Inhibition of the Vasodilator Effect of Hypercapnic Acidosis by Hypercalcemia in Dogs and Rats

By Enoch P. Wei, Marc D. Thames, Hermes A. Kontos, and John L. Patterson, Jr.

ABSTRACT

The vasodilator effect of local hypercapnic acidosis, produced by intra-arterial infusion of an acid buffer solution consisting of a mixture of glycine and hydrochloric acid, in the perfused hind limb and in the perfused gastrocnemius muscle of anesthetized dogs was examined at normal plasma calcium concentration and during elevation of the plasma calcium concentration produced by intra-arterial infusion of calcium chloride. Hypercalcemia inhibited the vasodilator action of hypercapnic acidosis so that the effect was not apparent until venous blood pH decreased below 7. The dilator effect of local application of acidified Ringer's solution on arterioles of the cremaster muscle of the rat was also inhibited by elevation of the plasma calcium concentration induced by intravenous administration of calcium chloride. The available evidence suggests that this inhibition of the dilator effect of hypercapnic acidosis by calcium ions is related to competition between calcium and hydrogen ions for binding at one or more cellular sites such as the sarcolemma, the sarcoplasmic reticulum, or the contractile proteins of vascular smooth muscle.

KEY WORDS

- local regulation of blood flow
- microcirculation
- vascular effects of electrolytes
- arterioles
- cremaster muscle
- calcium ion-hydrogen ion interactions

Hypercapnic acidosis decreases the contractility of cardiac (1, 2) and skeletal muscle (3). It has been suggested (4) that this action in cardiac muscle is related to displacement by hydrogen ions of calcium ions bound to contractile proteins. Cellular structures including the sarcolemma, the sarcoplasmic reticulum, and the contractile proteins have a high affinity for hydrogen ions, and the binding of calcium ions by these tissue preparations is strongly pH dependent (5-8). There is substantial evidence that the mechanism of muscular contraction in smooth muscle, particularly with regard to the role of calcium ions, is basically the same as that in skeletal and cardiac muscle (9). In the present study, we examined the influence of changes in calcium ion concentration in the extracellular fluid on the vasodilator effect of hypercapnic acidosis in the perfused hind limb and the perfused gastrocnemius muscle of the dog and in the arterioles of the cremaster muscle of the rat.

Methods

Experiments were performed on dogs and rats. Eighteen dogs were anesthetized with sodium pentobarbital (25 mg/kg, iv); heparin was used as the anticoagulant. The dogs were fitted with an endotracheal tube and ventilated with a positive-pressure respirator. In eight dogs, the perfused hind limb was studied. The peritoneal cavity was opened, and the vessels at the bifurcation of the aorta were exposed and dissected free. The hind limb was perfused, using a pulsatile occlusive pump, through the external iliac artery with arterial blood obtained from the carotid artery. The volume output of the pump was adjusted to maintain the perfusion pressure approximately equal to the systemic arterial blood pressure. The flow rate in this group of dogs averaged 71 ± 12.8 ml/min. Perfusion pressure was monitored with a Statham strain gauge. All infusions were given intra-arterially upstream from the pump so that blood flow was not disturbed. Blood flow to the distal part of the limb was eliminated by a tight ligature around the ankle. The aorta was ligated below the origin of the renal arteries to minimize collateral flow. Venous blood was obtained from the femoral vein through a Teflon catheter. In ten other dogs, gastrocnemius muscle was perfused...
through the popliteal artery with blood from the carotid artery in the same manner as that used for the entire hind limb. Blood flow in this group of dogs averaged 40 ± 8.5 ml/min. All arterial branches except those entering the gastrocnemius muscle were directly ligated. In addition, the aorta was ligated just above the bifurcation into the iliac arteries. Collateral flow in this preparation, measured as the venous outflow from the muscle with the perfusion pump stopped, was less than 5 ml/min. The popliteal vein was cannulated with a Teflon catheter leading to a Y-connection of dogs averaged 40 ± 8.5 ml/min. All arterial

carbon dioxide tension (Pco₂), hematocrit, and exposed testis was wrapped in saline-soaked gauze. The rat was then placed on a holder which supported the Lucite prism rod in a fixed position with respect to a light source. The scrotal skin lying over the prism and the Lucite rod was cut, and the connective tissue was removed to expose the cremaster muscle. In all cases the exposed area of the cremaster muscle was constantly kept moist with Ringer’s solution adjusted to a pH of 7.40.

