Intracoronary Adenosine Deaminase Reduces Canine Myocardial Reactive Hyperemia

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SUMMARY We employed intracoronary infusions of calf intestine adenosine deaminase (ADA) to test the hypothesis that adenosine regulates coronary blood flow during myocardial reactive hyperemia (RH). Infusions of 4.5 U ADA/min per kg body weight into the left circumflex coronary artery of 10 open-chest dogs reversibly reduced repayment of flow debt by 30-39% (P < 0.05) following 5-, 10-, 15-, 20-, and 30-second coronary occlusions, the percentage reduction being independent of occlusion length. ADA reduced peak RH flow rate (17%, P < 0.05) only after 5-second occlusions. Intracoronary infusions of [14C]ADA in seven dogs produced interstitial ADA concentrations between 1.2 and 13.1 U/ml in perfused myocardium and, in five of these dogs, 11I activity in the cardiac node was 1.8-35 times that of contiguous mediastinal tissue. Theophylline, a specific adenosine antagonist, reduced repayment of flow debt by 27-38% (P < 0.02) in eight dogs, an effect similar to that of ADA. In six other dogs, ADA plus theophylline did not reduce RH flow debt repayment below that produced by ADA alone. This experiment confirms the contribution of adenosine to myocardial RH but shows that this nucleoside accounts for but a third of volume flow. Other, as yet unidentified, factors are collectively more important. Circ Res 49: 1383-1387, 1981

TWO lines of evidence support the hypothesis that adenosine regulates coronary blood flow during myocardial reactive hyperemia: (1) adenosine appears in coronary venous blood during reactive hyperemia in quantities paralleling the duration of the preceding ischemic interval (Rubio et al., 1969), and (2) coronary flow rate changes concordantly during reactive hyperemia with changes in myocardial adenosine content (Olsson et al., 1978).

However, other evidence challenges this view. Methylxanthines strongly antagonize the coronary effects of exogenous adenosine (Afonso, 1970), yet only modestly reduce the reactive hyperemic response (Bittar and Pauly, 1971; Juhran et al., 1971; Curnish et al., 1972; Eikens and Wilcken, 1977). Coronary occlusions of one heart beat or less have no detectable effect on myocardial metabolism, yet elicit hyperemic responses (Eikens and Wilcken, 1974; Schwartz et al., 1980). Other vasodilatory substances such as K+ and CO2 (or H+) accumulate in ischemic hearts and also could participate in reactive hyperemia (Raberger et al., 1975; Case, 1976; Murray and Sparks, 1978).

This study estimates the contribution of adenosine to canine myocardial reactive hyperemia by employing adenosine deaminase (ADA) to destroy adenosine in the cardiac interstitium. This enzyme reduces but does not abolish this response, confirming that adenosine contributes to, but is not solely responsible for, post-ischemic coronary vasodilation.

Methods

Healthy mongrel dogs weighing 15.5-30 kg were anesthetized by sodium pentobarbital (30 mg/kg, iv) and artificially ventilated at rates maintaining arterial blood Po2 and Pco2 in the normal range (Feigl and D'Alecy, 1971). Catheters in the right femoral vein and aortic root served for drug infusions and monitoring coronary perfusion pressure, respectively. Thoracotomy through the left 5th interspace and construction of a pericardial cradle exposed the heart for implantation of an electromagnetic flowprobe, pneumatic occlusive cuff, and plastic intracoronary catheter (Elliott et al., 1968) near the origin of the left circumflex coronary artery.

