Role of Adenosine in the Maintenance of Coronary Vasodilation Distal to a Severe Coronary Artery Stenosis

Observations in Conscious Domestic Swine

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SUMMARY. The purpose of this study was to test the hypothesis that adenosine is required to maintain arteriolar vasodilation distal to a severe coronary stenosis. Eight closed-chest conscious pigs were prepared by placing a 7.5-mm long stenosis (82% lumenal diameter reduction) in the proximal left anterior descending coronary artery. Regional myocardial blood flow (microsphere technique) was measured at control 1, after 10 minutes of intracoronary infusion of adenosine deaminase (7-10 U/kg per min) distal to the stenosis, and 20-30 minutes after stopping adenosine deaminase infusion. Studies with 125I-labeled adenosine deaminase were conducted in six additional pigs to document the extent to which infused adenosine deaminase penetrated the interstitial space. 125I-labeled adenosine deaminase was infused for 10 minutes (10-11 U/kg per min) into the left anterior descending coronary artery. Calculated interstitial fluid concentrations of adenosine deaminase ranged between 71 and 272 U/ml and were at least one order of magnitude greater than that required to deaminate all the adenosine which would be released into the interstitium in response to 15–30 seconds of coronary occlusion. In the primary group of animals (n = 8), endocardial flow (ml/min per g) distal to stenosis at control 1 (1.15 ± 0.33) was reduced vs. endocardial flow in the nonobstructed circumflex zone (1.59 ± 0.38, P < 0.05). Flows in epicardial layers were comparable at control 1 (distal zone = 1.40 ± 0.36 vs. circumflex zone = 1.45 ± 0.41). Distal zone endocardial and epicardial flows did not change vs. control 1 in response to infusion of adenosine deaminase. However, the distal/circumflex epicardial flow ratio declined vs. control 1 (0.98 ± 0.14) during adenosine deaminase infusion (0.87 ± 0.17, P < 0.05). The distal/circumflex endocardial flow ratio during adenosine deaminase (0.72 ± 0.20) was unchanged vs. control 1 (0.76 ± 0.22) but was less than control 2 (0.80 ± 0.18, P < 0.05). Thus, destruction of all or most interstitial adenosine caused only slight relative reduction in regional myocardial blood flow distal to a severe coronary artery stenosis. Accordingly, adenosine contributes only modestly to maintenance of arteriolar vasodilation in this setting or else its absence is almost fully compensated for by another mechanism(s). (Circ Res 53: 42–51, 1983)

ADENOSINE is widely regarded as the primary physiological mediator of both reactive hyperemia and autoregulation of the coronary vascular bed (Berne and Rubio, 1979). Although several recent studies (Hester et al., 1982; Jones et al., 1982; Manfredi and Sparks, 1982; Saito et al., 1981) have undertaken tests of the adenosine hypothesis as it applies to reactive hyperemia or autoregulation in response to increased myocardial oxygen demand, none have addressed the role of adenosine in the maintenance of normal or only modestly reduced levels of myocardial blood flow distal to a severe coronary artery stenosis. Accordingly, the present investigation was performed to test the hypothesis that adenosine is required to decrease coronary arteriolar tone and thereby prevent a marked reduction in myocardial blood flow distal to a severe coronary artery stenosis. Studies were conducted in closed-chest, sedated, but conscious swine instrumented with an artificial intralumenal coronary artery stenosis which reduced vessel diameter by 82% (Gewirtz and Most, 1981; Gewirtz et al., 1982a, 1982b, [in press]). Infusion of the enzyme, adenosine deaminase into the coronary bed, distal to the stenosis was employed to destroy interstitial adenosine and thereby evaluate the role played by adenosine in the maintenance of coronary vasodilation distal to a severe coronary artery stenosis.

Methods

Animal Preparation

Eight farm bred pigs (mean weight 42.3 kg, range 34.9–53.8 kg) were premedicated with ketamine (25 mg/kg, im) and then anesthetized with halothane (0.5–1.5%) and nitrous oxide. Anesthetic gases were mixed with oxygen...
and delivered via an endotracheal tube with a volume-cycled respirator. Next, the animal was heparinized (225 IU/kg) and a 7F end-hole catheter was inserted into the left femoral artery and advanced to the arch of the aorta. This line was used to monitor pressure, obtain blood for pH, Po2, Pco2, and reference sample for determination of regional myocardial blood flow. The right femoral artery was used to insert an 8F angiographic catheter which was passed retrograde under fluoroscopic guidance into the left atrium via the left ventricle. This catheter was used to administer radioactive microspheres. A 7F "head hunter" catheter (USCI) inserted into the right internal jugular vein was advanced under fluoroscopic control into the coronary sinus and thence to the proximal portion of the anterior interventricular vein (AIV). This catheter was used to sample coronary venous blood draining myocardium in the distribution of the left anterior descending coronary artery. The animal's heart was paced at a constant rate throughout. A 1.4-mm diameter, 70-cm long plastic catheter had been inserted into the left femoral artery and advanced to the arch of the aorta. This wire was advanced under fluoroscopic control into the coronary artery (Gewirtz and Most, 1981). The wire was quickly removed leaving the stenosis in place. The stenosis contained a second lumen into which the distal end of the 1.4-mm diameter, 70-cm long plastic catheter had been attached prior to placement of the stenosis. The distal end of the catheter was open to the distal end of the stenosis and used to infuse enzyme into, and record pressure from, the distal left anterior descending coronary artery bed. This completed the preparation of the animal. After instrumentation had been accomplished, all cutdown sites were closed and anesthesia discontinued. Small doses (20–40 mg) of sodium thiamylol were then given intravenously throughout the study to insure that the animal was comfortable and rested quietly. Although sedated, the animal breathed spontaneously, was awake, and had brisk corneal reflexes.

