Role of Adenosine in Coronary Blood Flow Regulation after Reductions in Perfusion Pressure

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SUMMARY. We employed intracoronary infusion of adenosine deaminase to test the hypothesis that endogenous adenosine contributes to regulation of coronary blood flow following acute reductions in coronary artery pressure. In 16 closed-chest anesthetized dogs, we perfused the left circumflex coronary artery from a pressurized arterial reservoir and measured coronary blood flow following changes in perfusion pressure before and 10 minutes after the start of intracoronary adenosine deaminase, 5 U/min per kg body weight. Parallel studies showed that this dose of enzyme resulted in cardiac lymph adenosine deaminase concentrations of 3.2 ± 0.4 U/ml. Adenosine deaminase abolished the vasodilator response to intracoronary adenosine, 4 and 8 μg, but had no effect on the vasodilator response to intracoronary papaverine, 200 and 300 μg, demonstrating enzyme efficacy and specificity. Additional experiments demonstrated that adenosine deaminase reversibly attenuated myocardial reactive hyperemia following 5- and 10-second coronary occlusions by 30% (P < 0.05), evidence that the infused enzyme effectively degraded endogenous adenosine. However, adenosine deaminase did not alter the time course for coronary autoregulation or the steady state autoregulatory flow response over the pressure range between 125 and 75 mm Hg. Further, adenosine deaminase did not alter steady state coronary flow when perfusion pressure was reduced below the range for effective autoregulation (60–40 mm Hg). Such results show that adenosine is not essential for either coronary autoregulation or for the maintenance of coronary vasodilation when autoregulatory vasodilator reserve is expended.

(Circ Res 56: 517–524, 1985)

CORONARY autoregulation is the intrinsic ability of the heart to maintain its blood supply relatively constant in the face of fluctuations in perfusion pressure (Mosher et al., 1964; Cross, 1964). The mechanism underlying this fundamental adaptive flow response is unknown. According to the metabolic hypothesis, coronary autoregulation is initiated by flow-dependent changes in tissue levels of a metabolic substrate or a metabolite (Johnson, 1964; Rubio and Berne, 1975; Belloni, 1979; Feigl, 1983). Several studies have demonstrated increased adenosine production and release during conditions in which oxygen supply is reduced (Katori and Berne, 1966; Rubio and Berne, 1969; Rubio et al., 1974; Schrader et al., 1977). In one of these studies (Schrader et al., 1977), tissue adenosine content and adenosine release increased when perfusion pressure was lowered over the autoregulation range in isolated buffer-perfused guinea pig hearts. As the investigators point out, such a correlation only suggests, but does not prove, that adenosine is the primary determinant of changes in coronary resistance during autoregulation.

This study tests the hypothesis that endogenous adenosine is an essential mediator of coronary autoregulation. The hypothesis predicts that destroying interstitial adenosine should abolish autoregulation. Extending this inquiry to pressures below the autoregulation range tests a second hypothesis, namely, that endogenous adenosine mediates coronary flow only when autoregulatory vasodilator reserve is expended. In both sets of experiments, an intracoronary infusion of adenosine deaminase was used to destroy interstitial adenosine. Adenosine deaminase did not alter coronary pressure-flow relationships in either instance, new evidence that adenosine is not essential for coronary autoregulation.

Methods

Animal Preparation

Experiments were performed in 16 healthy closed-chest adult mongrel dogs (weight 25–35 kg). Animals were given morphine sulfate (3 mg/kg, sc) and anesthetized with α-chloralose (50 mg/kg, iv), with additional doses of chloralose given as needed throughout the experiment. The dogs were ventilated with oxygen-enriched room air by a positive pressure respirator to keep arterial oxygen tension between 100 and 125 mm Hg and carbon dioxide tension at 35 ± 5 mm Hg. Arterial pH was maintained at 7.40 ± 0.05 by an intravenous infusion of sodium bicarbonate (150 mm, 5 ml/kg per hr, iv) (Arfors et al., 1971).
The hematocrit averaged 38 ± 2%. Systemic pressure was measured in the aorta with a catheter passed retrograde from the femoral artery and a strain gauge pressure transducer. Rectal temperature was held at 37°C with heating pads. Heart rate was measured continuously with a cardiocotograph triggered from the electrocardiogram.

