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# Effect of pH on Calcium-Activated Potassium Channels in Pulmonary Arterial Smooth Muscle Cells of the Rabbit

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=ABSTRACT=

**Single smooth muscle cells of the rabbit pulmonary artery were isolated by treatment with collagenase and elastase. Using the patch clamp technique, potassium channel activity was recorded from the inside-out membrane patch. The channel had a single channel conductance of about 360 pS in symmetrical concentration of K on both sides of the patch, 150 mM, and had a linear current-voltage relationship. During the application of 10 mM tetraethylammonium (TEA) to the intracellular membrane surface, the amplitude of single channel current was reduced and very rapid flickering appeared. The open probability ($P_O$) of this channel was increased by increasing positivity of the potential across the patch membrane, with e-fold increase by 20 mV depolarization, and by increasing the internal $Ca^{2+}$ concentration. These findings are consistent with those of large conductance Ca-activated K channels reported in other tissues. But the shortening of the mean open time by increasing $[Ca^{2+}]_i$ was an unexpected result and one additional closed state which might be arisen from a block of the open channel by Ca binding was suggested. The $P_O$-membrane potential relationship was modulated by internal pH. Decreasing pH reduced $P_O$. Increasing pH not only increased $P_O$ but also weakened the voltage dependency of the channel opening. The modulation of Ca-activated K channel by pH was thought to be related to the mechanism of regulation of vascular tone by the pH change.**

**Key Words:** Vascular smooth muscle, Single channel recording, Ca-activated K channels, pH.

## INTRODUCTION

Potassium channels in vascular smooth muscle are considered to be important in regulating electrical activity and vascular resistance (Longmore & Weston, 1990). At least five types of K channels were found in vascular smooth muscles: Ca activated K channels (Benham et al, 1986; Bolton et al, 1986; Inoue et al, 1986), delayed rectifier K channels (Okabe et al, 1987), rapidly inactivating K channels (Okabe et al, 1987), inward rectifier K channels (Edwards & Hirst, 1988) and ATP-sensitive K channels (Standen et al, 1989). In order to evaluate the precise function of each current in the electrical behaviour and in the regulation of vascular tone, not only the properties of each type of channel but also the extent of their contribution to the electrical activity in different tissues must be elucidated. Experimental evidence is still lacking, but they are thought to vary with the type of tissue: vein or artery, spontaneously active or electrically quiescent vessels.

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Ca-activated K channels are found in most types of cells (Blatz & Magleby, 1987). It is now apparent that there are many types of Ca-activated K channels which differ in their conductances and sensitivity to activation by both Ca concentration and voltage (Haylett & Jenkinson, 1990). In some neurons Ca-activated K channels allow and modulate repetitive firing and in some exocrine and endocrine cells they contribute to the regulation of secretion (Gorman et al, 1981; Petersen, 1986). The presence of this channel in vascular smooth muscle has been demonstrated in rabbit portal vein (Inoue et al, 1985) and in guinea-pig mesenteric artery (Bolton et al, 1985; 1986). The function of this channel in vascular smooth muscle is thought to regulate pacemaker activity, to hyperpolarize the membrane when the calcium concentration increases and then to prevent further increase of calcium and cause relaxation. It has not been investigated whether this channel in electrically quiescent large vessel has the same properties as those in resistance vessels or veins.

Cytoplasmic pH is known to influence ionic currents and exchanger currents in the plasma membrane of various cells (Nonner et al, 1990; Irisawa & Sato, 1986; Earm & Irisawa, 1986). Inhibition of Ca-activated K channel by lowering pH has been demonstrated in carotid body cells (Peers, 1989), pancreatic $\beta$-cells (Cook et al, 1984), epithelium of choroid plexus (Christensen & Zeuthen, 1987), red blood cells (Stampe & Vestergaard-Bogind, 1985) and tracheal smooth muscle cells (Kume et al, 1990). In the vascular smooth muscle cells, the effect of pH on this current has not been studied, even though regulation of vascular tone by the pH change seemed to be one of the most important mechanisms in autoregulation of blood flow.

This study was performed in order to elucidate the properties of the potassium channel which is most abundant in pulmonary arterial smooth muscle of the rabbit and to investigate the effect of pH on this channel.

## METHODS

### Cell dispersal

Young rabbits (800-1200g) were anaesthetized with nembutal (30mg/kg). The main pulmonary artery and its primary branch were isolated. The arterial tissue was dissected free of the surrounding adventitia and connective tissue. The arterial strip was cut into small pieces of 1-2mm widths and bathed in a Ca-free physiological salt solution (PSS) for 10-15 mins. The pieces were bathed in Ca-free PSS containing elastase (Sigma, type II-A, 0.2mg/ml), collagenase (Wako, 1mg/ml) and dithioerythritol (1mg/ml) for 50-60 minutes. The digested arterial strips were kept in Krafts-Brühe (KB) solution at 4°C. To obtain dispersed single cells, the strip was teased with fine forcep in bath on working stage of inverted microscope.

