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Effect of pH on the ATP-sensitive K^+ Channel in Aortic Smooth Muscle Cells from Rats

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The effects of pH on K^+ currents were investigated in single smooth muscle cells isolated from the thoracic aorta of Wistar-Kyoto rats. Whole-cell K^+ currents were recorded in the conventional configuration of the voltage-clamp technique. Pinacidil (10uM) activated the whole-cell current and the pinacidil-activated current was completely inhibited by glibenclamide (10uM) , an inhibitor of ATP-sensitive K^+ channel (K_{ATP} channel). Pinacidil-activated current was reversed at near the K^+ equilibrium potential. This current was time- and voltage-independent and reduced by elevating intracellular ATP. Pinacidil-activated current was reduced by lowering the external pH. However, alteration of internal pH has controversial effects on pinacidil-activated current. When the single cell was dialyzed with 0.1 mM ATP, alteration of internal pH had no effect on pinacidil-activated K^+ current. In the contrast, when the single cell was dialyzed with 3 mM ATP, pinacidil-activated current was increased by lowering internal pH. Our results suggest that K^+ channel activated by pinacidil may be K_{ATP} channel and internal H^+ may reduce the inhibitory effect of ATP on K_{ATP} channel.

Key Words: pH, K^+-channel, Vascular smooth muscle, Pinacidil, Glibenclamide

INTRODUCTION

Regulation of vascular tone by pH alteration seems to be one of the most important mechanisms in autoregulation of blood flow. However, the mechanism underlying the effects of pH on vascular tone has not been established. The mechanism that cytoplasmic pH modulates the ionic currents such as Ca^{2+} current (Kloeckner & Isenberg, 1994) and K^+ current (Lee et al, 1991; Kume et al, 1990) is suggested as an important one.

Since voltage-gated Ca^{2+} channel is strongly regulated by membrane potential in vascular smooth muscle, membrane potential is an important regulator of vascular tone. In vascular smooth muscle, membrane potential is controlled by K^+ permeability through sarcolemmal K^+ channel. Activation of K^+ channel would increase the K^+ efflux, which hyperpolarizes the membrane potential. Hyperpolarization makes voltage-dependent Ca^{2+} channel close, decreasing Ca^{2+} entry, which leads to vasodilation. In vascular smooth muscle cells, various types of K^+ channels have been identified: 1) Ca^{2+}-activated K^+ (K_{Ca}) channel (Benham et al, 1986; Bolton et al, 1986; Inoue et al, 1986), 2) voltage-dependent K^+ (K_V) channel, 3) inward rectifier K^+ channel (Edwards & Hirst, 1988; Edwards et al, 1988), and 4) ATP-sensitive K^+ (K_{ATP}) channel (Standen et al, 1989). The modulation and expression of ion channels vary with types of vascular bed, and physiological roles of the K^+ channel also vary with different vessels. K_{Ca} channel and K_V channel are regulated in a voltage-dependent manner and are activated in response to membrane depolarization. Therefore, it has been suggested that K_{Ca} channel and K_V channel play a physiological role in limiting further depolarization of membrane. However, K_{ATP} channel is voltage independent. At resting state, K_{ATP} channel can be acti-

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vated by reduced cytoplasmic ATP, metabolic factors and endogenous vasodilators, leading to hyperpolarization of membrane potential and vasodilation (Standen et al, 1989), which causes the increase of local blood flow.

K_{ATP} channel has been identified in various kinds of cells including cardiac muscle (Noma, 1983), skeletal muscle (Spruce et al, 1985), pancreatic β-cell (Ashcroft et al, 1984; Cook & Hales, 1984) and vascular smooth muscle (Standen et al, 1989). And there are multiple evidences that intracellular H^+ apparently modulate gating of K_{ATP} channel in pancreatic β-cell (Misler et al, 1989), in skeletal muscle (Davies, 1990) and in cardiac muscle (Cuevas et al, 1991; Koyano et al, 1993). However, in vascular smooth muscle cells, the effect of pH on K_{ATP} channel is not reported yet. It has been proposed that vessel tone is autoregulated by metabolic factors such as H^+, K^+, adenosine and lactate. These factors seem to be involved in autoregulation of local blood flow via modulating the K_{ATP} channel.

