

## Fatty Acids Directly Increase the Activity of $\text{Ca}^{2+}$ -activated $\text{K}^+$ Channels in Rabbit Coronary Smooth Muscle Cells

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*The large conductance  $\text{Ca}^{2+}$  activated  $\text{K}^+$  channel (BK channel) has been considered to play an important role in the excitability and contractility of vascular smooth muscle cells. Activation of the BK channel causes the hyperpolarization and relaxation of vascular smooth muscle cells. It has been reported that fatty acids can affect the BK channel activity and its concentration is increased significantly during myocardial ischemia. These reports suggest that fatty acids may contribute to the ischemic coronary vasodilation by increasing the BK channel activity. However, the underlying mechanism of fatty acid-induced activation of the BK channel is still uncertain. In the present study, we measured the effect of fatty acids on the BK channel activity in rabbit coronary smooth muscle cells by using patch clamp method and also examined its underlying mechanism. Arachidonic acid (AA) dissolved in DMSO activated the BK channel in a dose-dependent manner (from 0.5 to 10  $\mu\text{M}$ ), and DMSO (0.1%) alone had no effect on the activity of the BK channel. Arachidonic acid activated BK channels in both cell-attached and inside-out patches, but the onset and recovery of this effect were slower in the cell-attached patch configuration. The BK channel activity was also increased by other fatty acids, including myristic acid, linoleic acid, palmitoleic acid and palmitic acid. Long chain fatty acids were more effective than short chain fatty acids (myristic acid), and there was no statistical difference between the effect of saturated (palmitic acid) and unsaturated fatty acids (palmitoleic acid) on the BK channel activity. The concentration of  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$  in the bathing solution had no appreciable effects on the AA-induced increase of BK channel activity. From the above results, it may be concluded that fatty acids directly increase the BK channel activity and may contribute to the ischemic coronary vasodilatation in rabbit coronary smooth muscle cells.*

**Key Words:** Rabbit coronary smooth muscle cells, ischemic metabolite, arachidonic acid,  $\text{Ca}^{2+}$ -activated  $\text{K}^+$  channels

Arterial tone, one of the important factors in blood flow regulation, is mainly determined by the cytosolic  $\text{Ca}^{2+}$  concentration of

vascular smooth muscle cells (Sato *et al.* 1988; Nelson *et al.* 1990). The concentration of intracellular  $\text{Ca}^{2+}$  is closely related to the magnitude of membrane potential of vascular smooth muscle cells (Mekata, 1986). When the membrane potentials of smooth muscle cells are hyperpolarized,  $\text{Ca}^{2+}$  influx via a  $\text{Ca}^{2+}$  channel and  $\text{Na}^+/\text{Ca}^{2+}$  exchange decrease. These effects cause a decrease of  $\text{Ca}^{2+}$ , and a subsequent relaxation of smooth muscles (Worley *et al.* 1986; van Breeman *et al.* 1987; Nelson *et al.* 1990).

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The large conductance Ca<sup>2+</sup>-activated K<sup>+</sup> channel (BK channel) has been identified in a number of excitable cells (Benham *et al.* 1986; Blatz & Magleby, 1987; Carl *et al.* 1989). In vascular smooth muscle cells, the BK channel has been considered to play an important role in its excitability and tone regulation (Cook, 1988; Hu *et al.* 1989; Carl *et al.* 1990b; Brayden & Nelson, 1992; Asano *et al.* 1993). A small change of the BK channel activity can affect the membrane potential greatly, because of the large number and large conductance of BK channels in vascular smooth muscle cells (Wiegel *et al.* 1979; Benham *et al.* 1986; Yamaguchi *et al.* 1988). Thus an agonist which changes the activity of the BK channel can affect the membrane potential and contractility of smooth muscle cells (Brayden & Nelson, 1992). The activity of the BK channel is thought to be regulated directly by the binding of Ca<sup>2+</sup> to the channel and also by the membrane potential (Blatz & Magleby, 1987). The BK channel activity is also subjected to many other factors such as neurotransmitters, nucleotides, and lipids (Cole *et al.* 1989; McManus & Magleby, 1991; Toro & Stefani, 1991).

Recently, it has been reported that a variety of fatty acids regulate the activity of ion channels, such as K<sup>+</sup> channels in cardiac and smooth muscles and Na<sup>+</sup> channels in neuronal cells (Takenaka *et al.* 1988; Kim & Clapham, 1989; Ordway *et al.* 1989; Hwang *et al.* 1990). The concentration of fatty acids increases significantly during myocardial ischemia (Chien *et al.* 1984), and it causes the hyperpolarization of the membrane potential of coronary smooth muscle cells (parkington *et al.* 1993). If fatty acids increase the BK channel activity in coronary smooth muscle cells, it can cause the hyperpolarization and subsequent relaxation of coronary smooth muscle cells. This effect may contribute to the compensatory change of coronary blood flow during myocardial ischemia.

In the present study, we measured the effect of fatty acids on the BK channel activity in single smooth muscle cells isolated from a rabbit's coronary artery by using the patch clamp method and also examined its underlying mechanism.

## MATERIALS AND METHODS

### Preparation of coronary smooth muscle cells

Albino rabbits (body wt, 2.0~2.5 kg) of either sex were anesthetised by injecting 60 mg/kg sodium pentobarbital into the ear vein, and they were exsanguinated by cutting a femoral artery. The left anterior descending coronary artery was dissected with neighboring cardiac muscle under a surgical microscope, and then moved to a preparation chamber. The surrounding connective tissue was carefully removed with fine scissors and a pincette under a surgical microscope.

The coronary artery strip was incubated in a plastic bottle containing Tyrode solution without Ca<sup>2+</sup> and Mg<sup>2+</sup>, at 37°C for 5 minutes. Thereafter the strip was transferred to another glass bottle containing collagenase solution (0 Ca<sup>2+</sup>, 0 Mg<sup>2+</sup> Tyrode solution +0.1% collagenase), and incubated for 20~25 minutes. After completion of enzyme digestion, the coronary strip was gently agitated using a blunt tipped glass pipette, until it was dispersed to the single cells. The single smooth muscle cells were resuspended in an albumin (0.1%) contained modified Tyrode solution (1 mM Mg<sup>2+</sup>, Ca<sup>2+</sup> free Tyrode solution) and stored at 4°C for further experiment.

