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Effects of Lemakalim, a Potassium Channel Opener, on the Contractility and Electrical Activity of the Antral Circular Muscle in Guinea-Pig Stomach

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= ABSTRACT =

Synthetic potassium channel openers (KCOs) are agents capable of opening K-channels in excitable cells. These agents are known to have their maximal potency in the smooth muscle tissue, especially in the vascular smooth muscle. Much attention has been focused on the type of K-channel that is responsible for mediating the effects of KCOs. As the KCO-induced changes are antagonized by glibenclamide, an K_{ATP} (ATP-sensitive K-channel) blocker in the pancreatic β-cell, K_{ATP} was suggested to be the channel responsible. However, there also are many results in favor of other types of K-channel (maxi-K, small conductance K_{Ca}, SK_{ATP}) mediating the effects of KCOs.

Effects of lemakalim, (-)enantiomer of cromakalim (BRL 34915), on the spontaneous contractions and slow waves, were investigated in the antral circular muscle of the guinea-pig stomach. Membrane currents and the effects on membrane currents and single channel activities were also measured in single smooth muscle cells and excised membrane patches by using the patch clamp method. Lemakalim induced hyperpolarization and inhibited spontaneous contractions in a dose-dependent manner. These effects were blocked by glibenclamide and low concentrations of tetraethyl ammonium (<2 mM). Glibenclamide blocked the effect of lemakalim on the membrane potential and slow waves. The mechanoinhibitory effect of lemakalim was blocked by pretreatment with glibenclamide. In a whole cell patch clamp condition, lemakalim largely increased outward K currents. These outward K currents were blocked by TEA, glibenclamide and a high concentration of intracellular EGTA (10 mM). Volatage-gated Ca currents were not affected by lemakalim. In inside-out patch clamp experiments, lemakalim increased the opening frequency of the large conductance Ca^{2+}-activated K channels (BK_{Ca}, Maxi-K).

From these results, it is suggested that lemakalim induces hyperpolarizatiom by opening K-channels which are sensitive to internal Ca and such a hyperpolarization leads to the inhibition of the spontaneous contraction.

Key Words: K channel opener, Gastric smoth muscle, Slow wave, Membrane current

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INTRODUCTION

Many different cellular processes have evolved to regulate the level of $[Ca^{2+}]i$ which is used as an intracellular modulator. Ca^{2+}-influx through the sarcoplasmic membrane of the smooth muscle is characterized by its voltage-dependence. K channels are suggested to modulate the Ca^{2+}-homeostasis by regulating the membrane potential. The membrane potential is determined by the ratio of intracellular and extracellular concentrations of ionic species and their relative permeabilities. K channels play an important role in determining the levels of membrane potentials and in the transmissions of electrical signals along the membrane of excitable cells. K channels provide a repolarization pathway for depolarized cells, and help to maintain the resting membrane potential. If cells become hyperpolarized due to the opening of K channels, Ca^{2+}-influx through voltage-dependent channels is blunted and Ca^{2+}-efflux through electrogenic Na-Ca exchange is stimulated, leading to net overall lowering of cytoplasmic Ca^{2+} levels (Cook & Quast, 1990).

Drugs or toxins which modulate the activities of the K channels have been investigated, and among those modulators, K channel-blockers are quite well known and some of them are used as anti-arrhythmic drugs. But only recently, a certain agent, BRL 34915 (cromakalim), has been found and reported to be able to open K channels in excitable tissues such as vascular smooth muscle (Hamilton, 1986; Bray et al, 1988). After these reports, many other kinds of substances have been suggested to be effective in activating K channels (Cook & Quast, 1990).

Because of its potent ability to depress the excitability of muscle cells, clinical applications of these KCOs have been pursued for treating hypertension (Cook, Weir & Danzeisen, 1988), or detrusor instability of the bladder smooth muscle (Foster & Brading, 1987; Foster et al, 1989), or airway hyperexcitability (Allen et al, 1986; Archs et al, 1988; Cook, 1988; Foster, 1989), or cardioprotection (Escande & Cavero, 1992). But the effect on the gastric smooth muscle has not been fully investigated and it is still uncertain whether KCOs open the ATP-sensitive K channel or Ca^{2+}-activated K channels in the smooth muscle (Cook & Quast, 1990).

So, in this study, we tried to investigate the effect of lemakalim, the most representative KCO, on the membrane potential and ionic currents recorded from guinea-pig gastric myocytes. During this investigation, we tried to elucidate what kind of K channels is responsible for the KCO-induced electrical changes in the gastric smooth muscle.

METHODS

Preparation of the tissue and intracellular recording of the electrical activity

Albino guinea-pigs of either sex ($n \fallingdotseq 60$), weighing 200-250 g, were stunned and bled. The stomach was isolated and cut in the longitudinal direction along the lesser curvature. The contents of the stomach were removed, and the mucosal layer was separated from the muscle layers in phosphate-buffered Tyrode solution (NaCl 147, KCl 4, $MgCl_2$ $6H_2O$ 1.05, $CaCl_2.2H_2O$ 2, NaH_2PO_4 $2H_2O$ 0.42, $Na_2HPO_4.12H_2O$ 1.81, glucose 5.5 mM, pH 7.35) at room temperature. Muscle strips (2 mm wide, 10 mm long) were cut parallel to the circular fibers, and set in a 100 ml vertical chamber. One end was fixed and the other was connected to a force transducer (Isometric Transducer, Harvard Bioscience, USA) to measure isometric contraction. Another strip was mounted in a 2 ml horizontal chamber. The strips were pinned out at one end with tiny pins on a rubber plate, and the other end

was connected to the force transducer. The strip was constantly perfused at a rate of 2 ~3 ml/min with tris-buffered normal Tyrode solution (NaCl 147, KCl 4, $CaCl_2.2H_2O$ 2, $MgCl_2.6H_2O$ 1.05, tris.HCl 5, glucose 5.5 mM, pH 7.35) bubbled with 100% O_2 and maintained at 35°C Electrical activities of smooth muscle cells were recorded by means of glass microelectrodes filled with 3 M KCl. Microelectrodes with a tip resistance of 40-80 MΩ were used. Mechanical and electrical responses of smooth muscle cells were simultaneously recorded on a pen recorder (MX-6, Device Ltd, Britain).

