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The mechanism of acidic pH-induced contraction in aortae from SHR and WKY rats enhanced by increasing blood pressure

[1]Ken-Ichi Furukawa, Junji Komaba, Hiroyuki Sakai & Yasushi Ohizumi

Department of Pharmaceutical Molecular Biology, Faculty of Pharmaceutical Sciences, Tohoku University, Aoba, Aramaki, Aoba-ku, Sendai 980, Japan

1 Effect of pH on vascular smooth muscle contraction was analyzed by use of biochemical and pharmacological techniques.

2 In the aorta isolated from spontaneously hypertensive rats (SHR) decreasing extracellular pH (pH_o) caused a rapid acidification of intracellular pH accompanied by a pH_o-dependent increase in tension. The contraction of the SHR aorta was remarkable compared with that of the Wistar Kyoto rat (WKY) aorta.

3 Removal of NH_4Cl caused a transient decrease in intracellular pH followed by a marked increase in tension.

4 Both contraction and intracellular Ca^{2+} mobilization induced by acidic pH_o were markedly inhibited by removal of extracellular Ca^{2+}, verapamil and adenosine, whereas these were not affected by tetrodotoxin or Gd^{3+}, a stretch-activated cation channel blocker. Furthermore, cromakalim (a K^+ channel opener) inhibited acidic pH_o-induced contraction (APIC).

5 Acidic pH_o induced a depolarization of cultured smooth muscle cells from SHR aorta.

6 Blood pressure elevated with increasing age of WKY and SHR accompanied by an increase in APIC. Feeding WKY with N^G-nitro-L-arginine, an inhibitor of nitric oxide synthases caused a marked elevation of their blood pressure followed by an increase in APIC.

7 These results suggest that APIC is caused by Ca^{2+} influx mediated through the activation of voltage-sensitive Ca^{2+} channels mainly due to acidic pH_o-induced depolarization of the plasma membrane of smooth muscle cells. It is also suggested that APIC is strengthened by the elevation of blood pressure.

Keywords: Spontaneously hypertensive rats; vascular smooth muscle; hypertension; blood pressure; acidosis; Ca^{2+} mobilization; voltage-sensitive Ca^{2+} channel; K^+ channel; fura-2; N^G-nitro-L-arginine

Introduction

Contractions of vascular smooth muscle are initiated by an increase in intracellular Ca^{2+} concentrations ($[Ca^{2+}]_i$) which follows various physiological stimuli (Somlyo, 1985). The contractile state can be modified by several factors including pH. Ischaemia causes various disturbances including hypoxia and acidosis in the cirulatory system (Levine, 1993). Although physiological pH of body fluid is maintained at around 7.4, ischaemia easily decreases the pH value to 6.5 or lower (Butwell *et al.*, 1993). Changes in extracellular pH (pH_o) affect intracellular pH (pH_i) (Yu *et al.*, 1991; Austin & Wray, 1993) and both pH_o and pH_i are known to alter vascular tone and thereby affect the circulation (Gaskell, 1980; Gardner *et al.*, 1988; Danthuluri *et al.*, 1989; Austin & Wray, 1993). pH has an important function in the regulation of intracellular events, so acidosis may cause functional disorders. However, effects of pH on vascular smooth muscle in physiological and pathophysiological conditions have been unclear. Adenosine is thought to be produced and released from various tissues as an endogenous protective agent in ischaemia, but the detailed protective mechanism is unresolved (Rudolphi *et al.*, 1993).

In spite of substantial studies including study of genes related to the disease, the mechanism that contributes to the aetiology and pathogenesis of essential hypertension remains unknown. The spontaneously hypertensive rat (SHR) is a useful model for studying mechanisms that may be responsible for essential hypertension. It has been reported that there are several functional abnormalities in vascular tissue of SHR compared to that of normotensive Wistar Kyoto rats (WKY) (Asano *et al.*, 1993; Batra *et al.*, 1993; Clark *et al.*, 1993; England *et al.*, 1993; Jaiswal *et al.*, 1993; Kuttan & Sim, 1993; Le Jemtel *et al.*, 1993; Orlov *et al.*, 1993; Schirner & Taube, 1993). Recent studies have revealed the abnormal Na^+/H^+ exchange in hypertension, suggesting the participation of abnormal pH control in the pathogenesis of hypertension (Rosskopf *et al.*, 1993).

The present study was carried out to clarify the effects of acidic pH_o on the rat thoracic aorta isolated from normo- and hypertensive rats. We have shown for the first time that the aorta from hypertensive rats is much higher sensitive to acidic pH_o than that from normotensive rats and that there is a close correlation between the elevation of blood pressure and hypersensitive contractile response to acidic pH_o. In addition, we discuss a possible mechanism of acidic pH_o-induced contraction of rat aorta. These results may contribute to the clarification of not only pathophysiological but also biochemical and physiological roles of pH in vascular smooth muscle functions.

Methods

Recording of blood pressure

The carotid arteries of rats were exposed and polyethylene tubes (PE 60; Clay Adams, Parsippany, U.S.A.) were inserted while under pentobarbitone anaesthesia (40 mg kg^{-1} body weight). The carotid arteries were connected to a pressure transducer (Nihon Koden, Tokyo, Japan) and the mean arterial blood pressures were recorded on a graph for at least 10 min until reaching a plateau.

[1] Author for correspondence.