**Coronary Perfusion System**

Pressure in the circumflex artery was controlled by an extracorporeal perfusion circuit (Dole et al., 1981). The circumflex artery was perfused from a pressurized arterial reservoir through a metal cannula advanced through the right carotid artery into the ascending aorta and wedged in the proximal circumflex artery under pressure and flow monitoring as described by Smith et al. (1974). The reservoir was heated to maintain the temperature of the blood entering the cannula at 37°C. Coronary artery pressure was measured at the cannula tip through an external auxiliary tube opening at the distal end of the perfusion cannula. Blood passed from the reservoir through an electromagnetic flow probe before entering the coronary cannula. The pressure in the reservoir was controlled by means of two compressed air tanks connected in parallel, permitting arbitrary alterations in perfusion pressure. The seal between the cannula tip and circumflex artery was confirmed by observing the response of cannula tip pressure to inflow occlusion and by injecting saturated Evans blue solution into the coronary cannula at the end of the experiment as described previously (Dole et al., 1981). The dye injection also served to delineate the area of perfused myocardium which was removed and weighed in order to calculate coronary flow per unit of muscle mass. Blood coagulation in the extracorporeal perfusion circuit was prevented by infusion of sodium heparin (initial dose 500 U/kg, iv, then 250 U/kg, iv, every hour). The flow meter zero was determined by frequent coronary inflow occlusions. The flow probe was calibrated by timed blood volume collections after each experiment with blood from the experimental animal.

We have previously shown that this perfusion system does not alter systemic hemodynamics. Furthermore, coronary reactive hyperemia and autoregulation are not impaired with the cannula in place (Dole et al., 1981).

**Penetration of 13I-Adenosine Deaminase into Cardiac Intestitium**

Experiments in five dogs evaluated the concentration of 13I-adenosine deaminase in cardiac lymph during the intracoronary infusion of this enzyme. In these experiments, we used mongrel dogs anesthetized with sodium thiopental, 18 mg/kg, iv, and maintained in surgical anesthesia by ventilation with O2-enriched air containing 0.5% halothane. Surgical preparation entailed thoracotomy with thiamylal, 18 mg/kg, iv, and maintained in surgical anesthesia by ventilation with O2-enriched air containing 0.5% halothane. Surgical preparation entailed thoracotomy with thiamylal, 18 mg/kg, iv, and maintained in surgical anesthesia by ventilation with O2-enriched air containing 0.5% halothane. Surgical preparation entailed thoracotomy with thiamylal, 18 mg/kg, iv, and maintained in surgical anesthesia by ventilation with O2-enriched air containing 0.5% halothane.

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The experimental animal.

**Functional Activity of Adenosine Deaminase**

Calf intestinal adenosine deaminase, type I, was obtained from Sigma as a suspension in 3.2 M (NH4)2SO4, pH 6. The enzyme solution for intracoronary infusion consisted of 4000 U adenosine deaminase and 12 mmol Na2HPO4 in a total volume of 30 ml 0.14 M NaCl. The pH of the enzyme solution was 7.4. This solution was filtered through a 0.44-µm cellulose acetate membrane immediately prior to use. A solution containing the same proportions of (NH4)2SO4 and Na2HPO4 in 0.14 M NaCl was infused into the coronary circulation during all control observations.

Two tests assessed the functional enzymatic activity of the adenosine deaminase. First, we determined the vasodilator response to a 4- and 8-µg intracoronary bolus of adenosine before, during, and at 120 minutes after the end of the adenosine deaminase infusion. Such a test ensures that the adenosine deaminase is catalytically active in our preparation. Second, we measured reactive hyperemic flow responses following 2-, 5-, and 10-second coronary inflow occlusions before, during, and at 60 minutes after discontinuing the adenosine deaminase infusion. The order of occlusions was randomized and each observation was the average of two occlusions for each duration. Occlusions were separated by at least 3 minutes. Such a test documents the penetration of the enzyme into the cardiac interstitial space. To allow the enzyme time to penetrate into the cardiac interstitium, all experimental observations began 10 minutes after the start of the adenosine deaminase infusion.