Solutions with a pH of 7.2, 7.0, and 6.5 were prepared by adding appropriate amounts of Na₂HPO₄ and lactic acid to the Ringer’s solution. A drop of each acidified Ringer’s solution from a micropipette (50 μl tip) was placed on the vessel under examination both before and after the administration of 0.1M calcium chloride solution. Small arteries with a diameter between 35 μ and 65 μ were examined with a Leitz microscope using a dry 6.5x objective lens. After application of each solution, the arterial diameter was continuously measured for 2 minutes with a Vickers imagesplitting device with the aid of a television camera and monitor (12). Diameter values during application of the solution with a pH of 7.4 were used as the control values.

**Results**

Table 1 summarizes the results from the eight dogs in which the perfused hind limb was studied. Before calcium chloride was infused, hypercapnic acidosis of increasing severity produced progressive decreases in perfusion pressure. When the calcium concentration was raised, hypercapnic acidosis of equal severity produced no significant change in perfusion pressure until the venous blood pH decreased below 7. Changes in hematocrit, both before and after calcium was infused, were minor. In six of these eight dogs, the response to an intra-arterial infusion of 1.2 μg/min of histamine was tested. Histamine phosphate in a concentration of 1 μg base/ml was prepared in 0.9% NaCl solution. Histamine was chosen because its vasodilator action is not influenced by changes in blood calcium concentration (13). At normal calcium concentration, histamine lowered perfusion pressure from 102.8 ± 3.1 mm Hg to 84.0 ± 3.1 mm Hg. When the calcium concentration was raised, histamine lowered perfusion pressure from 109.8 ± 7.2 mm Hg to 95.8 ± 5.1 mm Hg. The decreases in perfusion pressure of 18.8 ± 3.7 mm Hg and 16.2 ± 4.3 mm Hg produced by histamine at normal and high calcium concentration, respectively, were not significantly different from each other.

Four of the ten dogs in which the gastrocnemius muscle preparation was studied were...
TABLE 1

Vasodilator Effect of Hypercapnic Acidosis on Perfused Hind Limb at Normal and at High Calcium Concentrations

<table>
<thead>
<tr>
<th>Acid infusion rate (ml/min)</th>
<th>Perfusion pressure (mm Hg)</th>
<th>Venous blood pH</th>
<th>Venous blood Pco₂ (mm Hg)</th>
<th>Hematocrit (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Normal Plasma Calcium Concentration (8.54 ± 0.08 mM)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>98.6 ± 3.5</td>
<td>7.33 ± 0.02</td>
<td>40.0 ± 3.3</td>
<td>43.0 ± 2.8</td>
</tr>
<tr>
<td>1.2</td>
<td>95.9 ± 3.3*</td>
<td>7.30 ± 0.02</td>
<td>41.3 ± 3.1</td>
<td>43.4 ± 2.8</td>
</tr>
<tr>
<td>2.5</td>
<td>92.3 ± 4.0*</td>
<td>7.25 ± 0.02</td>
<td>45.0 ± 3.6</td>
<td>42.6 ± 3.1</td>
</tr>
<tr>
<td>4.9</td>
<td>86.6 ± 3.1*</td>
<td>7.17 ± 0.02</td>
<td>49.4 ± 2.7</td>
<td>44.1 ± 3.1</td>
</tr>
<tr>
<td>9.9</td>
<td>70.8 ± 3.7*</td>
<td>6.96 ± 0.05</td>
<td>66.6 ± 5.7</td>
<td>41.0 ± 2.7</td>
</tr>
<tr>
<td></td>
<td>High Plasma Calcium Concentration (8.56 ± 0.17 mM)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>101.8 ± 5.3</td>
<td>7.32 ± 0.02</td>
<td>39.7 ± 2.5</td>
<td>44.0 ± 3.3</td>
</tr>
<tr>
<td>1.2</td>
<td>103.4 ± 4.9</td>
<td>7.26 ± 0.01</td>
<td>41.5 ± 2.3</td>
<td>45.9 ± 2.5</td>
</tr>
<tr>
<td>2.5</td>
<td>104.5 ± 5.3</td>
<td>7.20 ± 0.01</td>
<td>44.2 ± 2.5</td>
<td>46.3 ± 3.1</td>
</tr>
<tr>
<td>4.9</td>
<td>98.5 ± 3.5</td>
<td>7.11 ± 0.02</td>
<td>51.8 ± 2.6</td>
<td>43.9 ± 3.1</td>
</tr>
<tr>
<td>9.9</td>
<td>95.1 ± 4.9*</td>
<td>6.96 ± 0.03</td>
<td>64.0 ± 3.3</td>
<td>42.5 ± 3.1</td>
</tr>
</tbody>
</table>

All values are means ± SE; pH, Pco₂, hematocrit, and calcium concentration were measured in femoral venous blood.

