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Pharmacological Evidence that Cromakalim Inhibits Ca^{2+} Release from Intracellular Stores in Porcine Coronary Artery

Byung Yong Rhim, Sun Hwa Hong, Chi Dae Kim, Won Suk Lee, and Ki Whan Hong

Department of Pharmacology, College of Medicine, Pusan National University, Pusan, Korea

In the present study, it was aimed to further indentify the intracellular action mechansm of cromakalim and levcromakalim in the porcine coronary artery. In intact porcine coronary arterial strips loaded with fura-2/AM, acetylcholine caused an increase in intracellular free Ca^{2+} ($[Ca^{2+}]_i$) in association with a contraction in a concentration-dependent manner. Cromakalim (1 μM) caused a reduction in acetylcholine-induced increased $[Ca^{2+}]_i$ not only in the mormal physiological salt solution (PSS) but also in Ca^{2+}-free PSS (containing 1 mM EGTA). In the skinned strips prepared by exposure of tissue to 20 μM β-escin, inositol 1,4,5-trisphosphate (IP_3) evoked an increase in $[Ca^{2+}]_i$, but it was without effect on the intact strips. The IP_3-induced increase in $[Ca^{2+}]_i$ was inhibited by cromakalim by 78% and levcromakalim by 59% (1 μM, each). Pretreatment with glibenclamide (a blocker of ATP-sensitive K^+ channels, 10 μM) and apamin (a blocker of small conductance Ca^{2+}-activated K^+ channels, 1 μM) strongly blocked the effect of cromakalim and levcromakalim. However, charybdotoxin (a blocker of large conductance Ca^{2+}-activated K^+ channels, 1 μM) was without effect. In addition, cromakalim inhibited the $GTP_\gamma S$ (100 μM, non-hydrolysable analogue of GTP)-induced increase in $[Ca^{2+}]_i$.

Based on these results, it is suggested that cromakalim and levcromakalim exert a potent vasorelaxation, in part, by acting on the K^+ channels of the intracellular sites (e.g., sarcoplasmic reticulum membrane), thereby, resulting in decrease in release of Ca^{2+} from the intracellular storage site.

Key Words: Intracellular Ca^{2+} release, K^+ channel opener, Vasorelaxation, Porcine coronary artery

INTRODUCTION

The vasorelaxant action of cromakalim is understood to be a consequence of its ability to stimulate K^+ conductance and in turn, to cause hyperpolarization of the plasma membrane and inactivate the voltage-dependent Ca^{2+} channels (Hamilton et al., 1986; Weir and Weston, 1986). Cromakalim has been reported to exert a direct inhibitory effect on the refilling of depleted intracelluler Ca^{2+} of the vascular beds (Bray et al., 1991; Quast, 1989) and the airway smooth muscle (Chopra et al., 1992). On the other hand, release of intracelullar Ca^{2+} from the sarcoplasmic reticulum is known to be regulated by IP_3 (IP_3-induced Ca^{2+} release) (Kobayashi et al., 1988; Somlyo et al., 1985). However, it is not clear as to whether cromakalim-induced relaxation is dependent entirely on the inhibition of extracellular Ca^{2+} influx through the plasma membrane by its ability to increase the K^+ conductance.

Recently, we have demonstrated that, in the permeabilized dispersed cells from canine coronary artery, cromakalim and pinacidil, the K^+ channel openers, caused a concentration-dependent inhibition of the contraction to IP_3 and phenylephrine, concluding that the K^+ channel openers exert a relaxation by acting on the intracellular membrane sites which is sensitive to IP_3 as well as the glibenclamide-sensitive K^+ channels of the plasma membrane (Rhim and Hong, 1994).

In the present study, to further explore the intracellular action mechanism of the K^+ channel openers in the porcine coronary artery, we observed the ihibitory effect of cromakalim and levcromakalim on the IP_3-and $GTP_\gamma S$-induced increase in $[Ca^{2+}]_i$ in the skinned strips.

Correspondence to Byung Yong Rhim

Further, we characterized the cromakalim sensitive K^+ channels of the intracellular site with glibenclamide (a blocker of ATP-sensitive K^+ channels) and apamin (a blocker of small conductance Ca^{2+}-activated K^+ channels).

MATERIALS AND METHODS

Preparation of arterial strips

Fresh porcine hearts were obtained from the slaughter house. The heart was immediately removed after a sacrifice and placed in the ice-cold physiological salt solution (PSS) saturated with 95% O_2-5% CO_2. The left anterior descending branches of the coronary artery were dissected and the connective tissues were carefully removed in a dissecting chamber under a stereoscope. Spirally cut strips of coronary artery (1 ~2 mm wide and 5~8 mm long) were prepared. The endothelium was removed by gentle rubbing of internal surface of the vessels with moistened cotton wood.

