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Potassium channel blockers and the effects of cromakalim on the smooth muscle of the guinea-pig bladder

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1 The K^+ channel blocking drugs tetraethylammonium Cl (TEA), procaine, 4-aminopyridine (4AP) and quinidine all produced concentration-dependent contractions of strips of smooth muscle from the guinea-pig urinary bladder. Apamin and glibenclamide caused little increase in the mechanical activity, and tolbutamide inhibited it.

2 TEA, procaine, 4AP, quinidine and apamin all increased the frequencies of spontaneous action potentials recorded with microelectrodes. TEA, quinidine and procaine all caused prolongation of the falling phase of the spike, and procaine and apamin completely abolished the after-hyperpolarization.

3 TEA and procaine increased K^+ efflux from the tissue, an effect blocked by nifedipine. TEA and apamin increased, whereas quinidine, procaine and 4AP decreased K^+ uptake.

4 Cromakalim caused a concentration-dependent hyperpolarization of the membrane, abolished spike activity, increased K^+ fluxes and relaxed the smooth muscle. The relaxant effect of cromakalim was unaffected by apamin, and in its presence the effects of cromakalim on membrane potential and K^+ fluxes were unchanged. Procaine abolished all the effects of cromakalim, and TEA at high concentrations reduced but did not abolish these effects. Quinidine reduced the effects of cromakalim on tension and membrane potential, but its actions were surmounted by higher concentrations of cromakalim. The effects of 4AP on tension and membrane properties were transitory, but it had some effects on the actions of cromakalim. Glibenclamide and tolbutamide reversed the relaxant effects of submaximal cromakalim concentrations, tolbutamide only transiently.

5 It is concluded that the channels opened by cromakalim are not those involved in generating the spike after-hyperpolarization. They have properties similar to the delayed rectifier K^+ channels responsible for spike repolarization, and also are similar to the ATP-dependent K channels in vascular smooth muscle.

Introduction

The excitable properties and spike shapes of smooth muscles vary widely from tissue to tissue, and it is likely that each smooth muscle possesses a unique set of K^+ channels, whether in channel type, or in the proportions of the various types present. One common feature of all smooth muscles so far studied, is the ability of cromakalim, a drug classified as a K^+ channel activator, to induce relaxation (Weston & Abbott, 1987; Cook, 1988). To investigate the types of K^+ channel present in smooth muscles, potassium channel inhibitors have been used as tools (Benham *et al.*, 1985; Inoue *et al.*, 1985; McCann & Welsh, 1987; Okabe *et al.*, 1987), although there is considerable overlap in the channels each inhibitor can block and there are few, if any, compounds which selectively block a single class of K^+ channel. At the time of writing, there have been two proposals as to the channels that can be activated by cromakalim in smooth muscle, the delayed rectifier channel (Beech & Bolton, 1987; Beech, 1989), and an ATP-dependent K^+ channel (Standen *et al.*, 1989).

There is at present little detailed information about the electrical properties of bladder smooth muscle, and less about the K^+ channels present. The best studied bladder is that of the guinea-pig, on which microelectrode techniques have been applied by Creed and her colleagues (Creed, 1971a,b; Callahan & Creed, 1981; 1986; Kurihara & Creed, 1972), Mostwin (1986) and Fujii (1988). Membrane currents have been recorded by Klöckner & Isenberg (1985) using a whole cell patch clamp. We have previously shown that cromakalim increases potassium permeability in the smooth muscle of the guinea-pig urinary bladder. This results in membrane hyperpolarization, cessation of spike activity and relaxation of the tissue. The channels opened by the drug in the guinea-pig bladder seem to be selectively more permeable to K^+ than to Rb^+ ions, and have similarities with channels involved in spike repolarization (Foster *et al.*, 1989).

In this paper we have investigated the effects of K^+ channel inhibitors on the mechanical and electrical activity in guinea-pig bladder and on transmembrane K^+ fluxes, and have examined the interaction of these inhibitors with the effects of cromakalim.

Methods

Details of the methods used are described in Foster *et al.* (1989), and are given in outline below.

Contractile studies

Isometric tension was recorded from strips of detrusor mounted in 0.2 ml chambers, continuously superfused at 1 ml min^{-1} with solutions warmed to 35–37°C, and equilibrated with 97% O_2, 3% CO_2. On mounting, tissues were stretched to an initial tension of 0.5 g wt. Drugs were administered in the desired concentrations in the superfusing solution.

Electrophysiology

Membrane activity was recorded from strips of detrusor mounted in a small organ bath and superfused at a rate of 2–3 ml min^{-1} with warmed (36°C), gassed solutions made hypertonic by addition of 12 g sucrose to 100 ml Krebs solution to reduce tissue movement. Microelectrodes had a tip resistance of 40–70 MΩ when filled with 3 M KCl. Potentials were displayed on a penwriting recorder (Gould Brush 220).

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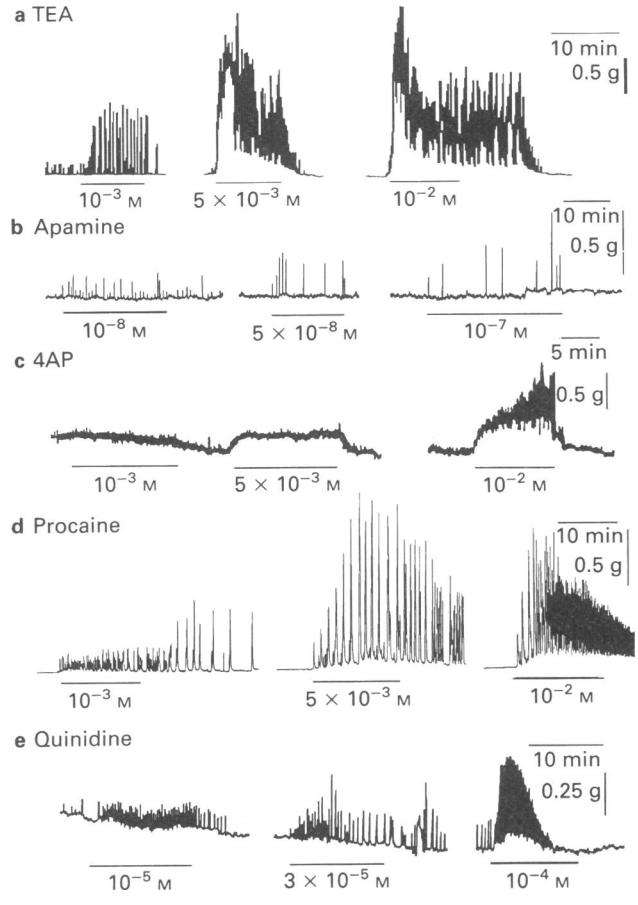

