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ATP-Sensitive K^+ Currents in Gastric Myocytes Isolated from Guinea-pig

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ATP-sensitive K^+ channels (K_{ATP}) were not identified in gastric smooth muscle cells. However, in tension recording of intact gastric circular muscle, lemakalim of K_{ATP} channels opener in other tissues suppressed mechanical contractions and this effect was blocked by glibenclamide, a specific inhibitor of K_{ATP} channels. The aims of this study were to investigate whether K_{ATP} channels exist in gastric smooth muscle of guinea-pig and to know its physiological role. Whole cell K^+ currents activated by lemakalim were recorded from freshly isolated cells with a 0.1 mM ATP, 140 mM KCl pipette solutions. Lemakalim (10 μM) increased inward currents of -224 ± 34 pA (n=13) at -80 mV of holding potential in bath solution contained 90 mM K^+. Bath-applied glibenclamide (10 μM) inhibited the lemakalim-activated inward currents by 91 ± 6% (n=5). These lemakalim-activated inward currents were reduced by increased intracellular ATP from 0.1 to 3 mM (-41 ± 12 pA) (n=5). The reversal potential of the glibenclamide-sensitive inward currents was -5.2 ± 2.4 mV (n=3) in external 90 mM K^+ and shifted to -14.8 ± 3.6 mV (n=3) in external 60 mM K^+, which close to equilibrium potential of K^+ (E_K). External barium and cesium inhibited the lemakalim-activated inward currents dose-dependently. The half-inhibitory dose (IC_{50}) of barium and cesium were 2.3 μM (n=5) and 0.38 mM (n=4), respectively. 10 mM tetraethylammonium (TEA) also inhibited the lemakalim-activated inward currents by 66 ± 15% (n=5). Both substance P (SP) (5 μM) and acetylcholine (ACh) (5 μM) inhibited lemakalim-activated inward currents. These results suggest that K_{ATP} channels exist in the gastric smooth muscle and its modulation by neurotransmitters may play an important role in regulating gastric motility.

Key Words: ATP-sensitive K^+ channels, Lemakalim, Glibenclamide, Substance P, Acetylcholine

INTRODUCTION

Potassium channels play an important role in regulating the membrane potential of smooth muscle and the cell excitability. The opening of potassium channels produces hyperpolarization, which closes voltage-dependent Ca^{2+} channels and decrease cell excitability. However, the closure of potassium channels produces depolarization, which opens voltage-dependent Ca^{2+} channels and increase cell excitability. Potassium channels are classified with 2 groups by biophysical properties and functions. First group is delayed rectifier potassium channels (K_V), A-type potassium channels (K_A) and calcium-dependent potassium channels (K_{Ca}), which are voltage-dependent and have a quiescence role of cell excitability. On the other hand second group is ATP-sensitive potassium channels (K_{ATP}) and inward rectifier potassium channels (K_{IR}), which are voltage-independent and stabilize resting membrane potential (Edward & Weston, 1993; Quayle et al, 1995). In gastrointestinal smooth muscle cells, the potassium channels of first group

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have been identified (Mitra & Morad, 1985; Noack et al, 1992; Carl et al, 1990; Mayer et al, 1990; Sanders, 1992), whereas the potassium channels of second group are well not identified. K_{ATP} channels have been characterized in cardiac muscle cells (Noma, 1983), skeletal muscle cells (Spruce et al, 1985), pancreatic β-cells (Cook & Hales, 1984), vascular smooth muscle cells (Clapp & Gurney, 1992; Kamouchi & Kitamura, 1994; Quayl et al, 1995), and urinary bladder smooth muscle cells (Bonev & Nelson, 1993). The characteristic finding is the openings of K^+ channels when the concentrations of intracellular ATP were depleted. The synthetic K^+ channel openers such as lemakalim, cromakalim, pinacidil, and diazoxide have known as openers of K_{ATP} channels in various cells (Edwards & Weston, 1993). And sulfonylurea drugs such as glibenclamide and tolbutamide (Ascroft & Ascroft, 1990) block these channels, which were known as specific inhibitors of K_{ATP} channels. In tension recording of intact gastric circular muscle of guinea-pig, lemakalim suppressed spontaneous mechanical contractions completely and this effect is blocked by glibenclamide. These findings suggest that K_{ATP} channels also may exist in smooth muscle of guinea-pig stomach. Therefore, to investigate whether K_{ATP} channels exist in gastric smooth muscle and to know its physiological role, we performed whole-cell patch clamp recording from isolated gastric myocytes of guinea-pig.

