Changes in pH Increase Perfusion Pressure of Coronary Arteries in the Rat

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Abstract. Stricture of coronary arteries is closely related to ischemic heart disease. The purpose of this study was to examine whether changes in pH caused contraction of rat coronary arteries, as determined using Langendorff perfused hearts. Changing the pH of the perfusate increased perfusion pressure as an indication of the contractile state of coronary arteries. Alkaline pH-induced increase of perfusion pressure in Wistar Kyoto rats (WKY) was almost identical to that of spontaneously hypertensive rats (SHR), whereas acidic pH-induced increase in SHR was much greater than that in WKY. Acidic pH-induced increase in perfusion pressure was inhibited by verapamil, cromakalim, and adenosine. Feeding WKY with N⁵-nitro-L-arginine resulted in hypertension followed by enhanced acidic pH-induced increase in perfusion pressure. These results suggest that acidic-pH induced contraction of rat coronary arteries is caused by Ca²⁺ influx through voltage-dependent Ca²⁺ channels and the contraction is enhanced by hypertension.

Keywords: acidosis, coronary perfusion pressure, hypertension, vascular smooth muscle, spontaneously hypertensive rat

Introduction

The contractile state of vascular smooth muscles can be modulated by several factors including pH. Although intracellular pH (pHᵢ) is maintained in the narrow range of 7.0 – 7.2 (1), disturbances including ischemia and hypoxia in the circulatory system cause acidosis (2, 3). Both extracellular pH (pHₑ) and pHᵢ are known to alter vascular tone and thereby affect the circulation (4 – 6). Vasoactive responses to acidification in vascular smooth muscles are often opposite, i.e., relaxation and contraction. Opposite responses are likely to be due to differences in vascular bed and species (3, 7, 8). However, effects of pHᵢ on vascular smooth muscles have been unclear.

A previous study showed that decreasing pHₑ caused intracellular acidification and contraction in rat aorta and aorta contraction in spontaneously hypertensive rats (SHR) is much greater than that from Wistar Kyoto rats (WKY) (9). It was also indicated that acidic pH-induced contraction was reinforced by elevation of blood pressure and was due to Ca²⁺ influx mediated through activation of voltage-sensitive Ca²⁺ channels (9). Furthermore, acidic pH induced an increase in tyrosine phosphorylation of phosphatidyl inositol 3-kinase, resulting in the myosin light chain phosphorylation-dependent contraction of SHR aorta (10). Vasocontractile responses induced by some agonists were enhanced in vascular smooth muscles in hypertensive rats (11 – 14). It is well known that both ischemia and hypoxia frequently cause acidosis in coronary arteries followed by angina pectoris and myocardial infarction and that hypertension is one of the most serious factors that cause these types of ischemic heart diseases. Therefore, it is important to investigate the effects of pH on contractility of the coronary artery. However, previous reports were controversial. While, it was reported that acidosis caused vasodilation in coronary arteries of the Sprague-Dawley rat (8), pig (15, 16), and dog (17), Wilson and Woodward (18) reported that acidosis induced coronary constriction in the rat heart. The animals used in these reports were all normotensive, and the effect of acidosis...
Materials and Methods

Animals
Male Wistar rats, WKY, and SHR were purchased from Charles River Japan (kanagawa). Rats were 10-week-old. Animals were treated in accordance with the principles and guidelines for the care and use of laboratory animals approved by The Japanese Pharmacological Society as well as the Law and Notification of the Japanese Government. WKY were fed following a plain commercial diet (F-2; Funabashi, Chiba) or one containing 0.02% L-nitro-L-arginine for 3 to 10 days before the start of the experiment. Food and water were available ad libitum.

Materials
Verapamil, cromakalim, and phentolamine were obtained from Sigma Chemical Co (St. Louis, MO, USA). Adenosine came from Wako Pure Chemical Industry (Tokyo), and L-nitro-L-arginine was from Aldrich Chemical Company (Milwaukee, WI, USA).

Recording of blood pressure
Following administration of heparin sodium (300 U), the carotid artery was exposed, and a polyethylene tube was inserted while the animal was under general anesthesia (50 mg/kg body weight pentobarbital). The carotid artery was connected to a pressure transducer (Nihon Kohden, Tokyo) and mean arterial blood pressure was recorded on a graph for at least 1 h before reaching a plateau. Mean blood pressure values of WKY and SHR were 111 ± 15 (n = 10) and 163 ± 18 (n = 10), respectively.