### Solutions

The PSS contained the following composition: 143mM NaCl; 5.4mM KCl; 0.5mM $MgCl_2$; 5mM N-2-Hydroxyethylpiperazine-N′-2 -ethanesulfonic acid (HEPES); 1g/l glucose, buffered to pH 7.4 with NaOH. The solution inside patch electrodes contained: 150mM KCl; 1mM $MgCl_2$; 10mM HEPES. The bathing solution contained (in mM): 150 KCl; 2.5 1,2-bis (Aminophenoxy)Ethane-N,N,N′,N″-tetraacetic acid (BAPTA); 10 HEPES; varying amounts of $CaCl_2$ for experiments where the effects of varying $[Ca^{2+}]_i$ were studied. Free Ca concentration was calculated by using apparent association constant $1.1 \times 10^7$ $M^{-1}$ between $Ca^{2+}$ and BAPTA in this combination. For bathing solution of pH 6.0, piperazine-N,N′-bis[2-ethane sulfonic acid] (PIPES) whose pK value is 6.8, was used instead of HEPES.

### Recording & analysis of results

Single channel currents were recorded in isolated inside-out patches detached from single cells of the pulmonary artery using the

patch clamp technique (Hamill et al, 1981). All the experiments were performed at 37°C. The signals were filtered at 5 kHz on an 4-pole Bessel filter, digitized by a pulse code modulator (Medical System) and recorded on video tape recorder. For kinetic analysis the data on video tape were transferred to a computer with a program of pClamp (Axon Instruments Inc.) at a sampling rate of 20kHz through Labmaster ADC interface. Open and close time were discriminated at 50% of the maximum open amplitude. The open probability ($P_O$) was expressed as a ratio of total open time to total recording time. When multiple open levels existed, the total open time was calculated as following:

$$\sum_{i=1}^{N}(i \text{ x total open time of i-th level})/N,$$

where N is total number of opening levels.

Using the nonlinear least square curve-fitting method based on the Levenberg-Marquardt algorithm, the open time distributions were fitted to several terms of exponential equation given by

$$f(t)=\Sigma(w/\tau)\exp(-t/\tau),$$

where w is weight factor and $\tau$ is time constant.

## RESULTS

Single channel activities were recorded in the inside-out membrane patch from the pulmonary arterial smooth muscle. From 34 trials of making patch, K channel activity which is sensitive to internal $Ca^{2+}$ concentration was recorded in 28 cases. When K concentration was 150 mM in both side of the patch membrane, single channel currents as shown in Figure 1 were recorded. The amplitude of the elementary current was increased linearly as increasing positive potential across the mem-

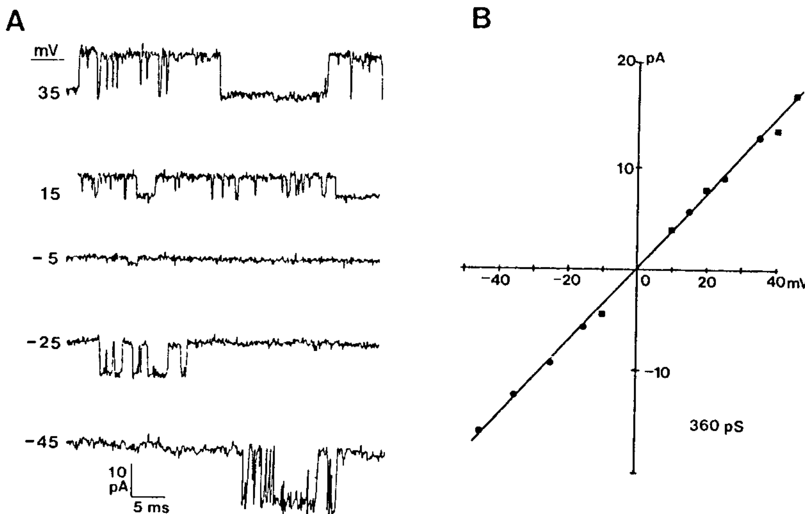



*Fig. 1. Singe channel current recordings of the activity of large conductance K channel at various potentials (A) and the current voltage relationship(B). Currents were recorded in inside-out membrane patch obtained from pulmonary arterial smooth muscle cells. K concentration was 150 mM in both side. Patch potentials were indicated left side of the current traces. The amplitude of the single channel currents was plotted against patch potentials in B(closed circles). Closed squares represented the results obtained when chloride was substituted by aspartate. The relationship was linear and the slope conductance was 360 pS.*