Therefore, this study was performed to investigate whether K_{ATP} channel exists in aortic smooth muscle cells of rat and alterations in intracellular and extracellular pH affect on K_{ATP} channel.

METHODS

Animal and cell dispersion

WKY rats, 16 ~20 weeks of age, were stunned by a blow to the head and exsanguinated. The thoracic aorta was quickly isolated and adhering adventitia and remaining fat were removed under a stereomicroscope in a physiological salt solution (PSS) containing (mM): NaCl 143, KCl 5.4, $CaCl_2$ 1.8, $MgCl_2$ 0.5, HEPES 5, and glucose 5, adjusted to pH 7.4 with NaOH. The aorta was cut into small pieces(1 mm x 3 mm) and transferred to the Ca-free PSS. The pieces of aorta were recovered in Ca-free PSS at 4℃ for 30 minutes. The pieces of aorta were digested for 40~50 min (at room temp.: 20~25°C) in Ca-free PSS consisting of (mg/ml): bovine serum albumin 1.5, dithiothreitol 1.5 and papain 0.3. The digested pieces of aorta were then transferred to Ca-free PSS without papain. Single smooth muscle cells were obtained by a gentle agitation with a wide-bore pipette in fresh Ca-free PSS. Cells were stored at 4~6°C until use. Patch-clamp experiments were performed within 8 h after cell dispersion.

Electrical recording

Whole-cell K^+ current was measured in the conventional configuration of the patch clamp technique. A drop of cell suspension was placed in a perfusion chamber (20~25°C) on the stage of an inverted microscope (Olympus, IMT-2). After cells adhered to the underlying glass coverslip, the chamber was superfused with an external solution containing (mM): NaCl 135 (or 80), KCl 5 (or 60), $MgCl_2$ 1, $CaCl_2$ 0.1, HEPES 10, glucose 10, adjusted to pH 7.4 with NaOH. Heat-polished borosilicate patch pipettes were filled with solution containing (mM): KCl 102, NaCl 10, EGTA 10, $CaCl_2$ 1, KOH 38, HEPES 10, GTP 0.2, ADP 0.1, adjusted to pH 7.2, and with either 0.1 mM ATP and 1 mM $MgCl_2$ or 3mM ATP and 3 mM $MgCl_2$ added. The tip resistances of patch pipettes filled with internal solution were 3~5 MΩ. With the help of 200x magnification, a hydraulic micromanipulator (Narishige, MO 788) was used to position pipettes on membranes of single aortic cells. High resistance seals (3~10 GΩ) were obtained by light suction, and sharper suction was applied to remove the pipette patch for whole-cell recording. Membrane potential was clamped and current was measured by a voltage-clamp amplifier (Axon Instruments, Axopatch 1D). Signals were low-pass filtered at 2 KHz. The amplifier and computer were interfaced by a Digidata 1200 board (Axon Instruments). Data acquisition was controlled by commercial p-Clamp software (Axon Instruments), and data were digitized and stored on a hard disk to permit analysis later.

Membrane area was estimated by integrating capacitive currents generated by 5 mV hyperpolarizing pulses after electronic cancellation of the patch-pipette capacitance. Glibenclamide-sensitive K^+ current amplitudes were individually calculated in pA/pF to normalize for difference in cell size.

Drugs

The drugs used were pinacidil (RBI) and glibenclamide (Sigma). Pinacidil (10 mM) was dissolved in 0.1 N HCl with further dilution in external solution before use. Glibenclamide (10 mM) was dissolved

100% DMSO with further dilution in external solution before use.

RESULTS

To investigate the existance of K_{ATP} channel in aortic smooth muscle cells, we first examined the whole-cell K^+ currents in smooth muscle cells enzymatically isolated from rat aortas. To minimize the Ca^{2+}-activated K^+ channels and voltage-dependent K^+ channels, Ca^{2+} in the pipette solution was buffered with 10mM EGTA and experiments were performed at steady negative membrane potential (−70 mV). Dialysis of single smooth muscle cells with a solution that contains 0.1 mM ATP increased an inward current since external K^+ was 60 mM and the holding potential was −70 mV (Fig. 1A). Pinacidil (10 uM) increased whole-cell currents and glibenclamide (10 uM), a selective inhibitor of K_{ATP} channels, inhibited the pinacidil-activated current as well as a large fraction of the steady-state current.

