ABSTRACT

In the presence of 8-azaguanine, an inhibitor of adenosine deaminase, adenosine appears in the perfusate of isolated cat and guinea pig hearts subjected to anoxia or severe hypoxia. With graded hypoxia the increase in coronary flow is roughly proportional to the sum of adenosine, inosine and hypoxanthine released from the hearts and inversely related to the oxygen tension of the perfusion fluid. Addition of epinephrine to oxygenated perfusion medium yields results similar to those obtained with hypoxia. These findings are compatible with the concept that vasodilation observed with hypoxia is due to adenosine formed from the breakdown of myocardial adenine nucleotides.

ADDITIONAL KEY WORDS coronary vascular resistance
cardiac vasoactive metabolites
isolated perfused hearts
adenosine deaminase inhibitor
graded myocardial hypoxia
regulation coronary blood flow
adenine nucleotide degradation
guinea pig
cat

Based upon the finding of degradation products of the adenine nucleotides in the effluent from the coronary circulation of hypoxic hearts, it has been proposed that the nucleoside adenosine is the mediator of the vasodilation observed with myocardial hypoxia and may be involved in the metabolic regulation of coronary blood flow.1 Experiments on ischemic cardiac muscle have shown that in this tissue the principal degradative pathway of the adenine nucleotides is via adenosine.2 However, only inosine and hypoxanthine have been found in the effluents from hypoxic isolated perfused cat hearts and from hypoxic in situ dog hearts.1 For the adenosine hypothesis to be valid it must be demonstrated that the adenosine formed during myocardial hypoxia is not completely converted to inosine and hypoxanthine within the myocardial cell. One reason for our failure to detect adenosine in the coronary sinus blood of the dog heart or in perfusates of anoxic cat hearts may be that adenosine deaminase, which is present in blood and perfusates, deaminates the adenosine in the sample before we can inactivate the enzyme. Therefore, the present study was undertaken to determine whether adenosine appears in cardiac effluents during hypoxia in the presence of an adenosine deaminase inhibitor and if so, what relationship the adenosine concentration bears to the increase in coronary fluid flow and to the degree of hypoxia imposed.

Methods

Experiments were performed on isolated guinea pig and cat hearts perfused by the Langendorff technique. Prior to rapid excision of their hearts, guinea pigs were struck a stunning blow on the head and cats were anesthetized with ether. Immediately after excision, the hearts were gently rinsed with warm oxygenated perfusion medium and fastened to the aortic cannula. The guinea pig hearts were perfused with a solution, each liter of which contained 7.4 g of NaCl, 0.4 g of KCl, 0.1 g of MgCl₂, 0.3 g of CaCl₂, 0.14 g of KH₂PO₄, 1.3 g of NaHCO₃, and 1.0 g of glucose. The cat hearts were perfused with a solution, each
ADENOSINE AND CORONARY FLOW

A liter of which contained 9.0 g of NaCl, 0.2 g of KCl, 0.1 g of MgCl2, 0.2 g of CaCl2, 0.048 g of NaH2PO4, 1.0 g of NaHCO3, and 1.0 g of glucose. When either O2 or N2 containing 5% of CO2 was bubbled through the perfusion medium at 36°C, its pH was 7.39. The perfusion pressure in all experiments was 40 cm of water.

The control collection periods were begun after 20 to 30 min of perfusion with oxygenated perfusion solution to ensure the washout of blood and the establishment of steady state conditions. During control and recovery periods, the perfusion fluid was equilibrated with 95% of O2 and 5% of CO2, whereas during anoxia the oxygen was replaced by nitrogen. In periods of severe hypoxia, previously oxygenated perfusion solution was bubbled with 95% of N2 and 5% of CO2 at a point just proximal to the aortic cannula so that the oxygen tension of the solution (as measured with a Clark pO2 electrode) reaching the heart had a partial pressure of oxygen of about 40 mm Hg. In each experimental period the hearts were perfused with 200 ml of solution which was collected in flasks immersed in ice. Twenty minutes of perfusion with oxygenated solution elapsed between termination of the period of anoxia and the start of perfusate collection in the recovery period. Prior to each collection period the adenosine deaminase inhibitor, 8-azaguanine, was injected into the tubing just proximal to the aortic cannula over a 2 min period at a rate calculated to give a final concentration of 10^-5 M in the perfusion medium. Since 8-azaguanine interferes with the enzymatic assay of adenosine and since difficulty was encountered in removing all of it from the perfusates, collection of the perfusate was not started until 3 min after the 8-azaguanine infusion was completed. The proteins in the perfusates were precipitated with perchloric acid and the adenine nucleotide derivatives were adsorbed on charcoal, eluted with ethanol and assayed enzymatically as previously described.1-3