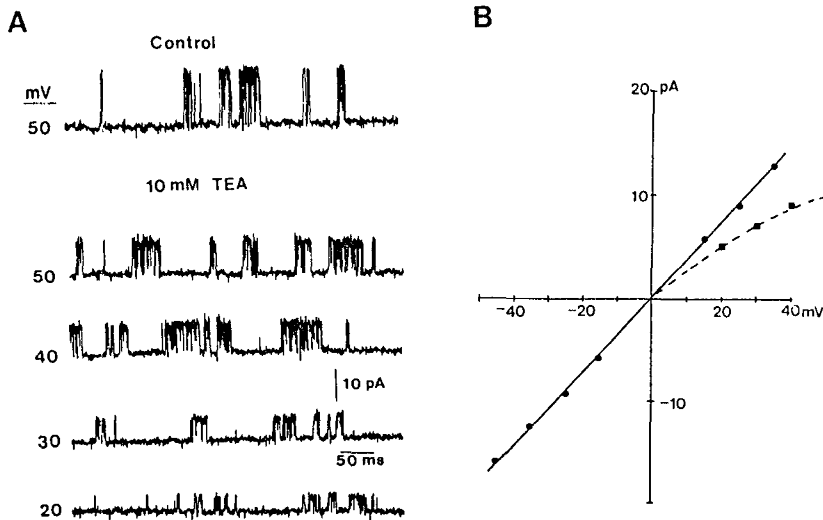



*Fig. 2. Effect of TEA on the K channel activity. TEA, 10 mM was applied to the inside of the membrane. Single channel currents obtained at each potential were shown in A and plotted in B. Control: closed circle; TEA: closed ssuare. The amplitude of the current was reduced by TEA.*

brane patch and there was no evidence for rectification. The current-voltage curve crossed the zero point as expected from symmetric concentration of KCl on both sides of the isolated membrane patch. The current amplitude and the reversal potential were not changed by the replacement of KCl with equimolar K-aspartate (data were not shown, but plotted by squares in Fig. 1B), so that the charge carrier through this channel was thought to be potassium ion. The single channel conductance calculated from Fig. 1 was 360 pS. This conductance corresponds to the maximum value of large conductance Ca-activated K channel. Channel activity was not inhibited by the application of 1 mM ATP in 34 trials except for one case. In that case, channel activity was not changed by varying the internal $Ca^{2+}$ concentration.

Application of 10mM TEA in bathing solution reduced the amplitude of the current through single channels and caused channel conductance to flicker (Fig. 2). But the effect on current amplitude may result from too short individual openings which are blurred

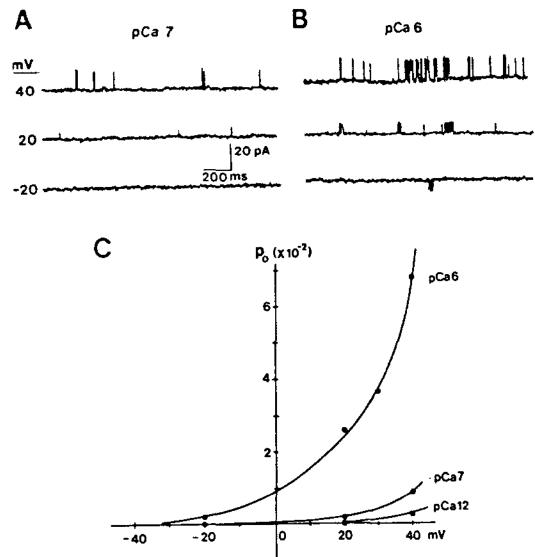



*Fig. 3. Effect of varying $[Ca^{2+}]_i$ and potential on K channel activity. K channel activities recorded at pCa 7 and pCa 6 were shown in A and B. Calibration bar applies to all traces. Open probability of this channel against the patch potential at various concentrations of $Ca^{2+}$ in internal surface(C). Duration of the sampling was 40 seconds for each condition.*

