**Contribution of Na⁺-K⁺ pump and Kir currents to extracellular pH-dependent changes of contractility in rat superior mesenteric artery**

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Kim, Moon Young, Guo Hua Liang, Ji Aee Kim, Seong Hoon Park, Jong Sik Hah, and Suk Hyo Suh. Contribution of Na⁺-K⁺ pump and Kir currents to extracellular pH-dependent changes of contractility in rat superior mesenteric artery. *Am J Physiol Heart Circ Physiol* 289: H792–H800, 2005. First published April 15, 2005; doi:10.1152/ajpheart.00050.2005.—We compared the branches and trunk of rat superior mesenteric artery (SMA) with respect to extracellular pH (pHe)-dependent changes in vascular contractility. Decreases in pHe from 7.8 to 6.4 significantly reduced apparent affinity (pD2) to norepinephrine (NE) and maximal contraction by NE, which were more prominent in larger-diameter arteries. On the other hand, decreases in pHl, significantly reduced Ba²⁺-sensitive K⁺-induced relaxation (which was evoked by elevation of extracellular K⁺ concentration from 6 to 12 mM) in the first branch and inhibited inwardly rectifying K⁺ (Kir) currents in cultured smooth muscle cells (SMCs) of SMA. RT-PCR revealed transcripts for Kir2.1 in the SMCs. Real-time PCR analysis revealed 6.1-, 3.3-, and 2.2-fold increases in the Kir2.1 mRNA-to-KIR current; vascular contractility; potassium channels; smooth muscle cells

THE CHANGES IN EXTRACELLULAR and intracellular pH (pHe and pHl, respectively) have been shown to modulate vascular contractility by affecting the activities of ion channels and pumps (1). H⁺ may interact with voltage-operated Ca²⁺ (VOC) channels both internally and externally (6, 7). In bovine pial and porcine coronary arteries, an increase in pHl potentiated the Ca²⁺ currents through L-type channels (6, 7). In addition, acidosis activated Ca²⁺-activated K⁺ channels in porcine coronary artery smooth muscle cells (SMCs; Ref. 4) and ATP-sensitive K⁺ channels in rat cerebellar arteries (5). These effects of H⁺ on Ca²⁺ and K⁺ channels could clearly account for the decrease in intracellular Ca²⁺ concentration and also for the decrease in contractility on acidification. On the other hand, decreases in pHl and pHo inhibited inwardly rectifying K⁺ (Kir) currents (10, 20, 22). Kir current activation would be expected to lead to hyperpolarization, and hence, vasorelaxation. It has been suggested that Kir channels are expressed at higher densities in small-diameter arteries (15). In porcine coronary artery, current densities were greater in cells isolated from smaller-diameter arteries. In contrast, arterial diameter was found to have little effect on the densities of voltage-dependent K⁺ currents (15). These results suggest that the modulation of vascular contractility by H⁺ varies depending on arterial diameter.

The present study was undertaken to elucidate the effect of arterial diameter on H⁺-induced vascular contractility. We provide evidence for activation of the Na⁺-K⁺ pump and Kir currents by extracellular alkalinization.

METHODS

The animals used in this study were treated in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publication No. 85-23, Revised 1996), and the experimental and animal care protocol was approved by the Animal Care and Use Committee of the Ewha Women's University. All experiments were performed at 37°C.

**Dissection of superior mesenteric arteries.** Sprague-Dawley rats (5–6 mo of age) of either gender were anesthetized by injection of pentobarbital sodium (150 mg/kg body wt ip) and killed by exsanguination. Superior mesenteric arteries (SMAs) were dissected out and divided into four regions including the trunk and the first, second, and third branches. An expanded explanation of SMA branches and trunk is available in an online supplement at http://ajpheart.physiology.org/cgi/content/full/00050.2005/DC1. When the second branch was long enough, its distal portion was included in the third branch, as much as the third branch was relatively short.