Skinned strip preparation

Chemically skinned strips of porcine coronary artery were made by exposure to 20 μM β-escin in the cytosolic buffer for 15 min at 37°C (Kobayashi et al., 1989). After skinning, the strips were washed twice with relaxing solution (cytosolic buffer) to remove the β-escin, and then mounted on the epifluorescence microscopy chamber. As fura-2 loading buffer in the skinned strips the Ca^{2+}-free cytosolic buffer containing 2 μM fura-2 was used. The Ca^{2+}-free cytosolic buffer contained 3 μM GTP, 10 μM antimycin A, 5.2 mM ATP, 5.0 mM creatine phosphate and 10 unit/ml creatine phosphokinase. Thereafter, the preparation was incubated in the cytosolic buffer containing 0.3 μM Ca^{2+} for 2 min and rinsed with Ca^{2+}-free cytosolic buffer for 30 sec and subsequently incubated in the fura-2 loading buffer for 2 min. The strips were exposed to agonists for 5 min unless otherwise stated.

Measurements of intracellular Ca^{2+} concentration

Coronary arterial strips were exposed to 40 μmol/l fura-2 acetoxylmethyl ester (fura-2/AM) for about 3 hours at 37°C. To help dissolve the fura-2/AM in PSS, the noncytotoxic detergent pluronic F-127(0.1% w/v) was included in the loading PSS. After the fura-2/AM loading, strips were rinsed with the normal PSS for approximately one hour and then used for experiments. Changes in fluorescence of the fura-2-Ca^{2+} complexes in the strips were measured by using a Spex Fluorog-2-Spectrofluorometer equipped with a dual wave length excitation device (Model CM 1TII1, Spex Industries, Edison, NJ, U.S.A.) connected to an inverted fluoromicroscope (TMD-8, Nikon, Tokyo, Japan). The fluoresecence image was obtained by focussing on the medial smooth muscle layer with a Nikon CF UV (Fluor) objective lens (×10). The muscle strip was placed horizontally in a temperature controlled 0.7-ml tissue chamber placed on the inverted microscope. The chamber was perfused with the PSS at a constant rate of 1 ml/min under resting tension of about 0.5 g. The light emitted from the muscle strip was collected alternatively with 340 nm and 380 nm light, and then was collected by a photomultiplier through a 510-nm filter. The signals were fed into a microcomputer for calculation of the ratio of the fluorescence intensities at 340-nm excitation (F_{340}) to that at 380 nm (F_{380}). Autofluorescence signals were determined by quenching fura-2 signals with 10 mM $MnCl_2$ and these were subtracted from the corresponding values of F_{340} and F_{380} obtained under conditions of fura-2 loading. Each experiment commenced with perfusion with 60 mM KCl-PSS for 3 min. At the end of experiment, the strips were treated with 50 μM ionomycin. Changes in the ratio were expressed as percentages of the differences between basal values and theose obtained with 60 mM KCl-PSS.

Solutions

The PSS used in the experiment contained (in mM) NaCl 130, KCl 4.7, NaH_2PO_4 1.18, $MgSO_4$ 1.17, $CaCl_2$ 1.6, $NaHCO_3$ 14.9, and dextrose 5.5, gassed with a mixture of 95% O_2-5% CO_2 and adjusted the pH to 7.5. The composition of the cytosolic buffer was as follows (in mM): NaCl 20, KCl 100, NaH_2PO_4 0.96, $NaHCO_3$ 25, and EGTA 4 with 2% bovine serum albumin and 0.3 μM $CaCl_2$ was added (pH 7.2 at 37°C). For a fura-2 loading buffer, 150 μM ethylene glycol-bis (b-aminoethylether) N,N,N_1,N_1-tetraacetic acid (EGTA), 3 μM GTP, 2 μM fura-2, 10 μM antimycin A, 5.2 mM ATP, 5.0 mM creatine phosphate and 10 unit/ml creatine phosphokinase were added into the Ca^{2+}-free cytosolic buffer (pH 7.2 at 37°C).