METHODS

Cell isolation

Guinea-pigs of either sex, weighing about 250~250 g, were stunned and bled. The stomach was isolated and cut in the longitudinal direction along the lesser curvature in Krebs-Ringer solution. The antral part of stomach was cut and the mucosal layer was seperated from the muscle layer. The circular muscle layer was dissected from the longitudinal muscle layer and cut into small segments in nominal Ca^{2+} physiological salt solution for 30 min at room temperature. Then, they were transferred to the same solution with containing 0.1% collagenase (Wako), 0.1% trypsin inhibitor (Sigma) and 0.2% bovine serum albumin (Sigma) and incubated for 20~30 min at 36°C. After digestion, the supernatant was discarded and muscle segments were transferred into the modified Kraft-Bruhe (K-B) medium (Isenberg & Klockner, 1982). Single cells were obtained by gentle agitation with wide-bored glass pipette. Isolated gastric myocytes were kept in the modified K-B medium at 4°C until use. All experiments were carried out within 12 hours of harvesting cells and performed at room temperature.

Recording of isometric contractions

Muscle strips (2~3 mm wide, 10~12 mm long) from antrum were cut parallel to the circular fiber, and mounted in a vertical chamber which had a capacity of 50 ml. Isometric contractions were record through a force transducer (Ugo, Italy) with Krebs-Ringer solution at 37°C.

Membrane currents recording

Isolated cells were transferred to a small chamber (400 μl) on the stage of an inverted microscope (IX-70, Olympus). The chamber was perfused with physiological salt solution (PSS, 2~3 ml/min). The standard whole-cell patch clamp technique was used to record currents (Hamill et al, 1981). Glass pipettes with resistance of 3~5 MΩ were used to increase rate of whole cell dialysis. Membrane currents were amplified by an Axopatch 1-D (Axon Instrument) and command pulses were applied using an IBM-compatible computer and pCLAMP 6.0 software (Axon Instrument). The data were filtered at 5 kHz and displayed on a oscilloscope (Hitatch), a computer monitor, and a pen recorder (Recorder 220, Gould). To minimize activities of voltage-dependent K^+ channels and Ca^{2+}-dependent K^+ channels, the experiments were performed at 80 mV of holding potential with intracellular Ca^{2+} buffered to lower levels.

Solutions

Krebs-Ringer solution contained (in mM): NaCl 118.3, KCl 4.7, $CaCl_2$ 2.5, $MgCl_2$ 1.2, KH_2PO_4 1.2, $NaHCO_3$ 24, EDTA 0.026, glucose 11.1 (pH 7.4, bubbled with 5% CO_2: 95% O_2). PSS contained (in mM): NaCl 135, KCl 5, $MgCl_2$ 1, $CaCl_2$ 1.8, Glucose 5, HEPES 5, and the pH was adjusted to 7.4 by Tris. $CaCl_2$ was simply omitted in the Ca-free PSS. The external pipette solution contained (mM): NaCl 52 (or 82), KCl 90 (or 60), $MgCl_2$ 1, HEPES 10, $CaCl_2$ 0.2,

and the pH was adjusted to 7.4 by NaOH. The internal pipette solution contained (mM): NaCl 10, KCl 102, $CaCl_2$, 1, GTP 1, HEPES 10, EGTA 10, ATP 0.1 (or 3), $MgCl_2$ 1, and the pH was adjusted to 7.2 by KOH (38 mM). Modified K-B solution contained (mM): L-glutamate 50, KCl 50, Taurine 20, KH_2PO_4 20, $MgCl_2$ 3, glucose 10, HEPES 10, EGTA (ethyleneglycol-*bis*(β -aminoethyl ether)-N,N,N',N'-tetraacetic acid) 0.5 and pH was adjusted with KOH.

Drugs

Lemakalim(Beecham Pharmaceuticals, Britian), glibenclamide, barium, cesium, tetraethyammonium (TEA), substance P, acetylcholine (all from Sigma) were used. Lemakalim and glibenclamide were dissolved in dimethylsulfoxide (DMSO).

RESULTS

Tension recording

The antral circular muscle of guinea-pig stomach produced spontaneous mechanical contractions. 1 mM tetraethyammonium (TEA) increased spontaneous mechanical contractions, and the effect was blocked by lemakalim(10 μM) completely. Glibenclamide (10 μM), an inhibitor of K_{ATP} channels, blocked the lemakalim-suppressed spontaneous mechanical contractions (Fig. 1A). However, lemakalim did not suppress the TEA-induced spontaneous mechanical contractions in the pretreatment of glibenclamide. Glibenclamide did not affect the mechanical contractions itself (Fig. 1B). These findings suggest that K_{ATP} channels may exist in gastric smooth muscle.

