*Kor J Physiol (1994) 28(2), 169-179*

# Effect of pH on the Vascular Tone and $^{45}Ca$ Uptake in the Aorta of Spontaneously Hypertensive Rats

**Seok Jong Chang, Byeong Hwa Jeon, Se Hoon Kim, Hoe Suk Kim and Hae Kun Park**

*Department of Physiology, College of Medicine, Chungnam National University*

## =ABSTRACT=

**The effect of extracellular and intracellular pH on vascular tone and $^{45}Ca$ uptake were investigated in aortic strips and dispersed single aortic smooth muscle cells of spontaneously hypertensive rats (SHR) and aged-matched Wistar-Kyoto rats (WKY).**

**The contraction produced by a change of extracellular pH (pHo) in the range of 6.5~8.3 was estimated by comparison with the level of vascular tone at pH 7.4. Contraction was induced below pHo 6.5 in WKY, pHo 7.1 in SHR, and over pHo 8.0 on both strains. The amplitude of contraction induced by high pHo (over pHo 7.7) was similar in SHR and WKY, but that induced by low pHo (below pHo 7.1) in SHR was greater than that in WKY. Either high pHo- or low pHo-induced contractions in WKY and SHR were not induced in the Ca-free Tyrode's solution and were induced by the addition of Ca. $^{45}Ca$ uptake increased progressively as pHo was increased from 6.8 to 8.0 in the single aortic smooth muscle cells of WKY and SHR.**

**$NH_4Cl$ induced a gradually developing contraction in a dose-dependent manner (5 mM~30 mM) and the removal of $NH_4Cl$ induced transient contraction was followed by profound relaxation in the aortic rings of both strains. The contractions induced by $NH_4Cl$ or by the removal of $NH_4Cl$ in SHR were significantly greater than that in WKY. These contractions were not induced in Ca-free Tyrode's solution. $^{45}Ca$ uptake was increased by $NH_4Cl$ (20 mM) and was not changed by the removal of $NH_4Cl$ (20 mM) in the aortic strips of WKY and SHR.**

**As a summary of above results, the vascular tone of SHR was more sensitive to the change pHi and pHo than that of WKY. The contractions induced by change of extracellular or intracellular pH depended on extracellular Ca in the aorta of SHR and WKY. However, the Ca uptake was in accord with the changes of contraction but increase in contraction by low pH was not accompanied by an increase in Ca uptake in both strains.**

**Key Words:** Extracellular pH, Intracellular pH, Vascular tone, Calcium, Ammonium chloride

## INTRODUCTION

Metabolic factors such as pH are well known as a major controller of local blood flow. It is generally well known that increase of hydrogen ion concentration (low pH) leads to decreased contractility and decrease of hydrogen ion concentration (high pH) leads to increased contractility in several types of smooth muscle and non-smooth muscle, such as skeletal muscle (Mainwood & Worsley-Brown, 1975) and cardiac muscle (Steenbergen et al, 1977).

"This paper was supported (in part) by NON DIRECTED RESEARCH FUND, Korea Research Foundation, 1993"

The tone of vascular smooth muscle can be altered by a change in extracellular or intracellular pH. It had been observed that the vasoconstrictor response to several agonists was decreased in low pH and increased in high pH in vascular smooth muscle (Tobian et al, 1959; Williamson & Moore, 1960; Vanhoutte & Clement, 1968). Decrease of contractility in low pH could explain why low pH has been shown to reduce calcium influx (van Breemen et al, 1972; Kohlhardt et al, 1976), directly inhibit myofilament contractility (Portzehl et al, 1969; Fabiato & Fabiato, 1978) and alter receptors on the cell surface (Flavahan & McGrath, 1981).

However, it has been reported that low pH increases the contractility of rabbit ear artery (Spurway & Wary, 1987) and human ureteral muscle (Cole & Fry, 1987). It has also been reported that Ca sensitivity decreased twofold as pH was increased from 6.5 to 7.1 in the skinned vascular smooth muscle of rat caudal artery (Gardner and Diecke, 1988). Thus the effects of acidosis on the contraction and relaxation properties of vascular smooth muscle are not well understood.

Change of intracellular pH (pHi) can affect the vascular tone of several vessels. pHi may regulate intracellular Ca, and thereby play a role in the regulation of vascular smooth muscle tone (Wray, 1988). It has been observed that intracellular alkalinization induced by $NH_4Cl$ leads to contraction, and intracellular acidification induced by the removal of $NH_4Cl$ leads to transient contraction in the vascular smooth muscle (Berk et al, 1987; Danthuluri & Deth, 1989). Siskind et al (1989) reported that intracellular alkalinization induced by $NH_4Cl$ in A7r5 vascular smooth muscle cells leads to a rise in intracellular calcium via release of stored intracellular Ca. Recently, Batlle et al (1993) reported that intracellular acidification induced by the removal of $NH_4Cl$ resulted in a rapid increase in intracellular Ca concentration in dispersed aortic smooth muscle cells.