Measurements of isometric contraction

After recording the blood pressure, rats were killed by cervical dislocation. The thoracic aorta was dissected and connective tissues were carefully removed. The endothelium was removed by gently rubbing the endothelial surface with cotton pellets. The lack of endothelium was checked by the abolition of acetylcholine-induced relaxation. The aorta was cut into helical strips approximately 1.5–2 mm in width and 20 mm in length. One end of the strip was secured to the glass tissue holder by a silk ligature and the other end was connected to a force-displacement transducer. The strip was suspended in a 20 ml organ bath containing HEPES-buffered Krebs solution of the following composition (mM): NaCl 120, KCl 4.8, $MgSO_4$ 1.3, glucose 5.8, $CaCl_2$ 1.2, KH_2PO_4 1.2, $NaHCO_3$ 12.6, HEPES 10. The solution was gassed with 95% O_2: 5% CO_2. pH of the solution was changed from a control value of 7.4 by addition of either NaOH or HCl and monitored from time to time with a handy type pH meter (Horiba, B-212). The tissues were equilibrated for 1 h under a resting tension of 1 g. Isometric contraction was then measured by the transducer. The strip was precontracted 3 times by adding KCl (final concentration was 60 mM). There was no significant difference in responses to KCl (60 mM) between hypertensive (SHR and N^G-nitro-L-arginine-treated WKY) and normotensive WKY rats. Therefore, we employed contraction induced by KCl (60 mM) as an internal standard and acidic pH_o-induced contraction (APIC) have been normalized to the KCl-induced contraction.

Simultaneous measurements of pH_i and contraction

The fluorescent pH indicator dye, 2,7-*bis*(carboxyethyl)carboxyfluorescein (BCECF) was used to monitor changes in pH_i. Spiral strips of aorta were loaded with BCECF by incubating with 10 μM BCECF-acetoxymethylester for 3–5 h at 37°C in the same HEPES buffered-Krebs solution as described above except that a non-cytotoxic detergent, cremophor EL (0.05%) was included. The strip was mounted horizontally in a bath (5 ml, 37°C) attached to a fluorimeter (CAF-100, Japan Spectroscopic, Tokyo, Japan). Contraction and BCECF fluorescence (excitation at 440 and 500 nm, emission at 530 nm) were measured simultaneously. To calculate the pH_i the ratio of two fluorescence intensities (R500/440) was calibrated using nigericin (6 mg ml^{-1}) in 120 mM K^+ buffer as described previously (Kurtz & Golchini, 1987).

Simultaneous measurements of $[Ca^{2+}]_i$ and contraction

The level of cytoplasmic Ca^{2+} was monitored by use of fura-2 as a fluorescent Ca^{2+} indicator. Spiral strips of aorta were loaded with fura-2 by incubating with 20 μM fura-2-acetoxymethylester for 3–5 h at 37°C in HEPES-buffered Krebs solution including cremophor EL (0.05%). After the strip was mounted horizontally in a bath (5 ml, 37°C) attached to a fluorimeter (CAF-100, Japan Spectroscopic, Tokyo, Japan), contraction and fura-2 fluorescence were measured simultaneously. The intensities of 500 nm fluorescence induced by excitation at 340 nm (F340) and 380 (F380) were measured. The ratio of these two fluorescence values (R340/380) was calculated as an indicator of the relative cytosolic Ca^{2+} level. The absolute $[Ca^{2+}]_i$ was not calculated because the dissociation constant of fura-2 and Ca^{2+} in cytosol may be different from that obtained *in vitro* (Karaki, 1989; Mitsui & Karaki, 1990).

Measurement of membrane potential of primary cultured vascular smooth muscle cells

Vascular smooth muscle cells (VSMCs) were isolated from rat aorta (250–300 g, SHR male rat) by enzymatic dispersion as described by Chamley *et al.* (1977). The resulting cells were seeded onto sterile glass cover slips laid in a 100 × 100 mm square dish for measurements of membrane potential. The cells were cultured for 4 to 5 days in Dulbecco's modified Eagle's medium supplemented with 10% heat-inactivated foetal calf serum, 10 units ml^{-1} penicillin, and 100 μg ml^{-1} streptomycin. After reaching confluency, cells were cultured in serum-free medium (Cosmedium 001) for additional 24 h to enhance differentiation. Membrane potential changes were monitored with a potential-indicator dye diS-C_3(5) (Sims *et al.*, 1974; Furukawa *et al.*, 1991) under conditions similar to those used for measurement of contraction. Cultured smooth muscle cells attached to glass coverslips were preincubated with 0.5 μM diS-C_3(5) at 37°C for 5 min in PBS before assaying fluorescence. Fluorescence measurements were carried out with excitation at 620 nm and emission at 680 nm, respectively, in a fluorimeter (F-2000, Hitachi, Tokyo, Japan). Fluorescence signal was calibrated by changing extracellular K^+ concentrations in the presence of 0.5–1.0 μM valinomycin, which enabled us to determine the extracellular K^+ concentration for which there was no change in fluorescence. The membrane potential was estimated using Nernst's equation by assuming that it equals the equilibrium potential for K^+ in the presence of valinomycin. The cytosolic K^+ concentration was assumed to be 156 mM (Jones, 1982).

Feeding

Rats were fed with plain commercial diet (SP; Funabashi Farm, Chiba, Japan) or that containing 0.023% N^G-nitro-L-arginine for 3 to 20 days before the start of the experiment. Food and water were available *ad libitum*.

Materials

Male normotensive Wistar-Kyoto (WKY) (strain: NCrj) and spontaneously hypertensive rats (SHR) (strain: NCrj) were purchased from Charles River Japan (Kanagawa, Japan). Nifedipine, verapamil, diltiazem, cromakalim and tetrodotoxin were from Sigma Chemical Co. (St. Louis, Missouri, U.S.A.) N^G-nitro-L-arginine was from Aldrich Chemical Company (Milwaukee, Wisconsin, U.S.A.). Gadolinium-hydrochloride, aspirin, adenosine and phentolamine were from Wako Pure Chemical Industry (Tokyo, Japan). Cremophor EL was from Nakarai Tesque Inc. (Kyoto, Japan). Other reagents used in this work were all analytical grade.

Statistics

The values given in figures are means ± s.e.means, unless otherwise stated, and the statistical analysis was performed by Student's *t* test. The *n* values indicate the number of animals.