Figure 1 The effect of K^+ channel blockers on contractile activity of strips of detrusor muscle from the guinea-pig bladder. Each row of recordings is taken from a different preparation. TEA, tetraethylammonium chloride; 4AP, 4-aminopyridine.

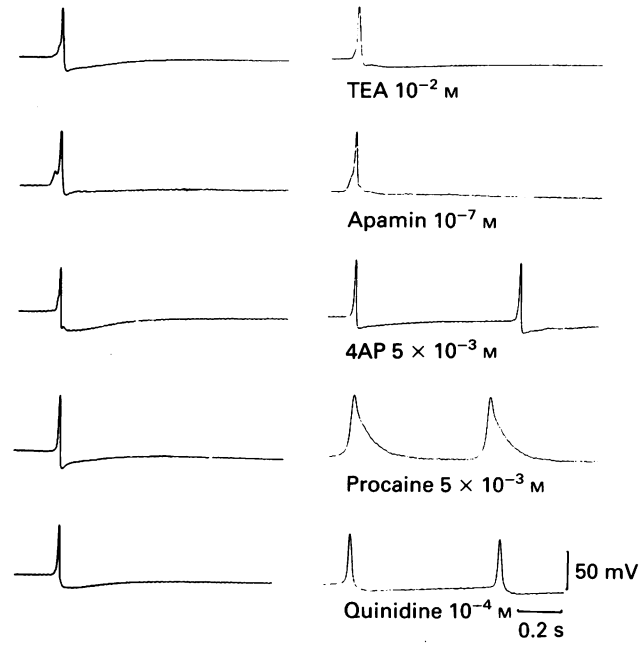

Figure 2 Detrusor muscle of guinea-pig urinary bladder: the effect of K^+ channel blockers on the action potential shape (membrane potential recorded with microelectrodes). In each row the left hand record shows a control action potential recorded in normal solution, and the right hand record shows an action potential after the maximum effect of the blocker on spike frequency had occurred during the same recording. Each row of traces is from a different tissue. Note the variability of shapes recorded in the control spikes, but the common fast repolarization and after-hyperpolarization. At the concentrations used, tetraethylammonium Cl (TEA) slows the repolarization and reduces the after-hyperpolarization. Apamin and 4-aminopyridine (4AP) have no effect on repolarization, but apamin completely abolishes and 4AP slightly reduces the after-hyperpolarization. Procaine greatly slows repolarization and abolishes the after-hyperpolarization. Quinidine slows repolarization and reduces the after-hyperpolarization.

$^{43}K^+$ fluxes

Strips of detrusor were mounted on stainless-steel holders. For uptake experiments tissues were exposed to isotope-containing solution for 10 min and then washed in ice cold Krebs solution for 5 min to remove extracellular isotope. The solution used to study tissue uptake of isotope contained 2–5 μCi ml^{-1} $^{43}K^+$. When K^+ channel inhibitors were studied, tissues were preincubated with the appropriate inhibitor for 10 min before exposure to isotope-containing solution also containing the inhibitor. In experiments where the effects of cromakalim (10^{-5} M) were studied, this agent was added to the isotope-containing solution. For efflux experiments, tissues were loaded in Krebs solution containing 1–3 μCi ml^{-1} $^{43}K^+$ for 4.5 h, and washed out in a superfusion apparatus with Krebs solution (35–38°C) at a flow rate of 1 ml min^{-1}. Two min samples were collected from the 10th min of the washout, and 30 min later cromakalim was applied for 10 min. K^+-channel inhibitors were applied 10 min before cromakalim and were present until the end of the washout. When nifedipine was used, this was present in all the washout solutions.

Solutions and drugs

The Krebs solution contained (mM): NaCl 120, KCl 5.9, $NaHCO_3$ 15.4, $MgCl_2$ 1.2, NaH_2PO_4 1, $CaCl_2$ 2.5, glucose 11 and was equilibrated with 97% O_2, 3% CO_2. For electrophysiological recordings, the osmolarity was increased by the addition of 12 g sucrose to 100 ml Krebs, to reduce tissue movement. Drugs used were: cromakalim (BRL 34915, (±)-6-cyano-3,4-dihydro-2,2-dimethyl-*trans*-4-(2-oxo-1-pyrrolidyl)-2H-benzo[b]pyran-3-ol), was a gift from Beecham Pharmaceuticals U.K. It was made up as a stock solution (10^{-2} M) with 70% ethanol, and diluted with the experimental Krebs solution to the desired concentration. Apamin, 4-aminopyridine (4AP), procaine and tetraethylammonium Cl (TEA) were obtained from Sigma, and made up as concentrated stock solutions in distilled water. Apamin was kept frozen and prepared freshly no more than an hour before it was required. Nifedipine was obtained from Bayer and PN200-110 (isradipine (isopropyl methyl-4-(4-benzofurazanyl)-1,4-dihydro-7,6-dimethyl-3,5-pyridinedicarboxylate) from Sandoz. Stocks of these two drugs and quinidine (Sigma), were prepared in 70% ethanol and the solutions containing nifedipine were shielded from light during use.