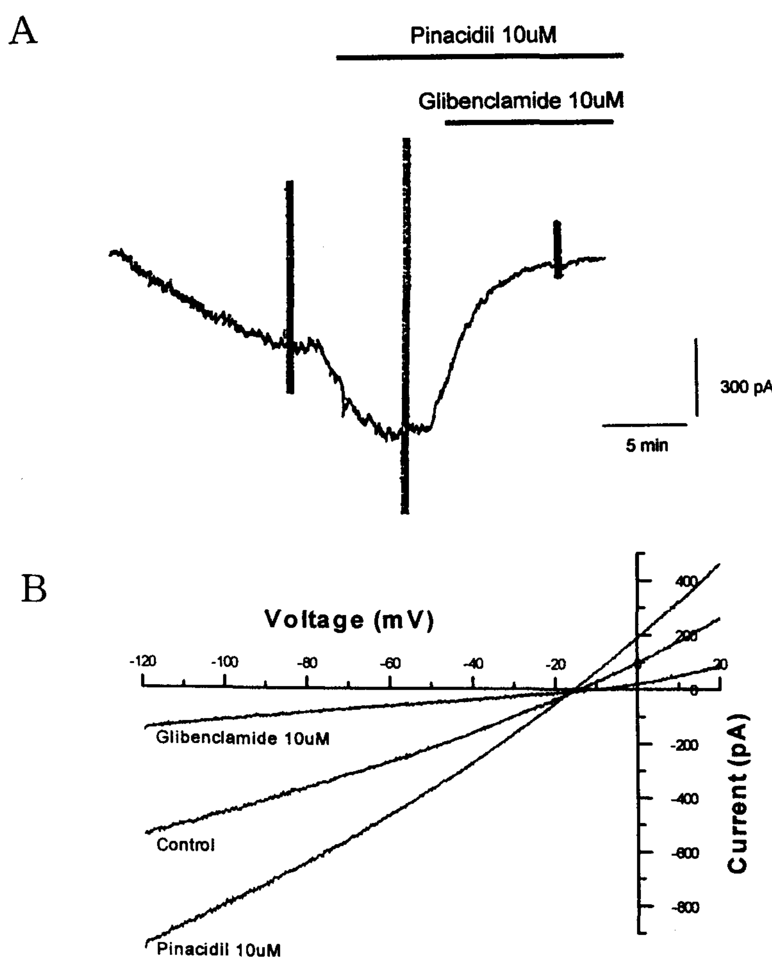

Fig. 1. Effects of pinacidil and glibenclamide on K^+ current. A: Original record illustrating effects of pinacidil (10 uM) and glibenclamide (10uM) on whole-cell currents. Holding potential was −70 mV. External and internal K^+ were 60 mM and 140 mM, respectively. Internal ATP was 0.1 mM. B: Voltage-dependence of K^+ currents. Voltage ramps of 250 ms duration were applied in the presence of pinacidil (10 uM) without and with glibenclamide (10 uM).

Current was recorded during application of a 250-millisecond voltage ramp from −120 to +20 mV (Fig. 1B). Before application of pinacidil, a linear steady-state current was recorded during dialysis of single smooth muscle cell with a solution containing 0.1 mM ATP. Pinacidil (10 uM) induced a large increase in the current slope and the pinacidil-activated current was nearly linear. Subsequent application of glibenclamide (10 uM) abolished the pinacidil-activated current as well as linear steady-state currents. The current traces obtained before and after application of pinacidil or glibenclamide intersected at about 16 mV, suggesting that the reversal potential of the pinacidil-activated current which is glibenclamide-sensitive was approximately −16 mV at 60 mM external K^+ (internal K^+: 140 mM).