Results

Effect of extracellular pH change on the tension in rat aortic strips

Effects of changes in extracellular pH (pH_o) on the aortic strips from WKY and SHR (11 weeks old) were first investigated (Figure 1). Decreasing pH_o from 7.4 to 6.5 caused a remarkable and sustained increase in tension in the SHR aorta being nearly equal to one induced by 60 mM KCl (Figure 1a). In the case of WKY the pH_o change caused a small but significant increase ($P<0.05$) in tension (Figure 1c). Increasing pH_o from 7.4 to 8.0 slightly increased the tension in both cases of WKY (data not shown) and SHR (Figure 1b). These responses of aortic strips to pH change were reproduced at least 3 times. As shown in Figure 2, the increase in tension induced by decreasing pH_o was clearly dependent on pH_o in both cases of WKY and SHR. The tension increased with decreasing pH_o at acidic pH_o lower than 7.5, having a peak at pH_o 6.5 and decreased by further decreasing pH_o. The tension in the SHR aorta was much greater than that in the WKY aorta over the range of pH_o from 5.5 to 7.0. The presence of endothelium did not affect the results described above (data not shown).

Relationship between tension and pH_i

Intracellular pH (pH_i) of the SHR (10 weeks old) aorta was monitored using BCECF as a fluorescent pH indicator. Resting pH_i was 7.3 ± 0.04 ($n=5$) at pH_o 7.4. Decreasing pH_o from 7.4 to 6.5 caused a rapid decrease in intracellular pH accompanied by an elevation of tension (Figure 3a, left traces). Figure 3b clearly shows the pH_o-dependency of pH_i. The ratio for the change in pH_i per unit change of pH_o was 0.89.

To investigate the effect of change in pH_i on contractile force more directly, NH_4Cl treatment was employed. Figure 4 shows representative traces of pH_i and contractile force. Resting pH_i was 7.35 ± 0.07 ($n=4$). Addition of 40 mM NH_4Cl caused an alkalinization of intracellular fluid (7.71 ± 0.12, $n=4$) accompanied by a faint increase in the tension. Removal of NH_4Cl resulted in a transient decrease in pH_i (6.95 ± 0.10, $n=4$) attended by a marked increase in the tension. DIDS, a representative blocker of Cl^-/HCO_3^- exchanger, inhibited both the decrease in pH_i and APIC induced not only by decreasing pH_o but also by the NH_4Cl-washout acidification (data not shown).

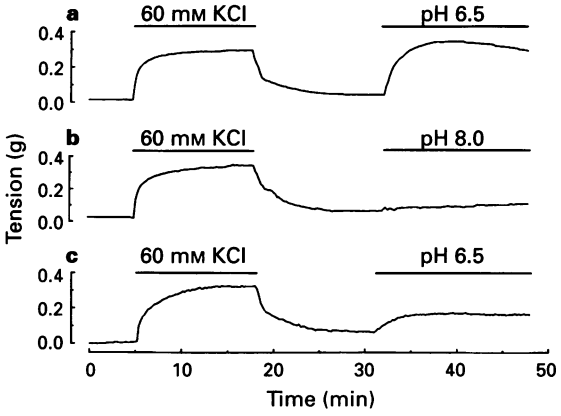

Figure 1 Typical recordings of the contractile effects of changing pH_o on resting strips of aorta. Spiral strips of thoracic aortae from 11-week-old SHR (a, b) and WKY (c) were precontracted 3 times by adding 60 mM KCl and then pH_o was changed. Three traces show the last trial of KCl (60 mM) and following pH_o change. External pH was changed from 7.4 to 6.5 (a and c) or 8.0 (b).

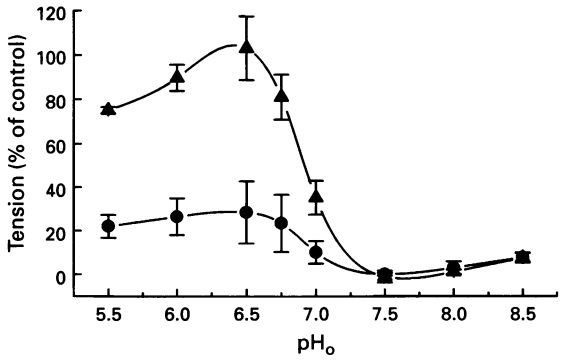

Figure 2 The relation between pH_o and contraction evoked by changing pH_o. Spiral strips of thoracic aortae from 11-week-old WKY (●) and SHR (▲) were precontracted 3 times by adding 60 mM KCl and then the contractions evoked by changing pH_o from 7.4 to various pH (5.5 to 8.0) were observed. The contractile response to the last trial of 60 mM KCl at pH 7.4 is expressed as 100%. $n=4$–10.

Effects of several drugs on APIC

Effects of several drugs on the APIC (pH 6.5) in SHR (11 weeks old) were investigated (Figure 5). Verapamil (1 μM), a representative blocker of voltage-sensitive Ca^{2+} channels and adenosine decreased APIC by approximately 90%. However, tetrodotoxin (10 μM), a potent Na^+ channel blocker, Gd^{3+} (100 μM), a blocker of stretch-activated cation channels, phentolamine (10 μM), an α-adrenoceptor antagonist and aspirin (1 mM), a cyclo-oxygenase inhibitor failed to inhibit