In another series of experiments, cat hearts were subjected to different degrees of hypoxia by perfusion with the salt solution equilibrated with 95, 65, 48, 40, 30, 20, 10 and 0% of oxygen. Each gas mixture contained 5% of CO2 and the balance was made up by N2. The administration of 8-azaguanine and the volume and method for collection of the perfusates were carried out in the same manner as for the first series of experiments. Four different degrees of hypoxia were imposed on each heart with approximately 20 min of perfusion with oxygenated salt solution between each period of hypoxia. Oxygen tensions were measured with a Clark electrode and recorded on a Honeywell Visicorder.

In two experiments cat hearts were perfused with oxygenated salt solution containing from 0.1 to 5.0 μg of epinephrine per ml. Between the periods of perfusion with epinephrine the hearts were perfused with oxygenated salt solution until flow and rate had returned to control levels. The order of the concentrations of epinephrine used was varied in each experiment.

Results

Anoxia and Severe Hypoxia

The effects of anoxia on coronary flow, heart rate and the release of adenosine, inosine and hypoxanthine from the guinea pig heart are illustrated in figure 1. Anoxia increased coronary flow approximately three-fold and reduced heart rate to between one fourth and one fifth of the control rate. Both returned to control levels during the recovery period. In the control and recovery periods adenosine was absent and only small amounts of either inosine or hypoxanthine or both were present in the perfusate. During the period of anoxia adenosine appeared in

![Figure 1](http://circres.ahajournals.org/)

Representative experiment showing the effects of anoxia on heart rate, coronary flow and the release of myocardial adenine nucleotide degradation products from the isolated perfused guinea pig heart. The arrows indicate the 2 to 3 min infusions of sufficient 8-azaguanine in the perfusion solution to give a final concentration of 10^-5 M in the perfusate.
**TABLE 1**

**Effect of anoxia and severe hypoxia on the appearance of adenosine, inosine and hypoxanthine in the perfusates of isolated guinea pig hearts in the presence of 8-azaguanine**

<table>
<thead>
<tr>
<th>Exp.</th>
<th>Coronary Flow</th>
<th>Heart Rate</th>
<th>Adenosine</th>
<th>Inosine</th>
<th>Hypoxanthine</th>
<th>Adenosine + Inosine + Hypoxanthine</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ml/min</td>
<td>mm/min</td>
<td>pmol</td>
<td>pmol</td>
<td>pmol</td>
<td>pmol</td>
</tr>
<tr>
<td>Cont</td>
<td>Mean</td>
<td>±SE</td>
<td>Mean</td>
<td>±SE</td>
<td>Mean</td>
<td>±SE</td>
</tr>
<tr>
<td>1</td>
<td>4.8</td>
<td>±.3</td>
<td>11.3</td>
<td>±1.5</td>
<td>3.9</td>
<td>±.9</td>
</tr>
<tr>
<td>2</td>
<td>4.8</td>
<td>±.3</td>
<td>14.6</td>
<td>±1.5</td>
<td>3.9</td>
<td>±.9</td>
</tr>
<tr>
<td>3</td>
<td>4.6</td>
<td>±.3</td>
<td>13.4</td>
<td>±1.5</td>
<td>3.9</td>
<td>±.9</td>
</tr>
<tr>
<td>4</td>
<td>3.6</td>
<td>±.3</td>
<td>10.8</td>
<td>±1.5</td>
<td>3.9</td>
<td>±.9</td>
</tr>
<tr>
<td>5</td>
<td>5.6</td>
<td>±.3</td>
<td>13.6</td>
<td>±1.5</td>
<td>3.9</td>
<td>±.9</td>
</tr>
</tbody>
</table>

