (1991). Cell preparation with this technique is extremely reproducible, and has been used for other vascular smooth muscle cells. In the case of large elastic arteries like the aorta, reproducibility is not attained with more conventional methods of cell isolation (discussed in Gurney, 1995). Briefly, thin strips of muscle were placed in the refrigerator (5–7°C) overnight in a low (160 μM) $CaCl_2$ dissociation medium, with ~0.3 mg ml^{-1} papain and 0.02% bovine serum albumin. The next morning the strips were incubated at 37°C for 10 min in the presence of the reducing agent dithiothrietol (1 mM). The strips were then transferred to fresh dissociation medium, and triturated with a wide bore Pasteur pipette to yield single cells. The ionic composition of the dissociation medium was as follows (mM): KCl 5, NaCl 110, NaH_2PO_4 0.5, $CaCl_2$ 0.16, $NaHCO_3$ 10, KH_2PO_4 0.5, HEPES 10, $MgCl_2$ 2, glucose 10, phenol red 0.03, taurine 10, EDTA 0.5, bubbled with 95% air/5% CO_2 to give a pH of 7.0.

Electrophysiological recordings and data analysis

Membrane potential and whole-cell currents were recorded by the conventional whole-cell configuration of the patch-clamp technique (Hamill *et al.*, 1981), with a Biologic RK300 amplifier (Intracel Ltd, Royston, Herts). Pipettes were pulled from borosilicate capillaries (Clark Electromedical Instruments, Berks.) and had resistances of 2–5 MΩ when filled with pipette solution. The junction potential (~2 mV) between the electrode and the bath solution was subtracted before obtaining a gigaseal. Corrections have not been made for changes in junction potential on obtaining the whole-cell configuration. Series resistances (R_s) were calculated for each cell from the decay time constant of the capacitative transient recorded in response to a 5 mV step. On average it was found to be 7±3 MΩ (n=100). R_s compensation (around 30–40%) was usually employed. The maximum voltage error in membrane potential resulting from R_s was 6 mV, calculated for the largest current of 0.8 nA recorded with an R_s of 8 MΩ. Current signals were filtered at 3 kHz for on-line data collection onto a Compaq 286 personal computer, and digitized at 10 kHz by use of the P-Clamp data acquisition software package with TL-1 interface (Axon Instruments, Burlingame, CA, U.S.A.). Data analysis was carried out by P-Clamp and Origin (Microsal Software Inc., Northampton, MA, U.S.A.) software. All results, unless otherwise stated, are expressed as mean± s.e.mean, and Student's t tests were used to assess statistical significance. Experiments were carried out at room temperature (~25°C), because the cells remained relaxed for a longer period than at higher temperatures.

Tension experiments

The aorta was cleared of endothelium by gentle rubbing of its inner surface with a cotton bud before every experiment, although its absence was not routinely tested for. The cleaned vessel was then cut into circumferential strips (1–2 mm wide), which were mounted in chambers (~0.2 ml) perfused with physiological solution bubbled with 95%/O_2/5% CO_2, and placed under 1 g of tension. Phentolamine 1 μM was added to the chamber in order to prevent the effects of noradrenaline released from sympathetic nerve terminals. Isometric tension recordings were made with a Harvard Apparatus (South Natick, MA, U.S.A.), model 60-2998 isometric transducer. Experiments were carried out at 37°C.

Drugs and solutions

Tension experiments were carried out in physiological solution of the following composition (mM): NaCl 112, KCl 5, $MgCl_2$ 1, $CaCl_2$ 1, NaH_2PO_4 0.5, KH_2PO_4 0.5, $NaHCO_3$ 15, HEPES 5, glucose 10 and phenol red 0.02. Solutions containing 50 mM K^+ were prepared by equimolar substitution of KCl for NaCl. Time- and voltage-dependent currents were recorded in a similar physiological solution which had the following composition (mM): KCl 5, NaCl 137, $MgSO_4$ 0.4, Na_2HPO_4 0.3, $CaCl_2$ 1.25, $NaHCO_3$ 4.2, KH_2PO_4 0.4, $MgCl_2$ 0.5, HEPES (N-2-hydroxyethylpiperazine-N'-2-ethanesulphonic acid) 5, glucose 5.5 and phenol red 0.03. All solutions were bubbled continuously with 95% O_2–5% CO_2 to give a pH of 7.3. The pipette (intracellular) solution had the following composition (mM): KCl 130, $MgCl_2$ 1, EGTA 1, sodium guanosine 5'-triphosphate (GTP, Sigma, Poole, Dorset, U.K.) 1 and HEPES 20, with pH adjusted to 7.3 with KOH. Cells or tissues were continuously perfused and drugs were applied via the perfusing solution. The following drugs were used: 4-aminopyridine (4-AP), imipramine hydrochloride, amitriptyline hydrochloride and phencyclidine hydrochloride (PCP, all from Sigma), tetraethylammonium chloride (TEA, Kodak, Rochester, N.Y. 16540), clofilium tosylate (Research Biomedical Incorporated, Natick, MA 01760), sotalol hydrochloride (Bristol-Myers Pharmaceuticals, Milton Road, Uxbridge UB10 8NS) and phentolamine mesylate (Ciba Laboratories, Horsham, Sussex). Clofilium was stored as a 10 mM stock solution in dimethylsulphoxide (DMSO), and all other drugs were made up as 10 mM stock solutions in water. Bath solution containing 4-AP was adjusted to pH 7.3 before use.

Results

Passive membrane properties

In normal physiological solution and with a K^+-based pipette solution, the zero current potential (i.e. resting potential) of single isolated aortic smooth muscle cells of the rabbit ranged from −39 to −51 mV with an average of −45±0.3 mV (n=100). These results are close to, but slightly lower than values obtained from intact aortic strips by use of intracellular microelectrodes; values of −50 mV, −52 mV and −55 mV have been obtained (Cauvin *et al.*, 1984; Bray *et al.*, 1988; Haeusler & De Peyer, 1989). The input resistance, measured from the change in current produced by a 10 mV hyperpolarizing step from −70 mV, was 11±2 GΩ (n=100). The capacitative transient decayed with an exponential time course, the time constant of which was 130±5 μs (n=100). The capacitance of the cell, which is proportional to the membrane surface area, was measured from the area under the transient, and found to be 19±0.3 pF (n=100).

Time- and voltage-dependent currents

Characteristics of outward currents Figure 1 illustrates typical voltage-clamp recordings of outward currents elicited from an

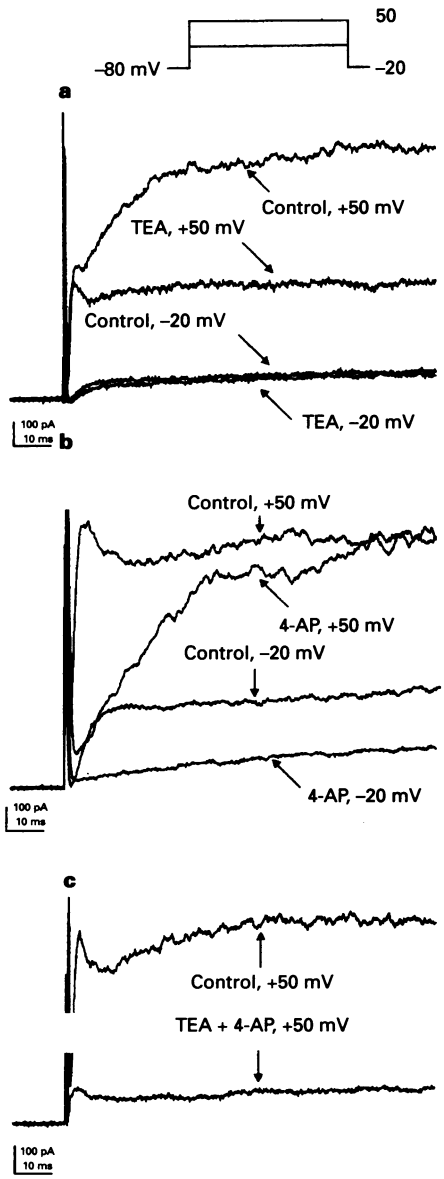

Figure 1 Currents stimulated at +50 mV and −20 mV in the absence and presence of (a) tetraethylammonium (TEA) 1 mM, (b) 4-aminopyridine (4-AP) and (c) at +50 mV in the absence and presence of TEA and 4-AP in combinations. All currents were elicited by stepping to the test potentials for 100 ms, from a holding potential of −80 mV. Peak currents were measured within the first 10 ms of the depolarizing pulse, and sustained currents within the last 20 ms.

