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Short communication

Extracellular acidosis results in higher intracellular acidosis and greater contraction in spontaneously hypertensive rat aorta

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Abstract

Acidic pH induces a contraction in aorta from spontaneously hypertensive (SHR) and Wistar–Kyoto (WKY) rats. The contractile response to acidic pH in SHR aorta is greater than that in WKY aorta. The purpose of this study was to investigate the correlation among extracellular pH (pH_o), intracellular pH (pH_i) and contraction in order to understand the exaggerated contractile response to acidic pH in SHR aorta. pH_i measurement showed that at pH_o 6.5, intracellular acidification was greater in SHR aorta than in WKY aorta. Decreasing pH_o further to 6.2 in WKY aorta produced intracellular acidification close to that achieved at pH_o 6.5 in SHR aorta, and at this level, the difference in contractile response between the two strains was also abolished. These results suggest that acidic pH_i, but not pH_o, is closely correlated with the contractile response and that the exaggerated contractile response in SHR aorta is due to a greater fall in pH_i.

Keywords: Acidosis, intracellular; Aorta; pH, acidic; Spontaneously hypertensive rat (SHR); Wistar–Kyoto rat (WKY)

1. Introduction

Intracellular pH (pH_i) regulates various cellular functions. Changes in extracellular pH (pH_o) produce changes in pH_i (Smith et al., 1998; Austin and Wray, 2000). The pH of the blood and extracellular fluid is maintained within narrow limits around 7.4, but it may be altered in pathophysiological conditions such as pulmonary edema, kidney damage and occlusive vascular disease (Smith et al., 1998). Ischemia and metabolic disorders, diabetes mellitus and renal dysfunction, for example, cause extracellular and intracellular acidification. Alterations in pH_i may play a significant role in alterations of vessel wall function and excitation–contraction coupling (Nagesetty and Paul, 1994; Chen and Rembold, 1995).

Despite numerous studies, the mechanisms that contribute to the etiology and pathogenesis of essential hypertension remain unclear. The spontaneously hypertensive rat (SHR) is a useful model for studying various aspects of hypertension. There are several differences between the vascular tissue of SHR and that of the normotensive Wistar–Kyoto (WKY) rat (Jaiswal et al., 1993; Le Jemtel et al., 1993; Asano et al., 1995). Previously, we have shown that acidic pH induces a contractile response that is significantly greater in SHR aorta than in WKY aorta (Rohra et al., 2002a). Since altered pH handling has been shown in hypertensive animals as well as in humans (Rosskopf et al., 1993), this study was carried out to test the hypothesis that changes in intracellular acidification following a decrease in pH_o may be different in SHR and WKY aortas. In this report, for the first time, we demonstrate that the magnitude of acidic pH-induced contraction is directly related to pH_i, and a fall in pH_o results in a higher intracellular acidosis and a greater contractile response in isolated aorta from SHR than in aorta from WKY rats.

2. Materials and methods

All animal procedures were designed in accordance with the Institutional Guidelines of Tohoku University, Sendai, for the care and use of laboratory animals.

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2.1. Tissue preparation

Male SHR and WKY (both 13–14 weeks old) of the NCrj strain 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 the failure of carbachol (1 μM) to cause relaxation of the 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). All experiments were carried out at 37 °C. The pH of the solution was changed from the control value of 7.4 by addition of HCl, and the tissues were treated with PSS with an acidic pH by exchanging the PSS of the organ bath. The tissues were adjusted to a preloaded resting tension of 1*g* and equilibrated for at least 1 h. Isometric contraction was measured with a force-displacement transducer (Nihon Kohden, Tokyo, Japan).

2.3. Simultaneous measurement of pH_i and contraction

The fluorescent pH indicator dye, 2,7-*bis*(carboxyethyl)-carboxyfluorescein (BCECF), was used to monitor changes in pH_i. Aortic strips were loaded with acetoxymethyl ester (AM) of BCECF (10 μM) for 2 h at 37 °C in PSS along with a noncytotoxic detergent, pluronic F-127 (0.025%). Later, the strips were mounted horizontally under a resting tension of 1 g in a bath attached simultaneously to a fluorometer (CAF-100, Japan Spectroscopic, Tokyo, Japan) and a force-displacement transducer. BCECF fluorescence (excitation at 450 and 500 nm, emission at 530 nm) was measured. To calculate the 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).

2.4. Solutions

The composition of the PSS was (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. PSS containing 64.8 mM KCl was made by replacing 60 mM NaCl with equimolar KCl.

2.5. Materials

Rats were purchased from Charles River (Kanagawa, Japan). Nigericin was from Sigma-Aldrich (St. Louis, MO, USA). BCECF-AM and pluronic F-127 were purchased from Dojindo (Kumamoto, Japan) and Molecular Probes (Eugene, OR, USA), respectively.