Study Protocol

After the preparation had stabilized for approximately 30 minutes, control measurements were obtained for (1) hemodynamics, (2) regional myocardial blood flow (microsphere technique, vide infra), (3) metabolic parameters (arterial and AIV oxygen and lactate content), and (4) arterial and AIV concentrations of adenosine deaminase.

Next, adenosine deaminase was infused into the coronary bed distal to the stenosis via the infusion catheter secured within the stenosis. The enzyme was dissolved in 0.3 M Tris buffer (pH 7.4, at 37°C) and delivered at a rate of 0.34 ml/min during the infusion at a rate of 0.34 ml/min. After final measurements had been obtained, approximately 3 × 106 radiolabeled microspheres (total activity, ~4 μCi) were injected into the perfusion catheter to objectively label myocardium distal to the stenosis that had been exposed to the enzyme infusion. The animal then was given a large intravenous dose of sodium thiamylol (200–300 mg) and killed 3–4 minutes later with a lethal dose of KCl. After this, the heart was removed and sectioned for determination of microsphere activity as described below. Prior to removing the heart from the thorax, we carefully noted the position of the distal end of the AIV catheter with respect to venous tributaries which may have drained into it from myocardium proximal to the stenosis. Two small tributaries were observed in each of three animals, one small tributary in each of three others, and no such tributaries in the remaining two animals.

Determination of Regional Myocardial Blood Flow

For each experimental condition, approximately 4 × 106 radiolabeled microspheres (15 μ DIA, 85–105 μCi total radioactivity) were injected via the left atrial catheter in order to determine regional myocardial blood flow (Domenech et al., 1969). A precisely timed, 2-minute reference collection of arterial blood was begun 15–30 seconds before injection of the radiolabeled microspheres. Blood was withdrawn at a constant rate (10 ml/min) from the thoracic aorta catheter into a 50-ml preweighed glass syringe by means of a Harvard pump. It should be noted that (1) a different radioisotope was chosen at random from a collection of seven isotopes (i.e., 43Sc, 103Ru, 253Nb, 105Cd, 113Sn, 54Cr, and 20Co) for each flow determination, and (2) the microspheres were suspended in 2 ml of 20% dextran with 0.01% Tween-80 and mechanically dispersed by repeated injection between stock vial and syringe for 2 minutes before each injection.

After the animal had been killed, the heart was removed and sectioned for determination of microsphere activity as previously described (Gewirtz and Most, 1981; Gewirtz et al., 1982a, 1982b). It should be noted, however, that in this study, the left ventricle was first cut into cubes weighing 1–3 g and the location of each carefully noted on a diagram of the free wall of the ventricle. Each cube was divided into endocardial and epicardial halves. Each endocardial and epicardial half was again divided in half in order to obtain endocardial and epicardial layers which represented, respectively, the innermost and outermost quarter of the left ventricular wall. Each quarter of the transmural cube weighed between 0.25 and 0.75 g. Flow results in endocardial and epicardial layers distal to the stenosis (distal zone), and in a region at the base of the heart perfused by the circumflex coronary artery (circumflex zone), are based on data obtained from the innermost and outermost quarters of the myocardial wall, respectively. However, transmural flow results are based upon activity of each isotope in all four quarters of each transmural cube. The value of transmural flow for each cube represents a weighted mean average of calculated flows for each of the four quarters comprising the cube.

Determination of Regional Myocardial Oxygen Consumption

Paired samples (2–3 ml) of arterial and AIV blood were obtained for determination of oxygen content (Lex-O2-CON Instrument, Lexington Instruments) during each
phase of the study. Samples were obtained immediately after each determination of regional myocardial blood flow. Blood samples were collected in heparinized glass syringes and immediately placed on ice for subsequent analysis at the end of the study. Oxygen content (volume percent) was determined in duplicate for each sample, and values accepted only if the difference between them was ≤0.2 ml O2/dL. 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-AIV oxygen difference.