isolated aortic smooth muscle cell in physiological solution and with K^+ pipette solution. Currents were stimulated by stepping to −20 mV or +50 mV from a holding potential of −80 mV, with an interpulse interval of 5 s. In all cells tested using this protocol, at least two components of time-dependent outward current could be distinguished, the amplitudes of both increasing with increasing depolarization. There was a transient component (I_t) which activated rapidly and inactivated within the first 15 ms of the command potential. This was followed by a noisy sustained component (I_{sus}), which failed to show significant inactivation at the end of the 100 ms test pulse. However, following the application of a longer (500 ms) voltage step, there were signs of inactivation developing (data not shown). Once the whole-cell configuration had been obtained, the amplitude of both I_t and I_{sus} increased or 'ran up' during the first few minutes of recording. On average, both I_t and I_{sus} increased 1.5 fold, with run up complete after about 1–2 min. Current run up of $I_{K(V)}$ in rabbit portal vein has been shown to depend on phosphorylation of the channel by protein kinase A (PKA, Aiello *et al.*, 1995). Although the patch pipette contained guanosine triphosphate (GTP) and not adenosine triphosphate (ATP), it is possible that run up observed in rabbit aorta occurs by a similar mechanism, owing to the presence of basal levels of ATP, adenosine 3′:5′-cyclic monophosphate (cyclic AMP) and/or free catalytic subunits of PKA. Current run up was not so prominent when experiments were performed in Ca^{2+}-free bath solution.

Previous studies on rabbit pulmonary artery (Clapp & Gurney, 1991) and portal vein (Beech & Bolton, 1989) demonstrated that a small net inward Ca^{2+} current precedes the outward K^+ current. In all cells examined from the rabbit aorta, a voltage-activated inward current was absent, implying that its amplitude must be small relative to the outward current. This is consistent with the inexcitability of the aorta under normal conditions.

Effects of K^+ channel blockers TEA blocks K^+ currents in many cells, but where potency of block from inside and outside have been investigated, striking differences are apparent between different cell types. Beech and Bolton (1989) found that TEA, when applied externally at low millimolar concentrations, was a selective inhibitor of Ca^{2+}-activated K^+ currents in rabbit isolated portal vein cells while leaving the delayed rectifier virtually unaffected. To determine whether the Ca^{2+}-activated current contributes to the macroscopic K^+ current in rabbit aorta, the effects of a low (1 mM) concentration of TEA were investigated on currents activated at −20 and +50 mV as illustrated in Figure 1a. TEA had little effect on the current at −20 mV, but markedly suppressed the noisier current activated at +50 mV. In contrast, I_t was only slightly reduced in the presence of TEA. This slight reduction presumably arose because I_t activation normally overlaps that of the TEA-sensitive current. Figure 2a and d show the voltage-dependence of TEA block of I_t and I_{sus}, respectively. TEA had little effect on I_t at any voltage tested, or on I_{sus} recorded at negative membrane potentials. Inhibition of I_{sus} by TEA was only observed at 0 mV or more positive potentials, implying that I_{KCa} was only activated over this voltage range. In 8 cells examined, I_t was reduced by 6±2%, and the amplitude of I_{sus} by 40±6% at +50 mV. The effects of TEA on I_{sus} could be completely reversed by washing the drug out. When the concentration of TEA was increased to 10 mM, no further effect on either current was observed, implying that the inhibition was maximal at 1 mM. Thus around 40% of I_{sus} at +50 mV appears to be carried through large conductance Ca^{2+}-activated K^+ channels.

The TEA-insensitive current appeared to have slow inactivation kinetics, and it was sustained for as long as the depolarizing steps were applied (up to 500 ms). In this respect it resembled delayed rectifier currents ($I_{K(V)}$) described in other excitable cells. Delayed rectifier and A currents have been shown to be sensitive to block by the convulsant 4-aminopyridine and its derivatives in a variety of excitable tissues, including rabbit portal vein (Aiello *et al.*, 1995), neurones (Grolleau & Lapied, 1995) and skeletal muscle (Brinkmeier *et al.*, 1991). The effects of 2 mM 4-AP on currents activated at −20 and +50 mV are illustrated in Figure 1b. At −20 mV, 4-AP caused a general depression of the outward current. At +50 mV, however, I_t was blocked almost completely, whereas a large proportion of I_{sus} remained. The voltage-dependence of 4-AP block of I_t and I_{sus} is illustrated in Figures 2b and e. 4-AP blocked I_t over the entire range of step potentials applied. It is likely that the slight relief of block of I_t at positive potentials reflects the activation of a component of I_{sus} which is normally superimposed on I_t. In contrast, block of I_{sus} showed voltage-dependence in that the block was substantially relieved at very positive potentials. In 6 cells examined, 2 mM 4-AP reduced I_t by 75±10%, and I_{sus} by 33±8% at +50 mV. Thus at least a third of I_{sus} at +50 mV appears to be composed of $I_{K(V)}$, although this may be an underestimate due to voltage-dependent effects.

In order to confirm that the TEA-insensitive outward current was carried principally by K^+, the tail current reversal potential was estimated for 4 cells using a double-pulse pro-

tocol (Figure 3). Cells were held at −80 mV and depolarized to +50 mV for 150 ms, to activate the voltage-dependent channels. Following this pre-pulse the membrane potential was stepped back for 150 ms to various more negative test potentials and the resulting tail current measured after leak subtracting the records. The reversal potential was estimated from a plot of the tail current amplitude against the test potentials, where it was equivalent to the point of intersection with the voltage axis and was -67 ± 2 mV ($n=4$; Figure 3b). In the experimental conditions employed, the reversal potential for K^+ calculated according to the Nernst equation was −80 mV. The measured value is close to but not identical to the estimated value. It is therefore likely that the outward current is carried mainly by K^+, although additional ions may contribute.

The combination of both TEA (1 mM) and 4-AP (2 mM) blocked almost all the outward current at +50 mV, leaving only a small residual current (Figure 1c). This implies that the TEA-insensitive component is probably carried mostly through delayed rectifier channels, and that I_{sus} reflects largely the combined activity of delayed rectifier and Ca^{2+}-activated K^+ channels. The current-voltage relationships for I_t and I_{sus} in the presence of 4-AP and TEA in combination are shown in Figure 2c and f. In both cases, the currents were markedly but not completely suppressed, with $71 \pm 5\%$ and $75 \pm 4\%$ ($n=4$) suppression of I_t and I_{sus}, respectively at +50 mV. These values suggest that block of I_t and I_{sus} by 4-AP and TEA are additive.

