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Strain-specific effects of acidic pH on contractile state of aortas from Wistar and Wistar Kyoto rats

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Abstract

The effects of acidosis were investigated on the resting and precontracted aortas from Wistar and Wistar Kyoto (WKY) rats. Decrease in pH from 7.4 to 6.5, having no effect on the resting tension of Wistar aorta, induced a marked contraction of WKY aorta. Acidic pH markedly relaxed the contraction to 300 nM phenylephrine in Wistar aorta, whereas in WKY aorta, it produced a biphasic response, an initial relaxation followed by potentiation of the contraction. In aortas loaded with fura 2-AM, phenylephrine caused an increase in intracellular Ca^{2+} ($[Ca^{2+}]_i$) and a contraction in both Wistar and WKY rats. pH 6.5 produced a decrease in $[Ca^{2+}]_i$ to a near-basal level and almost abolished the phenylephrine-induced contraction in Wistar rat aorta. However, in WKY aorta, a biphasic response, an initial decline and later a recovery of $[Ca^{2+}]_i$ level, was observed. Interestingly, at similar sustained $[Ca^{2+}]_i$, the contractile response to phenylephrine in WKY aorta was potentiated under acidic pH conditions. Acidic pH-induced inhibition of the contraction to phenylephrine was unaffected by iberiotoxin, 4-aminopyridine, and glibenclamide (Ca^{2+}-activated, delayed rectifier and ATP-sensitive K^+ channel inhibitors, respectively), in aortas from both Wistar and WKY. Decrease in extracellular pH was associated with a rapid fall in intracellular pH (pH_i) and the intracellular acidification profile was not different in both strains. All these results show that acidic pH induces strain-specific inhibitory and excitatory effects on the contractile state of aortas from Wistar and WKY rats, respectively. The sustained and transient relaxant responses to acidic pH in Wistar and WKY aortas, respectively, are due to decrease in $[Ca^{2+}]_i$ levels, but this decrease in $[Ca^{2+}]_i$ is independent of the activation of K^+ channels.

Keywords: Acidosis; Hyperpolarization; K^+ channel, ATP-sensitive; K^+ channel, Ca^{2+}-activated; K^+ channel, delayed rectifier; Aorta, Wistar rat; Aorta, Wistar Kyoto rat

1. Introduction

The tone of the vascular smooth muscle regulates the blood flow to organs and the blood pressure (Martens and Gelband, 1998). Abnormalities of smooth muscle function have been implicated in multiple diseases including hypertension (Kagota et al., 2001; Rossoni et al., 2002). The contractile state of vascular smooth muscle can be regulated by many factors, including pH (for a review, see Wray, 1988). pH of the blood and extracellular fluids is generally maintained at around 7.4, but it may decrease in a number of clinical situations, such as hypoxia, diabetes mellitus, and renal disorders (Austin and Wray, 2000).

Most of the studies have shown that the acidosis is associated with activation of K^+ channels, hyperpolarization, decrease in intracellular Ca^{2+} ($[Ca^{2+}]_i$) level, and relaxation of vascular tissue (Ishizaka and Kuo, 1996; Berger et al., 1998; Peng et al., 1998). However, contrary to these reports, we have shown that acidic pH induces a contraction in isolated aortas from spontaneously hypertensive (SHR) and Wistar Kyoto (WKY) rats (Furukawa et al., 1996; Rohra et al., 2002a). We have also observed that in Wistar rat aorta, acidic pH produces no effect on the resting tension (Rohra et al., 2002b). This indicates that the effects of acidosis on the vascular smooth muscle cannot be generalized to all strains. Given the complexity of the effects of pH on vascular smooth muscle cells, it is not surprising that the cellular mechanisms by which acidosis exerts its contrasting influence on vasculature in different strains are incompletely understood.

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Previously, we have described the effects of acidic pH on the resting level of isolated aortas from various strains (Rohra et al., 2002b). The present study was carried out to investigate the effects of acidosis on partially contracted aortas. In this paper, for the first time, we report the overlapping as well as contrasting strain-specific effects of acidic pH on the contractile state of aortas from Wistar and WKY rats. The findings of the present study show that acidosis results in the relaxation of phenylephrine-induced contraction in Wistar rat aorta, whereas in WKY, similar to Wistar, an initial relaxation occurs that is later overcome by the potentiation of contraction. Furthermore, it is shown for the first time that unlike peripheral vasculature, the relaxant effect of acidosis on aortas from Wistar and WKY rats due to a decrease in $[Ca^{2+}]_i$, is independent of the activation of K^+ channels.

