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The effect of cromakalim on the smooth muscle of the guinea-pig urinary bladder

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1 The actions of cromakalim were studied on the detrusor muscle from guinea-pig urinary bladder. Cromakalim reduced the frequency and amplitude of spontaneous contractile activity of the smooth muscle of the guinea-pig urinary bladder at 5×10^{-8} M and abolished the activity at concentrations above 5×10^{-7} M.

2 Electrophysiological experiments demonstrated that cromakalim increased membrane conductance, caused a dose-dependent hyperpolarization of the cell membrane and loss of spike activity. These events are consistent with the opening of K^+ channels.

3 The effects of 10^{-6} M and 10^{-5} M cromakalim on the contractile responses to carbachol, potassium and transmural nerve stimulation were studied. Cromakalim did not prevent the detrusor from responding to these agents, although it significantly reduced the contractile response to K^+ at concentrations below 70 mM.

4 Uptake and efflux experiments using $^{86}Rb^+$ were unable to demonstrate any significant effect on transmembrane movement produced by cromakalim (10^{-5} M).

5 $^{43}K^+$ efflux showed a dose-dependent increase in the rate constant on addition of cromakalim. The difference in the selectivity for K^+ over Rb^+ was confirmed in dual label uptake experiments.

6 Substitution experiments in which the K^+ ions in the tissue were gradually replaced by Rb^+ demonstrated that cromakalim had a progressively decreasing effect on spontaneous activity as internal K^+ was lowered. When all the K^+ was replaced by Rb^+, cromakalim no longer inhibited spontaneous activity, confirming that the channel opened by cromakalim appears relatively impermeant to Rb^+.

Introduction

Cromakalim (BRL 34915) is one of the new class of potassium channel activating drugs that has recently received much attention. It has been shown to hyperpolarize the membranes of various smooth muscles (vascular smooth muscle, Hamilton *et al.*, 1986; taenia coli, Weir & Weston, 1986a; Allen *et al.*, 1986). In these tissues the drug is thought to produce its effect by opening K^+ channels in the smooth muscle membranes, so moving the membrane potential towards the potassium equilibrium potential. Evidence for this was provided by experiments in which $^{86}Rb^+$ was used as a tracer for K^+. This has become accepted protocol due to the expense and short half life of potassium isotopes ($^{42}K^+$, $t_{1/2}$ = 11 h; $^{43}K^+$, $t_{1/2}$ = 23 h) compared with the relatively inexpensive and longer acting $^{86}Rb^+$ ($t_{1/2}$ = 19 days). The use of $^{86}Rb^+$ as a marker for K^+ has been investigated by many workers. (see e.g. Widdicombe, 1977; Mauger *et al.*, 1978; Imaizumi & Watanabe, 1981; Bolton & Clapp, 1984).

Cromakalim is a potent relaxant of vascular and tracheal smooth muscles, and the potential use of such drugs in the control of hypertension and asthma has been postulated. We are interested in functional abnormalities of the urinary bladder, and in particular the condition of detrusor instability in which the smooth mucle becomes hyperexcitable. In this condition the potassium channel activating drugs have a potential use in directly decreasing the excitability of bladder smooth muscle cells. The effects of cromakalim on the contractile responses, electrical activity and transmembrane K^+ and Rb^+ fluxes of normal guinea-pig bladder smooth muscle have therefore been examined.

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Methods

Contractile studies

Tension experiments were carried out on bladder smooth muscle strips measuring 1 × 1 × 7 mm. These were dissected from the detrusor under a binocular microscope. They were mounted in a 0.2 ml organ bath (Brading & Sibley, 1983) and continuously superfused with Krebs (35–37°C) solutions at a flow rate of approximately 1 ml min^{-1}. Recessed platinum ring electrodes in the organ bath were used for the stimulation of the intrinsic nerves (50 V, 0.05 ms, 5 s train, variable frequency). The apparatus allowed six strips to be studied simultaneously; the tension was measured isometrically on home mounted Akers transducers and the output was recorded on a six channel Watanabe pen recorder. Initially strips were placed under a 1 g resting tension and then allowed to equilibrate for at least 1 h. Contractile responses were expressed as a percentage of a control response to a 10 s application of 10^{-4} M carbachol, applied at the beginning of the experiment. For construction of dose-response curves agonists were applied and then washed off, leaving 5–10 min between doses.

Electrophysiology

Electrical activity was recorded either intracellularly with microelectrodes, or extracellularly, using the double sucrose-gap apparatus. For microelectrode studies, strips of smooth muscle 10 × 1 × 1 mm were dissected from the dome of the bladder, and mounted in a 2 ml organ bath. Tissues were superfused at a rate of 2–3 ml min^{-1} with warmed (36°C) Krebs solution made hypertonic by addition of 12 g sucrose to 100 ml Krebs to reduce tissue movement. Microelectrodes were filled with 3 M KCl, and had a tip resistance of between 40 and 70 mΩ. Potentials were displayed on a pen-writing recorder (Gould Brush 2200).

For the double sucrose-gap method, a strip of smooth muscle 15 mm by 1.5 mm was mounted in a recording chamber similar to the one described by Goto *et al.* (1974). The chamber consisted of five compartments separated by four rubber membranes with holes in the centre, through which the specimen was pulled. Both ends of the tissue were connected to threads, one fixed, and the other connected to a mechano-transducer for simultaneous tension measurement. The central compartment (0.5 mm width) was superfused with warmed (36°C) isotonic Krebs solution, and the adjacent chambers (2 mm) with isotonic sucrose solution. The two outer chambers (1 ml capacity) were filled with isotonic KCl solution. Electrical activity was recorded between the central and outer chamber with a Ag-AgCl electrode and a plate electrode. Electrical and mechanical activity were displayed on a pen-writing recorder (Gould Brush 2200). Electrical stimulation was applied via a Ag-AgCl electrode in the central chamber. Test solutions were applied to the central chamber.