### Patch-clamp experiments

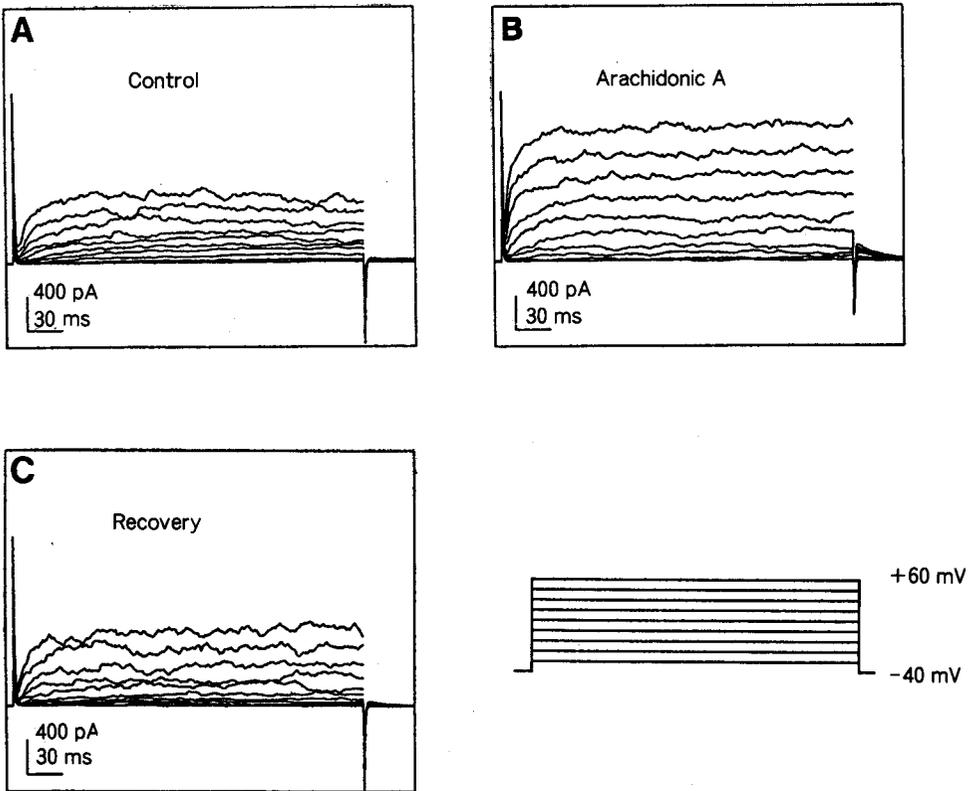
Recordings of single channel currents were made from cell attached and excised patches using standard patch-clamp techniques (Hamil *et al.* 1981). High resistance seals (>10 G ohm) were formed between the electrode and cell membrane by applying mild suction (-10 cm H<sub>2</sub>O). Excised inside-out patches were produced by pulling an electrode from the cell surface rapidly. Patch clamp studies were performed with a commercial amplifier (Axopatch 1-C), and data were stored on computer hard disk, and backed up with a tape backup system (Colorado Inc.). An A/D, D/A converter (Labmaster board) was employed for data acquisition (digitized at 5 kHz after filtering at 1 KHz) and for the generation of ramp voltage signals under the control of an IBM compatible computer using pClamp software. In the case of the ramp

pulse experiment (Carl & Sanders, 1990a), reversal potential and slope conductance were determined for each current response. Activation curves were calculated by averaging current responses to fifteen potential ramps and dividing each data point of the averaged current by the single channel current amplitude at that membrane potential. The leakage current was also subtracted during calculation of the activation curve. Single channel conductance was obtained as the slopes of linear regressions on single-channel current-voltage (*i*-*V*) plots. In the case of constant holding potential, single channel conductance was calculated by dividing a current amplitude, which was measured from long dwell time events, by driving force (membrane potential). The channel activity

(average number of open channels; *N*Po) was calculated by using an all point histogram