Recording of perfusion pressure
The heart was quickly excised, washed in ice-cold Krebs-Ringer solution, and weighed (0.903 ± 0.031 g for WKY, n = 10; 0.944 ± 0.0453 g for SHR, n = 10). According to the Langendorff procedure, a glass cannula was inserted into the aorta and the heart was perfused retrogradely via the aorta with Krebs-Ringer solution at a constant flow of about 12 ml/min using a microtube-pump (MP3; Rikadenki, Tokyo) for about 20 min equilibration. The Krebs-Ringer solution (pH 7.4) used as perfusate contained the following components: 120 mM NaCl, 4.8 mM KCl, 1.2 mM CaCl₂, 1.3 mM MgSO₄, 25.2 mM NaHCO₃, 5.8 mM glucose, 1.2 mM KH₂PO₄, and 20 mM HEPS, maintained at 37°C with a 95% O₂ : 5% CO₂ gas mixture. Perfusion pressure was measured through a branch of the aortic cannula using the pressure transducer. Perfusion pH was altered from an original value of 7.4 by the addition of either HCl or NaOH. The perfused heart was treated with various drugs by adding each drug to the pH-adjusted perfusate.

Measurements of isometric myocardial contraction
Hearts from SHR were quickly excised and washed in ice-cold Krebs-Ringer solution. The atrium was separated and the ventricle was sliced approximately 3 mm in width and 10 mm in length. One end of the cardiac muscles was secured to an acrylic tissue holder by a silk ligature, and the other end was connected to a force-displaced transducer (Nihon Kohden, Tokyo). Cardiac strips were suspended in a 20 ml organ bath containing Krebs-Ringer solution gased with 95% O₂ : 5% CO₂ (37°C) and were stimulated at 1 V with a duration of 10 ms and a frequency of 1 Hz. These strips were equilibrated for about 1 h under a resting tension of 1 g, and then isometric contractions were measured using a transducer and recorded on a polygraph. The pH of the solution was changed from the control value of 7.4 by the addition of either NaOH or HCl.

Statistical analyses
All data are reported as means ± S.E.M. and n refers to the number of animals used. Statistical significance of differences between the control and drug-response group (of the same species) was calculated using Student’s paired t-test, while statistical significance between two completely different animal groups (different species) was calculated using Student’s unpaired t-test. Results are considered statistically significant at P<0.05.

Results
Effects of changing perfusate pH on perfusion pressure
To investigate the effects of pH changes on the contractile response of coronary arteries, isolated rat hearts, mounted on the Langendorff perfusion apparatus were perfused at a constant flow rate of 12 ml/min, and perfusion pressure was monitored. Perfusion pressure at pH 7.4 for isolated hearts from WKY and SHR was 49.1 ± 9.14 (n = 6) and 53.4 ± 9.0 mmHg (n = 5), respectively. There was no significant difference between perfusion pressure for WKY and for SHR (P>0.05). Change in perfusate pH resulted in an increase in perfusion pressure. An example of the contractile...
response is shown in Fig. 1. Acidic pH-induced increase of perfusion pressure in SHR was much greater than that in WKY (Fig. 1: a and c). Alkaline pH-induced increase in SHR was almost identical to that in WKY (Fig. 1: b and d). Addition of NaCl instead of NaOH or HCl caused no change in perfusion pressure (data not shown). The relation between perfusate pH and increase in perfusion pressure of the coronary artery in WKY and SHR is shown in Fig. 2. Maximum response to alkaline pH was greater than that to acidic pH. Perfusion pressure increased in the range of pH 7.7 to 8.6 or at pH 6.5 in a pH-dependent manner. It was interesting to note that alkaline pH-induced increase of perfusion pressure in WKY was almost identical to that in SHR between pH 7.4 – 8.6, while acidic pH-induced increase of perfusion pressure in SHR was greater than that in WKY, especially at pH 6.5 – 6.8.

Effect of changing pH of the medium on myocardial contractility

To determine the effects on myocardial contractility using coronary perfusion pressure, the isometric tension of cardiac muscles was measured. Contractile forces of the left atrium and the right ventricle were suppressed by changing the pH from 7.4 to acidic pH 6.5 (Fig. 3), and that of the right atrium and the left ventricle did not change (data not shown). Increasing pH from 7.4 to 8.5 did not affect the contractility of any myocardium (data not shown).

Effects of a range of drugs on the increase in perfusion pressure induced by changing perfusate pH of the perfusate

Effects of a range of drugs on the increase in perfusion pressure induced by changing pH were investigated in SHR hearts (Fig. 4). Acidic pH (6.5)-induced increase in perfusion pressure was markedly inhibited by verapamil (1 – 5 \( \mu \)M), a blocker of voltage-sensitive Ca\(^{2+} \) channels; cromakalim (1 – 5 \( \mu \)M), an opener of K\(^+ \) channels; propranolol (1 – 5 \( \mu \)M), an inhibitor of beta-adrenergic receptors; and atenolol (1 – 5 \( \mu \)M), a blocker of beta-adrenergic receptors.
Effects of feeding with N\textsuperscript{G}-nitro-L-arginine on blood pressure and the increase of perfusion pressure induced by changing perfusate pH