We assessed the specificity of adenosine deaminase for adenosine in four animals by comparing the effects of intracoronary injections of adenosine and papaverine before and during adenosine deaminase infusion. The order of injections was randomized and each observation was the average of two injections for each duration. Occlusions were separated by at least 3 minutes. Such a test documents the penetration of the enzyme into the cardiac interstitial space. To allow the enzyme time to penetrate into the cardiac interstitium, all experimental observations began 10 minutes after the start of the adenosine deaminase infusion.

Then, we performed this experiment as described previously (Dole et al., 1981). The dye injection also served to delineate the area of perfused myocardium which was removed and weighed in order to calculate coronary flow per unit of muscle mass. Blood coagulation in the extracorporeal perfusion circuit was prevented by infusion of sodium heparin (initial dose 500 U/kg, iv, then 250 U/kg, iv, every hour). The flow meter zero was determined by frequent coronary inflow occlusions. The flow probe was calibrated by timed blood volume collections after each experiment with blood from the experimental animal.

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Since the assay of adenosine deaminase activity is carried out at 25°C, but the cardiac interstitium is at 37°C, we compared initial rates of deamination of 0.1 mM adenosine at these two temperatures. At 37°C, the catalytic activity of adenosine deaminase was 1.95 times higher than the activity at 25°C.

**Experimental Activity of Adenosine Deaminase**

The hematocrit averaged 38 ± 2%. Systemic pressure was measured in the aorta with a catheter passed retrograde from the femoral artery and a strain gauge pressure transducer. Rectal temperature was held at 37°C with heating pads. Heart rate was measured continuously with a cardiocotograph triggered from the electrocardiogram.

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from the dog's left common carotid artery. The circuit contained an in-line electromagnetic flow probe for measuring femoral blood flow.

**Experimental Protocol**

After the preparation had stabilized for 30-45 minutes, we recorded control heart rate, mean aortic pressure, coronary blood flow at a coronary perfusion pressure of 100 mm Hg, the coronary flow response to intracoronary adenosine, the coronary flow response to intracoronary papaverine, coronary reactive hyperemia after 2-, 5-, and 10-second inflow occlusions, and coronary artery pressure-flow curves. In nine animals, steady state coronary flow was determined at pressures of 125, 100, and 75 mm Hg. In seven of these animals, the dynamic flow response to a step change in pressure between 125 and 75 mm Hg was also recorded. In seven separate animals, steady state coronary artery pressure-flow relationships were obtained over the range of 100–40 mm Hg by decreasing pressure in 20 mm Hg steps. Flow was allowed to stabilize at least 2 minutes between each pressure change.

Following control measurements, adenosine deaminase was infused into the circumflex coronary bed at a rate sufficient to deliver 5 U/min per kg. At the 10th minute of enzyme infusion, we repeated all of the experimental observations. The adenosine deaminase infusion then was stopped and a final set of measurements of all experimental parameters was obtained 1 hour later. The responses to intracoronary injections of adenosine were also recorded at 120 minutes after infusion was discontinued.

At the completion of the experiment, Evans blue was injected into the perfusion cannula at the maximum coronary artery pressure used during the experiment, and the animal was killed by intravenous injection of saturated potassium chloride. The heart then was removed and the stained area weighed.

**Quantification of Autoregulation**

The degree of autoregulation was quantified by calculating the closed loop gain of the system (Norris et al., 1979) as

\[ G_A = 1 - \frac{\Delta F/F}{\Delta P/P} \]

where \( F \) is the steady state flow at pressure \( P \), and \( \Delta F \) is the change in flow for a given change in pressure \( \Delta P \). Values of \( G_A \) greater than zero indicate the degree of autoregulation with a maximum value of 1. Values of \( G_A \) ≤ 0 indicate a lack of autoregulation, i.e., a passive vascular bed.

We characterized the time course of the autoregulatory response by measuring \( t_{50} \), the time for mean coronary flow to return 50% of the way to control after a step change in pressure, and also by measuring the time for flow to reach a steady state.