Patch clamp experiments

Isolation of cells : The circular muscle layer was separated from the longitudinal layer and small segments of the tissues were made. The muscle segments were incubated in Ca^{2+}-free PSS (physiological salt solution) for 30 min at room temperature. Then, the muscle segments were incubated for 20-30min in Ca^{2+}-free PSS containing 0.1% collagenase, 0.1% trypsin inhibitor, and 0.2% bovine serum albumin at 35℃. After digestion, single cells were dispersed by gentle agitation with a wide-bored glass pipette in the Krafts-Brühe (KB) solution. Isolated gastric myocytes were kept in KB medium at 4°C. All experiments were carried out within 12 hours of harvesting cells and performed at room temperature.

Membrane currents measurement: Isolated cells were transferred to a small chamber on the stage of an inverted microscope (IMT-2, Olympus, Japan). The chamber was perfused with PSS (2~3 ml/min). Glass pipettes with a resistance of 2~5 M were used to make a giga seal of 5~10 GΩ Standard patch clamp techniques were used (Hamill et al. 1981). An axopatch-1C patch-clamp amplifier (Axon instruments, USA) was used to record membrane currents and command pulses were applied by using IBM-compatible AT computer and pClamp software v.5.51. The data were displayed on a digital oscilloscope (PM 3350, Philips, Netherlands), a computer monitor and a pen recorder (Recorder 220, Gould, USA).

Ensemble-average current: Ensemble-average currents were obtained from the responses of single channels to the ramp-pulses from 0 mV to 100 mV applied 15 times repeatedly (Fig. 10-A). Such depolarizing pulse activated many Maxi-K channels which are sensitive to the voltage as well as intracellular Ca^{2+}. Those current responses were arithmetically averaged (ensemble-average current) by using the computer software pCLAMP v. 5.5. The average current across the patch

$$I_{avg} = NP_o \times I_{channel}$$

where N is the number of channels in the patch and $I_{channgl}$ is the single-channel current; therefore, I_{avg} can be considered to reflect the state of open probability (P_o).

Solutions: Ca^{2+}-free PSS containing (mM) NaCl 134.8, KCl 6.2, $CaCl_2$ 0, glucose 12.2, HEPES 0.4 was adjusted to pH 7.3 by Tris. PSS was made by adding 2.3 mM $CaCl_2$ in the Ca^{2+}-free PSS. For recording the excised-patch single channel current, bath solution composed of 140 mM of KCl buffered with HEPES & Tris was used and the solution was Ca^{2+}-clamped to various levels by using the computer program from Fabiato & Fabiato (Fabiato & Fabiato, 1979)

KB solution containing (in mM) L-glutamate 50, KCl 50, Taurine 20, KH_2PO_4 20, $MgCl_2$ 3, glucose 10, HEPES 10, EGTA 0.5 was adjusted to pH 7.3 by KOH. Pipette solution consisted of (in mM) K-aspartate 110, Mg-ATP 5, di-Tris-creatine phosphate 5, KCl 20, $MgCl_2$ 1, ethylene glycol-bis(-aminoethyl ether)-N,N,N′,N′-tetraacetic acid (EGTA) 0.1, HEPES 5, pH 7.4. To exclude Ca^{2+}-activated K current, intracellular EGTA was raised to 10 mM. EGTA was used as an artificial Ca^{2+}-buffer in this kind of whole-cell patch clamp experiments, to exclude the Ca^{2+}-activated K current which is also voltage-dependently activated. Currents recorded in such a condition (Fig. 5-A) was considered to be mainly composed of the delayed-rectifier type

K current (iK_{del}, Kim et al, 1993; Rhee et al, 1993; Noack 1992) which was originally classified by Hodgkin & Huxly (Hodgkin & Huxley, 1952).For studies in which K currents were blocked, pipette solution contained (in mM) Cs-aspartate 110, Mg-ATP 5, di-Tris-creatine phosphate 2.5, di-Na-creatine phosphate 2.5, $MgCl_2$ 1, HEPES 0.1, tetraethyl ammonium (TEA)-Cl 20, EGTA 5, pH 7.4.

Drugs: Lemakalim ((-)enantiomer of cromakalim, BRL 34915) was kindly donated by *Beecham Pharmaceuticals* (Britain) and all the other drugs were purchased from SIGMA (U. S.A).

RESULTS

Effects of lemakalim on the spontaneous contractions of the antral circular muscle of guinea-pig

Spontaneous contractions were measured isometrically. Lemakalim weakened the spontaneous contractions in a dose-dependent manner (Fig. 1-A) and the inhibitory effect was prevented by pretreatment with gliben-clamide which is known as a K_{ATP} channel blocker in pancreatic β-cells (Fig.1-B).