Radio-tracer ion flux analysis

Slightly larger strips of detrusor were used for flux studies; they were mounted on stainless steel holders and allowed to equilibrate in normal Krebs solution for at least an hour before the experiment. For efflux experiments, the tissues were equilibrated with warmed Krebs solution containing 1.5–3 μCi ml^{-1} $^{43}K^+$ or 8–12 μCi ml^{-1} $^{86}Rb^+$ or both. To wash out, the holder and tissue were first dipped into a non-radioactive bath of Krebs to remove adhering radioactivity and then inserted into a superfusion apparatus. The superfusate was collected in 2 min samples and subsequently counted in a gamma counter. The last sample contained the tissue (Brading, 1967). $^{43}K^+$ and $^{86}Rb^+$ loss was analysed in terms of the rate coefficient. This was calculated by dividing the counts leaving the tissue during a 2 min interval by the mean counts in the tissue during that interval. In uptake experiments tissues were exposed for 10 min either to $^{43}K^+$ and $^{86}Rb^+$ simultaneously (dual label experiment), or to just one isotope (single label). They were then washed in ice cold Krebs for 5 min to remove extracellular tracer, (Aickin & Brading, 1983) before counting. It was possible to distinguish between the two isotopes because of their differing half lives.

Statistics

Results are expressed as the mean ± s.e. (except where s.d. is indicated) (n), and their significance was assessed by a paired t test. Values of P less than 0.05 are shown with a single *, and less than 0.001 with two **.

Solutions

The composition of the Krebs solution was (mM): NaCl 120, KCl 5.9, $NaHCO_3$ 15.4, $MgCl_2$ 1.2, NaH_2PO_4 1, $CaCl_2$ 2.5 and glucose 11. 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 Beechams U.K. It was made up as a stock solution of 10^{-2} M with 70% ethanol, and diluted appropriately with Krebs solution. Carbachol was supplied by Sigma. This was made up as a stock solution of 10^{-2} M in distilled water. In experiments where high potassium Krebs solutions were used, NaCl was replaced isosmotically with

KCl. In experiments where internal K^+ was replaced by Rb^+, RbCl was substituted for KCl in the above Krebs solution. All solutions were equilibrated with 97% O_2, 3% CO_2.

Results

Mechanical activity

The majority of guinea-pig detrusor strips exhibited spontaneous mechanical activity during the course of an experiment (81%, $n = 74$). In Figure 1 the effect of increasing concentrations (10^{-8}–5×10^{-5} M) of cromakalim on this spontaneous activity is shown: 10^{-8} M appeared to have no effect on this activity. In 2 out of 6 strips 5×10^{-8} M reduced the frequency of the spontaneous contractions with variable effects on the amplitude. Concentrations above 5×10^{-7} M invariably caused cessation of the spontaneous mechanical activity with the time taken for reappearance of the activity being concentration-dependent. After washout the spontaneous activity reappeared and a transient potentiation in the frequency and amplitude of myogenic activity was observed. This lasted for approximately 10 min before the spontaneous activity returned to resting level.

Due to the very phasic nature of bladder smooth muscle it was impossible to achieve a maintained degree of tension against which an inhibitory dose-response curve for cromakalim could be constructed, and so the effect of the compound on the response to agonists was investigated to observe whether or not it was preventing the muscle from reacting to its normal excitatory agonist (muscarinic stimulation), potassium depolarization, and intrinsic nerve activation.

Figure 2 shows that cromakalim (10^{-5} M) did not significantly inhibit the ability of the detrusor to

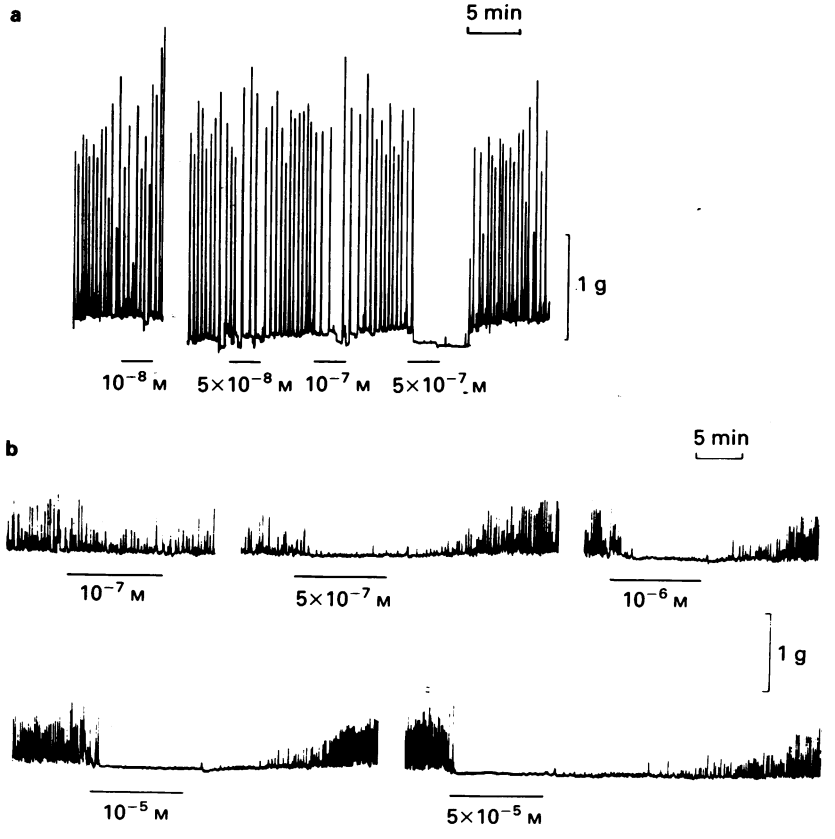

Figure 1 The effect of cromakalim on spontaneous activity from guinea-pig detrusor muscle. Little effect is seen at 10^{-8} M cromakalim, while 5×10^{-8} M reduces the frequency of the spontaneous activity. At 5×10^{-7} M, cromakalim totally inhibits the spontaneous contractions. There is little maintained tone in these strips. (a) and (b) are tissues from separate experiments.