**Determination of Regional Lactate Metabolism**

Lactate concentration in arterial (ART) and AIV blood was determined by means of a spectrophotometric method with a commercial reagent kit (Calbiochem Rapid Lactate Reagents). Samples of blood (5 ml) were immediately deproteinized by placing them in cold perchloric acid (8% vol/vol). Subsequently, the samples were centrifuged and the supernatant frozen for later analysis in duplicate. Regional extraction/production of lactate (%) was calculated in standard fashion. Thus:

\[
\% \text{ Extraction or production} = \frac{[\text{ lactate}]_{\text{ART}} - [\text{ lactate}]_{\text{AIV}}}{[\text{ lactate}]_{\text{ART}}} \times 100
\]

**Determination of Plasma Adenosine Deaminase Concentration**

Samples (2 ml) of blood were centrifuged at 5000 rpm × 10 minutes, after which the plasma layer was decanted and refrigerated at 4°C for subsequent analysis. Plasma enzyme assays were performed within 48-72 hours of completion of each experiment. Enzyme levels were determined by adding a 20-μl aliquot of plasma to a cuvette containing 12 mg of adenosine dissolved in 3.0 ml of 0.3 M Tris buffer (pH 7.5) and the change in light absorbance at the 265-nm photopoint of adenosine was determined with the aid of a Carey spectrophotometer. The concentration of enzyme in the plasma (U/ml) was calculated in standard fashion based upon the rate of change in light absorbance (Mavck and Davis, 1980). The lower limit of detectable plasma enzyme activity with this method was found to be on the order of 10⁻² U/ml.

**Validation Experiments**

Several closely related experiments were performed to establish the size and purity of Sigma type I adenosine deaminase, and to determine the extent to which it penetrated the cardiac interstitial space.

**Size, Purity, and Radiolabeling of Adenosine Deaminase**

The contents of six vials (~50 mg) of Sigma type I adenosine deaminase were collected by centrifugation and the precipitate redissolved in 3.0 ml of 20 mM Tris-HCl buffer (pH 7.4) containing 0.1 M NaCl. The adenosine deaminase then was chromatographed on a column of Sephadex G-75 Superfine (2.5 X 90 cm) at a flow rate of 20 ml/hour. Fractions (2.6 ml) were assayed for protein content (Bradford, 1976) with bovine serum albumin as standard. The void volume for this column was 78 ml.

The protein pellet was dissolved in 20 mM MOPS buffer (pH 7.4) containing 0.1 M NaCl. To remove residual ammonium sulfate, the enzyme was desalted on a Sephadex G-25 column (1.7 X 20 cm) with MOPS buffer. The peak of protein was pooled and labeled with 1.0 mCi of ¹²⁵I Bolton-Hunter reagent (Bolton and Hunter, 1973). After 15 minutes, the reaction mixture was chromatographed on the Sephadex G-25 column to remove unbound Bolton-Hunter reagent. Fractions were assayed for protein content, radioactivity, and enzyme activity. The fraction with maximal protein content (4.0 mg/ml) also contained maximal radioactivity (>10⁶ cpm/ml) and maximal adenosine deaminase activity (158 U/ml). Accordingly, this fraction was used in subsequent experiments as the source of tracer for unlabeled adenosine deaminase. An aliquot of this fraction also was saved for subsequent sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis and autoradiography.

**Estimation of Adenosine Deaminase Concentration in Cardiac Interstitial Fluid**

Iodine-125-labeled adenosine deaminase was used to estimate concentrations of adenosine deaminase attained in the interstitial fluid of the myocardium following intracoronary infusion of the enzyme. This was accomplished.
as follows. In six closed-chest, anesthetized (halothane 0.5–1.5% and nitrous oxide) pigs, the distal end of a 1.4-
mm diameter perfusion catheter was placed in the mid-
portion of the animal’s left anterior descending coronary
artery. The distal end of a 7F head hunter catheter was
placed under fluoroscopic control in the proximal portion
of the animal’s anterior interventricular vein. The animal’s
red blood cells (10 ml of whole blood) were labeled with
51Cr (200–400 μCi) according to standard technique (Baum
and Bramlet, 1975). A small aliquot (0.08–0.40 mg) of
radiolabeled enzyme was mixed with approximately 60 mg
of unlabeled adenosine deaminase. The mixture was in-
fused for 10 minutes into the animal’s left anterior de-
sceding coronary artery at a rate of 0.34 ml/min, and in
a concentration sufficient to deliver 10–11 U/kg per min.
At the end of 10 minutes, the infusion was discontinued.
Samples of arterial and AIV blood were obtained 30 and
60 seconds later for determination of enzyme activity, 125I,
and 51Cr radioactivity. Methylen blue then was injected
into the coronary perfusion catheter to demarcate myo-
cardium exposed to the adenosine deaminase infusion.
Following this, the animal was killed with lethal dose of
KCl. Next, the heart was removed and sectioned for
determination of 125I and 51Cr activity. The heart was
divided into two zones for this purpose: one at the base
of the heart remote from the infusion, and the other
demarcated by methylene blue. Fat and epicardial blood
vessels were trimmed away from each zone. Each zone
was subdivided into several transmural cubes weighing
approximately 1 g each. Each cube was in turn divided into
endocardial and epicardial halves. Tissue samples were
placed in preweighed plastic vials, weighed, and then
counted in a gamma well counter as described above. A
computer was used to correct for spillover of 51Cr counts
into the 125I window. Finally, to facilitate counting of 125I
activity in the blood, each sample of whole blood was
centrifuged, after which plasma was decanted off. One-
milliliter samples of plasma and red blood cells were
counted separately for determination of 125I and 51Cr ac-
tivity in each.

