Molecular and electrophysiological characteristics of K⁺ conductance sensitive to acidic pH in aortic smooth muscle cells of WKY and SHR

Hidekazu Kiyoshi,1 Daiju Yamazaki,1 Susumu Ohya,1 Mika Kitsukawa,1 Katsuhiko Muraki,1,2 Shin-ya Saito,3 Yasushi Ohizumi,3 and Yuji Imaizumi1

1Department of Molecular and Cellular Pharmacology, Graduate School of Pharmaceutical Sciences, Nagoya City University, Mizuho-ku, Nagoya, Japan; 2Cell Signaling and Ion Channel Research Group, Cellular Pharmacology, School of Pharmacy, Aichigakuin University, Nagoya, Japan; and 3Department of Pharmaceutical Molecular Biology, Graduate School of Pharmaceutical Sciences, Tohoku University, Aoba-ku, Sendai, Japan

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Changes in K⁺ conductance can contribute to the pathophysiological features of vascular smooth muscle cells in the settings of hypertension or stroke (13, 54). Under pathological ischemic conditions, where extracellular pH (pHₑ) can decrease to ~6.5, changes in pHₑ and intracellular pH are thought to be critical for the regulation of vascular smooth muscle tone (1, 18). Large-conductance Ca²⁺-activated (BK), voltage-dependent (KV), and ATP-sensitive (K_ATP) K⁺ channels regulate the membrane potential in many vascular smooth muscles, and their activities can be modulated under conditions of acidic stress (2, 10, 26). In the coronary and pulmonary circulations, acidosis elicits opposite responses; vasodilation and vasoconstriction, respectively (26, 52). Tissue-specific expression and regulation of vascular K⁺ channel α-subunits are, at least in part, responsible for the opposing effects of acidosis on their vascular muscle tone (6). Furukawa et al. (17) have shown that acidic pH produces a markedly enhanced contraction in aorta from spontaneously hypertensive rats (SHR) compared with that in Wistar-Kyoto (WKY) rats. They suggested that changes in K⁺ conductance may contribute to the enhanced contraction in SHR aorta. Additional mechanisms underlying the abnormal contraction in SHR aorta have also been suggested based on results obtained using tissue preparations. For example, Cl⁻ channel activation may also be involved in the mechanisms (44), and ryanodine receptor (RyR) contributes to the transient component of the acidic pH-induced contraction (45).

In vascular smooth muscles, BK channel consists of pore-forming α- and regulatory β-subunits, BK-α (KCNMA1) and BK-β₁ (KCNMB1), respectively. In combination, these subunits play a key role in a negative feedback mechanism for the regulation of myogenic tone with respect to Ca²⁺ sparks and Ca²⁺ influx in the resting state (7, 27). Previous works (33, 34) have indicated that K⁺ efflux through BK channels is increased in arterial smooth muscle cells from hypertensive rats and that the upregulation of BK channels in cell membranes during hypertension is regarded as a homeostatic mechanism for buffering vascular excitability. The upregulation of BK-α proteins in SHR aorta is interpreted as a compensatory mechanism in the setting of hypertension because this could reduce the enhanced Ca²⁺ influx through L-type Ca²⁺ channels (34, 37). Therefore, the overexpression of BK channels in cardiovascular pathologies, such as hypertension, can provide novel upregulation of disease-specific membrane targets for vasodilator therapies (19). Additionally, BK-β₁ subunit, which modulates Ca²⁺ and voltage sensitivity of BK-α subunit, appears to be associated with the regulation of blood pressure (7). Recent reports (4, 5) have shown, however, that downregulation of BK-β₁-subunit expression may be an integral component in the development of vascular dysfunction during hypertension. Additional studies are needed to address whether the expression of

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BK channel subunits is up- or downregulated in vascular smooth muscles of SHR.