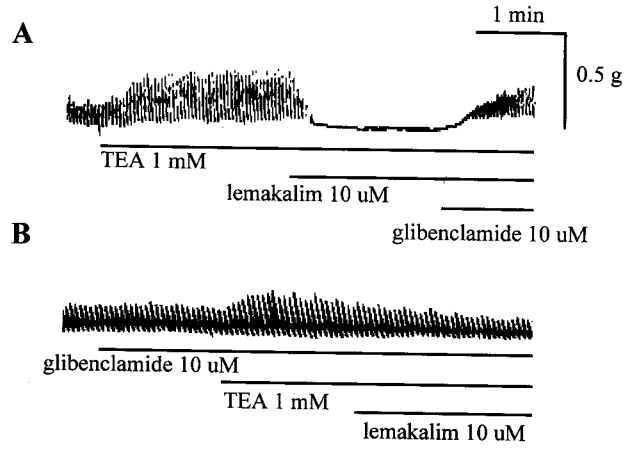

Fig. 1. Effects of lemakalim and glibenclamide on the spontaneous mechanical contractions in the antral circular muscle strip of guinea-pig stomach. The application of tetraethylammonium (TEA) (1 mM) increased the amplitude of spontaneous contractions. Under this condition, lemakalim (10 μM) suppressed spontaneous contractions completely. This lemakalim-induced suppression effect was blocked by glibenclamide (10 μM) (A). Spontaneous contractions were not affected by glibenclamide itself. In the pretreatment of glibenclamide to the resting state, lemakalim did not suppressed spontaneous contractions as like A (B).

Lemakalim activated glibenclamide-sensitive inward currents

To identify the possibility that K_{ATP} channels exist in gastric smooth muscle, we performed the whole-cell patch clamp method in gastric myocytes of

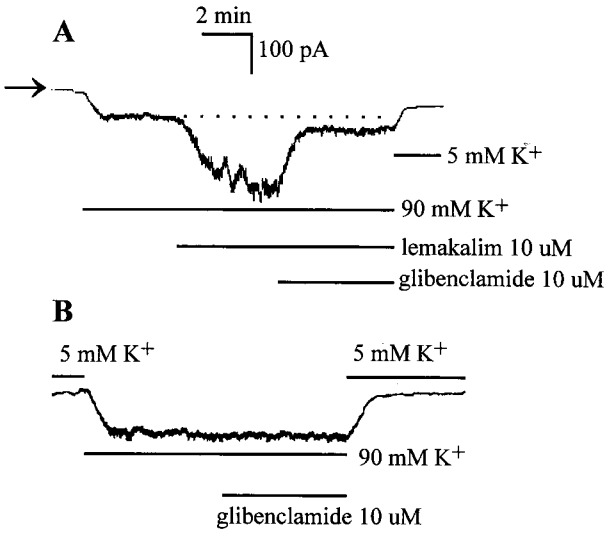

Fig. 2. Typical trace of glibenclamide-sensitive inward current induced by lemakalim in gastric antral myocytes of guinea-pig. Current was recorded at −80 mV of holding potential. Internal pipette solution contained 140 mM K^+ and 0.1 mM ATP. Exchanging of extracellular K^+ concentrations from 5 to 90 mM inward current was induced from zero current level (arrow). Dashed line indicates inward current level in the presence of external 90 mM K^+. Under this condition, lemakalim(10 μM) activated large inward current. Glibenclamide (10 μM) reversed lemakalim-activated inward current. But glibenclamide did not block up to the zero current level (A). Glibenclamide did not affect on external 90 mM K^+ -induced inward current in the absence of lemakalim (B).

guinea-pig. The holding potential was maintained at −80 mV to minimize activities of other voltage-dependent currents and cells were perfused with external 90 mM K^+ to increase driving force at this holding potential. Internal pipette solution contained 140 mM K^+ and 0.1 mM ATP. Under these conditions, the potassium equilibrium potential was −11 mV and potassium currents were inward. The exchanging of external K^+ concentrations from 5 to 90 mM induced the steady-state inward currents. The amplitude of steady-state currents was −90±23 pA (n=13). Under this condition, application of lemakalim (10 μM) activated further inward currents of −224± 34 pA (n=13) and glibenclamide (10 μM) blocked the lemakalim-activated currents by 91±6% (n=5) (Fig. 2A). However, glibenclamide had no effect on the steadystate current by 90 mM external K^+ (Fig. 2B).