2. Materials and methods

2.1. Tissue preparation

Male Wistar and WKY of NCrj strain (both 11–13 weeks old and 240–270 g in weight) were used in this study. The animals were stunned and killed. Open rings of approximately 3 mm width were made from the aorta. The endothelium was removed by gently rubbing the endothelial surface with cotton pellets. The lack of endothelium was confirmed by failure of carbachol (1 μM) to cause relaxation of phenylephrine (1 μM)-induced contraction.

2.2. Measurement of isometric contraction

The aortic strips were suspended vertically in a 6-ml organ bath, containing well-aerated (95% O_2/5% CO_2), HEPES-buffered physiological salt solution (PSS) of pH 7.4. All experiments were carried out at 37 °C. The tissues were adjusted to a preloaded resting tension of 10 mN and equilibrated for at least 1 h. Isometric contraction was measured by the force displacement transducer (Nihon Kohden, Tokyo, Japan). The aorta was contracted three times with 64.8 mM KCl to acclimatize the tissue. The pH of the solution was changed from control value of 7.4 to 6.5 by addition of HCl, and the tissues were treated with acidic pH by simply exchanging the bathing solution with the acidic PSS. In experiments investigating the effect of acidic pH on the contractile state of the aorta, the concentration of 300 nM for phenylephrine was selected in order to obtain a contractile response that should be 60–70% of the 64.8 mM KCl-induced contraction. The tissues showing <60% or >70% of the 64.8 mM KCl-induced contraction were discarded and not included in the study.

2.3. Simultaneous measurement of $[Ca^{2+}]i$ and contraction

The $[Ca^{2+}]_i$ level was monitored by using fura 2-AM as a fluorescent $[Ca^{2+}]_i$ indicator. Aortic strips were loaded with 5 μM fura 2-AM overnight at 4 °C in PSS containing a non-cytotoxic detergent pluronic F-127 (0.025%) and mounted horizontally in a bath attached to a fluorimeter (CAF-100, Japan Spectroscopic, Tokyo, Japan) and a transducer. Contraction and fura 2 fluorescence were measured simultaneously. The intensity of fluorescence at 500 nm induced by excitation at 340 and at 380 nm was monitored. 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 (Mitsui and Karaki, 1990). Since fura 2 leaks out from the rat aortic strips during the experiment, strict care was taken to finish the experiment before the mirror image response of 340 and 380 fluorescence signals was lost.

2.4. Simultaneous measurement of intracellular pH and contraction

The fluorescent intracellular pH (pH_i) indicator dye, 2,7-bis(carboxyethyl)carboxyfluorescein (BCECF) was used to monitor changes in pH_i. Aortic strips were loaded with BCECF-AM (10 μM) for 2 h at 37 °C in PSS along with a non-cytotoxic detergent, pluronic F-127 (0.025%). Later, the strips were washed twice with PSS and then mounted horizontally under a resting tension of 10 mN in a bath attached simultaneously to a fluorimeter (CAF-100, Japan Spectroscopic) and a force-displacement transducer. BCECF fluorescence (excitation at 450 and 500 nm, emission at 530 nm) was measured. To calculate the absolute pH_i, the ratio of two fluorescence intensities (R450/500) was calibrated using nigericin (10 μM) in 130 mM KCl solution as described elsewhere (Kurtz and Golchini, 1987).

2.5. Solutions

The composition of the PSS was as follows (in mM): NaCl, 120, KCl, 4.8, $MgSO_4$, 1.3, $CaCl_2$, 1.2, $NaHCO_3$, 25.2, glucose, 5.8, KH_2PO_4, 1.2, and HEPES, 20. A 64.8 mM sample of KCl was made by replacing 60 mM NaCl of PSS with equimolar KCl. The 130 mM KCl solution used for the calibration of pH_i consisted of (in mM) NaCl, 20, KCl, 130, $MgSO_4$, 1.3, $CaCl_2$, 1.2, glucose, 5.8, KH_2PO_4, 1.2, and HEPES, 20.