Sigma furnished type I calf intestine ADA as a suspension in 3.2 M (NH4)2SO4, pH 6.0. Analytical gel chromatography on an 0.9 x 50 cm column of Sephacryl S-200 equilibrated and eluted with 0.1 mM KH2PO4, pH 7.0, in 0.2 M NaCl showed that 90% of the deaminase activity had an inclusion volume equivalent to that of free catalytic subunits (Stokes radius 23 Å) and the remaining 10% emerged in the void volume. ADA for radiiodination was dialyzed against 0.05 M KH2PO4, pH 7.0, and was labeled by the chloramine T technique (Hunter and Greenwood, 1973). Chromatography of the reaction mix-
tute on an 0.7 x 35 cm column of Sephadex G-10 equilibrated and eluted with 0.1 M KHPO$_4$, pH 7.0, in 0.2 M NaCl completely separated radiiodinated enzyme from free $^{131}$I. All $^{131}$I activity in the enzyme peak was precipitable by trichloroacetic acid. Pharmacia supplied Sephacryl and Sephadex gels and New England Nuclear supplied Na $^{131}$I.

ADA for intracoronary infusion was dissolved in 0.14 M NaCl containing 0.012 M Tris-HCl, pH 7.5 ("Tris-NaCl"), to neutralize the (NH$_4$)$_2$SO$_4$. The extent of dilution and rate of infusion were adjusted to deliver 4.5 U ADA/min per kg body weight at infusion rates <1 ml/min. Theophylline for intracoronary infusion was dissolved in redistilled methyl sulfoxide and diluted in 0.15 M NaCl to a final concentration of 5 mM.

**Effect of ADA on Reactive Hyperemia**

Each experiment consisted of three sets of observations of the reactive hyperemia response to coronary occlusions lasting 5, 10, 15, 20, or 30 seconds. A set of observations included two occlusions of each length, i.e., a total of 10 hyperemic responses. The order of occlusions was independently randomized for each set by drawing lots. The first set of observations made during an intracoronary infusion of Tris-NaCl served as a control. The second set of observations consisted of occlusions made during ADA infusion. To afford the enzyme time to penetrate into the cardiac interstitium, this series of occlusions began 5 minutes after the start of ADA infusion. The third set of observations began approximately 10 minutes after the end of ADA infusion and during the administration of Tris-NaCl. When the third set of observations was complete, an intracoronary injection of Evans Blue marked the myocardium under study and the dog was killed by an intracardiac injection of KCl. The weight of dye-stained myocardium served as the basis for expressing coronary flow rate.

**Validation of Technique**

Three experiments assessed how completely intracoronary ADA infusions destroyed adenosine in the interstitial compartment. The first examined the effect of doubling the rate of ADA infusion on the reactive hyperemia response. Three randomized series of 10- and 20-second coronary occlusions during ADA infusion served as controls. The rate of ADA administration was doubled to 9 U/min per kg, and 5 minutes later an independently randomized series of occlusions commenced.

The second experiment compared the effect of ADA alone with that of ADA plus theophylline blockade of coronary artery adenosine receptors on the intensity of the hyperemic response to 5- and 20-second coronary occlusions. For these experiments, a Y tube connected the coronary infusion catheter to one syringe containing ADA and to a second syringe containing 5 mM theophylline. The first experimental period began with observations made 5 minutes after the start of ADA administration. When this series of randomized triplicate coronary occlusions was complete, theophylline infusion began at a rate of 0.206 ml/min as ADA infusion continued. An independently randomized set of coronary occlusions began 3 minutes later.

Last, regional accumulation of $^{[131]}$IADA during infusion of this enzyme and its appearance in the cardiac lymphatic drainage documented the penetration of this enzyme into the cardiac interstitium. The transepicardial insertion of a coronary infusion catheter into either the left anterior descending or left circumflex coronary artery prepared open-chest dogs for this experiment. Two precautions minimized disruption of the periarterial lymphatics draining the perfused region. An intramyocardial injection of 0.1 ml Evans Blue marked these channels so that catheter placement avoided them. Also, the catheter was inserted at a site devoid of subepicardial fat, thus avoiding dissection of the artery.