Statistics

Experimental results are given as means and standard errors of the mean, and significance was tested by Student's unpaired *t* test. *P* values of less than 0.05 are considered significant.

Results

Effect of K^+-channel inhibitors on mechanical and electrical activity of normal tissues

Figure 1 shows the effects of the five potassium channel blocking drugs on the contractile activity of strips of detrusor. With the exception of apamin, the tissue responded to the drugs with a concentration-dependent increase in contractile activity. TEA and procaine elicited vigorous contractile activity, and there were significant effects at concentrations of 10^{-3} M

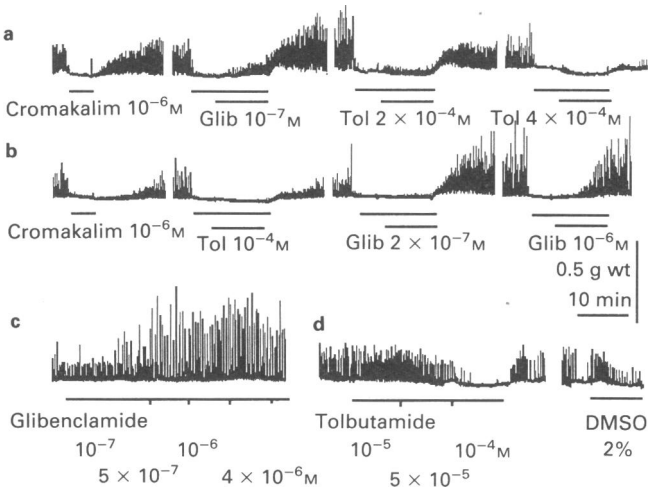

Figure 3 The effects of tolbutamide (Tol) and glibenclamide (Glib) on contractile activity of guinea-pig bladder strips, and their ability to reverse the actions of cromakalim (10^{-6} M). Traces (a) and (c) are from one tissue, traces (b) and (d) from two others. The effects of cromakalim were more easily reversed in tissue (a), where 10^{-7} M glibenclamide caused more recovery than did 2×10^{-7} M in tissue (b). Glibenclamide 10^{-6} M caused complete recovery of the spontaneous activity in all tissues tested. Tolbutamide caused a small recovery at 2×10^{-4} M, and a transient recovery at 4×10^{-4} M. Note the reduced spontaneous activity of the tissues after tolbutamide had been washed off, and the increased activity after washing off glibenclamide. In trace (c) the tissue had not completely recovered from previous exposure to tolbutamide by the beginning of the trace. The 10^{-4} M tolbutamide solution contained 1% dimethylsulphoxide (DMSO). The inhibitory effect of 2% DMSO shown at the end of trace (d) is clearly less than that produced by 10^{-4} M tolbutamide.

TEA and 10^{-3} M procaine. 4AP had a less dramatic effect, but in most tissues caused a contractile response at 10^{-3} M. Quinidine was more potent, eliciting contractile activity at 10^{-5} M, but even at 10^{-4} M the response was transient and not as striking as with TEA or procaine. Apamin had little effect. In 2 out of 8 strips it caused a small increase in contractile activity at 10^{-8} M, but in the rest it caused a slight inhibition. Since it has recently been reported that ATP-dependent K^+ channels may be involved in the relaxant effects of cromakalim on vascular smooth muscle (Quast & Cook, 1988; Cavero *et al.*, 1988; Standen *et al.*, 1989), the effects of the sulphonylureas tolbutamide and glibenclamide on contractile activity were also studied, as these drugs block such channels in the pancreatic beta cells (Ashcroft, 1988), although at much lower concentrations than needed to inhibited the cromakalim-activated channels (Standen *et al.*, 1989; Buckingham *et al.*, 1989). Glibenclamide at 10^{-7} M caused a small increase in the amplitude of the spontaneous activity, but increasing the concentration to 4×10^{-6} M had little further effect. Tolbutamide had no effect at 10^{-5} M, but suppressed the spontaneous activity at 10^{-4} M.

An investigation of the effect of the first five of the K^+-channel inhibitors on the electrical activity showed that they all increased the spike frequency with no detectable depolarization of the cell membrane, apart from procaine (5×10^{-3} M) which caused a small depolarization. Because of the variability of the spike shape even in the absence of drugs (see Figure 2), it is difficult to be precise about the effects of the K^+-channel inhibitors on it, but apamin (10^{-7} M) and 4AP (5×10^{-3} M) had no measureable effect on the rate of repolarization of the spikes, TEA (10^{-2} M) and quinidine (10^{-4} M) caused some prolongation of the falling phase, (the time for half repolarization increasing to 220% and 340% of the