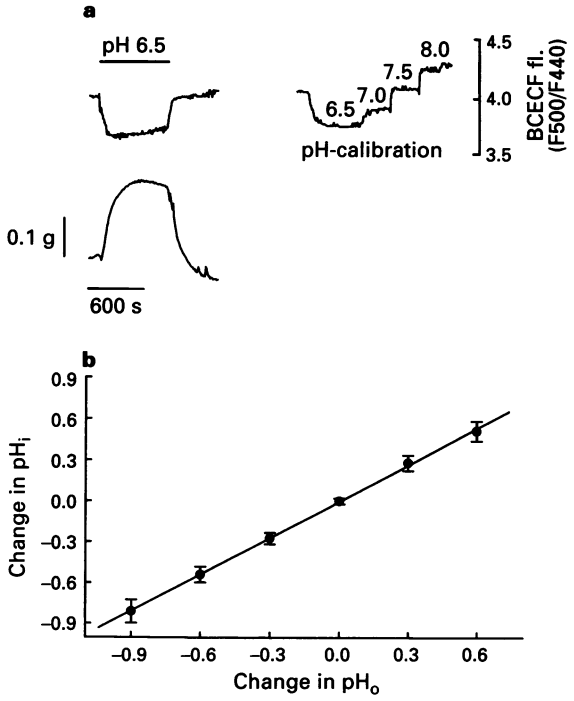

Figure 3 Intracellular pH change and contraction of aortic strips from SHR induced by acidic pH_o. Aortic strips from SHR (10 weeks old) were loaded with BCECF and then simultaneous measurements of intracellular pH and contraction were carried out. After precontraction with 60 mM KCl, the strips were contracted by changing pH_o from 7.4 to 6.5; then intracellular pH was calibrated by nigericin-high KCl method as described under Methods. (a) Representative traces of pH_i and contraction. Four preparations from different animals were used. (b) The relation between the change in pH_o and the subsequent change in pH_i ($n=6$). The regression line fitted has a correlation coefficient of 0.99 and a slope of 0.89 ± 0.01.

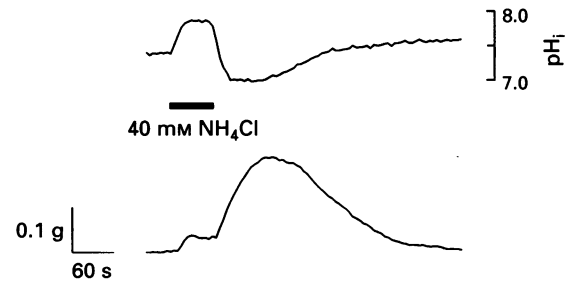

Figure 4 Effects of addition and washout of ammonium chloride on pH_i. Aortic strips from SHR (11 weeks old) were loaded with BCECF and then simultaneous measurements of intracellular pH (upper trace) and contraction (lower trace) were carried out. After precontraction with 60 mM KCl, the strip was incubated with 40 mM NH_4Cl for 1 min and then NH_4Cl was removed by washing with fresh buffer. Four preparations from different animals were used.

APIC. Contraction evoked by NH_4Cl-induced acidification showed the same responses to these drugs (data not shown). These results suggest that APIC is caused by membrane depolarization of the aorta. Therefore, the effect of cromakalim, a potent activator of K^+ channels was investigated to clarify the involvement of depolarization in the mechanism of APIC (Figure 6). Cromakalim inhibited APIC as well as KCl-induced contraction in a concentration-dependent manner with IC_{50} values of 0.23 and 0.50 μM, respectively.

Effects of acidic pH_o on $[Ca^{2+}]_i$

Contraction of vascular smooth muscle is regulated by several factors including $[Ca^{2+}]_i$. $[Ca^{2+}]_i$ in the SHR (10 weeks old) aorta was monitored by use of fura-2 as a fluorescent Ca^{2+} indicator. As shown in Figure 7a, acidic pH_o (pH 6.5) induced a rapid and sustained increase in $[Ca^{2+}]_i$ followed by the contraction. Returning to normal pH (7.4) abolished APIC. The application of EGTA (2 mM) to reduce extracellular Ca^{2+} below 1 μM eliminated both the Ca^{2+} mobilization and APIC.

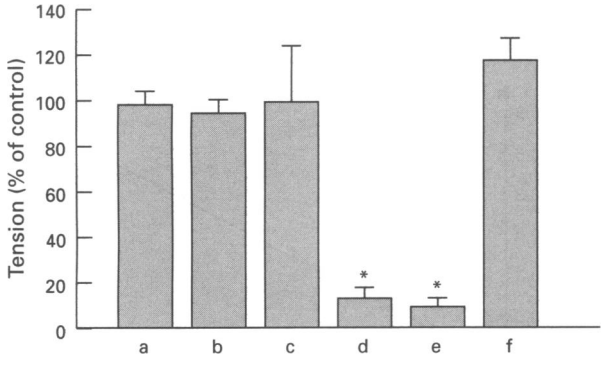

Figure 5 Effects of several drugs on contraction response of SHR aortae to acidic pH_o. Aortic strips from SHR (11 weeks old) were contracted by changing pH_o from 7.4 to 6.5. After reaching a plateau, several drugs were added. Tension was expressed as percentage of control value obtained in the absence of each drug ($n=5$). (a) Phentolamine 10 μM; (b) tetrodotoxin 10 μM; (c) Gd^{3+} 100 μM; (d) verapamil 1 μM; (e) adenosine 1 mM; (f) aspirin 1 mM. *Significantly different from control ($P<0.01$).

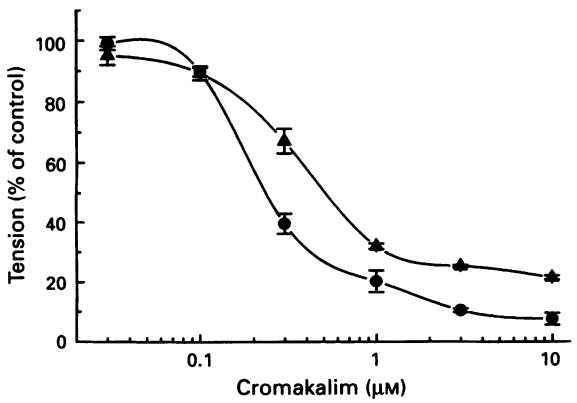

Figure 6 Effects of cromakalim on KCl- and acidic pH_o-induced contraction of aortic strips from SHR. Aortic strips from SHR (10 weeks old) were precontracted 3 times by adding 60 mM KCl. Then, the strips were contracted by changing pH_o from 7.4 to 6.5 (●) or adding 30 mM KCl (▲). Cromakalim was added cumulatively. Tension was expressed as percentage of control value obtained without cromakalim ($n=4$).