**Contraction measurement on isolated arterial rings.** A home-made myograph was used to record mechanical responses from the arterial ring segments (2.0–3.0 mm) from each region. An expanded explanation of the myograph and the technique used to mount the rings is available in the online supplement. The rings of the trunk and branches were threaded with two strands of stainless steel wire (trunk, 100 μm diameter; branches, 40 μm diameter); one was anchored in the organ bath chamber (1 ml), and the other was connected to a mechanotransducer (model FT-03; Grass). The chamber was perfused at a flow rate of 2.5 ml/min with oxygenated (95% O₂-5% CO₂) Krebs-Ringer bicarbonate solution using a peristaltic pump. The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

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composition (in mM) of the Krebs buffer was 118.3 NaCl, 4.7 KCl, 1.2 MgCl₂, 1.22 KH₂PO₄, 2.5 CaCl₂, 25.0 NaHCO₃, and 11.1 glucose, pH 7.4. The pH of the solution was changed by changing the HCO₃⁻ concentration. In experiments to examine the effects of pHo on K⁺-induced relaxation, oxygenated (100% O₂) HEPES-buffered solution was also used. The composition (in mM) of HEPES-buffered solution was 150 NaCl, 6 KCl, 1.5 CaCl₂, 1 MgCl₂, 10 HEPES, and 10 glucose, pH 7.4.

To abolish the influence of endothelium on contraction, endothelium was damaged by passing air through the lumen of the vessel (17) and pretreating the vessel with 30 µM Nω-nitro-L-arginine methyl ester (l-NAME). Optimal resting tension (0.5–1 g) was applied. Rings were contracted with norepinephrine (NE). When the contraction reached a steady state, extracellular K⁺ concentration ([K⁺]₀) was increased from 6 to 12 mM to evoke K⁺-induced relaxation.

Rat SMA McM culture. Vascular SMCs were isolated using a previously described procedure (16). Briefly, intact arteries were placed in a Ca²⁺-free extracellular solution that contained (in mg/ml) 1 papain, 1 bovine serum albumin, and 1 dithioerythritol and were incubated for 4–6 min at 37°C. Samples were then washed several times with ice-cold Ca²⁺-free solution and incubated in a digestive solution [in which 1 mg/ml collagenase (Wako), 1 mg/ml bovine serum albumin, and 1 mg/ml dithioerythritol were contained in Ca²⁺-free solution] (5 min for third and second branches, 10 min for first branch, and 15 min for trunk) at 37°C. The tissues were then washed several times with ice-cold extracellular solution to remove any enzymes and were agitated with a fire-polished Pasteur pipette to yield single SMCs.

Cells were grown in growth medium (100 ml) composed of 86 ml of DMEM, 10 ml of fetal bovine serum, 2 ml of penicillin-streptomycin (100 U/ml final), 1 ml of l-glutamine, and 1 ml of minimal essential amino acids. Cells were passaged and used up to the fifth passage for functional studies. To identify the nature of the cells, we used immunohistochemical techniques to detect smooth muscle α-actin (data not shown).

Cell cultures were maintained at 37°C in fully humidified air with 5% CO₂ atmosphere. Cells were detached by exposure to trypsin, reseeded on gelatin-coated coverslips, and maintained in culture for 2–4 days before use. Measurements were performed on nonconfluent cultures.

cDNA was generated from total RNA using BcaBEST polymerase (Takara Shuzo). The PCR conditionings used were as follows: initial denatured at 95°C for 10 min and then subjected to 50 cycles of two-step PCR (15 s at 95°C, 1 min at 60°C) on an ABI Prism 7000 sequence detection system (Applied Biosystems). Samples were amplified simultaneously in triplicate in one assay run. Standard curves were computed for all genes from a series of fivefold serial template dilutions from 3.125–100 ng (five concentrations). For each sample, the amounts of each target gene and of β-actin (endogenous control) were determined from the corresponding standard curves.

Electrophysiology. Membrane potential was monitored in current-clamp mode with an EPC-9 amplifier (HEKA Elektronik; Lambrecht, Germany) using a nystatin-perforated patch (100 µg/ml). Whole cell currents were measured using ruptured patches. Voltages were monitored in voltage-clamp mode with an EPC-9 amplifier. The holding potential for the whole cell experiment was −60 mV. We applied a voltage ramp from −150 to +100 mV every 10 s with a duration of 650 ms. Records were collected at a sampling rate of 1–4 kHz.