Effects of lemakalim on the slow waves

By using conventional intracellular microelectrode techniques, the membrane potential and its changes were recorded. Isometric contractions were also measured simultaneously. Circular smooth muscle of the antral region showed spontaneous electrical activity, which consisted of a slow depolarization, upstroke and plateau potential and repolarization, sometimes with superimposed spike potential (Fig. 2, Fig. 3, Fig. 4). The resting membrane potential values (the membrane potential between each slow wave) were between –60~ –65 mV. Low concentrations (<1 μM) of lemakalim induced a small hyperpolarization of the resting membrane potential and reduced the amplitude of slow waves (Fig. 2-C(a)). With higher concentrations of lemakalim (5 μM), the hyperpolarization reached -80 mV, and the slow waves were severely decreased or sometimes completely disappeared (Fig. 2-C (b)). Such effects occurred in a dose-dependent manner (Fig. 2-C, D) and were reversed by washing out with PSS (Fig. 3-A).

Lemakalim-induced changes in the resting membrane potential and slow waves were

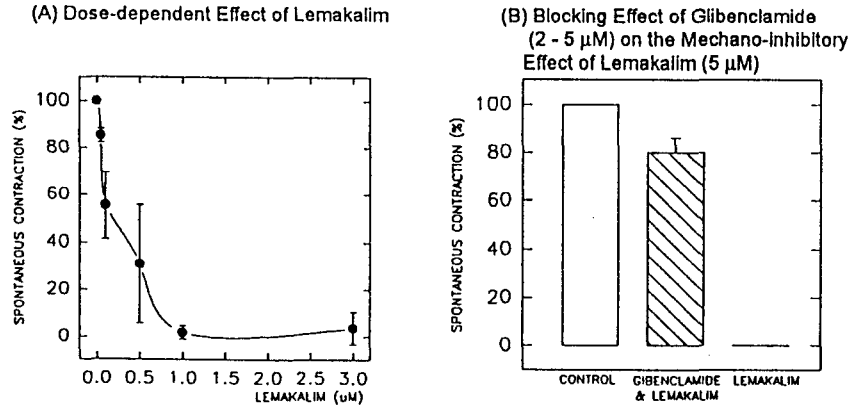

Fig. 1. Effects of lemakalim on the spontaneous isometric contractions. Dose-dependent changes of the amplitude of the spontaneous contractions are plotted in (A) by their mean value and standard error (n=4). The amplitudes of control contractions were regarded as 100%. In (B), the pretreatment with glibenclamide (5 μM) blocked the inhibitory effect of lemakalim (2~5 μM).

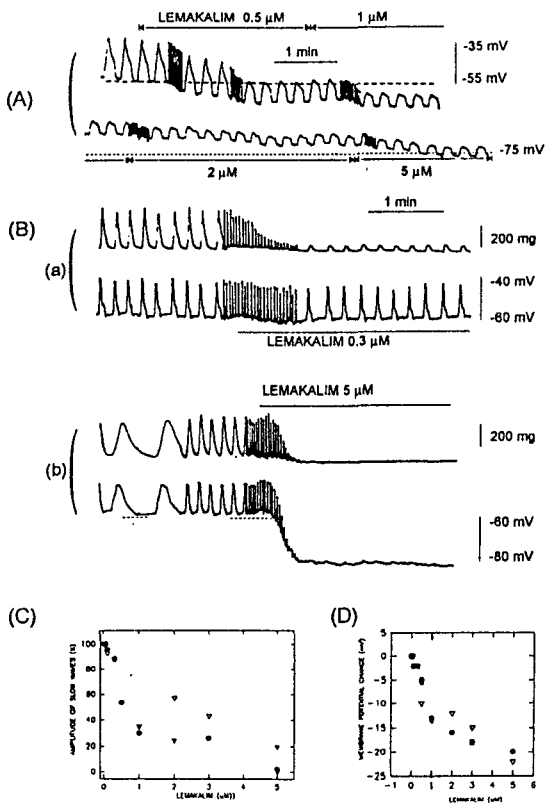

Fig. 2. Effects of lemakalim on the membrane potential and slow waves. (A) and (B) shows the dose-dependent effects of lemakalim on the membrane poteß ntial and slow waves. (C) and (D) were plotted from the result of three experiments (●, ▽, ▼). Lemakalim induced the hyperpolarization in a dose-dependent manner ((A), (B), (D)). The contour of the slow wave was also changed by lemakalim and the upstroke phase and plateau phase were more sensitively suppresed by lemakalim than the basic oscillation of the membrane potential ((A), (B), (C)). Isometric contractions were also measured simultaneously (each upper trace of (a) and (b) in (B)) and the representative effects of the low (0.3 μM) and high (5 μM) of lemakalim are shown in (B). (a) and (b) were recorded from one cell and (b) was recorded after the complete recorvery from (a).

almost completely abolished by the treatment with glibenclamide (Fig. 3-A, B). Also, the after-treatment with low concentrations of TEA (0.5~2 mM) in the presence of lemakalim (2 μM) could reverse the effect of lemakalim on slow waves and the inhibitory effect on mechanical contraction was also recovered by TEA (Fig. 4).

Effects of lemakalim on the ionic currents recorded from the single gastric myocytes

Delayed-rectifier type K current: Depolarizing test pulses between -45 mV and 30 mV with 15 mV increment from the holding potential of -65 mV elicited voltage-dependent outward currents (delayed-rectifier K current, iK_{del}) with the pipette solution of high K^+ containing 10 mM EGTA.

Lemakalim had no effect on this iK_{del} at 2 or 3 μM (Fig. 5-A, B), the concentration that was sufficient to induce a marked hyperpolarization during the conventional intracellular recordings (Fig. 2, Fig. 3, Fig. 4). But with much higher concentrations, say, above 10 μM, a little increase of the amplitude or the noise level was sometimes observed (3 out of 5 experiments, Fig. 5-C, D).