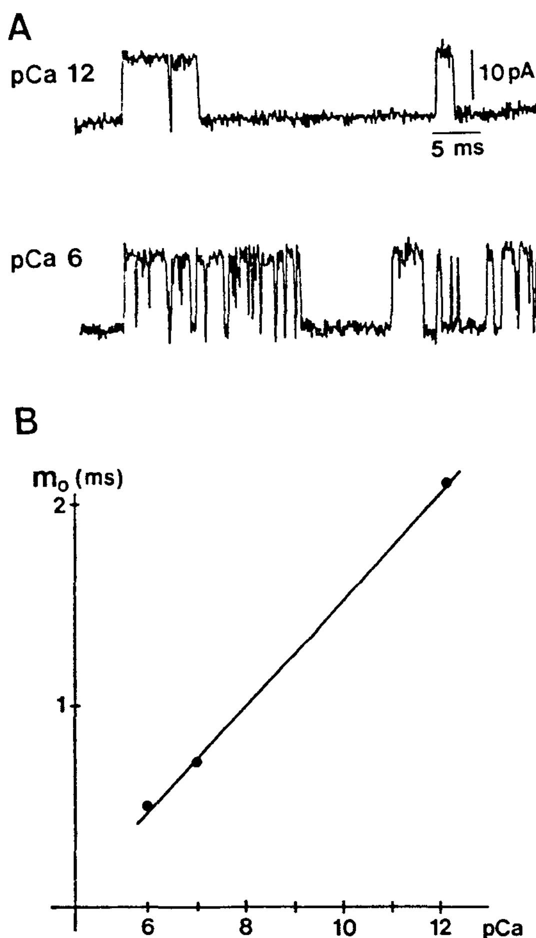



*Fig. 4. Effect of varying* $[Ca^{2+}]_i$ *on the channel kinetics. Typical records of channel activities obtained in low and high* $[Ca^{2+}]_i$ *(pCa 12 and 6) were shown in A. Patch potential was +40 mV. Rapid flickering of the channel activity was noticed at high concentration of* $[Ca^{2+}]_i$. *Mean open time*($m_o$) *was calculated by dividing total open time by number of opening and plotted in B against pCa.*

out by limited bandwidth of patch clamp amplifier.

The open state probability of this channel was dependent on the membrane potential and free Ca concentration of the bathing solution. The effect of varying $[Ca^{2+}]_i$ and the potential on K channel activity was shown in Fig. 3. When Ca was not added to the bathing solution containing 2.5 mM BAPTA (The calculated pCa was about 12. It may not be a real free calcium concentration, but this condition is indicated by pCa 12 in this paper.), the channel opening was rare but more often seen at positive potential. At higher $[Ca^{2+}]_i$, pCa 7 & 6, the $P_O$ increased exponentially by increasing the positivity of patch potential. An e-fold increase in $P_O$ occurred with about 20 mV depolarization. The effect of increasing $[Ca^{2+}]_i$ on the pattern of channel opening was shown in Fig. 4. Two types of closing events were observed. One type consisted of well-resolved closed-state events, while the second type consisted of short-lived flickers arising from the open state level in a burst pattern. Increasing $[Ca^{2+}]_i$ not only increased the burst duration but also increased the second type of closing during the burst. So the mean open time was shortened by $[Ca^{2+}]_i$ in a concentration dependent manner (Fig. 4B). This phenomenon can also be observed in open time distribution obtained from channel activities at pCa 6 and pCa 12 (Fig. 5). The open time distribution curve at pCa 12 showed two distinct components: short opening of less than 1 ms duration and long opening of several ms durations. By increasing $[Ca^{2+}]_i$ to pCa 6, the component of long opening was mainly affected.

The $P_O$ was modulated by pH of bathing solution as well as membrane potential and $[Ca^{2+}]_i$. The opening probability was plotted with respect to the applied potential across patch under conditions of various pH at constant $[Ca^{2+}]_i$, pCa 6 (Fig. 6). When pH was decreased, there was no significant effect on the conductance of single channel but it reduced the $P_O$. Increasing pH not only increased $P_O$ considerably but also changed the dependency of $P_O$ on the membrane potential. Channel activation occured from more negative potential, its voltage dependency was weakened and the relationship showed a saturation pattern. But the mean open time was not changed significantly.