In this experiment, 51Cr-labeled red blood cells served as
a marker for the intravascular space. To estimate more
accurately the interstitial fluid (ISF) concentration of the
enzyme, which is proportional to 125I activity in tissue
specimens, it is necessary to subtract 125I activity confined
to the myocardial intravascular space from total 125I ac-
tivity in the heart. This can be done by subtracting myocar-
dial 51Cr activity (vascular space marker) from total 125I
activity in the myocardium, after correcting myocardial
51Cr activity by the ratio of 125I to 51Cr activity in the blood.
The more 125I adenosine deaminase passes through the
myocardium and recirculates in the vascular space, the
greater the value of the blood 125I:51Cr ratio, thus, the
more 51Cr activity in the myocardium should be subtracted
from total myocardial 125I activity. Accordingly, the fol-
lowing model was employed to estimate concentration of
adenosine deaminase (ADA) in the interstitial fluid.

\[
\text{(ADA)}_{\text{ISF}} = \frac{1}{(125\text{I})_{\text{LV}} - [(51\text{Cr})_{\text{LV}} \times (125\text{I})_{\text{RED}}]} \times 0.217 \times \text{SA}_{\text{ADA}}
\]

where: \((\text{ADA})_{\text{ISF}} = \text{ADA concentration in ISF, U/ml; (125I)}_{\text{LV}} = \text{125I activity in left ventricle (LAD zone), cpm/g; (125I)}_{\text{RED}} = \text{125I activity in coronary venous blood, cpm/ml; (51Cr)}_{\text{LV}} = \text{51Cr activity in left ventricle (LAD zone), cpm/g; (51Cr)}_{\text{RED}} = \text{51Cr activity in coronary venous blood, cpm/ml; 0.217 = constant, ml of ISF/g left ventricle; and } \text{SA}_{\text{ADA}} = \text{specific radioactivity of ADA infusion, cpm/U.}

Stability of Bolton-Hunter Reagent/Adenosine Deaminase Complex

To assess the stability of the Bolton-Hunter reagent/ADA complex, an additional experiment was carried out. Approximately 7 weeks after preparation of the radiola-
beled protein, which had been stored at 4°C, a 0.8-mg sample was incubated in fresh pig blood (5 ml) at 37°C for 10 minutes. After 5 mg of bovine serum albumin had
been added to the blood, the entire mixture was pre-
cipitated with 5 ml of ice-cold 15% perchloric acid (PCA).
The mixture then was centrifuged (3500 rpm × 20 min-
utes), the plasma/PCA supernatant decanted off, and
centrifuged again (5000 rpm × 20 minutes). The original
protein precipitate was washed twice with normal saline
and then dried by placing it in an oven at 32°C for 12
hours. A 0.25-ml aliquot of the plasma/PCA mixture and
three 0.1-g samples of the dried protein precipitate were
counted with those in the precipitate by expressing each
on a per gram basis after accounting for dilution in each
phase.

Statistical Methods

All data are expressed as mean ± 1 sd. The significance
of group mean changes vs. control was assessed by means
of blocked one-way analysis of variance and Dunnett’s
test (Winer, 1971). P values < 0.05 were considered statisti-
cally significant.

Results

Physiological Study—Eight Animals with
Coronary Artery Stenosis

Plasma Adenosine Deaminase Levels

Under control conditions, arterial and AIV plasma
levels (U/ml) of adenosine deaminase were below
the limits of detection of the assay method employed
(i.e., <10–2 U/ml). In contrast, during infusion of
the enzyme, levels increased to 0.9 ± 0.3 in arterial
plasma and 6.2 ± 2.0 in AIV plasma. Twenty min-
utes after discontinuation of the infusion, adenosine
deaminase activity was still detectable in arterial and
AIV plasma (1.0 ± 0.2 and 0.9 ± 0.3, respectively).

Hemodynamics (Table 1)

As required by the study protocol, heart rate did
not change significantly during the investigation. In
response to enzyme infusion, there was no signifi-
cant change (vs. control 1) in (1) mean aortic pres-
sure, (2) distal coronary mean pressure, (3) stenosis
mean pressure gradient, or (4) mean left atrial pres-
sure. A modest but significant (P < 0.05) rise in both
aortic mean pressure and distal coronary mean pres-
sure did occur after discontinuation of the enzyme
infusion.