Drugs

D-myo-inositol 1,4,5-trisphosphate (IP_3, potassium

salt), acetylcholine, guanosine 5'-[γ-thio] triphosphate ($GTP_\gamma S$), gnanosine 5'-[β-thio] diphosphate ($GDP_\beta S$), glibenclamide, apamin, heparin (sodium salt, MW 4,000~6,000) and β-escin were purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.). Charybdotoxin was obtained from Peptide International, Inc. (Louisville, KY, U.S.A.). Fura-2 and fura-2 acetoxylmethyl ester (fura-2/AM) were obtained from Molecular Probes, Inc. (Eugene, OR, U.S.A.). Cromakalim was kindly donated from SmithKline Beecham Pharmaceuticals (Surrey, England) and levcromakalim was from Korean Research Institute of Chemical Technology (Daejeon, Korea). Cromakalim and levcromakalim were dissolved in ethanol-polyethyleneglycol (50: 50% vol/vol) to make a stock solution of 1 mM. Glibenclamide was sonicated in 0.1 N NaOH containing 5% dextrose to make 10 mM stock solution and the pH was adjusted to 7.4 by adding 0.1 N HCl. Apamin and charybdotoxin were dissolved in 0.1% bovine serum albumin to make stock solution of 0.1 mM.

Statistical analysis

Data are expressed as means±S.E. of the mean. Student's *t*-tests were used for comparisons of the paired and unpaired data. The probability value of less than 0.05 was considered statistically significant.

RESULTS

Effect of cromakalim and levcromakalim on acetylcholine effect in intact strips

Figure 1A shows a typical tracings of concentration-dependent increase in $[Ca^{2+}]_i$ by application of acetylcholine in intact strips of porcine coronary artery. Upper panel shows increase in fluorescence at 380 nm and decrease in fluorescence at 340 nm by adding acetylcholine. Lower panel shows the fluorescence ratio (F_{340}/F_{380}). The summarized data obtained from 4 preparations are presented in figure 1B. Acetylcholine (0.1, 1 and 10 μM) produced an increase in $[Ca^{2+}]_i$ in a dose-dependent manner. Cromakalim and levcromakalim (0.1 and 1 μM, each) inhibited the increased $[Ca^{2+}]_i$ induced by 1 μM acetylcholine in the normal PSS (Fig. 2). After washout of cromakalim and levcromakalim, the acetylcholine effect on $[Ca^{2+}]_i$ was fully recovered (data not shown).

When the extracellular Ca^{2+} was removed by perfusion with Ca^{2+}-free PSS containing 1 mM EGTA,

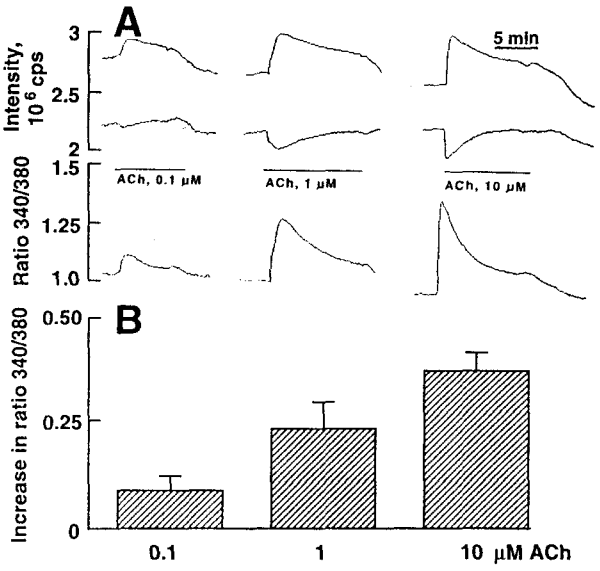

Fig. 1. Dose dedendent increase in $[Ca^{2+}]_i$ by acetylcholine (ACh) in intact strips of porcine coronary artery. A. Tracings are showing dose-dependent increase in $[Ca^{2+}]_i$ by acetylcholine. Upper panel shows increase in fluorescence at 380 nm and decrease in fluorescence at 340 nm by adding of acetylcholine. Lower panel shows the fluorescence ratio (F_{340}/F_{380}). B. the summarized data obtained from 4 preparations and each bar represents mean±S.E.M.

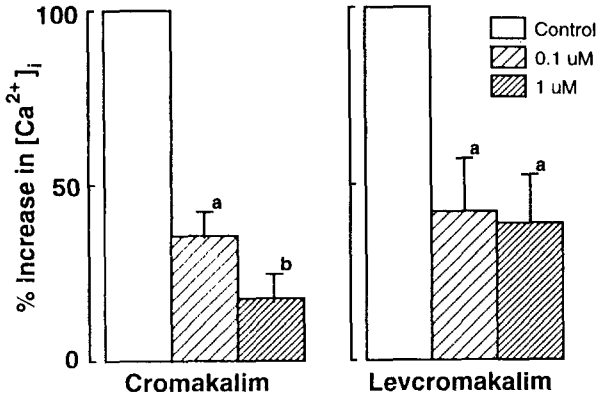

Fig. 2. Inhibitory effect of cromakalim and levcromakalim on the acetylcholine (1 μM)-induced increase in $[Ca^{2+}]i$ in the intact strips of porcine coronary artery. Each bar represents mean±S.E.M. from 4 experiments. a, $p<0.05$; b; $p<0.01$ vs. corresponding control.