Several other drugs were tested in an attempt to dissect out individual components of K^+ channel current from the macroscopic K^+ current. The drugs used were previously found to be specific blockers of particular K^+ channels in other preparations (Carmeliet, 1985; Arena & Cass, 1988; Delpon *et al.*, 1992; Reeve & Peers, 1992). In general however, these proved to be of little value in the characterization of K^+ channels in rabbit aorta.

Figure 4a shows that at a concentration of 10 μM, these drugs had little effect on I_t or I_{sus}, with the exception of PCP,

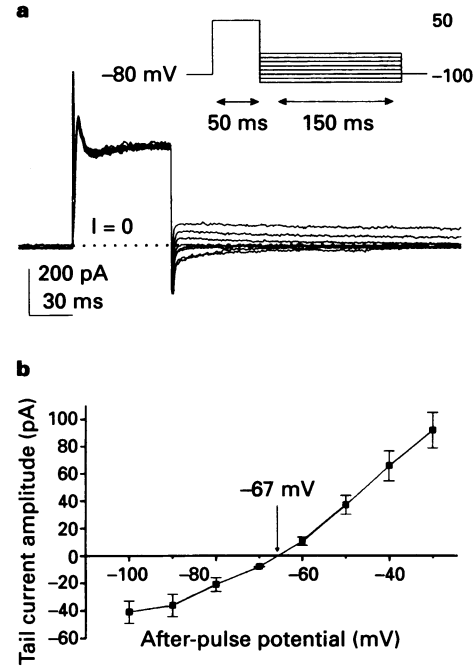

Figure 3 Tail current reversal potential for the 4-aminopyridine sensitive current ($I_{K(V)}$) of the outward K^+ current of rabbit isolated aortic smooth muscle cells. The tail current reversal potential was estimated (for 4 cells) by holding the cells at −80 mV and depolarizing to +50 mV for 150 ms (a). For further details see text. Values in (b) show mean ± s.e.mean ($n=4$).

which caused a substantial block of I_{sus} whilst I_t remained virtually unchanged. Figure 4b shows the effects of raising the concentration of each drug to 100 μM. In every case, I_{sus} was

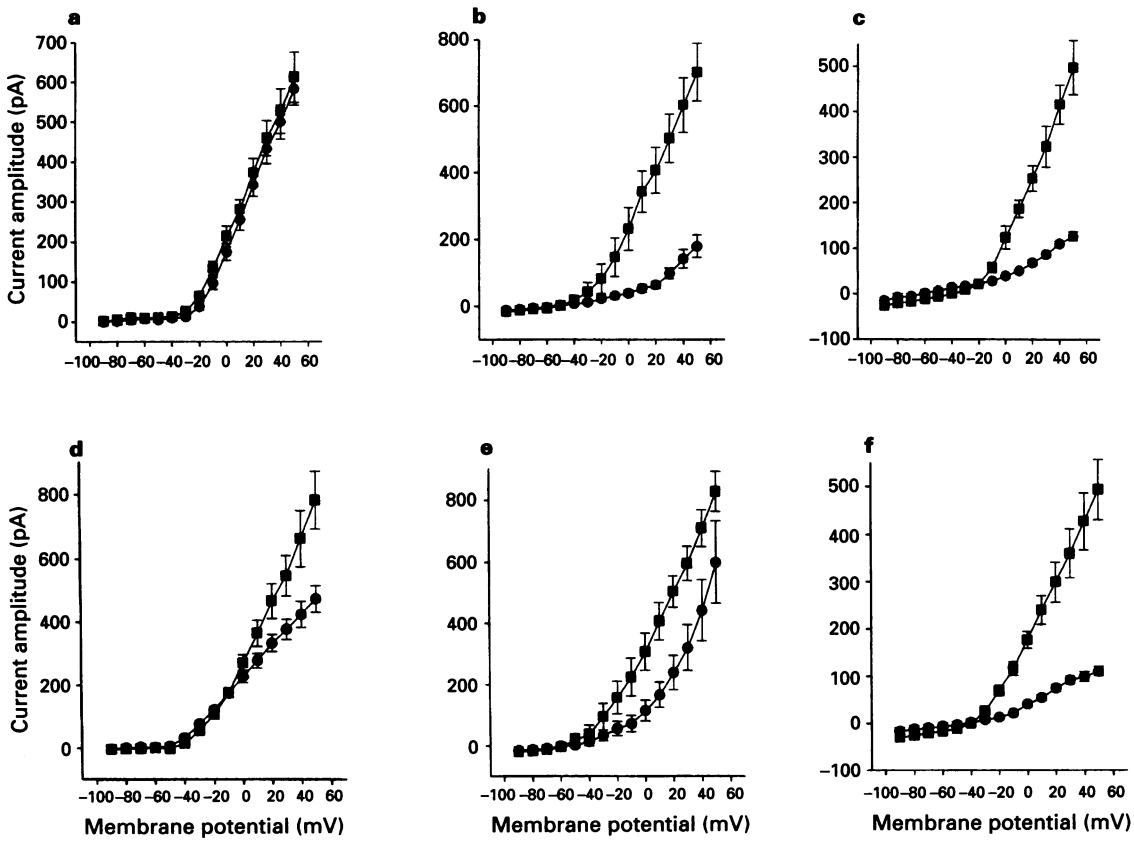

Figure 2 Current-voltage relationships for peak current (a,b,c) and sustained current (d,e,f), showing in each case the control current (■) versus the current in the presence (●) of either (a,d) tetraethylammonium (TEA) 1 mM, (b,e) 4-aminopyridine (4-AP) 2 mM or (c,f) TEA and 4-AP together. Values shown are means ± s.e.mean.

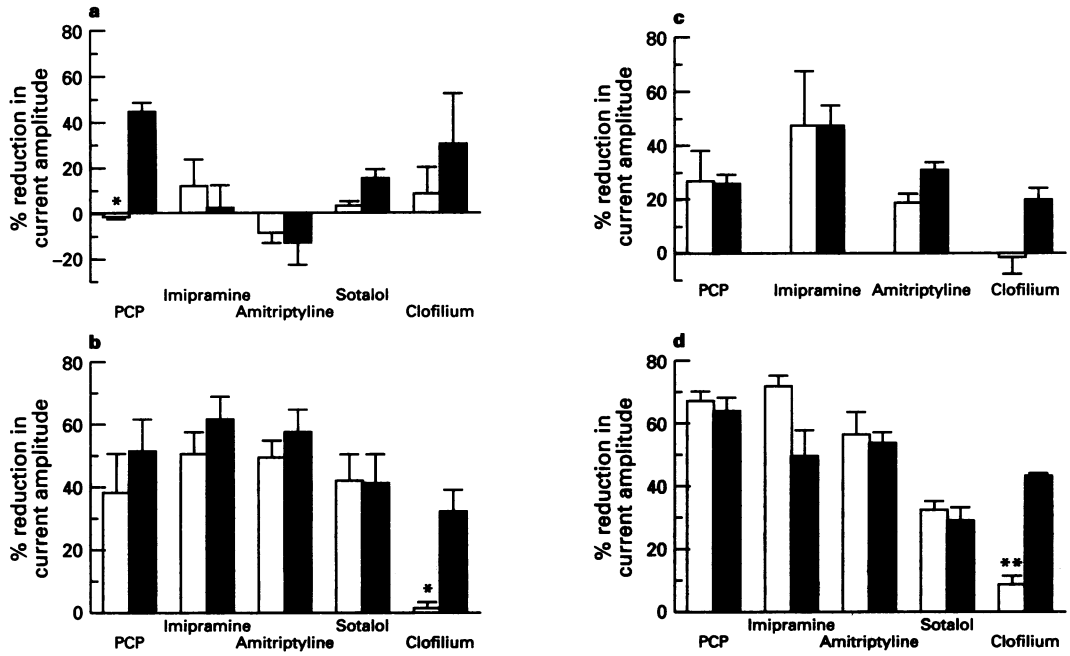

Figure 4 Effects of various K^+ channel blockers on the peak (open columns) and sustained (shaded columns) currents in the absence of tetraethylammonium (TEA) at 10 μM (a) and 100 μM (b), and in the presence of 3 mM TEA at 10 μM (c) or 100 μM (d). PCP, phencyclidine hydrochloride.

blocked substantially. With the exception of clofilium, which appeared to cause selective inhibition of I_{sus}, all drugs also caused substantial block of I_t.

