Br. J. Pharmacol. (1988), **95**, 795–804

Characteristics of cromakalim-induced relaxations in the smooth muscle cells of guinea-pig mesenteric artery and vein

[1]K. Nakao, K. Okabe, K. Kitamura, H. Kuriyama & [2]A.H. Weston

Department of Pharmacology, Faculty of Medicine, Kyushu University, Fukuoka 812, Japan

1 The effects of cromakalim (BRL 34915) on the smooth muscle cells of guinea-pig mesenteric artery and vein were investigated with microelectrode and tension recording methods.

2 Cromakalim (> 10 μM) produced membrane hyperpolarization with an increase in ionic conductance. The hyperpolarization occurred to a greater extent and lasted longer in the vein than in the artery.

3 The hyperpolarization induced by cromakalim in mesenteric vein comprised two components, one of which was Mn sensitive. In mesenteric artery, the hyperpolarization was relatively insensitive to Mn.

4 From the current-voltage relationship measured from arterial smooth muscle membranes, the reversal potential of cromakalim was estimated to be −80 mV. The cromakalim-induced hyperpolarization was not modified in Na- or Cl-deficient solution.

5 In both mesenteric artery and vein, cromakalim relaxed tissues precontracted with high K with (below 40 mM) or without (above 40 mM) hyperpolarization of the membrane.

6 In the mesenteric artery, action potentials evoked by electrical stimulation ceased before the generation of hyperpolarization.

7 Cromakalim produced a cross-desensitization with nicorandil on the evoked membrane hyperpolarization in mesenteric artery.

8 It is concluded that the relaxing actions of cromakalim result from the hyperpolarization which follows the opening of Ca-dependent K channels. The inhibition of a voltage-dependent Ca current may also be involved in this inhibitory effect.

Introduction

Cromakalim (BRL 34915; (±)-6-cyano-3,4-dihydro-2,2-dimethyl-*trans*-4-(2-oxo-1-pyrrolidyl) - 2H-benzo-[b]pyran-3-ol) is a novel antihypertensive agent (Ashwood *et al.*, 1986) which produces relaxation associated with membrane hyperpolarization in many visceral smooth muscles. It has been proposed that these actions of cromakalim result from the opening of membrane K channels (guinea-pig taenia caeci, Weir & Weston, 1986a; guinea-pig trachealis, Allen *et al.*, 1986a; rat portal vein, Hamilton *et al.*, 1986; rat aorta and portal vein, Weir & Weston, 1986b; guinea-pig portal vein, Quast, 1987).

In guinea-pig trachealis (Allen *et al.*, 1986a) and rat portal vein (Hamilton *et al.*, 1986) both the relaxation and the hyperpolarization occur within the same concentration ranges of cromakalim. In contrast, this drug produces relaxation of rat uterus with only a small membrane hyperpolarization and without a detectable change in K efflux estimated using ^{86}Rb (Hollingsworth *et al.*, 1987). Furthermore, in rat portal vein, Hamilton *et al.* (1986) demonstrated that spontaneous electrical discharges were inhibited by low concentrations of cromakalim without any detectable change in the membrane potential or ^{86}Rb efflux. Recently, Shetty & Weiss (1987) also found that in rat portal vein, a high concentration of cromakalim (5 μM) increased K efflux whereas a low concentration (0.5 μM) inhibited spontaneous mechanical changes with no modification of K exchange. These results suggest that factors additional to membrane hyperpolarization may be involved in cromakalim-induced relaxation of vascular tissues.

[1] Author for correspondence.
[2] Present address: Department of Physiological Sciences, Medical School, University of Manchester, Manchester M13 9PT.

The objective of the present experiments was to investigate further the role of K-channel opening in the smooth muscle relaxant actions of cromakalim. The effects of this agent were also compared with those of nicorandil, another smooth muscle relaxant with the ability to open K-channels (Sumimoto *et al.*, 1987). In these experiments changes in the electrical and mechanical properties of vascular smooth muscle were studied using guinea-pig mesenteric artery and vein, tissues in which the actions of nicorandil had previously been investigated (Itoh *et al.*, 1981; Karashima *et al.*, 1982).

Methods

Guinea-pigs of either sex (250–300 g) were stunned and bled. The superior mesenteric artery and vein (each about 100 μm in diameter) were excised together and isolated with fine forceps under a binocular microscope. The vessel tissue (0.1–0.15 mm in diameter and 10 mm in length) was pinned onto a rubber plate in a chamber (1.5 ml in volume) and superfused with a modified Krebs solution (32–35°C) at a flow rate of 3 ml min^{-1}.

Recording of electrical activity

To record the electrical activity of the mesenteric vascular muscle, glass capillary microelectrodes (borosilicate glass capillary; Hilgenberg Glass GmbH Co.) filled with 3 M KCl were used. Their tip resistance ranged between 40–80 MΩ. An electrode was inserted into a smooth muscle cell from the adventitial side of the vessel by means of a micromanipulator (MN-2, Narishige Sci. Inst. Lab) and electrical responses were displayed on a pen recorder (RKG-4024, Nihon Kohden Kogyo Co.). To record electrotonic and action potentials, the partition stimulating method was used (Abe & Tomita, 1968). An agar (2 M KCl) electrode was used as the indifferent recording electrode and in the experiments using a Cl-deficient solution the microelectrodes were filled with 2 M K-citrate.