The Ca^{2+} transient as well as APIC was also abolished by adding verapamil (1 μM), a Ca^{2+} channel blocker (Figure 7b). Adenosine caused a concentration-dependent inhibition of APIC with an IC_{50} value of 0.475 mM. As shown in Figure 8a, 1 mM adenosine markedly inhibited not only the contraction but also an increase in cytosolic Ca^{2+} concentrations evoked by changing pH_o to 6.5. Furthermore, pretreatment of the strip with 1 mM adenosine abolished both the APIC and Ca^{2+} transient without affecting pH_i (Figure 8b). Similar effects of these drugs were also observed in WKY (data not shown).

Effects of acidic pH_o on membrane potential of vascular smooth muscle cells

To assess the effect of acidic pH_o on membrane potential, primary cultures of vascular smooth muscle cells were prepared from SHR aorta by enzymatic dispersion and their membrane potential was measured by a potential-indicator dye diS-$C_3(5)$ (Figure 9). The value of the resting membrane potential at normal pH_o was -52 ± 5 mV ($n=3$). Addition of 60 mM KCl as a positive control induced a depolarization to -15.1 ± 4 mV ($n=3$). Acidic pH_o (pH 6.5) remarkably decreased membrane potential to -26.7 ± 7 mV ($n=3$) which was close to that obtained by adding 30 mM KCl (-23.8 ± 5 mV, $n=3$). Cromakalim markedly inhibited both KCl- and acidic pH_o-induced depolarization (data not shown).

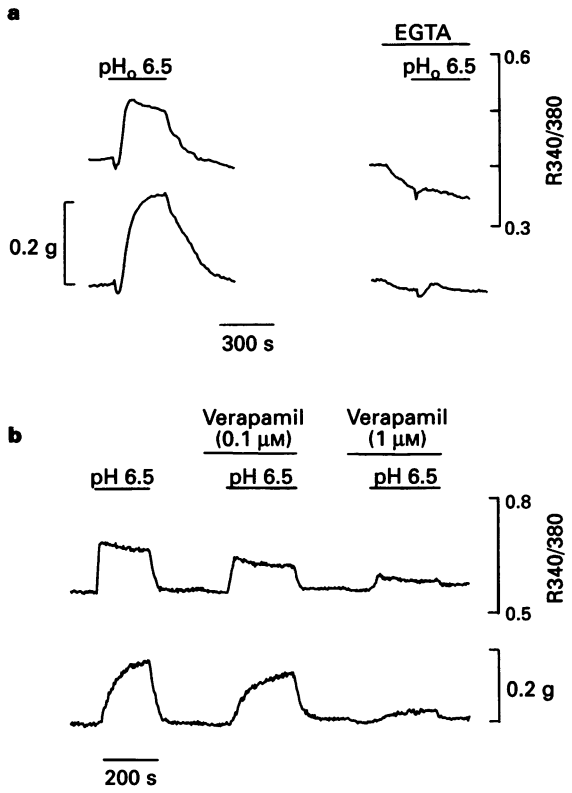

Figure 7 Acidic pH_o-induced contraction and elevation of cytosolic Ca^{2+} level of the rat aorta from SHR. Aortic strips from SHR (10 weeks old) were loaded with fura-2 and then simultaneous measurements of $[Ca^{2+}]_i$ and contraction were carried out. Upper traces show the ratio of intensity of the fluorescence induced by excitation at 340 nm to that at 380 nm as an index of relative cytosolic Ca^{2+} level. Lower traces show the contraction corresponds to the upper trace. Four preparations from different animals were used. (a). Left traces show contraction and Ca^{2+} mobilization evoked by decreasing pH_o from 7.4 to 6.5. Right traces show effects of 2 mM EGTA on APIC and intracellular Ca^{2+} mobilization. (b) Effects of verapamil (0.1 and 1.0 μM) on APIC and $[Ca^{2+}]_i$.

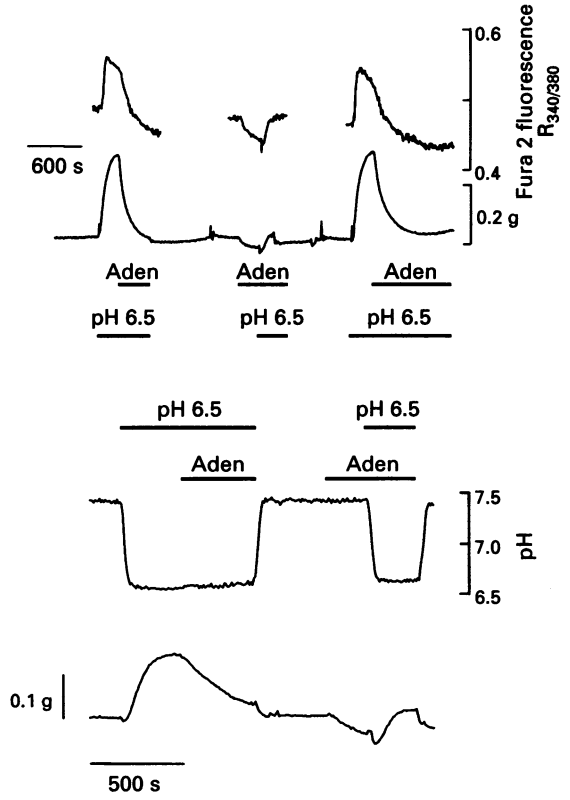

Figure 8 Effects of adenosine (Aden) on acidic pH_o-induced contraction, $[Ca^{2+}]_i$ and pH_i in aortic strip from SHR. Aortic strips from SHR (11 weeks old) were loaded with fura-2 (a) or BCECF (b) and then simultaneous measurements of $[Ca^{2+}]_i$ or pH_i and contraction were carried out. Aortic strips were contracted by changing pH_o from 7.4 to 6.5. Typical recording of the vasodilator effect of adenosine is shown (three different animals were used). One mM adenosine (Aden) was applied after the contraction reached a plateau. When the contraction returned to around the resting level after changing pH_o to 7.4, 1 mM adenosine was applied and then pH_o was changed to 6.5. Application of adenosine at pH 6.5 was repeated again in (a). Upper traces are $[Ca^{2+}]_i$ (a) or pH_i (b) and the lower traces are contraction (a and b).