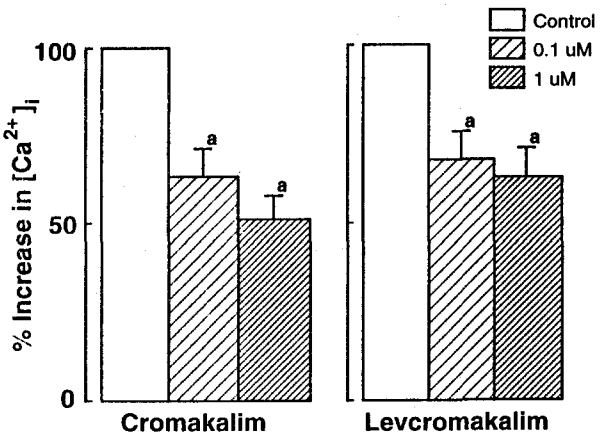

Fig. 3. Effect of removal of extracellular Ca^{2+} (Ca^{2+}-free) on the inhibition by cromakalim (CRK) and levcromakalim (L-CRK) to acetylcholine-induced increase in $[Ca^{2+}]_i$ in the intact strips of porcine coronary artery. Each bar represents mean±S.E.M. from 3~5 experiments. a, $p<0.05$ vs. control.

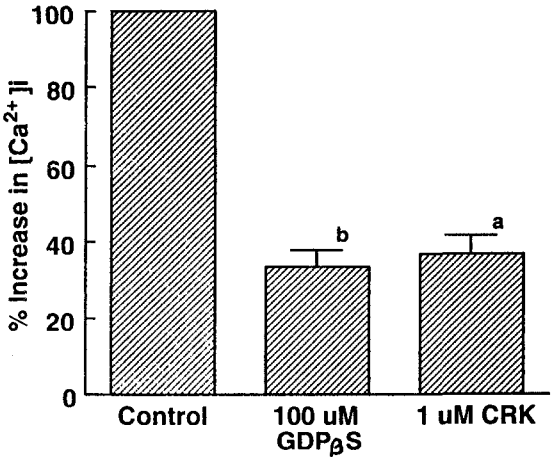

Fig. 4. Effect of cromakalim (1 μM) and $GDP_{\beta}S$ on $GTP_{\gamma}S$-induced increase in $[Ca^{2+}]_i$ in skinned strips of porcine coronary artery. a, $p<0.05$; b, $p<0.01$ vs. control value. Each bar represents mean±S.E.M. from 3~5 experiments.

the acetylcholine-induced increase in $[Ca^{2+}]_i$ was reduced by 34.3±6.1% (Fig. 3). In the Ca^{2+}-free PSS, cromakalim (1 μM) and levcromakalim (1 μM) reduced the acetylcholine-induced increase in $[Ca^{2+}]_i$ by 54.2±5.1% and 66.4±5.7%, respectively. Effect of both K^+ channel openers manifested not only in the normal PSS but also in the Ca^{2+}-free PSS (Fig. 3).

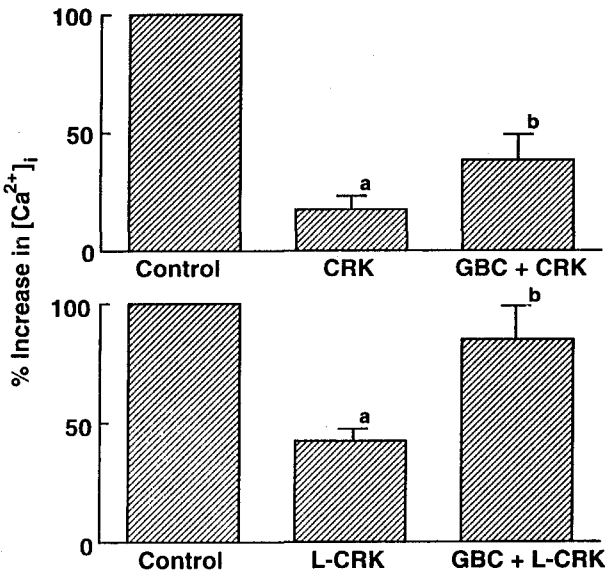

Fig. 5. Effect of glibenclamide (GBC, 10 μM), cromakalim (CRK, 1 μM)- and levcromakalim (L-CRK, 1 μM)-induced inhibition of Ca^{2+} release by IP_3 in skinned strips of porcine coronary artery. a, $p<0.05$ vs. control; b, $p<0.05$ vs. CRK and L-CRK, respectively. Each bar represents mean±S.E.M. from 4~6 experiments.