Within the past 5 years, it has been demonstrated that some of the background K⁺ conductances in various types of cells, including vascular smooth muscle cells, are due to two-pore domain K⁺ channels (KCNK superfamily) (31). Two-pore domain weakly inward rectifying K⁺ channel (TWIK)-like acid-sensitive K⁺ channels (TASK1–5) are sensitive to changes in pH, and are also sites of action for volatile anesthetics and neurotransmitters (49). TASK channels can also modulate a wide range of numerous physiological and pathophysiological processes (32). For example, recent studies (20, 21, 53) have shown that TASK channels can contribute to the resting potential of vascular smooth muscles, such as pulmonary and carotid artery. However, little is known about expression and function of TASK channels in aortic smooth muscle either under control conditions or chronic hypertension.

The present study demonstrates that the enhancement of contraction of aortic smooth muscle in the setting of acidic pH, is associated with membrane depolarization in SHR aortic smooth muscle. Our results demonstrate that the changes in membrane potential are strongly modulated by upregulation of BK channel expression in SHR aortic smooth muscle. Furthermore, we have found that TASK-like currents are functionally expressed in WKY aortic myocytes and that TASK1 channel expression is downregulated in SHR aorta.

METHODS

Measurement of transmembrane potential. Aortic smooth muscle was dissected from male rats (SHR or WKY rats, 10–12 wk old) (Japan SLC, Shizuoka, Japan). Male rats were anesthetized with ether and euthanized by bleeding. All experiments were carried out in accordance with the “Guiding Principles for the Care and Use of Laboratory Animals” (the Science and International Affairs Bureau of the Japanese Ministry of Education, Science, Sports, and Culture) and also with the approval of the Ethics Committee in Nagoya City University. After endothelial cells were removed, the smooth muscle layer (2 × 5 mm) was pinned to the bottom in a 1-mL chamber and was perfused at a rate of 2–4 mL/min with HEPES-buffered Krebs solution. All experiments were done at room temperature (23 ± 1°C). For RNA preparations, −50 mV by square command pulses from myocytes perfused with the standard solution. All experiments were done at room temperature (23 ± 1°C). For RNA preparations, −50 mV by square command pulses from myocytes perfused with the standard solution. All experiments were done at room temperature (23 ± 1°C). For RNA preparations, −50 mV by square command pulses from myocytes perfused with the standard solution. All experiments were done at room temperature (23 ± 1°C). For RNA preparations, −50 mV by square command pulses from myocytes perfused with the standard solution. All experiments were done at room temperature (23 ± 1°C). For RNA preparations, −50 mV by square command pulses from myocytes perfused with the standard solution. All experiments were done at room temperature (23 ± 1°C). For RNA preparations, −50 mV by square command pulses from myocytes perfused with the standard solution.
Western blot analysis. Membrane fractions of the rat tissues (brain, kidney, and aorta) and HEK-293 transfectants were prepared as previously reported (38), and protein contents were measured with protein assay kit (Bio-Rad, Hercules, CA) with BSA as a standard. The protein samples were subjected to SDS-PAGE on 10% polyacrylamide gel, and the proteins were then transferred to polyvinylidene difluoride membrane (Bio-Rad). After transferring total proteins to the filter membrane, we stained them with Ponceau S staining solution to identify that the band density of the several major proteins was almost identical in all lanes. The blots were incubated with the affinity purified polyclonal antibodies specific for BK-α (1098–1196, Alomone, Jerusalem, Israel), BK-β1 (N-15 and Y-16, Santa Cruz Biotechnology, Santa Cruz, CA), TASK1 (N-15, Santa Cruz Biotechnology), or TASK2 (G-14, Santa Cruz Biotechnology) overnight and then incubated with anti-rabbit or goat horseradish peroxidase-conjugated IgG (Chemicon International, Temecula, CA) for 1 h. An enhanced chemiluminescence detection system (Amersham Biosciences) was used for the detection of the bound antibody. Resulting images were analyzed by a LAS-1000 (Fujifilm, Tokyo, Japan), and the digitized signals were quantitated with Image Gauge software (Fujifilm).