**Data Analysis**

Hemodynamic data before and after adenosine deaminase were compared by Student's \( t \)-test for paired observations. Differences in pressure-flow data, responses to drugs, and reactive hyperemic flow responses before, during, and after adenosine deaminase were assessed by analysis of variance and Tukey's test for multiple comparisons (Snedecor and Cochran, 1980).

Reactive hyperemic flow—defined as the volume of flow greater than control flow rate—and peak reactive hyperemic flow were determined, and the data from two occlusions for each duration were averaged and expressed as a percent change from the control response.

Differences between means were considered to be statistically significant when \( P \) was less than 0.05. All data are expressed as mean ± SEM.

**Results**

Control hemodynamic parameters for all 16 animals at a circumflex perfusion pressure of 100 mm Hg were: heart rate 132 ± 7 beats/min, mean aortic pressure 115 ± 6 mm Hg, and mean coronary flow 78 ± 3 ml/min per 100 g. The corresponding values during infusion of adenosine deaminase were 128 ± 8 beats/min, 116 ± 6 mm Hg, and 77 ± 3 ml/min per 100 g; at 60 minutes after discontinuing adenosine deaminase, they were 130 ± 8 beats/min, 113 ± 8 mm Hg, and 80 ± 4 ml/min per 100 g. These changes are not significant.

**Effects of Adenosine Deaminase on Coronary Autoregulation**

Figure 1 shows the effects of adenosine deaminase on steady state coronary flow over the pressure range of 125 to 75 mm Hg in nine animals. Control flow averaged 80 ml/min per 100 g at a pressure of 100 mm Hg and was not altered by adenosine deaminase. The autoregulatory gain for a step reduction in pressure from 125 to 100 mm Hg averaged 0.53 ± 0.04 during the control period prior to adenosine deaminase and 0.50 ± 0.05 during adenosine deaminase infusion. These values were not significantly different. The control gain for a reduction in pressure from 100 to 75 mm Hg averaged 0.56 ± 0.06 and was not altered during adenosine deaminase infusion (\( G_A = 0.58 ± 0.06 \)).

Figure 2 shows the effects of adenosine deaminase on the coronary flow response to a step change in pressure between 125 and 75 mm Hg. Following a reduction in pressure, coronary flow initially decreased and then returned toward control, reaching...
a new steady state within 36 seconds. Following an increase in pressure, coronary flow rose transiently and then declined, reaching a new steady state within 25 seconds. Table 1 summarizes the effects of adenosine deaminase on the time course of coronary autoregulation in seven animals. Adenosine deaminase did not affect the dynamic flow response to a pressure change in either direction.

Effects of Adenosine Deaminase on Coronary Pressure Flow Relations below the Autoregulatory Range

Figure 3 shows the effects of adenosine deaminase on steady state coronary flow responses to 20 mm Hg reductions in pressure over the range between 100 and 40 mm Hg in seven animals. Whereas there was no difference in control flow when pressure was reduced from 100 to 80 mm Hg, pressure reductions below 80 mm Hg resulted in a progressive decrease in flow. Adenosine deaminase did not alter the flow response to pressure change even at pressures below the autoregulation range.

Figure 4 plots the degree of autoregulation expressed as the gain, \( G_A \), for each pressure step before and during adenosine deaminase infusion. There was a decrease in the degree of autoregulation when initial pressure was lowered from 100 to 80 mm Hg. Between 60 and 40 mm Hg, autoregulation was totally abolished, as indicated by the negative gain. Despite the progressive reduction in the degree of autoregulation, adenosine deaminase had no effect on the flow response to pressure change.

Cardiac Lymph \(^{13}I\)-Adenosine Deaminase

Lymph flow rate varied between about 6 and 20 \( \mu l/min \) as judged by the time necessary to fill a 50-\( \mu l \) capillary pipette. Accordingly, the number of lymph samples collected during the 20 minutes of \(^{13}I\)-adenosine deaminase infusion was three in 2 dogs, five in 2 dogs, and eight in 1 dog. As shown in Figure 5, lymph \(^{13}I\)-adenosine deaminase activity rose throughout the period of infusion, reaching an average value of \( 3.2 \pm 0.4 \) U/ml (range 2.2-4.5 U/ml). The time course of lymph \(^{13}I\)-adenosine deaminase activity showed an initial rapid rise followed by an asymptotic approach to the maximum value.