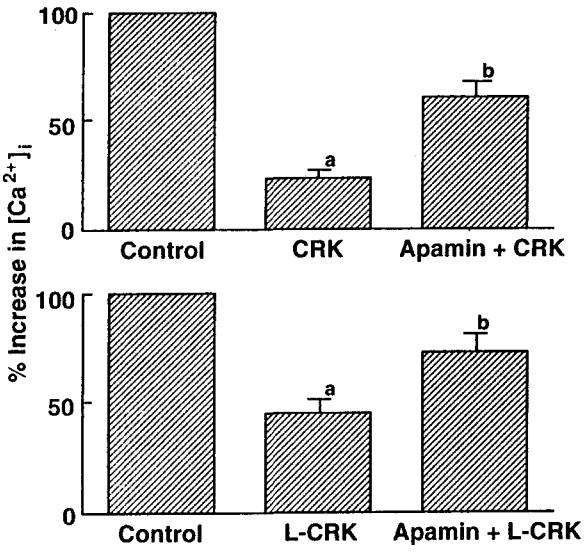

Fig. 6. Apamin (1 μM) inhibited the cromakalim (1 μM, CRK)- and levcromakalim (1 μM, L-CRK)-induced inhibition of Ca^{2+} release by IP_3 in skinned strips of coronary artery. a, $p<0.05$ vs. control; b, $p<0.05$ vs. CRK and L-CRK, respectively. Each bar represents mean± S.E.M. from 4~6 experiments.

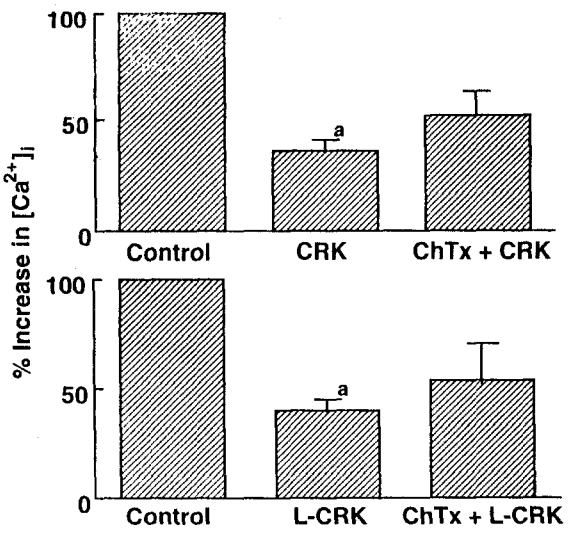

Fig. 7. Effect of charybdotoxin (ChTx, 1 μM) on the cromakalim (CRK)- and levcromakalim (L-CRK)-induced inhibition of IP_3-induced increase in $[Ca^{2+}]_i$ in skinned strips of porcine coronary artery. *, $p<0.05$ vs. control. Each bar represents mean±S.E.M. from 3~5 experiments.

Effect of cromakalim and levcromakalim on the IP_3 effect in the skinned strips

As a pilot study, we observed the consistent increase in Ca^{2+} signals upon repeated application of 1 μM IP_3 every 60 min in skinned strips of porcine coronary artery. The IP_3 effect on Ca^{2+} signals was confirmed with the inhibitory effect of heparin (IP_3 receptor antagonist, low molecular weight, M.W. 4,000~6,000) at concentration of 1 mg/ml (data not shown).

$GTP_\gamma S$ (100 μM), a non-hydrolysable GTP analogue, caused an increase in $[Ca^{2+}]_i$ in the skinned porcine coronary arterial strips which was also strongly inhibited cromakalim as well as by $GTP_\beta S$ (Fig. 4).

The IP_3-induced increase in $[Ca^{2+}]_i$ was inhibited by cromakalim (1 μM) by 78% and by levcromakalim (1 μM) by 59%, respectively (Figs. 5, 6).

Effect of K^+ channel blockers on the cromakalim action

As shown in figures 5 and 6, prior application of either glibenclamide (10 μM for 10 min) or apamin (1 μM for 10 min) blocked the inhibitory actions of cromakalim and levcromakalim on the IP_3-induced increase in $[Ca^{2+}]_i$. However, charybdotoxin (a blocker of large conductance Ca^{2+}-activated K^+ channels, 1 μM for 10 min) did not exert any blocking effect on the cromakalim action (Fig. 7).