*Difference from control is significant (P < 0.05).

used for preliminary experiments designed to determine whether the glycine-hydrochloric acid buffer solution had inherent vasoactivity and whether adjustments in the infusion rate of calcium chloride were necessary to maintain a constant calcium ion activity, despite changes in pH and dilution resulting from the changing rates of acid infusion. Infusion of the glycine-hydrochloric acid buffer, after the pH had been increased to 7.3-7.4 with 0.15N NaOH, at 4.9 ml/min produced less than a 5-mm Hg reduction in perfusion pressure in each of these four dogs. It was concluded that glycine had no inherent vasoactivity independent of changes in pH. Also, calcium ion activity when no acid was infused was equal to that during infusion of acid at 4.9 ml/min. This finding was confirmed when the complete experiment was carried out in the six remaining dogs in this group. Table 2 summarizes the results from these six dogs. Prior to the infusion of calcium chloride, progressively more severe hypercapnic acidosis produced decreases in perfusion pressure comparable in magnitude to those seen in the entire hind limb (Table 2).

TABLE 2

Vasodilator Effect of Hypercapnic Acidosis on Perfused Gastrocnemius Muscle at Normal and High Calcium Concentrations

<table>
<thead>
<tr>
<th>Acid infusion rate (ml/min)</th>
<th>Perfusion pressure (mm Hg)</th>
<th>Venous blood pH</th>
<th>Venous blood Pco₂ (mm Hg)</th>
<th>Hematocrit (%)</th>
<th>Total plasma calcium (mmoles/liter)</th>
<th>Blood calcium ion activity (mmoles/liter)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Normal Plasma Calcium Concentration</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>108.8 ± 7.6</td>
<td>7.32 ± 0.14</td>
<td>34.8 ± 1.6</td>
<td>36.8 ± 2.7</td>
<td>2.12 ± 0.04</td>
<td>0.99 ± 0.03</td>
</tr>
<tr>
<td>1.2</td>
<td>96.3 ± 4.3*</td>
<td>7.27 ± 0.07</td>
<td>38.1 ± 1.3</td>
<td>38.0 ± 2.3</td>
<td>2.03 ± 0.04</td>
<td>1.01 ± 0.06</td>
</tr>
<tr>
<td>2.5</td>
<td>89.3 ± 6.2*</td>
<td>7.17 ± 0.16</td>
<td>42.5 ± 1.7</td>
<td>37.8 ± 2.5</td>
<td>1.99 ± 0.03*</td>
<td>1.04 ± 0.04</td>
</tr>
<tr>
<td>4.9</td>
<td>69.1 ± 5.2*</td>
<td>7.03 ± 0.10</td>
<td>51.8 ± 4.2</td>
<td>37.6 ± 2.3</td>
<td>1.91 ± 0.00*</td>
<td>1.04 ± 0.01</td>
</tr>
<tr>
<td></td>
<td>High Plasma Calcium Concentration</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>105.8 ± 6.6</td>
<td>7.27 ± 0.10</td>
<td>37.1 ± 2.4</td>
<td>38.5 ± 2.4</td>
<td>3.64 ± 0.21</td>
<td>1.79 ± 0.06</td>
</tr>
<tr>
<td>1.2</td>
<td>103.8 ± 6.8</td>
<td>7.23 ± 0.07</td>
<td>39.8 ± 1.9</td>
<td>38.8 ± 2.5</td>
<td>3.52 ± 0.19</td>
<td>1.84 ± 0.14</td>
</tr>
<tr>
<td>2.5</td>
<td>102.3 ± 4.6</td>
<td>7.13 ± 0.07</td>
<td>46.6 ± 3.2</td>
<td>39.5 ± 2.4</td>
<td>3.51 ± 0.21*</td>
<td>1.96 ± 0.06</td>
</tr>
<tr>
<td>4.9</td>
<td>101.6 ± 11.7</td>
<td>6.99 ± 0.11</td>
<td>53.6 ± 4.1</td>
<td>38.0 ± 1.8</td>
<td>3.47 ± 0.23*</td>
<td>2.05 ± 0.16</td>
</tr>
</tbody>
</table>

All values are means ± SE; pH, Pco₂, hematocrit, calcium concentration, and calcium ion activity were measured in venous blood from the gastrocnemius muscle.