The standard external solution contained (in mM) 150 NaCl, 6 KCl, 1.5 CaCl₂, 1 MgCl₂, 10 HEPES, and 10 glucose. The osmolarity of this solution as measured with a vapor-pressure osmometer (Fiske; Norwood, MA) was 320 ± 5 mosM. The standard pipette solution contained (in mM) 40 KCl, 100 potassium aspartate, 1 MgCl₂, 0.1 or 5 EGTA, 4 Na₃ATP, 10 HEPES, pH 7.2 with KOH (290 mosM).

Chemicals. Iberitoxin, norepinephrine bitartrate (NE), ouabain, l-NAME, and tetrathylenammonium chloride (TEA) were purchased from Sigma. Nystatin was purchased from ICN Biomedicals and was applied from a stock solution in DMSO. The final concentration of DMSO was <0.05%.

Statistical analysis. Pooled data are presented as means ± SE. One-way ANOVA followed by the Turkey’s test and repeated-measures ANOVA was used for multiple comparisons of pHo-dependent changes of concentration-dependent responses to NE. In the other cases, statistical analysis was performed using Student’s t-test. Values were considered statistically significant at P < 0.05.

RESULTS

Effects of pHo on vascular contractility. In SMA branches and the trunk, NE induced concentration-dependent contractile responses. On decreasing pHo from 7.8 to 6.4, NE-induced contraction was significantly reduced, and the concentration-response curves to NE were shifted to the right (Fig. 1, A-D). The pHo-dependent changes of the contractions were more prominent in the trunk (P < 0.001; Fig. 1, C and D). In the second branch, pHo decreased from 7.4 to 6.4, respectively. In trunks, pD2 was increased to 5.44 ± 0.01 on increasing pHo to 7.8 and reduced to 5.28 ± 0.01 and 5.17 ± 0.01 on decreasing pHo to 6.9 and 6.4, respectively. In trunks, pD2 was increased to 5.95 ± 0.02 on increasing pHo to 7.8 and reduced to 5.47 ± 0.02 and 5.29 ± 0.02 on decreasing pHo to 6.9 and 6.4, respectively. Concentration-dependent responses to NE and the pD2 changes by pHo of the first and
the third branches were similar to those of the second branch (data not shown). In addition, maximal contraction by NE (30 \( \mu \)M) was also reduced on decreasing pHo (Fig. 1E). Compared with contraction at pHo 7.4, contractions of the third, second, and first branches and trunk were reduced to 89.98 ± 2.52, 89.13 ± 2.41, 83.28 ± 1.39, and 71.57 ± 5.79% on decreasing pHo to 6.4, respectively. Thus pHo-dependent pD2 and NE-induced contraction changes were more prominent in trunks than in branches. These data suggest that the modulation of vascular contractility by H\(^+\) depends on arterial diameters and that the decreases in vascular contractility by H\(^+\) are more prominent in larger-diameter arteries.

Comparative characteristics between SMA branches and trunk. Precontracted arterial rings were relaxed on increasing \([K^+]_o\) from 6 to 12 mM (Fig. 2, A and B), and this relaxation is known as K\(^+\)-induced relaxation. We compared the magnitudes of K\(^+\)-induced relaxation in SMA branches and the trunk. As shown in Fig. 2C, K\(^+\) evoked 92.66 ± 1.29, 60.34 ± 2.37, and 19.26 ± 1.35% relaxations in the third branch, the first branch, and the trunk, respectively. These results suggest that K\(^+\)-induced relaxation increases as the arterial diameter decreases.

Because Kir2.1 channels contribute K\(^+\)-induced relaxation (21), we examined the presence of Kir2.1 channels and compared the levels of their expression in SMA branches and the
trunk. We confirmed the presence of Kir2.1 transcripts in SMCs from SMA branches and the trunk by RT-PCR (Fig. 3, A and B), and significant differences in the transcript levels of Kir2.1 were found in branch and trunk SMCs (Fig. 3C). We observed 6.1-, 3.3-, and 2.2-fold increases in the Kir2.1 mRNA-to-β-actin mRNA ratios of SMCs of the third, second, and first branches, respectively, vs. the corresponding relative levels of trunk SMCs. There was a strong correlation between the transcript levels of Kir2.1 and K^+^-induced relaxation (Fig. 3D). In contrast, no significant differences in the transcript levels of α1- and α2-subunits of the Na^+^-K^+^-pump were found in branch and trunk SMCs (Fig. 3E). In addition, no significant differences in the transcript levels of α3-subunits of the Na^+^-K^+^-pump were found in branch and trunk SMCs (data not shown). These results suggest that KirR channel densities increase with reduced arterial diameter, which accounts for the difference in the magnitude of K^+^-induced relaxations between SMA branches and trunk.

