Tissue Acidosis
Role in Sustained Arteriolar Dilatation Distal to a Coronary Stenosis

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This study tested the hypothesis that myocardial tissue acidosis is responsible for maintenance of reduced arteriolar tone distal to a severe coronary arterial stenosis. Domestic swine (n=10) were instrumented with a coronary arterial stenosis that reduced vessel diameter 80%. Measurements of hemodynamic indexes were made 1) before stenosis, 2) at 5, 20, and 60 minutes after stenosis placement, and 3) after each of three, 20-minute NaOH infusions (0.05 M, 0.1 M, and 0.5 M) distal to the stenosis (group 1). Intracellular pH at the end of 30 minutes of 0.5 M NaOH infusion distal to the stenosis was measured in a second group (n=6) of swine (group 2). After stenosis placement in group 1, endocardial blood flow declined significantly, and evidence of regional acidosis (increased coronary venous Pco2 and decreased coronary venous pH) and ischemia (lactate production) developed. One hour later, evidence of acidosis persisted, though to a lesser extent. Myocardial oxygen and lactate metabolism exhibited similar patterns. Infusion of 0.5 M NaOH (0.38 ml/min) reduced (p<0.01) distal zone epicardial blood flow but did not change endocardial flow. Regional myocardial oxygen extraction (75±8%, mean±SD) and consumption (8.2±2.3 ml/min/100 g) also declined significantly (p<0.01) in response to 0.5 M NaOH infusion compared with 60 minutes after stenosis (86±4 and 12.4±2.8 ml/min/100 g respectively). The pH of coronary venous blood (7.41±0.05) was elevated (p<0.01) by 0.5 M NaOH above prestenosis (7.36±0.03) and 60-minute poststenosis levels (7.33±0.04). Measurement of intracellular pH (14C-DMO/3H-inulin technique) in group 2 animals showed that distal zone pH (7.00±0.11) after 0.5 M NaOH infusion was at a level similar to that in normal myocardium in a nonstenosed region of the heart. Thus, because endocardial blood flow was unchanged during 0.5 M NaOH infusion, even though pH of coronary venous blood reached systemic levels and intracellular pH increased significantly, tissue acidosis is probably not required for maintenance of arteriolar dilation distal to a severe coronary arterial stenosis. The data support the hypothesis that tissue acidosis capable of mediating acute changes in coronary arteriolar tone does not have a major role in the long-term maintenance of reduced vascular tone distal to a coronary arterial stenosis. (Circulation 1989;79:890–898)

For some time, acute changes in arterial blood pH and Pco2 have been known to influence coronary arteriolar tone and myocardial blood flow.1–15 The results of several recent studies, however, indicate that mediators of acute changes in coronary arteriolar tone may not have an important role in more long-term adjustments of the coronary circulation to sustained changes in either myocardial oxygen supply or demand.16–18 Thus, coronary vasodilation is maintained during sustained isoproterenol infusion even though adenosine release by the myocardium peaks rapidly during the initial phase of the infusion and then returns to control levels.16 In addition, adenosine deaminase inhibits, in part, the initial flow increase observed in response to isoproterenol but has no effect on the sustained flow response to the drug.17 The concentration of potassium ion also has increased in coronary venous blood in response to vasodilation stimuli (e.g., pacing and ischemia) and then returned to control levels even though coronary vasodilation persists.18 Whether or not similar changes occur in coronary venous pH and Pco2...
after imposing a severe coronary arterial stenosis is unknown. The purpose of this investigation, therefore, was to test the hypothesis that 1) acute changes in acid-base and aerobic metabolism after imposing a severe coronary arterial stenosis may revert toward baseline (i.e., prestenosis) levels over time despite constant regional myocardial blood flow and external determinants of myocardial oxygen demand and 2) tissue acidosis does not have an essential role in the long-term maintenance of arteriolar dilation distal to the stenosis. Experiments were performed in closed-chest, sedated domestic swine instrumented with a severe coronary arterial stenosis.17,19–21

Methods

Animal Preparation

Farm-bred domestic swine (n = 10; mean weight, 42 kg; range, 37–49 kg) were premedicated with ketamine (25 mg/kg i.m.) and sodium thiamylol (total dose, 0.5–1.0 g i.v.), intubated, and anesthetized with halothane (0.5–1.5%) and nitrous oxide (60:40 mixture with oxygen). The animals were ventilated with a volume-cycled respirator through which supplemental oxygen was delivered at 2–3 l/min mixed with room air and anesthetic gases. Arterial blood gases were monitored frequently and were maintained at appropriate levels (pH 7.38–7.45, Pco2 at 35–45 mm Hg, and Po2 at 100–125 mm Hg) throughout each study. After induction of anesthesia, each animal was anticoagulated with heparin (225 IU/kg i.v.). Full anticoagulation was maintained by administering approximately half the initial loading dose every 2–3 hours.