A solution of $^{[131]}$IADA then was infused for 20 minutes at a rate of 4.5 U ADA/min per kg body weight. During the 10th minute of this infusion, Evans Blue was injected into myocardium remote from the perfused zone in order to stain the cardiac node. At the end of the enzyme infusion, Evans Blue was injected into the coronary catheter and, after approximately 15 seconds, (>2 coronary transit times) the dog was killed by intracardiac KCl injection. The cardiac node and a piece of adjacent mediastinum were excised and weighed. Duplicate samples of myocardium (~0.5 g) from the $^{[131]}$IADA-perfused LV, from LV outside the perfused zone, and from the center of the RV free wall were also excised and weighed. These samples plus duplicate samples of arterial blood and aliquots of infusate were then counted for $^{131}$I activity.

**Effect of Theophylline on Reactive Hyperemia**

Experiments assessing the effect of theophylline on reactive hyperemia employed an animal preparation and experimental protocol similar to those of the ADA experiments. Theophylline dissolved in methyl sulfoxide was diluted with 0.14 M NaCl to a concentration of 5 mM for intracoronary infusion at 0.38 ml/min. Solutions of NaCl containing identical concentrations of methyl sulfoxide (0.5-1%, vol/vol) were infused during control observations. The coronary flow response to bolus injections of 100 μg adenosine into the left atrium prior to and during theophylline infusions assessed the extent to which this alkylxanthine antagonized the coronary vasoreactivity of adenosine.

**Data Analysis**

Peak reactive hyperemia flow rate was read directly from the oscillographic record and was expressed as a percentage of control. The volume of
reactive hyperemia flow, defined as flow greater than the control rate, was estimated by tracing that portion of the flow curve on paper and cutting out and weighing to the nearest 0.1 mg. Reference to the weight of a piece of paper of known area (equivalent to volume flow) yielded an estimate of reactive hyperemia flow. Repayment of flow debt was calculated as the quotient of reactive hyperemia flow divided by the product of control flow rate multiplied by the duration of occlusion.

Estimation of the concentration of $[^{131}I]$ADA in the interstitial compartment, $[ADA]_{ISF}$, employed the formula:

$$[ADA]_{ISF} = \frac{([^{131}I]_{PLV} - 0.105 \times [^{131}I]_{blood})}{(S.A.[^{131}I]ADA) \times 0.217 \text{ ml ISF/g LV}},$$

where the subscripts PLV and blood refer to $[^{131}I]$ activity in perfused myocardium and blood (cpm/g and cpm/ml, respectively) and S.A. $[^{131}I]$ADA is the specific activity of the radioiodinated enzyme (cpm/U). The coefficients 0.105 and 0.217 are the volumes of the vascular lumen and the interstitial space (sucrose space minus the space accounted for by the vascular wall and lumen; Frank and Langer, 1974). The rate of $^{131}I$ activity in the cardiac node divided by that in adjacent mediastinal tissue (both in cpm/g) assessed the presence of $[^{131}I]$ADA in cardiac lymph.

Data from each experimental period and each animal are the averages of the two or three reactive hyperemia responses. Grouped data are expressed as mean ± SEM. Student’s t-test for paired observations was used to assess the differences between responses during adenosine deaminase or theophylline infusion and the pretreatment controls. Observations made after adenosine deaminase infusions were used only to judge reversibility of the enzyme effect and displayed large variances. This suggests important animal-to-animal differences in the rate at which the enzyme was cleared, and so these data were not formally analyzed. Analysis of variance assessed the data on myocardial distribution of $[^{131}I]$ADA and also the stability of heart rate, coronary flow, and perfusion pressure during the course of an experiment.

**Results**

Ten dogs were used to assess the effects of ADA on reactive hyperemia. Control heart rates, coronary flow rates and blood pressure averaged, respectively, 158 ± 9 beats/min, 86 ± 4 ml/min per 100 g, and 117 ± 6 mm Hg. These variables did not change significantly during the course of an experiment. ADA infusion did not significantly change coronary flow rate, which averaged 94 ± 5% of control during enzyme infusion and 99 ± 3% of control after infusion.

