Effects of cromakalim on the electrical slow wave in the circular muscle of guinea-pig gastric antrum

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1 In circular muscle strips of the antrum of guinea-pig stomach, the effects of cromakalim were studied on mechanical activity and intracellular membrane potential.

2 Cromakalim inhibited mechanical activity at concentrations higher than 1 μM , accompanied by membrane hyperpolarization and a decrease in membrane resistance. The hyperpolarization was markedly potentiated in K^+ -free solution and was still observed in the absence of Na^+ .

3 Slow wave electrical activity was relatively resistant to cromakalim. Changes in its amplitude and frequency were not consistent but blockade of slow waves was never observed. In many preparations cromakalim induced spike-like potentials at the top of slow waves, or when spike-like potentials already existed they were potentiated. However, mechanical activity was always inhibited.

4 Inhibition by cromakalim of the phasic contractions associated with the slow waves, could not be reversed by increasing the external K^+ concentration (12–30 mM).

5 The results suggest that in guinea-pig stomach muscle mechanical suppression by cromakalim does not simply result from membrane hyperpolarization or from inhibition of slow waves. A clear dissociation was found between the mechanical and electrical activities. Slow waves, particularly their frequency, are relatively insensitive to membrane hyperpolarization.

Keywords: Cromakalim; slow wave; gastric muscle

Introduction

Cromakalim is considered to activate potassium (K^+) channels which are sensitive to intracellular ATP and which can be blocked by sulphonylureas, such as glibenclamide (Cook, 1988; Weston, 1989; Weston & Edwards, 1992). Cromakalim relaxes several types of smooth muscle including that from the gastrointestinal tract and this effect is also antagonized by glibenclamide (Cavero *et al.*, 1989; McPherson & Angus, 1990; Quast, 1992). Such relaxation is likely to be mainly due to a decrease in calcium (Ca^{2+}) influx resulting from membrane hyperpolarization (Weston, 1989), but in addition to this mechanism, the direct inhibition of Ca^{2+} influx (Nakao *et al.*, 1988; Okabe *et al.*, 1990; Bray *et al.*, 1991; Post *et al.*, 1991) and intracellular Ca^{2+} release (Bray *et al.*, 1991) may also be involved.

Spontaneous electrical activity, which takes the form of slow waves in the antrum region of the guinea-pig stomach, is known to be relatively insensitive to Ca^{2+} channel blockers, such as verapamil (Golenhofen & Lammel, 1972). The frequency and amplitude of the slow wave are also only very weakly voltage-dependent, when studied by the sucrose-gap method (Tomita, 1981). In the canine colon, the main effect of the racemate cromakalim or its (–)-enantiomeric component, levromakalim was to decrease slow waves duration. The frequency of slow waves was found to be either decreased (Post *et al.*, 1991) or remained constant (Farraway & Huizinga, 1991), during hyperpolarization caused by these K^+ channel openers. On the other hand, a marked decrease of amplitude in the slow wave of guinea-pig gastric muscle, was observed in the presence of 1 μM cromakalim, although the frequency was not significantly altered (Ito *et al.*, 1992). In the present experiments, we studied the effects of cromakalim on the electrical and mechanical activities in the circular muscle of guinea-pig gastric antrum to determine any correlation between mechanical inhibition and the amplitude of the slow wave. Some of these results have been reported in a preliminary form (Tomita & Brading, 1990).

Methods

The methods used were essentially the same as those described previously by Chihara & Tomita (1987). Hartley guinea-pigs (250–350 g) of either sex were killed by stunning and bleeding and the stomach was removed. After careful removal of the mucosa, muscle strips (1 mm $\times$ 5 mm) were dissected from the antral region in the direction of the circular muscle fibres. A strip was mounted horizontally in a small chamber (0.3 ml) by fixing one end with small pins and connecting the other end to a strain gauge with fine thread for recording tension development. The membrane potential was measured with a conventional intracellular microelectrode technique. In some experiments, a partition method (Abe & Tomita, 1968) was used to measure changes in electrotonic potentials by applying 1–2 s pulses of a constant potential field to the tissue between slow waves.

The chamber was perfused with a physiological solution (prewarmed to 34°C) at a rate of 2 ml min⁻¹. The normal solution contained (mM): NaCl 127, KHCO_3 6, CaCl_2 2.4, MgCl_2 1.2, glucose 12, Tris-HCl 10, the pH being adjusted to 7.4 at 34°C. Meclofenamate (0.3 μM) was added to the perfusate throughout the experiments to eliminate any contribution of endogenous prostaglandins (Syed *et al.*, 1990). Numerical values are expressed as the mean $\pm$ s.e.mean with the number of observations (*n*).