Recording of mechanical activity

To record the mechanical activity, a ring-shaped segment of either the mesenteric artery or vein (0.3–0.5 mm and 1.0–1.5 mm in length, respectively) was cannulated and fixed by a pair of fine L-shaped needles; one was fixed at the bottom of the recording chamber and the other was connected to a mechanotransducer (TB612; Nihon Kohden). Tension changes were measured isometrically. The excised vascular tissue was slightly stretched (0.5–1.0 mN tension level) and this allowed the tissue to maintain dimensions similar to those *in vivo*. The tissue was allowed to equilibrate for 1 h under these conditions, after which high K (39 mM) solution was applied every 30 min until the contraction amplitude became constant. All these procedures required 2–4 h.

Solutions and drugs

The basic ionic composition of the Krebs solution used was as follows (mM): Na^+ 134.7, K^+ 5.9, Ca^{2+} 2.6, Mg^{2+} 1.2, HCO_3^- 15.5, $H_2PO_4^-$ 1.2, Cl^- 134.0, glucose 12.0. The solution was aerated with 97% O_2 and 3% CO_2, and the pH was kept at 7.2–7.4. Solutions containing high concentrations of K were prepared by replacing NaCl with an equimolar amount of KCl up to 118 mM. For low K solutions, additional NaCl was used. Na-deficient solutions were prepared by replacing NaCl with Tris-Cl (15.5 mM Na containing solution). Cl-deficient solutions were prepared by replacing NaCl with an equimolar amount of sodium isethionate (12.1 mM Cl-containing solution). Ca-free solutions were prepared by replacing $CaCl_2$ with $MnCl_2$ or $LaCl_3$, and in some cases, 0.5 mM ethyleneglycol *bis* (β-aminoethyl ether)-N,N,N,N-tetraacetic acid (EGTA) was added to the Ca-free solution. Drugs used in the present experiments were: (±)-cromakalim (Beecham), nicorandil (2-nicotinamidoethyl nitrate; Chugai) and EGTA (Dozin).

Statistics

The results obtained were expressed as mean values ± s.d. of the mean of the individual observations (n). Statistical significance was estimated by use of Student's t test and a P value of less than 0.05 was considered to be statistically significant.

Results

Effects of cromakalim on electrical activities of smooth muscle cells of guinea-pig mesenteric artery and vein

The smooth muscle membranes of the mesenteric artery and vein were electrically quiescent and their resting membrane potentials were -69.5 ± 2.5 mV ($n = 120$, 30 preparations) and -59.5 ± 2.3 mV ($n = 100$, 20 preparations), respectively. Application of cromakalim (30 μM) hyperpolarized the arterial and venous smooth muscle membranes to -75.0 ± 2.5 mV ($n = 50$, 5 preparations) and -71.5 ± 2.6 mV ($n = 50$, 5 preparations), respectively (Figure 1). Changes in ionic conductance were estimated from changes in the amplitude of the electrotonic potential, by inserting a recording microelectrode into the cell at a distance of 0.1 mm from the stimulating electrode. During membrane hyperpolarization by 30 μM cromakalim, the amplitude of

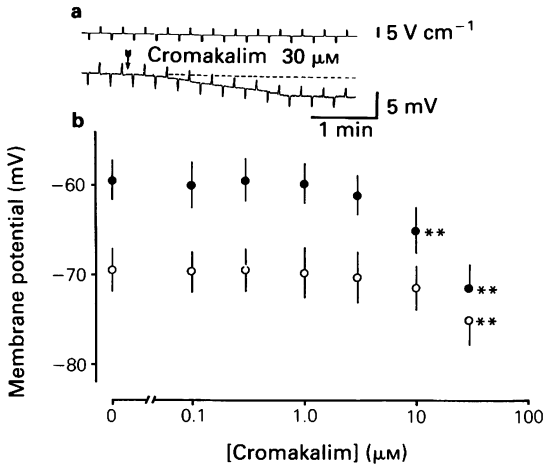

Figure 1 (a) Effects of cromakalim (30 μM) on membrane potential and electrotonic potentials induced by alternate application of inward and outward current at constant intensity from a smooth muscle cell of the mesenteric artery. (b) Effects of cromakalim on the membrane potential in smooth muscle cells of the mesenteric artery (○) and vein (●); $n = 50$, 5 preparations. The membrane potential was measured 5 min after application of cromakalim. The microelectrode was inserted into a cell located about 0.1 mm from the stimulating electrode. Symbols indicate mean and vertical lines show s.d. ** $P < 0.01$.