Abnormal Ca handling in the plasma membrane of arterial smooth muscle cells has been thought to play a role in hypertension. Hypertension is associated with a variety of disturbances of cell membrane function. This association has been attributed to changes in sodium, calcium and pH in the vascular smooth muscle (Haddy, 1983; Swales, 1990). pHi in the vascular smooth muscle of SHR were significantly more alkaline compared with WKY (Izzard & Heagerty, 1989; Davies et al, 1991). The activity of the Na/H exchange is higher in SHR vascular smooth muscle cells than in WKY cells and $Na^+$-dependent alkalinization was significantly higher in SHR than WKY cells (Alexander et al, 1990; Ellstrom et al, 1993). Increased Na/H exchange in SHR may increase the intracellular Na and Ca concentrations which may contribute to high basal tone in the artery of SHR.

The vascular smooth muscle of SHR exhibits high basal tone (Noon et al, 1978; Chang et al, 1994). High basal tone in the aortic strips of SHR is mainly due to an increase of Ca influx via potential-operated Ca channels (Lindner & Heinle, 1987; Chang et al, 1994) and an increase in the number of dihydropyridine-sensitive Ca channels (Chang et al, 1994). In vascular smooth muscle, the influx of Ca that is required for basal tone is thought to occur via potential-operated Ca channels (Nelson et al, 1990), which in cardiac muscles are known to be sensitive to pH (Kraft & Kass, 1988). Although the extracellular or intracellular hydrogen ion concentration has been implicated in the regulation of vascular tone, little is known about the effects of pH on vascular tone in the aorta of SHR.

Therefore, we investigated the effect of extracellular pH and $NH_4Cl$ on vascular tone and $^{45}Ca$ uptake in aortic rings and dispersed single aortic smooth muscle cells of SHR and WKY.

## METHODS

### Animals

All experiments were performed on 12~16 week-old SHR (Okamoto, 1969) and aged-matched normotensive WKY. The systolic blood pressure was measured in conscious restrained rats by the tail-cuff plethysmographic method (PE-300, Narco-Biosystems, Houston, Texas). Systolic blood pressure was 204±5 mmHg in SHR (n=20) and 136±8 mmHg in WKY (n=20).

### Measurement of isometric tension

The rats were stunned and exsanguinated. The aorta was quickly dissected and adhering adventitia and remaining fat were removed under a stereoscopic microscope. The aorta was allowed to recover for 2 hours at room temperature. The aorta was then carefully cut into rings (3~4 mm wide).

The aortic ring was inserted by two parallel straight stainless steel wires (0.3 mm in diameter, 5 mm in length). The lower end was anchored and the upper end was connected to a force transducer (F-60, Narco-Bio system) by glass filament. The organ bath was controlled thermostatically and filled with 50 ml of the Tris-buffered Tyrode's solution containing (mM): NaCl 158, KCl 4, $CaCl_2$ 2, $MgCl_2$ 1, Glucose 6, and Tris 5 (pH 7.4 at 37°C). The organ bath solution was maintained at 37°C and continuously bubbled with 100% $O_2$. The strip was suspended under a tension of 2 g. Each perparation was allowed to recover for at least one hour. Isometric tensions were recorded on a physiograph (MK-IV, Narco-Bio system) (Chang et al, 1990).

To avoid the possible influence of the endothelium, the endothelium was removed by gently rubbing the intimal surface with a cotton ball. Successful removal of the endothelium was confirmed later by the failure of acetylcholine ($10^{-6}$ M) to induce relaxation (Furchgott & Zawadzki, 1980).

### Preparation of dispersed single aortic smooth muscle cells

The aorta were excised, and the adventitia were removed in HEPES-buffered Tyrode's solution containing (mM): NaCl 140, KCl 4, $MgCl_2$ 1, $CaCl_2$ 1, HEPES 10, glucose 6, pH adjusted to 7.4 at 37°C. The endothelium was removed by gently rubbing the intimal surface with a cotton ball. The aorta was cut into small strip (2 mm wide and 10 mm long), and incubated in HEPES-buffered Tyrode's solution for one hour. Then small strips were incubated in a Ca-free HEPES-buffered Tyrode's solution containing 2 mg/ml collagenase (Wako), 2 mg/ml papain (Sigma), 1 mg/ml dithiotreitol (Sigma), and 5 mg/ml bovine serum albumin (Sigma) for 40 minutes. After digestion, the single cells were separated by gently pipetting the muscle strips through a wide-pore pasteur pipette. The suspension of single cells was filtered through double layers of nylon mesh (pore size: 0.5 mm). The suspension was centrifuged at 1000 rpm for 5 minutes to eliminate the connective tissue debris.