Regional Myocardial Blood Flow (Table 2 and Fig. 2)

Under control conditions, both endocardial and transmural flows distal to the stenosis were reduced signifi-}
cantly (P < 0.05) compared with respective
flows in the circumflex zone. Similarly, the endo-
cardial:epicardial flow ratio in myocardium distal to
TABLE 1

<table>
<thead>
<tr>
<th></th>
<th>Control 1</th>
<th>ADA</th>
<th>Control 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart rate (beats/min)</td>
<td>120 ± 21</td>
<td>123 ± 27</td>
<td>115 ± 19</td>
</tr>
<tr>
<td>Aortic mean pressure (mm Hg)</td>
<td>117 ± 15</td>
<td>122 ± 6</td>
<td>131 ± 9*</td>
</tr>
<tr>
<td>Distal coronary mean pressure (mm Hg)</td>
<td>77 ± 7</td>
<td>86 ± 9</td>
<td>86 ± 10*</td>
</tr>
<tr>
<td>Stenosis mean pressure gradient (mm Hg)</td>
<td>41 ± 11</td>
<td>42 ± 7</td>
<td>45 ± 10</td>
</tr>
<tr>
<td>Mean left atrial pressure (mm Hg)</td>
<td>4 ± 5</td>
<td>5 ± 4</td>
<td>4 ± 5</td>
</tr>
</tbody>
</table>

Results are expressed as mean ± 1 so.

* P < 0.05 vs. control 1.

The stenosis was significantly reduced (P < 0.05) compared with the endocardial:epicardial flow ratio in the circumflex region under control conditions. Epicardial blood flow distal to the stenosis under control conditions, however, did not differ significantly from that of epicardial flow in the circumflex zone under control conditions.

In response to adenosine deaminase infusion, there were no significant changes in absolute values of endocardial, epicardial, or transmural blood flow in myocardium distal to the stenosis, or in myocardium in the circumflex zone. However, the ratio of epicardial flow in the distal zone to epicardial flow in the circumflex zone (Fig. 2) declined significantly (P < 0.01) vs. control 1 in response to enzyme infusion. Likewise, the ratio of transmural blood flow (distal:circumflex zone) decreased significantly (P < 0.05) vs. control 1 in response to adenosine deaminase infusion. Finally, although the distal to circumflex flow ratio for endocardial specimens did not change significantly vs. control 1 in response to enzyme infusion, the value of the ratio during infusion was significantly less (P < 0.05) than that of the second control period. The second control value, however, did not differ significantly from that of the first.

Regional Lactate Metabolism (Table 3 and Fig. 3)

Arterial lactate levels did not change significantly during the course of the study. There was a modest rise vs. control 1 in (ATV) lactate levels in response to adenosine deaminase infusion. Although the value was not significantly greater than that of control 1, it was significantly greater (P < 0.05) than

![Figure 2](http://circres.ahajournals.org/)

**Figure 2.** Individual flow responses to adenosine deaminase (ADA) infusion are shown here. The data are expressed as the ratio of left anterior descending (LAD) coronary flow distal to the stenosis to flow in myocardium perfused by the circumflex (CX) coronary artery. The change from control 1 (C1) to ADA was statistically significant for epicardial (P < 0.01) and transmural (P < 0.05) samples. In the endocardium, only the change from ADA to control 2 (C2) was statistically significant (P < 0.05).
TABLE 3

Regional Myocardial Lactate Metabolism of Eight Animals
Given Intracoronary Adenosine Deaminase (ADA)

<table>
<thead>
<tr>
<th></th>
<th>Control 1</th>
<th>ADA</th>
<th>Control 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arterial lactate</td>
<td>1.12 ± 0.40</td>
<td>1.13 ± 0.61</td>
<td>0.92 ± 0.25</td>
</tr>
<tr>
<td>(mM/liter)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AIV lactate</td>
<td>0.77 ± 0.30</td>
<td>0.90 ± 0.38*</td>
<td>0.63 ± 0.20</td>
</tr>
<tr>
<td>(mM/liter)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Extraction (%)</td>
<td>30.5 ± 18.6</td>
<td>13.8 ± 23.7†</td>
<td>31.2 ± 10.7</td>
</tr>
</tbody>
</table>

Results are expressed as mean ± 1 SD.

*P < 0.05 vs. control 2; †P < 0.05 vs. control 1.

that of control 2. Control 1 and control 2 AIV lactate levels did not differ significantly. Regional lactate extraction declined significantly (P < 0.05) vs. control 1 in response to enzyme infusion. After discontinuation of the infusion, lactate extraction returned to control levels.

Regional Myocardial Oxygen Metabolism (Table 4)

Arterial oxygen content did not change significantly vs. control 1 at any time during the study. Similarly, neither AIV oxygen content nor calculated regional myocardial oxygen consumption changed significantly vs. control 1 at any time during the study. Finally, it should be noted that regional myocardial oxygen extraction was high (>85%) under control conditions and remained so throughout the study.