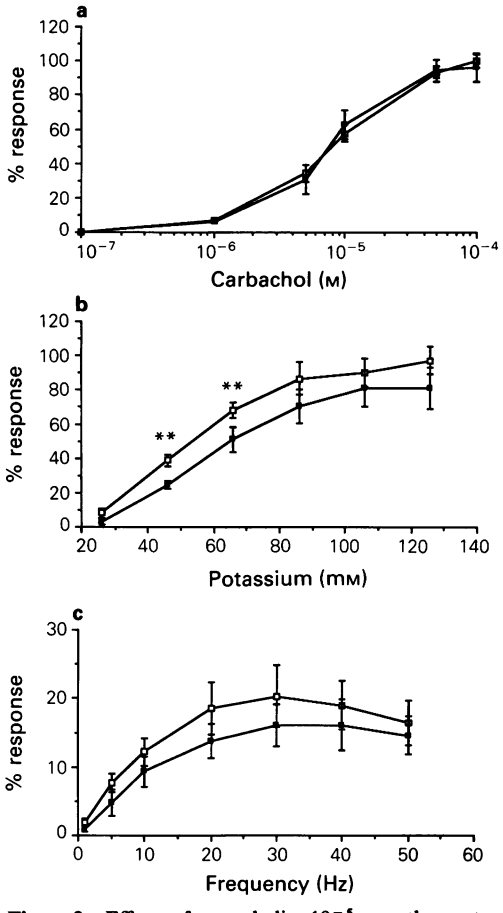

Figure 2 Effects of cromakalim 10^{-5} M on the contractile responses of guinea-pig detrusor. (a) Dose-response curve to 10 s applications of carbachol. (b) Dose-response curve to 4 min applications of K^+. (c) Frequency-response curve to transmural nerve stimulation (5 s train, 50 V, 0.05 ms). Controls (□); in the presence of cromakalim (■). Results are expressed as means with s.e.mean shown by vertical bars ($n = 12$).

Table 1 The effect of cromakalim on the frequency of spontaneous electrical activity and the associated hyperpolarization of the cell membrane

Cromakalim (M)	*Spike frequency* (min^{-1})	*Hyperpolarization* (mV)
Control	22.4 ± 3.9	0
10^{-7}	23.3 ± 1.5	0
5×10^{-7}	13.0 ± 3.0	0
10^{-6}	0	5.8 ± 1.9
5×10^{-6}	0	12.2 ± 3.0
10^{-5}	0	22.0 ± 5.0

respond to these agents, except at the lower concentrations of potassium where there was some small depression of the dose-response curve.

Electrical activity

The detrusor smooth muscle cells had a mean resting potential of −60.6 ± 3.2 mV (mean ± s.d., $n = 20$, 10 animals). Most cells showed spontaneous spike activity, either continuously, or in periodic bursts. Table 1 shows the effects of cromakalim on the membrane potential and spike frequency. At concentrations above 5×10^{-7} M, cromakalim caused a dose-dependent hyperpolarization, which at 10^{-5} M was such that the membrane potential must have been close to the potassium equilibrium potential. At a concentration of 5×10^{-7} M, the spike frequency was reduced, with no apparent change in the membrane potential. Higher concentrations of the drug abolished spike activity. These results are illustrated in Figure 3.

Figure 4 shows the effect of increasing the extracellular potassium to 20 mM after the membrane had been hyperpolarized by 5×10^{-6} M cromakalim and spike activity abolished. This concentration of K^+ was calculated to bring the potassium equilibrium potential close to the normal resting potential and, as can be seen, the spontaneous electrical activity returned in the continuous presence of cromakalim.

Figure 5 shows the effects of cromakalim (10^{-6}–10^{-5} M) on a strip of muscle mounted in the double sucrose gap apparatus. Alternate hyperpolarizing and depolarizing current pulses were applied to the node. Depolarizing pulses caused spike activity in the cells (not well recorded with this method, due to lack of synchronization of the spikes in the various muscle bundles in the node), and a phasic contraction. The size of the electrotonic potentials allowed assessment of changes in the membrane resistance. Cromakalim hyperpolarized the tissue, reduced the phasic tension responses and also decreased the membrane resistance. It was also found that cromakalim had little effect on the excitatory junction potentials evoked by brief current pulses. For example, in one experiment in the presence of 10^{-6} M cromakalim, the amplitude was reduced to 94.8 ± 3.84% of the control amplitude, and in 10^{-5} M to 77.6 ± 2.22% ($n = 10$). A reduction in amplitude would be expected even if transmitter release is unaffected, because of the decreased membrane resistance.