2.6. Materials

Wistar and WKY rats were purchased from Kumagai (Sendai, Japan) and Charles River (Kanagawa, Japan), respectively. Phenylephrine, iberiotoxin, glibenclamide, and nigericin were from Sigma-Aldrich (St. Louis, MO, USA), while 4-aminopyridine was obtained from Wako (Japan). Fura 2-AM and BCECF-AM were purchased from Dojindo (Kumamoto, Japan), and pluronic F-127 was obtained from Molecular Probes (Eugene, OR, USA).

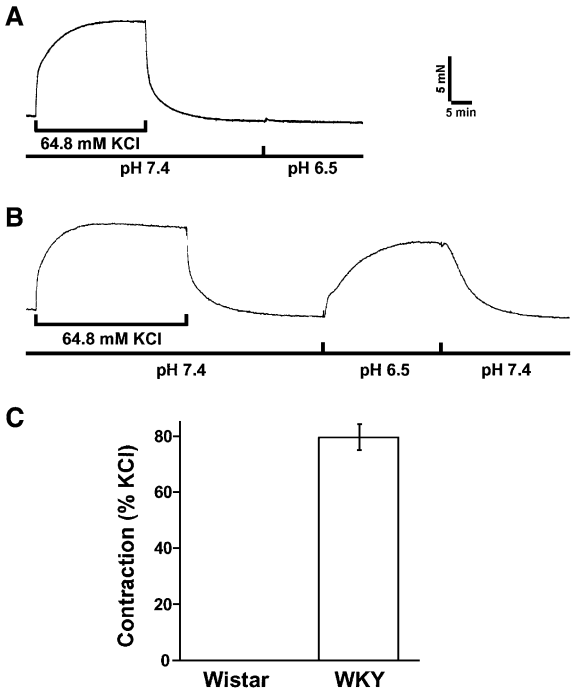

Fig. 1. Effects of acidosis on aortas from Wistar and WKY rats. Representative recordings showing the effects of acidosis on the resting tension of isolated aortas from Wistar (A) and WKY (B) rats. pH was changed from 7.4 to 6.5 by exchanging the bathing solution. (C) Quantification of the contractile response induced by acidic pH in Wistar and WKY aortas. The data represent the means ± S.E.M. $n = 8$ experiments from three to four different animals in each group.

Phenylephrine and iberiotoxin were dissolved in water, while glibenclamide, 4-aminopyridine, fura 2-AM, and BCECF-AM were dissolved in dimethyl sulphoxide.

2.7. Data analysis

Data from tension recording experiments are expressed as mean ± S.E.M. The relaxation or potentiation of phenylephrine-induced contraction caused by acidosis have been expressed as the percentage of the contraction observed at pH 7.4. *n* represents the number of experiments performed. Data were analysed by Student's *t*-test, and the differences were considered statistically significant at $P < 0.05$.

3. Results

3.1. Effect of acidosis on the resting tension of isolated aortas from Wistar and WKY rats

Decreasing pH from 7.4 to 6.5 by exchanging the bathing solution showed no effect on the resting level of aorta from Wistar rat (Fig. 1A), while acidosis induced a marked contraction of WKY aorta (Fig. 1B), consistent with our previous studies (Rohra et al., 2002a, 2003a). As shown in Fig. 1C, the magnitude of acidic pH-induced contraction was 79.4 ± 4.7% of the 64.8 mM KCl-induced contraction in WKY aorta ($n = 8$).

3.2. Effect of acidosis on precontracted aortas from Wistar and WKY rats

The aortas from both Wistar and WKY rats were contracted with submaximal concentration of phenylephrine (300 nM) at pH 7.4. When the response reached a steady level, the bathing solution was exchanged with the