In some experiments, only mechanical responses were recorded. For these experiments, preparations of a size similar to those used for electrical recordings were held vertically in a small tube (1 ml) and superfused with the physiological solution prewarmed to 34°C at a rate of 2 ml min⁻¹.

Drugs used were cromakalim (a gift from SmithKline Beecham Laboratories), meclofenamate monohydrate (Parke-Davis) and glibenclamide (Sigma). The stock (10 mM) solutions of cromakalim and glibenclamide were prepared in ethanol and dimethylsulphoxide, respectively.

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Results

Effects of spontaneous electrical and mechanical activity

The circular muscle of guinea-pig gastric antrum produced rhythmic electrical slow waves, each accompanied by a contraction. The configuration of the slow wave varied to some extent in different preparations and even in different regions of the same tissue. A notch was often noticeable in the falling phase of the wave, suggesting the presence of two components (cf. Tomita, 1981). Cromakalim at a concentration higher than $1\text{ }\mu\text{M}$ reduced muscle tone and inhibited phasic contractions, accompanied with membrane hyperpolarization, as shown in Figure 1. On average, the hyperpolarization was $7.4 \pm 1.5\text{ mV}$ ($n = 14$) with $10\text{ }\mu\text{M}$ cromakalim. Effects on amplitude and frequency were not consistent, but cessation of the slow wave was never observed. In many preparations, the lower slow part of the slow wave became smaller, whereas the spike-like potential when present at the upper part became larger, or started to appear during hyperpolarization, as shown in Figure 1a. The extremes in change in amplitude of slow waves were a decrease to 30% and an increase to 145% in 12 preparations, but in most preparations the change was less than 20% with $10\text{ }\mu\text{M}$ cromakalim. The slow wave frequency was not clearly modified in 4 preparations, it slightly (3–6%) increased in 3, or slightly (5–15%) decreased in 7 out of 14 preparations with $10\text{ }\mu\text{M}$ cromakalim.

Changes in electrotonic potential were examined in 3 preparations. An example of the result is shown in Figure 1b. The amplitude of electrotonic potentials was clearly reduced to $73 \pm 6\%$ ($n = 3$) during hyperpolarization produced by $10\text{ }\mu\text{M}$ cromakalim.

Figure 2a shows an atypical effect of cromakalim. In this preparation, $3\text{ }\mu\text{M}$ cromakalim increased the slow wave amplitude markedly (40%) and the slow wave frequency slightly (4%) during hyperpolarization. Mechanical activity was strongly inhibited in spite of the significant increase in amplitude of the slow wave and the appearance of a spike-like potential. The cromakalim-induced increase in amplitude and frequency was even greater when the temperature of the organ bath was lowered from 34°C to 24°C (Figure 2b), but the mechanical inhibition was still very clear.

Antagonism by glibenclamide

The effects of cromakalim were strongly antagonized by glibenclamide, as previously reported (Ito *et al.*, 1992). Glibenclamide itself did not show any noticeable effect on its own up to $3\text{ }\mu\text{M}$. However, as shown in Figure 3, cromakalim

Figure 1 Effects of cromakalim ($10\text{ }\mu\text{M}$; at horizontal bars) on mechanical (upper) and electrical activity (slow waves, lower trace) in the circular muscle of guinea-pig gastric antrum. Examples obtained from two different preparations (a and b) are shown. In (b), electrotonic potentials were produced by applying a constant potential field (2 s in duration) after every three slow waves using the partition methods.

Figure 2 Effects of cromakalim ($3\text{ }\mu\text{M}$; at horizontal bars) at different temperatures (34°C and 24°C). Records (a) and (b) were obtained from the same preparation, but from different cells.

Figure 3 Inhibition of cromakalim effect with glibenclamide. (a) Control, and (b) cromakalim ($3\text{ }\mu\text{M}$) was applied 5 min after application of glibenclamide ($0.3\text{ }\mu\text{M}$). Both recordings were obtained from the same cell.

Figure 4 Effects of cromakalim ($10\text{ }\mu\text{M}$; at horizontal bars) in the presence of normal K^+ (6 mM) (a) and nominally K^+ -free solution (b), obtained from different cells in the same preparation.