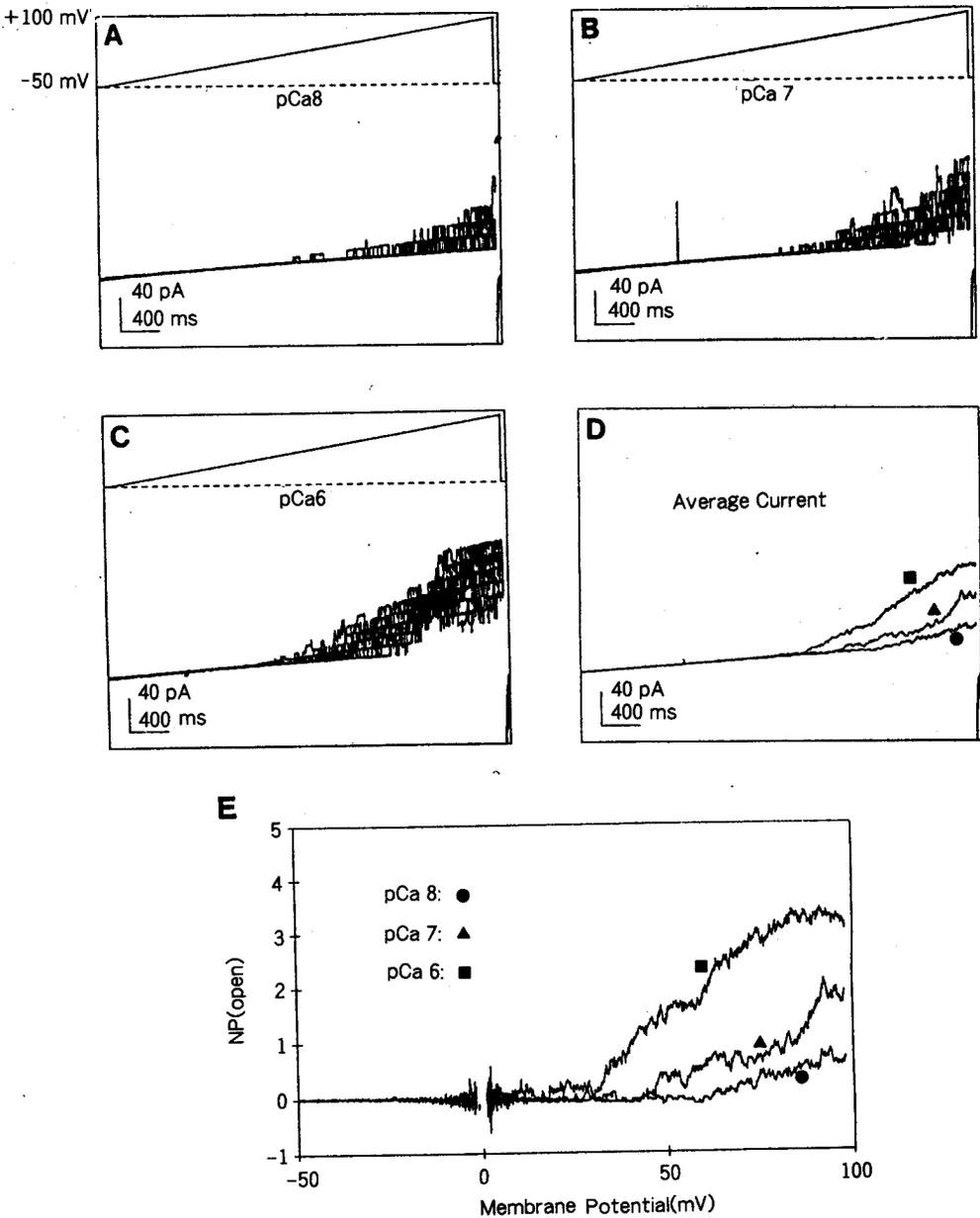
**Solution and Drug**

The standard pipette-filling solution for single channel recording was (in mM) 140 KCl, 10 Glucose, 10 HEPES, 1 mM EGTA (pH 7.4). The bath solution contained (in mM) 140 KCl, 10 Glucose, 10 HEPES, 1 mM MgCl<sub>2</sub>, and Ca<sup>2+</sup> concentration was varied from pCa8 to pCa6 by adding appropriate amounts of CaCl<sub>2</sub> and EGTA using the Fabiato formula (Fabiato & Fabiato, 1979). Fatty acid solutions were prepared by dispersal of concentrated fatty acid stocks in dimethyl sulfoxide into the bath solution. All drugs and chemicals used in this experiment were purchased from Sigma (Sigma Co, St. Louis, U.S.A.).



**Fig. 1.** Effect of arachidonic acid on outward current of coronary smooth muscle cells. Rabbit coronary smooth muscle cell was voltage clamped at  $-40$  mV and outward membrane currents in response to voltage steps spaced  $10$  mV from  $-30$  mV to  $+60$  mV was measured (A). Application of arachidonic acid ( $2$   $\mu$ M) to the perfusion solution caused a significant increase in outward membrane currents (B) and its effect was completely abolished by washout (C).

Activation of  $K^+$  Channel by Fatty Acid



**Fig. 2.** Calcium- and Voltage-dependent activation of  $K^+$  channels with ramp protocols. Patch membrane was linearly depolarized from  $-50$  mV to  $+100$  mV for 4 seconds by using a ramp voltage pulse. This ramp voltage pulse was repeated 15 times and the resulting current responses of an inside-out patch in symmetrical  $140/140$  mM KCl solution were recorded. The data points were clustered at certain current range, which corresponds to the opening of single channel. The amplitude of single channel current was linearly increased with driving force.  $Ca^{2+}$  concentration of perfusion solution was changed from  $10^{-8}$  M (A) to  $10^{-7}$  M (B),  $10^{-6}$  M (C), and current responses were recorded. D: averaged current traces from each 15 current traces (●:  $10^{-8}$  M  $Ca^{2+}$  solution, ▲:  $10^{-7}$  M  $Ca^{2+}$  solution, ■:  $10^{-6}$  M  $Ca^{2+}$  solution). E: open probability (NPo) was calculated from averaged current, after leakage current and driving force were corrected (●:  $10^{-8}$  M  $Ca^{2+}$  solution, ▲:  $10^{-7}$  M  $Ca^{2+}$  solution, ■:  $10^{-6}$  M  $Ca^{2+}$  solution).

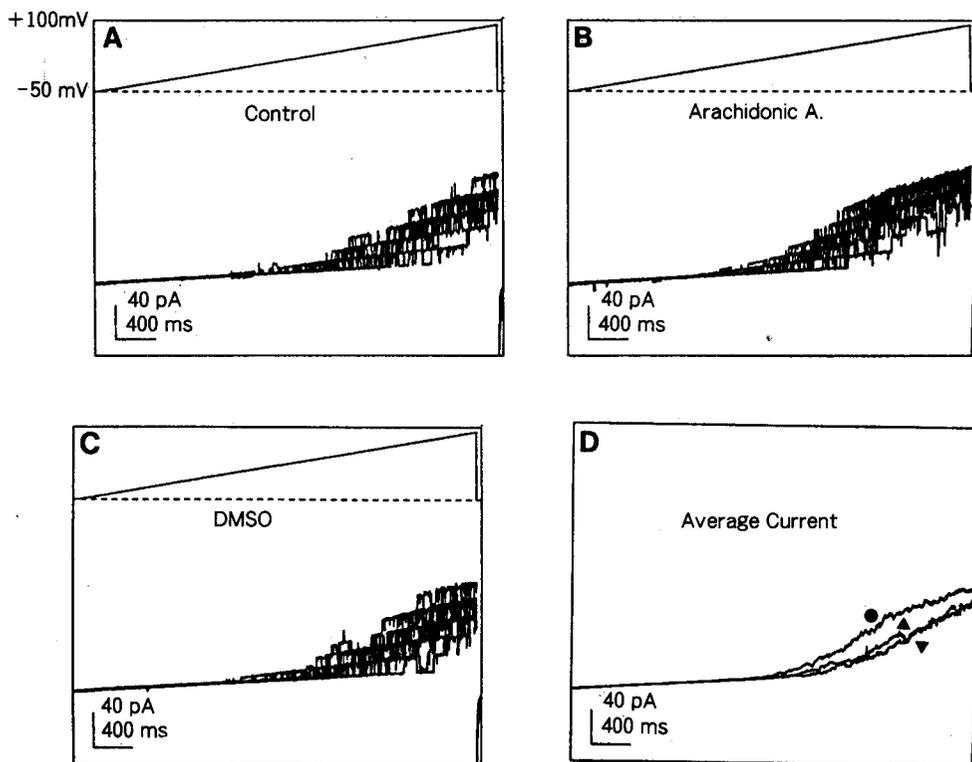
## RESULTS

### Changes of the BK channel activity by free fatty acids

All experiments were performed with spindle shaped relaxed smooth muscle cells. After making a whole cell mode, smooth muscle cells usually remained relaxed throughout the whole experiment. Depolarizing voltage steps from a holding potential of  $-40$  mV to  $+60$  mV elicited an oscillating outward current which did not decrease with time. The amplitude of the outward current was increased by depolarization and completely suppressed by external application of TEA