Figure 1 depicts the effect of ADA on reactive hyperemia in one dog and Table 1 summarizes the results from all 10 dogs. ADA infusion significantly reduced peak reactive hyperemia flow rate after 5-second occlusions (-17%, $P < 0.02$) but did not affect this index of vasodilation following longer occlusions. Irrespective of the duration of coronary occlusion, the enzyme consistently and significantly reduced the volume of reactive hyperemia by about one-third. The intensity of reactive hyperemia returned toward control levels after the enzyme infusion was stopped, evidence that the effect of the enzyme is reversible.

Experiments in two dogs assessed the effect of doubling the rate of ADA infusion on the volume of reactive hyperemia flow following 10- or 20-second coronary occlusions. These observations consisted of three trials at each occlusion length and ADA infusion rate in each dog. Flow debt repayment following 10-second occlusions averaged 218 ± 8 and 229 ± 10% during ADA infusion at 4.5 U ADA/min per kg and 222 ± 14 and 218 ± 16% at 9 U ADA/min per kg, respectively. Repayments following 20-second occlusions averaged 275 ± 9 and 294 ± 6% at the lower and 291 ± 9 and 292 ± 6% at the
higher ADA infusion rate. Thus, ADA concentration had no effect on flow debt repayment at either occlusion length.

The intracoronary infusion of ADA plus theophylline did not further diminish the intensity of reactive hyperemia below the level produced by ADA alone (Table 2). Indeed, during the combined administration of ADA plus theophylline, peak flow after 5 seconds of coronary occlusion averaged 17% higher (P < 0.01) and flow debt repayment averaged 15% higher (P > 0.2) than during ADA alone. The corresponding indices of vasodilation following 20-second coronary occlusions changed by averages of -1 and -5% during ADA plus theophylline. Between the control and experimental periods, blood pressure remained at 84±8 to 85±8 beats/min.

Table 3 summarizes the results of the experiments assessing the penetration of [131I]ADA into the cardiac interstitium. [131I] activities in RV and in LV remote from the perfused zone were significantly lower than [131I] activity in the perfused zone (P < 0.01). The calculated concentration of [131I]ADA in the interstitial space ranged between 1.2 and 13.1 U/ml, with a median value of 3.2 U/ml. The cardiac node of dog 2 was not found and that of dog 4 was not dye-stained. The [131I] activity in the cardiac nodes of the other five dogs ranged between 1.8 and 35 times that of adjacent mediastinal tissue.

Intracoronary theophylline infusions yielding concentrations between 0.04 and 0.07 mM in coronary plasma water reduced the coronary flow responses to intra-atrial injections of 100 μg adenosine by 83 ± 7%, yet reduced the volume of reactive hyperemia flow by only 27–36% (Table 4). Theophylline had a discernible effect on peak reactive hyperemia flow only after 5 seconds of ischemia. Persistent increases in control coronary flow rate consequent to cardio-acceleration precluded assessing the effects of higher theophylline concentrations.

### Discussion

These experiments employing intracoronary infusions of ADA catalytic subunits to destroy adenosine released into the cardiac interstitial space show that adenosine accounts for only a part of the coronary vasodilatation of myocardial reactive hyperemia. Thus, these observations reconcile evidence implicating adenosine in reactive hyperemia (Rubio et al., 1969; Olsson et al., 1978) with the contradictory evidence that aminophylline antagonizes the coronary vasoreactivity of adenosine but has either no effect or incompletely blocks reactive hyperemia (Bittar and Pauly, 1971; Juhran et al., 1971; Curnish et al., 1972; Eikens and Wilcken, 1977). Our evidence indicates that adenosine indeed contributes to myocardial reactive hyperemia but that other factors, perhaps physical as well as chemical, collectively exert a more important vasodilatory effect. This study does not identify these other factors.