Depolarizing and hyperpolarizing command potential were applied from a holding potential of −70 mV (Fig. 2). Depolarization to less than −20 mV evoked only a small background current. Depolarization to −20 mV or more positive potentials induced a voltage- and time-dependent outward current. Addition of pinacidil (10 uM) induced a downward shift in the holding current and increased the currents amplitude induced by each command potential. The pinacidil-activated currents were time-and voltage-independent. Subsequent addition of glibenclamide (10 uM) induced a upward shift in the holding current and inhibited the pinacidil-activated current, whereas the time and voltage-dependent current was unaffected by glibenclamide.

To characterize the ionic nature of the pinacidil-activated current, we examined the effect of extracellular K^+ on reversal potential (Fig. 3). The reversal

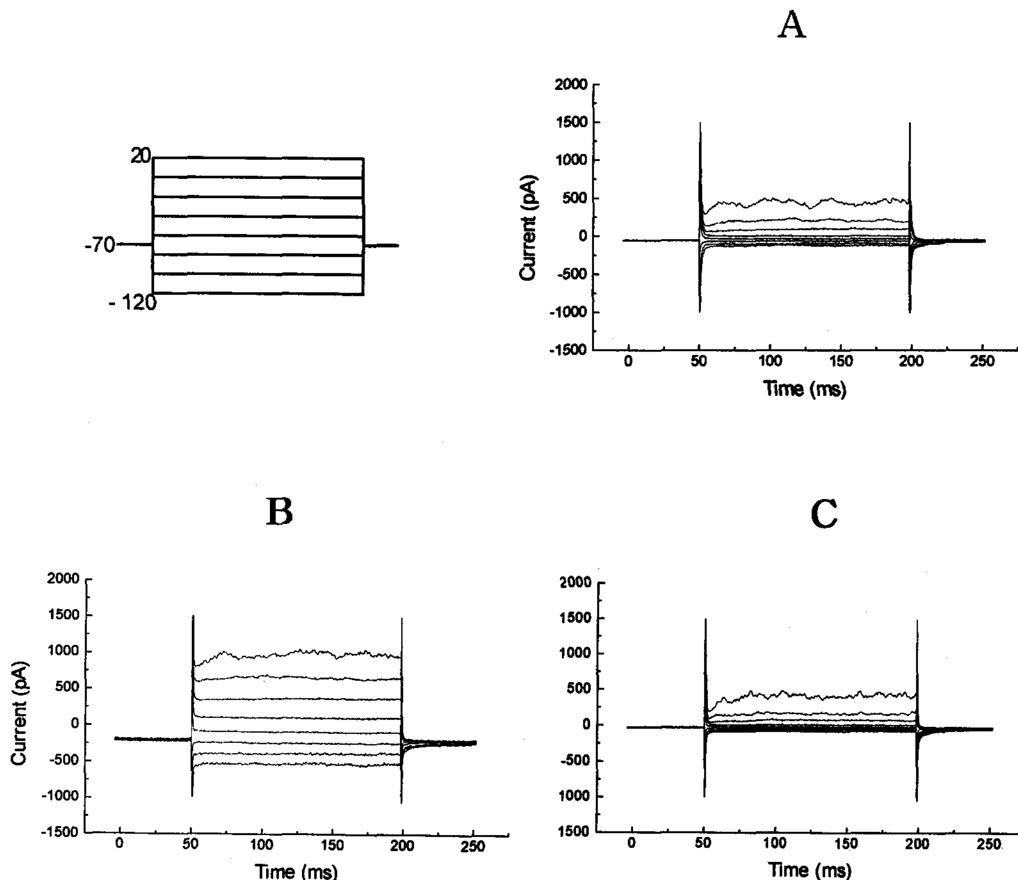

Fig. 2. Voltage and time dependence of K^+ currents. External and internal K^+ were 60 mM and 140 mM, respectively. Internal ATP was 3 mM. Voltage steps from −120 mV to 20 mV were applied in the absence (A) and presence (B) of pinacidil (10 uM), and in the presence of pinacidil and glibenclamide (10 uM) (C).

potentials of pinacidil-activated currents were 19.2± 1.3 mV (external K^+: 60 mM) and 68.7±0.9 mV (external K^+: 5 mM). The calculated K^+ equilibrium potentials with 60 mM and 5 mM external K^+ were −21.6 and −80 mV, respectively. The whole-cell current in the presence of pinacidil reversed near the K^+ equilibrium potential, indicating that pinacidil-activated current was induced through K^+ selective channels.