The animal was instrumented for the study as follows. A 7F catheter was advanced under fluoroscopic control into the left atrium. This catheter was used for administering radioactive microspheres for measurements of regional myocardial blood flow. Another 8F catheter was positioned in the aorta to monitor arterial blood gases and for reference withdrawal for microsphere determinations of regional myocardial blood flow. A pigtail catheter was placed at the apex of the left ventricle to monitor pressure. The femoral veins were cannulated bilaterally to administer fluids and medications during the study. Another 7F catheter was positioned in the midportion of the anterior interventricular vein (AIV). Next, a 7F pacing catheter was placed in the coronary sinus to maintain the animal’s heart rate at 135–140 beats/min throughout the study.

Before placement of the coronary arterial stenosis (see below), halothane and nitrous oxide were discontinued, and the animal was permitted to awaken sufficiently to breathe spontaneously and exhibit modest tremulousness. A constant intravenous infusion of sodium thiamylol was begun at 1–13 mg/min to maintain sedation and to ensure the animal was free of pain. Once the animal had stabilized for approximately 20 minutes, the experimental protocol was begun.

Experimental Protocol

A period of hemodynamic and arterial blood gas stability was documented by obtaining measurements of arterial and AIV blood gases with recordings of hemodynamic indexes (i.e., heart rate and arterial and left ventricular pressures) every 5 minutes for 15–20 minutes. After showing that three successive measurements of arterial and AIV pH differed by less than 0.1–0.2% (arterial vs. arterial, AIV vs. AIV), initial measurements of all experimental variables were obtained, which included hemodynamic output, regional myocardial blood flow (microspheres), arterial and AIV blood gases, and pH, lactate, and oxygen content of arterial and AIV blood.

Next, a rigid plastic stenosis that reduced vessel diameter by 80% was inserted within the lumen of the animal’s left anterior descending coronary artery as previously described.17,19–21 Experimental variables were measured again at 5, 20, and 60 minutes after stenosis placement. After completion of measurements at the 60-minute point, an intracoronary infusion of 0.05 M NaOH distal to the stenosis was begun at a rate of 0.38 ml/min. The infusion was continued for 20 minutes, and at the end of this period, all experimental variables were measured again. Arterial and AIV pH and blood gases and hemodynamic measurements also were obtained 10 minutes after beginning NaOH infusion. Once data acquisition at the 20-minute point had been completed, another 20-minute infusion was begun at 0.38 ml/min but with 0.1 M NaOH. Again, arterial and AIV pH and blood gases and all other experimental variables were measured at the midway point and at the end of the infusion. After this, the sequence was repeated again but with an infusion of 0.5 M NaOH.

Once the final set of measurements had been obtained, myocardium distal to the stenosis was marked by injection of radiolabeled microspheres through the coronary infusion catheter attached to the stenosis.17,19–21 Afterward, the animal was given a large intravenous dose of sodium thiamylol and killed 5 minutes later by intravenous injection of potassium chloride. Next, the chest was opened, and the location of the distal end of the AIV catheter was diagrammed in relation to the position of the stenosis in the left anterior descending coronary artery. The stenosis-catheter system was removed from the left anterior descending coronary artery, and the vessel itself was inspected for evidence of gross intimal damage. Afterward, the heart was rinsed thoroughly in tap water, refrigerated in buffered saline, and sectioned for determination of microsphere activity (see below).
Regional Myocardial Blood Flow

For each experimental condition, approximately $4 \times 10^6$ radiolabeled microspheres (15-μ diameter, 85–105 μCi total radioactivity) were injected into the left atrial catheter to determine regional myocardial blood flow.22 Seven determinations of flow were made in each animal. A different radiisotope was chosen at random from a collection of eight radiotracers ($^{51}$Cr, $^{55}$Fe, $^{85}$Kr, $^{88}$Y, $^{103}$Ru, $^{111}$In, $^{153}$Gd, and $^{125}$I) for each flow determination. A more detailed description of microsphere methodology used in our laboratory has been published.19

Coronary arteriolar resistance in endocardium distal to the stenosis was calculated by dividing distal coronary mean diastolic pressure minus mean left arterial pressure plus 7 mm Hg23 by distal zone endocardial blood flow. Distal zone epicardial and transmural resistances were calculated in the same fashion except that distal coronary mean pressure and distal zone epicardial and transmural flows, respectively, were used in the computation. Resistances in the circumflex zone were calculated with aortic instead of distal coronary pressure.

