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Effect of Cromakalim on the Smooth Muscle of the Cat Gastric Antrum

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Abstract—The effect of the K^+-channel opener cromakalim (BRL 34915) on the electrical and contractile activity of the smooth muscle of the cat gastric antrum has been studied. Cromakalim induced a concentration-dependent inhibition of the contractions and shortening of the sustained partial repolarization phase of the plateau action potential. High concentrations of cromakalim produced hyperpolarization and shortening of the repolarization and depolarization phases of the plateau action potential. The K^+-channel blockers 4-aminopyridine (10^{-2} M) and tetraethylammonium (10^{-2} M) decreased the effect of cromakalim on the phasic contractions, while glibenclamide (5×10^{-5} M) completely abolished it. We suggested that the inhibitory effect of cromakalim on the electrical and contractile activity of the gastric antrum smooth muscle is due to the cromakalim-induced increase of the outward K^+-current through glibenclamide-dependent K^+-channels.

Cromakalim (BRL 34915,(±)-(-6 cyano-3, 4-dihydro-2,2-dimethyl-trans-4-(2-oxo-1 pyrrolidyl) 2H-1 benzopyran-3-4-ol) is a benzopyran derivative which abolishes the spontaneous electrical and mechanical activity of different types of smooth muscles: vascular (Weir & Weston 1986a; Hamilton et al 1986; Nakao et al 1988), tracheal (Allen et al 1986), guinea-pig caecum (Weir & Weston 1986b; Den Hertog et al 1989), guinea-pig urinary bladder (Foster et al 1989; Fujii et al 1990) and rat uterus (Hollingsworth et al 1987). In the guinea-pig central nervous system (CNS) cromakalim inhibits synaptic transmission in CA_3 neurons and decreases the epileptiform activity (Alzheimer & ten Bruggencate 1988; Alzheimer et al 1989). A large body of research has indicated that the inhibitory effects of cromakalim in smooth muscles is produced via activation of the K^+-channels (Weir & Weston 1986a,b; Hamilton et al 1986; Taylor et al 1988). The extent of hyperpolarization depends on the tissue studied. Thus in the rat uterus cromakalim induces relaxation with only a small hyperpolarization (Hollingsworth et al 1987), while in the portal vein, low concentrations of cromakalim abolish the multispike complexes without a pronounced hyperpolarization, and high concentrations produce a dose-dependent hyperpolarization (Weir & Weston 1986a; Hamilton et al 1986). Bearing in mind the specificity of the excitation-contraction coupling in the different smooth muscles, we decided to examine the effect of cromakalim on the electrical and contractile activity of the smooth muscle of the cat gastric antrum.

Materials and Methods

Cats were anaesthetized with chloralose (100 mg kg^{-1}, i.p.) and the stomachs removed. After thorough washing with Krebs solution, the antral part of the stomach was removed and circular strips, 1·5 × 10 mm, from the anterior wall of the antrum were dissected under the microscope. In the first series of experiments, the strips were mounted in a 10 mL organ bath containing Krebs solution bubbled with 95% O_2–5% CO_2 at 37°C. The contractile activity was recorded under isometric conditions using a strain gauge, type M 1000 (Mikrotechna, Czechoslovakia). The strips were suspended under 1 g (10 mN) tension. There was a 60 min equilibration period before any measurements were made. In the second series of experiments the effect of cromakalim on the electrical and contractile activity of the smooth-muscle strips was studied by the sucrose gap technique.

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Solutions and drugs

Krebs solution comprised (mM); Na^+ 137·5, K^+ 5·94, Ca^{2+} 2·5, Mg^{2+} 1·2, Cl^- 134·15, HCO_3^- 15·5, $H_2PO_4^-$ 1·19, glucose 11·5. Cromakalim, (SmithKline Beecham) was dissolved in redistilled water, polyethylene glycol 400 and ethanol in a ratio of 6:3:1; tetraethylammonium (TEA) and glibenclamide were provided by Sigma and 4-aminopyridine was from Merck-Schuchardt.