Relationship between acidic pH_o-induced contraction and blood pressure

The results described above suggest that the contractile response to acidic pH_o is strengthened by the elevation of blood pressure. Therefore, mean blood pressure of WKY and SHR at various ages and APIC in their aortae were examined. As shown in Figure 10a, the mean blood pressure of WKY at 5 weeks old was 114 ± 4.5 mmHg. It increased gradually with increase in age (7% increase after 9 weeks from 5 to 14 weeks old). On the other hand, blood pressure of SHR at 5 weeks old was already much greater than that of WKY at the same age (131 ± 2.1 mmHg for SHR). It increased markedly with increasing age (50% increase after 10 weeks from 5 to 15 weeks old). As shown in Figure 10b, APIC (pH 6.5) was also age-dependent. APIC in the SHR aorta increased two times after 10 weeks from 5 to 15 weeks old. In the case of WKY it also increased with increase in age but the increase was small compared to SHR. Figure 10c indicates that there is a close correlation between the increase in APIC and the elevation of blood pressure ($r = 0.90$).

Effect of feeding with N^G-nitro-L-arginine on blood pressure and APIC in WKY

Ikeda *et al.* (1992) reported that feeding with N^G-nitro-L-arginine elevated the blood pressure of WKY. APIC was examined in the aorta from WKY fed with 0.023% N^G-nitro-L-arginine. Feeding with N^G-nitro-L-arginine elevated the blood pressure of WKY (14 weeks old) from 121 ± 8.1 to 132 ± 0.49 mmHg after 2 days and to 153 ± 8.5 mmHg after 9 days (Figure 11a). In these rat aortae APIC (pH 6.5) markedly increased from $34.6 \pm 11.3\%$ to $71.6 \pm 9.2\%$ after 2 days and to $96.1 \pm 17.7\%$ after 9 days in parallel to the blood pressure. Both the increase in blood pressure and the enhancement of APIC by prolonged oral administration of N^G-nitro-L-arginine disappeared 10 days after stopping the feeding. As shown in Figure 11b, there is a close correlation between the increase in APIC and the elevation of blood pressure ($r = 0.95$). *In vitro* treatment of aortae with N^G-nitro-L-arginine did not cause any change in APIC.

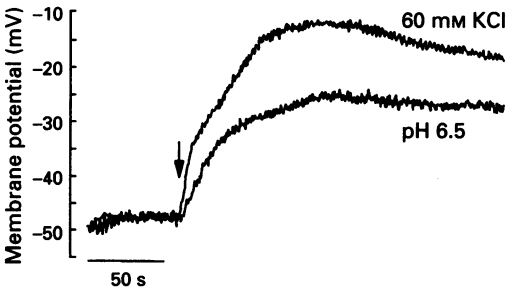

Figure 9 Effect of acidic pH_o on the membrane potential of vascular smooth muscle cells from SHR aortae. Cultured smooth muscle cells attached to glass coverslips were prewarmed at 37°C for 5 min and then incubated with 0.5 μM diS-$C_3(5)$ before assaying fluorescence. Measurements were carried out with excitation at 620 nm and emission at 680 nm, respectively. After the fluorescence intensity stabilized, HCl to change pH_o from 7.4 to 6.5 or 60 mM KCl was added. Representative traces are shown ($n = 3$).

Discussion

Decreasing pH_o caused a rapid and sustained decrease in pH_i followed by a contraction in rat aorta. Acidic pH_o-induced contraction (APIC) was not affected by an α-adrenoceptor blocker (phentolamine), a cyclo-oxygenase inhibitor (aspirin) and a Na^+ channel blocker (tetrodotoxin). The aortic strips used in the present experiments were without endothelium. These results would eliminate the possible involvement of an indirect action through the release of chemical mediators as the mechanism of action of acidic pH_o, suggesting the direct action of acidic pH_o. There was a linear relationship between the pH_o and steady-state pH_i over the pH_o range examined. There may be a rapid passage of H^+ across the plasma membrane of aortic cells. H^+ entry through Na^+/H^+ exchanger is thermodynamically unlikely, because the pH_o lower than 6.0 would be required for this mechanism under the normal condition of intracellular Na concentrations and pH_i. A possible mechanism is H^+ entry through Cl^-/HCO_3^- exchanger. This mechanism is supported by the observation that DIDS, a representative blocker of Cl^-/HCO_3^- exchanger, inhibited both the decreases in pH_i and APIC. Furthermore, the contraction was also induced by the decrease in pH_i by the removal of NH_4Cl. These results suggest that APIC is mainly caused by the decrease in pH_i. However, a small decrease in pH_i induced by the removal of NH_4Cl caused a large increase in tension compared with the acidic pH_o-induced decrease in pH_i. The addition of NH_4Cl did not cause any remarkable pH_o change. Therefore, it remains uncertain which the real trigger of the concentration is – absolute pH_i or ΔpH (the difference between pH_i and pH_o).