Viability of the single cells was assessed by the trypan blue exclusion test (Bagby et al, 1971; Johns & Riehl, 1982). The total number of stained and viable single smooth muscle cells was then determined. All experiments were carried out within 4 hours after preparation of the cell suspension.

### Measurement of $^{45}Ca$ uptake

$^{45}Ca$ uptake was measured in 0.5 ml of HEPES-buffered Tyrode's solution containing $^{45}Ca$ (4 $\mu$Ci/ml). $1\sim2\times10^5$ cells were incubated in the solution at 37°C for 15 minutes. Incubation was stopped by addition of 1 ml of ice-cold HEPES solution containing $La^{3+}$ (30 mM) and followed by centrifugation at 1000 rpm for 5 minutes. The supernatants were

removed and the cell pellets were washed 2 times with ice-cold HEPES solution containing $La^{3+}$ (30mM). Cell pellets were lysed with 0.5 ml of 0.5 N NaOH and added to 5.5 ml of scintillation cocktail (Luma-gel). Radioactivity was measured with a liquid scintillation counter (Tri-Carb 350C).

The rusults are expressed as means ± S.E. Student's t-tests were used for statistical analysis. P values of less than 0.05 were considered to be statistically significant.

Drugs used were HEPES, EGTA, lanthanum chloride, papain, dithiotreitol, bovine serum albumin (Sigma), collagenase (Wako), $^{45}Ca$ (specific activity, 18.7 mCi/mg, New England Nuclear).

## RESULTS

### Effects of extracellular pH on the vascular tone

The change in vascular tone induced by a change of extracellular pH($pH_0$) in the range of 6.5~8.3 was estimated by comparison with the level at $pH_0$ 7.4 in the aortic rings of WKY and SHR. Typical traces of contraction during the change of $pH_0$ in both strains was shown in Fig. 1. As shown in Fig. 1, the vascular tone in the aortic rings of WKY was not changed in the $pH_0$ range of 6.8~7.7, but it was increased below $pH_0$ 6.5 or over $pH_0$ 8.0. The vascular tone in the aortic rings of SHR was not changed in the $pH_0$ range of 7.4~7.7, but it was increased below $pH_0$ 7.1 or over $pH_0$ 8.0. The contractile response curves as a function of the change of pHo in both strains are shown in Fig. 2. Low pHo-induced contraction in SHR were markedly greater than that in WKY ($p<0.01$). High pHo-induced contractions were not significantly different between aortic rings of SHR and WKY ($p<0.8$).

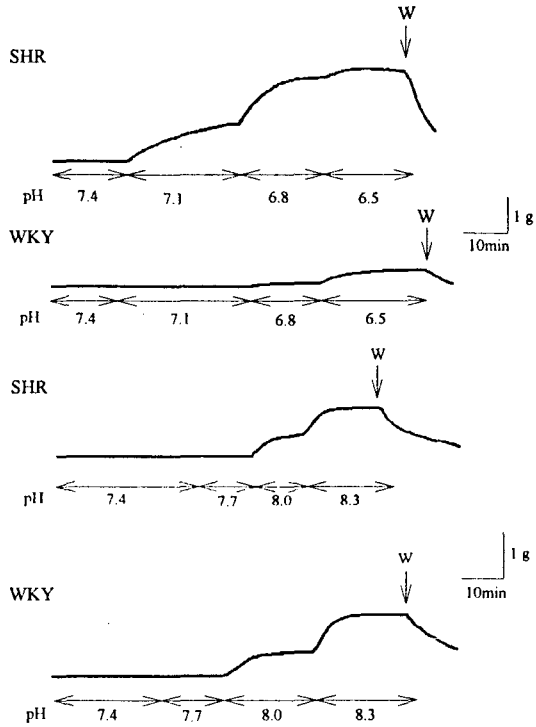


*Fig. 1. Typical traces of contraction induced by a change in extracellular pH in the aortic rings of spontaneously hypertensive rats (SHR) and Wistar-Kyoto rats (WKY).*