Effects of pH_o on K^+^-induced relaxation. In the first branch, K^+^-induced relaxation of 63.94 ± 3.74% at pH 7.4 was increased to 95.09 ± 1.91% on increasing pH_o to 7.8 and reduced to 21.72 ± 1.89 and 9.72 ± 1.56% on decreasing pH_o to 6.9 and 6.4, respectively (Fig. 4). We then examined the effects of the Kir channel blocker Ba^{2+} on K^+^-induced relaxation. K^+^-induced relaxations were completely inhibited by 30 μM Ba^{2+} at pH_o values of 6.4, 6.9, and 7.4 (Fig. 4, A-C). In contrast, K^+^-induced relaxation at pH_o 7.8 was not inhibited by Ba^{2+} but was completely inhibited by Ba^{2+} with ouabain (Fig. 4, D and E).
and contraction at pH values of 7.4 and 6.4 (E unaffected by TEA or IbTx (tations did not decrease with time and were unaffected by TEA or IbTx (B and D). In the trunk, TEA or IbTx caused no change in contraction at pH values of 7.4 and 6.4 (E and F). ***p < 0.001.

During agonist-induced contraction, K⁺ efflux occurs through large-conductance Ca²⁺-activated K⁺ channels in SMCs, and this can evoke K⁺-induced relaxation that is sufficient to suppress the level of contraction by 75% (3). Thus we examined the effects of pH₀ on the relaxation evoked by the released K⁺ through large-conductance Ca²⁺-activated K⁺ channels during NE-induced contraction. In the second branch, in which K⁺-induced relaxation is prominent, NE-induced contraction was spontaneously decreased with time, and this decrease was reversed by the Ca²⁺-activated K⁺ channel blockers TEA or iberiotoxin at pH₀ 7.4 (Fig. 5, A and C). The decreasing tension was significantly increased to 119.62 ± 4.07 and 132.95 ± 4.31% on application of TEA or iberiotoxin, respectively (Fig. 5C). In contrast, in the trunk, in which K⁺-induced relaxation is negligible, the spontaneous and TEA- or iberiotoxin-induced changes in contraction were not observed (Fig. 5E). These data suggest that K⁺-induced relaxation might be evoked by the released K⁺ through Ca²⁺-activated K⁺ channels during NE-induced contraction. The effects of pH₀ on the relaxation was then examined. When pH₀ was decreased to 6.4, NE-induced contraction was not spontaneously reduced, and no significant change in the contraction was detected on application of TEA or iberiotoxin in the second branch (Fig. 5, B and D) and trunk (Fig. 5F), although the release of K⁺ through Ca²⁺-activated K⁺ channels might be increased because Ca²⁺-activated K⁺ channels are activated by acidification (4). These data also suggest that a decrease in pH₀ inhibits K⁺-induced relaxation.

Inwardly rectifying currents were recorded from cultured SMCs of SMA branches (Fig. 6). Because inwardly rectifying currents were found to be inhibited by 300 μM Ba²⁺ (Fig. 6, A, B, D, and E), these Ba²⁺-sensitive currents may be Kir currents. These Ba²⁺-sensitive currents were significantly reduced on decreasing pH₀ from 7.4 to 6.4 (Fig. 6, A and C-E). These results suggest that a decrease in pH₀ inhibits Kir channels.

Effects of pH₀ on Na⁺-K⁺ pump. From the data shown in Fig. 4D, we suggested that an increase in pH₀ activates the Na⁺-K⁺ pump. For further evaluations, we examined the effects of pH₀ on Na⁺-K⁺ pump activity. As much as the relative contribution of the Na⁺-K⁺ pump to K⁺-induced relaxation might be greater in larger-diameter arteries, the SMA trunk was used to examine ouabain-sensitive K⁺-induced relaxation. Ouabain-sensitive K⁺-induced relaxation was markedly reduced on decreasing pH₀ from 7.4 to 6.4 (Fig. 7). K⁺-induced relaxations of 17.74 ± 1.35% at pH₀ 7.4 were reduced to 1.07 ± 0.26% on decreasing pH₀ to 6.4. We then examined the effects of ouabain on resting tensions in SMA trunk. The resting tensions were not altered on pH₀ changes. Then, ouabain was applied. On application of ouabain, the resting tensions were increased, and these increases were enhanced on increasing pH₀ from 6.4 to 7.8 (Fig. 8). Compared with 10 μM NE-induced contraction at pH₀ 7.4, ouabain increased the resting tension to 0.89 ± 0.89% at pH₀ 6.4, whereas the tension was increased to 30.74 ± 1.78% at pH₀ 7.8 (Fig. 8E). These findings suggest that ouabain-induced contraction decreases on acidification.