Validation Experiments

Purity of Commercial (Sigma, type I) Adenosine Deaminase

As shown in Figure 1, the commercial product eluted off the column essentially as a single peak of adenosine deaminase activity. There was a small initial protein spike which preceded the major protein peak by several fractions. Although this fraction clearly contained adenosine deaminase activity, it was not used in the radiolabeling experiment in order to obtain as pure a tracer as possible. Comparison of the distance migrated by a sample of the major protein fraction subjected to SDS polyacrylamide gel electrophoresis with distance migrated by proteins of known molecular weight permitted estimation of the molecular weight of adenosine deaminase contained in the commercial preparation.

Stability of 125I-Labeled Adenosine Deaminase

Comparison of the autoradiograph image of the SDS-polyacrylamide gel with the silver stain pattern demonstrated that the major protein band corresponded exactly with the region of exposure on the x-ray film. Accordingly, it is clear that 125I Bolton-Hunter reagent was covalently bound to adenosine deaminase.

TABLE 4

Regional Myocardial Oxygen Metabolism of Eight Animals Given Intracoronary Adenosine Deaminase (ADA)

<table>
<thead>
<tr>
<th></th>
<th>Control 1</th>
<th>ADA</th>
<th>Control 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arterial O2 content</td>
<td>12.0 ± 1.2</td>
<td>11.8 ± 1.3</td>
<td>11.6 ± 1.0</td>
</tr>
<tr>
<td>(vol %)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AIV O2 content</td>
<td>1.49 ± 0.43</td>
<td>1.36 ± 0.69</td>
<td>1.30 ± 0.52</td>
</tr>
<tr>
<td>(vol %)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A-V O2 difference</td>
<td>10.5 ± 1.4</td>
<td>10.4 ± 1.2</td>
<td>10.3 ± 1.0</td>
</tr>
<tr>
<td>(vol %)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>O2 extraction (%)</td>
<td>87.4 ± 4.0</td>
<td>88.5 ± 5.1</td>
<td>86.7 ± 4.2</td>
</tr>
<tr>
<td>O2 consumption</td>
<td>13.8 ± 2.4</td>
<td>14.2 ± 2.1</td>
<td>15.0 ± 2.4</td>
</tr>
<tr>
<td>(ml/min per 100 g)</td>
<td></td>
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Results are expressed as mean ± 1 SD.
Radiolabeled adenosine deaminase was incubated in porcine blood to determine if an enzyme in the animal's plasma might exist which could cleave the ADA/Bolton Hunter reagent bond and thereby cause dissociation of the radiolabel from adenosine deaminase. No evidence of this was found. [125I] activity (cpm/g) in the plasma/PCA supernatant (1.74 X 10^7 cpm/g) was 10^-4 times less than activity in the protein precipitate (4.18 X 10^10 cpm/g). Thus, the ratio of bound to unbound label was greater than 10,000:1.

### Estimation of Tissue Concentration of Adenosine Deaminase (Table 5)

Since arterial and coronary venous activities of [125I], [51Cr], and adenosine deaminase did not differ significantly from one another at 30 and 60 seconds after discontinuation of the enzyme infusion, values of each were pooled to obtain a more reliable estimate of each parameter. Calculated interstitial fluid concentrations of adenosine deaminase (based on transmural counts) ranged between 71 and 272 U/ml in the perfused myocardial zone. Recirculation of the enzyme resulted in calculated interstitial fluid concentrations between 0.8 and 2.5 U/ml in myocardium not directly exposed to adenosine deaminase infusion (remote zone).

### Discussion

We have shown in previous studies employing a porcine model instrumented with an artificial 82% coronary artery stenosis that epicardial vasodilatory reserve distal to the stenosis is considerably reduced and endocardial reserve essentially absent (Gewirtz et al., 1982a 1982b, [in press]). In the present study, under control conditions, endocardial and transmural flow distal to the stenosis was significantly reduced, compared with that of the unobstructed circumflex zone. Likewise, the endocardial:epicardial flow ratio distal to the stenosis was less than unity, and significantly reduced in comparison with that of the circumflex zone. The fact that epicardial flow distal to the stenosis under control conditions was not reduced vis-a-vis the circumflex zone, despite a reduction in mean coronary perfusion pressure of 40 mm Hg, indicates that an autoregulatory reduction in arteriolar tone must have occurred in this layer. Furthermore, since endocardial flow distal to the stenosis was reduced, it is likely that the autoregulatory reserve in this layer of the myocardium was exhausted. The adenosine hypothesis would predict, under these conditions, that interstitial fluid concentrations of the nucleoside should be elevated (Berne and Rubio, 1979), and that destruction of interstitial adenosine should cause a profound reduction in regional blood flow in both endo- and epicardial layers of the heart distal to the stenosis. However, contrary to the prediction of the adenosine hypothesis, destruction of interstitial adenosine failed to cause a substantial decline in regional blood flow in either layer. Indeed, in absolute terms, there was no significant change in regional blood flow distal to the stenosis, although there was a modest decrease in distal:circumflex zone flow ratios.