DISCUSSION

K^+ channel openers including cromakalim hyperpolarize the plasma membrane of vascular smooth muscles through opening of the K^+ channels and to close the Ca^{2+} channels (Bray et al., 1991; Cook, 1988; Cook et al., 1988; Yamagishi et al., 1992), subsequently leading to relaxation of the arterial smooth muscles (Coldwell and Howlett, 1987; Hamilton et al., 1986; Southerton et al., 1988; Weir and Weston, 1986). However, there appears to be a distinct quantitative difference between the concentrations of cromakalim eliciting relaxant effects and those inducing the apparent K^+ effluxes (Cook et al., 1988; Nakao et al., 1988; Quast and Baumlin, 1988; Shetty and Weiss, 1987). This divergence indicates that the mechanism by which these agents affect tension may not be identical to that for potenitation of K^+ efflux (Shetty and Weiss, 1987). Furthermore, the precontraction to noradrenaline was demonstrated to be relaxed by low concentrations of cromakalim (<1 μM) without accompanying any change in membrane potential (Bray et al., 1991).

In the present study with both intact and skinned strips, cromakalim and levcromakalim showed a significant inhibition of the increased $[Ca^{2+}]_i$ evoked by acetylcholine and IP_3. The fact that the tonic increase in $[Ca^{2+}]_i$ by acetylcholine was eliminated by removal of the extracellular Ca^{2+} ($[Ca^{2+}]_o$) indicates the importance of the influx of $[Ca^{2+}]_o$ in the contractile action of acetylcholine as reported with histamine (Mori et al., 1990) and U46619 (Yamagishi et al., 1992).

On the other hand, the phasic increase in $[Ca^{2+}]_i$ induced by acetylcholine in the absence of $[Ca^{2+}]_o$ is explained as a result of IP_3-induced Ca^{2+} release from the intracellular storage sites (Berridge and Irvine, 1984; Somlyo et al., 1988; Suematsu et al., 1984). As the second messenger, IP_3 (a metabolite of phosphatidylinositol 4,5-bisphosphate) is importantly involved in transmitter-induced Ca^{2+} release from the sarcoplasmic reticulum of the smooth muscle independent of change in membrane potential (Berridge and Irvine, 1984; Somlyo et al., 1988). However, in the

present study with skinned strips, IP_3 and $GTP_\gamma S$ caused a transient increase in $[Ca^{2+}]_i$ in the absence of extracellular Ca^{2+}. In agreement with our results, Quast and Baumlin (1991) have demonstated that cromakalim inhibits contractions induced by noradrenaline in the rat isolated mesenteric arteries.

In the present study, we have established that cromakalim acts on the IP_3-binding site of the sarcoplasmic reticulum in addition to the K^+ channels of the plasma membrane. Although it is not identified whether the glibenclamide-sensitive receptors are present in the sarcoplasmic membrane, the facts that cromakalim exerted inhibitory effect on the IP_3-induced increases in intracellular Ca^{2+} signals and the inhibition of IP_3 effect by cromakalim or levcromakalim was sensitively antagonized by glibenclamide indicate the presence of the glibenclamide-sensitive K^+ channels in the sarcoplasmic reticulum membrane.

A question arises as to how cromakalim inhibits the IP_3-induced increase in $[Ca^{2+}]_i$. Fink and Stephenson. (1987) have reported the presence of the K^+ channels in the sarcoplasmic membrane of skeletal muscle and demonstrated that those channels modify the amount of releasable Ca^{2+}. Otherwise, Yamagishi et al. (1992) have demonstrated that the hyperpolarization of the plasma membrane by the K^+ channel openers inhibits Ca^{2+} release from the intracellular storage site in accordance with the inhibition of the phosphatidylinositol turnover and a reduction of IP_3 synthesis. Recently, Rhim and Hong (1994) reported that cromakalim potently inhibited the IP_3-induced contraction without inhibition of the IP_3 synthesis within the concentration ranges for relaxation in the permeabilized cells from canine coronary artery. They further postulated that inhibition by cromakalim of the phenylephrine-induced contraction of the intact strips and of the IP_3-induced contraction of the permeabilized cells was ascribed to its action on the K^+ channels in sarcoplasmic reticulum membrane. In their experiment (Rhim and Hong, 1994), the involvement of hyperpolarization of the plasma membrane by cromakalim was rejected because the experiment with permeabilized cells was conducted in the buffer solution containing 100 mM KCl and 180 nM Ca^{2+} (Burgess et al., 1983). The nature and physiologic functions of the sarcoplasmic reticulum K^+ channels of the smooth muscle, however, remain to be examined electrophysiologically.

Does cromakalim require the K^+ conductance in the Ca^{2+} release from sarcoplasmic reticulum? A role for K^+ was demonstrated to act to compensate the charge flow to allow Ca^{2+} release from the intracellular Ca^{2+} stores in liver cells (Muallem et al., 1985) and brain microsomes (Shah and Pant, 1988). The conductive nature is allowing efflux of Ca^{2+} into the cytosol in exchange for K^+ uptake. In line with these speculations, it is likely that cromakalim and levcromakalim may increase the permeability of sarcoplasmic reticulum of the smooth muscle cells.