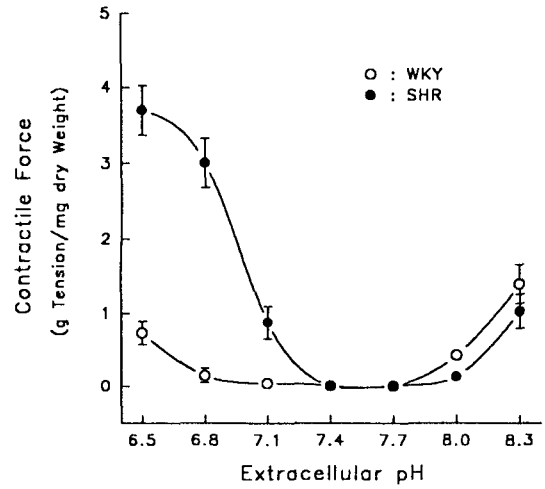


*Fig. 2. Dose-response curves of contractions induced by a change in extracellular pH in the aortic rings of Wistar-Kyoto rats (WKY) and spontaneously hypertensive rats (SHR).*

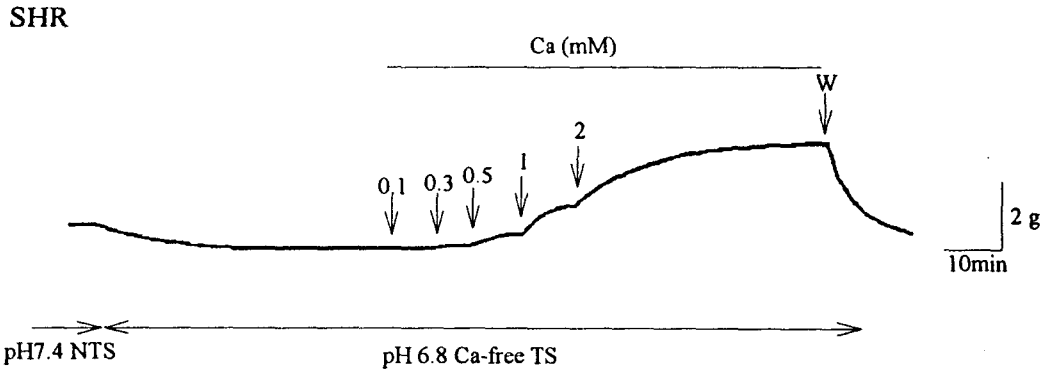


*Fig. 3. Extracellular Ca dependency of pH 6.8-induced contraction in the aortic rings without endothelium of spontaneously hypertensive rats (SHR). NTS: Normal Tyrode's solution, Ca-free TS: Ca-free Tyrode's solution*

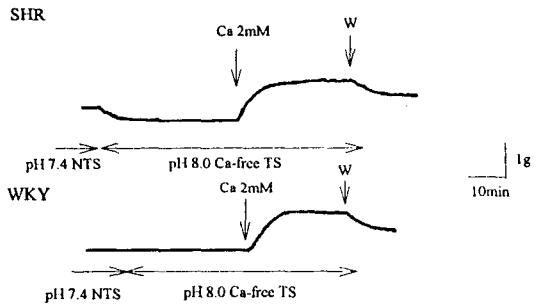


*Fig. 4. Extracellular Ca dependency of pH 8.0-induced contraction in the aortic rings without endothelium of spontaneously hypertensive rats (SHR) and Wistar-Kyoto rats (WKY). NTS: Normal Tyrode's solution, Ca-free TS: Ca-free Tyrode's solution*

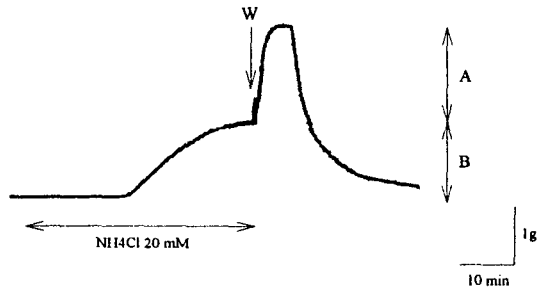


*Fig. 5. Typical trace of $NH_4Cl$ (20 mM)-induced contraction and transient contraction after $NH_4Cl$ removal in the aortic rings without endothelium of spontaneously hypertensive rats. A: Transient contraction after $NH_4Cl$ removal, B: $NH_4Cl$-induced contraction*