It has been indicated that contractions of rat mesentery and porcine coronary are associated with intracellular alkalinization (Austin & Wray, 1993; Nagesetty & Paul, 1995). We also confirmed their observation. However, in this study, contrac-

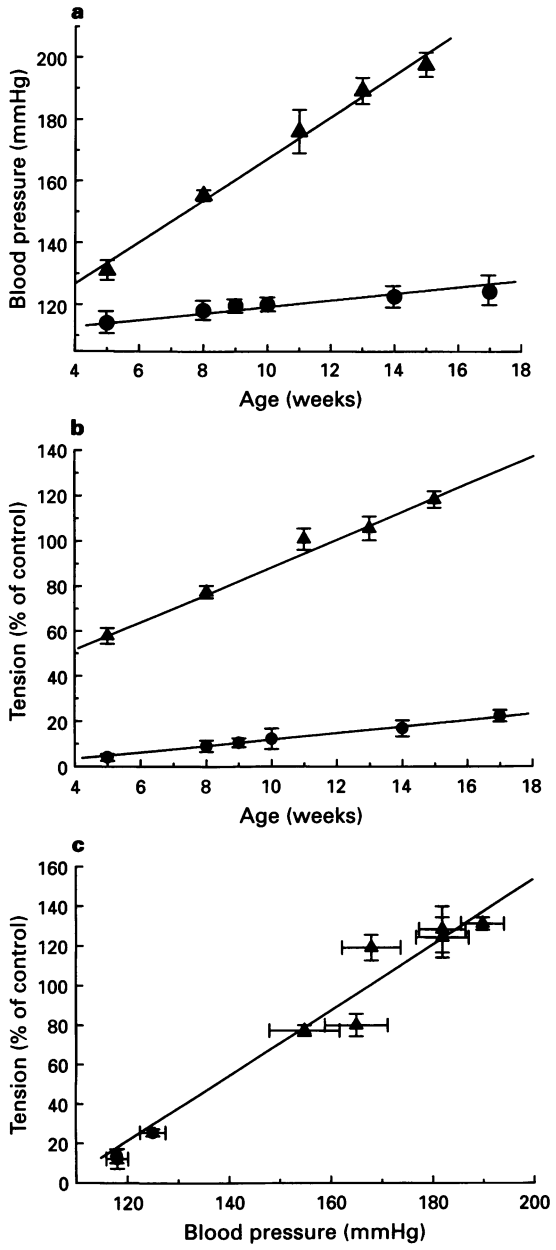

Figure 10 Relationships among age, blood pressure and acidic pH_o-induced contraction of SHR and WKY. (a) Development of blood pressure by aging; (b) development of acidic pH_o-induced contraction by aging. Tension induced by changing pH_o from 7.4 to 6.5 is expressed as percentage of contraction induced by 60 mM KCl at pH 7.4; (c) relationship between the blood pressure and the contractions. (●) WKY; (▲) SHR. The regression line fitted has a correlation coefficient of 0.90 and a slope of 1.65 ± 0.43 ($n = 4-6$).

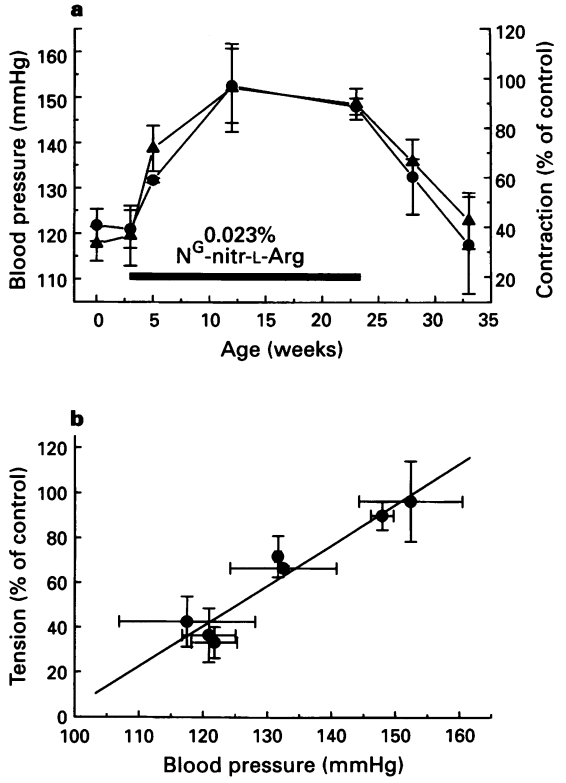

Figure 11 Effects of feeding with N^G-nitro-L-arginine on blood pressure and acidic-pH_o-induced contraction in WKY. WKY (14 week old) were fed with 0.023% N^G-nitro-L-arginine for 20 days. (a) Increase in blood pressure (●) and enhancement of acidic pH_o-induced contraction (▲) by feeding with N^G-nitro-L-arginine. Contraction induced by changing pH_o from 7.4 to 6.5 is expressed as percentage of that induced by 60 mM KCl at pH 7.4. (b) Relationship between blood pressure and contraction. The regression line fitted has a correlation coefficient of 0.96 and a slope of 1.79 ± 0.24 ($n = 5$).

tion of rat aorta is associated with intracellular acidification. Rabbit aorta shows almost the same contractile response to acidic pH (our unpublished observation). Battle *et al.* (1993) showed that intracellular acidification increases Ca^{2+} from an intracellular store(s) in rat aortic vascular smooth muscle cells. Blood vessels from distinct vascular regions may display different sensitivities to changes in extracellular pH.