Statistics

The amplitude of contractions was expressed as percent of the amplitude of the maximal contractions before cromakalim. The effect of cromakalim on the amplitude of the contractions induced by 4-aminopyridine-tetraethylammonium (4-AP), TEA or glibenclamide was also determined. The duration of the plateau of the plateau action potential was measured at the level of 1/3 of the amplitude of the initial depolarization phase of the plateau action potential. The data were assessed for statistical significance using Student's t-test at $P < 0.05$. The means ± s.e.m. are presented.

Results

Smooth-muscle strips isolated from the antral part of the cat stomach were characterized by spontaneous phasic contractions. Cromakalim administered either cumulatively or at single concentrations of 10^{-9}–10^{-6} M exerted no effect on the resting tone of the strips but reduced the amplitude of the spontaneous contractions (Fig. 1A). The threshold concentration varied from 5×10^{-9} to 3×10^{-8} M. The inhibitory

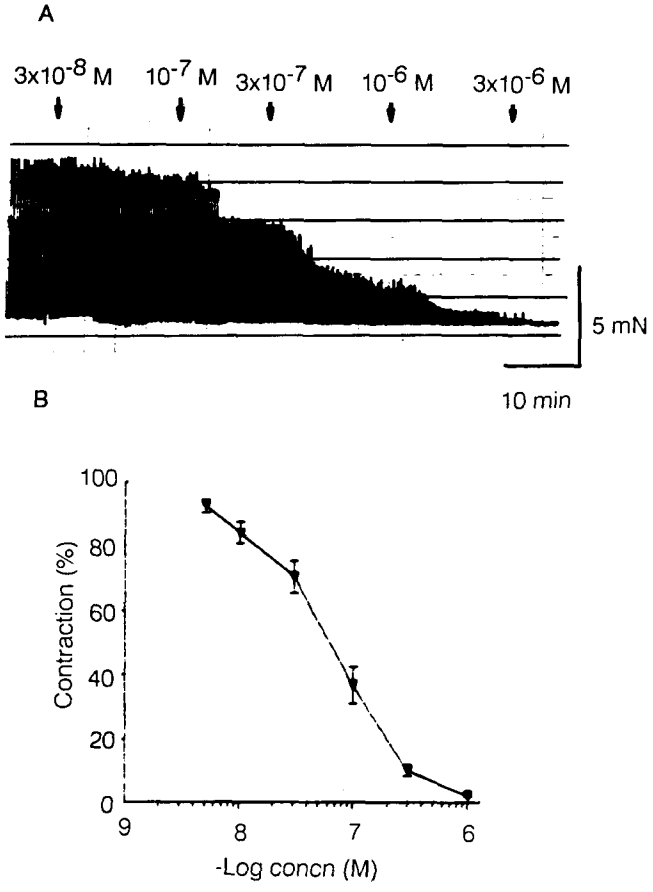

FIG. 1. The concentration-dependent effect of cromakalim on the amplitude of the spontaneous contractions of strips isolated from the cat antrum. A. Inhibition of the contractions on cumulative application of cromakalim. B. Concentration-response curve for cromakalim. Each point is the mean ± s.e.m. (n = 7).

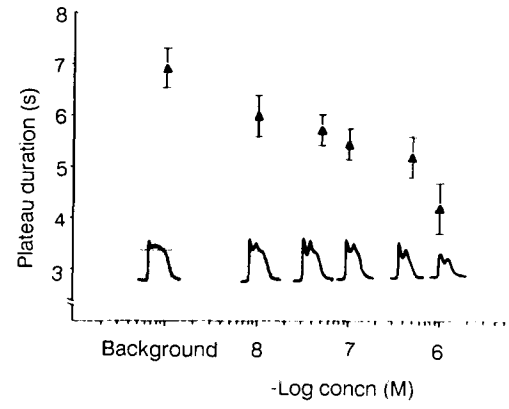

FIG. 2. The concentration-dependent effect of cromakalim on the duration of the plateau action potential generated in the smooth muscle of the gastric antrum. Each point is the mean of the duration of the plateau action potential recorded 10 min after cromakalim ± s.e.m. An original record of single plateau action potentials demonstrating the changes in their repolarization phase and amplitude is also shown.