($3\text{ }\mu\text{M}$) did not produce hyperpolarization and inhibition of mechanical activity in the presence of glibenclamide ($0.3\text{ }\mu\text{M}$). In 2 out of 4 preparations, there was a small cromakalim-induced depolarization and an increase in muscle tone. A glibenclamide-resistant inhibitory effect of cromakalim has been reported (Ito *et al.*, 1992), but we failed to observe this phenomenon using $1\text{ }\mu\text{M}$ glibenclamide and $10\text{ }\mu\text{M}$ cromakalim.

Effects of changing $[\text{K}^+]$

Hyperpolarization produced by cromakalim was increased significantly in K^+ -free solution (Figure 4). When K^+ was removed, the membrane was slowly depolarized, as previously observed with the sucrose-gap method (Ohba *et al.*, 1977). Cromakalim ($10\text{ }\mu\text{M}$), applied 4 min after K^+ removal, produced 13 mV hyperpolarization from the control level (b), compared with 5 mV in normal solution (a).

The relaxant effect of cromakalim was reduced by increas-

ing the external K^+ concentration, but a clear mechanical inhibition was still observed when cromakalim ($3 \mu M$) was applied during a steady contracture in $40 \text{ mM } K^+$ solution, in which rhythmic contractions were blocked. Figure 5 shows the effects of excess K^+ ($12\text{--}30 \text{ mM}$) on mechanical activity in the presence and absence of cromakalim ($3 \mu M$). Excess K^+ raised muscle tone concentration-dependently. Phasic contractions were increased with $12 \text{ mM } K^+$ (Figure 5a), but reduced with K^+ higher than 24 mM (Figure 5 e.g). Cromakalim abolished phasic contractions and reduced the K^+ -contracture slightly. In the presence of cromakalim, no recovery of phasic contractions was observed by increasing K^+ concentrations, although a tonic contraction was produced concentration-dependently, which was smaller than the tonic component in the absence of cromakalim. The same results were confirmed in two other preparations.

Effects of cromakalim were also studied in the absence of

Figure 5 Mechanical activity in response to excess K^+ in the absence (left) and in the presence of cromakalim (right). Excess K^+ ($12\text{--}30 \text{ mM}$) was applied every 30 min, and cromakalim ($3 \mu M$) was applied 4 min before the second application of a given concentration of K^+ , as indicated above the recordings.

Figure 6 Effects of cromakalim in normal Na^+ and $0 \text{ mM } Na^+$ solution. After observing the control response to cromakalim ($10 \mu M$) (a), $127 \text{ mM } Na^+$ was replaced with $63.5 \text{ mM } MgCl_2$ and 63.5 mM sucrose (Na^+ -free) and 4 min later cromakalim was applied (b). (a) and (b) are from different cells in the same preparation.

Na^+ (Figure 6). In this experiment, $127 \text{ mM } NaCl$ was replaced with $63.5 \text{ mM } MgCl_2$ plus 63.5 mM sucrose, keeping the Cl^- concentration constant (134 mM).

In Na^+ -free solution, the membrane was depolarized and the slow waves were reduced in amplitude. Mechanical activity was strongly inhibited in excess Mg^{2+} (Na^+ -free) solution. When *N*-methyl-D-glucamine was used as a Na^+ substitute, the change in membrane potential was essentially the same, but a greater tension developed, making stable intracellular recording difficult. Cromakalim repolarized the membrane and restored the amplitude of the electrical rhythmic activity (Figure 6b). The frequency of this electrical activity was higher than that of the slow waves in normal solution and a hump could not be observed in the falling phase. In two preparations, cromakalim also repolarized the membrane in the presence of ouabain ($5 \mu M$) which slowly depolarized the membrane by $5\text{--}10 \text{ mV}$ over a period of 10 min (not shown).

Discussion

In the circular muscle of guinea-pig gastric antrum, cromakalim ($3\text{--}10 \mu M$) hyperpolarized the membrane and this was accompanied by a decrease in membrane resistance, as judged by the decrease in the amplitude of the electrotonic potentials. The hyperpolarization could be readily blocked by glibenclamide, confirming the previous report by Ito *et al.* (1992). In contrast, the hyperpolarization was markedly potentiated in K^+ -free medium. Furthermore, cromakalim could still produce hyperpolarization in the absence of Na^+ or in the presence of ouabain. These results strongly suggest that cromakalim increases the K^+ conductance of the plasma membrane, through activation of glibenclamide-sensitive K^+ channels, as reported for many other smooth muscles (Weston & Edwards, 1992).