Effects of pH₀ on membrane potential. The mean resting membrane potential of cultured SMCs from trunk was −55.56 ± 6.44 mV at pH₀ 7.4 (n = 8), and this was significantly depolarized by −46.22 ± 6.63 mV on decreasing pH₀ to 6.4 (Fig. 9, A and B). Ouabain depolarized membrane potential at pH₀ 7.4 (Fig. 9C), whereas membrane potential was unaffected by ouabain treatment at pH₀ 6.4 (Fig. 9D). These
findings suggest that ouabain-induced depolarization decreases on acidification.

**DISCUSSION**

This study provides evidence to indicate that the Na\(^+\)/K\(^+\) pump and K\(_{IR}\) channels participate in the pH\(_o\)-dependent control of vascular contractility. The results of the present study indicate that 1) an increase in pH\(_o\) potentiates vascular contractility; 2) the pH\(_o\)-dependent control of vascular contractility is more prominent in larger-diameter arteries, on the contrary; 3) a decrease in pH\(_o\) inhibits K\(_{IR}\) currents; 4) K\(_{IR}\) channel densities are significantly greater in the SMCs of smaller-diameter branches than in those of larger-diameter branches; and 5) an increase in pH\(_o\) potentiates, and a decrease in pH\(_o\) inhibits, the Na\(^+\)/K\(^+\) pump activity. From these results, we conclude that extracellular alkalization activates K\(_{IR}\) channels and the Na\(^+\)/K\(^+\) pump, which in turn reduces vascular contractility and thereby attenuates the increased vascular contractility induced by extracellular alkalization. Inasmuch as K\(_{IR}\) channel densities are significantly greater in smaller-diameter than in larger-diameter arteries, the attenuation is more pronounced in smaller-diameter arteries.

K\(^+\)-induced relaxation is evoked via the Na\(^+\)/K\(^+\) pump and K\(_{IR}\) channel activation (11, 21). K\(_{IR}\) channel conductance, which is dependent on membrane potential, is very small at potential positive to the calculated equilibrium potential for K\(^+\) (E\(_K\)) and starts to increase as membrane potential hyperpolarizes to \(-E_K\). When SMA is contracted by NE (30 \(\mu\)M), membrane potential may depolarize because the resting membrane potential of rat SMA was approximately \(-60\) mV and depolarized to approximately \(-40\) mV by phenylephrine (10 \(\mu\)M) (19). Inasmuch as E\(_K\) at 6 mM [K\(^+\)]\(_o\) is approximately \(-85\) mV (150 mM [K\(^+\)]\(_i\) and 37°C), the conductance of K\(_{IR}\) channels may be negligible during NE-induced contraction. On [K\(^+\)]\(_o\) elevation in a millimolar range, E\(_K\) is shifted to the more positive potential (the calculated E\(_K\) is approximately \(-65\) mV at 12 mM [K\(^+\)]\(_o\)), and the conductance of K\(_{IR}\) channels increases. In addition, [K\(^+\)]\(_o\) elevation activates the Na\(^+\)/K\(^+\)

**Fig. 6.** Inhibition of inwardly rectifying K\(^+\) (K\(_{IR}\)) currents by extracellular acidification. Time course of the membrane currents is shown (A). Data points were obtained at 50 and \(-140\) mV during repetitive ramps from \(-150\) to \(+100\) mV. Current-voltage (I-V) relations were obtained at the points marked in A (B and C). Difference currents before and after application of Ba\(^{2+}\) (2–1) or decreasing pH\(_o\) from 7.4 to 6.4 (3–4) are shown (D). Inwardly rectifying currents were inhibited by Ba\(^{2+}\) (B and D) and by extracellular acidification (C and D). Current density, measured at \(-140\) mV (E), was significantly reduced by Ba\(^{2+}\) or extracellular acidification. V\(_M\), membrane voltage. *P < 0.05.