**Effects of Ca removal and Ca addition on the pH-induced contractions**

To study the dependence on extracellular Ca of low or high pHo-induced contraction, aortic rings were exposed to Ca-free Tyrode's solution for 30 minutes and then calcium was added to the bath solution. As shown in Fig. 3 and 4, the vascular tone in sHR was slowly decreased in the Ca-free Tyrode's solution. Low pHo (pH 6.8)-induced contraction was not induced in the Ca-free Tyrode's solution and was increased by the addition of Ca as a dose-dependent manner (0.1 mM~2 mM) in the aortic rings of SHR (Fig. 3). The effect of Ca on the low pHo (pHo 6.8)-induced contraction in the aortic rings of WKY was not examined because of the very small contraction at pHo 6.8. High pHo (pHo 8.0)-induced contraction was not induced in the Ca-free Tyrode's solution and was increased by the addition of Ca (2 mM) in the aortic rings of

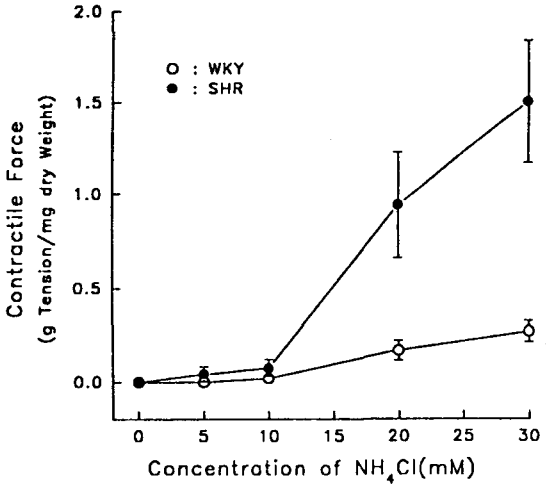


*Fig. 6. Dose-response curve of contractile force to $NH_4Cl$ in the aortic rings without endothelium of Wistar-Kyoto rats (WKY) and spontaneously hypertensive rats (SHR).*

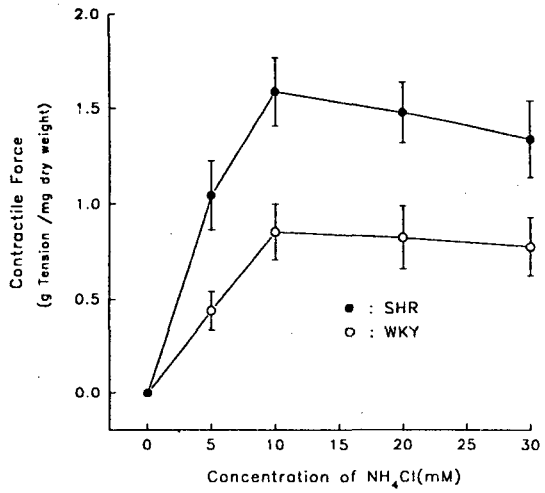


*Fig. 7. Dose-response curves of transient contractions by the removal of $NH_4Cl$ in the aortic rings without endothelium of the Wistar-Kyoto rats (WKY) and spontaneously hypertensive rats (SHR).*

both strains (Fig. 4).

### Effects of $NH_4Cl$ on the vascular tone

We studied the effect of $NH_4Cl$, which is employed as a tool to directly change the pHi, on the vascular tone in the aortic rings of both strains. A typical trace of contraction induced by $NH_4Cl$ (20mM) and transient contraction induced by removal of $NH_4Cl$ is shown in Fig 5. $NH_4Cl$ induced a gradually developing contraction after a latent period of 20~30 minutes and the romoval of $NH_4Cl$ induced transient contraction followed by profound relaxation.

The dose response curves of contractile force to $NH_4Cl$ in the aortic rings of both strains are shown in Fig. 6. $NH_4Cl$ induced contraction in a dose-dependent manner (5~30 mM) in both strains. Contractile responses to $NH_4Cl$ in SHR were greater than that in WKY ($p<0.01$) (Fig. 6). The transient contraction induced by removal of $NH_4Cl$ after the aorta was exposed to $NH_4Cl$ (5 mM~30 mM) is shown in Fig. 7. As shown in Fig. 7, transient contraction by removal of $NH_4Cl$ was initiated at a concentration of 5 mM $NH_4Cl$ and reached its maximum at a concentration of 10 mM $NH_4Cl$ in both strains. The transient contraction by removal of $NH_4Cl$ in SHR was greater than that in WKY ($p<0.05$).