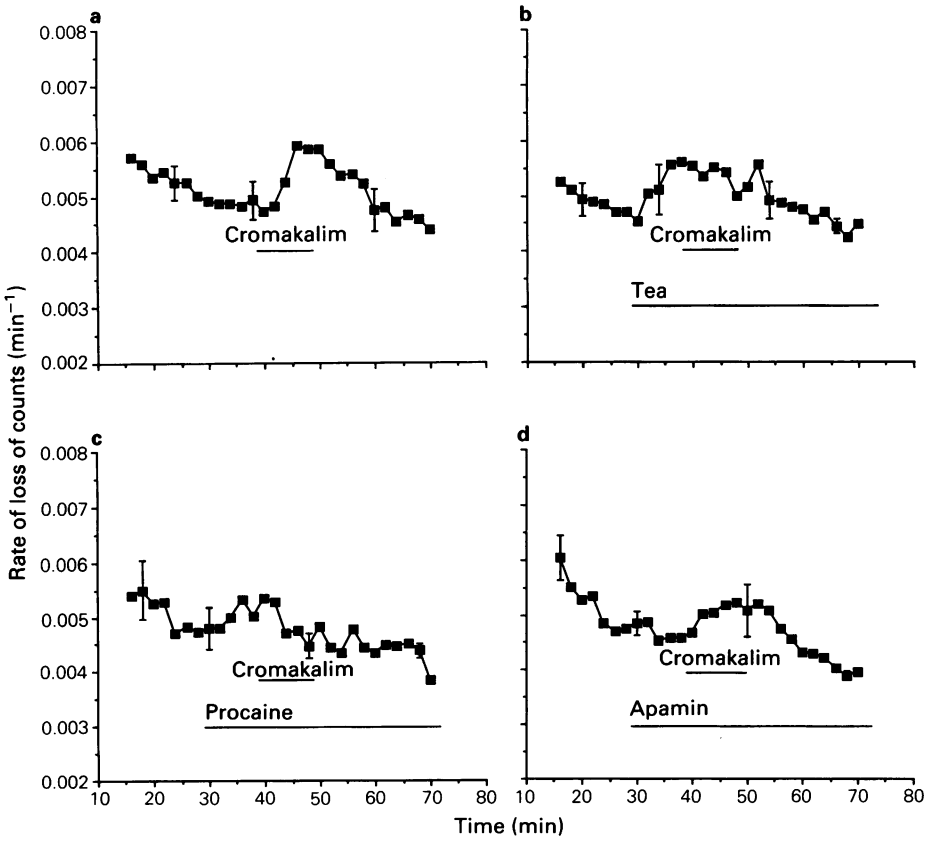

Figure 4 The effect of cromakalim (10^{-5} M) on the rate of loss of $^{43}K^+$ from strips of guinea-pig bladder smooth muscle, on its own, and in the presence of tetraethylammonium Cl (TEA, 5×10^{-3} M), procaine (5×10^{-3} M) or apamin (10^{-7} M). Procaine and TEA cause an increase in the efflux rate which obscures the effects of cromakalim. Values are means with s.e.mean shown by vertical bars, $n = 3$ tissues (from 2 animals).

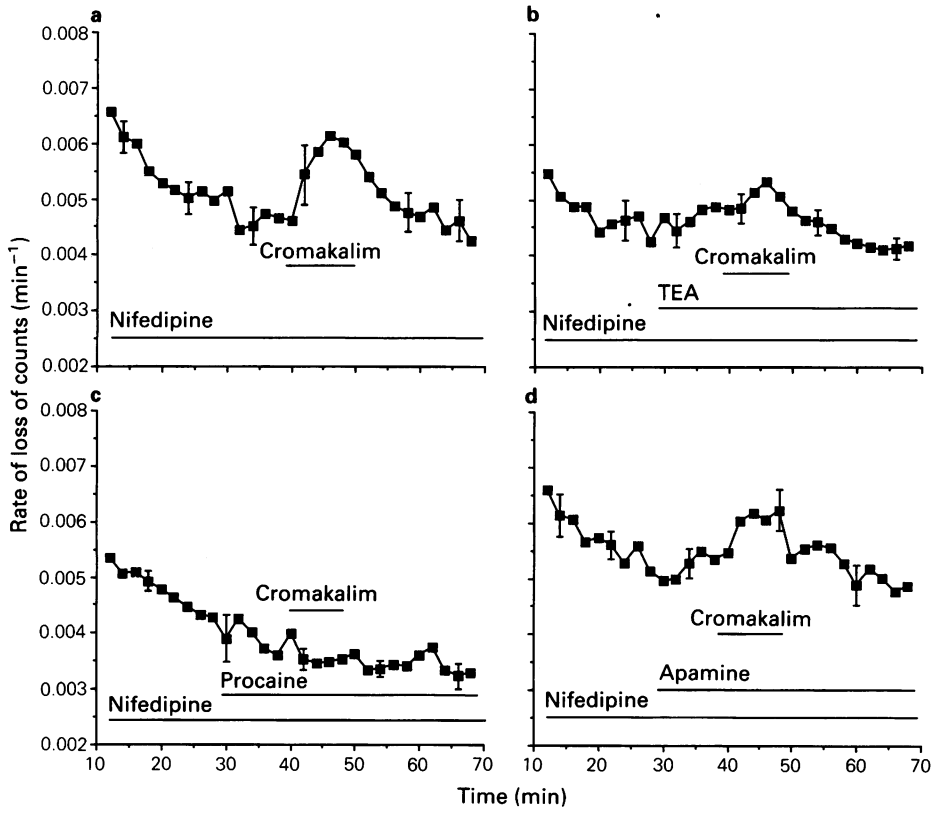

Figure 5 The effect of cromakalim (10^{-5} M) on the rate of loss of $^{43}K^+$ from strips of guinea-pig bladder smooth muscle in the presence of nifedipine (5×10^{-7} M). Nifedipine abolishes the increase in rate of loss of $^{43}K^+$ induced by tetraethylammonium Cl (TEA, 5×10^{-3} M) and procaine (5×10^{-3} M), and it is apparent that TEA reduces, and procaine abolishes the stimulation of the efflux produced by cromakalim. Apamin (10^{-7} M) has no significant effect. Values are means with s.e.mean shown by vertical bars, $n = 3$ tissues (from 2 animals).

control in the examples shown). Procaine (5×10^{-3} M) caused a marked prolongation of the falling phase, which took more than 10 times the control for half repolarization. The drugs also had variable effects on the pronounced after-hyperpolarization seen in normal tissues. Procaine and apamin completely eliminated this phase of the spike, 4AP and TEA had little effect, and quinidine reduced the after-hyperpolarization. Figure 2 shows a typical control spike, and spikes in the presence of the five K^+-channel blockers. An unexpected finding was that the effect of 4AP on spike frequency was transient. The frequency, after an initial large rise, fell to control levels after about 10 min in the continuous presence of the drug, and remained low even on further application of 4AP from a freshly made-up solution. A similar phenomenon was also seen in its effects on contractile activity.