**Fig. 7.** Effects of extracellular acidification on ouabain-sensitive K\(^+\)-induced relaxation. Tracings show responses to pH\(_o\) values of 7.4 (A) and 6.4 (B). In SMA trunk, K\(^+\)-induced relaxation was inhibited by ouabain (a Na\(^+\)/K\(^+\) pump inhibitor), and ouabain-sensitive relaxation was reduced by extracellular acidification (C). ***P < 0.001.
pump. $K_{IR}$ channel conductance increase and $Na^+\cdotK^+$ pump activation hyperpolarize membrane potential. This hyperpolarization inhibits voltage-gated $Ca^{2+}$ channels and relaxes the vascular smooth muscle. In contrast, vascular smooth muscle contracts in response to $[K^+]_o$ elevation to $>25$ mM. Thus 12 or 15 mM $K^+$ may be enough to evoke $K^+$-induced relaxation (8, 21). Among the $Na^+\cdotK^+$ pump and $K_{IR}$ channels, $K_{IR}$ channels may be a main contributor in $K^+$-induced relaxation, because Kir2.1 gene expression in arterial smooth muscle is required for $K^+$-induced relaxation (21).

**Role of $K_{IR}$ channels in $pH_o$-dependent control of vascular contractility.** The magnitude of $K^+$-induced relaxation varies depending on arterial diameter; the relaxation is prominent in small-diameter arteries such as resistant arteries and SMA branches, whereas it is relatively small or negligible in large-diameter arteries such as the SMA trunk. Kir2.1 gene expression in arterial SMCs is required for $K_{IR}$ currents and $K^+$-induced relaxation (21). Real-time PCR analysis revealed significant differences in Kir2.1 transcript levels in SMA branches and trunks, and the smallest-diameter branch showed the highest Kir transcript expression. In addition, the magnitude of $K^+$-induced relaxation was greater in smaller-diameter branches. These findings suggest that $K_{IR}$ channel densities are higher in smaller-diameter arteries, which is consistent with previous work (2, 15). The highest Kir2.1 transcript levels were found in SMCs from the smallest-diameter artery in the rat (2), and $K_{IR}$ current densities were higher in SMCs from smaller-diameter porcine coronary arteries (15).

**Fig. 8.** Effects of $pH_o$ on ouabain-induced contractions in SMA trunks. Ouabain-induced contractions of trunk were significantly enhanced on increasing $pH_o$ values from 6.4 (A) to 6.9 (B), 7.4 (C), and 7.8 (D). Magnitudes of the contractions were expressed as a percentage of 10 µM NE-induced contraction at $pH_o$ 7.4 (E).

**Fig. 9.** Effects of $pH_o$ and ouabain on membrane potential of cultured smooth muscle cells from SMA trunk. A decrease in $pH_o$ from 7.4 to 6.4 (A and B) or treatment with ouabain at $pH_o$ values of 7.4 (C) and 6.6 (D) significantly depolarized resting membrane potentials. ***$p < 0.001$. 

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In SMA branches and trunks, differences in KIR channel densities may have caused the observed differences in pHo-dependent changes in vascular contractility (see Fig. 1). In agreement with previous studies (22), the present study showed that a decrease in pHo inhibited KIR currents. A decrease in pHo inhibits KIR currents by affecting mainly single channel conductance (2). They showed that Kir2.3 current inhibition by extracellular acidification started at pH 7.0 and plateaued at pH 6.0 with a pK of 6.7. Our finding that Ba2+-sensitive K+ induced relaxation was markedly reduced at pHo values of 6.9 and 6.4 is consistent with this finding. KIR current activation would be expected to lead to hyperpolarization and, hence, to relaxation. Thus when pHo is increased, KIR current activation would be expected to attenuate the increase of vascular contractility. Inasmuch as the channel densities are higher in SMCs of smaller-diameter arteries, the attenuation may be more prominent in smaller-diameter arteries, and therefore the pHo-dependent changes in pD2 and NE-induced contractions were less pronounced in smaller-diameter arteries.