### Effects of Ca on the $NH_4Cl$-induced contraction

To study the dependence of extracellular Ca on the $NH_4Cl$-induced contraction, aortic rings were exposed to Ca-free Tyrode's solution and then to $NH_4Cl$ and then calcium was added to the bath solution (Fig. 8). $NH_4Cl$ (20 mM) did not induce any contraction in Ca-free Tyrode's solution and it induced contraction at the addition of 2 mM Ca in both strains (Fig 8A). To study the dependence of extracellular Ca on the transient contraction induced by the removel $NH_4Cl$, aortic rings were exposed to $NH_4Cl$ (20 mM)-containing Ca-free Tyrode's solution and $NH_4Cl$ was then removed by washing with Ca-free or normal Tyrode's solution (Fig. 8B, C).

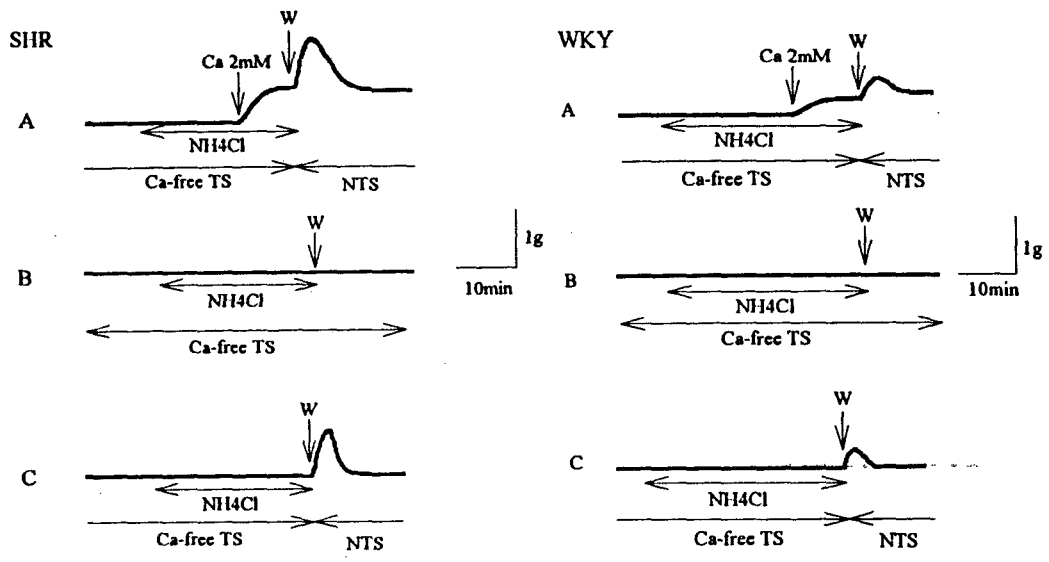


*Fig. 8. Effect of extracellular Ca on the $NH_4Cl$ (20 mM)-induced contraction and transient contraction induced by removal of $NH_4Cl$ in the aortic rings without endothelium of spontaneously hypertensive rats (SHR) and Wistar-Kyoto rats (WKY). NTS: Normal Tyrode's solution, Ca-free TS: Ca-free Tyrode's solution. A: Adminstration of the $NH_4Cl$ (20 mM) and Ca 2 mM in the Ca-free Tyrode's solution and wash with normal Tyrode's solution, B: Administration of $NH_4Cl$ (20 mM) in the Ca-free Tyrode's solution and wash with Ca-free Tyrode' solution. C: Adminstration of $NH_4Cl$ (20 mM) in the Ca-free Tyrode's solution and wash with normal Tyrode's solution.*

Transient contraction induced by the removal of $NH_4Cl$ was not induced by washing with Ca-free Tyrode's solution (Fig 8B), but this contraction was induced by washing with normal Tyrode's solution (Fig. 8C).

### Effects of extracellular pH on $^{45}Ca$ uptake

To study the effect of pHo on $^{45}Ca$ uptake, we used dispersed single aortic smooth muscle cells. The single aortic smooth muscle cells were immersed in solutions of different pH (pH 6.8, 7.4, 8.0) and were incubated with $^{45}Ca$ (4 $\mu$Ci/ml) for 15 minutes. $^{45}Ca$ uptake in each solution of different pH is shown in Fig. 9. $^{45}Ca$ uptake was increased with the increase of pHo in the single aortic smooth muscle cells of both strains. $^{45}Ca$ uptake in the single aortic smooth muscle cells of SHR was greater than that of WKY in solutions of the same pH.