Perfusion pressure increase in SHR induced by acidic pH seemed to be age-dependent or blood pressure-dependent. Mean blood pressure of 10-week-old and 15-week-old SHR rats was 163 ± 18 (n = 10) and 205 ± 21 mmHg (n = 5), respectively. On the other hand, the increase in perfusion pressure evoked by exposure to acidic pH (7.0) was 45.2 ± 4.1 (n = 10) and 73.4 ± 8.9 mmHg (n = 5), respectively. To further investigate the relation between blood pressure and increase in perfusion pressure, changing-pH induced increase of perfusion pressure was examined in hearts from WKY fed with 0.02% N\textsuperscript{G}-nitro-L-arginine, an inhibitor of nitric oxide synthase. Feeding with N\textsuperscript{G}-nitro-L-arginine elevated blood pressure in WKY from 111.2 ± 14.4 mmHg (n = 7) to 129.2 ± 13.5 mmHg (n = 9) after 3 days, to 141.0 ± 6.3 mmHg (n = 5) after 6 days, and to 177.0 ± 10.4 mmHg (n = 5) after 10 days (Fig. 5a). In contrast, feeding with the normal diet for 10 days did not cause any marked elevation of blood pressure in WKY (122.5 ± 12.5 mmHg, n = 6). Resting pressure recorded at the beginning of the perfusion in normal WKY was almost identical to that in SHR and WKY fed with N\textsuperscript{G}-nitro-L-arginine (Fig. 5b). Acidic pH (6.5)-induced increase of perfusion pressure in control WKY (normal diet) was 24.1 ± 4.2 mmHg. After feeding with N\textsuperscript{G}-nitro-L-arginine for 3, 6, and 10 days, increase in perfusion pressure was markedly enhanced (51.3 ± 10.7, 73.6 ± 10.8, and 97.6 ± 23.1 mmHg, respectively, n = 5–9) (Fig. 5c). There was no correlation between alkaline pH (8.5)-induced increase in perfusion pressure and blood pressure (Fig. 5d). Perfusate containing N\textsuperscript{G}-nitro-L-arginine (10 mM) did not affect resting pressure (data not shown), suggesting the lack of a direct effect of N\textsuperscript{G}-nitro-L-arginine on perfusion pressure.

Discussion

In the present study, we attempted to investigate the effects of pH changes on the contractile state of coronary arteries in normotensive and hypertensive rats. Changing the pH of the perfusate from 7.4 to alkaline pH resulted in an increase of coronary perfusion pressure in both WKY and SHR, confirming previous reports (5, 19). However, effects of acidic pH on coronary arteries in normotensive rats have been controversial (8, 18). Moreover, previous reports paid no attention to the blood pressure in rats. We observed that acidic pH induced an increase in coronary perfusion pressure in SHR and this was greater than that in WKY. This result suggests that coronary arteries in SHR are highly sensitive to acidic pH compared with WKY. Furthermore, hypertensive WKY produced by NOS inhibition showed a similar high sensitivity to acidic pH. These results may explain some of the disagreements concerning the response to acidic pH between previous reports.

The cardiac endothelium produces a variety of vasoactive factors including nitric oxide (NO). Impaired endothelium-dependent vasodilatation can be often linked to a decrease in endothelium-derived nitric oxide, because administration of L-arginine has been shown to reduce systemic blood pressure in some forms of experimental hypertension (20, 21). Furthermore, we confirmed in this report that administration of N\textsuperscript{G}-nitro-L-arginine raises blood pressure and alters vascular nature (9, 22–25). Endothelial dysfunction and reduced NO production represent prominent pathophysiological abnormalities associated with hypertensive cardiovascular diseases (21). Therefore, high sensitivity to
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Acidic pH being prominent in hypertensive rats may be due to endothelial dysfunction.

Coronary circulation is impaired during systole because of the extravascular compression by cardiac contractions (26, 27). To exclude this possibility as a cause of the increase in coronary perfusion pressure, the effects of acidic pH on cardiac muscles were investigated. Contractility of the left atrium and the right ventricle was suppressed by acidic pH, while that of the right atrium and the left ventricle was not affected by it (Fig. 3). It has been indicated that myocardial contractility decreases during ischemia, hypoxia, and intracellular acidosis (28, 29). Therefore, it is likely that the increase in perfusion pressure in response to pH change resulted from coronary artery contraction rather than from an increase in myocardial contractility.

