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Effect of Acidosis on Vasoconstrictors and Vasodilators in Rat Aortic Rings

Shinichi Dozaki*, Yoshihiro Ohta*, Osamu Kemmotsu*

Abstract

Although acidosis induces vasodilation and attenuation of the effects of several cardiovascular drugs, a quantitative analysis of the effect of acidosis on vasoconstrictors and / or vasodilators has not been conducted. In this study we used rat aortic rings to evaluate the effect of pH on vascular contraction following administration of phenylephrine, dopamine and potassium chloride (KCl) and also on the vasodilating action of prostaglandin E1 (PGE1) and nicardipine.

Thoracic aortic rings obtained from male Sprague-Dawley rats (N=20) were suspended in an organ bath containing HEPES solution (37℃) aerated with 100% O_2. Contractile responses to phenylephrine 3×10^{-7}M, dopamine 3×10^{-5}M and KCl 40mM were obtained in each pH solution (7.4, 7.0, 6.6 and 6.2). Relaxation responses with increasing doses of PGE1 and nicardipine concentrations were also obtained.

The contractive response in pH6.2 solutions was 17 ±4.5%, 37±6.3% and 71±4.9% of control which the tension obtained at pH7.4 was regarded as 100 % in the phenylephrine, dopamine and KCl groups, respectively. PGE1 produced dose dependent vasodilation in pH6.2 solution only, which suggested that acidosis could not attenuate the vasodilating action of PGE1. Nicardipine exhibited a vasodilatory effect in each pH solution. The results demonstrated that acidosis itself attenuated the vasoconstrictive effects of phenylephrine and dopamine, and that KCl and nicardipine could maintain their pharmacological actions but PGE1 showed a complicated action in acidosis.

Key words : Acidosis, Aortic ring, Rat, Vasoconstrictors (phenylephrine, dopamine, KCl), Vasodilators (prostaglandin E1, nicardipine)

*Department of Anesthesiology and Intensive Care, Hokkaido University School of Medicine, Sapporo, Japan

Introduction

There are many reports describing the effect of acidosis on cardiac contractility following the administration of cardiovascular drugs. In severe acidosis the pressor response of vascular smooth muscle to catecholamines is decreased[1,2]. Small changes in pH have been shown to cause reductions of vascular smooth muscle contractility[3,4]. It is known that the contractile response of blood vessels is reduced by the application of CO_2[5,6] and also by decreasing the pH of the buffer solution[7,8]. Acidosis decreases the contractility of microvascular smooth muscle by decreasing adrenergic responsiveness through a selective action on α2-mediated constriction[9]. Although the mechanism by which a decrease in the pH induces alterations in smooth muscle contractility has been defined[10], the effect of acidification on vascular tone when vasodilators are administered has not been investigated. In this study using rat aortic rings we evaluated the effect of pH on vascular contraction following administration of phenylephrine, dopamine and potassium chloride (KCl) and on vasodilation following administration of prostaglandin E1(PGE1) and nicardipine.

Methods

After obtaining the approval of our institutional animal research committee, 20 male Sprague-Dawley rats (BW 398±15 gm, mean±SEM) were used. Rats were anesthetized with ether. The thoracic aorta was removed from the diaphragm to the heart then placed in oxygenated Krebs-Henseleit (K-H) solution and dissected free of fat and connective tissue taking care not to damage the endothelial cell layers or stretch the vessels. Aortic rings, cut into 3 mm wide segments, were mounted between 2 stainless steel wires and placed in 40 ml organ baths containing a modified K-H solution of the following composition (mM): KCl 4.75, KH_2PO_4 1.19, $MgSO_4 \cdot 7H_2O$ 1.19, $CaCl_2 \cdot 2H_2O$ 2.54, NaCl 119, $NaHCO_3$ 25 and glucose 11, pH7.4. The solution was continously aerated with a gas mixture of 95% O_2 and 5% CO_2, and maintained at 37 ℃. Tissues were equilibrated for 2 hours under a resting tension of 2 grams by changing of bath fluids every 15 minutes. Isometric tension was measured with a force-displacement transducer (TB-612T, EF-650G, Nihon Koden; Tokyo, Japan) and recorded with a polygraph (RM-6000, Nihon Koden; Tokyo, Japan).