To investigate the influence of extracellular pH on pinacidil-activated current, whole-cell K^+ currents were measured in the presence of pinacidil (10 uM), by changing the extracellular pH (Fig. 4). Lowering the extracellular pH reduced the whole-cell K^+ currents (9.24±1.06 pA/pF at pH 8.0, 6.38±0.88 pA/pF at pH 7.4 and 4.96±0.75 pA/pF at pH 6.8). Effects of intracellular pH on pinacidil-activated currents were also examined. Dialyzing smooth muscle cell with a solution containing 3mM ATP or 0.1 mM ATP, the pinacidil-activated currents were measured. Figure 5 shows the effects of intracellular pH on pinacidil-activated currents. When single smooth muscle cell was dialyzed with a solution containing 0.1 mM ATP, intracellular acidification reduced pinacidil-activated current. The current amplitude was reduced from 17.16±1.75 pA/pF (pH 7.8) to 15.24± 1.61 pA/pF (pH 6.6). However, when the single smooth muscle cells were dialyzed with a solution con-

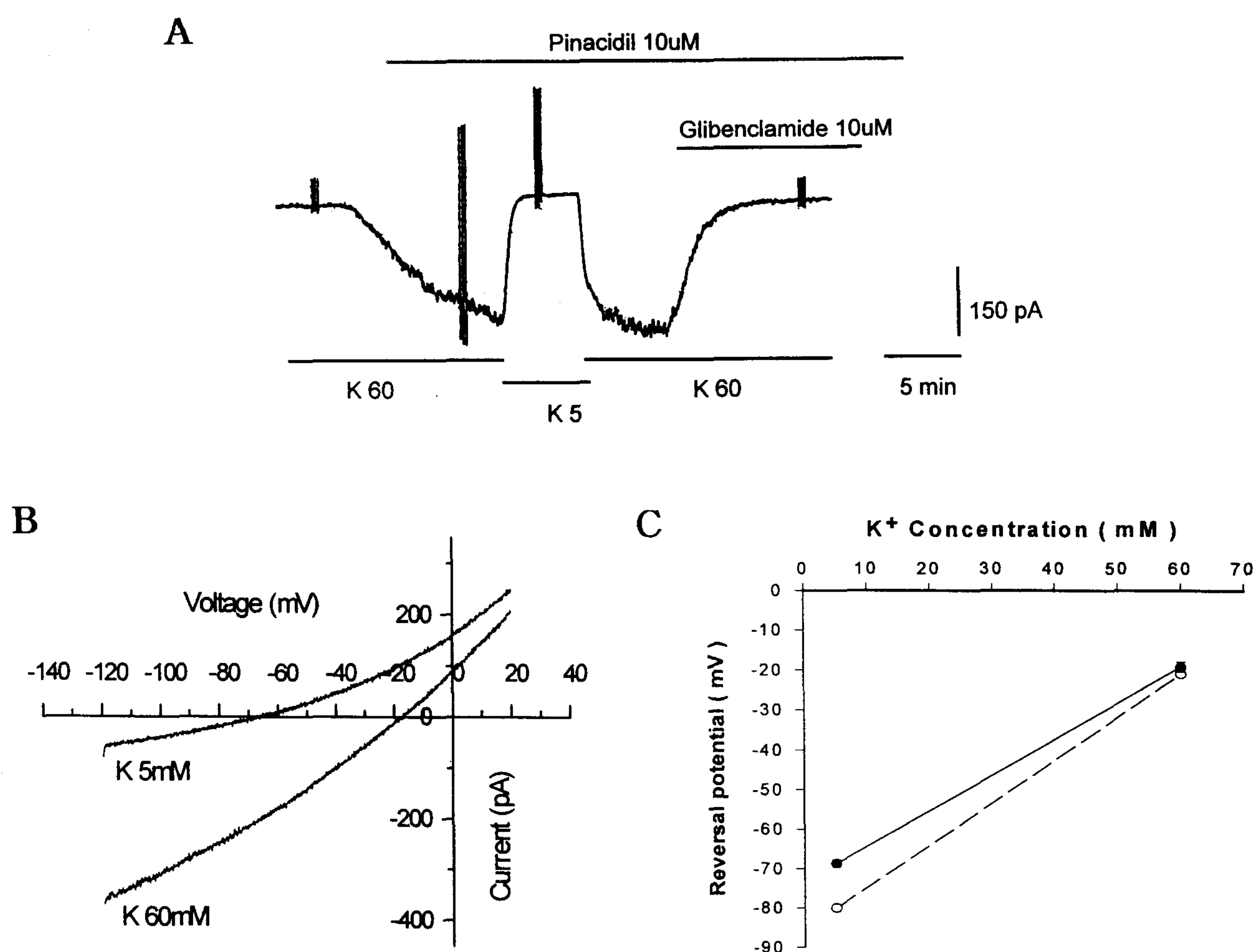