Table 1

<table>
<thead>
<tr>
<th>( \Delta P ) (mm Hg): 125 to 75</th>
<th>( \Delta P ) (mm Hg): 75 to 125</th>
</tr>
</thead>
<tbody>
<tr>
<td>( t_{1/2} ) (sec)</td>
<td>Duration (sec)</td>
</tr>
<tr>
<td>Control</td>
<td>2.7 ± 0.3</td>
</tr>
<tr>
<td>ADA</td>
<td>2.6 ± 0.2</td>
</tr>
</tbody>
</table>

Results are expressed as mean ± SEM, \( n = 7 \). Abbreviations: \( \Delta P \), step change in pressure; ADA, adenosine deaminase; \( t_{1/2} \), time for flow to return 50% of the way to control after \( \Delta P \).
value. Thus, by the 10th minute of enzyme infusion, lymph $^{131}$I-adenosine deaminase concentration averaged 84% of maximum (range 74–95%). Lymph $^{131}$I activity declined rapidly after the end of the enzyme infusion, but levels were still appreciable, approximately 0.5 U/ml, 20 minutes later (data not shown).

**Functional Activity of Adenosine Deaminase**

Figure 6 (panels A and B) summarizes the effects of adenosine and papaverine in the coronary bed before, during, and after intracoronary administration of adenosine deaminase. During control conditions, adenosine (4 and 8 μg) increased coronary flow by 124 ± 9% and 200 ± 24%, respectively. Adenosine deaminase totally abolished the vasodilator response to intracoronary adenosine during the enzyme infusion and for as long as 120 minutes after discontinuing the enzyme infusion. Papaverine in doses of 200 and 300 μg increased control coronary flow by 90 ± 15% and 120 ± 22%, respectively. The coronary vasodilator response to papaverine was unchanged during infusion of adenosine deaminase, demonstrating enzyme specificity. The intra-arterial injection of 10- and 20-μg doses of adenosine into the femoral bed increased mean femoral flow by 79 ± 14% and 118 ± 14%, respectively. In contrast to responses in the coronary bed, the vasodilator response to adenosine in the femoral bed (Fig. 6, panel C) was attenuated only during the enzyme infusion, and returned to control levels 120 minutes after the enzyme infusion was stopped. Thus, the prolonged inhibition of adenosine-induced coronary vasodilation was not due to adenosine deaminase still circulating in the blood, but rather reflected the action of residual enzyme in the heart.

The effects of adenosine deaminase on coronary reactive hyperemia are summarized in Table 2. During control conditions, the ratio of peak reactive hyperemic flow to basal flow averaged 1.5 ± 0.1 for 2-second occlusions, 2.4 ± 0.1 for 5-second occlusions, and 3.3 ± 0.3 for 10-second occlusions. Peak reactive hyperemic flow was modestly reduced (10%, $P < 0.05$) only with 5-second occlusions. Adenosine deaminase reduced reactive hyperemic flow by approximately 30% ($P < 0.05$) following 5- and 10-second coronary occlusions and by 8% ($P < 0.05$) following 2-second occlusions.

**Figure 4.** Effects of adenosine deaminase (ADA) on the autoregulatory gain $G_a$ for 20 mm Hg reductions in perfusion pressure over the range between 100 and 40 mm Hg. Closed bars indicate pre-ADA control values. Open bars indicate values during ADA infusion. ADA had no effect on $G_a$ despite a progressive reduction in the degree of autoregulation at the lower initial pressures. Values are mean ± SEM, n = 7.