Potassium selectivity of lemakalim-activated inward current

K_{ATP} channels from vascular (Kleppisch & Nelson, 1995) and urinary bladder smooth muscle (Bonev & Nelson, 1993) are K^+ selective. To identify the ionic selectivity of the lemakalim-activated inward currents, ramp pulse of 250 ms duration from −90 mV to 20 mV was applied in the absence of drugs, in the presence of lemakalim, and in the presence of lemakalim and glibenclamide with external 90 mM and 60 mM K^+. Under these conditions, the calculated K^+

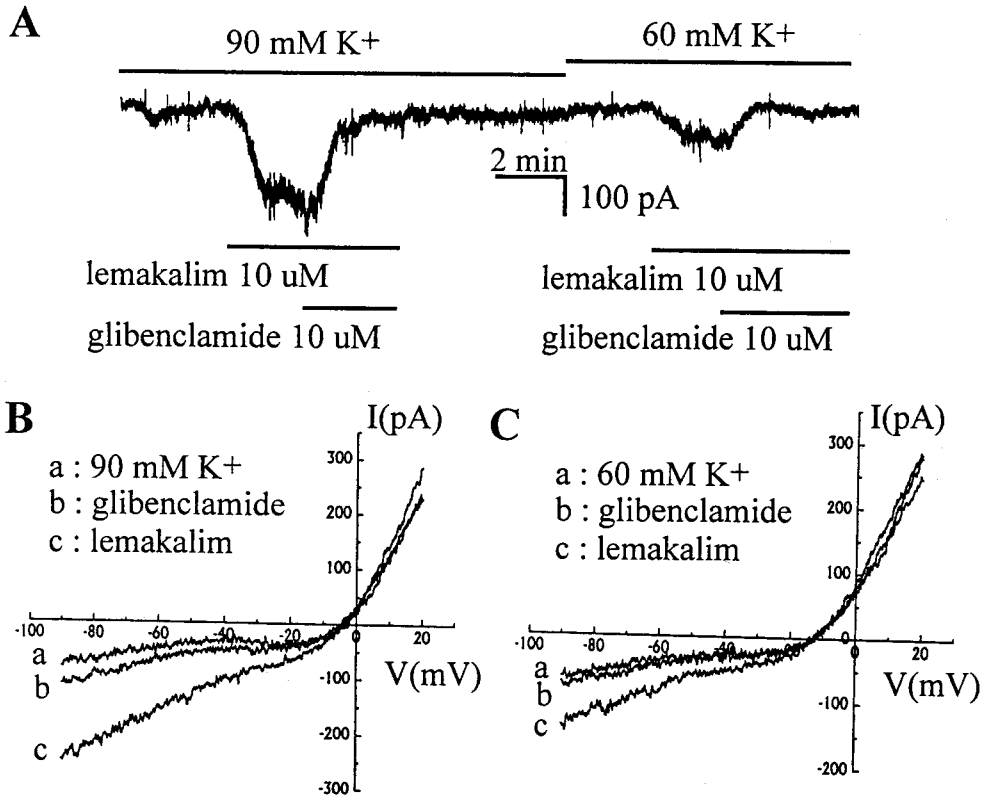

Fig. 3. Ionic selectivity in the presence of lemakalim and glibenclamide. A:The effect of glibenclamide on the current induced by lemakalim (10 μM) with external 90 and 60 mM K^+ at −80 mV of holding potential. Internal pipette solution contained 140 mM K^+, 0.1 mM ATP. The amplitude of the lemakalim-induced current in external 60 mM K^+ was smaller than that in external 90 mM K^+. Current-voltage relationships obtained from (A) using a 250 ms ramp pulse from −90 to 20 mV. The calculated equilibrium potentials for K^+ values with 90 and 60 mM external K^+ were −11 and −21 mV, respectively. The real reversal potential in 90 mM external K^+ was −5.2 mV (B) and shifted to −14.8 mV in 60 mM external K^+ (C). These reversal potentials were not changed in the presence of lemakalim and glibenclamide.

equilibrium potentials were −11 and −21 mV, respectively. Fig. 3A shows the consecutive current record induced by lemakalim with external 90 mM and 60 mM K^+ in one cell. Current amplitude in external 60 mM K^+ was much smaller than in external 90 mM K^+. Fig. 3B, C show current-voltage relationships obtained from Fig. 3A using a ramp pulse. The mean reversal potentials of the lemakalim-activated currents with 90 and 60 mM external K^+ were −5.2±2.4 mV (n=3) (Fig. 3B) and −14.8±3.6 mV (n=3) (Fig. 3C), respectively. The lemakalim-activated inward currents were reversed near to E_K, indicating that the currents were K^+ selective. The current-voltage curve exhibited linear relationship between 90 to 0 mV, which suggest that the lemakalim-activated currents are not voltage-dependent and that glibenclamide did not affect the outward currents above 0 mV.