On the other hand, cromakalim-induced inhibition of IP_3-induced increase in $[Ca^{2+}]_i$ in skinned strips was sensitively antagonized by apamin (bee venom toxin) (Habermann, 1984) but not by charybdotoxin (venom of lsraeli scorpion) (Miller et al., 1985). These results suggest the presence of the apamin-sensitive K^+ channels in the sarcoplasmic membrane but not of the large conductance Ca^{2+} activated K^+ channels. These results are consistent with our previous observation with canine coroanry arterial cells in that cromakalim inhibited the IP_3-induced contraction in the permeabilized arterial smooth cells and the inhibition by cromakalim of the IP_3-induced contraction was sensitively antagonized by apamin and less sensitively by glibenclamide (Rhim and Hong, 1994). It is not clear two subtypes of the K^+ channels exist in the membrane.

Guanine nucleotide-binding proteins (G proteins) have been shown to be involved in the functional coupling of receptors to phospholipase C-mediated increases in IP_3 and Ca^{2+} release from the endoplasmic reticulum of the non-muscle cells (Cockcroft and Gomperts, 1985), and guanine nucleotide-stimulated hydrolysis of phosphatidylinositol 4,5-bisphosphate has been demonstrated in the smooth muscle cells (Sasaguri et al., 1985). In addition, $GTP_\gamma S$ (a non-hydrolysable analogue of GTP) releases Ca^{2+} from the sarcoplasmic reticulum of the skinned skeletal muscle (DiVirgilio et al., 1986). In the present study, we identified that cromakalim as well as $GDP_\beta S$ exerts the inhibitory effect on the GTP_γ S-induced increased release of $[Ca^{2+}]_i$. These results provided the evidence to support the hypothesis that cromakalim, the K^+ channel opener, has the intracellular action site.

Taken together, it is suggested that, in the sarcoplasmic reticulum membrane, the small conductance Ca^{2+}-activated K^+ channels and ATP-sensitive K^+ channels are present and they are activated by cromakalim and levcromakalim, the benzopyran derivative K^+ channel openers. The K^+ channel openers have multi-

ple sites of action in causing the vasorelaxation: the plasma membrane and the intracellular site sensitive to IP_3.

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REFERENCES

Berridge MJ and Irvine RF. Inositol trisphosphate, a novel second messenger in cellular signal transduction. *Nature* 312: 315−321, 1984

Bray KM, Weston AH, Duty S, Newgreen DT, Longmore J, Edwards G and Brown TJ. Differences between the effects of cromakalim and nifedipine on agonist-induced responses in rabbit aorta. *Br J Pharmacol* 102: 337−344, 1991

Burgess GM, McKinney JS, Fabiato A, Leslie BA and Puthey JWJr. Calcium pools in saponin-permeabilized guinea-pig hepatocytes. *J Biol Chem* 258: 15336−15345, 1983

Chopra LC, Twort CHC and Ward JPT. Direct action of BRL 38227 and glibenclamide on intracellular calcium stores in cultured airway smooth muscle of reabbit. *Br J Pharmacol* 105: 259−260, 1992

Cockcroft S and Gomperts BD. Role of guanine nucleotide binding protein in the activation of polyphosphoinositide phosphodiesterase. *Nature* 314: 534−536, 1985

Coldwell MC and Howlett DR. Specificity of action of the novel antihypertensive agent, BRL34915, as a potassium chanel activator: comparison with nicorandil. *Biochem Pharmacol* 36: 3663−3669, 1987

Cook NS, Quast U, Hof RP, Baumlin Y and Pally C. Similarities in the mechanism of action of two new vasodilator drugs: pinacidil and BRL 34915. *J Cardiovasc Pharmacol* 11: 90−99, 1988

Cook NS. The pharmacology of potassium channels and their therapeutic potential. *Trends Pharmacol Sci* 9: 21−28, 1988

DiVirgilio F, Salviati G, Pozzan T and Volpe P. Is a guanine nucleotide-binding protein involved in excitation-contrction coupling in skeletal muscle? *EMBO Journal* 5: 259-262, 1986

Fink RH and Stephenson DG. Ca^{2+}-movements in muscle modulated by the state of K^+-channels in the sarcoplasmic reticulum membranes. *Pflügers Archives* 409: 374−380, 1987

Habermann E. Apamin. *Pharmac Ther* 25: 255−270, 1984

Hamilton TC, Weir SW and Weston AH. Comparison of the effects of BRL 34915 and verapamil on electrical and mechanical activity in rat portal vein. *Br J Pharmacol* 88: 103−111, 1986