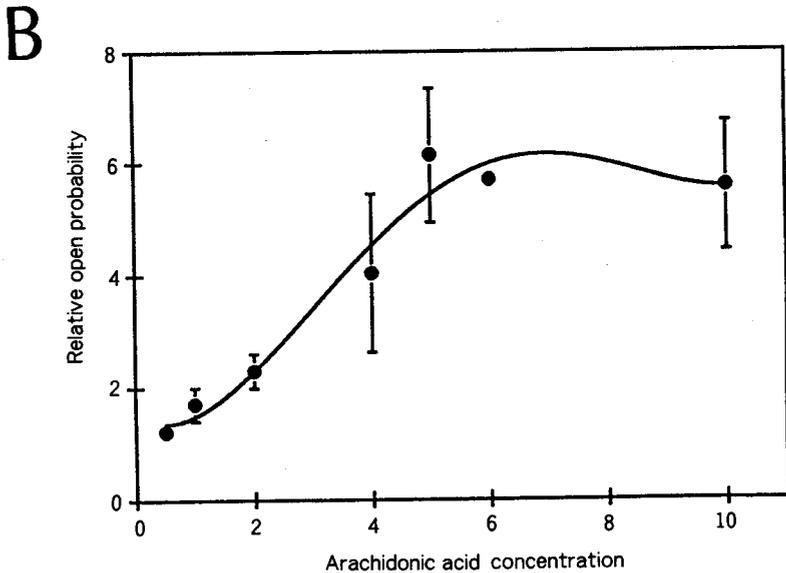
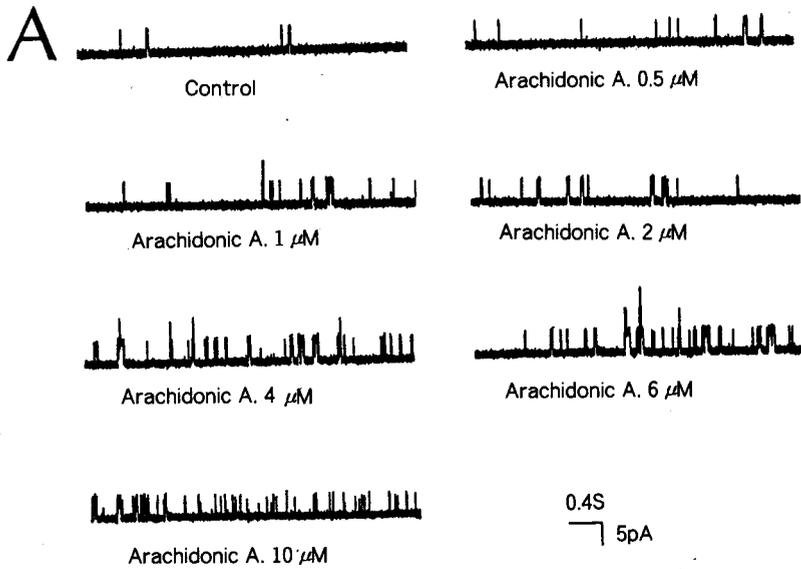
( $5$  mM) (data not shown). Application of  $2$   $\mu$ M arachidonic acid to the bath solution apparently increased the amplitude of the outward current. After removal of AA, the activity of the outward current returned to the control level (Fig. 1).

After making an inside-out patch, a large conductance  $K^+$  channel activity was recorded. Its activity increased in proportion to the depolarization of the membrane potential (from  $-50$  mV to  $+100$  mV) and to the cytosolic  $Ca^{2+}$  concentration. The conductance of channels was studied by measuring the amplitudes of unitary currents at a holding potential from  $-50$  mV to  $+100$  mV. Data were fitted by the least square method and the single channel conductance was  $240 \pm 2.1$  ( $n = 5$ ). There was no change in single channel



**Fig. 3.** Effect of arachidonic acid and DMSO on  $Ca^{2+}$ -activated  $K^+$  channel activity. Application of arachidonic acid ( $2$   $\mu$ M) to the cytoplasmic side increased the  $Ca^{2+}$ -activated  $K^+$  channel activity (B) compared to control (A). After washout of arachidonic acid, DMSO ( $0.1$  %) was applied to the bath solution. In this patch DMSO alone had no effect on  $Ca^{2+}$ -activated  $K^+$  channel activity (C). D: averaged current traces from each 15 current traces ( $\nabla$ : control,  $\blacktriangle$ : DMSO,  $\bullet$ : arachidonic acid  $2$   $\mu$ M).