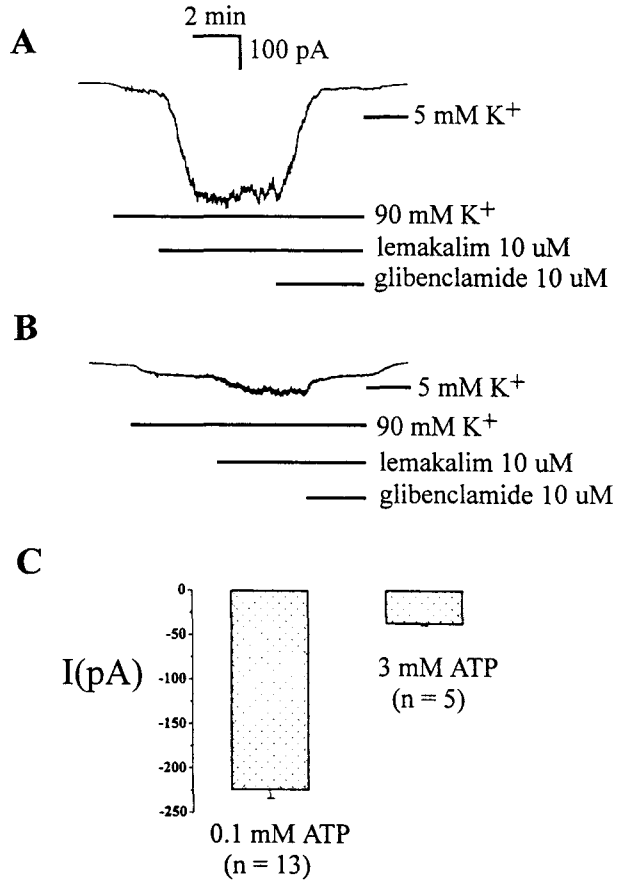

Fig. 4. Effects of intracellular ATP on the lemakalim-activated inward currents. Currents were recorded by lemakalim (10 μM) with pipette containing 0.1 mM ATP (A) and 3 mM ATP (B) at −80 mV of holding potential. Internal pipette and bath K^+ were 140 and 90 mM. C: Mean amplitude of the lemakalim-induced inward currents with pipette containing 0.1 (n=13) and 3 mM ATP (n=5).

ATP inhibition of lemakalim-activated K^+ currents

To investigate the ATP-dependency of the lemakalim-activated K^+ currents, cells were dialyzed with 3 mM ATP. Under this condition, the mean amplitude of lemakalim-activated K^+ currents was reduced from −224±34 (n=13) to −37.8±3.9 pA (n=5) (Fig. 4A, B, C). This finding indicates the lemakalim-activated K^+ currents are dependent upon intracellular ATP.

Barium, cesium and TEA effects on K_{ATP} channels

The ATP-sensitive K^+ channels were blocked by barium and cesium in skeletal muscle (Spruce et al, 1988) and urinary smooth muscle (Bonev & Nelson, 1993). In gastric myocytes, barium and cesium also suppressed ATP-sensitive K^+ currents. Fig. 5A & 5B show original current records blocked by barium and cesium, dose-dependently at −80 mV of holding potential. The half-inhibitory doses (IC_{50}) of barium and cesium were 2.3 μM (n=5) and 0.38 mM (n=4), respectively. TEA is an effective inhibitor of calcium-dependent K^+ channels in other tissues. In contrast, TEA shows relative ineffective inhibition in K_{ATP} channels. For example, in skeletal muscle, TEA blocks K_{ATP} channels with a IC_{50} of 6.7 mM. In the present study, TEA (5 and 10 mM) inhibited the lamakalim-activated K^+ currents (Fig. 6A). 10 mM TEA inhibited by 66±15% (n=5) (Fig. 6B).

Modulation of K_{ATP} by substance P (SP) and Acetylcholine (ACh)

We next examined the effect of substance P and acetylcholine on the lemakalim-activated K^+ currents. Fig. 7A shows the effect of substance P on the lemakalim-activated K^+ current. At −80 mV of holding potential lemakalim activated K^+ current to 230 pA. Application of substance P (5 μM) suppressed K^+ current by about 50%. This current was further inhibited by glibenclamide. Substance P suppressed the lemakalim-activated K^+ currents by 50±5% in 7 cells. Acetylcholine also suppressed leamkalim-activated K^+ currents (Fig. 7B). 5 μM acetylcholine suppressed lemakalim-activated K^+