Ca^{2+}-activated K current: In this experiment, currents were elicited by a similar protocol shown above but with a low concentration (0.1 mM) of EGTA in the pipette. Many largely fluctuating outward currents were recorded under these conditions and these currents were considered to be Ca^{2+}-activated K currents (iK_{Ca}) superimposed upon the delayed rectifier-type K current described above (Fig. 6-A).

Lemakalim (2 μM) markedly increased the outward current in these conditions (Fig. 6-B & D), and such an increase was blocked by the application of glibenclamide (Fig. 6-C). The effects of lemakalim on the outward current recorded at each membrane potential and the blocking effect of glibenclamide were also plotted (Fig. 6-D).

Not only glibenclamide but also TEA (2 mM) could block the effect of lemakalim on the K currents considered as iK_{Ca} (Fig. 7). By pretreatment with TEA, the application of lemakalim could not increase the outward K

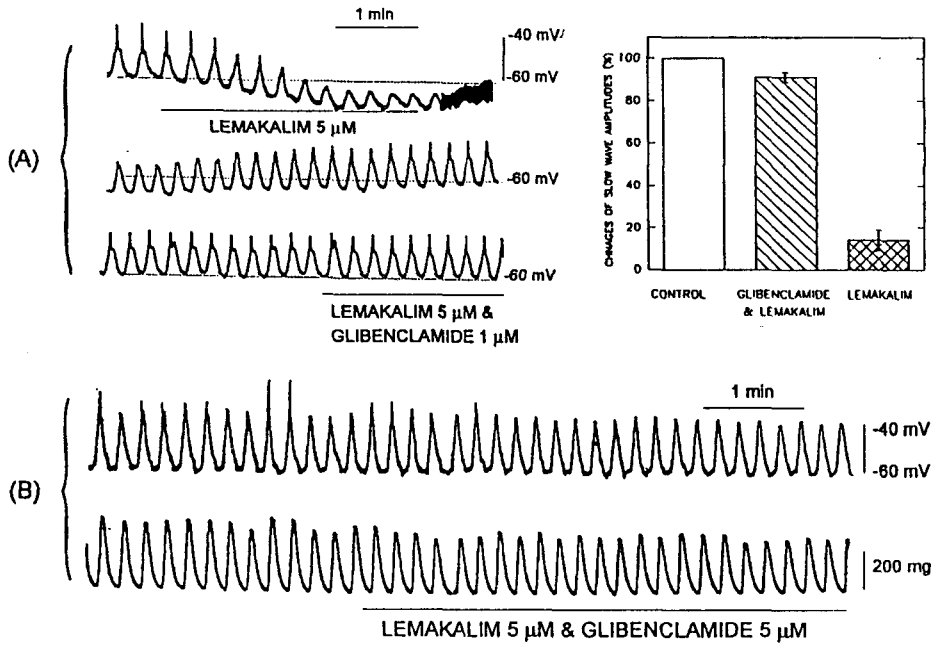

Fig. 3. Effects of glibenclamide on the lemakalim-induced changes of slow waves and spontaneous contractions. Effects of lemakalim on the membrane potential and slow waves were almost completely blocked by the concomitant application of glibenclamide ((A), the upper trace of (B)). The mechano-inhibitory effect was also blocked by glibenlclamide (the lower trace of (B)). The blocking effect of glibenclamide (1~5 μM) on the changes in the amplitude of slow waves by lemakalim (5 μM) is plotted by the mean value and the standard error in the inset.

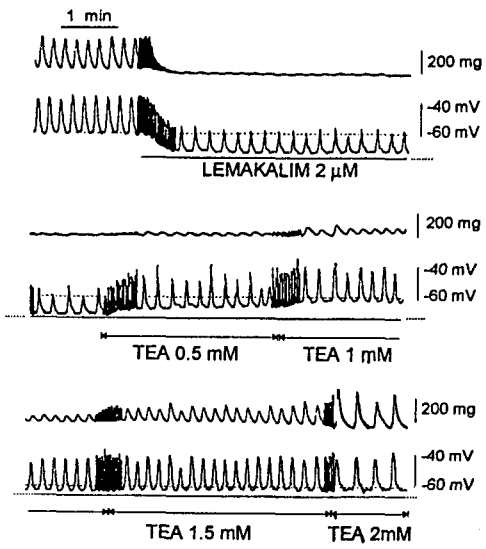

Fig. 4. Effects of tetraethylammomium (TEA) on the lemakalim-induced changes of slow waves and spontaneous contractions. Slow waves and the isometric contraction were recorded simultaneously. Each upper trace from the first, second, and the third paired traces represents the spontaneous isometric contraction. Each lower trace represents the membrane potential and slow waves. Both the hyperpolarization and changes in the contour of slow waves were reversed by the after-treatment with TEA (0.5 mM ~ 2 mM, lower traces). The inhibition of the isometric contraction by lemakalim was also reversed by TEA (upper traces).