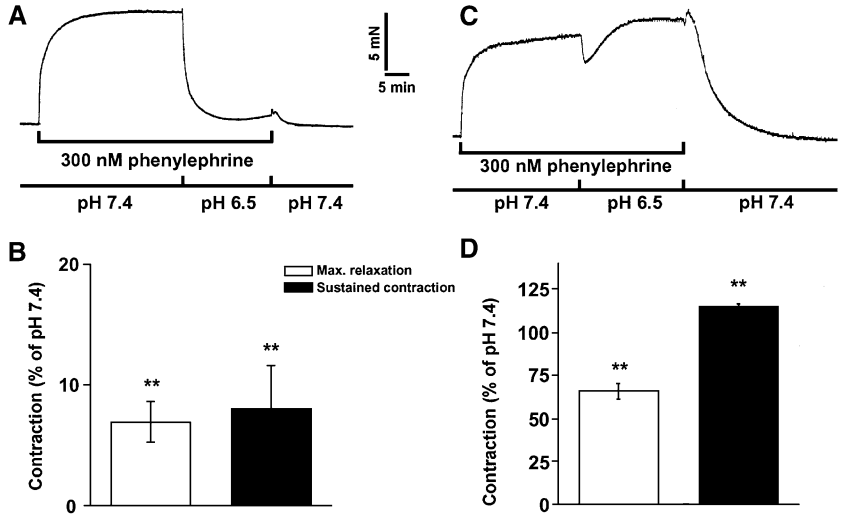

Fig. 2. Effects of acidosis on the phenylephrine-contracted aortas from Wistar and WKY rats. Wistar (A) and WKY (C) aortas were contracted with 300 nM phenylephrine at pH 7.4. After the peak response was achieved, pH of the bathing solution was decreased to 6.5 as described in Results. (B) and (D) show the quantification of the effects of acidosis on contractile responses to phenylephrine in Wistar and WKY aortas, respectively. The contraction to phenylephrine at pH 7.4 was considered as 100%. The "Max. relaxation" represents the minimum level of contraction attained after decreasing pH to 6.5, while "Sustained contraction" is the level of maximum maintained contraction at acidic pH. The data represent the means ± S.E.M. $n = 5–7$ experiments from three to four different animals in each group. $^{**}P < 0.01$ versus contractile response at pH 7.4 in the respective group.

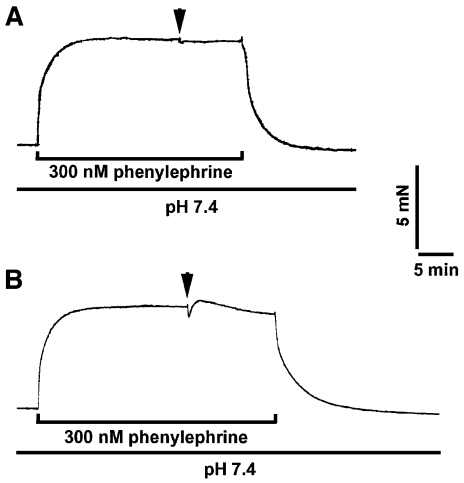

Fig. 3. Representative recordings showing the effect of changing the bathing solution without altering the pH. Wistar (A) and WKY (B) aortas were contracted with 300 nM phenylephrine at pH 7.4. After the peak response was achieved, bathing solution was drained and replenished with the fresh one, containing the same concentration of phenylephrine and of the same pH 7.4. Arrowheads indicate the points at which bathing solution was changed. Recordings are the representatives of five to six experiments from two to three different animals.

pre-warmed, aerated solution containing the same concentration of phenylephrine, but this time, the pH was adjusted to 6.5. As shown in Fig. 2A and B, acidosis nearly abolished the contraction induced by phenylephrine in Wistar aorta, and this acidic pH-induced relaxation was persistent over an observed period of 20 min. However, in three out of seven preparations, a mild and slow recovery was observed. Interestingly, acidosis induced a biphasic response in WKY aorta precontracted with phenylephrine. Like Wistar, acidosis initially caused a relaxation of the phenylephrine-induced contraction of the WKY aorta that was followed by a slow recovery and a sustained contraction (Fig. 2C). In contrast to Wistar, the magnitude of sustained contraction at pH 6.5 was more than that observed at pH 7.4 (Fig. 2D). In separate experiments, the effects of acidic pH on the contractile state of aortas from Wistar and WKY rats were monitored at multiple pH points. The acidic pH-induced relaxation/potentiation of the contractile response to phenylephrine in Wistar and WKY aortas was dependent upon the level of acidosis (data not shown).

In order to exclude the possibility of any artifact, experiments were done to check whether the exchange of bathing solution itself has any effect on the contractile state of the aorta. The aortas from Wistar (Fig. 3A) and WKY (Fig. 3B) rats were contracted with 300 nM phenylephrine at pH 7.4. When the response attained a peak level, the bathing solution was drained and replenished with a fresh one containing the same concentration of phenylephrine and of the same pH 7.4. As shown in Fig. 3, there was no change in the level of contraction after the exchange of solution.