## Activation of $K^+$ Channel by Fatty Acid

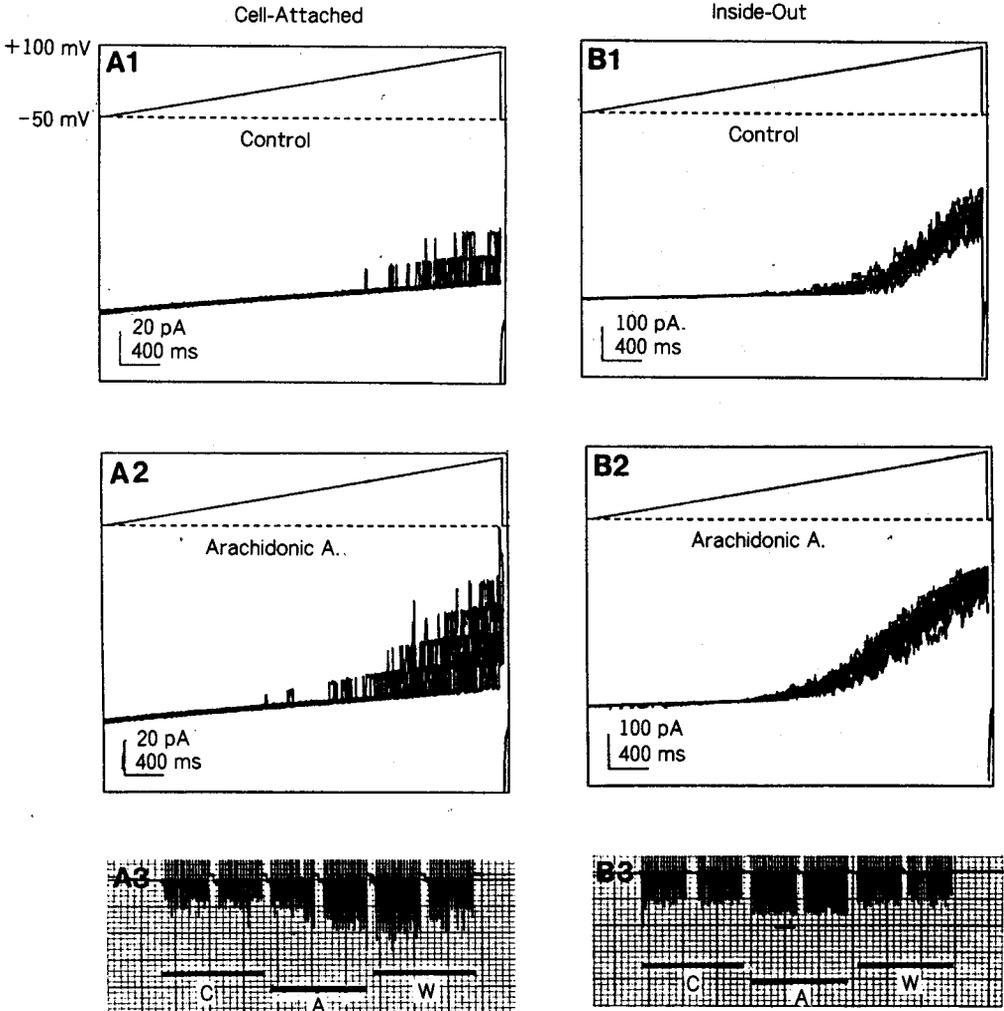


**Fig. 4.** Dose-dependent increase of  $Ca^{2+}$ -activated  $K^+$  channel activity by arachidonic acid in inside-out patch. A) After making an inside-out patch, the membrane potential was held at +20 mV and resulting single channel currents were recorded (control). Concentration of arachidonic acid in the bath solution was increased stepwise from 0.5  $\mu\text{M}$  to 10  $\mu\text{M}$ .  $Ca^{2+}$ -activated  $K^+$  channel activity was increased along with the concentration of arachidonic acid from 0.5 to 6  $\mu\text{M}$ , but the response to 10  $\mu\text{M}$  arachidonic acid was smaller than that to 6  $\mu\text{M}$  arachidonic acid. Concentration of  $Ca^{2+}$  in the bath solution was clamped at  $10^{-7}$  M. The current traces were filtered at 1 K Hz and digitized by 5 K Hz. B) Relation between relative open probability and arachidonic acid concentration. Channel activity of control was taken as an open probability of 1 and effect of arachidonic acid on the channel activity were expressed as the ratio of control. Data were averaged from each patch ( $n=5$ ) and the curve between data points was fitted by computer program.

conductance following depolarization or increase of  $\text{Ca}^{2+}$  concentration. Activation voltage of the BK channel was shifted to the hyperpolarization potential level by increasing the cytoplasmic  $\text{Ca}^{2+}$  concentration (from pCa 8 to pCa 6) (Fig. 2).

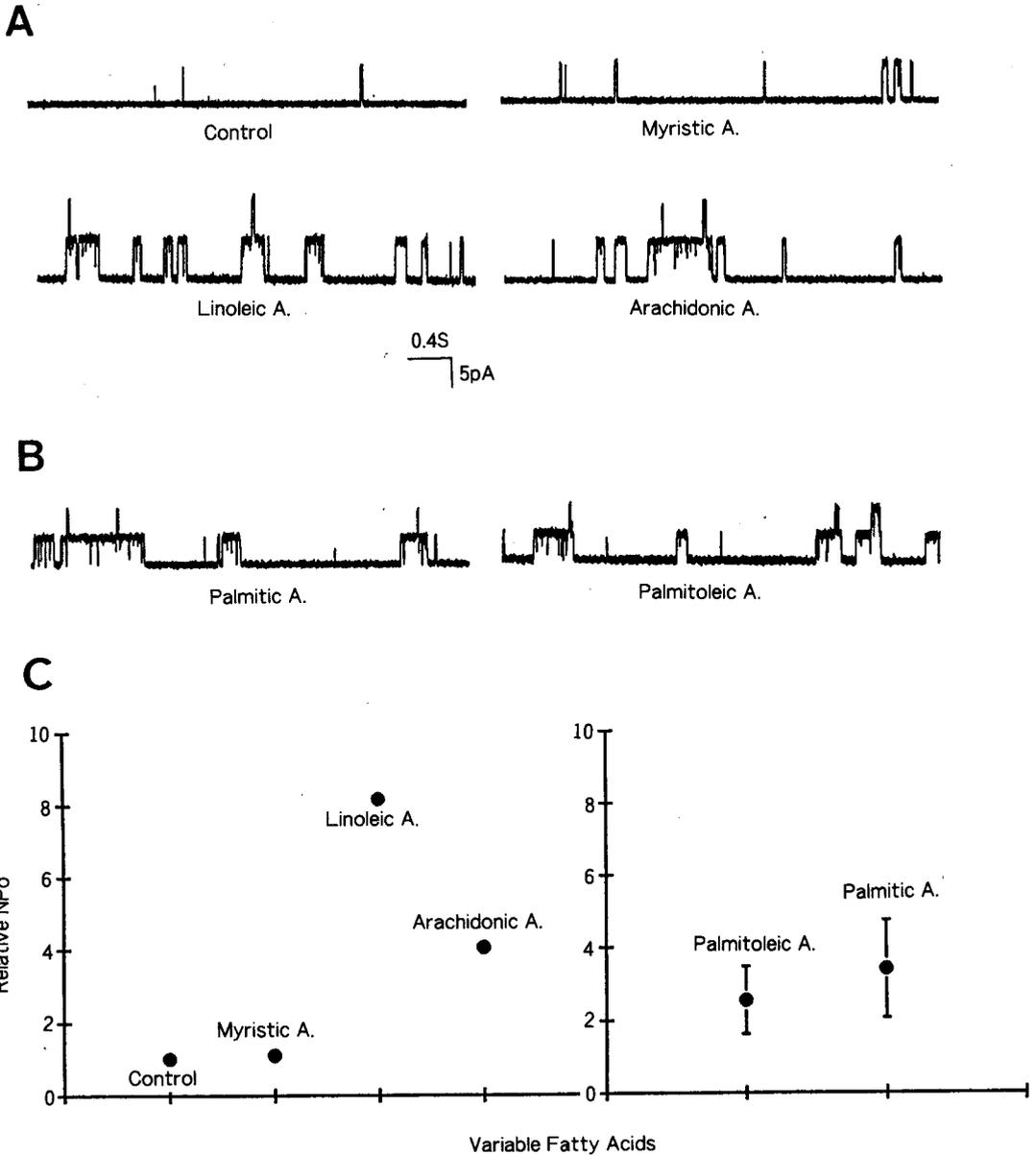
Application of AA ( $2\ \mu\text{M}$ ) to the bath ap-

parently increased the  $\text{K}^+$  channel activity in all potential ranges. The activation voltage of the BK channel was also shifted to the hyperpolarization potential, which is similar to the effect of high cytoplasmic  $\text{Ca}^{2+}$  concentration. Application of DMSO (0.1%) alone, which was used as a solvent for AA, had no



**Fig. 5.** Effect of arachidonic acid on  $\text{Ca}^{2+}$ -activated  $\text{K}^+$  channel activity in cell-attached and inside-out patch configuration. In both cell-attached (A) and inside-out patches (B) application of arachidonic acid ( $2\ \mu\text{M}$ ) to the bath solution ( $10^{-7}\ \text{M}\ \text{Ca}^{2+}$ ) increased the current response to ramp voltage pulse compare to control. Membrane potential was linearly changed from  $-50\ \text{mV}$  to  $+100\ \text{mV}$  for 4 seconds Current response to ramp voltage pulse were also displayed in the form of continuous chart record (A3: cell-attached, B3: inside-out patch) and Bar in the lower part represents the exposed time (C: control, A: application of  $2\ \mu\text{M}$  arachidonic acid, W: wash out of arachidonic acid).

Activation of  $K^+$  Channel by Fatty Acid



**Fig. 6.** Effect of arachidonic acid and other fatty acids on  $Ca^{2+}$ -activated  $K^+$  channels. After making an inside-out patch, the membrane potential was held at  $+30$  mV and resulting single channel current were recorded in  $pCa$  7 bath solution.