*Difference from control is significant (P < 0.05).
CALCIUM ION–HYDROGEN ION INTERACTIONS

1). Following elevation of the calcium ion activity by the infusion of calcium chloride, hypercapnic acidosis of comparable magnitude produced no significant change in perfusion pressure. The infusion of acid at 2.5 ml/min or at a higher rate produced a significant decrease in total calcium concentration as a result of dilution. However, calcium ion activity remained constant because the increase in calcium ion activity resulting from a decrease in pH counterbalanced the effect of dilution.

Table 3 shows that acidosis dilated arterioles of the rat cremaster muscle when plasma calcium concentration was normal and that this effect was inhibited by raising the plasma calcium concentration.

Discussion

The results reported in the present paper show that the vasodilation induced by hypercapnic acidosis in the perfused hind limb and the perfused gastrocnemius muscle of the dog and in the arterioles of the cremaster muscle of the rat is inhibited by increasing the calcium ion activity in the extracellular fluid. The results do not disclose the precise mechanism of this inhibitory reaction. Certain possibilities, however, can be reasonably excluded. Increased serum calcium ion activity produces modest vasoconstriction (14, 15) that remains fairly constant beyond a calcium ion activity of 2 mmoles/liter (15). Since vasodilator influences should be depressed in the presence of a vasoconstrictor stimulus, the possibility that the inhibition of the vasodilator action of hypercapnic acidosis by hypercalcemia is a manifestation of such a nonspecific influence must be considered. This possibility is unlikely for several reasons. The resting vascular resistance during hypercalcemia before the administration of acid was not different from the vascular resistance when normal calcium concentration prevailed. Similarly, the caliber of arterioles in the rat cremaster muscle was not significantly affected by elevating the calcium ion concentration. In addition, the vasodilator effect of histamine was not inhibited by hypercalcemia in the same dogs in which the inhibitory reaction of hypercalcemia on the vasodilator effect of hypercapnic acidosis was evident.

Another possible mechanism that can be reasonably dismissed as contributing to the inhibition of the vasodilator effect of hypercapnic acidosis by hypercalcemia is related to the effects of changes in pH on calcium ion activity. A decrease in pH raises the calcium ion activity in protein-containing solutions such as blood and presumably also extracellular fluid (11, 15). It is possible, therefore, that as pH decreased calcium ion activity increased progressively and thus provided an increasingly more powerful vasoconstrictor influence. However, in the gastrocnemius muscle it was shown by direct measurement that such an increase in activity did not occur, presumably because the effect of dilution from the higher infusion rate of acid counteracted the effect of decreasing pH. It is likely that similar conditions prevailed in the isolated hind limb, but since calcium ion activity was not measured in this preparation we cannot be certain. Even if a progressive increase in calcium ion activity did occur with decreasing pH, it is unlikely that it would have been powerful enough to account for the inhibitory effect of hypercalcemia. Two reasons support this conclusion. First, the increase in calcium ion activity that could have occurred in this preparation because of the reduction in pH was relatively small. Second, the vasoconstrictor effect of hypercalcemia is fairly constant beyond a calcium ion activity of 2 mmoles/liter (15). Since the calcium ion activity in the present study was in the range in which a fairly constant vasoconstrictor action results from

TABLE 3

Vasodilator Effect of Application of Acid Solution on Arterioles of Rat Cremaster Muscle at Normal and High Calcium Concentrations

<table>
<thead>
<tr>
<th>Solution pH</th>
<th>Arterial diameter (μ)</th>
</tr>
</thead>
<tbody>
<tr>
<td>7.4</td>
<td>42.8 ± 4.6</td>
</tr>
<tr>
<td>7.2</td>
<td>48.2 ± 4.5*</td>
</tr>
<tr>
<td>7.0</td>
<td>50.7 ± 5.2*</td>
</tr>
<tr>
<td>6.5</td>
<td>54.3 ± 4.8*</td>
</tr>
</tbody>
</table>

Normal Calcium Concentration (1.9 ± 0.2 mM)

| 7.4         | 43.8 ± 5.5            |
| 7.2         | 44.8 ± 5.5            |
| 7.0         | 44.7 ± 5.6            |
| 6.5         | 43.0 ± 5.2            |

High Calcium Concentration (3.0 ± 0.1 mM)

All values are means ± SE obtained from six rats. Diameters represent peak values following application of the test solution.