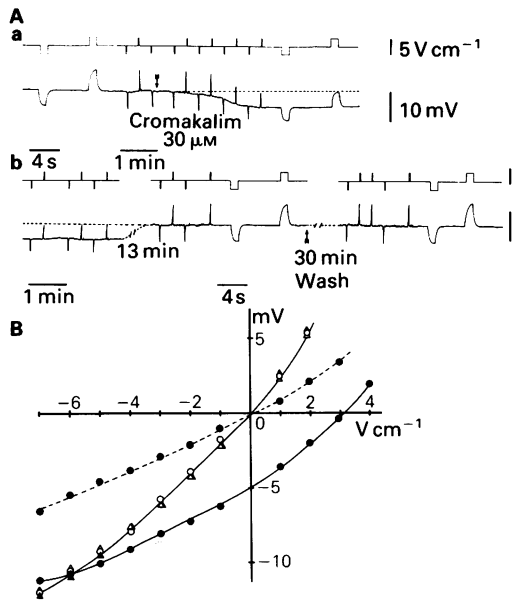

Figure 2 Changes in membrane potential and electrotonic potential observed from the mesenteric artery before, during and after application of cromakalim. (A) Inward and outward currents were applied at constant intensity (a,b) before and during the application of 30 μM cromakalim, and (b) after removal of cromakalim. (B) The current-voltage relationships observed from a smooth muscle cell of the mesenteric artery; (○) control; (●———●) 3 min after application of 30 μM cromakalim; (●- - - -●) 5 min after application of 30 μM cromakalim; (▲) 15 min after application of 30 μM cromakalim; (△) 60 min after removal of cromakalim. (------) After the membrane was hyperpolarized by cromakalim, the membrane potential was displaced to the resting level by outward current injection and the current-voltage relationship was re-assessed. The resting membrane potential of this cell was −67 mV.

the electrotonic potential was reduced to about 0.7 times the control (Figure 1a). Figure 1b represents a summary of the membrane potential changes in the presence of various concentrations of cromakalim. Cromakalim above 10 μM significantly hyperpolarized the membrane in venous smooth muscle cells and a concentration of 30 μM BRL 34915 was required to hyperpolarize the arterial cells. The absolute values of hyperpolarization were larger in the venous smooth muscle cells than in the arterial preparations.

To investigate in more detail the conductance changes which occurred during the membrane hyperpolarization induced by cromakalim in mesenteric artery, a constant intensity or various intensities of inward and outward current pulses were applied alternately. With relatively weak stimulation, the response evoked by outward current pulses was larger than that evoked by an inward current due to the anomalous rectifying property of the membrane (Figure 2a). The majority of muscle cells ($n = 40$) did not produce an active response on application of outward current pulses and only a few cells ($n = 3$) generated an action potential (see later). The hyperpolarization induced by cromakalim was accompanied by an increase in the ionic conductance of the membrane. However, this hyperpolarization was relatively transient and after about 15 min in the continued presence of cromakalim (30 μM) the membrane potential had returned to control levels. The electrotonic potential also returned to the level observed before application of the drug.

Figure 2B shows the current-voltage (I-V) relationships observed before, during application of cromakalim (after 3 min and 15 min) and 60 min after removal of the drug. When various intensities of inward current pulse were applied, an upwardly concaved I-V curve was observed. The slope of this relationship determined 3 min after application of 30 μM cromakalim was less steep than that of the control curve. Following restoration of the membrane potential to control levels 15 min after application of cromakalim, the slope was the same as that