**Figure 5.** Time course of $^{131}$I-adenosine deaminase ($^{131}$I-ADA) activity in the cardiac lymph. Symbols represent data from individual dogs.
we believe that our measurements of lymph aden-
more nearly represents the true value. Nevertheless,
from regions not receiving enzyme directly, and thus
lymph is unlikely to be diluted by lymph draining
Unlike lymph sampled at more remote sites, the
perfusion field receiving 131 I-adenosine deaminase.
probe the composition of the interstitial space of the
phatic channel probably is the most direct way to

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**Table 2**

<table>
<thead>
<tr>
<th>Duration of occlusion (sec)</th>
<th>Peak RH flow rate (Δ% from control)</th>
<th>RH flow (Δ% from control)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>During ADA (60 min)</td>
<td>After ADA</td>
</tr>
<tr>
<td>2</td>
<td>-2 ± 2</td>
<td>0 ± 1</td>
</tr>
<tr>
<td>5</td>
<td>-10 ± 3*</td>
<td>-4 ± 3t</td>
</tr>
<tr>
<td>10</td>
<td>-2 ± 2</td>
<td>-2 ± 2</td>
</tr>
</tbody>
</table>

* Results are expressed as mean ± SEM, n = 9. Abbreviations: RH, reactive hyperemia; ADA, adenosine deaminase.
*† Significantly different from zero, P < 0.05.
*† Significantly different from 2-second occlusion during ADA, P < 0.05.
*† Significantly different from corresponding values during ADA, P < 0.05.

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**Discussion**

The major finding in this study is that intracorono-
ary infusion of adenosine deaminase at a concen-
tration sufficient to attenuate myocardial reactive
hyperemia did not affect the coronary artery pressure-
flow relationship when pressure was reduced over
the autoregulation range or to levels even below
this range.

Additional experiments demonstrated that aden-
osine deaminase was efficacious, selective, and had
penetrated into the cardiac interstitial space. An in-
tracoronary infusion of the enzyme abolished the
coronary vasodilator response to adenosine but did
not alter the response to papaverine, evidence of
enzyme efficacy and specificity. Further, a compar-
ison of the vasodilator responses to exogenous aden-
osine in the coronary and femoral beds 2 hours after
the intracoronary adenosine deaminase infusion had
been discontinued showed that the coronary re-
sponse was still markedly attenuated, whereas the
femoral response was fully restored. This finding
indicated that suppression of the coronary response
resulted from enzyme retained in the heart rather
than from enzyme in the blood. The observation
that adenosine deaminase attenuated myocardial
reactive hyperemia following 5- and 10-second oc-
closures by 30%, provides functional evidence that
infused enzyme penetrated into the cardiac intersti-
tium. Finally, the studies of 131 I-adenosine deami-
nase confirm quantitatively that during intracoro-
nary adenosine deaminase infusion, substantial
amounts of the enzyme penetrate into the cardiac
interstitium.

The collection of lymph from an epicardial lym-
phatic channel probably is the most direct way to
probe the composition of the interstitial space of the
perfusion field receiving 131 I-adenosine deaminase.
Unlike lymph sampled at more remote sites, the
lymph is unlikely to be diluted by lymph draining
from regions not receiving enzyme directly, and thus
more nearly represents the true value. Nevertheless,
we believe that our measurements of lymph aden-
osine deaminase concentration are a minimum esti-
mate of the catalytic activity of the enzyme in the
interstitial space. Owing to the transit time of 131 I-
adenosine deaminase from the interstitium to the
sampling site and the averaging effect of sample
collections lasting several minutes, the concentration
in lymph at any moment will be less than that in
the interstitium. Additionally, lymph adenosine de-
aminase activity does not reflect the extent to which
enzyme binds to adenosine deaminase binding pro-

tins (Daddona and Kelley, 1977; Schrader and
Stacy, 1977) on the surface (Kornfield, 1983) of the
cells which constitute the boundary of the interstitial
space. Adenosine deaminase thus bound retains cat-
alytic activity and augments that of enzyme in so-
lution. Finally adenosine deaminase activity is, by
convention, measured at 25°C. The catalytic activity
in interstitium at 37°C was 1.95 times higher.