A) Fatty acids with variable chain length ( $10 \mu M$  myristic acid,  $10 \mu M$  linoleic acid,  $10 \mu M$  arachidonic acid) were applied to the cytoplasmic side and their effects on  $Ca^{2+}$ -activated  $K^+$  channel were examined.

B) Effect of saturation of fatty acid on  $Ca^{2+}$ -activated  $K^+$  channel was examined by applying a palmitic ( $2 \mu M$ ) and palmitoleic acid ( $2 \mu M$ ) to cytoplasmic side, and the membrane potential was held at  $+20$  mV.

C) Relation between relative open probability and types of fatty acid. Changes in channel activity was expressed as a ratio of control, and there was no statistical difference between the effect of palmitic and palmitoleic acid ( $n=5$ ,  $p=0.31$ ).

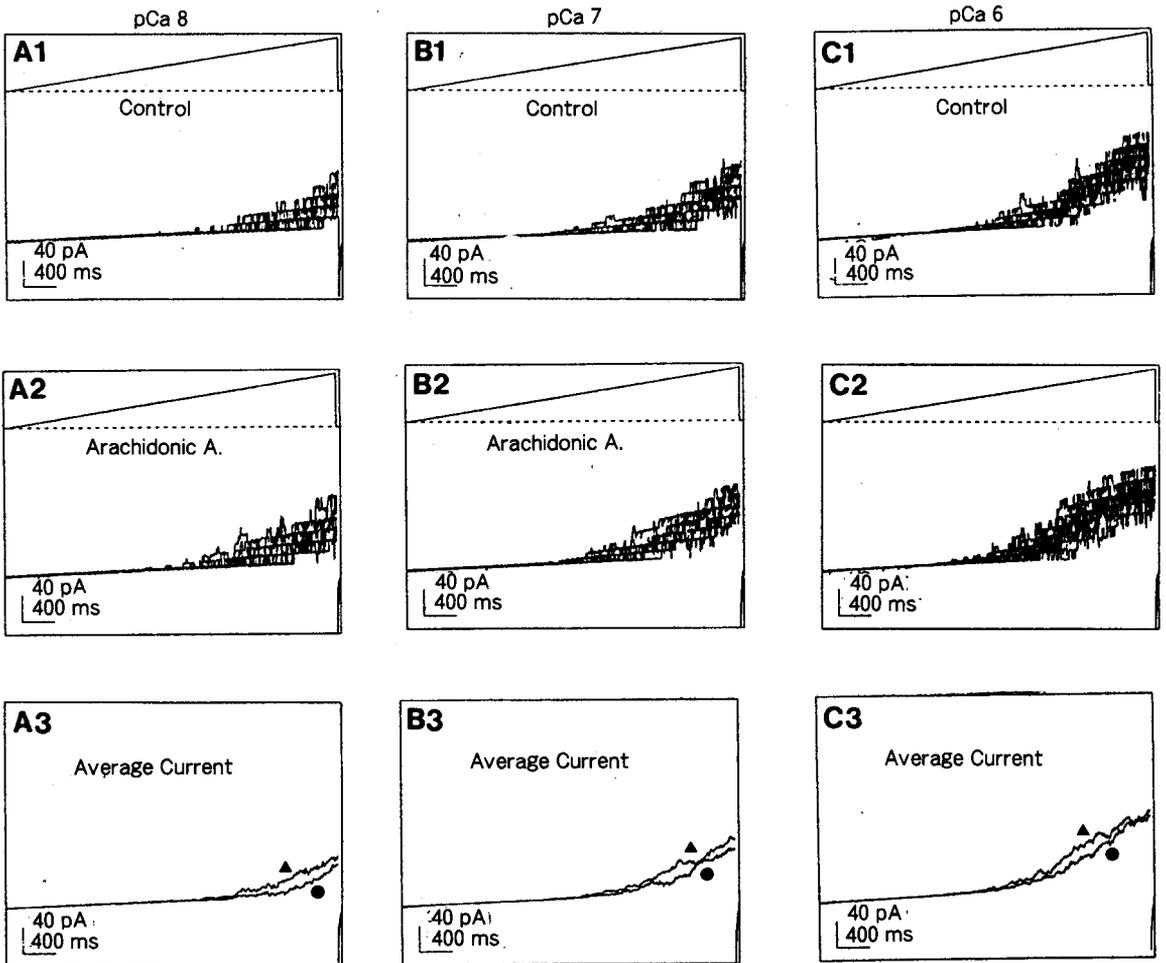
effect on the BK channel activity compared to the control (Fig. 3).

When the concentration of AA was increased stepwise from 0.5 to 10  $\mu\text{M}$ , there was a linear increase in BK channel activity up to 5  $\mu\text{M}$ . An AA-induced increase of BK channel activity showed a saturation phenomena in the range of more than 5  $\mu\text{M}$  of

AA in the bath (Fig. 4).

**Effects of patch configuration and type of fatty acids on BK channel activity**

To determine whether activation of the BK channel by fatty acid requires a signal transduction system or cytosolic enzyme, we examined the effect of AA in both cell-at-