Immunocytochemistry. Isolated myocytes of the rat aorta were seeded onto glass-bottom dishes. Before being stained, isolated myocytes were fixed, permeabilized, and blocked as previously reported (38). Cells were then exposed to anti-TASK1 or TASK2 polyclonal antibody (1:50 dilution) for 12–16 h at 4°C. Excess primary antibody was removed by repeated washing with PBS, and the cells were exposed to Alexa Fluor 488 donkey anti-goat IgG antibody (1:200 dilution, Molecular Probes, Eugene, OR). Digital images were viewed on a scanning confocal microscope (LSM510, Carl Zeiss, Esslingen, Germany). As negative controls, cells were preincubated with excess antigen before the addition of primary antibody.

Statistics. Data were expressed as means ± SE. Statistical significance between two and among multiple groups was evaluated using Student’s t-test or Sheffe’s test after F-test or ANOVA. In some analyses, Williams test was used after ANOVA. In the figures, statistical significance is shown at P values of <0.05 and <0.01, as indicated.

RESULTS

Effects of acidic pH on membrane potential in SHR and WKY rats. To compare the effects of extracellular acidic pH on the membrane potential of smooth muscle cells in WKY and SHR aortas, measurements using conventional microelectrodes were performed on isolated, superfused tissue segments. As shown in Fig. 1A, the RMP in SHR aortas was −45.8 ± 0.9 mV, which was more depolarized than that in WKY aortas (−49.1 ± 0.6 mV, n = 8 for each, P < 0.05), as has been reported previously in another vascular smooth muscle (8). Progressive extracellular acidification from pH7.4 to 7.1, 6.8, and 6.5 resulted in membrane depolarization in both SHR and WKY aortas (Fig. 1A). The changes in RMP occurred immediately after the exchange of perfusion solutions and reached the steady state within a few minutes. RMP at pH7.5 was −43.2 ± 0.5 and −36.5 ± 0.8 mV in WKY and SHR aortas, respectively (n = 8 for each, P < 0.01), and the magnitude of membrane depolarization by acidification was significantly larger in the SHR (9.3 ± 1.3 mV) than in the paired WKY aortas (5.9 ± 0.4 mV, n = 8 for each, P < 0.05) (Fig. 1B).

Effects of acidic pH on outward currents in SHR and WKY aortic myocytes. When aortic myocytes were exposed to acidic external solution (pH6.5), the amplitude of outward currents elicited by depolarization was substantially reduced in both WKY and SHR groups (WKY, Fig. 2A, a and b; SHR, Fig. 2A, c and d). This effect was reversed on return to pH7.4 (not shown). The corresponding I-V relationships in aortas of WKY and SHR are shown in Fig. 2B, a and b, respectively. Average cell capacitance in aortic myocytes of SHR was 20.8 ± 1.8 and not significantly different from that of WKY; 20.2 ± 1.8 pF (n = 18 for each). The I-V relationships of differential outward currents between pH7.4 and 6.5 in Fig. 2B, a and b, were illustrated in Fig. 2C. The acidic pH-sensitive outward currents in SHR tended to be larger than those in SHR, but the difference was not statistically significant.

Effects of acidic pH on BK currents in SHR and WKY aortic myocytes. The contribution of the BK channel to total K+ conductance in vascular smooth muscles during hypertension is a topic of considerable interest, because of somewhat controversial results (4, 5, 11, 16, 34). In the present study, we first attempted to identify a K+ current component susceptible to 1 μM penitrem A, a specific blocker of BK channel (16), in SHR and WKY aortic myocytes. Application of 1 μM penitrem A decreased outward currents in both WKY and SHR (Fig. 3, A and B); note, however, that this decrease was significantly larger in SHR (5.30 ± 0.62 pA/pF) than in WKY rats (1.88 ± 0.62 pA/pF).
0.74 pA/pF at +40 mV, n = 8 for each, P < 0.05). It was, therefore, confirmed that the BK channel current was larger in aortic myocytes of SHR than those of WKY rats at pH 7.4.