2.6. Statistics

Data from isometric tension recording experiments are expressed as means ± S.E.M. percentages of the KCl-induced contraction. *n* represents the number of experiments performed. Data were analyzed by Student's *t*-test and the differences were considered statistically significant at $P < 0.05$.

3. Results

Decreasing pH_o from a control value of 7.4 to 6.5 induced a sustained contraction in isolated aortas from both SHR and WKY. Consistent with our previous studies (Rohra et al., 2002a,b), the magnitude of the contractile response was significantly greater ($P < 0.01$) in SHR aorta (110.7 ± 5.4% of the 64.8 mM KCl-induced contraction, $n = 8$) than in WKY aorta (79.6 ± 1.5% of the 64.8 mM KCl-induced contraction, $n = 8$).

In simultaneous pH_i and tension measurement experiments, pH_i at pH_o 7.4 in isolated aortas of SHR and WKY were found to be 7.31 ± 0.02 ($n = 6$) and 7.32 ± 0.01 ($n = 7$), respectively. Changing pH_o to 6.5 caused a rapid decrease in pH_i, and after a delay (1–2 min), a contraction was initiated in aortas from both SHR and WKY. The decrease in pH_i in SHR aorta was significantly greater than that observed in WKY aorta (Fig. 1). The steady-state pH_i achieved following extracellular acidification was fairly persistent over a time range of 18 min in both SHR and WKY aortas (Fig. 1). Consistent with the results of isolated tension recording experiments, the contractile response to pH_o 6.5 in this protocol also was significantly greater in SHR aorta than in WKY aorta (Fig. 2A). Interestingly, a further fall in pH_o to 6.2 in WKY aorta resulted in a corresponding further fall in pH_i and a contractile response that was very close to that observed in SHR aorta at pH_o 6.5 (Fig. 2B).

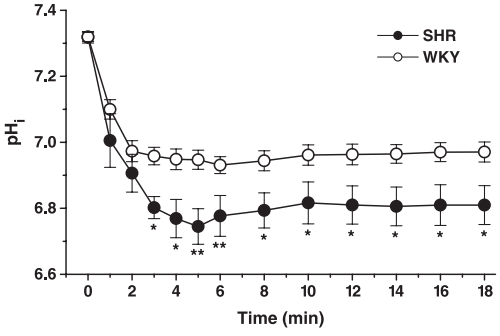

Fig. 1. Effect of acidic pH_o on pH_i in isolated aortas from spontaneously hypertensive (SHR) and Wistar–Kyoto (WKY) rats. Aortic strips were loaded with BCECF-AM and simultaneous measurements of pH_i and contraction were done. Changes in pH_i following a decrease in pH_o against time in SHR and WKY aortas. '0' on the *x*-axis indicates the time when the pH of the bathing solution was changed from 7.4 to 6.5. $n = 6–7$ in each group. $^*P < 0.05$, $^{**}P < 0.01$ between SHR and WKY aortas.

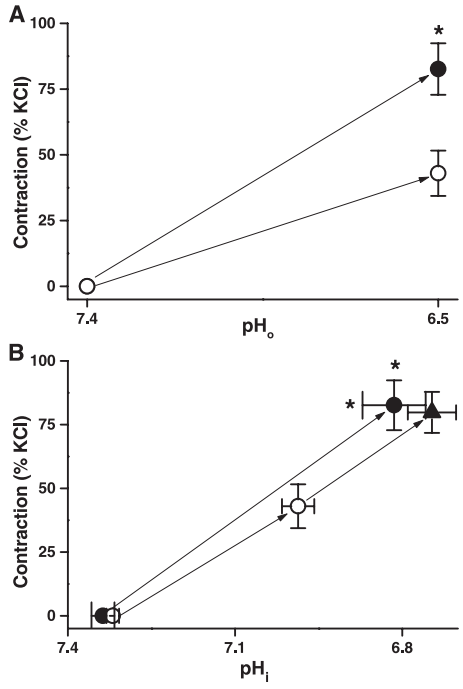

Fig. 2. Interrelationships among pH_o, pH_i and contraction in isolated aortas from spontaneously hypertensive (SHR) and Wistar–Kyoto (WKY) rats. (A) Interrelationship between pH_o and contraction induced by decreasing pH_o to 6.5 in isolated aortas from SHR (●) and WKY (○). *$P<0.05$ showing the difference in contractile response between SHR and WKY aortas. $n=6–7$ in each group. (B) Interrelationship between pH_i and contractile response induced by decreasing pH_o to 6.5 in SHR (●) and WKY (○) aortas and to pH_o 6.2 in WKY aorta (▲). *$P<0.05$ showing the differences in both pH_i and contraction between SHR and WKY aortas at pH_o 6.5. $n=6–7$ in each group.