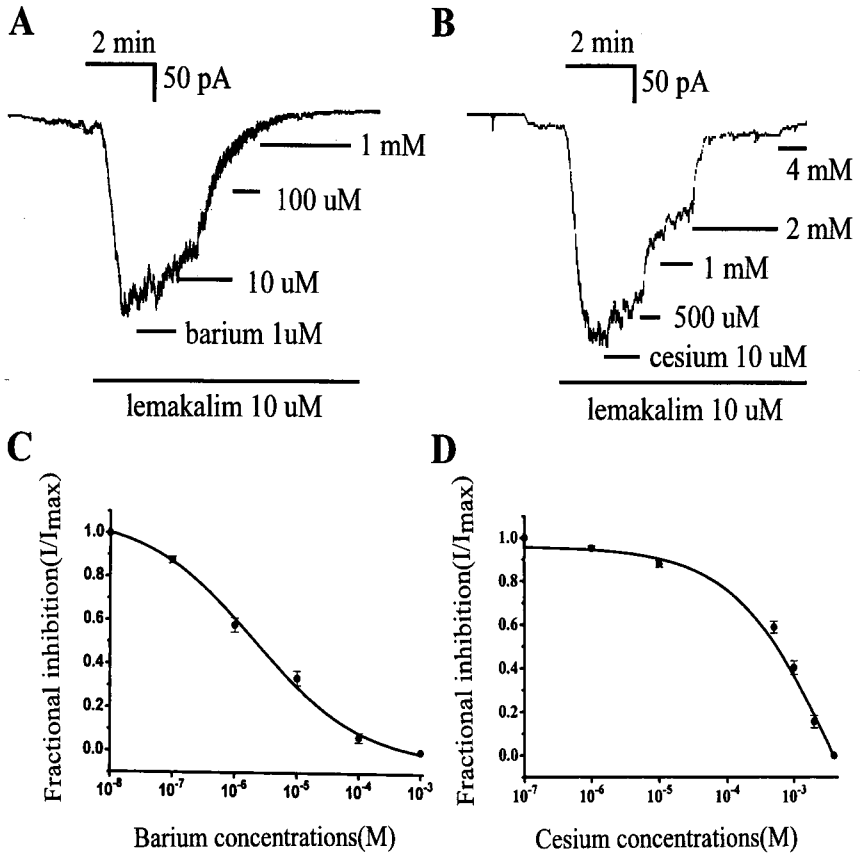

Fig. 5. Effects of external barium and cesium on the lemakalim-activated inward currents. Lemakalim-induced inward currents were blocked by barium (A) and cesium (B). Holding potential was −80 mV. Internal pipette solution contained 140 mM K^+, 0.1 mM ATP. C and D: Dose-dependency of barium and cesium in the inhibition of the lemakalim-activated inward currents. Solid lines are fit to data by a non-regression equation ($1/\{1 + ([\text{barium or cesium}]/IC50)^n\}$). The half-inhibitory dose (IC_{50}) of barium and cesium were 2.3 μM (n=5) and 0.38 mM(n=4), respectively. Values are means±SE.

currents by 83±12% in 4 cells.

DISCUSSION

In the present study, we have identified the presence of ATP-sensitive K^+ (K_{ATP}) channels in the gastric smooth muscle cells of guinea-pig and the suppression of these channels by substance P and acetylcholine.

It is well known that lemakalim, cromakalim, pinacidil, and diazoxide, synthetic K^+ channel openers, activate K_{ATP} current in cardiac muscle (Escande et al, 1988), skeletal muscle (Weik & Neumcke, 1990), and vascular smooth muscle cells (Standen et al, 1989) and that glibenclamide blocks the current. Thus, we used lemakalim and glibenclamide to study K_{ATP} channel in gastric myocytes. In exchanging of external K^+ from 5 to 90 mM, inward steady-state currents were produced from zero current level. Lemakalim activated further large inward currents from steady-state level. Lemakalim-activated large inward currents were reversed by glibenclamide. However, glibenclamide did not inhibit these currents up to the steady-state current level in 90 mM K^+, completely. 10 μM glibencalmide blocked lemakalim-activated currents by 91±6%, and glibenclamide has no effect on the steady-state currents in the absence of lemakalim in external 90 mM K^+. The steady-state and the remaining currents after the administration of glibenclamide were reversed to the zero current level by 2 mM of barium (not shown). These findings were different from those vascular smooth muscle cells. In vascular smooth muscle cells, inward steady-state