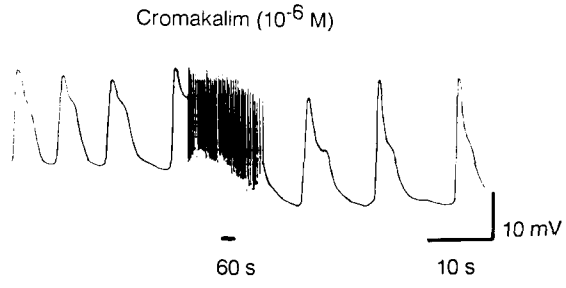

FIG. 3. Hyperpolarization produced by a high (10^{-6} M) concentration of cromakalim. The 6-min record after the administration of cromakalim was made at a paper speed of 5 mm min^{-1}.

effect of cromakalim increased in a concentration-related manner, with 100% inhibition observed at a concentration of 10^{-6} M (Fig. 1B). These changes were reversible. After several washes of the strips with Krebs solution, their spontaneous contractile activity was restored to control values.

It is known that the smooth muscle of the cat gastric antrum generates spontaneous plateau action potentials consisting of an initial depolarization phase followed by a sustained partial repolarization forming the plateau of the plateau action potential. The depolarization phase triggers the phasic contraction and the duration of the plateau determines the amplitude and duration of the contraction (Papasova & Boev 1976). Cromakalim at low concentrations mainly affected the duration of the plateau (Fig. 2). At a concentration of 10^{-8} M, cromakalim shortened the repolarization phase of the plateau action potential. The same was observed with a concentration of 5×10^{-8} M. The high concentration of cromakalim (10^{-6} M) led to a decrease in the amplitude of the initial depolarization phase of the plateau action potential. This latter change was preceded by a pronounced hyperpolarization (10–12 mV) (Fig. 3).

We also studied the interactions between cromakalim and the K^+-channel blockers 4-AP, TEA or glibenclamide. Both 4-AP and TEA produced a concentration-dependent increase in the amplitude of the phasic contractions. The effect of cromakalim on the 4-AP and TEA-induced contractions depended on the concentrations of the blockers. Thus at a concentration of 10^{-3} M of 4-AP and TEA, cromakalim (10^{-6} M) completely eliminated the contractions (Table 1). When the concentration of the K^+-channel blockers was increased to 10^{-2} M, the inhibitory effect of cromakalim decreased from 20·0 ± 2·5 mN in normal Krebs solution to 12·2 + 2·0 mN in the presence of 4-AP and from 14·2 ± 3·4 to 5·7 ± 1·2 mN in the presence of TEA.

The blocker of ATP-dependent K^+-channels, glibenclamide (5×10^{-5} M), had no significant effect on the amplitude of spontaneous contractions and furthermore cromakalim had no effect on this response.

Discussion

The observed inhibitory effect of cromakalim on the amplitude of the spontaneous contractions of the antral smooth muscle mimicked its effect on other smooth muscles (vascular, tracheal, urinary bladder, uterus). This inhibitory effect of cromakalim occurred via specific receptors, through inhibition of the inward Ca^{2+}-currents or through activation of the outward K^+-currents. However, evidence for the existence of cromakalim receptors is still lacking. The suggestion that cromakalim acts through inhibition of

Table 1. Effect of cromakalim on the amplitude of spontaneous contractions of smooth muscle strips in the absence and in the presence of K^+-channel blockers.