Flux studies

Although cromakalim had very consistent and repeatable effects on the tension and electrical

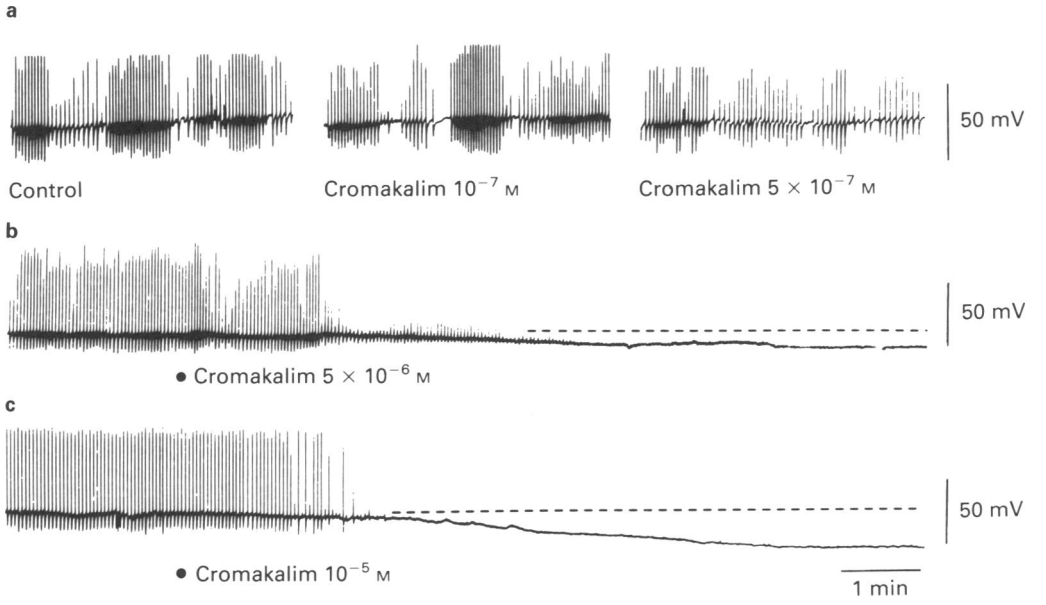

Figure 3 The effects of cromakalim on membrane potential and spontaneous spike activity (micro-electrode recording). (a) Drug concentrations up to 5×10^{-7} M had no effect on the resting potential (−60 mV) but reduced spike frequency at 5×10^{-7} M. (b and c) Higher concentrations hyperpolarized the membrane (by 12 mV at 5×10^{-6} M and 25 mV at 10^{-5} M) and abolished spontaneous activity. In 10^{-5} M cromakalim, the membrane potential was close to the estimated E_K (−85 mV).

properties of guinea-pig bladder smooth muscles, the effects of Rb^+ and K^+ fluxes were much more variable.

Efflux experiments When $^{86}Rb^+$ was used as a tracer for K^+, cromakalim at 10^{-5} M had no effect on the rate of loss of $^{86}Rb^+$ from 8 strips from five guinea-pigs, but in another 4 strips from three different guinea-pigs, there was a very slight increase in the efflux rate. In comparison, when $^{43}K^+$ was used as the tracer, out of 20 strips from 14 guinea-pigs, only 3 strips from 2 particular guinea-pigs did not respond to cromakalim at this concentration. Some strips responded to lower concentrations. Examining all the results to date, 47 strips from 14 guinea-pigs responded to concentrations of cromakalim between 5×10^{-6} M and 10^{-4} M with an obvious (but variable) increase in the rate of loss of K^+. Figure 6 illustrates an experiment in which strips from the same animal were loaded in Krebs solutions containing either $^{43}K^+$ or $^{86}Rb^+$ as tracer, washed out in normal Krebs solution, and exposed to 10^{-5} M cromakalim for 10 min. In this experiment the drug caused a clear increase in the rate of loss of $^{43}K^+$, but had no effect on the loss of $^{86}Rb^+$. Figure 7 shows the results of an experiment with strips from three bladders, in which the effect of varying the concentration of cromakalim was studied on the rate of

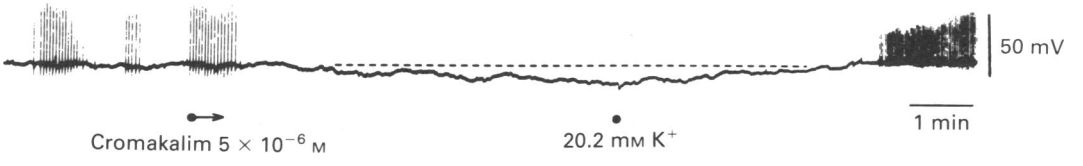

Figure 4 The effects of raising extracellular K^+ concentration ($[K^+]_o$) on the action of cromakalim, (microelectrode recording). Raising $[K^+]_o$ to 20.2 mM reversed the membrane hyperpolarization and the spontaneous activity returned, suggesting that under the influence of cromakalim, the membrane potential moves toward E_K (E_K in 20.2 mM $[K^+]_o$ is about −55 mV, close to the resting membrane potential).