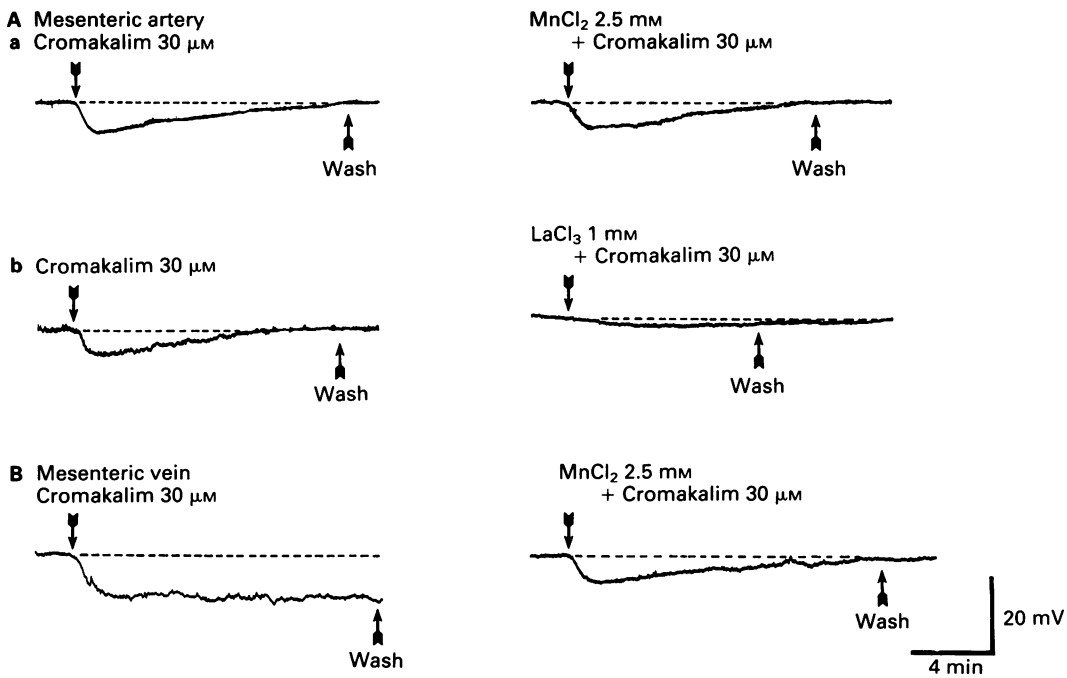

Figure 3 Effects of cromakalim on the membrane potential recorded from smooth muscle cells of (A) mesenteric artery and (B) mesenteric vein. At first, cromakalim was applied in normal Krebs solution; this was then replaced with a solution containing zero Ca + 2.5 mM $MnCl_2$ (Aa and B) or $LaCl_3$ 1 mM + 1.5 mM $CaCl_2$ (Ab). The cells were exposed to zero Ca or Ca^{2+}-deficient solution for 15 min before application of cromakalim. The resting membrane potentials were −68 mV (Aa), −69 mV (Ab) and −60 mV (B).

observed in control conditions. When the I-V curves in the presence (hyperpolarizing period) and absence of cromakalim were compared, both lines crossed at approximately −80 mV ($n = 3$). To detect the relative change in ionic conductance during application of cromakalim, the membrane potential was displaced to control levels by current injection and the I-V relationship was again observed. The slope of this relationship was consistently less steep than in controls.

Effects of external Ca on the cromakalim-induced hyperpolarization

As already stated cromakalim transiently hyperpolarized the membrane in the mesenteric artery but in the mesenteric vein it produced a sustained hyperpolarization over a 15 min period. As shown in Figure 3Aa, when nominally Ca-free solution containing 2.5 mM $MnCl_2$ was superfused, the amplitude and duration of the hyperpolarization induced by cromakalim were not modified in the mesenteric artery (control: 6.2 ± 1.3 mV, $n = 12$; Ca-free + Mn solution: 5.8 ± 1.1 mV, $n = 13$). However, when the same solution was superfused in mesenteric vein, the peak amplitude of hyperpolarization was greatly reduced (control: 11.5 ± 1.6 mV, $n = 13$; Ca-free + Mn solution: 7.0 ± 1.5 mV, $n = 11$), the duration of hyperpolarization was markedly shortened and its time-course resembled that observed in mesenteric artery (Figure 3B). Thus, in mesenteric vein the hyperpolarization induced by cromakalim comprises two components. One of these is Mn-resistant and is the dominant component in the mesenteric artery. In the presence of 1 mM $LaCl_3$, the membrane was slightly depolarized (3.3 ± 0.4 mV, $n = 3$) and the Mn-resistant cromakalim-induced hyperpolarization was markedly inhibited in the mesenteric artery (Figure 3Ab). When EGTA (0.5 mM) was included in the Ca-free solution, the membrane was depolarized to −40.0 ± 2.8 mV ($n = 12$, 3 preparations) and no cromakalim-induced hyperpolarization was observed in either mesenteric artery or vein (data not shown).

Effects of cromakalim on membrane potential and contraction in various concentrations of K

In mesenteric artery increasing the K concentration from 5.9 mM to 90 mM produced an approximately linear membrane depolarization. When the K con-