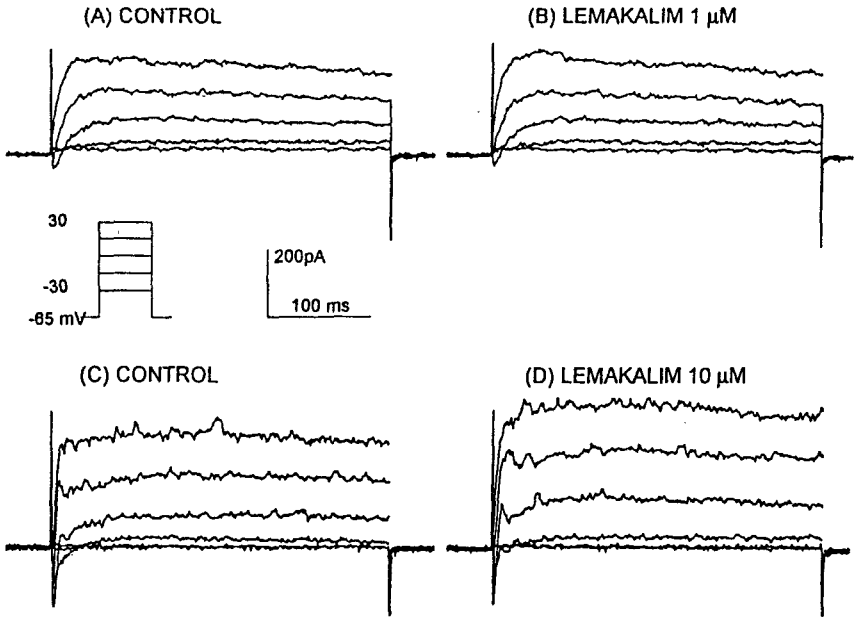

Fig. 5. *Effects of lemakalim on the delayed-rectifier K currents. Currents were elicited by depolarizing pulses between –30 and 30 mV with 15 mV increment from the holding potential of –65 mV, using a high K^+ solution containing 10 mM of EGTA in the pipette and normal Tyrode's in the bath. No change was induced by 2 μM of lemakalim (A, B). But with much higher concentrations of lemakalim (>10 μM), a little increase of outward currents was observed sometimes (C, D).*

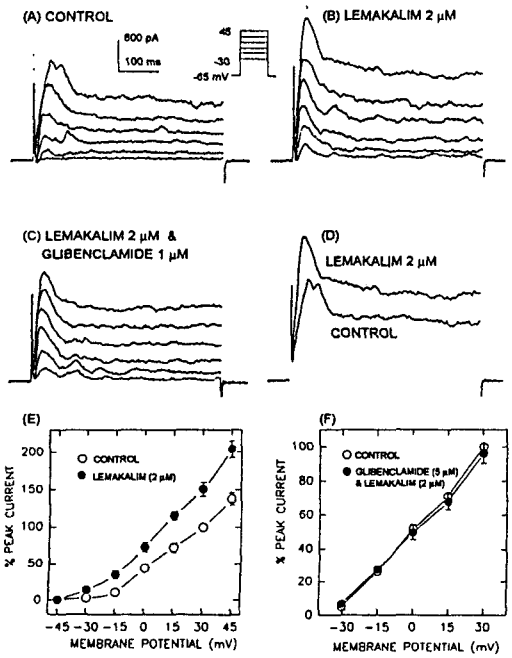

Fig. 6. *Effects of lemakalim on the Ca^{2+} -dependent K currents. Currents were elicited by the pulse protocol shown in the figure, using a high K^+ solution containing 0.1 mM of EGTA in the pipette and PSS in the bath. Outward currents were increased by 2 μM of lemakalim (A, B). These effects were reversed by the addition of glibenclamide (C). In (D), the largest currents from (A) and (B) were traced together. In (E) and (F), collected results from 4 experiments were plotted by their mean values and the standard errors.*

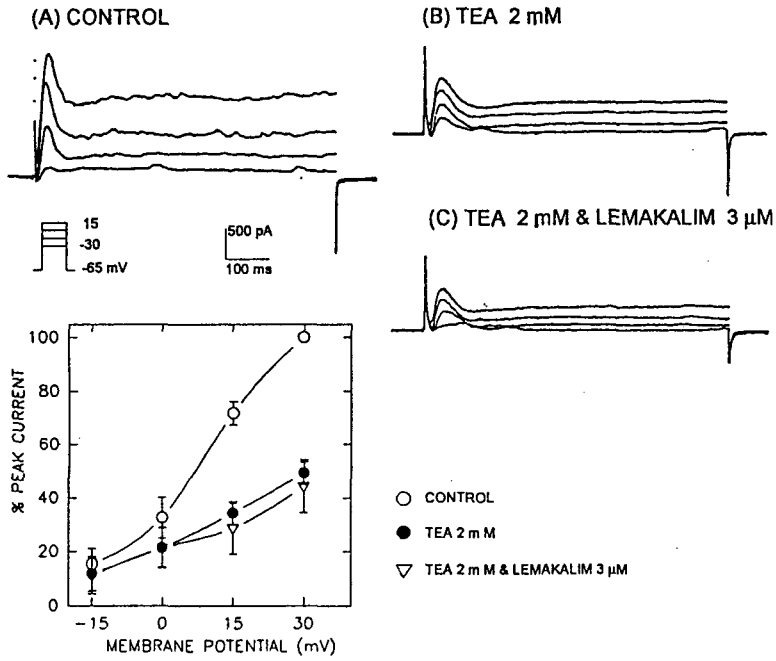

Fig. 7. Blocking effect of TEA on the effect of lemakalim on the Ca^{2+}-dependent K currents. Outward currents were elicited by the pulse protocol shown in the figure and using a high K^+ solution containing 0. 1 mM of EGTA in the pipette and PSS in the bath. TEA (2 mM) markedly decreased the outward current (B), and in this condition, the addition of lemakalim (3 μM) could not increase the outward K current.

currents.

Voltage-activated Ca current: For recording only inward currents, we used cesium-aspartate solution as described in the Methods. Mainly inward currents were observed by clamping membrane potentials from the holding potential (-60 mV) to various levels (-40~30 mV). Lemakalim (2 μM) had no effect on these Ca currents (Fig. 8-B) in most cases (5 out of 7 experiments. The others showed a small increase or decrease).