Treatment (M)	Amplitude of spontaneous contraction (mN)	Cromakalim (10^{-6} M) (mN)	Number of observations	P
—	6·1±0·8	0·2±0·02	12	<0·01
4-Aminopyridine (10^{-3})	10·0±1·5	0·2±0·02	7	<0·01
Tetraethylammonium (10^{-3})	9·2±1·3	0·2±0·02	10	<0·01
4-Aminopyridine (10^{-2})	20·0±2·5	12·2±2·0	5	>0·05
Tetraethylammonium (10^{-2})	14·2±3·4	5·7±1·2	4	>0·05
Glibenclamide	7·8±1·3	6·4±1·2	12	>0·05

The values are presented as means±s.e.m.

inward Ca^{2+}-currents can be ruled out because the effect of cromakalim is unchanged either in Ca^{2+}-free medium (Wilson & Cooper 1989) or after blocking the Ca^{2+}-influx by lanthanum (Coldwell & Howlett 1986) or isradipine (Quast 1987). That nifedipine completely inhibits the spontaneous contractions of the canine heart muscle and cromakalim partly inhibits them led Yanagisawa et al (1988) to believe that cromakalim does not affect the inward Ca^{2+}-currents but increases the outward K^+-currents. In support of this possibility are the data showing that blockade of the Ca^{2+}-influx by lanthanum does not change the effect of cromakalim on ^{86}Rb-efflux. On the other hand, cromakalim-induced ^{86}Rb-efflux is completely inhibited by the K^+-channel blockers 4-AP (Allen et al 1986) and TEA (Coldwell & Howlett 1986). In the present experiments on the antral smooth muscle, 4-AP and TEA partly antagonized the inhibitory effect of cromakalim on the phasic contraction amplitude. Direct evidence for the participation of K^+ in the effect of cromakalim is the observation of sustained outward K^+-current in the membrane of isolated cells from the portal vein after application of 10 mM of cromakalim (Beech & Bolton 1989). Beech & Bolton (1989) reported a smaller effect of TEA than 4-AP on cromakalim-produced outward K^+-current. The present studies support the finding that only the high concentration (10^{-2} M) of 4-AP and TEA partly antagonized cromakalim.

There are data that the phasic contractions of the cat stomach correlates with the amplitude and duration of the repolarization phase of the plateau action potential (Papasova & Boev 1976). From Fig. 2 it is seen that cromakalim concentration-dependently shortened the duration of the plateau action potential. This was associated with inhibition of the phasic contractions over the same range of concentrations. The changes in the plateau duration occurring at low concentrations of cromakalim are not likely to be due to changes in the resting membrane potential because cromakalim at concentrations lower than 10^{-6} M produced only a slight hyperpolarization of the cell membrane (1–2 mV at 30% short-circuit of sucrose gap). We would like to suggest that the changes in the duration of the repolarization phase of the plateau action potential might be determined by a cromakalim-effect on a different outward K^+-current than the K^+-current involved in the resting membrane potential. Osterrieder (1988) has described a similar shortening of the repolarization phase in isolated cardiac myocytes by low concentrations of cromakalim. Den Hertog et al (1989) have suggested that cromakalim affects the glibenclamide-sensitive K^+-channels in the smooth muscle of the guinea-pig taenia caeci. According to McPherson & Angus (1990), glibenclamide antagonizes the cromakalim effect in both vascular and non-vascular muscles. Farraway & Huizinga (1990) have reported shortening of the plateau duration of the plateau action potential in the canine colonic smooth muscle and a decrease in the amplitude of the related contraction in the presence of cromakalim. The authors ascribe this effect to some activation of the glibenclamide sensitive K^+-channels. The fact that the effect of cromakalim was completely antagonized by glibenclamide suggests that the changes in the electrical and contractile activity of the gastric smooth muscle could be due to the involvement of the ATP-sensitive K^+-channels. These data should be considered when using cromakalim in clinical practice.

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