The interrelationships among pH_o, pH_i and the contractile response are summarized in Fig. 2. It is evident that at pH_o 6.5, the magnitude of the contraction was significantly different in aortas from SHR and WKY (Fig. 2A), with the difference being pH_o-dependent. However, when the contraction was plotted against pH_i, SHR and WKY aortas showed a similar pH_i–contraction relationship (Fig. 2B).

4. Discussion

Decreasing pH_o caused a sustained contractile response in isolated aortas from both SHR and WKY. However, the magnitude of the contraction was significantly greater in the former than in the latter at pH_o 6.5. The SHR is a useful model to study various aspects of human hypertension. The greater contractile response to acidic pH in SHR aorta suggests an important predisposing factor for vasospastic conditions, such as coronary artery disease, which are more prevalent in hypertensive subjects.

While investigating the mechanism of acidic pH-induced contraction, we had already attempted to understand the difference in the contractile response between SHR and WKY. For example, the possible involvement of Cl^- channels and voltage-dependent Ca^{2+} channels (Rohra et al., 2002a), but these data proved too inconclusive to provide clue regarding the difference between the two strains. At a molecular level, tyrosine phosphorylation of phosphatidylinositol 3-kinase was observed to be stimulated at acidic pH, resulting in the acidic pH-induced contraction in SHR aorta (Rohra et al., 2002b), but again we observed the same mechanism to contribute to the acidic pH-induced contraction in WKY aorta (unpublished observation). Therefore, it is reasonable to believe that the mechanism(s) underlying the acidic pH-induced contraction is(are) essentially similar in both SHR and WKY, albeit exaggerated in the former.

Since differences in pH_i-regulating mechanisms have been reported between hypertensive and nonhypertensive humans (Diez et al., 1992) and animals (Izzard and Heagerty, 1989), it is reasonable to compare the relationships between pH_i and contraction in SHR and WKY. Although pH_o was held at 6.5, pH_i was lower in SHR aorta than in WKY aorta and, in parallel, the contractile response induced in SHR aorta was greater. Interestingly, the pH_i attained at pH_o 6.2 in WKY aorta was close to that observed at pH_o 6.5 in SHR aorta and at this point the difference in the magnitude of the acidic pH-induced contraction between SHR and WKY aortas was abolished. From these findings, it is concluded that it is the steady level of pH_i that correlates with the magnitude of contraction, and that the difference in the acidic pH-induced contraction between SHR and WKY aortas is related to the difference in the degree of intracellular acidification achieved following a decease in pH_o.

The mechanism underlying the difference in the steady-state pH_i attained in SHR and WKY aortas following a fall in pH_o has yet to be evaluated. Steady-state pH_i at any given time reflects the balance between the acid-loading and the acid-extrusion mechanisms (Wray, 1988). The Na^+/H^+ exchanger is the most important pH_i-regulating mechanism in vascular smooth muscle cells. Enhanced expression (Garciandia et al., 1995; LaPointe et al., 1997) and increased activity of this exchanger have been reported in hypertensive subjects (Rosskopf et al., 1993) as well as in animal models of hypertension (Izzard and Heagerty, 1989; Silverman et al., 1995). Indeed, we have also observed that amiloride, an inhibitor of the Na^+/H^+ exchanger, produces intracellular acidification and a greater contractile response in SHR aorta (unpublished observation). Therefore, it is unlikely that a blunted activity of Na^+/H^+ exchanger is detrimental to the lower pH_i observed in SHR aorta than in WKY aorta at pH_o 6.5. However, the roles of other pH_i-regulating mechanisms, such as the Na^+/HCO_3^- co-transporter (Neylon et al., 1990) as well as acid-loading mechanisms, in the greater fall in pH_i in SHR aorta have yet to be evaluated. The Cl^-/HCO_3^- exchanger acts as an acidifying mechanism in vascular smooth muscle (for review, see Chipperfield and Harper, 2000), and the activity of this protein has been shown to be increased in hypertension (Diez et al., 1992). Therefore, it is possible that an altered

activity of the Cl^-/HCO_3^- exchanger may be a factor resulting in the higher intracellular acidification observed in the SHR aorta.

In conclusion, the results of the present study demonstrate that pH_i, but not pH_o, is closely correlated with the contractile response in isolated aortas from SHR and WKY. Furthermore, it is shown for the first time that at acidic pH_o, intracellular acidification is greater in isolated aorta from SHR than in isolated aorta from WKY, resulting in a greater contractile response.

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