*Difference from control is significant (P < 0.05).

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hypercalcemia, small variations in calcium ion activity would have had very little effect on vascular resistance.

It is more likely that the inhibition of the vasodilator effect of hypercapnic acidosis by hypercalcemia is related to interactions between hydrogen and calcium ions. There is strong evidence supporting the occurrence of competition between calcium ions and hydrogen ions for binding on cellular structures including the sarcolemma, the sarcoplasmic reticulum, and the contractile proteins. Competition for binding at one or more of these sites could readily explain the inhibitory effect of hypercalcemia on the vasodilator effect of hypercapnic acidosis. The mechanisms by which competition at each one of these sites could explain the inhibitory action of hypercalcemia on the vasodilator effect of acidosis will be discussed in relation to the mechanism by which acidosis relieves vascular smooth muscle. It must be emphasized, however, that because the precise mechanism of vascular smooth muscle relaxation by acidosis is not known no definitive choice between the various possibilities can be made at this time.

The sarcolemma binds significant quantities of calcium in the resting state (9). It has been suggested (9) that this bound calcium is released inside the cell during the action potential and contributes significantly to augmentation of the force of contraction. Calcium and hydrogen ions compete for binding sites on cell membrane fragments (8). Therefore, it is possible that, by displacing calcium ions from these membrane binding sites, hydrogen ions cause a reduction in the force of contraction of the smooth muscle cells. Such a mechanism could explain not only the relaxing effect of acidosis on vascular smooth muscle but also its inhibition by increases in the calcium ion concentration in the extracellular fluid. If this mechanism is the main mode of action of hypercapnic acidosis, then the relaxing effect should depend on changes in extracellular fluid pH. The available evidence shows that this theory is only partially true. Alexander (16) has found that strips of portal veins relax in response to the addition of strong acids to the bath, in the absence of a change in PCO₂ tension. Moreover, the action of CO₂ on cerebral vascular smooth muscle depends on changes in extracellular pH (17). However, in contrast to these findings, the vasodilator action of hypercapnic acidosis in the human forearm (18) and the mesenteric (19) and renal (20) vascular beds of anesthetized animals and the effect of hypercapnic acidosis on myocardial contractility (2) depend on increases in PCO₂ rather than on decreases in extracellular fluid pH, suggesting that they are mediated through some intracellular mechanism. It is possible that differences in vascular smooth muscle from various vascular beds accounts for these apparent discrepancies.

A significant source of calcium ions for activation of contraction in smooth muscle is the sarcoplasmic reticulum (21). The amount of sarcoplasmic reticulum in different types of vascular smooth muscle varies and, therefore, the dependence of various vascular smooth muscles on calcium from extracellular sources is correspondingly different. The sarcoplasmic reticulum in vascular smooth muscle has not been studied extensively. However, in skeletal muscle, it has been shown that the sarcoplasmic reticulum possesses binding sites with different affinities for calcium (22). Also, hydrogen ions compete for binding on the same sites on the sarcoplasmic reticulum of skeletal muscle as do calcium ions (7). Therefore, any increase in the concentration of hydrogen ions in the interior of the cell should result in displacement of calcium ions from binding sites on the sarcoplasmic reticulum. This displacement would limit the number of calcium ions released from the sarcoplasmic reticulum by activation and thus result in a corresponding decrease in the force of contraction. Both the vasodilator action of hypercapnic acidosis with its apparent dependence on intracellular pH in at least some types of vascular smooth muscle and the inhibition of this vasodilator action by changes in the calcium ion concentration could thus be explained.

A third site at which interaction between hydrogen ions and calcium ions could explain the findings observed in this work is the contractile proteins themselves. Like skeletal and cardiac muscle, smooth muscle is probably activated by the binding of calcium to troponin, thus releasing the inhibitory effect of this protein on actomyosin adenosinetriphosphatase (ATPase) activity (23). Lowering the pH decreases the calcium sensitivity of glycerol-extracted muscle and the ATPase activity of extracted myofibrils (24,
25). Fuchs and his colleagues (26) have also found that lowering the pH decreases both the affinity of troponin for calcium and the stability of the calcium-troponin complex. Therefore, it is possible that intracellular acidosis could diminish the binding of calcium to troponin and thus inhibit the subsequent activation of actomyosin ATPase. The net effect of these actions would be to decrease the force of the ensuing contraction.

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