Fig. 3. Ion selectivity of K^+ currents. External K^+ was 60 mM or 5 mM and internal K^+ was 140 mM. Internal ATP was 3 mM. A: Original record illustrating effects of extracellular K^+ concentration on pinacidil-activated K^+ current. B: Voltage ramps of 250ms duration were applied in the presence of pinacidil (10 uM). C: Reversal potentials of pinacidil-activated current in single aortic smooth muscle cells (●). Data points represent mean±SE (n=4). Dot line represents theoretical equilibrium potential for K^+ obtained from the Nernst equiation (○).

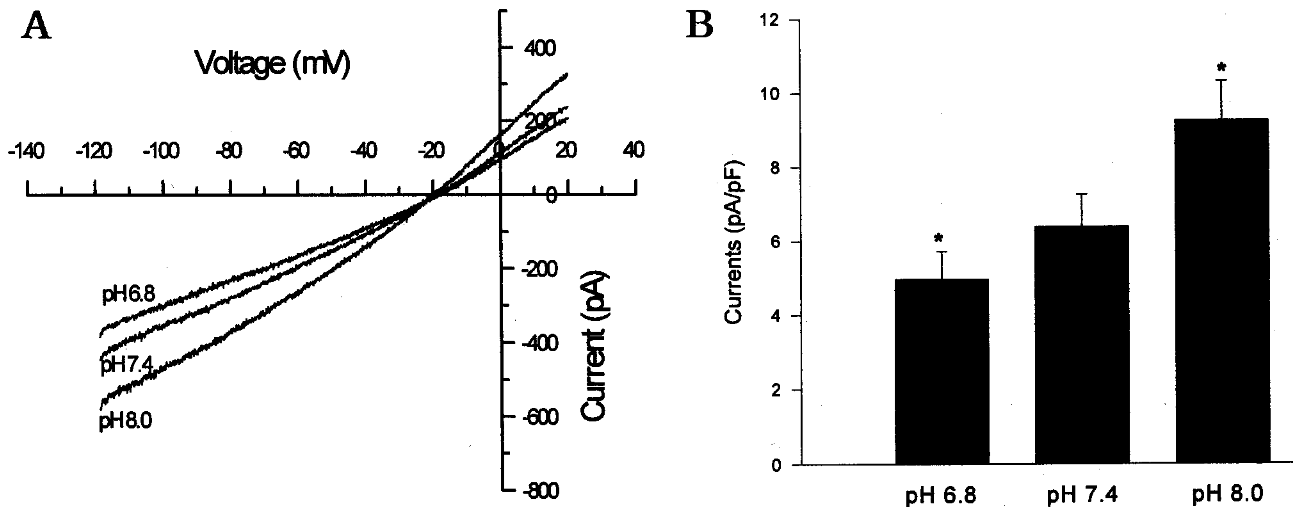

Fig. 4. Effect of extracellular pH on pinacidil-activated K^+ currents. Holding potential was -70 mV. External and internal K^+ were 60 mM and 140 mM, respectively. Internal ATP was 3mM. In the presence of pinacidil (10 uM), extracellular pH was altered. A: Voltage ramps of 250ms duration were applied, changing the extracellular pH in the presence of pinacidil (10 uM), B: Inhibition of pinacidil-activated K^+ current by extracellular acidic pH. Summurized data from 5 cells with 3 mM internal ATP. * Difference in pinacidil-activated K^+ currents at different extracellular pH was statistically significant ($P<0.05$).