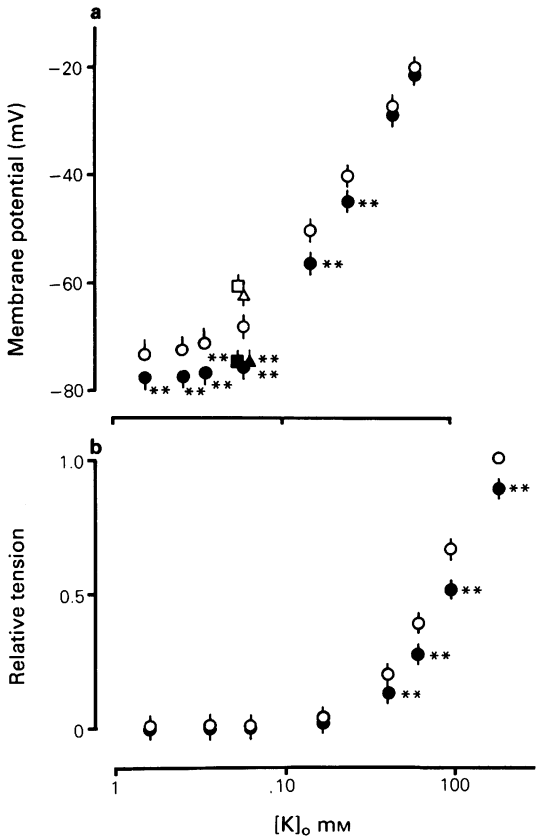

Figure 4 Effects of cromakalim (30 μM) on the membrane potential (a) and contraction (b) evoked by various concentrations of K in smooth muscle cells of the mesenteric artery. In (a), the effects of cromakalim were observed in normal Krebs (○, ●) and in high and low K (n = 15–20, 3 preparations), Na-deficient (15.5 mM) (△▲; n = 10, 2 preparations) or Cl-deficient solution (12.1 mM) (□■; n = 12, 2 preparations). (○, △, □) control responses; (●, ▲, ■) effects of cromakalim. Individual preparations were excised from different animals. The maximum hyperpolarization and relaxation were plotted. In (b), the amplitude of the 118 mM K-induced contraction was normalised as 1.0 and the contractions induced by lower K concentrations in the absence (○) and presence (●) of cromakalim were expressed as relative tension. Results were obtained from 5 preparations (n = 15–20) each from a different animal. Vertical lines indicate s.d. ** $P < 0.01$.

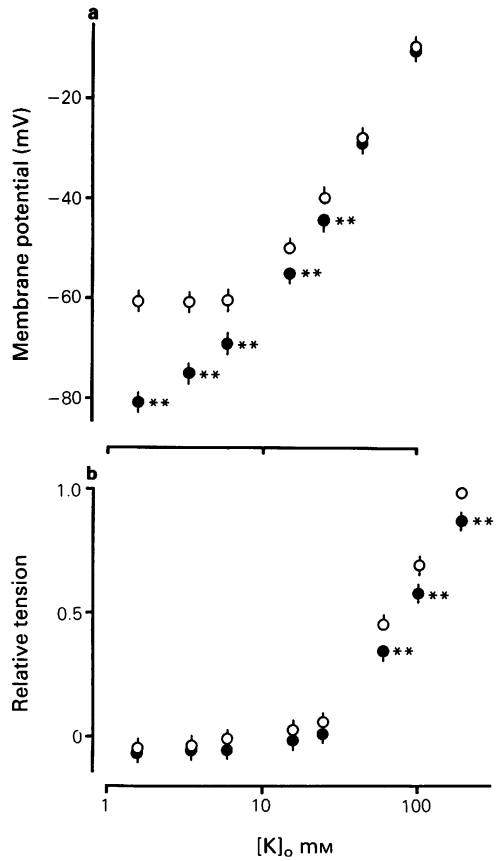

Figure 5 Effects of cromakalim (30 μM) on the membrane potential and contraction evoked by various concentrations of K in smooth muscle cells of the mesenteric vein. Experimental procedures were the same as those described in Figure 4. In (a) 3 preparations (n = 15–20) and in (b) 5 preparations (n = 13–15) were excised from different animals. (○) Control responses; (●) responses in the presence of cromakalim (30 μM). Vertical lines indicate s.d. ** $P < 0.01$.

centrations were reduced below 5.9 mM the membrane was hyperpolarized, but to a relatively smaller extent (Figure 4a). On addition of 30 μM cromakalim, the membrane was hyperpolarized only at K concentrations below 40 mM (Figure 4a), while K-induced contractions were consistently inhibited at K concentrations higher than 40 mM (Figure 4b). Thus, dissociation between the hyperpolarization and relaxation occurred.

In low Na (15.5 mM Na) or low Cl (12.1 mM Cl) solutions, the membrane was depolarized to -61.5 ± 2.7 mV (n = 10) or -60 ± 2.4 mV (n = 12), respectively. When 30 μM cromakalim was added under these conditions, the membranes were hyperpolarized to the same extent as that observed in normal Krebs solution (15.5 mM Na: -74.1 ± 2.5 mV, n = 10; 12.1 mM Cl: -73.8 ± 2.6 mV, n = 12).

In the mesenteric vein, cromakalim consistently hyperpolarized the membrane at K concentrations

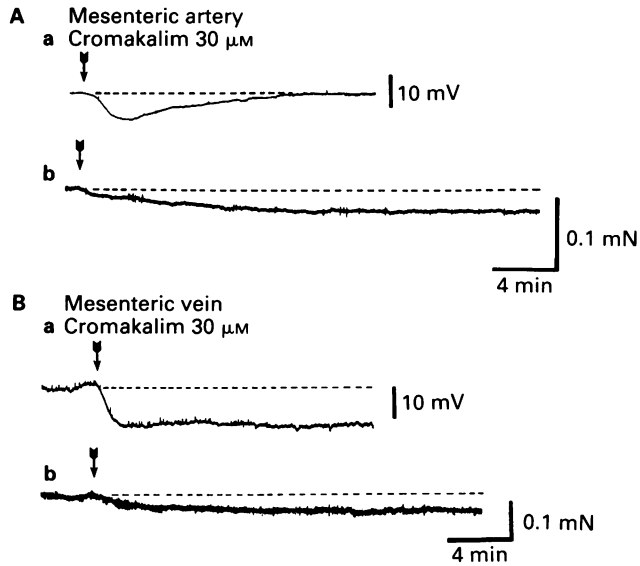

Figure 6 Effects of cromakalim (30 μM) on the membrane potential and tension of tissues precontracted with 11.8 mM K. (A), Mesenteric artery and (B) mesenteric vein. (a) and (b) were recorded from different tissues. Dotted lines indicate the resting membrane potential (a) and the tension level (b) before application of cromakalim. The membrane potentials of the mesenteric artery and vein in 11.8 mM K solutions were −57 mV and −55 mV, respectively.