The latter observation demonstrates that enzyme infusion did cause a biological effect, albeit small. In this regard, it is important to consider the possibility that inosine (produced by deamination of adenosine) caused coronary vasodilation (Jones and Mayer, 1980) and, thus, largely offset the effects of adenosine deaminase infusion. This hypothesis seems unlikely, however, for the following reason. No more inosine could have been produced by adenosine deaminase infusion than an amount equal to the quantity of adenosine destroyed by the enzyme. However, inosine is only 1/400th as potent to the quantity of adenosine destroyed by the enzyme. However, inosine is only 1/400th as potent as adenosine (Olsson et al., 1979). Since arterial and coronary venous activities of [125I] and [51Cr] in the perfused zone were 10^-4 times less than that in the infusate, it seems unlikely that inosine (produced by deamination of adenosine) caused coronary vasodilation. The latter observation demonstrates that enzyme infusion did cause a biological effect, albeit small.

### Table 5

<table>
<thead>
<tr>
<th>Animal no.</th>
<th>SADA</th>
<th>Infusate activity (cpm/g) U/ml</th>
<th>Remote zone activity (cpm/g) U/ml</th>
<th>Calculated interstitial fluid ADA (U/ml)</th>
<th>Remote zone activity (cpm/g) U/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>204</td>
<td>0.045</td>
<td>5195</td>
<td>117</td>
<td>0.9</td>
</tr>
<tr>
<td>2</td>
<td>224</td>
<td>0.020</td>
<td>13199</td>
<td>272</td>
<td>2.5</td>
</tr>
<tr>
<td>3</td>
<td>248</td>
<td>0.049</td>
<td>9148</td>
<td>171</td>
<td>0.8</td>
</tr>
<tr>
<td>4</td>
<td>561</td>
<td>0.201</td>
<td>23165</td>
<td>184</td>
<td>0.8</td>
</tr>
<tr>
<td>5</td>
<td>1038</td>
<td>0.165</td>
<td>28662</td>
<td>127</td>
<td>1.5</td>
</tr>
<tr>
<td>6</td>
<td>959</td>
<td>0.163</td>
<td>14798</td>
<td>71</td>
<td>1.8</td>
</tr>
<tr>
<td>Mean</td>
<td>539</td>
<td>0.107</td>
<td>15567</td>
<td>157</td>
<td>1.4</td>
</tr>
</tbody>
</table>

SADA = specific activity of ADA infusate (cpm/U); ratio = ratio of [125I]/[51Cr] activity in blood; (ADA)isf = interstitial fluid ADA concentration (U/ml); cpm/g = counts/min per gram.
than that of adenosine. Since regional blood flow distal to the stenosis did not change during enzyme infusion, and since some inosine would be converted to hypoxanthine in the blood, an increase in inosine concentration of this magnitude (i.e., 400\times) would require an even greater rise in adenosine production. However, available data indicate that myocardial adenosine production increases by at most, 15- to 25-fold over baseline levels in response to a near-maximal vasodilatory stimulus (McKenzie et al., 1980; McKenzie et al., 1982). Even these increments in adenosine production probably would not be attainable under conditions of the present study, because some of this reserve would have been expended, in order to compensate for the presence of the stenosis. Thus, it is unlikely that the amount of inosine derived from destruction of adenosine would have been adequate to obscure whatever effects adenosine deaminase might otherwise have had on regional myocardial blood flow.

The possibility that reduced endocardial blood flow distal to the stenosis (during the time period prior to beginning collection of data) resulted in sufficient accumulation of inosine to maintain steady levels of flow over the remainder of the study also seems unlikely. Substantial accumulation of inosine in severely ischemic myocardium has been reported (Jones et al., 1976), but only in an animal model with multiple acute coronary artery ligations. Whereas it is true that endocardial flow was reduced in animals reported in the present study, the relative flow reduction vis-a-vis normally perfused myocardium was not severe (Table 2). Moreover, net extraction of lactate \( \geq 10\% \) was present in six of eight animals. In addition, epicardial layers distal to the stenosis were not underperfused at all. Accordingly, it seems unlikely that the degree of myocardial ischemia present in our study was of sufficient intensity to generate inosine in amounts adequate to maintain steady levels of flow despite destruction of interstitial adenosine.

The results of this study indicate, therefore, that maintenance of increased interstitial fluid levels of adenosine are not required to sustain coronary vasodilation distal to a severe stenosis. This observation suggests either that adenosine plays only a very small role to begin with in this regard, or that other compensatory mechanisms are rapidly and almost completely able to take over for adenosine if the nucleoside is removed from the interstitial space. We recognize that the available evidence does not allow us to distinguish between these alternatives. Finally, the data obtained do not rule out the possibility that adenosine may play a more important role in the initiation as opposed to the maintenance of coronary vasodilation in response to an abrupt drop in coronary perfusion pressure.