In the next set of experiments, the pH-sensitive K⁺ currents were divided into two components: penitrem A-sensitive and -insensitive components. In the presence of penitrem A, the acidification to pHo 6.5 tended to decrease slightly the peak outward current in both WKY and SHR (Fig. 4A). Figure 4B, a and b, shows the I-V relationships of outward current components altered by acidification of the superfusate in the absence (closed squares) and presence (open squares) of 1 μM penitrem A in WKY and SHR, respectively. Acidic pH-sensitive BK currents at pHo 40 mV are 6.5 times larger in SHR aortic myocytes (2.6 pA/pF) than in WKY myocytes (0.4 pA/pF). In contrast, the contribution of non-BK-type K⁺ current component to the total acidic pH-sensitive current was much larger in WKY (76.8%) than in SHR (31.4%).

A recent report (4) has concluded that the downregulation of BK-β₁ is a cause of hypertension in arterial smooth muscles of angiotensin II-infused hypertensive rats. Moreover, the expression level of BK-β₁ is higher in the arterial smooth muscles of normotensive Sprague-Dawley rats than in those in WKY and SHR (5). In the present study, we determined BK-β₁ and -β₂ expression in SHR and WKY aortas using real-time PCR and Western blot analysis (Fig. 5). To quantify the transcriptional expression of BK channel subunits, we first performed the conventional RT-PCR, and we confirmed no nonspecific bands, such as primer dimmers and the specificity of PCR products (not shown). The expression levels of BK-α and -β₁ transcripts were not significantly different between SHR and WKY aortas (Fig. 5A). In contrast, the immunoreactive signal of the BK-α proteins in SHR aorta was 1.54 ± 0.15-fold larger than the corresponding signal in WKY aorta (n = 4 for each, P < 0.05), whereas that of BK-β₁ protein was not significantly different (1.05 ± 0.17-fold, n = 4 for each, Fig. 5B). When specific antibodies were preincubated with peptides against which the antibodies were generated, the immunoreactive signals were faint (not shown). These findings indicate that upregulation of BK-α proteins in SHR aortic myocytes is responsible for the increase in BK channel current and that the expression of β₁-subunit protein is not changed and, therefore, is not involved in the mechanism.

Membrane potential changes by acidic pHo under block of BK channels. The results shown above suggest that the acidic pH-induced membrane depolarization in aortas is mainly attributable to the decrease in BK channel activities and that the larger depolarization in SHR than in WKY rats is due to larger contribution of BK channels to RMP in SHR. In the next series of experiments, other K⁺ channel current components, which might be also responsible for the regulation of RMP by pHo,
Fig. 3. Effects of a large-conductance Ca\(^{2+}\)-activated K\(^+\) (BK) channel blocker penitrem A on outward K\(^+\) currents in WKY and SHR aortic myocytes. A: I-V relationships of averaged data show changes in outward current density in the absence (○) and presence (▲) of 1 μM penitrem in aortic myocytes of WKY (a) and SHR (b) at pH\(_6\), 7.4. Currents were elicited by 10-mV depolarizing steps from −60 to +40 mV (n = 8 for each). B: I-V relationships show penitrem A-sensitive K\(^+\) current density calculated from A in aortic myocytes of WKY and SHR. *P < 0.05 and **P < 0.01 vs. pH\(_6\), 7.4.

Fig. 4. Analyses of acidic pH-sensitive outward currents using penitrem A in WKY and SHR aortic myocytes. A: I-V relationships were obtained in the presence of 1 μM penitrem A at pH\(_6\), 7.4 and 6.5 in aortic myocytes of WKY (a) and SHR (b). B: acidic pH-sensitive outward current components in the absence (●) and presence (▲) of 1 μM penitrem A in WKY (a) and SHR (b). Acidic pH-sensitive outward current components in the absence of penitrem A were derived from data in Fig. 2C. The component in the presence of penitrem A was obtained by subtracting value of closed circle from that of open triangle at each voltage in A, a and b. *P < 0.05 vs. pH\(_6\), 7.4.
Fig. 5. Expression of BK-α and BK-β₁ subunits in SHR and WKY aortas. A: quantitative PCR for expression of BK-α and BK-β₁ mRNA relative to that of GAPDH in SHR and WKY aortas. B: expression of the BK-α (a) and BK-β₁ (b) proteins was analyzed by Western blot analysis. Membrane proteins extracted from SHR and WKY aortas were immunoblotted with anti-BK-α and anti-BK-β₁ antibodies. Bc: molecular mass of standards (BK-α, 120 kDa; and BK-β₁, 25 kDa). Bc: amount of expressed BK-α and BK-β₁ proteins was evaluated using arbitrary units of optical density, and expression levels were compared between WKY aorta and SHR aorta (n = 4 for each). BK-α protein expression was significantly higher in SHR aorta compared with WKY aorta. *P < 0.05 vs. WKY BK-α.