These experiments were repeated in the presence of 3 mM TEA (Figure 4c and d), in order to block the Ca^{2+}-activated K^+ currents and compare the influence of the different drugs on $I_{K(V)}$ and A-like currents. Under these conditions, 10 μM PCP, imipramine or amitriptyline caused significant blockade of both transient and sustained currents. Imipramine, in particular, was much more effective in the presence of TEA than in its absence, implying that it has a preferential action on delayed rectifier channels. PCP was slightly less effective at reducing the current in the presence of TEA, suggesting that it also inhibits Ca^{2+}-activated K^+ currents. Clofilium had no effect on I_t. The pattern of non-selective blockade of I_t and $I_{K(V)}$ was also apparent when the drug concentrations were raised to 100 μM. Although, at 100 μM, the effect of PCP was slightly larger in the presence of TEA than in its absence, imipramine, amitriptyline and sotalol all appeared to cause a similar degree of block whether or not TEA was present. Again, clofilium appeared to cause a selective blockade of $I_{K(V)}$. However, since the degree of block was little affected by the presence of TEA, it did not appear to select between the delayed rectifier or Ca^{2+}-activated K^+ currents.

The experimental protocol used to test the effects of these drugs involved measuring the currents evoked during depolarizing steps applied at 5 s intervals. While perfusing the drug onto the cell, once the change in amplitude of the macroscopic current had reached a steady level, it was assumed that the drug had reached its maximum concentration at the channel pore. In the case of clofilium however, it was noted that once steady-state block of the current had been attained, cessation of stimulation for a 2 min period without removing the drug, resulted in recovery of the current amplitude almost to the level observed before the drug was applied. Upon resumption of the stimulation protocol, current amplitude was reduced again back to the steady-state block level. This suggested that the block of the K^+ channels by clofilium was use-dependent. This was investigated further by applying a series of depolarizing steps with varying inter-pulse intervals, in the presence of TEA (1 mM) to suppress $I_{K(Ca)}$. With a brief (1 s) interval, both I_t and $I_{K(V)}$ were reduced to a similar extent once steady state block had been obtained (58±10%, $n=3$; 76±6%, $n=3$ respectively, Figure 5a). This reduction did not reflect run-down of the currents due to the rapid stimulation, because repetition of this protocol in the absence of drug showed that the current amplitudes were unaltered. Increasing the interval to 5 s

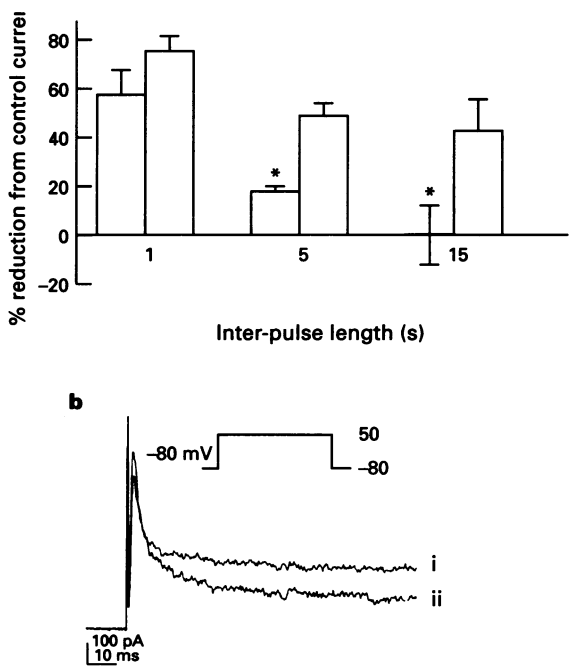

Figure 5 (a) The effects of clofilium 10 μM in the presence of tetraethylammonium (TEA) 3 mM, on the peak (left columns) and sustained (right columns) currents. Currents were stimulated by stepping to +50 mV for 100 ms from a holding potential of −80 mV, with either 1, 5 or 15 s intervals in between the depolarizing steps. All current amplitudes shown are measured at steady state block. (b) The control current (i) and the current at steady state (ii) after block by clofilium 10 μM in the presence of TEA 3 mM, with 15 s intervals in between pulses.

caused a significant difference between the degree of block of I_t (18±2%; $n=3$) and $I_{K(V)}$ (49±5%, $n=3$), the latter being blocked significantly more than I_t. Increasing the inter-pulse interval to 15 s caused $I_{K(V)}$ to be blocked to approximately the same extent as with a 5 s interval, while the block of I_t was essentially absent. In some experiments an increase in I_t was observed during stimulation with a 15 s interpulse interval in the presence of 10 μM clofilium, as shown in Figure 5b. Despite the enhanced amplitude of I_t, its inactivation appears to have been accelerated by clofilium. A similar acceleration of I_t inactivation was apparent in all cells examined.

From these results it seems that apparent selectivity of clofilium for $I_{K(V)}$ compared with I_t is in fact dependent upon the frequency of test pulses applied; the differential effect of the drug disappeared as the stepping frequency was increased.

Inactivation of I_t and I_{sus} In a further attempt to separate $I_{K(V)}$ from I_t, we examined the voltage-sensitivity of inactivation of these two components of current. These experiments were carried out in the presence of 1 mM TEA to suppress $I_{K(Ca)}$. A two-pulse protocol, which involved stepping to a test pulse of +50 mV from progressively more depolarized pre-pulse potentials of 60 s duration was used, in order to examine the steady state inactivation of these components. In 4 cells examined, I_t showed half-maximal inactivation at −51±3 mV while half-maximal inactivation of $I_{K(V)}$ occurred at −47±2 mV (Figure 6c). Following a pre-pulse to −30 mV there was no significant difference between the inactivation of I_t and $I_{K(V)}$ with both components almost completely inactivated (Figure 6a and c). This protocol was therefore not helpful in separating the 2 components.

However, in addition to the potential of the pre-pulse its duration is an important factor in determining inactivation. Decreasing the duration of the pre-pulse at −30 mV to 100 ms resulted in minimal inactivation of $I_{K(V)}$, but caused pronounced inactivation of I_t (Figure 6b,d,). Indeed, the inactivation of $I_{K(V)}$ was so minimal that the data could not be fitted to a Boltzman inactivation curve, unlike I_t (Figure 6d). The extent of inactivation of I_t was probably underestimated, because this current was superimposed upon $I_{K(V)}$, which developed almost immediately during the test pulse, probably because it was already partially activated during the pre-pulse to −30 mV. Even so, Figure 6b and d show that I_t and $I_{K(V)}$ were partially separable on the basis of their differential rates of inactivation at −30 mV.