Regional Myocardial Oxygen Metabolism

Paired samples (2–3 ml) of arterial and AIV blood were obtained for determining oxygen content (Lex-O$_2$-CON Instrument, Lexington Instruments, Waltham, Massachusetts) during each phase of the study. Oxygen content (vol%) was determined in duplicate for each sample, and values were accepted only when the difference between them was 0.2 ml O$_2$/dl or less. Regional myocardial oxygen consumption (ml/min/100 g) was calculated as the product of transmural regional myocardial blood flow distal to the stenosis and the arterial and AIV oxygen difference.

Regional Myocardial Lactate Metabolism

Lactate concentration in arterial and AIV blood was determined by a spectrophotometric method with commercially available kits (Calbiochem Rapid Lactate Reagents, Calbiochem-Behring, La Jolla, California). Samples of blood (5 ml) were immediately deproteinized by placing them in cold perchloric acid (8% vol/vol). The samples were centrifuged, and the supernatant was frozen for subsequent analysis in duplicate. Regional lactate consumption was calculated as the product of transmural regional myocardial blood flow distal to the stenosis and the arterial and AIV lactate difference.

Regional Myocardial Acid and Base Metabolism

A Corning blood gas machine (Corning, New York) was used to measure pH, Po$_2$ and Pco$_2$ in arterial and AIV blood. Blood samples were obtained simultaneously (artery and AIV) under anaerobic conditions, immediately placed on ice, and then analyzed as rapidly as possible (within 10–20 minutes).

Calculation of pH and Pco$_2$ in left anterior descending coronary artery blood. The extent to which NaOH infusion altered the pH and Pco$_2$ of arterial blood in the left anterior descending coronary artery distal to the stenosis was determined in three separate in vitro studies. Known quantities of NaOH were added to arterial blood under anaerobic conditions, and resulting pH and Pco$_2$ were measured. Next, pH (or Pco$_2$) was plotted as a function of micromoles of NaOH added per liter of blood. The data from each in vitro study were fit to a second-order polynomial with a commercially available computer program (Sigmamplot, Jandel Scientific, Sausalito, California). The coefficients for the first- and second-order terms were very similar for each curve and so were averaged to provide a general model for predicting arterial pH (or Pco$_2$) based on micromoles of NaOH added per liter of arterial blood.

The models used were as follows:

$$pHe = pHo + 2.2 \times 10^{-2}(NaOH_a) + 3.0 \times 10^{-4}(NaOH_a)^2$$ (1)

$$Pco_2 = Pco_2o + 1.3 \times (NaOH_a) + 1.4 \times 10^{-3}(NaOH_a)^2$$ (2)

where pHo is corrected pH; pHo is measured pH; Pco$_2$o is corrected Pco$_2$; Pco$_2$o is measured Pco$_2$; and NaOH$_a$ is final, added NaOH concentration in arterial blood.

Because concentration of NaOH in the syringe and its infusion rate were known and because transmural blood flow distal to the stenosis was measured, it was possible to estimate micromoles of NaOH added per liter of arterial blood perfusing myocardium distal to the stenosis.

Measurements of intracellular pH. A separate group of six domestic swine was studied to measure the effect of intracoronary NaOH (0.5 M) infusion on myocardial intracellular pH. Intracellular pH was measured by a modification of the $^{14}$C-5,5-dimethyl-2,4-oxazolidinedione (DMO) $^3$H water method of Scholerb and Grantham24 and was calculated as follows:

$$pHi = pH + LOG_{10}[(A - B)/B]$$ (3)

where pH is intracellular pH; pK' is DMO dissociation constant, temperature corrected; A is ([$^{14}$C-DMOTotal-$^{14}$C-DMOeff]/H$_2$Ototal-H$_2$Oeff); and B is ($[^{14}$C-DMOeff]/[10$^{pHeff+pK'}$]+1).