Effects of lemakalim on the single channel activities of Maxi-K recorded in excised inside-out patch clamp experiment

The large conductance Ca^{2+}-activated K channels (so called Maxi-K channels) are very densely expressed in the membrane of smooth muscle cells and also in these gastric myocyte. Their single channel conductance was around 300 pS with identical concentrations of K^+ on both sides of the membrane and the opening frequency was sharply dependent upon the concentration of Ca^{2+} on the cytoplasmic side of the membrane (Fig. 9-A). At a constant concentration of Ca^{2+} (pCa = 7.5), lemakalim applied to the inside of the membrane increased the opening frequency of the membrane (Fig. 9-B).

To evaluate the effect of lemakalim on the activity of Maxi-K channels, ensemble-average currents were obtained (see Methods). This ensemble-average current was also increased by the application of lemakalim (10 μM) to the cytoplasmic side of the membrane (Fig. 10-B). Higher concentrations (10 μM) of lemakalim than were effective in conventional microelectrode recordings or whole cell patch clamp experiments were needed to increase the opening frequency of Maxi-K channels, and

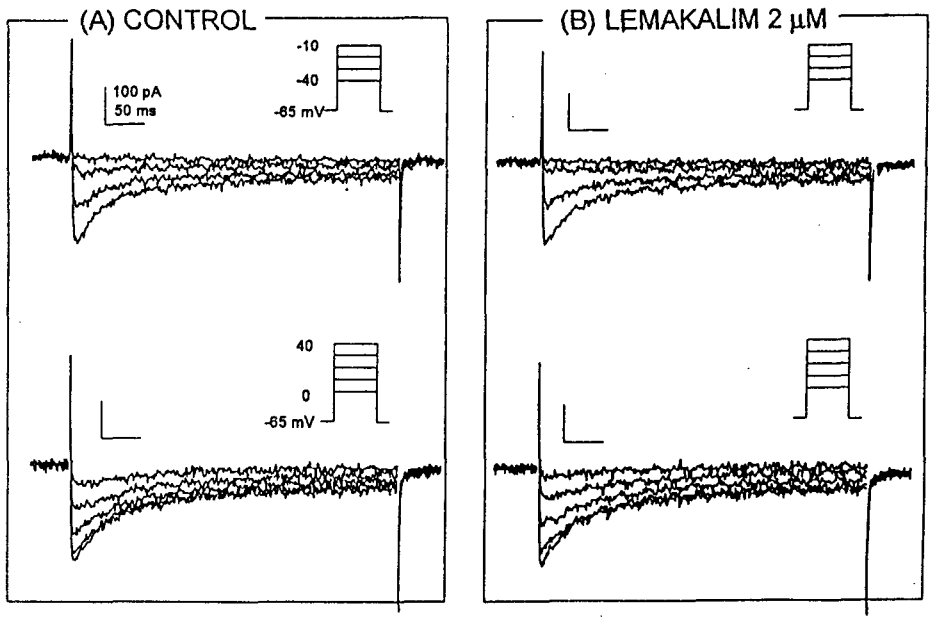

Fig. 8. Lemakalim showed no effect on the voltage-activated Ca current. The inward Ca currents were activated by the pulse protocol shown in the figure using a Cs-TEA solution containing 5 mM of EGTA in the pipette (A). These currents were not changed by the application of lemakalim (2 M, B).

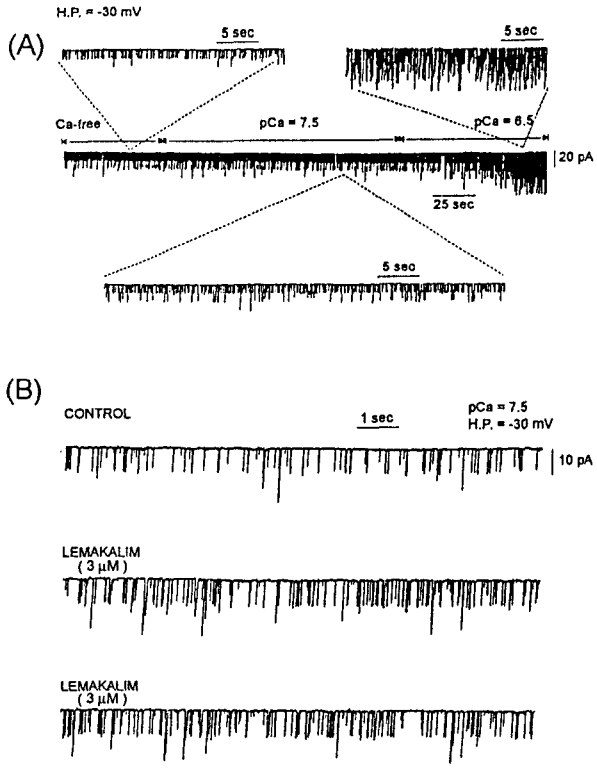

Fig. 9. Effects of lemakalim on the activity of Maxi-K channels. Single channel activities of the large conductance Ca^{2+}-activated K channels (Maxi-K channels) are observed using the inside-out mode patch clamp technique. Solutions of both sides of membrane were composed of symmetrical high K^+ (140 mM/140 mM). The number of open channels and the opening frequency were sensitively increased according to the cytoplasmic Ca^{2+} concentration at the same membrane potential (A). The application of lemakalim to the internal side of the membrane markedly increased the opening frequency of these channels at the same $[Ca^{2+}]$ and same membrane potential (B).