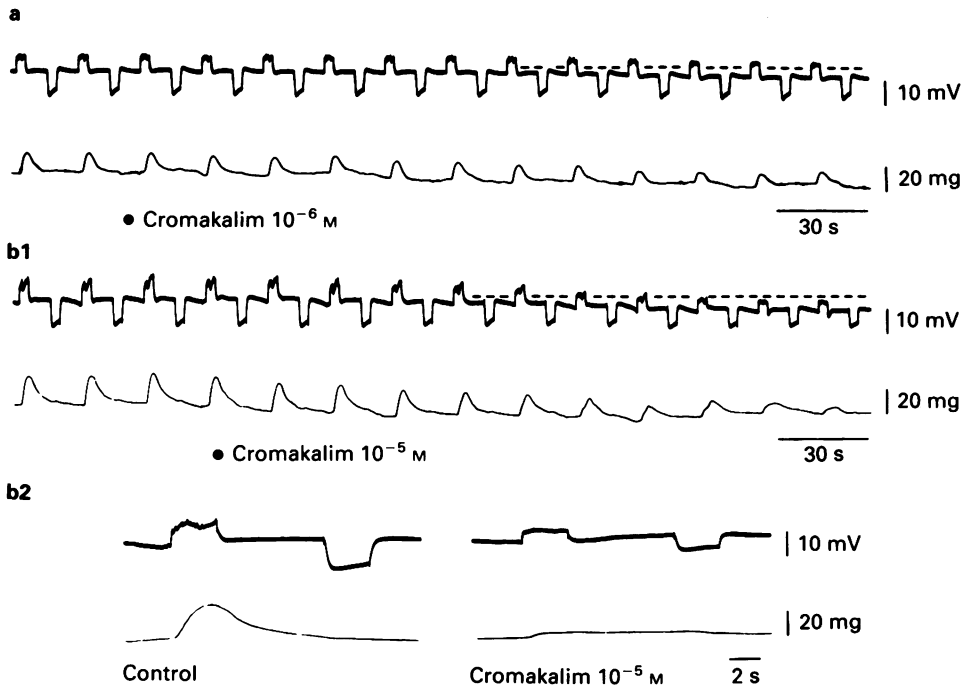

Figure 5 The effects of cromakalim on membrane potential and membrane conductance (double sucrose gap recording). In each pair, the upper trace shows electrical activity and the lower trace mechanical activity. Electrotonic potentials were evoked by inward and outward current pulses (3 s, 0.1 Hz). Depolarizing electrotonic potentials lead to contractions. (a and b1) Cromakalim 10^{-6} M (a) and 10^{-5} M (b1), hyperpolarized the membrane, increased the conductance and subsequently reduced the contraction. (b2) Electrotonic potentials (faster time scale) before and during exposure to cromakalim (10^{-5} M).

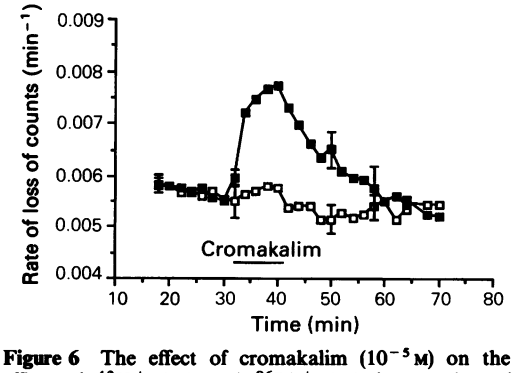

Figure 6 The effect of cromakalim (10^{-5} M) on the efflux of $^{43}K^+$ (■) and $^{86}Rb^+$ (□) from strips of guinea-pig detrusor muscle. The results shown are the means with s.e.mean shown by vertical bars ($n = 8$ for each curve). These results were obtained in the same experiment, under the same conditions using the same solutions. The channels opened by cromakalim appear to be selective for K^+ over Rb^+.

loss of $^{43}K^+$. In this case there was a clear dose-dependency of the effect.

Uptake experiments We used 10 min exposure to the radioactive label to assess the effect of cromakalim on the uptake of $^{43}K^+$ or $^{86}Rb^+$, since this time is on the linear part of the uptake curve, and is long enough to get significant uptake, but sufficiently short to prevent much backflux of tracer. Cromakalim (10^{-5} M) had little effect on the uptake of $^{86}Rb^+$, but normally caused a significant increase in the uptake of $^{43}K^+$, although the effect was rather variable. The largest increase seen in one experiment was 43%, and in another experiment there was no significant increase. In a group of dual label experiments, in which the uptake baths contained both $^{43}K^+$ and $^{86}Rb^+$, there was no significant difference in the uptake of the two isotopes in normal Krebs solution, (the tissue took up 73.2 ± 3.0% ($n = 24$ tissues) of the activity of an equivalent volume of the bathing solution), but if this is taken as 100%, in the presence

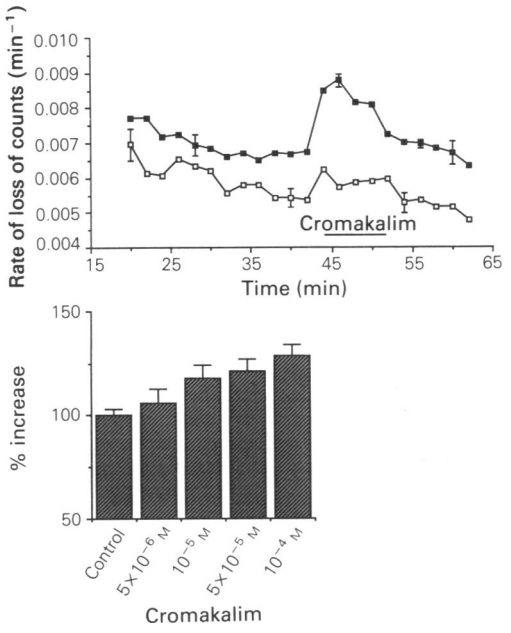

Figure 7 The effect of different concentrations of cromakalim on the $^{43}K^+$ efflux from stips of guinea-pig detrusor muscle ($n = 3$ for each concentration shown). Full efflux curves of the mean (with s.e.mean shown by vertical bars) are given showing the effect of 5×10^{-6} M (□) and 5×10^{-5} M (■). The histograms show the mean increase (s.e.mean shown by vertical bars) in efflux rate during 10 min exposure to the drug, expressed as a percentage of the control (the average of the previous 5 values).

of 10^{-5} M cromakalim there was an increase in the uptake of $^{43}K^+$ to $114.2 \pm 3.1\%$ (52) whereas the $^{86}Rb^+$ uptake ($103.6 \pm 2.5\%$ (30)) was not significantly increased.