3.3. Effect of acidosis on $[Ca^{2+}]i$ and contraction

Phenylephrine caused a rapid increase in the $[Ca^{2+}]_i$ level followed by contraction in fura 2-loaded aortas from both Wistar (Fig. 4A) and WKY (Fig. 4B) rats. When the pH of bathing solution was decreased to 6.5 by the method already described, a rapid and monophasic fall in $[Ca^{2+}]_i$ reaching to almost basal level was observed in Wistar rat aorta. This decrease in $[Ca^{2+}]_i$ was attended by a near-complete relaxation of contraction (Fig. 4A). In contrast, pH 6.5 induced a biphasic response in WKY aorta. An initial decrease in $[Ca^{2+}]_i$ attended by the relaxation of phenylephrine-induced contraction was followed by an increase in $[Ca^{2+}]_i$ level reaching almost to the pre-acidosis level.

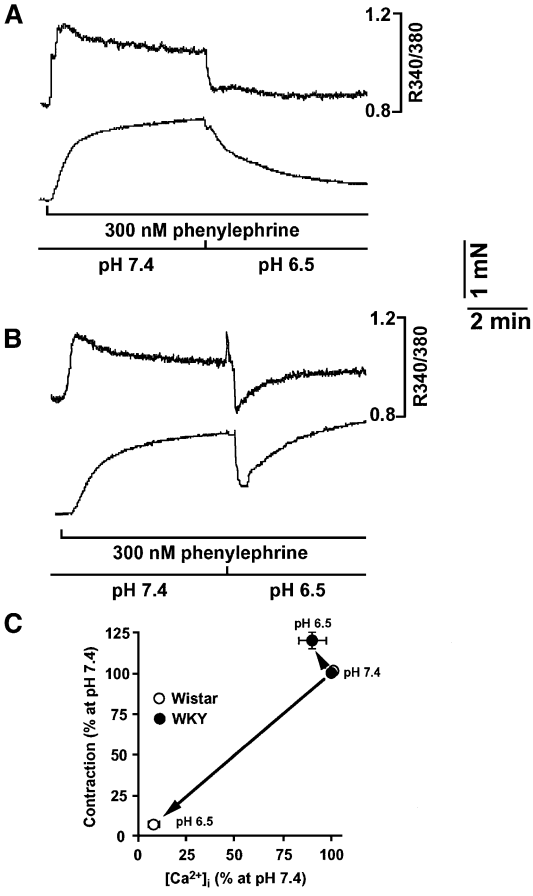

Fig. 4. Effects of acidosis on phenylephrine-induced $[Ca^{2+}]_i$ mobilization and contraction in aortas from Wistar and WKY rats. Aortic strips from Wistar or WKY rats were loaded with 5 μM fura 2-AM and $[Ca^{2+}]_i$, and contraction were measured simultaneously. Aortas were contracted with 300 nM phenylephrine and when the $[Ca^{2+}]_i$ and contraction attained a steady level, pH of the bathing solution was changed to 6.5 as described in Results. Typical recordings showing the effects of acidic pH on the phenylephrine-induced $[Ca^{2+}]_i$ mobilization (upper traces) and contraction (lower traces) in Wistar (A) and WKY (B) aortas. (C) shows the quantification of the effects of acidosis on the contractile response and $[Ca^{2+}]_i$ mobilization produced by phenylephrine in Wistar and WKY aortas. The contraction and $[Ca^{2+}]_i$ increase caused by phenylephrine at pH 7.4 were considered as 100%. The data represent the means ± S.E.M. $n = 5–6$ experiments from three different animals in each group.