Cell phenotype may change during cell culture. However, it is unlikely that the recorded KIR current is a culture artifact for a couple of reason. First, transcript for Kir2.1 was also detected in freshly isolated cells from rat SMA (2). Thus transcript for Kir2.1 is detected not only in cultured SMCs but also in freshly isolated cells. Second, K+ -induced relaxation was inhibited by Ba2+. This suggests that KIR channel contributes K+ -induced relaxation in rat SMAs. In addition, there was a strong correlation between the transcript levels of Kir2.1 and the magnitude of K+ -induced relaxation. These results suggest the presence of KIR channels and a functional role for KIR channels to evoke K+ -induced relaxation in rat SMAs.

Role of Na+-K+ pump in pHo-dependent control of vascular contractility. The contribution of Kir channels to K+ -induced relaxation would depend on Kir channel densities and thus would be greater in smaller-diameter arteries. On the contrary, no significant differences in the Na+-K+ pump densities were found in SMC branches and the trunk. Therefore, we suggest that Na+-K+ pump expression is independent of arterial diameter and that the relative contribution of the Na+-K+ pump to K+ -induced relaxation may be greater in larger-diameter arteries.

Acidification depolarized the membrane potential of cultured SMCs from the SMA trunk, which is consistent with previous findings (13, 14). They showed that normocapnic acidosis caused depolarization in intact arteries of the rat. However, they did not examine the mechanism of acidification-induced depolarization. In this study, we suggest that acidification-induced depolarization is evoked by inhibition of the Na+-K+ pump and KIR channel. Compared with the Na+-K+ pump, the contribution of KIR channels to acidification-induced depolarization may be relatively small in the resting or agonist-stimulated conditions, because the membrane potential of SMCs is positive to the EK in both conditions.

Ouabain depolarizes membrane potential by inhibiting the Na+-K+ pump. Inasmuch as depolarization may evoke contraction by increasing Ca2+ influx through VOC channels, ouabain-induced contraction might be at least in part evoked by ouabain-induced depolarization. In contrast, a decrease in pHo, which also depolarized membrane potential, did not evoke contraction. This absence of acidification-induced contraction can be clearly explained by a previous report showing that acidification inhibits VOC channels (6, 7). This effect of H+ on VOC channels can also explain why ouabain-induced contractions decrease on acidification. In addition, inasmuch as ouabain did not induce depolarization at pHo 6.4, we suggest that ouabain-induced depolarization is decreased on acidification and that the decrease in ouabain-induced depolarization contributes to a decrease in ouabain-induced contraction on acidification.

Na+-K+ pump expression and activity is regulated by a variety of moieties that include the cytoskeleton, endogenous inhibitors, and protein kinases (9). Membrane potential also affects Na+-K+ pump activity. Depolarization would increase the amplitude of the Na+-K+ pump currents, because membrane potential moves further from the reversal potential (12). However, when pHo was decreased to 6.4, ouabain failed to evoke depolarization, because H+ inhibits the Na+-K+ pump. The mechanism of Na+-K+ pump inhibition by H+ remains to be investigated.

Physiological implications. Here, we propose that the Na+-K+ pump and KIR channels play important roles in pHo-dependent control of vascular contractility in smaller-diameter arteries. Acidification decreases Ca2+ influx through VOC channels and Ca2+ sensitivity of contractile proteins (9) in SMCs and thus decreases vascular contractility. On the contrary, acidification inhibits the Na+-K+ pump and KIR channels, and vascular contractility can be increased by the inhibition. Therefore, the Na+-K+ pump and KIR channels may inhibit excessive changes in vascular contractility by pH and sensitively control blood flow. In pathological conditions such as hypoxia, pHlo decreases. Hence Kir channel inhibition is likely to impact on the extent of blood flow increases under hypoxic conditions and appears to be an important negative-feedback process that limits blood flow increases.

The results of the present study show that rat mesenteric artery SMCs possess Kir2.1 channels that evoke K+ -induced relaxation, and the channel expression increases as arterial diameter decreases. Furthermore, we show that an increase in pHo potentiates and a decrease in pHo inhibits Kir currents and Na+-K+ pump activity. Thus the increase of vascular contractility by extracellular alkalization is more pronounced in larger-diameter arteries.

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