After evaluation of the steady state (60 s) inactivation the protocol was repeated in the absence of TEA, where Ca^{2+}-activated K^+ channels should contribute to the outward current; the steady state inactivation appeared to be virtually identical to that recorded in the presence of TEA. Half maximal inactivation of I_t occurred at −50±2 mV, and that of I_{sus} at −45±4 mV. This may reflect inactivation of voltage-dependent Ca^{2+} channels during the pre-pulse, which would occur at depolarized potentials and lead to a reduction of Ca^{2+} entry and subsequent reduction of Ca^{2+}-activated K^+ channel activation. Inactivation of the outward current was never complete, even using very depolarized pre-pulses. With a 60 s pre-pulse to −10 mV, 18±5% ($n=4$) of the maximum current

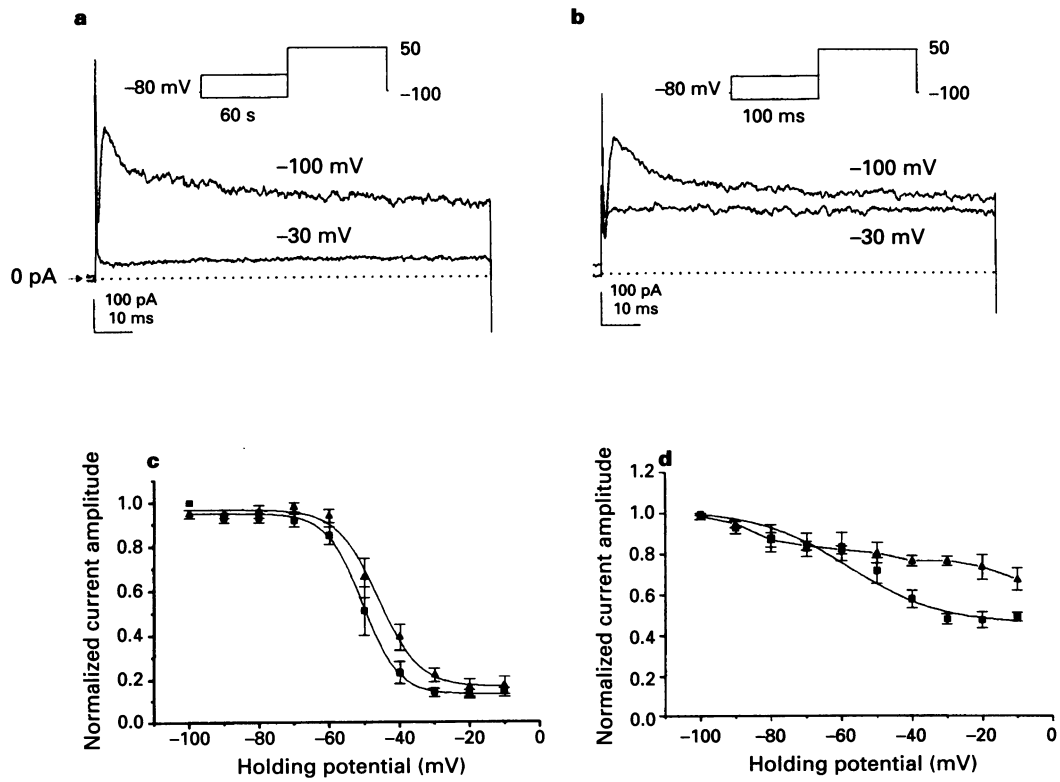

Figure 6 Voltage-dependent inactivation of the transient (I_t) and sustained (I_{sus}) components of the outward K^+ currents in the presence of tetraethylammonium (TEA) 1 mM. (a) Currents were evoked by holding the membrane potential for 60 s at −100 or −30 mV, and stepping to the test potential of +50 mV for 100 ms. (b) The same protocol as (a), except that the pre-pulse duration was 100 ms. (c) Normalized current amplitudes plotted against pre-pulse potential for peak (■) and sustained (▲) currents with a 60 s pre-pulse. The curves are fits to each set of data of the Boltzmann equation:

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

where I_{max} and I_{min} are the maximum and minimum of the curve, V_h the pre-pulse potential giving 50% inactivation and V_s the slope factor. For I_{sus}: $V_h=47\pm2$ mV, $V_s=5.8$ and $I_{min}=0.17$. For I_t: $V_h=-51\pm3$, $V_s=5.1$ and $I_{min}=0.13$ (d) as in (c) with a 100 ms pre-pulse. Values in (c) and (d) are means±s.e.mean.

remained in the presence of TEA and 18±3% ($n=4$) in its absence. Thus almost 20% of the outward current appears to be non-inactivating. The nature of this component has not been investigated further in this study, but it could reflect a non-inactivating component of $I_{K(V)}$, such as has recently been described by Overturf *et al.* (1994) in colonic circular smooth muscle.

Tension studies

In addition to their effects on the macroscopic K^+ currents recorded from isolated aortic cells, the effects of K^+ channel blockers on aortic tone were also investigated (Figure 7, Table 1). Each drug was applied separately and the resulting contraction was measured as a percentage of the contraction induced by 50 mM K^+ in the same tissue. This concentration of K^+ has previously been shown to cause maximal contractions in rabbit aortic rings (Cook *et al.*, 1988). PCP (Figure 7, Table 1) and imipramine (Table 1), both applied at 100 μM, caused only minor increases in basal tension while clofilium (100 μM) caused slightly larger increases. TEA (1 mM) and 4-AP (1 mM) caused greater force development, 4-AP sometimes causing the tension to oscillate (Figure 7). However, the effects of 4-AP and TEA were very variable between preparations as indicated by the range of values in Table 1. When applied together, TEA and 4-AP caused near maximal contractions, the amplitudes of which were larger than the sum of the two individual contractions (Figure 7). Contractions caused by all of the K^+ channel blocking drugs were transient in nature, the amplitude of contractions diminishing before the drugs were washed off (Figure 7).

When applied in the presence of 50 mM K^+, all of the drugs tested caused partial relaxation, with the exception of TEA and 4-AP which enhanced the high K^+ contracture (Table 2). This suggests that PCP, imipramine and clofilium interfere with Ca^{2+} entry into the cell, since depolarization caused by high K^+ is thought to reflect mainly Ca^{2+} entry through voltage-gated Ca^{2+} channels. The effects of 4-AP (1 mM), TEA (1 mM) and PCP (100 μM) were readily reversible on washout. Recovery from imipramine block took at least 30 min while clofilium block persisted even longer but did eventually reverse (1–2 h).

Table 1 Influence of K^+ channel blocking drugs on basal tension of rabbit aortic muscle strips

Drug	*Concentration*	*% of 50 mM K^+ contraction* (Mean ± s.e.mean)	(Range)	*No. of animals**
4-AP	1 mM	26±7	7–96	8
TEA	1 mM	18±5	3–58	8
4-AP+TEA	1 mM each	89±22	42–127	3
Imipramine	100 μM	7±2	0–20	5
PCP	100 μM	4±2	0–9	4
Clofilium	100 μM	14±1	6–22	4

*Each drug was tested on two muscle strips from each animal.
Abbreviations used: 4-AP, 4-aminopyridine; TEA, tetraethylammonium; PCP, phencyclidine hydrochloride.