Note: 1) measured concentration of $^{14}$C-DMO and $^3$H-inulin in coronary venous plasma was used to approximate extracellular space (ecf) concentration; 2) pHecf is measured coronary venous pH; 3) H$_2$Ototal is tissue wet weight–tissue dry weight; 4) H$_2$Oeff is $^3$H-inulin total/($^3$H-inulin ecf); and 5) $^{14}$C-DMO ecf is ($^{14}$C-DMO ecf)/$^3$H$_2$O ecf. The protocol used was as follows:

First, the animal was anesthetized with halothane and nitrous oxide and then instrumented for the study as described above. Before stenosis place-
TABLE 1. Hemodynamics Indexes

<table>
<thead>
<tr>
<th></th>
<th>Prestenosis</th>
<th>Poststenosis (min)</th>
<th>NaOH dose (M)</th>
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</thead>
<tbody>
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<td></td>
<td></td>
<td>5</td>
<td>20</td>
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<tr>
<td>Heart rate (min)</td>
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<td>135 ± 2</td>
<td>134 ± 2</td>
</tr>
<tr>
<td>Aortic mean pressure (mm Hg)</td>
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<td>117 ± 17</td>
<td>126 ± 8</td>
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<td>LAD mean pressure (mm Hg)</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>LA mean pressure (mm Hg)</td>
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<td>4 ± 5</td>
<td>7 ± 4</td>
</tr>
<tr>
<td>Tension time index (mm Hg*sec)</td>
<td>29 ± 3</td>
<td>27 ± 4</td>
<td>29 ± 3</td>
</tr>
</tbody>
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LAD, left anterior descending coronary artery; LA, left atrium.
*p < 0.05 vs. 60 minutes poststenosis; †p < 0.05 vs. 5 minutes poststenosis; ‡p < 0.01 vs. 60 minutes poststenosis.

ment, paired samples of arterial and AIV blood were obtained for pH, blood gas, and background liquid scintillation counting measurements. Next, the artificial stenosis (80% lumenal diameter reduction) was positioned in the animal's left anterior descending coronary. Five minutes after stenosis placement, repeat arterial and AIV blood samples were obtained for blood gas and pH measurements, and hemodynamic indexes were recorded. Next, 75 μCi of 14C-DMO and 50 μCi 3H-inulin were injected intravenously. An intracoronary infusion (0.38 ml/min) of 0.5 M NaOH distal to the stenosis was begun immediately after isotope injection. The infusion was continued for 30 minutes to allow for tracer equilibration.25 Hemodynamic indexes were monitored continuously and remained stable during the infusion period. At the end of 30 minutes, repeat samples of arterial and AIV blood were obtained for measurements of all experimental variables. Next, the chest was opened, and the heart was exposed to obtain a transmural biopsy (about 1.5 x 2.5 cm) of myocardium distal to the stenosis.

Myocardial tissue was immediately blotted dry, cut into four layers (from endocardium to epicardium) each weighing 0.1–0.2 g, placed in preweighed vials, reweighed, and processed for liquid scintillation counting. Tissue processing included decolorization (30% H2O2), digestion (0.6 N NCS, Amersham, Arlington Heights, Illinois), and pH adjustment followed by temperature and dark adaptation before counting.

To calculate intracellular pH in myocardium distal to the stenosis (distal zone) certain assumptions were used. First, because interstitial fluid pH and tracer concentration could not be measured directly, we used AIV plasma pH and tracer activities in the calculation of distal zone intracellular pH. Others have used the same assumption in conjunction with this method.26 Second, in preliminary studies involving overnight drying of tissue samples (n = 181) at 50°C the dry:wet weight ratio was determined to be 0.23 ± 0.01 (mean ± SD). Accordingly, in all but one experiment of the present series, an assumed dry:wet weight ratio of 0.23 was used in calculating intracellular pH. The weighted mean value of intracellular pH for each tissue sample in the distal zone was used to compute an average transmural value of intracellular pH for the entire zone.

Statistical Methods

The significance of differences in group mean values was assessed by blocked, one-way analysis of variance and Newman-Keuls or Dunnett’s test.26 Results were considered statistically significant at a p value less than 0.05. All values are expressed as mean ± SD.

Results

Hemodynamics

In comparison with prestenosis levels, no changes occurred in heart rate, mean aortic pressure, mean left atrial pressure, or tension time index during the 1st hour after stenosis insertion (Table 1). Mean pressure distal to the stenosis exhibited a modest but statistically significant (p < 0.05) increase at 20 and 60 minutes after stenosis placement compared with values observed 5 minutes after stenosis insertion.