Fig. 6. Effects of pHo on RMP in the presence of penitrem A (PenA). A: RMP at pHo 7.4, 7.1, 6.8, and 6.5 in WKY and SHR in the presence or absence of 1 μM penitrem A. RMP was measured with conventional microelectrodes in superfused tissue segments. After penitrem A was added, the pHo was changed from 7.4 to 6.5 and to 8.0 in each preparation. **P < 0.05 and ***P < 0.01 vs. WKY. #P < 0.05 and ##P < 0.01 vs. RMP in control of each strain. $P < 0.05 and $$$P < 0.01 vs. RMP in the presence of penitrem A at pH 7.4 in each strain. Six preparations were used from each WKY and SHR. B: difference between RMPs at pHo 7.4 and those pHo 6.5 or 8.0 in the presence of penitrem A. Results were replotted using data shown in A. *P < 0.05 and **P < 0.01 vs. WKY.
by TEA and 4-AP as has been shown in experiments based on heterologous expression of these cloned channels (15). Alkalization to pHo 8.0 from 7.4 increased the currents at −100 mV to 210 ± 15 and 263 ± 37% of the control in WKY and SHR aortic myocytes, respectively (n = 7 for each, P < 0.01 vs. control) (Fig. 7B). The acidification to pHo 6.5 decreased the currents in WKY aortic myocytes to 49 ± 8% (n = 7, P < 0.01 vs. control). The values of current density at −100 mV in three different pHo solutions were summarized in Fig. 7C.

Halothane-sensitive K⁺ channel currents in SHR and WKY aortic myocytes. To confirm that the currents recorded at potentials negative to −30 mV were mainly due to TASK channels, we took advantage of the fact that they can be activated by volatile anesthetics, e.g., halothane (41). Results obtained using the same ramp-voltage protocol were shown in Fig. 8. In the external bathing solution, 300 μM Cd²⁺, 1 μM glibenclamide, and 1 μM penitrem A were added to the 140 mM K⁺ external solution. Application of 1 mM halothane at pHo 7.4 increased the inward currents in negative potential range in WKY rats (Fig. 8A,a). The change in current density at −100 mV, following application of 1 mM halothane, was 0.41 ± 0.18 and 0.16 ± 0.05 pA/pF in WKY and SHR (Fig. 8A,b, n = 6). The relative increase in the current at −100 mV was significant in WKY rats (181 ± 21% of the control, n = 6, P < 0.05 vs. control) but not in SHR (51 ± 25%, n = 6). Figure 8B shows summarized data of changes in halothane-induced currents by acidification (pHo 6.5) and alkalization (pHo 8.0) in WKY and SHR. The current amplitude at −100 mV and pHo 7.4 in the absence of halothane was taken as 1.0.

At pHo 8.0, 1 mM halothane increased the currents to 425 ± 89% (n = 6) at −100 mV in WKY aortic myocytes, and halothane also increased the currents to 188 ± 24% at pHo 7.4 and 129 ± 31% (n = 6 for each) at pHo 6.5. In contrast, although pH-sensitive conductance was present in SHR aortic myocytes, they were resistant to 1 mM halothane. In combination, these results suggest that TASK-like currents have a substantial role in acidic pH-sensitive background K⁺ channel currents in aortic myocytes in WKY rats, and the currents in SHR tend to be substantially smaller than those in WKY rats.

In Fig. 9, effects of halothane on RMP in WKY and SHR were examined using a microelectrode technique. Application of 1 mM halothane resulted in membrane hyperpolarization (2.1 ± 0.5 and 1.3 ± 0.2 mV in WKY and SHR, respectively), which was effectively inhibited by the addition of 3 μM methanandamide, a potential TASK channel blocker (35). It is notable that the addition of methanandamide did not lead the RMP to the level more depolarized than the initial one before the application of halothane.