### Effect of $NH_4Cl$ on $^{45}Ca$ uptake

To study the effect of $NH_4Cl$ and the removal of $NH_4Cl$ on $^{45}Ca$ uptake, we used aortic rings of SHR and WKY. To examine effect of $NH_4Cl$ on $^{45}Ca$ uptake, aortic rings were immersed in the $NH_4Cl$ (20 mM) containing solution (pHo 7.4) and were incubated with $^{45}Ca$ (4 $\mu$Ci/ml) for 15 minutes. To examine effect of $NH_4Cl$ removal on $^{45}Ca$ uptake, aortic rings were pretreated with $NH_4Cl$ (20 mM) for 30 minutes and were transferred to $NH_4Cl$-free solution with $^{45}Ca$ (4 $\mu$Ci/ml) and incubated for 15 minutes. The effect of $NH_4Cl$ on the $^{45}Ca$ uptake in the aortic rings of both strains is shown in Fig. 10. As shown in Fig. 10, $^{45}Ca$ uptake was increased by the addition of $NH_4Cl$ (20 mM), but it was not affected by the removal of $NH_4Cl$ in either strain. $^{45}Ca$ uptake in the presence or

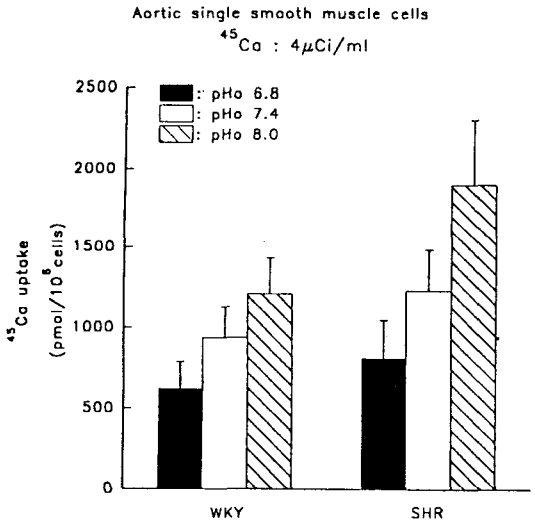


*Fig. 9. Effects of extracellular pH (pHo) on the $^{45}Ca$ uptake of the single aortic smooth muscle cells of the Wistar-Kyoto rats (WKY) and spontaneously hypertensive rats (SHR).*

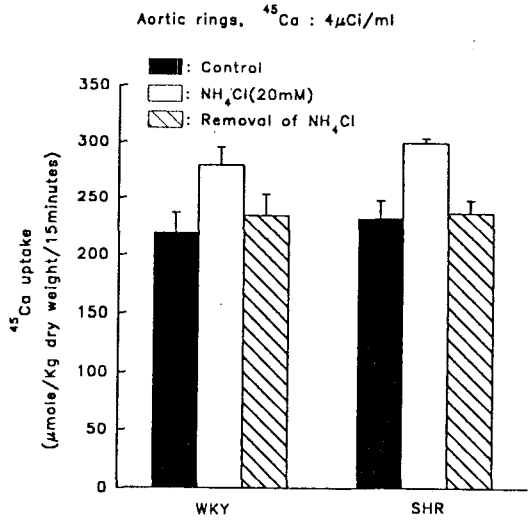


*Fig. 10. Effects of $NH_4Cl$ (20mM) and removal of $NH_4Cl$ on the $^{45}Ca$ uptake of the aortic rings without endothelium of the Wistar-Kyoto rats (WKY) and spotaneoulsy hypertensive rats(SHR).*

removal of $NH_4Cl$ in the aortic rings of SHR did not significantly differ from that of WKY.

## DISCUSSION

In the present study, we demonstrated that the change of pHo affected vascular tone in the aorta of SHR and WKY. In WKY, contraction was not induced in the range of pHo 6.8~7.7 and was induced below pHo 6.5 or over pHo 8.0. In SHR, contraction was not induced in the range of pHo 7.4~7.7 and was induced below pHo 7.1 or over pHo 8.0. The range of pH which did not change the vascular tone was more narrow in SHR than in WKY. These findings suggested that the vascular tone of SHR was more sensitive to change of pHo than that of WKY. Low pHo- or high pHo-induced contractions in the aortic strips of SHR and WKY were not induced in the Ca-free Tyrode's solution and were induced by addition of Ca. This finding suggested that Ca source for low pH- or high pH-induced contraction in SHR or WKY was extracellular Ca.

$NH_4Cl$, a weak base, is used as an experimental tool for directly elevating intracellular pH(pHi). During exposure to $NH_4Cl$, $NH_3$ rapidly enters into the cell and combines with $H^+$ to from $NH_4^+$, thereby raising pHi. In the present study, $NH_4Cl$ induced a gradully developing contraction (Fig. 5). $NH_4Cl$-induced contractions were completely dependent on the presence of extracellular Ca (Fig. 8). These results suggest that intracellular alkalinization induced by $NH_4Cl$ induced the tonic contraction, and contraction induced by $NH_4Cl$ is dependent on extracellular Ca. Siskind et al (1989) reported that extracellular Ca might contribute to the alkaline-induced rise in intracellular Ca by Ca influx via voltage-sensitive Ca Channels in vascular smooth muscle cells.