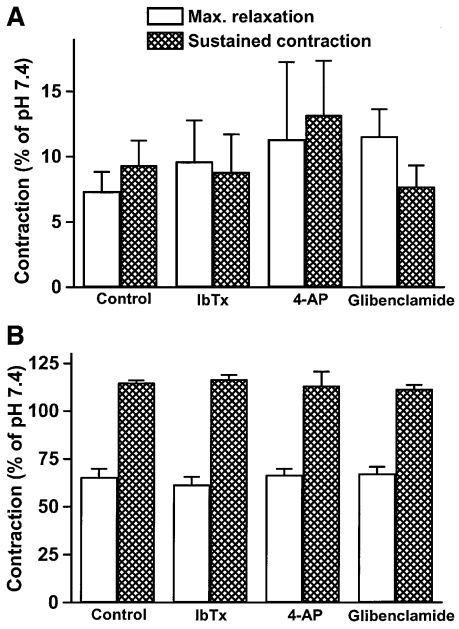

Fig. 5. Effects of acidosis on phenylephrine-contracted aortas in the presence of K^+ channel inhibitors. Aortas from Wistar (A) and WKY (B) rats were contracted with 300 nM phenylephrine. After the peak response was achieved, K^+ channel inhibitors, iberiotoxin (100 nM), 4-aminopyridine (1 mM) or glibenclamide (5 μM) were added to the bathing solution. 10 min later, pH of the solution was changed to 6.5 as described in Results. The contractile response to phenylephrine at pH 7.4 was considered as 100%. Since iberiotoxin mildly increased the contractile response to phenylephrine, the final level of contraction after addition of iberiotoxin was adjusted to 100%. The "Max. relaxation" represents the minimum level of contraction attained after decreasing pH to 6.5, while "Sustained contraction" is the level of maximum maintained contraction at acidic pH. The data represent the means ± S.E.M. $n = 6–8$ experiments from three to four different animals in each group.

Although the sustained $[Ca^{2+}]_i$ levels before and after acidosis were not much different, in two out of five experiments, the sustained level of $[Ca^{2+}]_i$ was even lower at acidic pH, but interestingly, the contractile response to phenylephrine observed at pH 6.5 was significantly higher compared to that at pH 7.4 (Fig. 4B). The interrelationship between $[Ca^{2+}]_i$ and contraction at pH 7.4 and 6.5 is depicted in Fig. 4C.

3.4. Effects of K^+ channel inhibitors on the acidic pH-induced relaxation

In order to evaluate further whether the acidic pH-induced sustained relaxation in Wistar aorta and the initial relaxation phase in WKY aorta are due to activation of K^+ channels, experiments were performed using iberiotoxin (a Ca^{2+}-activated K^+ [K_{Ca}] channel inhibitor), 4-aminopyridine (a delayed rectifier K^+ channel inhibitor) and glibenclamide (an ATP-sensitive K^+ [K_{ATP}] channel inhibitor). The aortas from both Wistar and WKY rats were contracted with 300 nM phenylephrine at pH 7.4, and when the response reached a stable level, iberiotoxin (100 nM), 4-aminopyridine (1 mM) or glibenclamide (5 μM) were added to the bathing solution. Iberiotoxin itself caused a small increase in the level of contraction induced by phenylephrine (3.8 ± 1.1% of the contraction to 300 nM phenylephrine). After 10 min, the pH of the solution was decreased to 6.5 in a similar way, as shown in Fig. 2, but this time, the solution also contained K^+ channel inhibitors. As shown in Fig. 5A, none of the K^+ channel inhibitors was able to inhibit the relaxation of phenylephrine-induced contraction induced by acidic pH in Wistar rat aorta. Similarly, in WKY aorta, neither the initial relaxation nor the maintained contraction were affected by any of the K^+ channel inhibitors used (Fig. 5B).

3.5. Effect of decrease in extracellular pH on intracellular pH

We have shown previously that the magnitude of acidic pH-induced contraction is closely correlated with the pH_i (Furukawa et al., 1996; Rohra et al., 2003b). Therefore, intracellular acidification profiles of both strains were investigated after decreasing pH_o. pH_i at resting conditions (pH_o 7.4) was found to be 7.30 ± 0.02 and 7.31 ± 0.01 in aortas from Wistar and WKY, respectively ($n = 5–6$ in each group). Changing pH_o to 6.5 produced a rapid fall in pH_i in both strains (Fig. 6A and B) and a corresponding contraction in WKY (Fig. 6B). The steady-state pH_i