Four lines of evidence exclude the possibility that the failure of ADA to block reactive hyperemia

### Table 2 Effect of ADA vs. ADA Plus Theophylline on Reactive Hyperemia

<table>
<thead>
<tr>
<th>Dog</th>
<th>ADA</th>
<th>ADA + The</th>
<th>ADA</th>
<th>ADA + The</th>
<th>ADA</th>
<th>ADA + The</th>
<th>ADA</th>
<th>ADA + The</th>
</tr>
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<td>319</td>
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<td>224</td>
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<td>206</td>
<td>331</td>
<td>315</td>
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<td>214</td>
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<tr>
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<td>197</td>
<td>226</td>
<td>511</td>
<td>429</td>
<td>214</td>
<td>300</td>
<td>238</td>
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<td>215</td>
<td>310</td>
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<td>186</td>
<td>218</td>
<td>310</td>
<td>275</td>
<td>264</td>
<td>289</td>
<td>261</td>
<td>224</td>
</tr>
<tr>
<td>Mean</td>
<td>208</td>
<td>249*</td>
<td>368</td>
<td>363</td>
<td>206</td>
<td>237</td>
<td>321</td>
<td>306</td>
</tr>
</tbody>
</table>

**Abbreviations:** ADA, adenosine deaminase; RH, reactive hyperemia; The, theophylline.

* Significantly different from control, 0.01 > P > 0.001.
completely is due to incomplete degradation of adenosine. First, doubling the rate of ADA infusion did not further reduce the intensity of reactive hyperemia. Second, superimposing theophylline blockade of coronary adenosine receptors atop ADA did not further reduce the intensity of reactive hyperemia below that produced by ADA alone. Third, the concentrations of [131I]ADA achieved during intracoronary infusion of this enzyme are sufficient to deaminate the adenosine formed during the ischemic interval. The lowest concentration of [131I]ADA in the cardiac interstitium of any dog was 1.2 U/ml. By definition, 1 U of ADA will deaminate 1 /nmol adenosine/min at 25°C and a rate at which it is cleared via the lymphatic drainage is displayed only a slight tendency to cluster around a median value of 3.2 U/ml. We believe this broad range is the resultant of the rate at which the enzyme penetrates into the interstitium and the rate at which it is cleared via the lymphatic drainage. The rate of penetration of a molecule as large as ADA is relatively slow and probably does not vary greatly from one animal to another. Lymmp flow rates are also low, but do vary widely, between 0.068 and 0.30 mg/min per g heart weight in one study (Drinker et al., 1940) and in another between 0.45 and 5.6 ml/hr (Michael et al., 1979). Although we did not measure lymph flow rates, we believe that such a wide range in the rates of enzyme clearance is a plausible explanation for the variability of interstitial [131I]ADA levels. A similar variability in the [131I]ADA activity in the cardiac nodes of these animals and the very variable rates which the enzyme was diluted in coronary plasmas could also make a smaller contribution to this variability.

In these experiments, intracoronary ADA infusions had a negligible effect on coronary resistance. This observation independently supports other ev

### Table 3 Penetration of [131I]ADA into Cardiac Interstitium

<table>
<thead>
<tr>
<th>Dog</th>
<th>Perf. LV</th>
<th>Rem LV</th>
<th>RV</th>
<th>Med.</th>
<th>CN</th>
<th>Blood</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3175</td>
<td>9041</td>
<td>*</td>
<td>602</td>
<td>487</td>
<td>864</td>
</tr>
<tr>
<td>2</td>
<td>3175</td>
<td>1299</td>
<td>417</td>
<td>430</td>
<td>1</td>
<td>4200</td>
</tr>
<tr>
<td>3</td>
<td>3175</td>
<td>1307</td>
<td>471</td>
<td>426</td>
<td>522</td>
<td>1686</td>
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<td>17570</td>
<td>477</td>
<td>6303</td>
<td>343</td>
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</tr>
<tr>
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<td>20792</td>
<td>3792</td>
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<td>268</td>
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<td>1327</td>
<td>1371</td>
<td>625</td>
<td>1818</td>
</tr>
<tr>
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<td>16744</td>
<td>12402</td>
<td>2568</td>
<td>2330</td>
<td>524</td>
<td>2732</td>
</tr>
</tbody>
</table>