Experiments on Rb tissues

Since the flux experiments suggest that the channels opened by cromakalim are significantly less permeant to Rb^+ than to K^+, we performed a series of experiments on tissues which had been exposed for 5–7 h to K^+-free Krebs, in which the KCl had been replaced with RbCl. Figure 8 shows the time course of uptake of Rb^+ in tissues bathed in K^+-free (Rb^+) solution, with $^{86}Rb^+$ as a tracer. Extrapolation of the graph suggests that the tissues will be virtually fully loaded with Rb^+ in about 7 h.

Contractile studies Figure 9 shows how the effect of cromakalim (10^{-5} M) on spontaneous mechanical activity varies with time of exposure to K^+-free (Rb^+) solution. As can be seen, there is a progressive

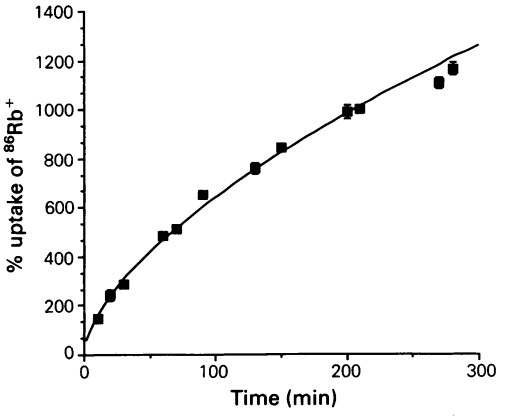

Figure 8 Graph to show the time course for replacement of cell K^+ with Rb^+ in strips bathed in zero potassium (Rb^+) Krebs solution. Means of $n = 7$; s.e.mean shown by vertical bars.

decrease in the inhibitory effect of cromakalim; this effect was seen in every tissue we tried (16). The response to cromakalim was virtually abolished after 7 h. Significant recovery of the effect occurred after an hour of bathing in normal Krebs solution.

Electrophysiology A similar reduction in the effect of cromakalim was seen on membrane potential and spike activity. This is shown in Figure 10. During

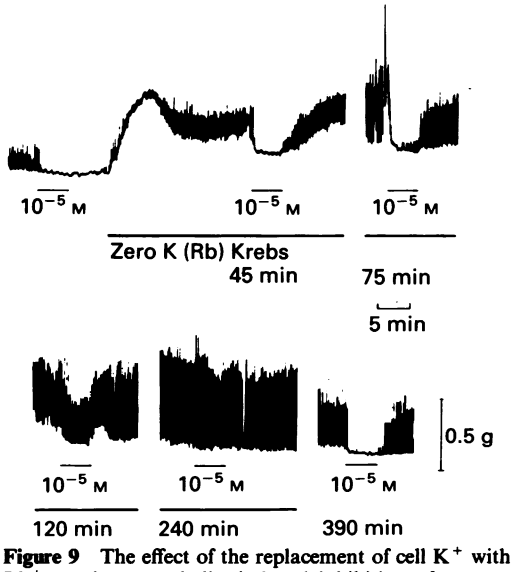

Figure 9 The effect of the replacement of cell K^+ with Rb^+ on the cromakalim-induced inhibition of spontaneous mechanical activity. After 7 h of exposure to zero K^+ (Rb^+) Krebs cromakalim had no effect. After 150 min perfusing with normal Krebs the response to cromakalim returns.

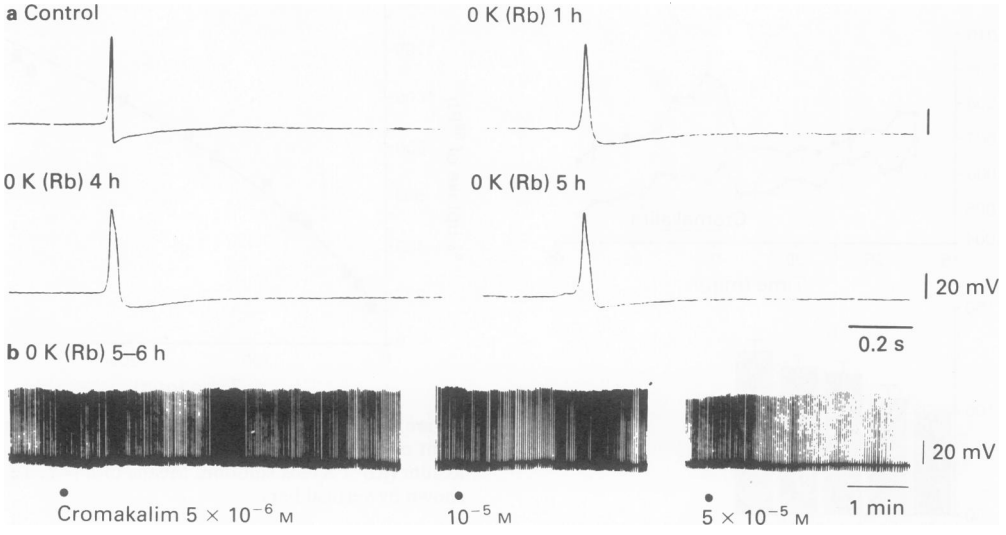

Figure 10 The effects of zero K^+ (Rb^+) solution (microelectrode recording) on the shape of the action potential, and the effects of cromakalim on spike frequency. During exposure to zero K^+ the spike duration was progressively prolonged (a), and after 5–6 h the effect of cromakalim was greatly reduced (b).

exposure to K^+-free (Rb^+) solution there was a progressive lengthening of the duration of the spikes (due to a reduction in the rate of spike repolarization). In one experiment the time taken for spike repolarization (from peak to maximum after-hyperpolarization), increased from 10–11 ms in the control, to 32–40 ms after 2 h in K^+-free (Rb^+) Krebs, and 40–44 ms after 4 h in K^+-free (Rb^+) solution. After 5–6 h in this solution the effects of cromakalim on the membrane potential and spike activity were virtually abolished.