Mechanical inhibition by cromakalim is probably due in part to hyperpolarization and a decrease in slow wave amplitude. These factors should reduce Ca^{2+} influx, the basic mechanism believed to underlie cromakalim-induced relaxation in other smooth muscles (Weston, 1989; Quast, 1992). However, some additional factor seems to be involved in the cromakalim-induced changes in gastric circular muscle. In some preparations, mechanical activity was strongly inhibited by cromakalim even when the slow wave amplitude was not reduced or even increased. In gastric antral muscle, the spike-like component at the top of the slow wave is considered the main determinant of the magnitude of the phasic contraction (Golenhofen & Lammel, 1972). Verapamil inhibits the spike-like component with little effect on slow waves and suppresses mechanical activity. Since cromakalim often increased the spike-like component, one might expect that this change would be accompanied by an increase in contraction amplitude, such as occurs on exposure of the guinea-pig gastric muscle to adrenaline (Chihara & Tomita, 1987) or with tetraethylammonium in the canine gastric muscle (Szurszewski, 1978). However, no such mechanical potentiation was actually observed in the presence of cromakalim.

If the inhibition of mechanical activity by cromakalim were simply due to membrane hyperpolarization, contractions would be expected to recover when the membrane is depolarized by excess K^+ . However, in the presence of $10 \mu M$ cromakalim, no such recovery was observed when $[K^+]_o$ was between 12 and 30 mM . Since cromakalim had no effect on Ca^{2+} -induced contraction in chemically skinned muscle strips (guinea-pig trachea: Allen *et al.*, 1986; rat myometrium: Hollingworth *et al.*, 1987), it is very likely that cromakalim exerts some inhibitory action on excitation-contraction coupling processes.

There is some evidence that cromakalim may inhibit Ca^{2+} influx directly and that this mechanism may be involved in relaxation, in addition to the inhibition of Ca^{2+} influx which occurs as a consequence of membrane hyperpolarization (rat

portal vein: Hamilton *et al.*, 1986; guinea-pig mesenteric artery and vein: Nakao *et al.*, 1988). In single muscle cells of rat portal vein (Okabe *et al.*, 1990) and in canine colon (Post *et al.*, 1991), inhibition of inward Ca^{2+} current by cromakalim has been demonstrated with patch-clamp experiments. It is therefore possible that the mechanical inhibition of cromakalim in gastric muscle observed in the present study is partly due to this mechanism, but enhancement of the spike-like potential by cromakalim and the accompanying inhibition of mechanical activity rather contradict this possibility. The ionic mechanisms underlying these paradoxical effects need further investigation. Modification of the function of intracellular Ca^{2+} stores might also be responsible for the cromakalim-induced mechanical inhibition, since some evidence has been presented in rabbit aorta that cromakalim interferes with the refilling of intracellular Ca^{2+} stores and impairs the ability of noradrenaline to release Ca^{2+} from the stores (Bray *et al.*, 1991).

If spontaneous activity in guinea-pig stomach is generated by a voltage-dependent ionic mechanism, its frequency should be decreased, or even completely inhibited, as the membrane becomes hyperpolarized. It has been reported that cromakalim blocks spontaneous spike activity during hyperpolarization in guinea-pig taenia caeci (Weir & Weston, 1986;

Den Hertog *et al.*, 1989) and rat portal vein (Hamilton *et al.*, 1986) or slow rhythmic electrical activity in guinea-pig trachealis (Allen *et al.*, 1986). On the other hand, the frequency of slow waves in the guinea-pig gastric antrum is not significantly affected by the hyperpolarization caused by cromakalim (Ito *et al.*, 1992; present study). Such results are consistent with those of previous studies obtained with the sucrose-gap method that slow wave frequency is only weakly voltage-dependent (Tomita, 1981). In the canine colon (circular muscle), it has been reported that cromakalim (10 μM) has either no consistent effect on the slow wave frequency (Faraway & Huizinga, 1991) or that it reduces the frequency by 30% with a hyperpolarization of 7 mV (Post *et al.*, 1991). It may be that the slow wave in guinea-pig stomach is less dependent on membrane potential than that in the canine colon. Nevertheless, in general, the pacemaker activity of slow wave in gastrointestinal muscles seems much more stable in response to changes in membrane potential than that of slow wave in guinea-pig airways muscle (Allen *et al.*, 1986) and spike-like activity in other smooth muscles (Hamilton *et al.*, 1986; Weir & Weston, 1986; Den Hertog *et al.*, 1989) suggesting that different mechanisms underlie the pacemaker processes which trigger these electrical activities.

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