Previous studies showed that in aortic smooth muscles, acidic pH-induced contraction was markedly inhibited by treatment with verapamil, a blocker of Ca^{2+} channels; cromakalim, an opener of K^+ channel; and chloride channel inhibitors including niflumic acid and diisothiocyanatostilbene-2,2'-disulfonic acid (DIDS) (9, 30). Acidic pH-induced increase of perfusion pressure in coronary arteries was inhibited by treatment with verapamil and cromakalim (Fig. 4). Niflumic acid and DIDS partially inhibited increase in perfusion pressure (unpublished own observations). Activation of K^+ channels and inhibition of Cl^- channels may cause hyperpolarization of the plasma membrane and render the membrane potential more negative and then suppresses voltage-dependent Ca^{2+} channels which are activated by depolarization of the plasma membrane (9, 30, 31). These observations suggest that acidic pH causes activation of voltage-dependent Ca^{2+} channels.
through depolarization of the plasma membrane of the coronary artery smooth muscles.

It is interesting to discuss the relation between hypertension and diseases that result from acidosis during ischemia or hypoxia. The degree of perfusion pressure response to alkaline pH in normotensive WKY was almost identical to that in SHR and hypertensive WKY fed with Nω-nitro-L-arginine (Fig. 5d). Interestingly, response to acidic pH in the hypertensive rats was much larger than that in normotensive ones, especially at pH 6.5 – 6.8 (Figs. 2 and 5c). These results suggest that hypertension is a serious factor that causes contraction of coronary arteries in weak acidosis. It is also suggested that acidic pH-induced increase in perfusion pressure is enhanced by elevation of blood pressure in genetic and acquired hypertensive rats. Hypertension is associated with hypertrophy of the coronary vessels in some cases resulting in reduction of the lumen diameter. This would lead to an increase in perfusion pressure by a purely physical process as described by Poiseuille’s Law. If this is true, the physiological process underlying the constrictor response does not necessarily have to change in order to account for the enhanced constriction. However, perfusion pressure before changing pH in SHR was not significantly different from that in WKY. Furthermore, the extent of alkaline pH-induced increase of perfusion pressure in SHR was almost identical to that in WKY. Therefore, an increase in response to acidic pH in hypertensive rats may not be due to a reduction in the lumen diameter of the coronary vessels at least in SHR rats younger than 10-week-old.

Several functional abnormalities have been found in vascular smooth muscle of hypertensive model animals such as SHR. Differences in voltage-dependent Ca2+ channels between smooth muscles in WKY and SHR have been reported (32, 33). It is well known that plasma membrane permeability of K+ is increased in arteries of SHR (13, 34). Open probability, Ca2+ sensitivity, and gene expression of Ca2+-activated K+ channels are enhanced in SHR arteries compared with those in WKY. This is thought to be due to a compensatory mechanism limiting arterial contraction in hypertension (11, 35 – 37). Ca2+-activated K+ channels are sensitive to pH, and acidic pH decreases the probability of channel opening (13, 16, 38). In this study, acidic pH-induced increase in perfusion pressure was enhanced by hypertension, and hyperpolarization caused by cromakalim or direct inhibition of voltage-dependent Ca2+ channels by treatment with verapamil markedly suppressed acidic pH-induced increase in perfusion pressure of the coronary artery. Therefore, it is possible that Ca2+-activated K+ channels in coronary arteries of hypertensive rats, which are highly activated to maintain the resting tone, are inhibited by acidic pH, resulting in enhancement of acidic pH-induced contraction.

Alkaline pH induced a marked increase of perfusion pressure in coronary arteries as compared with aorta (9), suggesting a high sensitivity to alkaline pH of coronary arteries. However, alkaline pH-induced increase in perfusion pressure was slightly suppressed by drugs that inhibit acidic pH-induced response (Fig. 4). It has been reported that intracellular alkalization causes Ca2+ release from intracellular Ca2+ stores (1, 39) followed by a contraction in vascular smooth muscles (6, 40). In addition to these observations, the alkaline pH-sensitive site of the contractile system seems to be located in the intracellular Ca2+ stores but not in the plasma membrane.

Adenosine is an important metabolite involved in vasodilation, and is released from various tissues as an endogenous protective agent in ischemia (41 – 43). It has been reported that endogenous adenosine contributes significantly to coronary vasodilation during ischemia (44). ATP-sensitive K+ channel activation may play an important role in adenosine-induced vasodilation (42, 45). It was interesting to observe that acidic pH-induced increase in perfusion pressure was inhibited by treatment with adenosine (Fig. 4). Therefore, it is possible that adenosine acts as a protective agent in diseases such as acidosis resulting from ischemia or hypoxia.

In summary, acidic pH-induced contraction of rat coronary arteries is enhanced by elevation of blood pressure. Contractions may be caused by Ca2+ influx through voltage-dependent Ca2+ channels via depolarization of the plasma membrane of coronary artery smooth muscles. The nature of vascular smooth muscles would change by elevation of blood pressure.

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