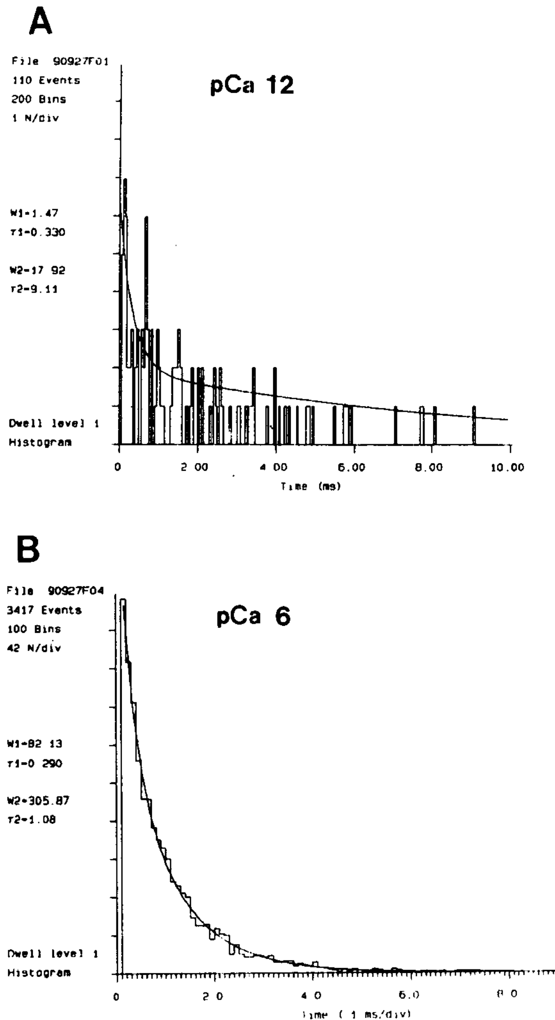



*Fig. 5. Effect of varying $[Ca^{2+}]_i$ on the open time distribution. When the distribution was fitted by the equation described in methods, sum of two exponentials was best fitted. $w_1$, 1.47; $\tau_1$, 0.330; $w_2$, 17.92; $\tau_2$, 9.11 in pCa 12. $w_1$, 82.13; $\tau_1$, 0.290; $w_2$, 305.87; $\tau_2$, 1. 08 in pCa=6. Number of events was 110 in pCa 12 and was 3417 in pCa=6.*

The Hill number at pH 7.4 was about unity suggesting only one molecule of Ca is bound to the channel protein. But as the internal $H^+$ concentration was increased, the Hill coefficient was reduced, and vice versa (Fig. 7). This result suggests that protons are compet-

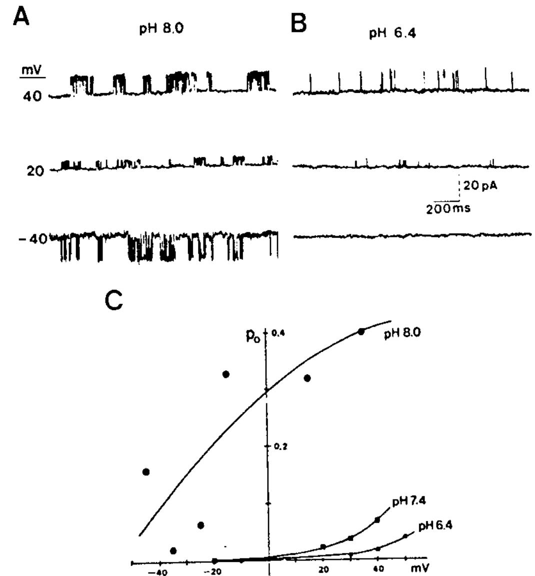



*Fig. 6. Effect of pH on the Ca-activated K channel activity. $Ca^{2+}$ concentration inside of the membrane was 1 $\mu M$. Channel activities recorded at various potentials in pH 8.0 and pH 6.4 were shown in A and B. Open probabilities($P_O$) were calculated and plotted against patch potentials in C.*

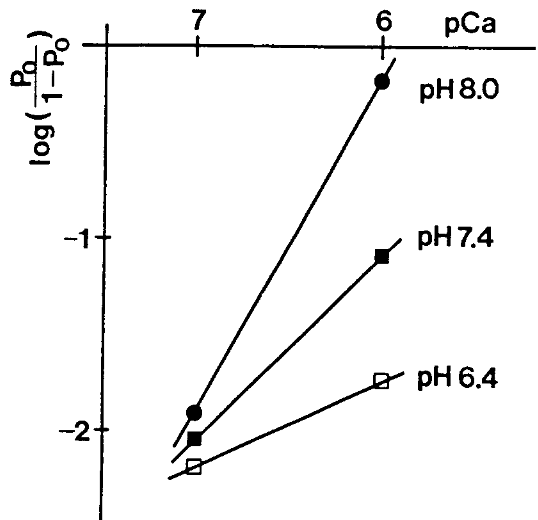



*Fig. 7. Hill plot of open probability($P_O$) with respect to pCa. Hill coefficient at pH 7.4 was about unity, increased at pH 8.0 and decreased at pH 6.4. $P_O$ was obtained from the record at +40 mV.*

ing with Ca to the same binding site of channel protein and affect the gating mechanism operated by internal Ca.