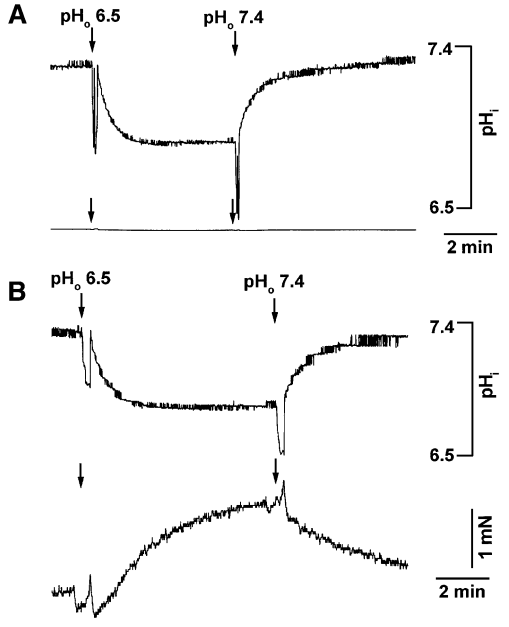

Fig. 6. Effect of decrease in extracellular pH (pH_o) on intracellular pH (pH_i) and resting tension in Wistar and WKY rat aortas. Aortic strips from Wistar and WKY rats were loaded with BCECF-AM as described in Materials and methods and simultaneous measurements of pH_i (upper traces) and contraction (lower traces) were done. pH_o was changed from 7.4 to 6.5 by changing the bathing solution. Representative recordings showing the effect of extracellular acidosis on pH_i and resting tension in aortas from Wistar (A) and WKY (B) rats. $n = 5–6$ experiments from three different animals in each group.

achieved at pH_o 6.5 was not different in aortas from Wistar (7.01 ± 0.03, $n = 5$) and WKY (7.03 ± 0.04, $n = 6$).

4. Discussion

The results of this study demonstrate the differential effects of acidic pH on the aortas from Wistar and WKY rats. Acidosis showed no effect on the resting state of Wistar rat aorta, but a marked contraction was observed in WKY aorta. Since we have shown that the contraction to acidic pH is dependent on pH_i (Furukawa et al., 1996; Rohra et al., 2003b), intracellular acidification profiles of Wistar and WKY aortas were evaluated following decrease in pH_o to exclude the possibility that a difference in pH_i might be responsible for the differential effects of acidosis on the two strains. The results of these experiments showed that the steady-state pH_i attained at pH_o 6.5 was not different in the aortas from Wistar and WKY rats; hence, this parameter does not contribute to the strain-specific effects of acidic pH.

The studies on the effects of acidic pH on the contractile state of vascular tissue are not abundant; nevertheless, a general consensus is that the acidosis relaxes the vessels (Kinoshita and Katusic, 1997; Berger et al., 1998). While investigating this notion further in our experimental conditions and using two different strains, we observed that the initial event after decreasing pH was the relaxation of the phenylephrine-contracted aortas from both Wistar and WKY rats, in agreement with the above studies. However, what follows later does not fully support the previous studies. The sustained phase of phenylephrine-induced contraction was indeed potentiated by acidosis in WKY aorta, in contrast to that from Wistar aorta, where the phenylephrine-induced contraction was inhibited by acidification. To the best of our knowledge, there is no other report to document the effects of acidic pH on the contractile state of WKY aorta. From these findings, it is suggested that the effects of acidic pH on vascular tissue cannot be generalized; rather, these effects are strain specific.

We attempted to investigate the mechanism of sustained and initial transient relaxation of phenylephrine-induced contraction in Wistar and WKY aortas, respectively. Since a respective increase or decrease in $[Ca^{2+}]_i$ is a prerequisite for smooth muscle contraction or relaxation in most of the cases (for a review, see Karaki et al., 1997), $[Ca^{2+}]_i$ profiles of fura 2-loaded aortas were evaluated under the acidic pH conditions. In the aorta from Wistar rat, both the decrease in $[Ca^{2+}]_i$ and relaxation of phenylephrine-induced contraction caused by acidosis were complimentary. A sustained decrease in $[Ca^{2+}]_i$ was consistent with a sustained relaxation of contraction. However, in WKY aorta, a transient decrease in $[Ca^{2+}]_i$ attended by a transient relaxation was followed by a recovery that never attained a level higher than the pre-acidosis conditions. Interestingly, the contractile response was significantly potentiated by acidosis at similar or even less that pre-acidosis $[Ca^{2+}]_i$ levels. All these findings suggest that the relaxant effect of acidic pH is related to a decrease in $[Ca^{2+}]_i$ in both Wistar and WKY rats, but the contractile effect of acidosis in WKY aorta may be dissociated from the $[Ca^{2+}]_i$ level in some part, the latter suggesting the presence of Ca^{2+}-sensitisation mechanism. However, further studies are required to determine whether this effect was the result of increasing Ca^{2+} sensitivity in contractile elements.