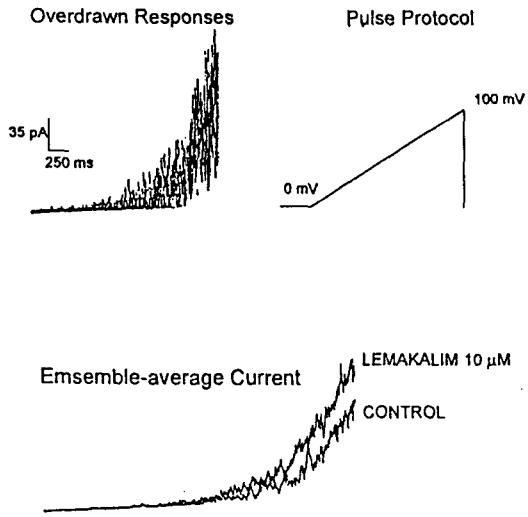

Fig. 10. Effects of lemakalim on the emsemble-average current through the Maxi-K channels. In the inside-out mode patch clamp condition, continuously depolarizing pulses (the ramp pulse) were applied repeatedly (15 times in this case, upper) and averaged (lower). Lemakalim increased this emsemble-average current.

such results were not found consistently (only 3 out of 8 cases showed positive results).

DISCUSSION

Effects of lemakalim on the membrane potential and slow waves in gastric smooth muscle

In this experiment, effects of lemakalim on slow waves and ionic currents have been investigated in the guinea-pig gastric smooth muscle. We might have to firstly clarify if this KCO (lemakalim) purely activates K channels and has no other nonspecific effect. But the obtained results that K channel blockers, glibenclamide or TEA, could reverse the effect of lemakalim (Fig. 3, Fig. 4, Fig. 6) support such an assumption that changes in membrane potential and slow waves are only caused by the changes in the activities of ionic channels.

It has been widely known that KCOs induce a prominent hyperpolarization in vascular smooth muscle. In the gastric smooth muscle, which shows electrical automaticity (slow waves), inhibitory effects of lemakalim on slow waves were also shown with characteristic changes in the contours of slow waves (Fig. 2, Fig. 3, Fig. 4, see also Itoh et al, 1992). With a little hyperpolarization, a decrease of the amplitude of slow waves was induced and such changes look as if the upper half of each slow wave was erased by hyperpolarization induced by lemakalim (Fig. 2, Fig. 3). Tomita (1981) has described that the slow wave recorded in guinea-pig stomach could be ascribed to the combination of three components which are 1) voltage-independent basic oscillation, 2) voltage dependent depolarizing upstroke, and 3) spike potentials superimposed upon 1) & 2). The result that the upper component disappeared, sensitive to chromakalim, and the basic oscillation remained quite persistently (Fig. 2, Fig. 3), strongly support such an idea. But with higher concentrations of lemakalim, abrupt and severe hyperpolarization almost to the level of -80 mV was induced and even the basic oscillation was also eliminated under these conditions (Fig. 2-B(b)). However, such a complete elimination of slow wave cannot be solely ascribed to the opening of K channels and it is not certain whether it is an indirect result or a non-specific effect.

Hyperpolarization induced by KCOs may prevent Ca^{2+} entry through voltage-operated Ca^{2+} channels, but also many other possible consequences have been reported so far. 1) Hyperpolarization favors Ca^{2+} extrusion through Na/Ca exchange (Mullins, 1979), 2) Hyperpolarization might interfere with the release of membrane-bound Ca^{2+} (Lodge & van Breemen, 1985) 3) Hyperpolarization prevents the refilling of intracellular Ca^{2+} stores (Bray et al, 1988) 4) Hyperpolarization inhibits caffeine-induced or IP_3-induced Ca^{2+} release from intracellular store (Quast, 1993) 5)

Hyperpolarization reduces Ca^{2+} sensitivity (Rasmussen, Kelly & Douglas, 1990). Apart from these hyperpolarization-induced effects, there is also evidence that KCOs can act via mechanisms not linked to K channel opening. For example, a phenomenon called 'the concentration paradox' which implies that 3-5 times more cromakalim and lemakalim were required to induce K channel opening than to induce 50% relaxation, has been reported repeatedly (Hamilton, Weir & Weston, 1986; Quast & Baumlin, 1988; Greenwood & Weston, 1993). Mechanisms of smooth muscle relaxation not linked to the opening of plasmalemmal K channels are not fully understood yet.

Related with the lemakalim-induced relaxation and suppression of slow waves, it should be confirmed whether lemakalim has any direct inhibitory effect on Ca currents. It has been reported that cromakalim showed an inhibitory effect upon the voltage-operated Ca current in canine colonic myocytes, while its (–) enantiomer, lemakalim had no effect (Post et al, 1991). In our result, lemakalim had no effect upon the size or voltage-relationship of Ca currents (Fig. 8). So the possibility of direct inhibition of voltage-dependent Ca-influx by lemakalim could be excluded in this smooth muscle.

Which kind of K channel is responsible for the lemakalim-induced hyperpolarization ?

In the smooth muscle, outward K currents recorded in usual whole cell clamp condition are mainly composed of Ca^{2+} & voltage-activated current (Haylett & Jenkinson, 1989) and voltage-activated Ca^{2+}-independent outward currents. The former Ca^{2+}-activated K current is large in size and shows a fluctuation or oscillation at a constantly depolarized membrane potential (Fig. 6). The fluctuations of these currents are usually ascribed to the oscillation of intracellular Ca^{2+} concentrations due to insufficient Ca^{2+}-buffering power of the low concentration of EGTA (Bolton & Lim, 1989). The latter K current is considered to be the delayed-rectifier type (Fig. 5) although the class of delayed-rectifier type is not a homogenous one (Hille, 1992). Other classes of K currents, such as the fast transient current or the inward rectifier or the ATP-sensitive K current are only exceptionally reported in some smooth muscle types (Bolton & Beech, 1992) and seemed to show a weak contribution to the total outward currents in this gastric myocytes of guinea-pig (unpublished observations).