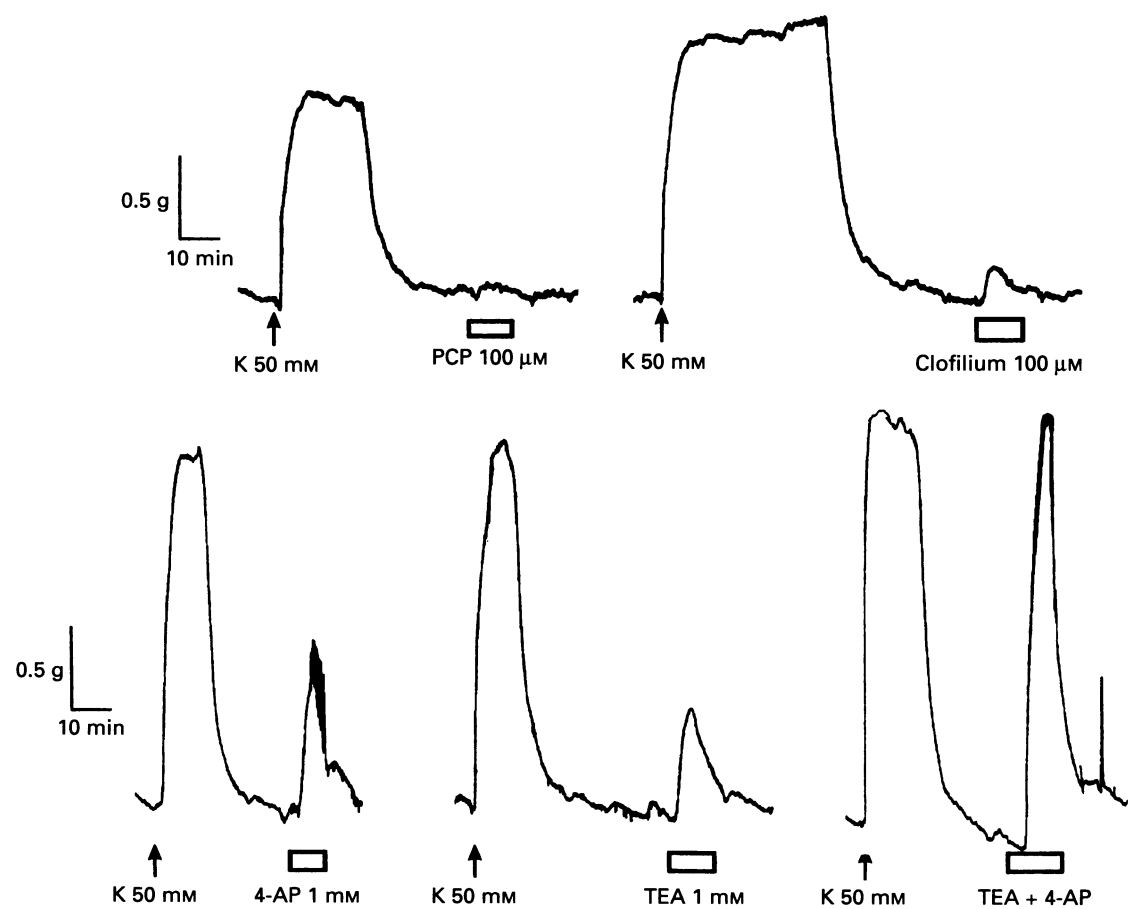

Figure 7 The effects of various K^+ channel blockers on tension recorded from a strip of rabbit aorta. Application of 50 mM K^+ is shown by arrows. Following recovery of basal tension, after returning to physiological K^+ solution, K^+ channel blockers were applied for the periods indicated by the bars. Abbreviation used: PCP, phencyclidine hydrochloride; 4-AP, 4-aminopyridine; TEA, tetraethylammonium.

Table 2 Effect of drugs on contractions induced by 50 mM K^+

Drug	Concentration	% facilitation	% block	No. of animals
TEA	1 mM	48 ± 22	–	3
4-AP	1 mM	37 ± 16	–	3
4-AP	2 mM	52 ± 22	–	3
PCP	100 μM	–	37 ± 2	4
Imipramine	100 μM	–	89 ± 4	5
Clofilium	100 μM	–	82 ± 5	5

Data shown are means ± s.e.mean.
For key to abbreviations used see legend of Table 1.

Discussion

The present study demonstrated the existence of both Ca^{2+}-activated and time- and voltage-dependent K^+ current components in rabbit aortic smooth muscle. The classical K^+ channel blocking drugs TEA and 4-AP, as well as a series of other drugs which have a much broader pharmacological spectrum of activity, were used in the characterization of these currents. Because these drugs have been shown to block particular K^+ currents in various tissues, albeit in addition to other ionic channels, we investigated the possibility that they might have specific blocking actions on K^+ channels in rabbit aortic smooth muscle cells.

It has been shown previously that low concentrations of TEA (<3 mM) are effective at blocking large-conductance Ca^{2+} activated K^+ channels (Inoue *et al.*, 1985). These channels are activated upon stepping to potentials positive to 0 mV and the macroscopic currents they give rise to are typically noisy owing to a large single-channel conductance (Beech & Bolton, 1989). It seems likely then that the component of I_{sus} that was blocked by TEA was a Ca^{2+}-activated K^+ current, since TEA reduced the current only at potentials above 0 mV, with concomitant reduction in the current noise. On this basis, approximately 40% of the outward K^+ current at +50 mV was composed of $I_{K(Ca)}$ in the rabbit aorta.

In the presence of TEA, the residual current showed well-defined transient and sustained components, with the voltage-dependent characteristics of 'A'-type and delayed rectifier currents. The transient current (I_t) observed upon stepping to a relatively depolarized potential had rapid kinetics, and was blocked by low concentrations of 4-AP but was virtually insensitive to block by TEA (≤10 mM). It was also highly sensitive to the holding potential, with 50% inactivation occurring at −50 mV. In all these respects, I_t closely resembled the transient K^+ current of rabbit pulmonary artery (Clapp & Gurney, 1991).

The sustained component recorded in the presence of TEA, was also markedly reduced upon application of 4-AP, suggesting that it was carried through delayed rectifier channels (Beech & Bolton, 1989; Smirnov & Aaronson, 1992). At +50 mV this $I_{K(V)}$ contributed approximately one third of the total sustained outward K^+ current, while at 0 mV it was clearly predominant. Its block by 4-AP was partially relieved at positive potentials. This may be explained in terms of the location of the 4-AP-binding site, and also by the positive charge which the 4-AP molecule carries. It has been suggested (Yeh *et al.*, 1976) that 4-AP does not alter the normal kinetic behaviour of the channel, but that channels which have the drug bound do not conduct. At negative potentials favourable electrostatic interactions between the positively charged 4-AP molecule and the binding site within the membrane's electrical field help to maintain 4-AP within the K^+ channel pore. The exact location of the binding site is unknown, but if it is towards the cytoplasmic side of the membrane, then with increasing depolarization the 4-AP molecule would be repelled away from the binding site and the block relieved. The residual current observed at positive potentials when TEA and 4-AP were applied together may, therefore, reflect the partial relief of 4-AP block of $I_{K(V)}$, although we cannot rule out the possibility that it represents an additional component of current not sensitive to TEA or 4-AP.

A number of voltage-dependent K^+ currents have been identified in cardiac myocytes, including inward rectifier K^+ channels (I_{KI}), transient outward channels (I_{to}) and a delayed rectifier ($I_{K(V)}$) which can be further subdivided into rapidly and very slowly activating components (Sanguinetti, 1992). Several class III antidysrhythmic drugs are relatively selective cardiac K^+ channel blockers. For example, clofilium tosylate has been shown to cause a voltage-dependent block of $I_{K(V)}$ in cardiac myocytes, but is without effect on I_{KI} (Arena & Kass, 1988). In addition, sotalol exhibits selective block in that it inhibits $I_{K(V)}$ by 50% at 10 μM but is without effect on I_{to} or I_{KI} at this concentration (Sanguinetti, 1992). The tricyclic antidepressant, imipramine, has been shown to cause a concentration-dependent block of the transient outward K^+ current in rabbit atria, but is without effect on the more slowly inactivating I_{K1} found in guinea-pig ventricular myocytes (Delpon *et al.*, 1992). Despite these drugs having specific blocking actions on various types of K^+ channels, however, they can in other respects be considered non-specific. For example, imipramine (Delpon *et al.*, 1992) and clofilium (Arena & Kass, 1988) both block Ca^{2+} channels, whilst sotalol causes β-adrenoceptor block (Groh *et al.*, 1995), which may disrupt phosphorylation-dependent regulation of certain K^+ channels (Aiello *et al.*, 1995).