Intracoronary infusion of NaOH at 0.05 and 0.1 M doses was not associated with significant changes (vs. 60 minutes after stenosis) in any hemodynamic index. In contrast, infusion of 0.5 M NaOH resulted in modest but statistically significant changes in heart rate (increase 2%, as a result of a slight increase in sinus rate above the paced rate, p < 0.05 vs. 60 minutes after stenosis), tension time index (decrease 10%, p < 0.01 vs. 60 minutes), and aortic mean pressure (decrease 6%, p < 0.05 vs. 60 minutes after stenosis).

Regional Myocardial Blood Flow

Before stenosis insertion, flows were similar in respective layers of the distal and circumflex zones (1.62 ± 0.56, 1.28 ± 0.49, and 1.48 ± 0.53 ml/min/g; endocardium, epicardium, and transmural, respectively). Circumflex zone flows did not change compared with control flows at any time during the study. Five minutes after placement of the stenosis, there was a significant reduction (p < 0.01) in distal zone endocardial and transmural flows compared
with control levels (Figure 1). In contrast, epicardial flow in the distal zone did not change significantly when comparing control values with values during the 1st hour after stenosis placement. Both endocardial and transmural flow distal to the stenosis increased between 5 and 60 minutes after stenosis. The changes, however, were not statistically significant.

Endocardial flow distal to the stenosis did not change when comparing the 60-minute poststenosis level with levels during or after NaOH infusion at any dose. Distal zone epicardial blood flow also did not change when comparing the 60-minute poststenosis value with the levels in response to either 0.05 or 0.10 M NaOH infusion. In contrast, infusion of 0.5 M NaOH distal to the stenosis resulted in a significant reduction ($p<0.01$) in epicardial flow compared with the 60-minute poststenosis value.

**Coronary Vascular Resistance**

Distal zone endocardial resistance declines significantly ($p<0.01$) when comparing prestenosis levels with 5-minute poststenosis levels, and after 5 minutes, resistance was unchanged for the remainder of the study. NaOH infusion did not change endocardial resistance either 5 or 60 minutes after stenosis at any dose level.

Epicardial resistance in the distal zone also declined significantly ($p<0.01$) when comparing prestenosis values with 5-minute poststenosis values, and after 5 minutes, resistance was unchanged for the remainder of the 60-minute observation period. Infusion of 0.05 and 0.1 M NaOH doses failed to cause any change compared with 60-minute poststenosis levels in epicardial resistance. However, epicardial resistance did increase significantly ($p<0.05$) when comparing the 60-minute poststenosis value to the value at 0.5 M NaOH. Finally, circumflex zone resistance in each myocardial layer remained constant when comparing respective prestenosis levels (endocardium, 78±38; epicardium, 103±45; transmural, 88±40 mm Hg/ml/min/g); for the entire study.

**Regional Myocardial Acid-Base Balance**

Arterial and AIV oxygen tension remained unchanged when comparing prestenosis values (113±20 and 19±3 mm Hg, respectively) with 60-minute poststenosis values. Similarly, arterial CO$_2$ tension remained constant when comparing prestenosis levels (43±4 mm Hg) with the 60-minute poststenosis levels. In contrast, AIV CO$_2$ tension increased significantly ($p<0.05$) when comparing the prestenosis levels with the 5-minute poststenosis values (Figure 2). During the next 55 minutes, CO$_2$ tension in AIV blood returned to values that did not differ significantly from prestenosis values.

The pH of arterial blood did not differ significantly when comparing prestenosis levels (7.43±0.02) with poststenosis levels throughout the entire study. In contrast, the pH of AIV blood was reduced significantly when comparing prestenosis values to poststenosis values at 5 ($p<0.01$), 20 ($p<0.01$), and 60 ($p<0.05$) minutes (Figure 2).

There was no change when comparing the 60-minute poststenosis values in arterial oxygen and in CO$_2$ tension (117±17 and 42±4 mm Hg, respectively) during NaOH infusion. AIV blood gas tension and pH were unchanged by 0.05 M NaOH compared with respective levels 60 minutes after stenosis. Similar results were obtained in response to 0.1 M NaOH infusion (Figure 2).
Infusion of 0.5 M NaOH significantly ($p<0.01$) reduced CO$_2$ tension measured in the AIV (Figure 2) and calculated for left anterior descending coronary artery blood ($41 \pm 4$ to $35 \pm 4$ mm Hg). In addition, the pH and PO$_2$ of AIV blood increased significantly compared with 60 minutes after stenosis (Figure 2) as did calculated pH of left anterior descending coronary artery blood ($7.42 \pm 0.03$ to $7.54 \pm 0.08$).