When $NH_4Cl$ was removed from the extracellular medium by replacing the $NH_4Cl$

solution with normal Tyrode's solution, pHi rapidly dropped to below basal levels because of rapid efflux of accumulated $NH_4^+$ in the form of $NH_3$. Spurway and his colleagues (Ighoroje & Spurway, 1984, 1985) found that intracellular acidosis by the removal of $NH_4Cl$ induced contraction in the rabbit ear arteries. We also observed that transient contraction of the aortic strips was induced by the removal of $NH_4Cl$ after loading of $NH_4Cl$. This contraction was not induced by washing with Ca-free Tyrode's solution and was induced by washing with normal Tyrode's solution. This finding suggests that the intracellular acidification induced the contraction in the aortic rings of both strains and the contraction induced by intracellular acidification depend on extracellular Ca.

Low pHo-induced contraction in SHR was markedly greater than that in WKY(Fig. 2). The contraction induced by $NH_4Cl$ and the removal of $NH_4Cl$ after the loading of $NH_4Cl$ in SHR was greater than that in WKY (Fig 6, 7). It was reported that the activity of Na/H exchange and protein kinase C in SHR were increased in SHR as compared to WKY (Alexander et al, 1990; Ellstrom et al, 1993). pHi in the vascular smooth muscle of SHR was significantly more alkaline compared with WKY(Izzard & Heagerty, 1989; Davies et al, 1991). It was known that alkaline pHi could be attributed to the increase of Ca sensitivity of the myofibrillar proteins in vascular smooth muscle (Danthuluri & Deth, 1989) and in skeletal muscle (Westerblad & Allen, 1993). Although the mechanism of increased contractile response by pH change in SHR was unknown, we suggested that it might be due to the increase of Ca sensitivity in the vascular smooth muscle of SHR.

$^{45}Ca$ uptake increased progressively as pHo was increased from 6.8 to 8.0 in single aortic smooth muscle cells of WKY and SHR (Fig 9). This finding suggested that hydrogen ions($H^+$) inhibited Ca uptake in the single aortic vascular smooth muscle cells of both strains. Van Breemen et al(1972) and Kohlhardt et al(1976) suggested that $H^+$ reduced $^{45}Ca$ influx, presumably by protonating anionic sites on calcium translocating proteins and channels. Webb and Bohr(1982) reported that passive diffusion of Ca into cells appears to be in competition with $H^+$. Also a decrease in pHo caused a strong decrease in Ca inward current, with near complete block at pHo 5.7, whereas an increase in pHo caused an increase in Ca inward current (West et al, 1992). $^{45}Ca$ uptake in the aortic rings was increased by $NH_4Cl$ and was not changed by the removal of $NH_4Cl$ (Fig. 10). These findings suggest that intracellular alkalinization may stimulate Ca influx, and intracellular acidification may not affect Ca influx in rat aorta.

In the present study, low pHo or low pHi induced a contracion which was dependent on extracellular Ca. However, low pH did not induce an increase of $^{45}Ca$ uptake in both strains. It is well known that contraction results from the increase of intracellular free Ca concentration in vascular smooth muscle. It is suggested that low pH-induced contraction results from the increase of intracellular Ca concentration although the mechanism of the increase of intracellular Ca concentration at low pH has not been fully defined. Kim & Smith (1988) proposed that low pHi increases intracellular Ca concentration indirectly via modulation of transmembrane changes mediated by Na-Ca exchange. Enhanced Na influx via Na/H exchange, activated as a result of low pHi, could either augment Ca influx or slow Ca efflux via Na-Ca exchange, and Batlle et al (1993) proposed that low pH-induced contraction may be due to increace of intracellular Ca as $H^+$ ions displace bound Ca in the vascular smooth muscle cells. However, further evaluation is necessary to clarify the relationship between the contraction induced by low pH and $^{45}Ca$ influx in the vascular smooth muscle.

In summary, the vascular tone of SHR was more sensitive to the change of pHi and pHo than that of WKY. The contractions induced by

change of extracellular pH or intracellular pH depended on extracellular Ca in the aorta of SHR and WKY. However, though the Ca uptake alone was in accord with the changes of contraction, increase in contraction by low pH did not go with increase in Ca uptake in both strains.

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