After a 2 hour equilibration period, contraction of the preparation with 3×10^{-7}M phenylephrine was used to test the integrity of endothelium. This concentration of phenylephrine produces submaximal tone (50－70 % of maximum) as previously determined in our laboratory[11]. Acethylcholine (10^{-5}M) was then added to the bath. If the relaxation was more than 50%, the endothelial ring was considered to be intact. Then the bath fluids were changed to HEPES solution of the following composition (mM): KCl 4.0, NaH_2PO_4 1.25, $MgCl_2$ 1.5, $CaCl_2 \cdot 2H_2O$ 2.0, NaCl 145, glucose 10.0 and HEPES 6.0, pH7.4 adjusted with 1N NaOH using a pH meter (Autocal pH meter PHM83, Radiometer Inc.; Copenhagen, Denmark). A bicarbonate-CO_2-free buffer was chosen to avoid the necessity of altering P_{CO_2} or HCO_3 in order to change pH. The solution was continuously aerated with 100% O_2, and maintained at 37℃. Contractile responses to phenylephrine 3×10^{-7}M, dopamine 3×10^{-5}M and KCl 40mM were obtained in each pH solution (7.4, 7.0, 6.6 and 6.2) and pH5.8 only in the dopamine group titrated with 1N NaOH and 1N HCl. Results were expressed as the percent change from the control value in pH7.4 which was regarded as 100 %. Relaxation responses with increasing PGE_1 and nicardipine concentrations were expressed as the percentage tension decrement from the contractile force elicited by KCl 40mM which was regarded as 100% in each pH.

All data are expressed as the mean ± SEM. Individual statistical comparisons were performed following analysis of variance for repeated measures. Concentration-relaxation curves for PGE_1 and nicardipine were fitted by nonlinear regression. Differences among means were considered significant when $P<0.05$.

Results

Vasoconstriction

There was no difference among the vasoconstrictors in absolute tension after contraction at pH7.4 or in aortic ring weight (Table 1).

Actual recordings of the contraction response in different pH solutions are shown in figure 1. In the top panel acidification from pH7.4 to 7.0 produced 18 % relaxation, from pH7.0 to 6.6 produced 32% relaxation and from pH6.6 to 6.2 produced nearly complete relaxation of aortic rings that had been previously contracted with phenylephrine. In the middle panel maximum tension showed no change in tension by acidification from pH7.4 to 7.0, but showed a significant relaxation from pH7.0 to 6.6 and almost complete relaxation at pH6.2 and 5.8 in aortic rings previously contracted with dopamine. In the bottom panel there was no change in the contraction of aortic rings at pH from 7.4 to 6.6, but acidification to pH6.2 produced 23

Table 1. Absolute tension after contraction at pH7.4 and aortic ring weight

Group	Tension(mg)	Weight(mg)
Phenylephrine (3×10^{-7}M)	1943±214	4.7±0.55
Dopamine (3×10^{-5}M)	2284±148	4.7±0.55
KCl (40 mM)	2345±177	4.7±0.31

All data are expressed as the mean±SEM. Each group has 8 preparations.

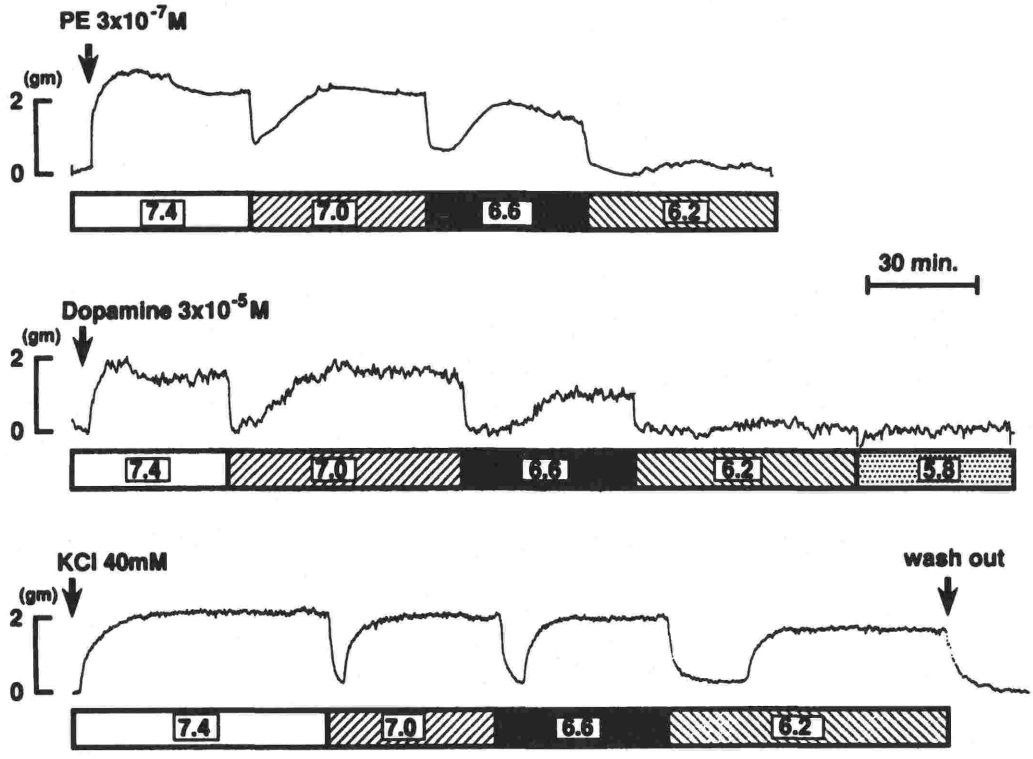

Figure 1 *Actual recordings of the contraction response in multiple pH solutions*

Top panel: Acidification from pH7.4 to 7.0 produced 18 % relaxation; pH7.0 to 6.6 produced 32% relaxation; pH6.6 to 6.2 produced nearly complete relaxation of aortic rings contracted with phenylephrine (PE).