**Intracellular pH Measurements**

After 30 minutes of intracoronary infusion of 0.5 M NaOH distal to the stenosis, the average value of distal zone intracellular pH (transmural) was $7.00 \pm 0.11$. The value is within the range of baseline levels for normal myocardium as determined both by the DMO method and by phosphorus nuclear magnetic resonance. Also of note, the value of distal zone interstitial fluid volume determined with $^3$H-inulin ($0.238 \pm 0.044$ ml/g left ventricle) agreed very closely with the value of this constant (0.217) determined by other methods.

**Regional Myocardial Oxygen Metabolism**

Compared with prestenosis levels, no significant change occurred in arterial (13.4 $\pm$ 1.5 ml/dl) or AIV (2.2 $\pm$ 0.6 ml/dl) oxygen content during the 1st hour after stenosis placement. Regional myocardial oxygen extraction distal to the stenosis also remained unchanged during the 1st hour after stenosis placement compared with prestenosis levels (84 $\pm$ 5%). In contrast, regional myocardial oxygen consumption declined significantly ($p<0.01$) when comparing prestenosis levels with levels 5 minutes after placement of the stenosis but did not change significantly during the remainder of the 60-minute observation period (Figure 3). The decline in regional myocardial oxygen consumption occurred exclusively as a result of the reduction in transmural myocardial blood flow after stenosis insertion.

There was no change when comparing 60-minute poststenosis values for regional myocardial oxygen consumption and extraction and for arterial and AIV oxygen content with values for intracoronary infusion of 0.05 M NaOH. Although arterial and AIV oxygen content and myocardial oxygen extraction during intracoronary infusion of 0.1 M NaOH remained unchanged compared with 60-minute poststenosis levels, regional myocardial oxygen consumption declined significantly ($p<0.01$). Infusion of 0.5 M NaOH resulted in a significant reduction ($p<0.01$) compared with 60 minutes after stenosis in both regional myocardial oxygen consumption (12.4 $\pm$ 2.8 to 8.2 $\pm$ 2.3 ml/min/100g) and extraction (86 $\pm$ 4 to 75 $\pm$ 8%). A modest but statistically significant ($p<0.05$) decline in arterial oxygen content occurred compared with 60-minute poststenosis levels during 0.5 M NaOH (0.5 $\pm$ 1.3 ml/dl) along with a relatively large increase ($p<0.01$ vs. 60 minutes after stenosis) in AIV oxygen content (1.9 $\pm$ 0.6 to 3.2 $\pm$ 1.2 ml/dl).

**Regional Myocardial Lactate Metabolism**

Arterial lactate levels remained unchanged for the duration of the study compared with prestenosis levels (0.75 $\pm$ 0.23 mM). AIV lactate concentration increased significantly ($p<0.01$) 5 minutes after...
stent placement (1.43 ± 0.76) compared with pre-stent placement concentrations (0.40 ± 0.10). Similarly, lactate concentration occurring before stent placement changed to production 5 minutes after stent insertion (p < 0.01) (Figure 3). During the next 55 minutes, both AIV lactate concentration (0.85 ± 0.24 at 60 minutes) and myocardial lactate production declined significantly compared with respective values at 5 minutes after stent placement. Infusion of NaOH at each dose level produced no significant change compared with 60 minutes after stent placement in AIV lactate concentration or myocardial lactate production.

Discussion

The role of pH and PCO2 in the regulation of myocardial blood flow has been considered by previous investigators.1-15 In addition, a comprehensive review of the subject has been written by Feigl.29 Important differences between earlier investigations and the present one should be reviewed, however, before summarizing the findings of previous studies. First, all of the published work has involved experimental models with normal coronary circulation. We are unaware of any data on this subject specifically pertaining to the stenosed coronary circulation. Second, previous studies frequently have used open-chest, anesthetized models often with pump-perfused coronary circulation and with exposure to extremes of pH or PCO2 or both. Accordingly, the results of such experiments may not be applicable to more intact preparations.29 Third, the focus of earlier studies has been on the role of pH or PCO2 or both as acute mediators of coronary tone. The role, if any, of these factors in more long-term adjustments of the coronary circulation, particularly in the stenosis setting, has been given relatively little attention.