Expression of TASK transcripts and proteins in SHR and WKY aortas. To obtain molecular biological evidence supporting the functional expression of TASK channels in aortic myocytes, we first performed RT-PCR analyses in isolated aortic myocytes of SHR and WKY rats (multicell PCR). TASK1–4 but not TASK5 signals were detected in rat aortic myocytes of SHR and WKY rats (Fig. 10A). Similar results were obtained from four separate experiments. The specificity of each PCR product was confirmed by DNA sequence anal-
ysis. Thus it is clear that TASK1–4 mRNAs were present in aortic myocytes.

Quantification of steady-state transcript levels was then performed using real-time PCR. cDNAs were prepared from SHR and WKY aortas, and steady-state transcripts were determined relative to an endogenous control housekeeping gene (GAPDH). In SHR aorta, the expression of TASK1–5 relative to GAPDH was $0.015 \pm 0.001$, $0.018 \pm 0.001$, $0.005 \pm 0.001$, $0.005 \pm 0.001$, and $0.005 \pm 0.001$, respectively ($n = 6$ for each, Fig. 10B). In WKY aorta, the expression was $0.029 \pm 0.004$, $0.019 \pm 0.004$, $0.006 \pm 0.002$, $0.020 \pm 0.002$, and $0.005 \pm 0.001$, respectively ($n = 6$ for each). These results suggest that TASK1, -2, and -4 transcripts are predominantly expressed in rat aorta, and the expression level of TASK1 in SHR is $50\%$ of that in WKY rats ($P < 0.05$). As positive controls, the expression levels of TASK1, -2, and -3 transcripts were also determined in rat heart, kidney, and brain, respectively, and were $0.067 \pm 0.009$, $0.061 \pm 0.004$, and $0.082 \pm 0.004$, respectively (not shown, $n = 5$ for each).

To complement and strengthen these findings, we determined the expression of TASK proteins in aortas of SHR and WKY rats by Western blot analyses using antibodies specific

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**Fig. 8.** Halothane sensitivity of TASK-like currents in SHR and WKY aortic myocytes. A: application of 1 mM halothane enhanced inward currents elicited by ramp depolarization from $-100$ to $+40$ mV for 1,000 ms in aortic myocytes from WKY (a) and SHR (b) at pH 7.4 in 140 mM K$^+$ external solution. Data (b) show that current density increased by application of 1 mM halothane was measured at $-100$ mV ($n = 6$ for each). B: current amplitude at pH 6.5, 7.4, and 8.0 in WKY (a) and SHR (b) aortic myocytes in the absence (○) and presence (△) of 1 mM halothane. Each current amplitude at $-100$ mV is shown as relative values, taking current density at pH 7.4 in the absence of halothane as 1.0 ($n = 6$ for each). *$P < 0.05$ vs. control.

**Fig. 9.** Halothane-induced hyperpolarization in WKY and SHR aortas. A: RMP was measured with conventional microelectrodes in superfused tissue segments. Application of 1 mM halothane induced hyperpolarization in WKY and SHR aortas that was blocked by 3 μM methanandamide (methAEA). B: effects of halothane and methAEA.

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for TASK1 or -2. The band recognized by the anti-TASK1 antibody was 60 kDa in both heart and aorta membranes (Fig. 10C, top blot). The densitometric analysis revealed that TASK1 protein levels were 1.52 ± 0.26-fold more abundant in WKY aorta than in SHR aorta (n = 4, P < 0.05). Similarly, the band recognized by the anti-TASK2 antibody was 50 kDa in kidney membranes and was detected at almost the same molecular size in aorta membranes (Fig. 10C, bottom blot). Densitometric analysis revealed that the expression of TASK2 proteins in SHR aortas was similar to that in WKY aortas (n = 4). These bands were specifically blocked when the anti-TASK1 or anti-TASK2 antibody was preincubated with the fusion protein against which the antibody was generated (not shown). These results are consistent with the expression levels of TASK1 transcripts determined by using real-time PCR.