Middle panel: Maximum tension showed no change by acidification from pH7.4 to 7.0, but showed 36 % relaxation from pH7.0 to 6.6 and almost complete relaxation at pH6.2 and 5.8 in aortic rings contracted with dopamine.

Bottom panel: There was no change on the contraction of aortic rings in pH from 7.4 to 6.6, but acidification at pH 6.2 produced 23%relaxation of aortic rings contracted with KCl.

%relaxation of aortic rings previously contracted with KCl.

Percent of control for the contractile values in the phenylephrine group (n=8) were 91 ±6.2% at pH7.0, 71 ±4.3% at pH6.6 and 17±4.5% at pH6.2. In the dopamine group (n=8) at pH7.0, 6.6, 6.2 and 5.8 (only in this group) the values were 112±6.1%, 71±8.0%, 37±6.3% and 37±7.1% of control. In the KCl group (n=8), they were 100±5.5%, 97±5.2% and 71±4.9 % of control. In both the phenylephrine and dopamine groups the contractile values at pH6.6 and 6.2 decreased significantly compared to values obtained at pH7.4 and 7.0. In the KCl group only the value at pH6.2 decreased significantly compared to values at any other pH. The contraction values in the KCl group were significantly higher than those in the phenylephrine and dopamine groups at pH6.6 and 6.2 (Fig.2).

Vasodilation

There were no differences at any pH among the drugs to contraction with KCl 40mM. Contractions at pH6.6 and 6.2 were significantly decreased compared to those at pH7.4 and 7.0 (Table 2). Only the change of relaxation of PGE_1 group (n=6 for each pH) at pH6.2 was significant, and there was no effect in other pH solutions (Fig.3). Nicardipine (n=7 for each pH exceptpH7.0; n=6) relaxed the aortic rings in a dose

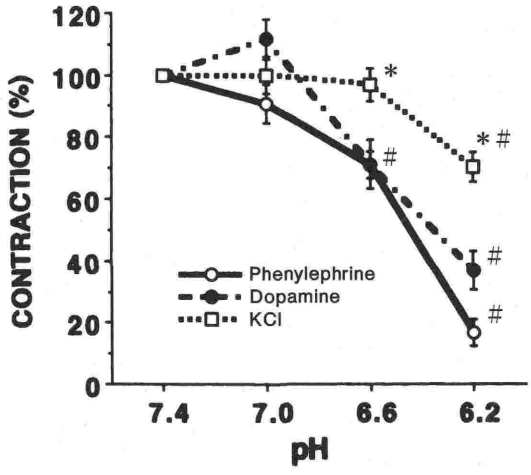

Figure 2 Contraction response in multiple pH solutions

The graph indicates the change of maximum tension in multiple pH solutions when the maximum tension at pH7.4 is regarded as 100 %. Although phenylephrine and dopamine demonstrate significant reductions at pH6.6 and 6.2 solutions, KCl maintaines the maximum tension at pH6.6 and shows a significant decrease at pH6.2. * $P<0.05$ versus phenylephrine and dopamine groups. # $P<0.05$ versus pH7.4 solution. In each curve, n=8.

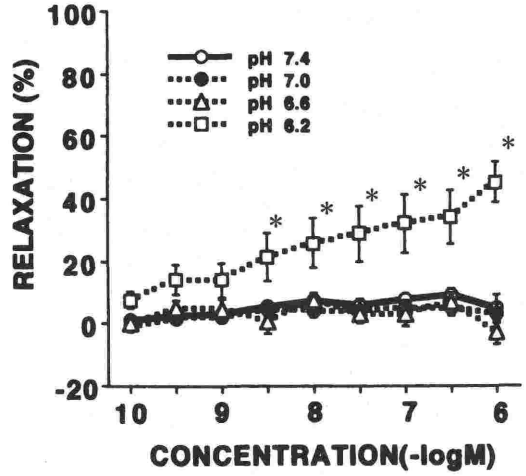

Figure 3 Relaxation response of prostaglandin E1 in multiple pH solutions

At pH7.4, 7.0 and 6.6 solutions prostaglandin E1 (PGE1) produces no effect, but in pH6.2 solution it shows concentration dependent relaxation. * $P<0.05$ versus pH7.4, 7.0 and 6.6 solutions. In each curve, n=6.