Effects of lemakalim are blocked by glibenclamide (a hypoglycemic sulfonylurea that blocks ATP-sensitive K channel (K_{APT}) in the heart, skeletal muscle, and pancreatic β-cells) with wide variety of potencies in almost every kind of tissue (Cook & Quast, 1990) including smooth muscle (Buckingham et al, 1989). Such a characteristic blockade by glibenclamide led many investigators to a simple conclusion that the K_{ATP} is the target site for the action of lemakalim or other KCOs (Ito et al, 1992). That scheme might be well fit to the situations in the experiments with cardiac myocyte, skeletal muscle or pancreatic β-cells where the typical K_{ATP}s have been characterized and are sensitively blocked by glibenclamide (Ashford, 1990). But in the case of the smooth muscle, the situation is quite complex (Xiong, Kuriyama & Kitamura, 1991) and a number of K channels have been implicated in the effect of KCOs including large conductance Ca^{2+}-activated K channels (Gelband & McCullough, 1993; Post et al, 1991), K_{ATP} channels (Standen et al, 1989), delayed rectifier K channels (Beech & Bolton, 1989) and various other kinds of small conductance K channels (Kajioka, Oike & Kitamura, 1990; Noack et al, 1992).

It has been reported that large (135pS) or medium (20 pS) conductance K_{ATP} channels which are not Maxi-K channels may exist in some vascular smooth muscle, although in other vascular muscles or gastrointestinal smooth muscle where cromakalim produces an outward current and hyperpolarization, ATP-sensitive channels are difficult to demonstrate (Bolton & Beech, 1992). Moreover, in the

gastrointestinal smooth muscle, no direct evidence has been reported which shows the existence of K_{ATP}, although lemakalim or other KCOs are potent relaxants and produce prominent electrical changes in this smooth muscle (Fig. 2, Fig. 3, Fig. 4).

In our results, glibenclamide was also found to antagonize the effect of lemakalim in the guinea-pig gastric smooth muscle (Fig. 3, Fig. 6). These results suggest that K_{ATP} channels may exist in this gastric myocyte. But other data suggest that the membrane hyperpolarization by lemakalim is related to an increase in K conductance through Ca^{2+}-sensitive K channels (Fig. 5, Fig. 6). Ca^{2+}-sensitive K channels are known to be composed of at least three kinds and two of them (a small conductance-, apamin-sensitive K channel (SK_{Ca}) and a large conductance-channel (Maxi-K channel)) are suggested to be present in the gastrointestinal smooth muscle (Bolton & Beech, 1992). Apamin, a specific blocker for the SK_{Ca} could not block the hyperpolarization induced by lemakalim (data not shown), so the Maxi-K channels seemed to be a more plausible site for lemakalim (Fig. 6, Fig. 9, Fig. 10). Maxi-K channels are known to be sensitively blocked by TEA at low (<2 mM) concentrations before other kinds of K channels are non-selectively blocked by higher concentrations of TEA (Haylett & Jenkinson, 1989). Those results that lemakalim-induced hyperpolarization or lemakalim-activated outward current were blocked by the treatment with low concentration (<2 mM) of TEA (Fig. 4, Fig. 7) supported the possibility of Maxi-K as the target site for lemakalim in gastric smooth muscle. However, it is also usually known that the normal activities of Maxi-K channels are not affected by glibenclamide (Haylett & Jenkinson, 1989). Such paradoxical results lead us to the suggestion that glibenclamide acts by competing for the lemakalim binding site rather than directly blocking the K channels. Such an assumption was also suggested by Beech & Bolton in their experiments with rabbit portal vein (Beech & Bolton, 1989). In addition, there also is an interesting report that glibenclamide binds to either a separate protein, or to a separate domain on the channel in order to effect channel inhibition, and this domain is functionally disconnected from the channel by trypsin-, or chymotrypsin treatment (Nichols & Lopatin, 1993). This means that the sulfonylurea regulatory site is not a constitutive part of the conducting pore, and if the same or similar domain is also present in other kind of channel protein or linked to it, it could be the common target site for lemakalim and competitive inhibition by glibenclamide (Eltze, 1989). As there is no direct evidence for this suggestion in guinea-pig gastric myocyte, it is not certain if Ca^{2+}-activated K channel may have such a constitutive regulatory site.

There are many electrophysiological studies that have shown the ability of KCOs to open Ca^{2+}-dependent K channels in vascular smooth muscle (Kusano et al, 1987; Gelband et al, 1989; Trieschman et al, 1988; Gelband, Lodge & van Breeman, 1989; Kajioka, Oike & Kitamura, 1990; Gelband & McCullough, 1993), colonic smooth muscle (Post et al, 1991). In those studies, it has been demonstrated that cromakalim doubled the P_0 (open probability) of BK channels, and this was manifested by a decrease in the mean closed time without a change in the mean open time.

So we tried to investigate the effects of lemakalim on the activity of K channels in guinea-pig smooth muscle (Fig. 9, Fig. 10). Nevertheless, Maxi-K channels are so densely distributed that activities of other kind of K channels are overshadowed by Maxi-K channels and could not be identified under these conditions, so further experiments about effects on other kinds of K channels were not done. Moreover, effects of lemakalim on the activity of Maxi-K channels were somewhat inconsistent and much higher concentrations (>10 μM) of lemakalim were needed (Fig. 10) to show a stimulatory effect (3 out of 8 cases). As the studies upon the single channel activities of

Maxi-K did not give us a a definite answer, studies on the single channel activities of K channels in gastric myocyte of guinea-pig and the precise effects of KCOs on the single channel activities of K channels remained as a subject for further study.

From the above results, it is suggested that lemakalim, a potassium channel opener, induces hyperpolarization through the increase of Ca^{2+}-sensitive K currents in guinea-pig gastric myocytes, which is blocked by glibenclamide or TEA.

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