The use of adenosine deaminase to probe the
participation of adenosine in coronary autoregula-
tion raises a critical question: Does enough enzyme
penetrate into the interstitial space to destroy aden-
osine as fast as it is formed? We do not know of a
method for directly measuring the rate of release of
adenosine into the cardiac interstitium. However, it
is possible to calculate how much adenosine pro-
duction would have to increase in order to maintain
adenosine concentrations constant in the presence
of adenosine deaminase. Such calculations make
allowances for the temperature differential between
the cardiac interstitium and the in vitro assay, as-
sume that the interstitial space volume is 0.217
ml/g (Frank and Langer, 1974), and accept the basal
interstitial adenosine concentration of 0.2 μM (Ols-
son et al., 1982). To maintain the adenosine concen-
tration of 0.2 μM in the presence of 3.2 U adenosine
dehydrogenase per ml, the average of our measurements,
adenosine production would have to increase sub-
stantially by 9.3 nmol/min per g of left ventricle.
Because, under these conditions, the enzyme is op-
erating at a substrate concentration of only 0.7% of
Km, any increase in the rate of adenosine production
will elicit a proportional increase in catalytic activity.
Such that adenosine concentration will remain es-
tentially constant. The catalytic activity of the en-
zyme will not begin to limit the rate of degradation
until the substrate concentration is well above the
Km of the adenosine deaminase, 29 μM (Rockwell
and Maquire, 1970). We know of no evidence that
cardiac interstitial adenosine concentrations ever at-
tain such high levels. Accordingly, the finding that
adenosine deaminase affected neither the static nor
the dynamic autoregulatory flow response implies
that adenosine is not essential for coronary autoreg-
ulatation. Furthermore, since adenosine deaminase
did not alter the coronary pressure-flow relationship
when pressure was reduced below the autoregula-
tory range, it seems unlikely that adenosine is essen-
tial for the maintenance of coronary vasodilation at
these lower perfusion pressures.
In these experiments, circumflex artery pressure was regulated independently of aortic pressure; thus, pressure gradients could have resulted in intracoronary collateral flow. Although early work by Driscoll et al. (1964) demonstrated that the pattern of coronary flow in response to changes in perfusion pressure was not altered by sizeable pressure gradients between major vessels, more recent data suggest that collateral flow may influence the steady state pressure-flow relationship in the circumflex artery at low perfusion pressures (Messina et al., 1983). Although coronary flow may have been underestimated at the lower perfusion pressures in the present study, the gradient for collateral flow was the same before and during adenosine deaminase infusion. Thus, the failure of adenosine deaminase to alter coronary flow responses to pressure change cannot be attributed to differences in collateral flow.

Our experiments in blood perfused in situ dog hearts lead to a conclusion diametrically opposite to that reached by Schrader et al. (1977) who studied isolated, buffer-perfused guinea pig hearts. Differences in the characteristics of autoregulation, perhaps related to the preparations or to the species, and also a fundamental difference between the experimental designs, may explain the discordant conclusions. Coronary autoregulation in the guinea pig preparation occurred between pressures of 60 and 20 cm H2O (44–15 mm Hg), which is below the pressure range for autoregulation which we and others have observed in the blood-perfused dog heart (Mosher et al., 1964; Cross, 1964; Guyton et al., 1977; Rouleau et al., 1979; Dole et al., 1981, 1982).

An inverse correlation between adenosine release rate and coronary perfusion pressure in the guinea pig was the major element of support for the hypothesis that adenosine mediates coronary autoregulation. In our study, destruction of cardiac interstitial adenosine provides a direct test of the role of this nucleoside in coronary autoregulation.

Our conclusion that adenosine is not essential for coronary vasodilation following reductions in perfusion pressure is consistent with recent work by Gewirtz et al. (1983) in a closed chest conscious pig model. In that study an intracoronary infusion of adenosine deaminase had no effect on regional myocardial perfusion pressure, which is the resultant of several, interacting mediators. In such a case, blocking or destroying one mediator could elicit a compensatory increase in the contribution of another such that the response is unaltered. Accordingly, we recognize the possibility that coronary autoregulation or myocardial reactive hyperemia may result from the concerted action of adenosine and other, as yet unidentified, vasodilator mechanisms which are capable of compensating when adenosine is destroyed.

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