Data obtained from earlier studies, nevertheless, indicate that acutely increasing PCO2 or lowering pH of arterial blood perfusing the coronary circulation or both results in coronary vasodilation.3-5,12 However, lowering pH or increasing PCO2 of arterial blood will produce a rightward shift (Bohr effect) of the oxygen-hemoglobin dissociation curve. In turn, a rightward shift of the curve would be expected to cause coronary vasoconstriction because more oxygen would be released from blood, thereby increasing myocardial oxygen delivery, at any given tissue oxygen tension.29 The fact that acidosis generally elicits coronary vasodilatation and not vasoconstriction, therefore, underscores the need to perform experiments to determine the effects of altering blood pH and PCO2 on coronary flow regulation.

Alkalizing arterial blood, whether by base infusion or hyperventilation, would be expected to cause an acute left shift of the oxygen-hemoglobin dissociation curve and thereby elicit coronary vaso-dilation. Although such a result was observed in an isolated heart-lung model,10 hyperventilation in dogs1,14 and humans11 caused coronary vasoconstriction. In addition, although alkalization of arterial blood with sodium bicarbonate caused an increase in myocardial blood flow in one study,14 heart rate and tension time index increased substantially and could have been responsible, at least in part, for augmentation of myocardial blood flow. Furthermore, because bicarbonate infusion causes intracellular acidosis along with extracellular alkalosis, the former also may have contributed to the observed increase in myocardial blood flow. Thus, the results of previous experiments in this area do not always conform to theoretical expectations, and additional work is required, particularly in the realm of tissue acidosis as a long-term mediator of coronary tone in the stenosis setting.

The results of this study show that abrupt imposition of a severe stenosis on the coronary circulation may result in a reduction in endocardial blood flow with no change or even an increase in epicardial flow. The reduction in endocardial blood flow was accompanied by evidence of tissue acidosis as manifested by increase in coronary venous PCO2 and reduction in coronary venous pH. A decline in myocardial oxygen consumption and a change from lactate consumption to lactate production (i.e., metabolic evidence of myocardial ischemia) also was documented. Both endocardial and epicardial resistance decreased (vs. prestenosis) after insertion of the stenosis, although the reduction in endocardial resistance was less than maximal.20,30 Accordingly, the data are consistent with a role for tissue acidosis in the acute reduction of arteriolar tone immediately after stenosis placement.

By observing the animals over time, it was possible to gain some insight into the potential role of tissue acidosis as a more long-term mediator of reduced arteriolar tone distal to a severe coronary arterial stenosis. As noted earlier, very little information is available in this regard. The data obtained in the present study and an earlier one describing the natural history of myocardial ischemia in this animal model31 show that metabolic evidence of myocardial ischemia and tissue acidosis declines over the course of an hour but does not resolve fully (Figures 2 and 3). Between 1 and 3 hours after stenosis placement in the natural history study,31 there was evidence of additional improvement in tissue acidosis and reduction in myocardial ischemia, although the changes were modest compared with those that occurred between 5 and 60 minutes after stenosis. The fact that arteriolar tone distal to the stenosis remained unchanged over time despite evidence of a reduction in both tissue acidosis and metabolic evidence of myocardial ischemia suggests either that tissue acidosis plays only a modest role in the chronic reduction of arteriolar tone in the stenosis setting or that once a certain degree of acidosis is attained, further reductions in arteriolar tone are not produced even if the acidosis increases. If the latter hypothesis were correct, a spontaneous or induced lessening of tissue acidosis would not be accompanied by an increase in coronary arteriolar
tone unless acidosis were corrected beyond some "critical level."

Information obtained in the second half of this study during which NaOH was infused into the coronary circulation distal to the stenosis is helpful in choosing between the hypotheses outlined above. Distal zone endocardial blood flow failed to decline in response to NaOH at any dose level tested. It should be recalled that infusion of 0.5 M NaOH was sufficient to raise distal zone intracellular pH to levels that have been reported for normal, nonstenosed myocardial regions.25,27 In addition, the pH of coronary venous blood was elevated to systemic levels (i.e., 7.41) during 0.5 M NaOH infusion. Thus, NaOH infusion (0.5 M) was capable of raising intracellular and tissue pH and could do so to an extent that should have removed the stimulus for vasodilatation if tissue acidosis functioned in that role. Because endocardial blood flow and resistance were not influenced by NaOH infusion, it is unlikely that tissue acidosis has an essential role in more long-term adjustments of the coronary circulation to the presence of a severe stenosis.