Kobayashi S, Somlyo AV and Somlyo AP. Heparin inhibits the inositol 1,4,5-trisphosphate-dependent, but not the independent, calcium release induced by guanine nucleotide in vascular smooth muscle. *Biochem Biophys Res Commun* 153: 625−631, 1988

Kobayashi S, Kitazawa T, Somlyo AV and Somlyo AP. Cytosolic heparin inhibits muscarinic and α-adrenergic Ca^{2+} release in smooth muscle. *J Biol Chem* 264: 17997−8004, 1989

Miller C, Moczydlowski E, Lotorre R and Phillips M. Charybdotoxin, a protein inhibitor of single Ca^{2+}-activated K^+ channels from mammalian skeletal muscle. *Nature* 313: 316−318, 1985

Mori T, Yanagisawa T and Taira N. Histamine increases vascular tone and intracellular calcium level using both intracellular and extracellular calcium in porcine coronary arteries. *Jap J Pharmacol* 52: 263−271, 1990

Muallem S, Schoeffield M, Pandol S and Sachs G. Inositol 1,4,5-trisphosphate modification of ion transport in rough endoplasmic reticulum. *Proc Natl Acad Sci USA* 82: 4433−4437, 1985

Nakao K, Okabe K, Kitamura K, Kuriyama H and Weston AH. Characteristics of cromakalim-induced relaxations in the smooth muscle cells of guinea-pig mesenteric artery and vein. *Br J Pharmacol* 95: 795−804, 1988

Quast U and Baumlin Y. Cromakalim inhibits contractions of the rat isolated mesenteric bed induced by moradrenaline but not caffeine in Ca^{2+}-free medium: evidence for interference with receptor-mediated Ca^{2+} mobilization. *Eur J Pharmacol* 200: 239−249, 1991

Quast U and Baumlin Y. Comparison of the effuxes of $^{42}K^+$ and ^{86}Rb elicited by cromakalim (BRL 34915) in tonic and phasic vascular tone. *Naunyn-Schmiedeb Arch Pharmacol* 338: 319−326, 1988

Quast U. Cromakalim inhibits phasic contractions of noradrenaline in the rat isolated mesenteric bed even in Ca^{2+}-free medium. *Br J Pharmacol* 96: 144P, 1989

Rhim BY and Hong KW. Relaxation by cromakalim and pinacidil of the isolated smooth muscle cells from canine coronary artery - Multiple sites of action. *Arch int Pharmacodyn* 328: 67−81, 1994

Sasaguri T, Hirata H, ltoh T, Koga T and Kuriyama H. Guanine nucleotide binding protein involved in muscarinic responses in the pig coronary artery is

sensitive to islet-activating protein. *Biochem J* 239: 567−574, 1985

Shah J and Pant C. Potassium-channel blockers inhibit inositol trisphosphate-induced Ca^{2+} release in the microsomal fractions isolated from the rat brain. *Biochem J* 250: 617−620, 1988

Shetty SS and Weiss GB. Dissociation of actions of BRL 34915 in the rat portal vein. *Eur J Pharmacol* 141: 485−488, 1987

Somlyo AV, Bond M, Somlyo AP and Scarpa A. Inositol triphosphate-induced calcuim release and contraction in vascular smooth muscle. *Proc Natl Acad Sci USA* 82: 5231-5235, 1985

Somlyo AP, Walker JW, Goldman YE, Trentham DR and Kobayashi S. Inositol trisphosphate, calcium and muscle contraction. *Philos Trannsact Royal Soc B*320: 399−414, 1988

Southerton JS, Weston AH, Bray KM, Newgreen DT and Taylor SG. The potassium channel opening action of pinacidil: studies using biochemical, ion flux and microelectrode technique. *Naunyn-Schmiedeb Arch Pharmacol* 338: 310−318, 1988

Suematsu E, Hirata M, Hashimoto T and Kuriyama H. Inositol 1,4,5-triphosphate release Ca^{2+} from intracellular store sites in skinned single cells of porcine coronary artery. *Biochem Biophys Res Commun* 120: 481−485, 1984

Weir SW and Weston AH. The effects of BRL34915 and nicorandil on electrical and mechanical activity and on ^{88}Rb efflux in rat blood vessels. *Br J Pharmacol* 88: 121−128, 1986

Yamagishi T, Yanagisawa T and Taira N. K^+ channel openers, cromakalim and Ki4032, inhibit agonist-induced Ca^{2+} release in canine coronary artery. *Naunyn-Schmiedeb Arch Pharmacol* 346: 691−700, 1992