Effect of K^+-channel inhibitors on the response of the detrusor to cromakalim

In the guinea-pig detrusor, it proved impossible to establish a dose-response relationship for the relaxant effects of cromakalim on smooth muscle strips, since the spontaneous activity consisted of contractions which rose from and returned to zero basal tension. Cromakalim at concentrations above 5×10^{-7} M simply abolished this activity. As it was not possible to study the effect of the K^+-channel inhibitors on the dose-response curve, their ability to block the effect of 10^{-6} M, and in some cases 10^{-5} M cromakalim was studied. Cromakalim 10^{-6} M is a sub-maximal concentration for both the hyperpolarization and increase in K^+ flux (Foster *et al.*, 1989). TEA was relatively ineffective at blocking the relaxant effect of cromakalim, and even at 10^{-2} M only partially inhibited the effect of 10^{-6} M cromakalim. Apamine did not antagonize the effect of 10^{-6} M cromakalim at any concentration applied (up to 10^{-7} M). 4AP 10^{-3} M was ineffective, cromakalim still causing a relaxation in its presence, but at higher concentrations of 4AP, although cromakalim (10^{-6} M) slowed the frequency of the contractions, it increased their amplitude. Procaine (10^{-4} M) completely blocked the relaxant effect of 10^{-6} and 10^{-5} M cromakalim. Quinidine blocked the effect of 10^{-6} M cromakalim partially at 10^{-5} M, and completely at 5×10^{-5} M, but was less effective against higher concentrations. This series of K channel blockers all clearly enhanced the spontaneous activity on their own. Glibenclamide had little effect on its own, but was able to block the relaxant effect of 10^{-6} M cromakalim partially at a concentration of 2×10^{-7} M, and completely at 10^{-6} M, although these concentrations did not prevent the action of 10^{-5} M cromakalim. Tolbutamide had a supressant effect on the spontaneous activity on its own at 10^{-4} M, but showed some evidence of partially blocking the effects of 10^{-6} M cromakalim, and more clearly, but transiently antagonized the effect at 4×10^{-4} M. The effects of glibenclamide and tolbutamide are shown in Figure 3.

Cromakalim (10^{-5} M) caused an increased rate of loss of $^{43}K^+$ from the tissue. The effect of the potassium channel inhibitors apamin, procaine and TEA on this action of cromakalim is shown in Figure 4. Apamine itself did not increase the $^{43}K^+$ efflux rate, and in the presence of apamin (10^{-5} M) cromakalim retained its ability to promote $^{43}K^+$ efflux. Both procaine (5×10^{-3} M) and TEA (10^{-2} M) themselves caused an increase in efflux of $^{43}K^+$, and cromakalim caused no further increase. Since the stimulation of the efflux observed on addition of TEA and procaine is probably a result of the increased contractile activity (for instance, an increase in intracellular Ca ions might open Ca^{2+}-dependent K^+ channels), the experiments were repeated in the presence of the Ca^{2+}-channel blocker nifedipine (5×10^{-7} M). In the presence of nifedipine, TEA and procaine no longer caused an increase in

the $^{43}K^+$ efflux, and it could then be seen that TEA partially antagonized and procaine completely abolished the effects of cromakalim. In the presence of both nifedipine and apamin (10^{-7} M), cromakalim retained its ability to promote $^{43}K^+$ efflux (Figure 5).

The effects of the first five inhibitors on the uptake of $^{43}K^+$ and the stimulatory effect of cromakalim on this uptake are illustrated in Figure 6. The results in this figure came from 6 experiments in which a clear stimulation of the $^{43}K^+$ uptake was seen by cromakalim on control tissues. In another 12 experiments analysis of the results showed no effect of cromakalim on the $^{43}K^+$ uptake and we did not include the results of the K^+ channel inhibitors from these experiments. Figure 6 shows that apamin and TEA significantly increased the uptake of $^{43}K^+$ on their own (to 114.8 ± 3.8 and 119.0 ± 10.8% of the control), quinidine and procaine cause a small but significant reduction (to 85.8 ± 7 and 83.8 ± 8%), and 4AP a large reduction (to 62.4 ± 1.7% of the control). With the exception of apamin, all the K^+-channel inhibitors reduced the stimulatory effect of cromakalim on the uptake.

In case the variability of the response to cromakalim of the normal tissues was due to differences in the spontaneous activity of the tissues, the experiments were also conducted in the presence of the Ca-channel antagonist PN 200-110. This treatment did not increase the proportion of tissues in which cromakalim induced significant $^{43}K^+$ uptake. Out of a total of 12 experiments carried out in the presence of PN 200-110, in only 4 did cromakalim cause a significant increase of $^{43}K^+$ uptake. PN200-110 abolished the spontaneous spikes of the tissue, but did not affect the response of the tissue to 30 mM K^+ or to cromakalim measured with microelectrodes, as shown in Figure 7. The membrane was depolarized in 30 mM K^+ and cromakalim 10^{-5} M still caused a hyperpolarization of the membrane by about 20 mV.

The effects of the K^+-channel inhibitors on the electrical response of the tissues to cromakalim is shown in Figure 8. In this figure the effects of 5×10^{-6} M cromakalim are shown. As can be seen, this concentration of cromakalim produced membrane hyperpolarization and loss of spike activity even in the presence of 10^{-2} M TEA, 10^{-7} M apamin and 5×10^{-3} M 4AP, whereas it had no effect at all in the presence of 5×10^{-3} M procaine and only caused a slowing of the spike frequency in the presence of 10^{-4} M quinidine. Procaine (5×10^{-3} M) was

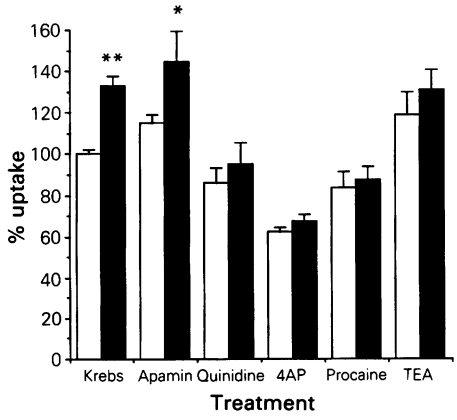

Figure 6 The effect of cromakalim (10^{-5} M) and K^+ channel blockers on the 10 min uptake of $^{43}K^+$ by strips of smooth muscle from guinea-pig bladder. Open columns show the uptake (as a percentage of the counts present in the control tissues) in the absence of cromakalim, and the solid columns in its presence. The concentrations of the K^+ channel blockers were: apamin, 10^{-7} M; quinidine, 3×10^{-5} M; 4-aminopyridine (4AP) 5×10^{-3} M; procaine, 5×10^{-3} M; tetraethylammonium Cl (TEA) 5×10^{-3} M. For each blocker $n = 6$ strips from 3 different guinea-pigs. For the control group, $n = 32$ strips from 7 different guinea-pigs. The uptakes in the presence of the blockers are all significantly different from the control uptake, but cromakalim significantly increases the uptake only in normal Krebs solution or in the presence of apamin.