The fact that epicardial blood flow distal to the stenosis declined in response to a high dose of (0.5 M) NaOH does not necessarily contradict the argument advanced above. Myocardial oxygen consumption and extraction declined significantly during 0.5 M NaOH, an observation that indicates that primary myocardial depression (induced by NaOH) was responsible for epicardial flow reduction. Primary vasoconstriction should have been associated with either no change or an increase in myocardial oxygen extraction, and so attributing the decline in epicardial flow to that mechanism is difficult. A leftward shift of the oxygen-hemoglobin dissociation curve could not have been responsible for the results because both myocardial oxygen extraction and blood flow declined. A leftward shift by itself should have caused an increase in epicardial flow (which is possible in this model20,30,31) with no change in myocardial oxygen consumption. Furthermore, the change in coronary arterial pH (from 7.41 to 7.54) and Pco2 (from 42 to 35 mm Hg) induced by 0.5 M NaOH would have reduced the P90 of arterial blood only modestly (i.e., from about 27 mm Hg to about 24 mm Hg19). The fact that myocardial ischemia failed to worsen with the decline in epicardial flow also supports the hypothesis that primary myocardial depression (as opposed to primary vasoconstriction) was induced by NaOH. Finally, failure of endocardial flow to decline during infusion of 0.5 M NaOH may reflect the fact that depression of myocardial function was more severe in the endocardium and that as a result, endocardial flow was less susceptible to further depression by NaOH.

The mechanism responsible for apparent NaOH-induced depression of myocardial function cannot be stated with certainty. An earlier natural history study,31 however, excluded the possibility of a time-dependent spontaneous deterioration of distal zone function as an alternative explanation for the observed decline in regional myocardial oxygen consumption during NaOH infusion. Similarly, although a high-dose base infusion was associated with a reduction in certain external determinants of myocardial oxygen demand (Table 1), the absolute changes were small and were not associated with autoregulatory reductions in circumflex zone blood flow. Accordingly, reduced distal zone epicardial blood flow and oxygen consumption during 0.5 M NaOH infusion probably cannot be explained by observed small changes in external determinants of oxygen demand. Further, because NaOH was administered by slow (0.38 ml/min) infusion into the blood (an excellent buffer) and because calculated pH of blood in the left anterior descending coronary artery increased to no more than 7.55, a direct caustic effect of NaOH on the myocardium or vasculature probably would not account for the result. Rather, it is more likely that myocardial contractility was adversely affected by the pH effect of base infusion, which presumably interfered with availability of calcium to the contractile apparatus.

In conclusion, even though tissue acidosis may be chronically present under resting conditions in the setting of a severe coronary arterial stenosis, data obtained in this study show that increased hydrogen ion concentration or Pco2 tension or both is not essential for long-term maintenance of reduced arteriolar tone distal to the stenosis. Although other mediators may substitute for low pH or elevated Pco2, results of earlier studies conducted in our laboratory indicate that sustained elevation of interstitial fluid adenosine concentration19 or increased prostacyclin production32 also are not essential for long-term maintenance of reduced arteriolar tone distal to a severe coronary arterial stenosis. Thus, the results of the present investigation are best understood in the context of a series of experiments,16-19,31,32 which indicate that known mediators of acute changes in coronary arteriolar tone may not have an important role (either alone or in combination) in the long-term maintenance of reduced arteriolar tone distal to a severe coronary arterial stenosis. The mechanism(s) involved in maintenance of reduced coronary arteriolar tone in this setting is unknown but could involve alterations in one or more proteins responsible for regulation of vascular smooth muscle contraction and relaxation. Additional biochemical studies are required to test this hypothesis.

Acknowledgments

We thank the staff of the Cardiac Research Laboratory, Rhode Island Hospital, for excellent technical assistance and Mrs. Christine Abatiello for the preparation of the manuscript. Donald Jackson, PhD, (Brown University) provided helpful advice and criticism.
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Key words: coronary flow regulation • pH • pCO₂ • coronary flow • maintenance of arteriolar dilation
Tissue acidosis: role in sustained arteriolar dilatation distal to a coronary stenosis.
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_Circulation_. 1989;79:890-898
doi: 10.1161/01.CIR.79.4.890
_Circulation_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1989 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7322. Online ISSN: 1524-4539

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