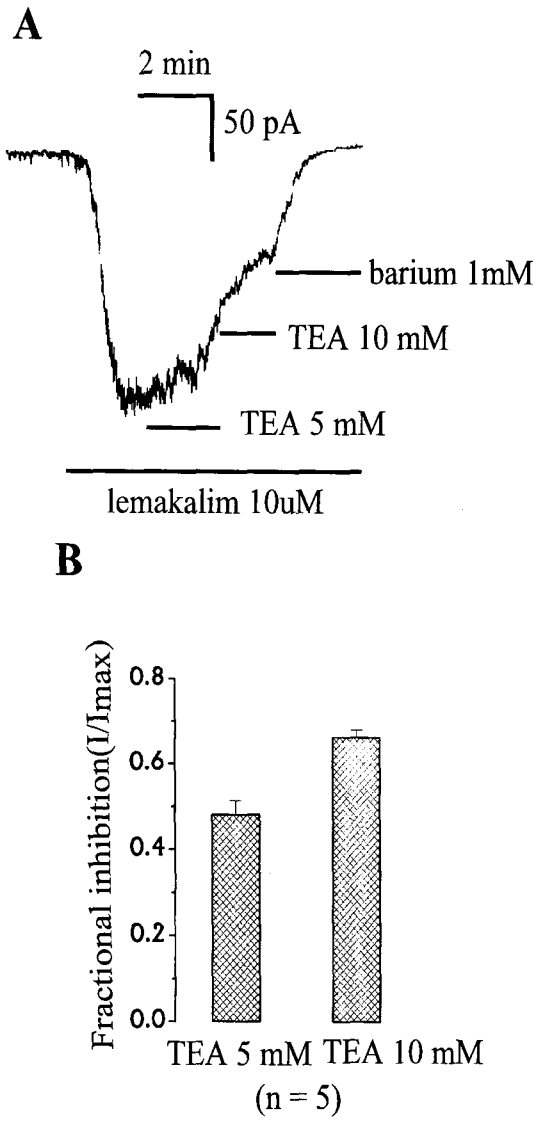

Fig. 6. Effects of external tetraethylammonium (TEA) on the lemakalim-activated inward currents. Lemakalim-activated inward current was blocked by TEA (5 and 10 mM) (A). Current was recorded by lemakalim with pipette containing 0.1 mM ATP at −80 mV of holding potential. Internal pipette and bath K^+ were 140 and 90 mM. Barium (1mM) suppressed the current further. C:Mean fractional inhibition of the current by TEA (5 and 10 mM) (n=5).

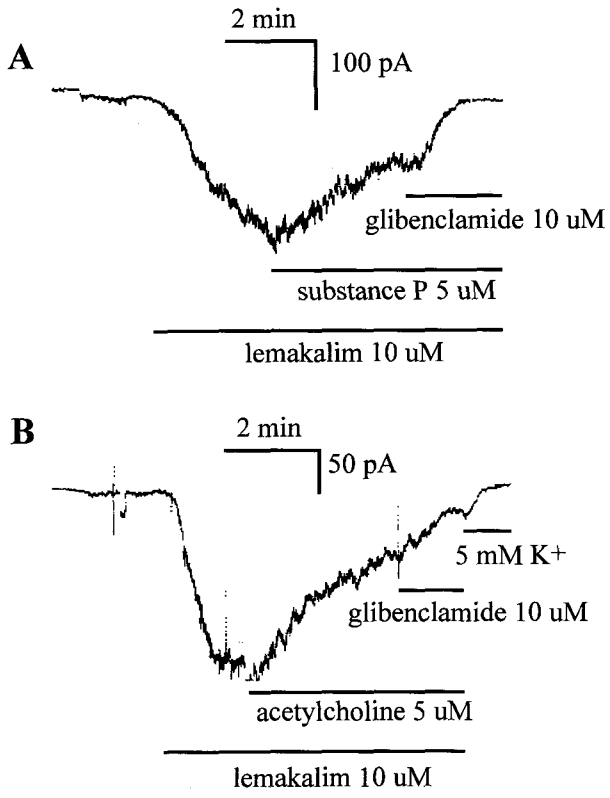

Fig. 7. Effect of substnace P (SP) and acetylcholine (ACh) on the lemakalim-activated inward currents. Both SP (5 uM) (A) and ACh (5 uM) (B) suppressed the lemakalim-activated inward current. Currents were recorded by lemakalim with pipette containing 0.1 mM ATP at −80 mV of holding potential. Internal pipette and bath K^+ were 140 and 90 mM, respectively.

current in the absence of lemakalim is very sensitive to glibenclamide and also, lemakalim-activated current is inhibited by glibenclamide, completely. We don't know the reason for the discrepancy. Vascular hyperpolarization and dilatation by high external K^+ were mediated by inward rectifier K^+ currents (Knot et al, 1996). These currents have shown barium-sensitive. Therefore, we think that inward rectifier K^+ current might be contained in the inward stady-state current recorded from gastric smooth muscle cells. However, further investigations will be required to identify this current.