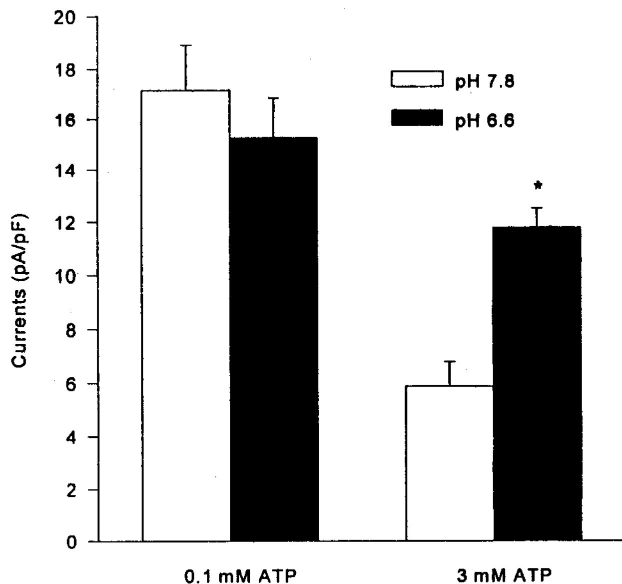

Fig. 5. Effect of intracellular pH on pinacidil-activated K^+ current. Holding potential was -70 mV. External and internal K^+ were 60 mM and 140 mM, respectively. Internal ATP was 0.1 or 3mM. Holding potential was $-$70 mV. Whole-cell K^+ currents were measured from different cells at different intracellular pH. Summurized data from 5 cells with 0.1 or 3 mM internal ATP. * Difference in pinacidil-activated K^+ currents at different pH was statistically significant ($P<0.05$).

taining 3 mM ATP, intracellular acidification increased the pinacidil activated current from 5.90 ± 0.91 pA/pF (pH 7.8) to 11.78 ± 0.72 pA/pF (pH 6.6).

DISCUSSION

Physiological and pharmacological characteristics of K_{ATP} channel have been examined in various tissues. A number of K^+ channel openers such as pinacidil, cromakalim and nicorandil have been shown to directly activate K_{ATP} channels in vascular smooth muscle and cardiac muscle (Quast & Cook, 1989; Weir & Weston, 1986). A common feature of K_{ATP} channels present in those tissues is that the channel activity is enhanced by reduced intracellular ATP. K_{ATP} channels in smooth muscle are inhibited by the antidiabetic sulfonylurea drugs such as glibenclamide and tolbutamide (Ashcroft & Ashcroft, 1990; Nelson et al, 1990; Faraci & Heistad, 1993). Glibenclamide inhibits whole-cell

K_{ATP} currents in coronary artery (Xu & Lee, 1994), mesenteric artery (Quayle et al, 1994) and portal vein (Russell et al, 1992). Furthermore, K_{ATP} channels in the various tissues are time-independent and voltage-independent (Noma, 1983; Qin et al, 1989). In the

present study, the current was increased when the single aortic smooth muscle cell was dialyzed with a solution containing 0.1 mM ATP in the absence of pinacidil, K^+ channel opener. The current was K^+ selective and increased by the addition of pinacidil. These steady state and pinacidil-activated K^+-selective currents were sensitive to glibenclamide. The pinacidil-activated K^+ current was time-independent, and the current was voltage independent because current-voltage relationship appeared to be linear. These characteristics indicate that the pinacidil-activated current in rat aortic cells is carried by K_{ATP} channel. There have been a number of reports that large conductance K_{Ca} channel is affected by hyperpolarizing vasodilators (Gelband et al, 1989; Hu et al, 1990; Kloeckner et al, 1989). However, because the currents were recorded from the single cell of which intracellular Ca^{2+} was buffered by 10mM EGTA and membrane potential was clamped at a holding potential of -70 mV, and the currents were blocked by glibenclamide, the pinacidil-activated current seems not to be carried by K_{Ca} channel but by K_{ATP} channel.