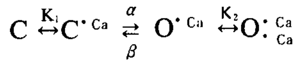
## DISCUSSION

In the present study we characterized the potassium channel which is most abundant in smooth muscle cells of the rabbit pulmonary artery and showed it is large conductance Ca-activated K channel. We could record this channel activity in more than 90% of trials (n =34), while small conductance K channel could be observed in less than 10% of trials and ATP-sensitive K channel in only one case. Furthermore activities of two or more channels were recorded even though we used very small pipettes (tip resistance was more than 20 MΩ). Therefore we could conclude that large conductance Ca-activated K channel is the most densely distributed channel in this tissue. Since the information about the channel density in other vascular muscles was hardly available, it was not possible to compare whether the density of K channel differs from tissue to tissue.

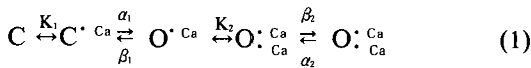
Generally, the characteristics of the channel appeared similar to those reported in rabbit portal vein (Inoue et al, 1985) and in guinea-pig mesenteric artery (Benham et al, 1986). The linear current-voltage relation at symmetrical K concentration, the increase of open probability by Ca or depolarization, and the blocking pattern by TEA were consistent well with other reports. But there are some differences in the value of single channel conductance, the sensitivity to Ca concentration and the change of channel kinetics by Ca. Benham et al, (1986) reported 200 pS with symmetrical 126 mM-K solution and Inoue et al, (1985) reported 273 pS with 142 mM-K, which was roughly in the same range as that which was observed in other cell membranes (mouse parotid acinar cell, 250 pS, Maruyama et al, 1983; bovine chromaffin cell, 180 pS, Marty, 1983; rat muscle cell, 187 pS, Magleby & Pallota, 1983). Our value of 360 pS with 150 mM K in both side of the membrane is larger than any other reports. The sensitivity of Ca-activated K channels to calcium concentration was known to vary from tissue to tissue. The $[Ca^{2+}]_i$ required to produce $P_O=0.5$ at 0 mV was $5x10^{-6}$M in cultured rat muscle (Barret et al, 1982) and $5x10^{-5}$ M when these channels were incorporated into lipid bilayers (Moczydlowski & Latorre, 1983). In contrast, secretory cells have much more sensitive channels, the comparable concentrations being $10^{-8}$ M (mouse lacrimal gland, Findlay, 1984) and $2x10^{-8}$ M (pig pancreatic acinar cells, Maruyama et al, 1983). In vascular smooth muscle cell, Benham et al (1986) reported the comparable concentration was $5x10^{-7}$ M. The channel recorded in this experiment showed much lower sensitivity to Ca, thus open probability was only 0.01 at 0 mV with $10^{-6}$ M Ca.

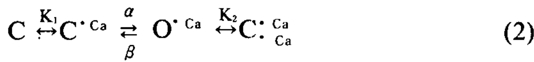
Many authors have investigated the gating behavior of large conductance Ca-activated K channels and proposed different models of channel gating mechanism (Magleby & Pallotta, 1983; Moczydlowski & Latorre, 1983). In this study, we could not analyse the effect of $Ca^{2+}$ on the single channel kinetics, as we failed to obtain a single-channel membrane patch. Instead the $[Ca^{2+}]_i$ effect on the open time distribution was analyzed. We observed the mean open time decreased with increasing $[Ca^{2+}]_i$, while Magleby & Pallota (1983), Moczydlowski & Latorre (1983), and Benham et al (1986) observed the increase of the mean open time. According to the model proposed by above authors, it was predicted that increasing $[Ca^{2+}]_i$ increased the mean open time. But rapid flickering of channel activity appeared at high $[Ca^{2+}]_i$ in our experiment (Fig. 4A), which resulted in the shortening of open duration. In order to interpret this phenomenon, it is necessary to add at least one additional closed state which arose from a fast block of the open channel by Ca binding. Theoretically, the addition of blocked state caused by the second binding of agonist, $Ca^{2+}$, to the following model which proposed by

Moczydlowsky & Latorre (1983)



can explain the paradoxically appearing phenomenon, the opposite directional change of opening probability and mean open time with increment of $[Ca^{2+}]_i$. Therefore, following possible models could be proposed based on our data.





The stochastically predicted $P_O$ and mean open time ($m_o$) in each of above models are given by

$P_O=(C^2+C \cdot K_2)/(C^2(1+R_2)+C \cdot K_2(1+R_1)+K_1 \cdot K_2 \cdot R_1)$,

$m_o=(K_2+C)/(\beta_1 \cdot K_2+\beta_2 \cdot C)$, in case of (1)

and

$P_O=C \cdot K_2/(C^2+K_2 \cdot C(1+R)+K_1 \cdot K_2 \cdot R)$,

$m_o=K_2/(\beta \cdot K_2+C)$, in case of (2)

where $C=[Ca^{2+}]$, $R_1=\beta_1/\alpha_1$, $R_2=\beta_2/\alpha_2$ and K's are dissociation constants (Colquhoun & Hawkes, 1977). In both schemes, the $P_O$ and $m_o$ shows paradoxical behavior to increment of $[Ca^{2+}]_i$, if the range of $[Ca^{2+}]_i$ and constants were selected appropriately (for example, $\beta_2>\beta_1$ in model (1)). But the above models are not the only possible model, since the similar results can be obtained in model that the blocked state is coupled to the state $C^{\cdot Ca}$ at the same time in model (1).