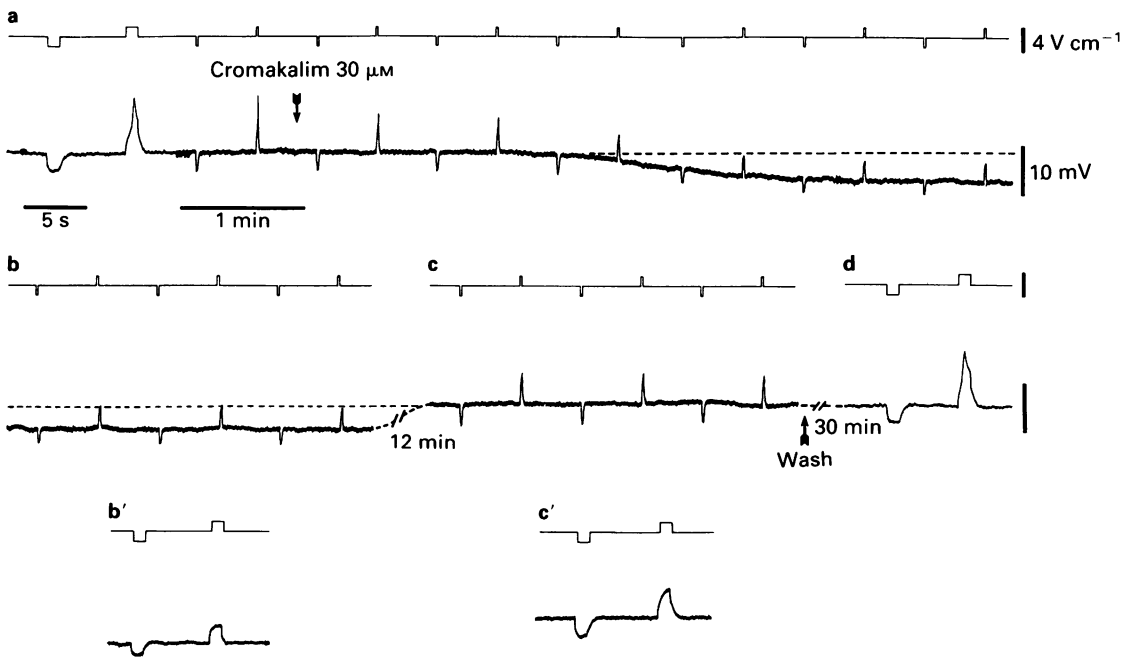

Figure 7 Effects of cromakalim on the action potential, membrane potential and electrotonic potential recorded from a smooth muscle cell of the mesenteric artery. (a) and (b) Segments of a continuous recording; (c) and (d) were recorded 12 min and 30 min after (b) and (c), respectively. (b') and (c') show segments of (b) and (c), respectively, recorded at a faster chart speed. Cromakalim was applied at the time indicated by the arrow. Dotted line indicates the resting membrane potential (−69 mV).

below 40 mM and the relationship between membrane potential and K concentration (above 15 mM) was linear (Figure 5a). In contrast, cromakalim only inhibited contractions evoked by high concentrations of K (above 40 mM) (Figure 5b). On superfusion with low K concentrations (below 3.6 mM), tissue resting tension was reduced in the presence or absence of cromakalim. Thus dissociation between hyperpolarization and relaxation of precontracted tissues similar to that observed in the mesenteric artery also occurs in the mesenteric vein.

To investigate further the action of cromakalim on membrane hyperpolarization and relaxation of tissues precontracted by high K (11.8 mM), changes in membrane potential and tension were measured simultaneously in both the mesenteric artery and vein (Figure 6A and B). In the former, both hyperpolarization and relaxation were observed during the initial exposure period, but after 13 min superfusion with cromakalim, the relaxation remained in the absence of membrane hyperpolarization (Figure 6A). However, in the mesenteric vein the hyperpolarization and relaxation occurred in a parallel manner (Figure 6B).

Effects of cromakalim on the action potential evoked by outward current pulses

As stated previously, outward current pulses evoked an action potential in some smooth muscle cells of the mesenteric artery and typical effects of cromakalim (30 μM) on such cells are shown in Figure 7. When cromakalim was perfused, the evoked action potential was rapidly attenuated before the appearance of any hyperpolarization. Subsequently membrane hyperpolarization and a reduction in the amplitude of electronic potentials occurred (Figure 7a). On prolonged superfusion with cromakalim the hyperpolarization was attenuated and both membrane potential and the amplitude of electronic potential returned to control levels. However, under these conditions no action potential could be evoked (Figure 7b,c) and recovery of such potentials required re-superfusion with normal Krebs solution (Figure 7d). This indicates that the inhibitory actions of cromakalim on the action potential are not causally related to the membrane hyperpolarization.