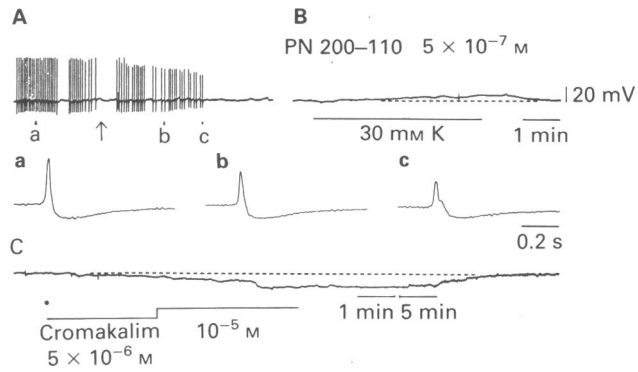

Figure 7 Detrusor muscle from guinea-pig urinary bladder: microelectrode recording of membrane potential activity. (A) The effect of the Ca-antagonist PN 200-110 (10^{-7} M) added at the arrow. The second line shows action potentials on an expanded time-scale. (B) and (C) are in the presence of 5×10^{-7} M PN 200-110. (B) Depolarization still occurs when the K^+ is elevated to 30 mM. (C) Cromakalim still causes hyperpolarization of the membrane. Note reduction in chart speed during latter portion of trace (C).

capable of blocking the effects of higher concentrations of cromakalim. In the presence of 10^{4} M quinidine, however, although the effect of 5×10^{-6} M cromakalim was virtually abolished, if the concentration of cromakalim was increased, the effects of quinidine were progressively antagonized. At 10^{-5} M cromakalim, the spike frequency was reduced, and the

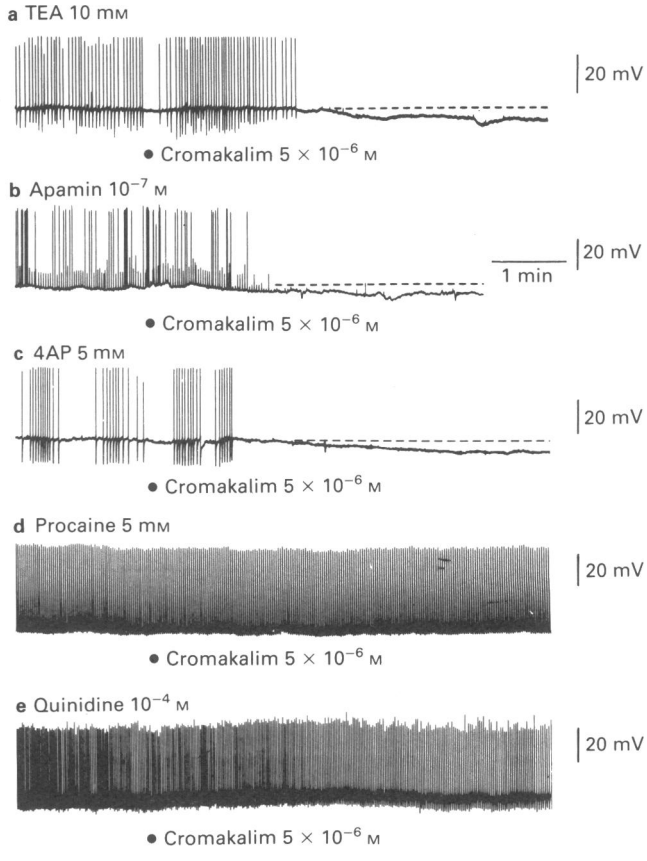

Figure 8 Detrusor muscle from guinea-pig urinary bladder: microelectrode recordings of the effects of cromakalim (5×10^{-6} M) in the presence of K^+ channel blocking drugs. Each tracing comes from a different tissue. Note the lack of an after-hyperpolarization to the spikes in the presence of apamin and procaine. At the concentrations used only procaine (d) and quinidine (e) abolish the action of cromakalim.

prolongation of the falling phase of the action potential (often leading to double spikes) and the reduction in the size of the after-hyperpolarization were both partially reversed, as shown in Figure 9. At 5×10^{-5} M cromakalim, the spike activity was abolished.

Discussion

The results in this paper suggest that there are at least two classes of K^+ channels present in the membranes of the smooth muscle of the guinea-pig urinary bladder and involved in action potential production: one that is responsible for the after-hyperpolarization and is blocked by apamin and procaine and partially blocked by quinidine, and another responsible for the falling phase of the spike which is resistant to apamin and 4AP, but blocked by procaine, TEA and quinidine. The channels that are responsible for the after-hyperpolarization do not appear to be involved in the response of the tissue to cromakalim, since apamin, at a concentration which abolishes the after-hyperpolarization and increases contractile activity, has no effect on the ability of cromakalim to hyperpolarize the cell membranes or relax the tissue. Apamine also fails to prevent the increase in efflux or uptake of $^{43}K^+$ induced by 10^{-5} M cromakalim. Several pieces of evidence suggest that the channels responsible for the repolarization of the spike may have similar properties to the channels opened by cromakalim. Procaine, at a concentration which causes the greatest prolongation of the spike repolarization, is also the most effective at blocking the effects of cromakalim on the tension, membrane potential and the K^+ fluxes. Potassium channel inhibitors which do not, or at concentrations which do not prolong the falling phase of the spike, are not effective in antagonizing the effects of cromakalim on tension, spike activity and membrane hyperpolarization. In an earlier paper (Foster *et al.*, 1989) we also showed that replacing the intracellular K^+ with Rb^+ blocked the effects of cromakalim and caused a significant prolongation of the falling phase of the spike. This suggests that the channels opened by cromakalim have a similar ionic selectivity to the channels responsible for spike repolarization.