Flux studies Figure 11 shows the results from an experiment in which the effect of cromakalim on $^{86}Rb^+$ efflux was studied using Rb^+ tissues (which had been for 5.5 h in K^+-free (Rb^+) solution at the start of the washout) and compared with its effects on the $^{43}K^+$ efflux from normal tissues. As can be seen, in this experiment there was no significant effect of cromakalim (10^{-5} M) on the K^+ flux from normal tissues, but there was a significant decrease in the rate of loss of Rb^+ from the Rb^+ tissues. The effect of cromakalim on $^{43}K^+$ and $^{86}Rb^+$ uptake into Rb^+ tissues is shown in Figure 12. In this case cromakalim increased the uptake of $^{43}K^+$, but not of $^{86}Rb^+$.

Discussion

The evidence presented in this paper is consistent with the view that cromakalim opens potassium channels in the membranes of smooth muscle cells of the guinea-pig detrusor. Electrophysiological experiments demonstrate a clear dose-related hyperpolarization and increase in membrane conductance. The hyperpolarization is reversed when the potassium equilibrium potential is altered to a value close to the original membrane potential. The loss of spike activity induced by cromakalim correlates with the

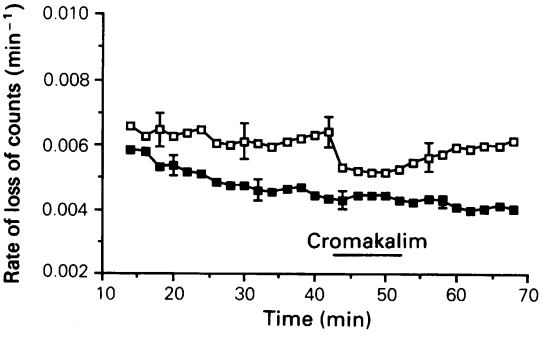

Figure 11 The effect of cromakalim (10^{-5} M) (■) on the efflux of $^{43}K^+$ from normal tissues; the results from tissues in which most of the K^+ had been replaced by Rb^+ (exposed to zero K^+ for 5 h) shown by (□). The results are the means with s.e.mean shown by vertical bars ($n = 8$ for each curve). Cromakalim decreased the rate of loss of Rb^+ from Rb^+ tissues but did not affect K^+ loss from normal tissues.

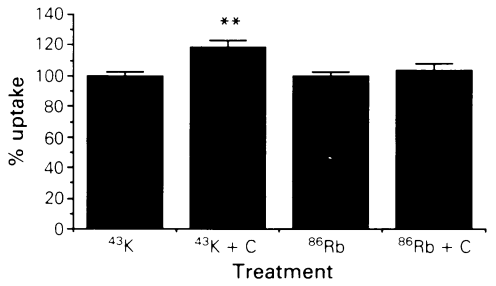

Figure 12 The effect of cromakalim (10^{-5} M) on the 10 min uptake of $^{43}K^+$ and $^{86}Rb^+$ (dual label experiment) by Rb^+ tissues (5.5 h in $0K^+$ (Rb^+) Krebs). Uptake is measured as c.p.m./mg of wet wt. as a percentage of the counts in the uptake solution (c.p.m./mg). Results are the mean with s.e.mean shown by vertical bars ($n = 6$).

reduction of the spontaneous mechanical activity recorded from tissue strips.

The effects of cromakalim on the contractile responses of the tissue to increasing extracellular K^+, application of carbachol and stimulation of intrinsic excitatory nerves are also consistent with this mode of action of cromakalim. The responses to increasing K^+ concentration were reduced significantly in the lower range of K^+ concentrations. In this range, the membrane potential is still probably significantly less negative than the potassium equilibrium potential (as has been shown in other smooth muscles, e.g. Casteels & Kuriyama 1966), and cromakalim will still be able to hyperpolarize the membrane, whereas at the higher K^+ concentrations cromakalim would have no significant effect on the membrane potential.

There was no significant effect of cromakalim on the contractile response to carbachol. This was a little surprising, since in many smooth muscles, muscarinic receptor activation leads to a marked depolarization (Bolton, 1979), which would have been reduced by the opening of extra potassium channels. However, recent work in our laboratory (Fujii, 1987; 1988) has shown that in the guinea-pig bladder, muscarinic receptor activation, even at maximally effective concentrations of carbachol or acetylcholine, has relatively little effect on the membrane potential, the contraction being mediated almost entirely through pharmaco-mechanical coupling.

Cromakalim caused a slight reduction in the response to intramural nerve stimulation. The results with muscarinic agonists suggest that this diminished response is unlikely to be due to effects on cholinergic transmission. This possibly demonstrates an effect on the non-adrenergic, non-cholinergic transmission that is responsible for about 70% of the response to transmural nerve stimulation in the guinea-pig bladder (Brading, 1987). Fujii (1987, 1988) has shown that the excitatory junction potentials (e.j.ps) that occur on intrinsic nerve stimulation are unaffected by muscarinic antagonists, but abolished after treatment with the P_2 receptor-desensitizing drug α,β-methylene ATP. Cromakalim does not abolish these excitatory junction potentials but does reduce their size, probably because of the lowered input resistance of the cell membrane following the opening of the K^+ channels. This would account for the slight reduction in the response to transmural nerve stimulation. It seems therefore that cromakalim is probably not significantly affecting the release of the excitatory transmitter responsible for the e.j.ps, although our data are not sufficiently precise to exclude a presynaptic effect of the drug.