K^+ channels of gastric smooth muscle cells are mainly delayed rectifier K^+ (K_V) channels and Ca^{2+}-dependent K^+ (K_{Ca}) channels (Mitra & Morad, 1985; Noack et al, 1992). Therefore, to minimize the activities of K_V and K_{Ca}, we performed the whole-cell patch clamp at 80 mV of holding and intracellular Ca^{2+} buffered to 20 nM by 10 mM EGTA. Also, high external K^+ was used to increase K^+ conductance. Under these conditions, K^+ currents are inward. This protocol has been used in vascular (Quayle et al, 1995; Kleppisch & Nelson, 1995) and urinary bladder smooth muscle cells (Bonev & Nelson, 1993). Generally K_{Ca} channels are blocked by

relatively low concentrations of external TEA (IC_{50}: 200 μM in vascular smooth muscle) (Langton et al, 1991), whereas K_{ATP} channels are blocked by relatively high concentrations of TEA (IC_{50}: 7 mM in skeletal muscle) (Davies et al, 1989). On the other hand, K_{ATP} channels are blocked by relatively low concentrations of external barium (IC_{50}: 100 μM at 62 mV in skeletal muscle), whereas K_{Ca} and K_V channels are blocked by high concentrations of barium (IC_{50}: 10 and 0.5 mM, respectively) (Miller et al, 1987; Hirst & Edwards 1989). K_{Ca} and K_V channels are voltage-dependent. However, in this study the lemakalim-activated inward currents were suppressed by relatively high concentrations of TEA (by 66% in 10 mM) and the half- inhibitory dose of barium was 2.3 μM and current-voltage relationship showed voltage-independent fashion. Glibenclamide blocked K_{ATP} channels in various tissues (Edwards & Weston, 1993), but does not block K_{Ca} and K_V currents (Bonev & Nelson, 1993). K_{Ca} and K_V currents are not inhibited by increased intracellular ATP concentrations (Kajioka et al, 1991). In our study lemakalim-activated inward currents were about 5-fold smaller in 3 mM intracellular ATP and glibenclamide did not affect K_V currents. From these findings, we think that glibenclamide-sensitive K^+ currents described here are different from K_{Ca} and K_V currents. These results are consistent with K_{ATP} currents reported in vascular smooth muscle and urinary smooth muscle (Bonev & Nelson, 1993; Quayle et al, 1995; Kleppisch & Nelson, 1995). There are several types of K_{ATP} channels (Edwards & Weston, 1993). Type 1 K_{ATP} channels are calcium- and voltage-independent, K^+-selective, and inhibited by increasing intracellular ATP. They exhibit inward rectification, and very sensitive to glibenclamide. K_{ATP} channels recorded from gastric myocytes in the present study are well correlated with the above findings, which suggest gastric smooth muscle cells also have a Type 1 K_{ATP} channels.

Gastrointestinal smooth muscles are regulated by neurotransmitters released from intrinsic and extrinsic nervous system. Acetylcholine (ACh) and substance P (SP) are major excitatory neurotransmitters in gastrointestinal smooth muscles. ACh and SP depolarize the membrane potential, resulting in contractions (Huzinger et al, 1984). The mechanisms of ACh- and SP-induced depolarization are explained with the suppression of K^+ currents in toad gastric myocytes (M-currents) (Sims et al, 1986) and mammalian colonic muscles (Cole et al, 1989), and with the activation of voltage-dependent Ca^{2+} currents (Clapp & Gurney, 1987; Mayer et al, 1990) and non-selective cationic currents (Inoue & Isenberg, 1991; Nakazawa et al, 1990; Sims, 1992; Lee et al, 1995). However, M-current is not exist in mammalian smooth muscle cells and the activation of voltage-dependent Ca^{2+} channel is controversial (Unno et al, 1996). So, in present, major mechanism of depolarization was explained with the activation of non-specific cationic channels. In this study we found that ACh and SP suppress K_{ATP} currents. The density of K_{ATP} channels is high, thus, activation of less than 0.1% of these channels will double the resting conductance for K^+ (Standen et al, 1989). And it is reported that the suppression of K_{ATP} channel induce membrane depolarization in pancreatic β-cells (Ashcroft et al, 1984). Therefore, these results suggest the suppression of K_{ATP} by Ach and SP may be a possible mechanism of membrane depolarization.

In summary, we have demonstrated that K_{ATP} channels exist in gastric myocytes. Both SP and ACh suppress the K_{ATP} currents. The inhibitory action of ACh and SP may be a possible depolarizing mechanism in gastrointestinal smooth muscle cells.

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