In vascular smooth muscle (Standen *et al.*, 1989), ATP-dependent K^+ channels have been demonstrated which are activated by cromakalim and blocked by glibenclamide and tolbutamide, and glibenclamide shifts the concentration-relaxation curves for cromakalim to the right (Buckingham *et al.*, 1989). Using whole cell recording techniques in guinea-pig detrusor we have so far been unable to demonstrate ATP-dependent K^+ channels (R. Inoue, unpublished results). However, the fact that glibenclamide and tolbutamide are able to reverse the effects of 10^{-6} M cromakalim on the contractile activity in the same range of concentrations as they reverse its effects in vascular smooth muscle, and that TEA is relatively ineffective in both tissues, suggests that the channels are very similar. The effects of the other potassium channel blockers are much less clear cut, and it is difficult to come to any conclusions without investigating a wider range of concentrations of the inhibitors. One or two interesting points emerge. TEA and quinidine are both capable of prolonging the falling phase of the spike; however, in the presence of concentrations of the

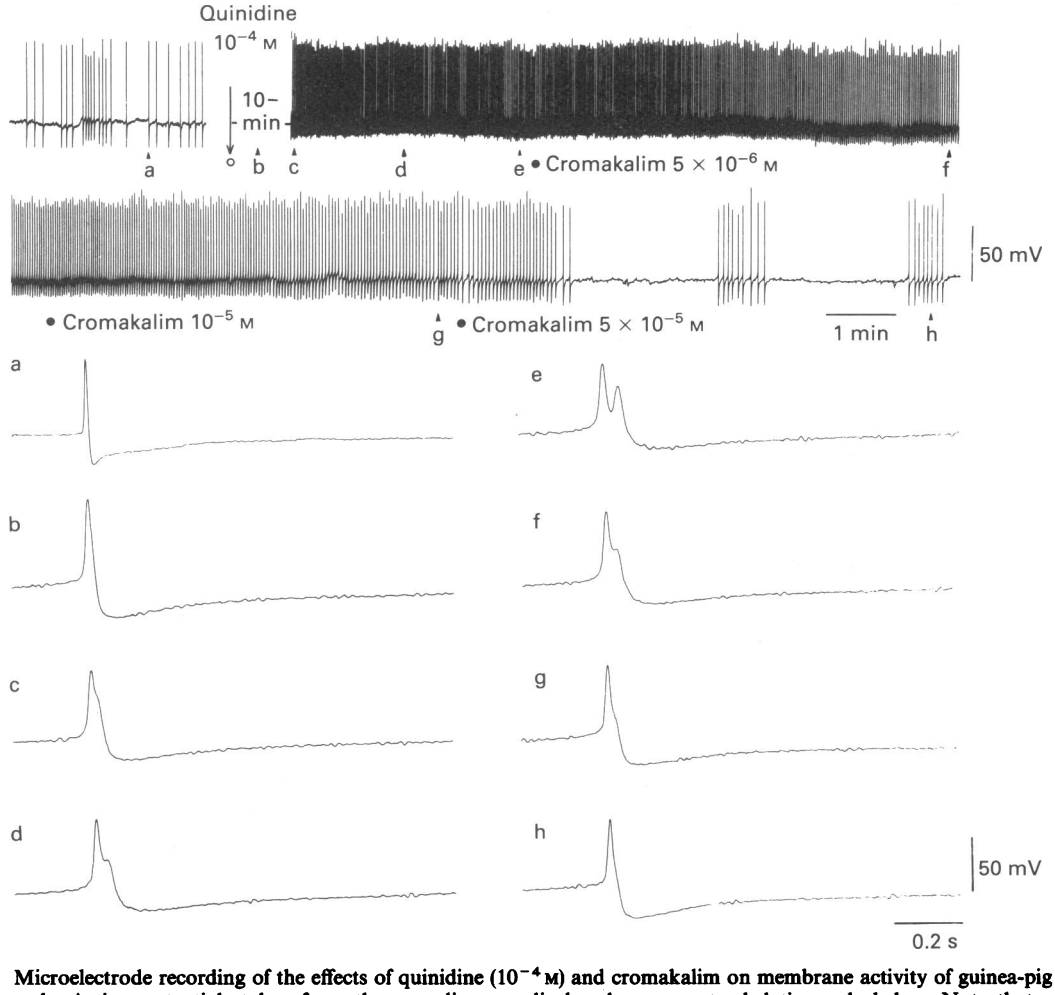

Figure 9 Microelectrode recording of the effects of quinidine (10^{-4} M) and cromakalim on membrane activity of guinea-pig bladder smooth muscle. Action potentials taken from the recording are displayed on an extended timescale below. Note that quinidine increases the spike frequency, reduces the size of the after-hyperpolarization and prolongs the repolarization phase of the action potential leading to double spikes. Increasing doses of cromakalim antagonize these effects.

inhibitors that produce similar prolongations, cromakalim (5×10^{-6} M) still caused a cessation of spike activity and hyperpolarized the membrane in the presence of TEA, but had little effect in the presence of quinidine. Cromakalim also caused an increase in efflux in the presence of TEA (although less marked than in the control tissues). In the presence of quinidine, increasing the concentration of cromakalim, without causing any apparent hyperpolarization, caused a reduction in spike frequency, shortened the spike repolarization and caused a return of the full sized after-hyperpolarization. This suggests that there might be some competition for binding between cromakalim and quinidine. The effects of 4AP are difficult to interpret because of the transience of its effect on spike frequency, and the atypical effect of cromakalim on tension in its presence. 4AP does, however, consistently and significantly reduce the tissue uptake of $^{43}K^+$ after a 10 min exposure, suggesting that it is an effective blocker of some K^+ channels at the concentrations used.