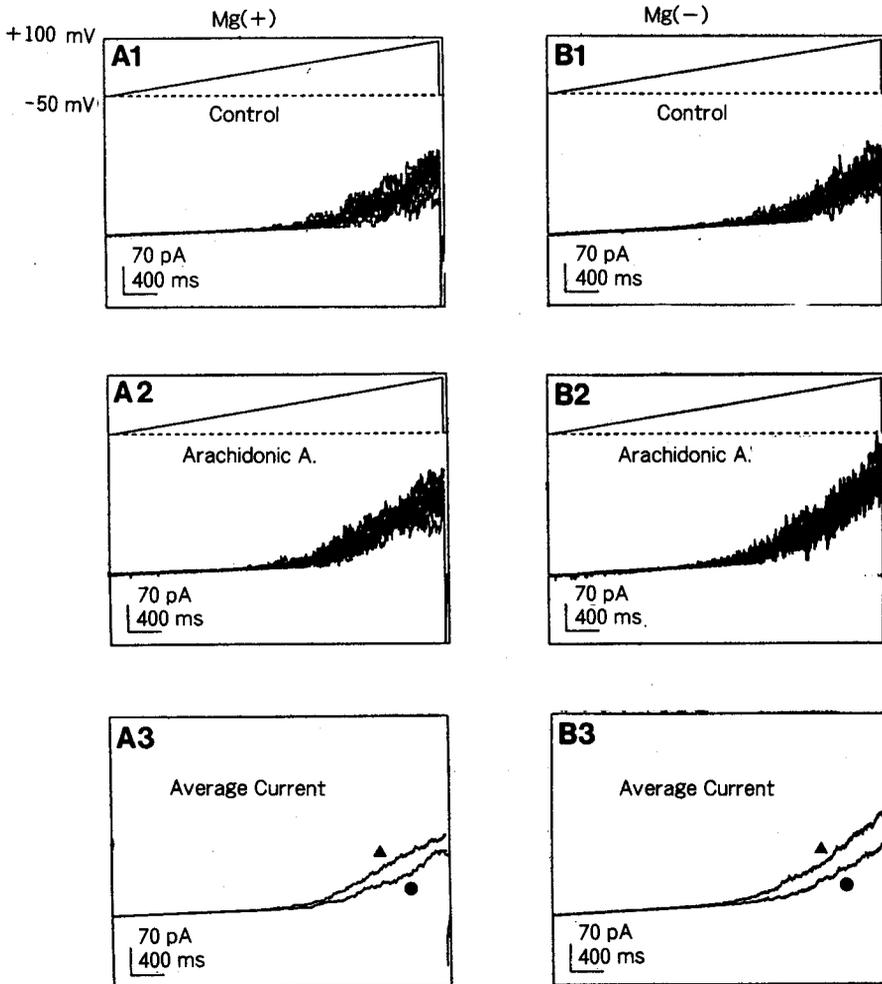
**Fig. 7.** Effect of Arachidonic acid on  $\text{Ca}^{2+}$ -activated  $\text{K}^{+}$  channel in different bath  $\text{Ca}^{2+}$  concentration. After making an inside-out patch, membrane potential was linearly changed from  $-50\text{ mV}$  to  $+100\text{ mV}$  for 4 seconds by ramp voltage pulse and current responses were recorded at different bath  $\text{Ca}^{2+}$  concentration (A1; pCa 8, B1; pCa 7, C1: pCa 6). Effect of arachidonic acid ( $2\ \mu\text{M}$ ) on  $\text{Ca}^{2+}$ -activated  $\text{K}^{+}$  channel was also examined at each different bath  $\text{Ca}^{2+}$  concentration (A2; pCa 8 + arachidonic acid, B2: pCa 7 + arachidonic acid, C3; pCa 6 + arachidonic acid). Averaged current traces were obtained from each 15 current traces (●; control, ▲; arachidonic acid, A3: pCa 6, B3; pCa 7, C3; pCa 6).

tached and cell-free, inside-out patches.

In the cell-attached patch, application of AA slowly increased the BK channel activity, and the increased BK channel activity was maintained after removal of AA from bath solution. In the inside-out patch, application of AA readily increased the BK channel activity and this effect disappeared when

perfused with the control solution. There was no discrepancy in the AA effect on BK channel activity between the cell-attached patch and inside-out patch (Fig. 5).

Fatty acids (myristic acid and palmitic acid) which are not the substrates for cyclooxygenase or lipoxygenase increased BK channel activity compared to the control.



**Fig. 8.** Effect of  $Mg^{2+}$  on the arachidonic acid-induced changes in  $Ca^{2+}$ -activated  $K^+$  channel activity.  $Ca^{2+}$ -activated  $K^+$  channel activity was examined in an inside-out patch in the presence (B1) and absence (A1) of  $Mg^{2+}$ .  $Ca^{2+}$  concentration of bath solution was  $10^{-7} M$  and membrane potential was linearly changed from  $-50 mV$  to  $+100 mV$  for 4 seconds. Effect of arachidonic acid ( $2 \mu M$ ) was also examined in the presence (A2) and absence (B2) of  $Mg^{2+}$ . A3: averaged current traces from 15 current traces of control (●) and of arachidonic acid (▲) in  $Mg^{2+}$ -containing solution. B3: averaged current traces of control (●) and arachidonic acid (▲) in  $Mg^{2+}$ -free bath solution.

Other fatty acids (linoleic acid and palmitoleic acid) which are the substrate for cyclooxygenase were also effective. The long chain fatty acid was more effective than short chain fatty acid. There was no statistical difference between saturated and unsaturated fatty acids in their effects on the BK channel activity (Fig. 6).

#### Effect of cytosolic $Mg^{2+}$ and $Ca^{2+}$ on arachidonic acid-induced changes in BK channel activity

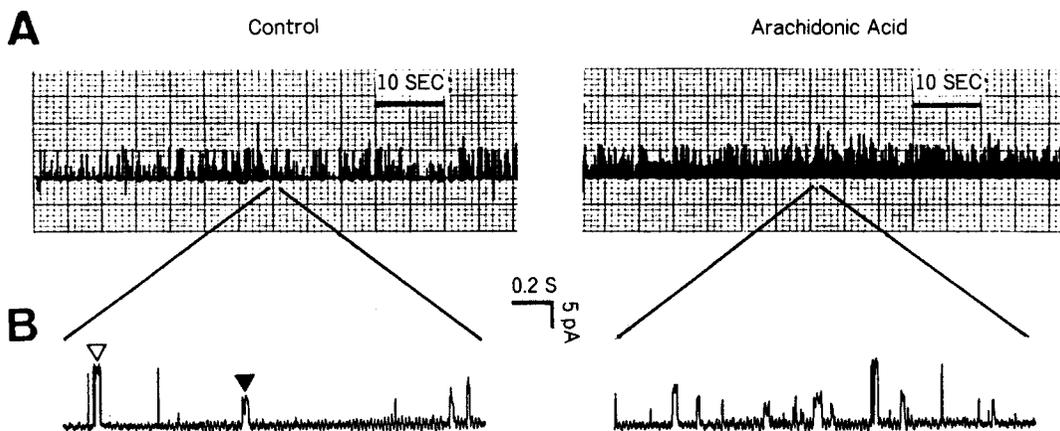
In myocardial ischemia, both divalent cations and free fatty acids are known to be increased significantly (Chien *et al.* 1984; Murphy *et al.* 1989; Freudenrich & Liberman, 1991). An increased cytosolic  $Mg^{2+}$  and  $Ca^{2+}$  could affect arachidonic induced BK channel activity change (Golowash *et al.* 1986; Blatz & Magleby, 1987). To test this possibility, we measured BK channel activity in the presence of both fatty acid and divalent cations. The effect of  $Ca^{2+}$  was examined by varying a bath  $Ca^{2+}$  concentration from pCa 8 to pCa6. When the  $Ca^{2+}$  was increased from pCa8 to pCa6, there was a significant increase in BK channel activity and the shifting of an activation voltage of the BK

channel to the hyperpolarized potential. Application of AA ( $2\ \mu M$ ) increased the BK channel activity significantly irrespective of bath  $Ca^{2+}$  concentrations (Fig. 7).

Addition of  $1\ mM\ Mg^{2+}$  into the bath caused no significant difference in the BK channel activity compared to the activity obtained in the absence of  $Mg^{2+}$ . In both cases application of AA increased the BK channel activity to a similar extent, but no appreciable discrepancy was found. In this experiment, bath  $Ca^{2+}$  concentration was fixed to  $10^{-7}\ M$  by using a Fabiato formula (Fig. 8).