Abbreviations: Sp. act., specific activity; Perf. LV, perfused left ventricle; Rem LV, remote left ventricle; RV, right ventricle; Med., mediastinum; CN, cardiac node; and ISF, interstitial fluid.

* Remote LV not identified with certainty.
† Cardiac node not found.

### Table 4 Effect of Intracoronary Theophylline on Myocardial Reactive Hyperemia

<table>
<thead>
<tr>
<th>Duration of occlusion (sec)</th>
<th>Peak RH flow (% control)</th>
<th>Repayment of flow debt (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Theophylline</td>
</tr>
<tr>
<td>5</td>
<td>105 ± 8</td>
<td>128 ± 4†</td>
</tr>
<tr>
<td>10</td>
<td>290 ± 14</td>
<td>269 ± 20</td>
</tr>
<tr>
<td>20</td>
<td>357 ± 9</td>
<td>351 ± 11</td>
</tr>
<tr>
<td>30</td>
<td>366 ± 7</td>
<td>362 ± 6</td>
</tr>
</tbody>
</table>

* Significantly different from control, 0.02 < P < 0.01, 0.01 < P < 0.001.
ence (Schrader and Gerlach, 1976; Olsson et al., 1981) that in mammalian heart the interstitial adenosine compartment is quite small and that an intracellular compartment accounts for nearly the entire cardiac pool.

We found that intracoronary infusions of ADA or theophylline reduced reactive hyperemia by very nearly the same amount. Earlier reports on the effect of aminophylline on myocardial reactive hyperemia are conflicting. This drug has had no effect on this response in some experiments (Eickens and Wilcken, 1977), whereas, in others, it reduced the response by as much as 40% (Curnish et al., 1972). This inconsistency may be due to differences in experimental preparation, e.g., conscious vs. anesthetized dog, and the route and method of administration, i.e., systemic vs. close arterial administration and continuous infusion vs. bolus injection. The use of aminophylline in some of these experiments rather than theophylline further complicates interpretation because aminophylline is a 2:2 complex of ethylene diamine, a strong organic base, with theophylline. What effect the administration of 2 equivalents of base with each equivalent of theophylline might have on coronary reactivity is unknown. Because of the limited solubility of theophylline in physiological saline, we employed nethyl sulfoxide to facilitate solution. It is possible that this agent influenced the hyperemic response. However, the effects of this solvent on vascular smooth muscle are evident only at concentrations of 0.14 M and higher (Jackson et al., 1979), so that it seems quite unlikely that the concentrations in coronary plasma water attained in our experiments (≤1 mm) had any effect.

Intra-arterial infusion of enzymes small enough to traverse the coronary capillary wall appears to be a useful experimental technique for modifying the chemical composition of the myocardial interstitial space. These experiments show that adenosine deaminase catalytic subunits, which have a Stokes radius of 23 Å, attain concentrations of >1 U/ml in the cardiac interstitium during intracoronary infusion. We wish to emphasize that these results depend on the use of a specific kind of calf thymus adenosine deaminase which consists almost entirely of free catalytic subunits. In many tissues, adenosine deaminase catalytic subunits are bound to a protein whose molecular weight is about 300,000 (Schrader and Stacy, 1977; Daddona and Kelley, 1978). This form of the enzyme would obviously be unsuitable for the present application.

References


Jackson CV, Karow AM, Carrier GO (1979) Influence of dimethyl sulfoxide (Me2SO) on vascular smooth muscle. Arch Int Pharmacodyn 237: 4–15


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