Table 2. Absolute tension after contraction with KCl 40mM in multiple pH solutions

Group	Tension (mg)			
	ph 7.4	7.0	6.6	6.2
PGE1 (n=6)	2655±287	2532±218	1708±145*	1485±135*
Nicardipine (n=7)	2313±266	2410±241	1663± 55*	1526±147*

All data are expressed as the mean±SEM. *$P<0.05$ vs pH 7.4 and pH 7.0.

dependent manner, and there was no difference among the subgroups (Fig.4).

Discussion

The concentrations of phenylephrine, dopamine and KCl used for vasoconstriction were thought to be adequate because they were within doses used clinically and there was no difference among the absolute contraction tension precontracted at pH7.4.

It has been found that small changes in pH do not alter vascular tension significantly but can induce changes in intracellular Ca^{2+} concentration ($[Ca^{2+}]i$)[12~15]. There have been reports that the changes in vascular smooth muscle contractility paralleled intracellular pH(pHi) and $[Ca^{2+}]i$[13] or were related to extracellular pH (pHo) rather than pHi[14]. In interactions between temperature and acidosis, acidosis has been reported to predominantly affect vascular tone[15]. On the other hand, alkilinization using NH_4Cl produces an increase in vascular tension and $[Ca^{2+}]i$, while acidosis due to NH_4Cl withdrawal reduces vascular tension and $[Ca^{2+}]i$[16,17]. All these investigations have been conducted over a small range of pH. In our study pH was changed from 7.4 to 6.2.

Although Loutzenhiser et al[10] adminstered higher concentrations of norepinephrine and KCl following

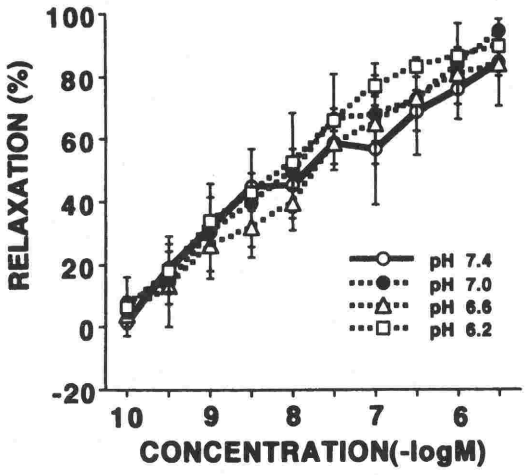

Figure 4 Relaxation response of nicardipine in multiple pH solutions

In all pH solutions, nicardipine relaxes the aortic rings in a dose dependent manner. There was no difference among the pHs. In each curve, n=7 except pH 7.0, n=6.

slight changes in pH, our results with phenylephrine, instead of norepinephrine, and KCl were consistent with their results within the same range of pH. In addition, in our study dopamine was also examined and a more acidotic solution was used.

Since the pharmacologic activity of dopamine and phenylephrine does not change in solutions above pH4.0 (comments from pharmaceutical companies), the reduction of vasoconstrictive force in the solution below pH6.6 seemed to be due to a direct action of acidosis on vascular endothelial cells and/or vascular smooth muscle. However, in contrast to Rinaldi et al[18], acidosis (pH7.1) decreased a canine coronary tone by 30%, in this study vasoconstriction due to KCl was decreased only at pH6.2 to 71 % of the control value. This difference might be related to the mechanism of the vasoconstrictive drugs: by extracellular Ca^{2+} influx through voltage gated Ca^{2+} channels for KCl and receptor operated intracellular Ca^{2+} release and Ca^{2+} channels for phenylephrine and dopamine[19].

PGE1 produces venodilation rather than arteriodilation[20], so there was no effect in the solutions of pH7.4, 7.0 or 6.6, but vasodilation was observed at pH6.2 in a dose dependent manner. This discrepancy can not be explained by acidosis only. If it were due to the difference of control value which contractions at pH6.6 and 6.2 were significantly decreased compared to those at pH7.4 and 7.0, we should change KCl dose to adjust the control value. However, the relaxation response was different significantly between at pH6.6 and at pH6.2. An extent acidosis might facillitate the vasodilating action of PGE1. Nicardipine showed a dose dependent vasodilatory effect in the presense of acidosis and there was no difference at different pH values. Because nicardipine is a calcium channel blocker, the aortic rings contracted with KCl might be relaxed independently of pH.

In conclusion, the results demonstrate that acidosis attenuates the vasoconstrictive effects of phenylephrine and dopamine, and that KCl and nicardipine maintain their pharmacological actions but PGE1 demonstrates a complicated action in the presense of acidosis.

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