#### Effect of arachidonic acid on other type of $K^+$ channels

The dominant channel activity recorded in this experiment was from large conductance  $K^+$  channels, which showed an increase in the activity when the membrane was depolarized. Less frequently, the activity of small conductance  $K^+$  channels were also observed. After making an inside-out patch, membrane potential was held at  $-50\ mV$  to reduce the BK channel activity. As shown in Fig. 9, the activity of the small conductance  $K^+$  channel was also increased after application of AA ( $2\ \mu M$ ) to the bath.



**Fig. 9.** Effect of arachidonic acid on large and small conductance  $K^+$  channels in inside-out patches.  $Ca^{2+}$  concentration of bath solution was  $10^{-7}\ M$  and the membrane potential was held at  $-50\ mV$ . **A:** Application of arachidonic acid ( $2\ \mu M$ ) to bath solution increased both the large and small conductance  $K^+$  channel activity. **B:** Current activated by arachidonic acid was shown at expanded time scale. ( $\nabla$ : large conductance  $K^+$  channel,  $\blacktriangledown$ : small conductance  $K^+$  channel)

## DISCUSSION

In this study, we examined if the activity of the BK channels in rabbit coronary smooth muscle cells could be affected by free fatty acids. Our results indicate that the activity of the BK channel is increased by AA and other fatty acids. These effects of fatty acids could be seen irrespective of the presence of cis-double bonds in their chemical structure. And also the efficacy is not related to their degree of saturation. In addition, the effect of AA can be seen in cell-free, inside-out patch. These data show that it is AA itself, not a metabolite of AA, which causes the activation of the BK channel in rabbit coronary smooth muscle cells.

1) Does arachidonic acid increase the BK channel activity by increasing membrane fluidity?

It has been reported that membrane fluidity plays an important role in membrane-associated protein function and that enhancement of lateral motion of lipids by fatty acids or other detergents causes the change in ion channel activity (Takenaka *et al.* 1986; Bregestovski *et al.* 1989; Gleason *et al.* 1991). The activity of Ca<sup>2+</sup>-activated K<sup>+</sup> channels are also affected by the change of membrane fluidity (Bolotina *et al.* 1989). In this experiment, we used DMSO as a solvent for AA which can increase the membrane fluidity by its detergent property. Application of a high concentration of DMSO (0.1%, 10 times higher concentration that used as a solvent for arachidonic acid) alone to the bath did not cause any change in the BK channel activity (Fig. 3). This result indicates the effect of AA on BK channel activity was due to AA itself, not to DMSO.

The fatty acid itself can increase the membrane fluidity at high concentration, and the effect of fatty acids on ion channel activity may be mediated by the effect of fatty acids on membrane fluidity (Takenaka *et al.* 1988). But the concentration of fatty acids to induce the change of membrane fluidity is usually in millimolar concentrations, which is 100 times higher than the concentration of

AA used in our experiment. And the time required to produce the maximal effect of AA on BK channel activity was less than 10 ~20 seconds (Fig. 5), which is very fast compared to the time required to change the membrane fluidity and ion channel activity in nerve cells (Takenaka *et al.* 1986; 1988; Bregestovski *et al.* 1989).

It has been reported that the fatty acid binding protein is present in the cell, and the micromolar concentration of fatty acids (similar concentration of fatty acid used in our experiment) can directly activate the cytosolic enzymes without involvement of the lipid bilayer (McPhail *et al.* 1984; Waldman & Murad, 1987; Sohmiya *et al.* 1992). These reports support the hypothesis that AA may increase the BK channel activity, not by increasing the membrane fluidity, but by binding to the BK channel directly.

2) Is there a substrate specificity for the activation of BK channel?

The effect of fatty acids on channel activity varies with their chemical natures. As an example, the cardiac K<sup>+</sup> channels are activated by cis-polyunsaturated fatty acids, but not by saturated fatty acids (Kim & Clapham, 1989; Ordway *et al.* 1989; Ordway *et al.* 1991). In our experiment, there was no difference in the effect of fatty acids between saturated and unsaturated fatty acids (Fig. 6-B). It suggests that fatty acids bind to the BK channel nonspecifically. The efficacy was more potent in long chain fatty acids (myristic acid versus palmitic acid, Fig. 6-A), because hydrophobicity of long chain fatty acids can enhance the interaction with a hydrophobic part of channel protein or lipid binding sites (Ordway *et al.* 1991).

3) Modulating factors for the effect of fatty acids on BK channel.

Many ion channels are regulated directly by G protein or by channel phosphorylation (Yatani *et al.* 1987; Walsh & Kass, 1988), and the activity of the BK channel in smooth muscle cells is also dependent on the state of channel phosphorylation (Kume *et al.* 1989; Fan *et al.* 1993). These reports raise the possibility that the fatty acid can increase the activity of the BK channel via G-protein or a cytosolic factor, such as protein kinase. AA could increase the BK channel activity in the

inside-out patch in the absence of GTP and other nucleotides and this effect could be reproduced during a series of experiments (Fig. 5). This result indicates that soluble cytosolic factors or nucleotides are not necessary for the AA-induced activation of the BK channel in rabbit coronary smooth muscle cells, as in the effect of fatty acids in toad stomach smooth muscle cells (Ordway *et al.* 1989). Thus, AA may directly increase the BK channel activity, not by G-protein or cytosolic factors.

The activity of BK channel is regulated by cytoplasmic divalent cations (Bolton *et al.* 1985; Moczydlowski & Latorre, 1983; Golowash *et al.* 1986; Kajioka *et al.* 1991), and its concentration is increased by myocardial ischemia (Murphy *et al.* 1989; Freudenrich & Liberman, 1991). Increasing the concentration of  $\text{Ca}^{2+}$  from pCa8 to pCa6 increased the BK channel activity at more hyperpolarized membrane potentials. However, application of AA caused a significant increase in channel activity in all conditions and there was no discrepancy of AA-induced change in pCa8, pCa7 and pCa6 solutions (Fig. 7). Some researchers reported that cytoplasmic  $\text{Mg}^{2+}$  can modify the BK channel activity by changing its  $\text{Ca}^{2+}$  dependency in some circumstance (Golowash *et al.* 1986; Bregestovski *et al.* 1989). But changing the  $\text{Mg}^{2+}$  concentration from 0 mM to 1 mM caused no change in the BK channel activity (Fig. 9, Carl *et al.* 1989) and also the application of AA to each solution in the same patch had no appreciable effect (Fig. 8). These results indicate that the AA does not affect the binding of  $\text{Ca}^{2+}$  or  $\text{Mg}^{2+}$  to the BK channel, and there is no cooperation between AA and divalent cations.

In summary, arachidonic acid, which is increased during myocardial ischemia, can directly activate the BK channel of rabbit coronary smooth muscle cells. This effect may be responsible for AA-induced hyperpolarization (Parkington *et al.* 1993), and may contribute to the protection of ischemia by dilating coronary vessels.

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