To confirm that TASK1 proteins are expressed on the surface membranes in aortic myocytes, the subcellular localization of TASK1 proteins was examined by using an immunocytochemical approach. Freshly isolated myocytes from aortas of SHR and WKY rats were stained with anti-TASK1 antibody, and the local distribution of immunoreactivity was visualized with laser-scanning confocal microscopy. The staining patterns for anti-TASK1 antibody were localized along cell membrane in both types of myocytes (Fig. 10D). TASK1 signals were completely removed by preincubation with the excess antigen (not shown). On the other hand, the staining pattern for anti-TASK2 antibody was faint and diffused over whole cell area in both types of myocytes. In control experiments, TASK2 proteins were detected at cell membrane in HEK-293 cells transfected with TASK2 cDNA (not shown). These results suggest that TASK1 is a major type of TASK subfamily expressing in aortic myocytes and that the down-regulation of TASK1 in SHR aorta may result in the lower density of halothane-sensitive background K⁺ currents in SHR aortic myocytes than those in WKY aortic myocytes.

**DISCUSSION**

**Involvement of BK channel inhibition in acidic pH-induced contraction.** It is well known that vascular tone is strongly modulated by membrane potential in a number of different vascular smooth muscles. In many of these preparations, membrane potential is predominantly controlled by K⁺ conductances (37). These K⁺ conductances may be altered in some pathological conditions, such as hypertension, stroke, and/or atherosclerosis. These primary electrophysiological defects are, in part, responsible for changes in cellular function in these diseases (47).

The present results confirm that aortic smooth muscle cells of SHR are more depolarized than those of WKY rats at rest. Although this net depolarization is small, it is significant and may be important functionally. Moreover, the depolarization induced by acidification is larger in SHR than in WKY rats. These results strongly suggest that the resting K⁺ conductance is reduced in aortic smooth muscle cells of SHR compared with that of WKY rats. Previously, the expression of Kv channels,
which are responsible for the RMP and membrane excitability (12), has been reported to be lower in SHR than in WKY rats (13).

In contrast, a number of different patterns of BK channel activity in the arterial smooth muscles of hypertensive rats have been reported. Liu et al. (33, 34) have concluded that upregulation of BK channel activity and BK-α protein expression is responsible for homeostatic regulation of the resting tone of aorta during chronic hypertension. In contrast, it has been also reported that downregulation of BK-β1 expression is associated with the abnormal vasoconstriction under pathological conditions, such as in the setting of hypertension and cerebral vasospasm (3–5). In the present study, Western blot analyses confirmed upregulation of BK-α expression and no reduction of BK-β1 expression in SHR aorta, and our electrophysiological measurements confirmed functional upregulation of BK channel current. Moreover, the fact that the depolarization induced by 1 μM penitrem A in aortic smooth muscles of SHR was significantly larger than that of WKY rats supports the larger contribution of BK channels to the RMP regulation in aortas in SHR than in WKY rats.

It has been well established that BK channel activity is inhibited by intracellular acidification in several types of smooth muscles (22, 30). The augmented depolarization after acidification of the superfusate in SHR aorta may be attributable to the increased expression and activity of BK channels compared with that in controls (WKY). The membrane depolarization that occurs in response to acidification in aorta may be partly due to the block of the BK channels. Although the contribution of BK channel activity to RMP in aorta is thought to be larger in SHR than WKY rats at standard pH, the overall resting K+ conductance is smaller in SHR, which could account for the more depolarized RMP in SHR than in controls. The contribution of BK channel activity to RMP is based on underlying temporally linked bursts of Ca2+ release from sarcoplasmic reticulum, so called Ca2+ sparks that elicit activation of nearby BK channels. The resulting spontaneous transient outward currents play a significant functional role in modulation of vascular tone (23, 25, 37). The corresponding changes of RyR gating or permeability after acidosis in rat aorta and the interrelationship between RyR and BK channels in SHR remain to be determined but are unlikely to be strongly modulated by pH+.