Local blood flow is regulated by metabolic factors. In cardiac and skeletal muscle, metabolites such as lactate, adenosine and H^+ are produced and diffused out during sustained muscle activity. These metabolites modulate the tone of vessel which distribute the cardiac and skeletal muscle. Furthermore, in hypoxic state, metabolites are increased from vascular smooth muscle cell itself. These factors are also involved in regulation of local blood flow as a metabolic factor. There are multiple mechanisms by which pH can modulate contractile state of the vascular smooth muscle cells. Intracellular acidosis induces relaxation of the vascular smooth muscle via the decrease of the Ca^{2+} sensitivity of the myofilament (Ruegg, 1986) or decrease of Ca^{2+} release from the sarcoplasmic reticulum (Fabiato & Fabiato, 1978; Schulz et al, 1989). In addition, increased H^+ concentration in intra- and extracellular medium is known to influence on sarcolemmal ion channel proteins. L-type Ca^{2+} channel activity is decreased when intracellular pH lowers, and this inhibits the influx of Ca^{2+} and induces the relaxation of vascular smooth musle (Kloeckner & Isenberg, 1994[a,b]). H^+ is also known to influence on the various types of K^+ channels such as Ca^{2+} activated K^+ channel (Kume et al, 1990; Lee et al, 1991), inward rectifying K^+ current (Moody & Hagiwara, 1982), delayed rectifying K^+ current (Wanke et al, 1979), and K_{ATP} channel (Cuevas et al, 1991; Davies, 1990; Kuyano et al, 1993).

K_{ATP} channel may be involved in the metabolic autoregulation of local blood flow. It is activated at the conditions of increased blood demand, eg, in hypoxia, either by release of vasodilators from the surrounding tissue or as a direct result of hypoxia on the vascular smooth muscle cell. Hypoxia decreases resistance to blood flow in many vascular bed, presumably as a means of increasing blood flow to the hypoxic region. In the coronary, cerebral, renal and skeletal muscle circulation, this hypoxic vasodilatation is attenuated by glibenclamide, suggesting a possible role of K_{ATP} channels in the response (Daut et al, 1990; Jackson, 1993; Taguchi et al, 1994). Hypoxia could activate K_{ATP} channels in arterial smooth muscle cells through a reduction in intracellular ATP. K_{ATP} channel could also be activated by intracellular acidification due to hypoxia, or vasodilator substance such as adenosine or lactate which diffused out from surrounding tissue including cardiac muscle and skeletal muscle. In cardiac and skeletal muscle, lowering of cytoplasmic pH increased the open probability of K_{ATP} channel in inside-out patches (Cuevas et al, 1991; Davies, 1990; Kuyano et al, 1993). In the present study, lowering the internal pH increased the glibenclamide-sensitive K^+ current when the single aortic smooth muscle cell was dialyzed with 3 mM ATP. This result supports the hypothesis that K_{ATP} channels are activated by reduced intracellular ATP concentration and acidification in hypoxic state, leading to membrane potential hyperpolarization, which causes the increase of blood flow. However, in single smooth muscle cells which are dialyzed with 0.1 mM ATP, glibenclamide-sensitive K^+ current was reduced by lowering internal or external pH. These results suggest that augmentation of glibenclamide-sensitive K^+ current by lowering internal pH has a close relation to intracellular ATP concentration. Davies (1990) reported that, in skeletal muscle, intracellular acidification leads to an increase in the average current flowing through the K_{ATP} channels in the presence of Mg^{2+} and ATP but intracellular acidification had a little effect on the open probability of K_{ATP} channel in the absence of Mg^{2+} and ATP. He suggested that pH may modulate ATP sensitivity of K_{ATP} channel in skeletal muscle, so that acidosis may lead to K_{ATP}

channel activation in fatigue. In cardiac muscle, intracellular acidification also increased the mean open time and the open-state probability in the presence of Mg^{2+} and ATP (Cuevas et al, 1991). These results also suggest that intracellular acidification modulates the K_{ATP} channel properties and reduces the sensitivity of K_{ATP} to inhibitory effect of ATP.

Our results are consistent with the hypothesis that K_{ATP} channel may be involved in metabolic autoregulation of local blood flow. However, further experiments are required to fully unravel the underlying mechanism for regulation of vascular tone by pH in resistance-sized arteriole.

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