APIC was abolished by elimination of extracellular Ca^{2+} or treatment with verapamil, a potent inhibitor of voltage-dependent Ca^{2+} channels. Increase in $[Ca^{2+}]_i$ induced by acidic pH_o was suppressed by the application of verapamil or Ca^{2+}-free medium. These results suggest that APIC is triggered by Ca^{2+} influx through voltage-dependent Ca^{2+} channels. Voltage-dependent Ca^{2+} channels are activated by the depolarization of the plasma membrane. It has been reported that cromakalim activates K^+ channels to hyperpolarize the plasma membrane resulting in relaxation of smooth muscle (Yanagisawa *et al.*, 1990). To confirm the participation of depolarization in the mechanism of APIC, the effect of cromakalim on APIC was investigated. APIC was diminished by cromakalim in a concentration-dependent manner with an IC_{50} value similar to that in concentrations induced by 30 mM KCl. Furthermore, acidic pH_o induced depolarization of primary cultured smooth muscle cells from SHR aorta. Cromakalim markedly inhibited both KCl- and acidic pH_o induced depolarization. These results suggest that acidic pH_o depolarizes the plasma membrane of smooth muscle cells and then voltage-dependent Ca^{2+} channels are activated to cause APIC. However, APIC at pH 6.5 was much larger than that caused by 30 mM KCl, although the depolarization induced by acidic pH_o (6.5) was close to that obtained by adding 30 mM KCl. Therefore, another mechanism contributing to APIC may also be present.

It is well known that the membrane potential of smooth muscle cells is determined by the permeability of K^+ as well as Ca^+ and both K^+ and Ca^{2+} channels are inhibited by acidic pH_i (Harder, 1982). Acidic pH_o cannot directly activate voltage-dependent Ca^{2+} channels, indicating that Ca^{2+} channels are not the first determinant of the resting membrane potential of the aorta in acidic pH_o. Therefore it is probable that inhibition of K^+ channels is responsible for depolarization in the rat aorta induced by acidification of extracellular fluid. Various types of K^+ channels in smooth muscle cells have been extensively studied by numerous investigators (Bolton &

Beech, 1992). The pH-sensitive K^+ channel is sensitive to pH_i and has a K_d value of 7.28 similar to the resting pH_i (Suzuki *et al.*, 1994). Acidic pH_o caused a rapid decrease in pH_i of the rat aorta. Furthermore, DIDS, an inhibitor of Cl^-/HCO_3^- exchanger partially inhibited APIC, suggesting that APIC is at least partly caused by H^+ entry through the mechanism. It is likely that intracellular H^+ reduces the open probability of K^+ channels of the aortic smooth muscle or competes with K^+ for the cation efflux pathway to cause membrane depolarization. It has been reported that Ca^{2+}-activated K^+ channels are highly activated in the resting state of arteries from SHR (Asano *et al.*, 1993; England *et al.*, 1993). An attractive explanation is that the K^+ channel that determines the membrane potential of aortic smooth muscle cells is enhanced in SHR to provide a counter-regulatory mechanism to limit arterial smooth muscle excitability. However, the alteration may easily cause APIC in acidosis.

The responsiveness to acidic pH was more evident in SHR than in WKY over the range of pH_o from 5.5 to 7.5. Both APIC and blood pressure markedly increased with increasing age of SHR, but in WKY both the parameters increased gradually. Prolonged oral administration of N^G-nitro-L-arginine in WKY caused a remarkable increase in APIC in parallel to the elevation of blood pressure. The increase in both parameters disappeared after stopping the administration of N^G-nitro-L-arginine. Interestingly, there was a close relationship between the elevation of blood pressure and the increase in APIC in all cases. Furthermore, acute hypertension augments the contractile response of the vascular smooth muscle (Sofola *et al.*, 1993; Griffith *et al.*, 1994). These results suggest that the increase in APIC is due to the elevation of blood pressure but not to hereditary factors. It is also suggested that acidosis becomes a crucial risk factor and may cause spasm of blood vessels in hypertension.

Mechanical stress often modulates characteristics of circulation systems (Kulik & Alvarado, 1993; Schwartzkopf *et al.*, 1993; Shida & Isoyama, 1993; Sterpetti *et al.*, 1993). Some functional change that continues after dissection may occur in smooth muscle cells in vessel walls under mechanical stress, i.e. stretching the smooth muscle cells or some hormonal stimulation during exposure to high blood pressure. Stretch-activated cation channels have been found in various types of cells including vascular endothelial and smooth muscle cells and may play important roles in regulating the vascular tone (Naruse & Sokabe, 1993; Kirber *et al.*, 1988; Hisada *et al.*, 1993). It is likely that these channels become sensitive to acidic pH due to high blood pressure. However, APIC was not inhibited by Gd^{3+}, a specific blocker of the channels (Yang & Sachs, 1989; Naruse & Sokabe, 1993). Therefore, stretch-activated cation channels may not contribute to the mechanism by which high blood pressure accelerates APIC.

Adenosine is an important metabolite associated not only with cellular metabolism but also with vasodilatation (Collis & Hourani, 1993; Ngai & Winn, 1993). Furthermore, several lines of evidence suggest that ischaemia often causes acidosis that may affect various functions of vascular smooth muscle and that adenosine is an endogenous protective agent in ischaemia (Berne *et al.*, 1974; Rudolphi *et al.*, 1993; Laxson *et al.*, 1993). The detailed protective mechanism of adenosine is as yet unresolved; the most likely hypothesis is that reduction in the intracellular cyclic AMP level by binding of adenosine to A_1 receptors results in decreasing Ca^{2+} influx (Rudolphi *et al.*, 1993). Adenosine caused a concentration-dependent inhibition of APIC and intracellular Ca^{2+} transient. Our results suggest that acidosis stimulates the activity of voltage-dependent Ca^{2+} channels through depolarization of the plasma membrane of smooth muscle. It is probable that adenosine decreases intracellular cyclic AMP concentrations and then inhibits Ca^{2+} influx to cause vasodilatation. This is consistent with the accepted role of adenosine in ischaemia.

In summary, APIC in the rat aorta is increased by the elevation of blood pressure. The contraction may be caused by Ca^{2+} influx through voltage-sensitive Ca^{2+} channels mainly due to acidic pH_o-induced depolarization of the plasma membrane of smooth muscle cells.

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