A consistent finding was that there was no clear relationship between the membrane potential of the cells and the spike frequency. Most of the K^+-channel inhibitors increased the spike frequency in the absence of any recordable depolarization of the membrane. Cromakalim on its own or in the presence of those inhibitors which did not block its effect, usually blocked spike activity at a time when there was no obvious change in membrane potential, the hyperpolarization beginning a few seconds later. The ionic mechanisms responsible for spike initiation in the bladder are unknown, but conceivably involve channels that are sensitive both to the K^+-channel inhibitors and to cromakalim.

We have again come up against the problem of lack of consistency of the effects of cromakalim on the K^+ fluxes, and this is more marked when looking at the uptake of $^{43}K^+$ than its efflux. We were only able to record an effect of 10^{-5} M cromakalim on K^+ uptake in 6 out of 15 experiments. In comparison, as we have previously reported (Foster *et al.*, 1989) we found an increased efflux in 17 out of 20 tissue strips, and have always seen cessation of mechanical and electrical activity on exposure to cromakalim at this concentration. The changes in membrane potential and spike frequency caused by cromakalim may themselves alter the pattern of opening of several types of K^+ channels, and the net effect will vary depending on the profile of channels that each strip possesses. The hyperpolarization will also reduce the driving force for K^+ efflux, but theoretically should increase the driving force for K^+ influx. The effects of cromakalim should therefore be seen more consistently on the uptake. The fact that the reverse is the case, could be due to some intrinsic properties of the channels opened by cromakalim. It has been suggested for instance, that the delayed rectifier K^+ channels in squid giant axon may show 'single file' effects, which would occur if several ions could occupy the channel simultaneously (Hodgkin & Keynes, 1955; Hille, 1984), which would alter the flux ratio in favour of efflux.

In conclusion, the results described in this paper support the suggestion put forward by Beech (1989) that the channels opened by cromakalim may be similar to the delayed rectifier K^+ channels that have been demonstrated in many smooth muscles, and whose presence in guinea-pig bladder has been suggested by Klöckner & Isenberg (1985). They also suggest that the channels are similar to the ATP-dependent K channels recently demonstrated in vascular smooth muscle (Standen *et al.*, 1989). More conclusive evidence awaits patch clamp analysis from detrusor smooth muscle cells.

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References

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

BEECH, D.J. (1989). Pharmacology of voltage dependent ion channels in rabbit vascular smooth muscle. *Ph.D. Thesis London University.*

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

BENHAM, C.D., BOLTON, T.B., LANG, R.J. & TAKEWAKI, T. (1985). The mechanism of action of Ba^{2+} and TEA on single Ca^{2+}-activated K^+-channels in arterial and intestinal smooth muscle cell membranes. *Pflügers Arch.*, **403**, 120–127.

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

CALLAHAN, S.M. & CREED, K.E. (1981). Electrical and mechanical activity of the isolated lower urinary tract of the guinea-pig. *Br. J. Pharmacol.*, **74**, 353–358.

CALLAHAN, S.M. & CREED, K.E. (1986). Non-cholinergic neurotransmission and the effects of peptides on the urinary bladder of guinea-pigs and rabbits. *J. Physiol.*, **374**, 103–115.

CAVERO, I., MONDOT, S., MESTRE, M. & ESCANDE, D. (1988). Haemodynamic and pharmacological mechanisms of the hypotensive effects of cromakalim in rats: blockade by glibenclamide. *Br. J. Pharmacol.*, **95**, 643P.

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

CREED, K. (1971a). Membrane properties of the smooth muscle membrane of the guinea-pig urinary bladder. *Pflügers Arch.*, **326**, 115–126.

CREED, K. (1971b). Effects of ions and drugs on the smooth muscle cell membrane of the guinea-pig urinary bladder. *Pflügers Arch.*, **326**, 127–141.

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

FUJII, K. (1988). Evidence for adenosine triphosphate as an excitatory transmitter in guinea-pig, rabbit and pig urinary bladder. *J. Physiol.*, **404**, 39–52.

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

HODGKIN, A.L. & KEYNES, R.D. (1955). The potassium permeability of a giant nerve fibre. *J. Physiol.*, **128**, 61–88.

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

KLÖCKNER, U. & ISENBERG, G. (1985). Action potentials and net membrane currents of isolated smooth muscle cells (urinary bladder of the guinea-pig). *Pflügers Arch.*, **405**, 329–339.

KURIHARA, S. & CREED, K.E. (1972). Changes in membrane potential of the urinary smooth muscle cells of the guinea-pig urinary bladder in the various environments. *Jpn. J. Physiol.*, **22**, 667–683.

McCANN, J.D. & WELSH, M.J. (1987). Neuroleptics antagonize a calcium-activated potassium channel in airway smooth muscle. *J. Gen. Physiol.*, **89**, 339–352.

MOSTWIN, J.L. (1986). Electrical and mechanical aspects of bladder contractility. *D. Phil. Thesis, Oxford.*

OKABE, K., KITAMURA, K. & KURIYAMA, H. (1987). Features of 4-aminopyridine sensitive outward current observed in single smooth muscle cells from the rabbit pulmonary artery. *Pflügers Arch.*, **409**, 561–568.

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

STANDEN, N.B., QUAYLE, J.M., DAVIES, N.W., BRAYDEN, J.E., HUANG, Y. & NELSON, M.T. (1989). Hyperpolarizing vasodilators activate ATP-sensitive K^+ channels in arterial smooth muscle. *Science*, **245**, 172–180.

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

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