Cross-desensitization of the hyperpolarization induced by cromakalim and by nicorandil in the mesenteric artery

As shown in Figure 8a, on prolonged superfusion with cromakalim (30 mM), the initial hyperpolarization in mesenteric artery gradually waned and within 15 min it had ceased. Subsequent exposure to nicorandil 100 μM in the continuing presence of cromakalim had no effect on membrane potential. When the application sequence was reversed, 30 μM cromakalim had no effect on membrane potential (Figure 8b). Thus, the effects of cromakalim and nicorandil on membrane hyperpolarization exhibit the phenomenon of cross-densitization.

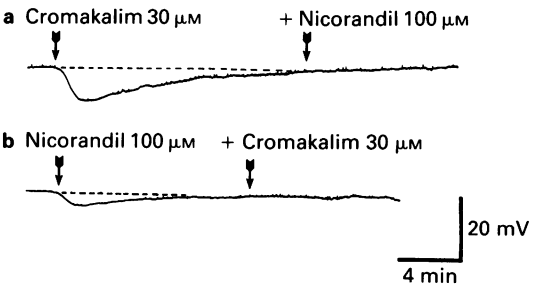

Figure 8 Effect of successive applications of cromakalim and nicorandil on the membrane potential in a smooth muscle cell of the mesenteric artery. (a) Cromakalim (30 μM) was initially applied; when the hyperpolarization had waned nicorandil (100 μM) was applied in the continuing presence of cromakalim. (b) The application sequence shown in (a) was reversed. Dotted lines indicate the resting membrane potential (−69 mV). Both records from the same preparation.

Discussion

The results of the present study have shown that hyperpolarization induced by cromakalim in the smooth muscle of guinea-pig mesenteric artery and vein can be accounted for by an increase in the K permeability of the membrane as demonstrated in many other smooth muscle tissues (see Introduction). In the rat portal vein, cromakalim hyperpolarized the membrane to about −90 mV (Hamilton *et al.*, 1986), and much the same hyperpolarization was observed in guinea-pig mesenteric and portal veins on application of nicorandil (Karashima *et al.*, 1982). The reversal potential for cromakalim in guinea-pig mesenteric artery as estimated from the I-V relationship was −80 mV, and the maximum hyperpolarization observed in the presence of 30 μM cromakalim was −75 mV. An agonist-induced reversal potential can be estimated from the following equation:

$$E_{rev} = E_m - \Delta V/\{1 - (p'/p)^2\},$$

where E_{rev} (mV) is the reversal potential for the agonist, E_m (mV) is the resting membrane potential, ΔV (mV) is the change in the membrane potential induced by the agonist and p'/p is the average change in the amplitude of the electronic potential (Ginsborg 1967; 1973; Bolton, 1972). Using the

above equation, the present experiments showed that the reversal potential for cromakalim was about −80 mV. In Na-deficient or Cl-deficient solutions, the smooth muscle membranes of the mesenteric artery were depolarized to a small extent but cromakalim hyperpolarized the cells to the same level as that observed in normal Krebs solution. Therefore, these results indicate a selective action of cromakalim on K channels in the guinea-pig mesenteric artery and vein. The larger hyperpolarization observed in the mesenteric vein compared with that in the artery may partly be due to a relatively low membrane potential and to contributions of the Mn-sensitive K current in the vein.

In mesenteric artery, the hyperpolarization produced by cromakalim was short-lived and the membrane potential returned to the control value within 15 min. Furthermore, repetitive exposure to cromakalim at short intervals (10 min) had no effect on membrane potential after the initial membrane hyperpolarization. However, in the mesenteric vein, no attenuation of the hyperpolarization occurred during 15 min exposure to cromakalim. In this tissue, the membrane hyperpolarization induced by cromakalim consisted of Mn-sensitive and Mn-resistant hyperpolarization. However, in the mesenteric artery, cromakalim activated solely a Mn-resistant hyperpolarization. Such Mn-sensitive and Mn-resistant hyperpolarization has been observed in a variety of tissues with other drugs (neurotensin: guinea-pig ileal circular smooth muscles, Yamanaka *et al.*, 1987; ATP: guinea-pig coronary artery, Takata & Kuriyama, 1980).

Cromakalim has been shown to increase ^{86}Rb efflux in many smooth muscles (rat portal vein, Hamilton *et al.*, 1986; guinea-pig trachea, Allen *et al.*, 1986a; guinea-pig taenia caecum, Weir & Weston, 1986a; guinea-pig portal vein, Quast, 1987; rabbit aorta, Kreye *et al.*, 1987a,b; rabbit mesenteric artery, Coldwell & Howlett, 1987). Kreye *et al.*, 1987a,b; found that in the rabbit aorta, increases in the ^{86}Rb efflux induced by cromakalim were inhibited by D600, nifedipine or trifluoperazine. Therefore, they concluded that the K channel opened by cromakalim was Ca-dependent. In the present experiments, both Mn (Ca) sensitive and Mn (Ca) resistant hyperpolarization was observed. However, following superfusion of LaCl (1 mM) any hyperpolarization induced by cromakalim was markedly attenuated. Furthermore, in Ca-free solution containing 0.5 mM EGTA, hyperpolarization to cromakalim was not observed. Therefore, both components of the increase in membrane potential induced by cromakalim may result from the opening of Ca-dependent K channels, but each component seems to possess a different sensitivity to Ca. Since several types of Ca-dependent K channel have been described in smooth muscle (Benham *et al.*, 1985; Inoue *et al.*, 1985; 1986), more detailed experiments are needed to clarify the types of K channel affected by cromakalim.