Because of their dissimilar actions on several voltage-gated cardiac K^+ current these drugs were applied in the presence of 3 mM TEA in an attempt to dissect I_t and $I_{K(V)}$ in rabbit aorta. Little selectivity was, however, observed when these drugs were applied at either 10 or 100 μM, except in the case of clofilium, which blocked the $I_{K(V)}$ to a much greater extent than I_t. In addition 10 μM imipramine and amitriptyline were more effective blockers of the current in the presence of TEA than in its absence, suggesting that these drugs had some selectivity for $I_{K(V)}$ and I_t over $I_{K(Ca)}$.

In the absence of TEA, 10 μM PCP caused a selective block of the sustained current, presumably dominated by $I_{K(Ca)}$. This may have been secondary to blockade of voltage-dependent Ca^{2+} channels, as PCP has been shown to block both Na^+ and Ca^{2+} channels in guinea-pig ventricular myocytes (Hadley & Hume, 1986). Although the inward Ca^{2+} current in rabbit aortic myocytes was small relative to outward current, its blockade may significantly reduce Ca^{2+} entry into the cell, and hence reduce the stimulation of Ca^{2+}-activated K^+ channels.

These experiments with clofilium in the presence of TEA suggested that it was selectively blocking $I_{K(V)}$, leaving I_t unaffected. However, as previously found (Reeve & Peers, 1992), the blocking action of this drug was on closer inspection clearly use-dependent, implying that it interacted with channels only after they were activated by depolarization (Figure 5). The accumulation of block at higher stimulation frequencies could be explained if clofilium dissociates from its binding site slowly, over a period of seconds. Thus a 1 s inter-pulse interval would not be long enough to allow all of the channels to become unblocked between pulses. Increasing the interval between depolarizing pulses would allow a higher proportion of clofilium molecules to dissociate from the channels between steps, so that once an equilibrium was established, the net block of the channels would be lower. A second feature of the block by clofilium which suggested that it is an open-channel blocker, was that although the mean amplitude of I_t was unaffected by the drug at low frequency stimulation, the rate of its inactivation was accelerated (Figure 4b). As stimulation frequency was increased, the amplitude of I_t was also reduced. These properties are consistent with the drug entering open I_t channels, leading to progressive block. Open-channel blockade by cloflium has also been demonstrated in a human delayed rectifier K^+ channel (Malayev *et al.*, 1995). The lack of effect of clofilium on the amplitude of I_t at low stimulation frequencies is in marked contrast to the effect of the drug on the

transient current in cloned cardiac potassium channels (Kobayashi *et al.*, 1995). In this case, clofilium enhanced the transient current from a holding potential of −60 mV, as well as accelerating current inactivation. The former effect was attributed to the presence of an extracellular binding site, which was separate from the binding site causing channel inactivation. Although the effects of clofilium on outward current at −60 mV in the rabbit aorta were also investigated (data not shown), no consistent augmentation of I_t was observed. We observed a slight augmentation in 3/4 cells held at −80 mV but did not study this effect further.

The non selective actions of the other drugs tested, as well as the use-dependent properties of the actions of clofilium meant that we were not able to achieve a pharmacological dissection of I_t and $I_{K(V)}$. It was, however, possible to dissect partially the two currents using an inactivation protocol with a short prepulse duration, providing some evidence that I_t and $I_{K(V)}$ were mediated by separate K^+ channels. It has, however, recently been suggested that delayed rectifier K^+ channels in smooth muscle exist as heteromultimers (Russell *et al*, 1994), i.e. they could be assembled from different K^+ gene products, giving rise to hybrid channels with a range of different kinetic and pharmacological properties. This possibility has been demonstrated using a human cardiac K^+ channel isoform, human Kv1.4 (Po *et al.*, 1993) which, when expressed in *Xenopus* oocytes, has properties similar to the cardiac transient outward current. The native channel recovers from voltage-dependent inactivation much faster than the cloned channel, the explanation being that the native channel exists as a heterotetramer, with 'A' current subunits as well as delayed rectifier subunits present. Although the existence of such heterotetrameric K^+ channels *in vivo* has not been proven yet, this phenomenon has been proposed to explain the K^+ channel diversity in the mammalian nervous system (Beckh & Pongs, 1990). One possible explanation for the lack of selectivity of these drugs in blocking I_t and $I_{K(V)}$ is that these represent somewhat different heterotetramers which have similar pharmacological and activation properties, but differ slightly in their voltage-dependent inactivation.

Although all of the K^+ channel blocking drugs examined had pronounced effects on K^+ currents, these effects were not in every case reflected in the tension studies. 4-AP and TEA by themselves clearly produced an increase in tension. It is interesting that TEA was effective despite the finding that it only blocked currents activated above 0 mV. This suggests that in intact tissues, where the intracellular environment is undisturbed, Ca^{2+}-activated K^+ channels are active at more negative potentials. The brief nature of the contractions produced by these K^+ channel blockers suggest that when the tissue is depolarized in response to blockade of one type of channel, the others are effective in counteracting the depolarization and returning the membrane potential back toward the resting level. Contractions produced when TEA and 4-AP were present together were greater than the sum of the individual contractions, and were partially, although not completely, sustained during drug application. Thus blocking two types of K^+ channel simultaneously is more effective, possibly because the depolarization cannot be completely counteracted. The response to TEA plus 4-AP did eventually wane, despite the continued presence of the drugs. The reason for this is not clear, but it could reflect voltage-dependent inactivation of the Ca^{2+} channels through which Ca^{2+} enters to promote contraction.

Unlike TEA and 4-AP, the other K^+ channel blockers failed to cause significant increases in tension at 100 μM, although they had pronounced effects on all components of current. It may be relevant that these drugs caused block of the contractions induced by 50 mM K^+, suggesting that they may also have been blocking voltage-gated Ca^{2+} channels. Block of Ca^{2+} channels by PCP, imipramine and clofilium has in fact been demonstrated in other tissues (Hadley & Hume, 1986; Arena & Kass, 1988; Delpon *et al.*, 1991), indicative of a non-specific blocking action by these drugs. Thus, the non-selectivity of these drugs may have obscured any correlation between the actions of the drugs on K^+ currents investigated and their effects on tension of intact aorta.

This work was supported by the British Heart Foundation. We thank Ms Alpa Ahir for carrying out the tension experiments.

References

AIELLO, E.A., WALSH, M.P. & COLE, W.C. (1995). Phosphorylation by protein kinase A enhances delayed rectifier K^+ current in rabbit vascular smooth muscle cells. *Am. J. Physiol.*, **268,** H926–H934.

ARENA, J.P. & KASS, R.S. (1988). Block of heart potassium channels by clofilium and its tertiary analogs: relationship between drug structure and type of channel blocked. *Mol. Pharmacol.*, **34,** 60–66.

BECKH, S. & PONGS, O. (1990). Members of the RKC potassium channel family are differentially expressed in the rat nervous system. *EMBO J.*, **9,** 777–782.