K_{ATP} channels in canine basilar arteries (Kinoshita and Katusic, 1997) and porcine coronary arterioles (Ishizaka et al., 1999) and voltage-gated K^+ channels in rat coronary vascular smooth muscle cells (Berger et al., 1998) have been shown to be activated by acidic pH leading to hyperpolarization, decrease in $[Ca^{2+}]_i$, and relaxation. More recently, intracellular acidosis has been reported to activate cloned K_{ATP} channels directly (Xu et al., 2001). All these reports compelled us to evaluate the involvement of various types of K^+ channels in acidic pH-induced fall in $[Ca^{2+}]_i$ and relaxation in Wistar and WKY aortas. An additional question that we aimed to address by using K^+ channel inhibitors was if K^+ channels do serve to antagonise and limit the magnitude of acidic pH-induced potentiation of contraction in WKY aorta. K_{Ca} channel inhibitor, iberiotoxin, delayed rectifier K^+ channel inhibitor, 4-aminopyridine, and K_{ATP} channel inhibitor, glibenclamide, were used in this study, and none of them was able to modify the sustained or transient relaxation of the contractile response to phenylephrine induced by acidic pH in Wistar and WKY aortas, respectively. Moreover, neither of these compounds did have any effect on the sustained contraction of WKY aorta. The concentrations of iberiotoxin (Edwards et al., 2001), 4-aminopyridine (Sobey and Faraci, 1999), and glibenclamide (Teramoto et al., 2000) used in the present study were sufficient to inhibit K_{Ca} and delayed rectifier and K_{ATP} channels, respectively. These results suggest that like the strain specificity, the effects of acidosis are vascular tissue specific as well, and unlike peripheral vasculature, activation of K_{Ca}, delayed rectifier or K_{ATP} channels is not a mechanism underlying the decrease in $[Ca^{2+}]_i$ and relaxation caused by acidification in both Wistar and WKY aortas. Besides, these channels do not limit the extent of acidic pH-induced potentiation of contraction.

The precise mechanism about how the acidosis causes a decrease in $[Ca^{2+}]_i$ resulting in relaxation of the phenylephrine-induced contraction is yet to be elucidated. Apart from K^+ channels, a direct inhibitory effect of acidosis on L-type Ca^{2+} channels in vascular smooth muscle has been reported (Klockner and Isenberg, 1994; Smirnov et al., 2000). From the results of these previous reports and the lack of involvement of K^+ channels in the current study, it is reasonable to speculate that the decrease in $[Ca^{2+}]_i$ and relaxation of phenylephrine-induced contraction caused by acidosis is due to inhibition of Ca^{2+} channels.

Here, it is emphasized that the aorta from WKY exhibits the dual reactivity towards acidification, an initial inhibition,

shared with Wistar rat aorta, that is later overcome by stimulation. This later stimulation in WKY is responsible for the strain-specific effects of acidosis observed. We have discussed various aspects of the contractile effects of acidosis on WKY and SHR aortas at length in our previous reports that release of Ca^{2+} from sarcoplasmic reticulum (Rohra et al., 2003a, c) and activation of Cl^- channels leading to depolarization and Ca^{2+} influx via voltage-dependent Ca^{2+} channels (Rohra et al., 2002a) at least, in part, cause contraction in acidic pH condition in SHR and WKY. Furthermore, we have found that acidosis increased tyrosine phosphorylation of proteins, which does not occur in Wistar rat aorta (Rohra et al., 2002b). Since WKY might have been spontaneously isolated from Wistar rat, it is reasonable to speculate that mutation in a single or multiple genes might be responsible for the difference in phenotype as observed from the various contractile mechanisms operating under the acidic pH conditions. Since diabetes mellitus is frequently associated with metabolic acidosis (Fearon and Steele, 2002, Halperin et al., 1981), the next interesting area will be to study the pathophysiological consequences of acidosis in WKY phenotype in the presence of diabetes mellitus and other metabolic disorders. In normal rats, diabetes is associated with enhanced relaxation (Downing et al., 1982), similar to the present study, where acidosis relaxed the aorta of Wistar rats. It is speculated from these findings that diabetes and the consequent acidosis in WKY phenotype rats might make the vasculature susceptible to contraction and thus have serious consequences in this setting. Further studies are required to elucidate this phenomenon.

In conclusion, the results of the present study demonstrate for the first time that acidosis exerts relaxation and relaxation followed by potentiation of contractions to phenylephrine in Wistar and WKY aortas, respectively. Furthermore, acidic pH-induced relaxation of aortas from both the strains is due to a decrease in $[Ca^{2+}]_i$, but is independent of the activation of K_{Ca}, delayed rectifier or K_{ATP} channels.

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