It is thought to be important to clairify whether the another Ca binding site which cause channel blocking is present and what is the cause of discrepancy between tissues. One of the possibilities is that the flickering was too fast to be resolved by their system and was regarded as a long opening.

It has been reported that Ca-activated K channel is inhibited by lowering pH (Cook et al, 1984; Christensen & Zeuthen, 1987; Stampe & Vestergaard-Bogind, 1985; Cornejo et al, 1989; Kume et al, 1990). In those studies $[Ca^{2+}]_i$-$P_O$ relationship was shifted to the right by lowering pH and the relationship between pH and $[Ca^{2+}]_i$ necessary for 50% activation of channel was almost linear. The membrane potential-$P_O$ relation was also modified by pH. Increasing pH not only increased open probability but also reduced the voltage dependence of the channel opening. From those results, competition between $Ca^{2+}$ and $H^+$ to the same binding site was proposed as an inhibitory mechanism of the channel opening by $H^+$. Our observation shown in Fig. 6 and 7 were consistent with the above reports qualitatively. But there is a discrepancy in the range of pH where the channel activity was affected significantly. If we compare $P_O$ at 0 mV and 1 $\mu$M $[Ca^{2+}]_i$, $P_O$ in pH 7.4 is 0.01 and that in pH 8.0 is 0.30 in our result, whereas $P_O$ in pH 7.0 is negligible and that in 7.4 is 0.82 in Kume et al (1990). Direct comparison with the other results can not be made since other authors did the experiment with a higher concentration of Ca, but the tendency is the same. Such a difference could be explained if the relative affinity of $H^+$ to $Ca^{2+}$ to the binding site have been higher in our experimental condition. If this is the case, it may be the reason why the open probability of our channel is lower than others in the physiological concentration of $H^+$. At this moment, when interpreting this discrepancy, two possibilities could be raised. One possibility is the difference in temperature at which the experiments have been done. Other investigators performed the experiments at room temperature (Cornejo et al, 1989; Kume et al, 1990, 22–24°C; Cook et al, 1984, 22–24°C), whereas we did at 37°C. If temperature increases the binding affinity of hydrogen ions to the activation site of the K channel more than that of calcium ions to the same site, then the inhibition of channel opening by $H^+$ will be dominant in a higher temperature. The other possibility is that the sensitivity of the channel to $H^+$ can be different from tissue to tissue like the Ca sensitivity, as already discussed. Whatever the mechanism is, it seems important to notice the fact that activity of

Ca-activated K channel in the pulmonary artery is greatly suppressed at physiological pH in the physiological range of $[Ca^{2+}]_i$ and it can be greatly increased by alkalinization. It could be suggested that regulation of the channel activity by pH might be more important in electrically quiescent vesseles because voltage sensitivity of the channel activation tend to be diminished by increasing pH and the channel could be activated even in the resting potential. The investigation of the relationship between this property and the effect of pH on the vascular tone will be very important.

## REFERENCES


Barrett JN, Magleby KL & Pallotta BS (1982) Properties of single calcium-activated potassium channels in cultured rat muscle. *J Physiol* **331,** 211-230

Benham CD, Bolton TB, Lang RJ & Takewaki T (1986) Calcium-activated potassium channels in single muscle cells of rabbit jejunum and guinea-pig mesenteric artery. *J Physiol* **371,** 45-67

Blatz AL & Magleby KL (1987) Calcium-activated potassium channels. *Trends Neurosc* **10** (11), 463-467

Bolton TB, Lang RJ, Takewaki T & Benham CD (1985) Patch and whole-cell voltage clamp of single mammalian visceral and vascular smooth muscle cells. *Experientia* **41,** 887-894

Bolton TB, Lang RJ, Takewaki T & Benham CD (1986) Patch and whole-cell voltage clamp studies on single smooth muscle cells. *J Cardiovasc Pharmacol* **8** (Suppl 8), 520-524

Christensen O & Zeuthen T (1987) Maxi $K^+$ channels in leaky epithelia are regulated by intracellular $Ca^{2+}$, pH and membrane potential. *Pflügers Arch* **408,** 249-259

Colquhoun D & Hawkes AG(1977) Relaxation and fluctuations of membrane currents that flow through drug-operated channels. *Proc R Soc Lond B* **199,** 231-262