In addition to BK channels, other KV channels may also play a substantial role in the regulation of RMPs in different types of smooth muscle. Some of these subtypes (e.g., Kv1.4 and Kv1.5) can be inhibited significantly by acidic conditions (9, 48). Several recent reports, including a previous study from Imaizumi’s laboratory (40), have shown that Kv1.2, Kv1.5, Kv2.1, and Kv9.3 are predominant components of K+ currents in rat aorta. However, Kv1.2, which is insensitive to acidosis (48), exhibits the highest expression level in SHR and WKY aortas (13). Our present study showed that the Kv current density in SHR aortic myocytes, which was evaluated based on the K+ current component resistant to penitrem A, was not significantly different in SHR myocytes compared with that in WKY myocytes (Fig. 3). This result suggests that Kv currents may not be involved in the enhancement of acidic pH-induced contraction in SHR aorta. In contrast, acidosis-induced vasodilation is mediated by the activation of KAATP channels in canine basilar artery (29) and by activation of BK channels in porcine coronary artery (22). In the present study, we used penitrem A to determine the contribution of BK channels to acid-sensitive current components in WKY and SHR. The pharmacological profile is advantageous, such as selectivity to BK channels, the potency, and the interaction with α-subunit of BK channels; nevertheless, a further line of evidence could be required for total understanding of the contribution of BK channels.

Possible involvement of TASK channels on pH-sensitive K+ currents. Recent studies have suggested that TASK-like channels may contribute to the maintenance of the RMP in pulmonary artery smooth muscles (20), as well as in several other tissues (31). Gurney et al. (21) have shown that TASK1 channels are major contributors to the resting potential in pulmonary arterial smooth muscle and have argued that these channels are responsible for hypoxic pulmonary vasoconstriction. The present study shows that the activation of halothane-induced membrane hyperpolarization and corresponding K+ currents in aortas of WKY rats. The alkalization also induced large-membrane hyperpolarization and markedly enhanced the halothane-induced current in the presence of BK channel blocker in WKY aortas. However, the acidification did not induce significant depolarization in WKY aortas under the block of BK channels. These results strongly suggest that TASK-like K+ channels are functionally available in WKY aortas and that these channels may not significantly contribute to RMP at pH+ 7.4 but do regulate RMP when substantially activated by alkalization and/or halothane.

Maingret et al. (35) have reported that anandamide is a selective blocker of TASK1. In the present study, the halothane-induced membrane hyperpolarization in WKY aortas was completely blocked by methanandamide, suggesting the functional expression of TASK1 in aorta. It is also notable that the addition of methanandamide removed the halothane-induced hyperpolarization but did not induce further depolarization. This finding gives a support for the interpretation that TASK1 does not substantially contribute to RMP regulation in physiological conditions at pH+ 7.4. However, anandamide is also known as a potential blocker of delayed rectifier KV channels in vascular smooth muscles (50, 51). Indeed, Poling et al. (42) have shown that Kv1.2, which is the KV channel mainly expressed in rat aorta, is significantly inhibited by anandamide. Therefore, pharmacological tools selective to TASK subtypes are required for the further analyses of TASK-like current functions. In contrast to WKY rats, the acidification induced significant depolarization in the presence of BK channel blocker in SHR. The pH+-sensitive current in SHR aorta was not activated by halothane. These pH+-sensitive non-BK channel current components in SHR remain to be determined.

To elucidate the molecular mechanism underlying differences in TASK-like current density in aortas of WKY and SHR, we also documented differential expression of TASK genes. Overall, the TASK1, TASK2, and TASK4 transcripts were abundantly expressed in aortas of both strains. Their relative abundance was TASK1 > TASK2 = TASK4 >> TASK3 >> TASK5 in WKY rats and TASK1 = TASK2 = TASK4 >> TASK3 >> TASK5 in SHR. The expression level of TASK1 was significantly lower in SHR aorta compared with WKY aorta, and no significant differences in TASK2–5 expression were found between the strains. We
have also focused on the expression of other KCNK members and determined the expression of several members of KCNK: KCNK1 (TWIK1), -2 (TWIK-related K+ channel opener, in vascular smooth muscle. J Clin Invest 112: 717–724, 2003.


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