In the mesenteric artery, the time course of the cromakalim-induced hyperpolarization and relaxation did not occur in parallel while in the mesenteric vein both responses showed a similar time-course. Hamilton *et al.* (1986) found that the spontaneous electrical discharges of rat portal vein were inhibited by cromakalim with no detectable change in the membrane potential. In rat uterus, Hollingsworth *et al.* (1987) also observed that spontaneous discharges were inhibited by cromakalim with only a small hyperpolarization. Therefore, these groups of workers concluded that the inhibitory effects induced by cromakalim were probably due to increases in two types of K permeability, resulting in inhibition of pacemaker potentials and hyperpolarization, respectively.

In the mesenteric artery, action potentials evoked by outward current pulses ceased before the initiation of hyperpolarization and in high K solution, a small but significant inhibition of contraction occurred without any membrane hyperpolarization. Such results suggest, therefore, that cromakalim can inhibit a voltage-dependent Ca channel independently of any increase in Ca-dependent K permeability. Cain & Metzler (1985) also showed that cromakalim is capable of reducing the slow inward (Ca) current in guinea-pig papillary muscle. Further support for an action on Ca channels is provided by evidence that the inward current evoked by depolarization during the whole cell voltage clamp procedure is inhibited by 30 μM cromakalim in the smooth muscle cells of rat portal vein (Okabe, unpublished observations).

However, several pieces of evidence suggest that inhibition of a Ca current plays little, if any, role in the inhibitory action of cromakalim. In rat uterus, the study by Hollingsworth *et al.* (1987) found no evidence for the inhibition of such an inward current by cromakalim. Coldwell & Howlett (1987) have shown that cromakalim does not interact with 1,4 dihydropyridine binding sites in rabbit mesenteric artery. Furthermore, if cromakalim were exerting a major inhibitory effect on a voltage-dependent Ca current, a marked inhibition of the contractions produced by exposure of mesenteric artery and vein to high K solutions would have been expected in the present study. Such a marked inhibition was not observed. In guinea-pig bladder, a tissue in which cromakalim opens a Rb-impermeable K channel, replacement of intracellular K with Rb leaves tissue electrical and mechanical activity largely unaffected, but abolishes the inhibitory effects of cromakalim (Brading, personal communication). In rat portal

vein, the K channel blocker glibenclamide, in concentrations which have no effect on spontaneous electrical and mechanical activity, blocks the inhibitory effects of cromakalim on such discharges (Weston, unpublished observations). Clearly, further studies on the effects of cromakalim on electrical spike activity are required to clarify these anomalies.

Nicorandil is known to hyperpolarize smooth muscle cells and to relax precontracted tissues (dog mesenteric artery and trachea; Inoue *et al.*, 1983; guinea-pig and porcine coronary arteries; Furukawa *et al.*, 1981; guinea-pig and porcine mesenteric arteries; Itoh *et al.*, 1981; guinea-pig trachea; Allen *et al.*, 1986b; rat aorta; Weir & Weston, 1986b; guinea-pig intestine; Yamanaka *et al.*, 1985). The inhibitory effects of nicorandil result from a combination of K-channel opening and activation of guanylate cyclase (Sumimoto *et al.*, 1987). Although cromakalim does not activate guanylate cyclase (Coldwell & Howlett, 1987; Taylor *et al.*, 1988), data obtained in the present study of mesenteric artery showed that prior exposure to cromakalim prevented nicorandil-induced hyperpolarization and *vice versa*. Such results suggest that the action of both drugs on K channels may occur via the same mechanism.

From the present results we conclude that cromakalim relaxes vascular smooth muscle by inducing hyperpolarization due to a Ca-dependent increase in K permeability which may be identical to that activated by nicorandil. In the mesenteric vein, this response consists of two components, separable by use of manganese. Data were also obtained indicating that the cromakalim-induced loss of action potentials in mesenteric artery resulted from the inhibition of a voltage-dependent Ca current. Further studies are in progress to characterize these actions of cromakalim.

This work was supported by Grants in Aid for Scientific Research from the Minisitry of Education, Science and Culture, Japan and The Ministry of Health and Welfare, Japan. Cromakalim was a gift from the Beecham Pharmaceutical Co., Japan. A.H.W. was supported by grants from the Japan Society for the Promotion of Science and from the Royal Society.

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(Received March 26, 1988
Revised May 20, 1988
Accepted June 8, 1988)