Cook DL, Ikeuchi M & Fugimoto WT(1984) Lowering of pH inhibits $Ca^{2+}$ activated $K^+$ channels in pancreatic $\beta$-cells. *Nature* **311,** 269-271

Cornejo M, Guggino SE & Guggino WB(1989) $Ca^{2+}$-activated $K^+$ channels from cultured renal medullary thick ascending limb cells: effects of pH. *J Memb Biol* **110,** 49-55

Earm YE & Irisawa H(1986) Effects of pH on the $Na^+$-$Ca^{2+}$ exchange current in single ventricular cells of the guinea pig. *Jpn Heart J* **27** (Suppl), 153-158

Edwards FR & Hirst GDS(1988) Inward rectification in submucosal arterioles of the guinea-pig ileum. *J Physiol* **404,** 437-455

Findlay I(1984) A patch-clamp study of potassium channels and whole-cell currents in acinar cells of the mouse lacrimal gland. *J Physiol* **350,** 179-195

Gorman ALF, Hermann A, & Thomas MV(1981) Intracellular calcium and the control of neuronal pacemaker activity. *Fed Proc* **40,** 2233-2239

Kume H, Takagi K, Satake T, Tokuno H & Tomita T(1990) Effects of intracellular pH on calcium-activated potassium channels in rabbit tracheal smooth muscle. *J Physiol* **424,** 445-457

Hamill OP, Marty A, Neher E, Sakmann B & Sigworth F(1981) Improved patch-clamp techniques for high resolution current recording from cells and cell free membrane patches. *Pflügers Arch* **391,** 85-100

Haylett DG & Jenkinson DH(1990) Calcium-activated potassium channels. In: Cook NS (ed) *Potassium Channels.* Chapter 3. Ellis Horwood Limited, p 70-95

Inoue R, Kitamura K & Kuriyama H(1985) Two Ca-dependent K channels classified by tetraethylammonium distribute on smooth muscle membranes of the rabbit portal vein. *Pflügers Arch* **405,** 173-179

Inoue R, Okabe K, Kitamura K & Kuriyama H (1986) A newly identified $Ca^{2+}$ dependent $K^+$ channel in the smooth muscle membrane of single cells dispersed from the rabbit portal vein. *Pflügers Arch* **406,** 138-143

Irisawa H & Sato R(1986) Intra- & extracellular actions of proton on the calcium current of isolated guinea-pig ventricular cells. *Circ Res* **59,** 348-355

Longmore J & Weston H(1990) The role of $K^+$ channels in the modulation of vascular

smooth muscle tone. In: Cook NS(ed) *Potassium channels.* Chapter 9. Ellis Horwood Limited, p 259-278

Magleby KL & Pallotta BS(1983). Calcium dependence of open and shut interval distributions from calcium-activated potassium channels in cultured rat muscle. *J Physiol* **344,** 585-604

Marty A(1983) $Ca^{2+}$-dependent K channels with large unitary conductance. *Trends Neurosc* **6,** 262-265

Maruyama Y, Gallacher DV & Petersen OH (1983) Voltage and $Ca^{2+}$ activated $K^+$-channel in baso-lateral acinar cell membranes of mammalian salivary glands. *Nature* **302,** 827-829

Moczydlowski E & Latorre R(1983) Gating kinetics of $Ca^{2+}$-activated $K^+$ channels from rat muscle incorporated into planar lipid bilayers. *J Gen Physiol* **82,** 511-542

Nonner W, Spalding C & Hille B(1980) Low intracellular pH and chemical agents slow inactivation gating in sodium channels of muscle. *Nature* **284,** 360-363

Okabe K, Kitamura K & Kuriyama H(1987) Features of 4-aminopyridine sensitive outward current observed in single smooth muscle cells from the rabbit pulmonary artery. *Pflügers Arch* **409,** 561-568

Peers C(1989) Selective effect of lowered extracellular pH on potassium currents in type I carotid body cells of the neonatal rat. *J Physiol* **417,** 82

Petersen OH(1986) Calcium-activated potassium channels and fluid secretion by exocrine glands. *Am J Physiol* **251,** G1-G13

Stampe P & Vestergaard-Bogind B(1985) The $Ca^{2+}$-sensitive $K^+$-conductance of human red cell membrane is strongly dependent on cellular pH. *Biochem Biophys Acta* **815,** 313-321

Standen NB, Quayle JM, Davies NW, Brayden JE, Huang Y & Nelson MT(1989) Hyperpolarizing vasodilatorsactivate ATP-sensitive $K^+$ channels in arterial smooth muscle. *Science* **245,** 177-180