Some of the more interesting observations we have made, come from the analysis of the effects of cromakalim on the uptake and efflux of Rb^+ and K^+ isotopes, and the effects of replacing intracellular K^+ with Rb^+. Many workers have used $^{86}Rb^+$ as a tracer for K^+ ions, as it is a more convenient isotope (half-life 18 days) and cheaper than the available K^+ isotopes $^{42}K^+$ and $^{43}K^+$ which have half-lives of only 11 and 23 h. It has also been shown that cromakalim increases the efflux of Rb^+ from rat (Weir & Weston, 1986b) and guinea-pig (Quast, 1987) blood vessels. Initial experiments were therefore carried out on the efflux of $^{86}Rb^+$ from strips of detrusor but there was little if any effect of cromakalim. Theoretically, an increase in K^+ permeability of the membrane leading to hyperpolarization will have two opposing actions on the efflux of cations that can pass through the channels. An increase in the number of ions leaving the cell might occur because of the larger permeability, but also there will be a decrease in the driving force due to the hyperpolarization of the cell membrane, which would attenuate the increase in efflux. Both factors work in the same direction however when examining the uptake of tracer. For this reason the subsequent experiments examined the effect of cromakalim on the uptake of $^{86}Rb^+$, but again there were no significant effects.

Further investigations were then undertaken using K^+ isotopes, and significant increases in both uptake and efflux were recorded. However, the concentrations of cromakalim required to produce these effects were high: we found no significant effects at less than 5×10^{-6} M cromakalim, whereas spontaneous electrical and mechanical activity is inhibited at 10^{-7} M. Another problem with these experiments has been the variability of the size of the response to cromakalim. Cromakalim 10^{-5} M was routinely used for the majority of experiments, and its effect on K^+ fluxes varied markedly from experiment to experiment.

There are several possible reasons for this variability. The normal procedure was to distribute tissues from each bladder used in an experiment through all the protocol groups. The means of the results from each group were used to compare treatments. In a recent experiment we compared the effects of cromakalim on the uptake of tissues using controls from the same bladder, in order to compare two guinea-pigs of different sizes. We found a very large difference in the uptake of K^+ in normal Krebs, the smaller guinea-pig had almost double the uptake per unit tissue weight compared with the larger. Tissues from the smaller animal were also less sensitive to cromakalim. Large inter-animal variation could have masked the effects of the drug in the pooled experiments. This variation could be due to differences in the proportions of the various K^+ channels that occur in these membranes, and also to differences in the amount of spontaneous electrical activity occurring (which involves activation of various K^+ channels).

When Rb^+ and K^+ fluxes were examined in both single and dual labelled experiments, the results confirmed that cromakalim has a much greater effect on the K^+ fluxes than Rb^+ fluxes. This suggests strongly that the channels opened by this drug are much more permeable to K^+ than to Rb^+. The differential permeability of the cromakalim-activated channels to K^+ and Rb^+ has also been suggested by Quast (1988) although the selectivity in the rat aorta and guinea-pig portal vein was not as great as in the detrusor.

When Rb^+ was used as a tracer, the total amount of Rb^+ in the intra and extracellular solutions was very small, and interactions between unlabelled K^+ and $^{86}Rb^+$ could have occurred. We therefore carried out an experiment on $^{86}Rb^+$ efflux from Rb^+ tissues, in which much of the intracellular K^+ had been replaced by Rb^+, and compared this with $^{43}K^+$ efflux from normal tissues. In this experiment, cromakalim had no significant effect on normal tissues, but caused a reduction of the efflux of $^{86}Rb^+$ in the Rb^+ tissues. This result could be explained if there were still enough K^+ in the Rb^+ tissues for cromakalim to cause hyperpolarization (E_K would be very negative, as there is no external K^+), and if Rb^+ was unable to penetrate the channels opened by cromakalim. In this case both the hyperpolarization and the possible shutting of voltage-sensitive K^+ channels through which Rb^+ could pass, would act to reduce the Rb^+ efflux. In normal tissues the lack of effect of cromakalim would be due to the balancing effects of factors tending to reduce the efflux and the increased K^+ efflux that would occur through the channels opened by the drug.

Further support for the idea that Rb^+ ions do not pass easily through the channels opened by cromakalim comes from the time-dependent decrease in the effectiveness of cromakalim on the spontaneous mechanical activity after the external K^+ had been replaced by Rb^+. The inhibitory effect of cromakalim is totally abolished at a time when all the intracellular K^+ would be replaced by Rb^+. As expected, this was also seen with electrical activity. However the unexpected result here was the progressive lengthening of the action potential that occurred, suggesting that some of the channels responsible for the repolarization phase of the spike are also more permeable to K^+ than to Rb^+.

In conclusion, an attempt has been made to correlate the effect of cromakalim on spontaneous activity, electrical activity and ionic fluxes. As low concentrations of cromakalim have an effect on spontaneous electrical activity without causing any hyperpolarization it is a possibility that the drug might also be effective in blocking pacemaker mechanisms. Also at concentrations which increase potassium permeability there is a selectivity for the channel opened by cromakalim for K^+ over Rb^+, a fact which has also been noted, to a lesser extent, in the rat aorta and guinea-pig portal vein (Quast, 1988).

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