Acceptance of these conclusions requires proof that infused adenosine deaminase remains active in porcine blood, and is capable of penetrating the cardiac interstitial space in amounts sufficient to destroy all or most adenosine present therein. A number of lines of evidence are available to support both points. First, previous investigators have shown that administration of adenosine deaminase specifically antagonizes the effects of adenosine on renal blood flow in a canine preparation (Scott et al., 1979). Second, Saito and co-workers (1981) have demonstrated that \(^{131}\)I-labeled adenosine deaminase penetrates the cardiac interstitial space and achieves concentrations ranging between 1 and 13 U/ml of interstitial fluid when administered by the intracoronary route. Validation experiments reported here are consistent with those of Saito et al. (1981). The fact that substantially higher interstitial concentrations of enzyme were obtained in the present study could be related to a variety of factors, including species differences, differences in duration and dose of enzyme administered, and differences in amount of myocardium exposed to enzyme infusion. Additional evidence that protein molecules comparable in size to that of adenosine deaminase penetrate the interstitial space of the myocardium is available from at least two other investigations. Thus, Schrader et al. (1977b) have shown that adenosine monophosphate complexed to carbonic anhydrase (mol wt = 30,000) is capable of eliciting coronary vasodilation when adenosine monophosphate-protein complex is administered by the intracoronary route. Similarly, Karmovsky (1967) has shown that horseradish peroxidase (mol wt = 40,000) penetrates the cardiac interstitial space after intravenous administration of the enzyme.

It is also important to note that the assumption that infused adenosine deaminase is uniformly distributed in the interstitial fluid may not be correct and may, if anything, lead to an underestimation of the actual concentration of enzyme attained in the vicinity of coronary resistance vessels. Most tissues contain a protein whose only known function is to bind two adenosine deaminase catalytic subunits. Subunits thus bound retain catalytic activity (Dadonna and Kelley, 1980). Recent reports indicate that the protein is located on the cell surface and that, under normal conditions, most adenosine deaminase binding sites are unoccupied and therefore able to bind catalytic subunits (Andy and Kornfeld, 1982; Trotta, 1982). Accordingly, it is possible that most of the adenosine deaminase retained in the hearts of the animals reported in this study may have been bound to cell surfaces. However, it is unlikely that binding of adenosine deaminase to cells vitiates the use of this enzyme to probe the participation of adenosine in the autoregulatory response studied here. That a much lower concentration reduced canine myocardial reactive hyperemia (Saito et al., 1981) argues that large amounts observed in the present study were even more likely to be effective. Further, if the enzyme were distributed asymmetrically as a result of binding to cells bordering the interstitial space, one would expect the highest concentrations to be in the vascular wall,
The influence of adenosine deaminase infusion on regional myocardial lactate metabolism also was evaluated in this study. Under control conditions, net transmural myocardial uptake of lactate was present. This probably reflects the fact that endocardial hypoperfusion distal to the stenosis was not sufficiently intense to cause enough lactate production to raise AIV concentrations (which reflect tissue lactate levels in both endocardial and epicardial layers of the heart) above arterial levels. Since epicardial blood flow distal to the stenosis was not reduced, net lactate extraction would be expected in that layer. Furthermore, since blood flow distal to the stenosis did not change significantly vs. control 1, and since major external determinants of myocardial oxygen consumption also remained constant during adenosine deaminase infusion, one would anticipate that regional lactate metabolism also would remain unchanged. Nevertheless, in six of eight animals, regional extraction of lactate declined in response to enzyme infusion (Fig. 3). Moreover, in four of the animals, extraction declined to levels which generally are considered abnormal [i.e., < 10% extraction (Neill, 1968)]. Unfortunately, the reason for apparent impairment of myocardial aerobic metabolism in these animals during adenosine deaminase infusion cannot be defined from the data obtained. Additional studies in which a regional index of myocardial contractility is obtained might be helpful in resolving this issue, since it is theoretically possible that an increase in myocardial contractility associated with enzyme infusion worsened the balance between the myocardial oxygen supply and demand in some of the animals.

In conclusion, the results of this study demonstrate that adenosine is not required for maintenance of arteriolar vasodilation in the setting of a severe coronary artery stenosis. It is possible that adenosine may play a more important role in initiating coronary vasodilation in response to an acute decline in coronary perfusion pressure. However, once vasodilation has been initiated, adenosine either plays only a minor role in maintaining it or else its function is rapidly and almost fully replaced by some other mediators(s) when the nucleoside is removed from the interstitial space.

We wish to acknowledge the assistance of Christine Abatiello and Janice Bordieri in the preparation of the manuscript. The expert technical assistance of Thomas DeVone, Ronald D’Amico, Patricia Mastrofrancesco, James O’Neill, and Lorraine Schofield is also acknowledged.

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Received January 27, 1983; accepted for publication May 19, 1983.

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INDEX TERMS: Adenosine • Adenosine deaminase • Coronary artery stenosis • Coronary autoregulation
Role of adenosine in the maintenance of coronary vasodilation distal to a severe coronary artery stenosis. Observations in conscious domestic swine.

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doi: 10.1161/01.RES.53.1.42

*Circulation Research* is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7330. Online ISSN: 1524-4571

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