BEECH, D.J. & BOLTON, T.B. (1989). Two components of potassium current activated by depolarization of single smooth muscle cells from the rabbit portal vein. *J. Physiol.*, **418,** 293–309.

BOLTON, T.B., LANG, R.J., TAKEWAKI, T. & BENHAM, C.D. (1985). Patch and whole-cell voltage clamp of single mammalian visceral and vascular smooth muscle cells. *Experientia*, **41,** 887–894.

BRAY, K.M., WESTON, A.H., MCHARG, A.D., NEWGREEN, D.T. & SOUTHERTON, J.S. (1988). Comparison of the inhibitory effect of BRL34915 and nifedipine on noradrenaline- and KCl-induced contractions in rabbit aorta. *Br. J. Pharmacol.*, **93,** 205P.

BRINKMEIER, H., ZACHER, E. & RUDEL, R. (1991). Voltage-dependent K^+ channels in the sarcolemma of mouse skeletal muscle. *Pflugers Arch.*, **419,** 486–491.

CARMELEIT, E. (1985). Electrophysiological and voltage clamp analysis of the effects of sotalol on isolated cardiac muscle and Purkinje fibres. *J. Pharmacol. Exp. Ther.*, **232,** 817–825.

CASTEELS, R., KITAMURA, K., KURIYAMA, H. & SUZUKI, H. (1977). The membrane properties of the smooth muscle cells of the rabbit main pulmonary artery. *J. Physiol.*, **271,** 41–61.

CAUVIN, C., LUKEMAN, S., CAMERON, J., HWANG, O., MEISHERI, K., YAMAMOTO, H. & VAN BREEMAN, C. (1984). Theoretical bases for vascular selectivity of Ca^{2+} antagonist. *J. Cardiovasc. Pharmacol.*, **6,** (Suppl. 4), S630.

CLAPP, L.H. & GURNEY, A.M. (1991). Outward currents in rabbit pulmonary artery cells dissociated with a new technique. *Exp. Physiol.*, **76,** 677–693.

COOK, N.S., WEIR, S.W. & DANZEISEN, M.C. (1988). Antivasoconstrictor effects of the K^+ channel opener cromakalim on the rabbit aorta - comparison with the calcium antagonist isradipine. *Br. J. Pharmacol.*, **95,** 741–752.

DELPON, E., TAMARGO, J. & SANCHEZ-CHAPULA, J. (1992). Effects of imipramine on the transient outward current in rabbit atrial single cells. *Br. J. Pharmacol.*, **106,** 464–469.

DROOGMANS, G., RAEYMAEKERS, L. & CASTEELS, R. (1977). Electro- and pharmacomechanical coupling in the smooth muscle cells of the rabbit ear artery. *J. Gen. Physiol.*, **70,** 129–48.

GROH, W.J., GIBSON, K.J., MCANULTY, J.H. & MAYLIE, J.G. (1995). β-adrenergic blocking property of dl-sotalol maintains class III efficacy in guinea-pig ventricular muscle after isoproterenol. *Circulation*, **92,** 262–264.

GROLLEAU, F. & LAPIED, B. (1995). Separation and identification of multiple K^+ currents regulating the pacemaker activity of insect neurosecretory cells. *J. Neurophysiol.*, **73,** 160–171.

GURNEY, A.M. (1996). Single cell techniques In *Pharmacology of Vascular Smooth Muscle*, ed. Garland, C.J. & Angus, J. pp 42–69. Oxford: Oxford University Press.

HADLEY, R.W. & HUME, J.R. (1986). Actions of phencyclidine on the action potential and membrane current of single guinea-pig myocytes. *J. Pharmacol. Exp. Ther.*, **237**, 131–136.

HAEUSLER, G. & DE PEYER, J.-E. (1989). Rabbit aorta: electrical properties and agonist-induced depolarization. *Eur. J. Pharmacol.*, **166**, 175–182.

HALLIDAY, F.C., AARONSON, P.I. & GURNEY, A.M. (1994). K^+ currents in isolated rabbit aortic smooth muscle cells. *J. Physiol.*, **477**, 44P.

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. *Pflugers Archiv.*, **391**, 85–100.

HUME, J.R. & LEBLANC, N. (1989). Macroscopic K^+ currents in single smooth muscle cells of the rabbit portal vein. *J. Physiol.*, **413**, 49–73.

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. *Pflugers Archiv.*, **405**, 173–179.

KOBAYASHI, Y., MIKALA, G. & YATANI, A. (1995). A novel enhancing effect of clofilium on transient outward-type cloned cardiac K^+ channel currents. *Br. J. Pharmacol.*, **114**, 1222–1226.

LONGMORE, J. & WESTON, A.H. (1990). In *Potassium Channels–Structure, Classification, Function and Therapeutic Potential* ed. Cook, N.S. pp 259–278. Chichester. Ellis Horwood Ltd.

MALAYEV, A.A., NELSON, D.J. & PHILIPSON, L.H. (1995). Mechanism of clofilium block of the human $K_v1.5$ delayed rectifier potassium channel. *Mol. Pharmacol.*, **47**, 198–205.

MEKATA, F. (1974). Current spread in the smooth muscle of the rabbit aorta. *J. Physiol.*, **242**, 143–155.

NEYLON, O.P., AVDONIN, P.V., LARSEN, M.A. & BOBIK, A. (1994). Rat aortic smooth muscle cells expressing charybdotoxin-sensitive potassium channels inhibit proliferative responses. *Clin. Exp. Pharmacol. Physiol.*, **21**, 117–120.

OVERTURF, K.E., RUSSELL, S.N., CARL, A., VOGALIS, F., HART, P.J., HUME, J.R., SANDERS, K.M. & HOROWITZ, B. (1994). Cloning and characterization of a Kv1.5 delayed rectifier K^+ channel from vascular and visceral smooth muscles. *Am. J. Physiol.*, **267**, C1231–1238.

PO, S., ROBERDS, S., SNYDERS, D.J., TAMKUN, M.M. & BENNETT, P.B. (1993). Heteromultimeric assembly of human potassium channels. *Circ. Res.*, **72**, 1326–1336.

REEVE, H.L. & PEERS, C. (1992). Blockade of delayed rectifier K^+ currents in neuroblastoma × glioma hybrid (NG 108-15) cells by clofilium, a class III antidysrhythmic agent. *Br. J. Pharmacol.*, **105**, 458–462.

RUSSELL, S.N., OVERTURF, K.E. & HOROWITZ, B. (1994). Hetero-tetramer formation and charybdotoxin sensitivity of two K^+ channels from smooth muscle. *Am. J. Physiol.*, **267**, C1729–1733.

SANGUINETTI, M.C. (1992). Modulation of potassium channels by antiarrhythmic and antihypertensive drugs. *Hypertension*, **19**, 228–236.

SMIRNOV, S.V. & AARONSON, P.I. (1992). Ca^{2+}-activated and voltage-gated K^+ currents in smooth muscle cells from isolated human mesenteric arteries. *J. Physiol.*, **457**, 431–454.

YEH, J.Z., OXFORD, G.S., WU, C.H. & NARAHASHI, T. (1976). Dynamics of aminopyridine block of potassium channels in squid axon membrane. *J. Gen. Physiol.*, **68**, 519–535.

ZHANG, H. & COOK, D. (1994). Cerebral vascular smooth muscle potassium channels and their possible role in the management of vasospasm. *Pharmacol. Toxicol.*, **75**, 327–336.

(Received June 8, 1995
Revised August 10, 1995
Accepted August 24, 1995)