**In each control, experimental, and recovery period the hearts were perfused with 200 ml of saline solution and the quantities of adenosine, inosine and hypoxanthine are those which were recovered from the entire perfusate. The mean control values in each column are for all the hearts.**

**Notes:**
- Cont = control; Anox = anoxia; Recov = recovery.
- *pO₂ ca. 40 mm Hg.

**In each control, experimental, and recovery period the hearts were perfused with 200 ml of saline solution and the quantities of adenosine, inosine and hypoxanthine are those which were recovered from the entire perfusate. The mean control values in each column are for all the hearts.**
Effect of Severe Hypoxia on the Appearance of Adenosine, Inosine and Hypoxanthine in the Perfusates of Isolated Cat Hearts in the Presence of 8-Azaguanine

<table>
<thead>
<tr>
<th>Exp.</th>
<th>Coronary Flow</th>
<th>Heart Rate</th>
<th>Adenosine Cont</th>
<th>Hypoxanthine Cont</th>
<th>Inosine Cont</th>
<th>Adenosine Recov</th>
<th>Hypoxanthine Recov</th>
<th>Inosine Recov</th>
</tr>
</thead>
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<tr>
<td>1</td>
<td>13.6</td>
<td>69.0</td>
<td>150</td>
<td>161</td>
<td>110</td>
<td>62</td>
<td>90</td>
<td>90</td>
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<tr>
<td>2</td>
<td>13.0</td>
<td>51.4</td>
<td>116</td>
<td>189</td>
<td>94</td>
<td>46</td>
<td>102</td>
<td>102</td>
</tr>
<tr>
<td>3</td>
<td>13.0</td>
<td>51.4</td>
<td>116</td>
<td>189</td>
<td>94</td>
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<tr>
<td>Mean</td>
<td>13.0</td>
<td>51.4</td>
<td>116</td>
<td>189</td>
<td>94</td>
<td>46</td>
<td>102</td>
<td>102</td>
</tr>
</tbody>
</table>

In each control, hypoxia and recovery period, the hearts were perfused with 200 ml of saline solution and the quantities of adenosine, inosine and hypoxanthine were those which were recovered from the entire perfusate.

The perfusate and the perfusate concentration of inosine and hypoxanthine increased. The administration of the 8-azaguanine resulted in a transient increase in heart rate and coronary flow in each of the experimental periods. Despite considerable variation in the quantities of adenosine, inosine and hypoxanthine released by different hearts subjected to the same experimental conditions, the directional changes in the amounts of the released compounds were essentially the same for all experiments (table 1). Severe hypoxia in the guinea pig hearts yielded similar results but the magnitude of the sum of the released inosine, hypoxanthine and adenosine was less than that observed with anoxia (table 1). In the cat hearts that were subjected to severe hypoxia, the pattern of the release of the adenine nucleotide degradation products resembled that obtained with the guinea pig hearts (table 2).

**GRADED HYPOXIA**

Progressive reduction in the tension of oxygen in the salt solution perfusing isolated cat hearts produced an increase in the concentration of inosine, hypoxanthine and adenosine in the perfusates. A maximal level of these compounds was reached when the oxygen tension fell below 200 mm Hg. Coronary flow increased in a manner roughly proportional to the increase in concentration of the adenine nucleotide degradative products and inversely proportional to the oxygen tension of the perfusate (fig. 2). A semilog plot of the increase in coronary flow against the sum of inosine, hypoxanthine and adenosine released during graded hypoxia yields a regression line, \( y = 116.1X + 183.2 \) with a correlation coefficient of \( r = 0.73 \) (fig. 3).

**EPINEPHRINE**

In the presence of 8-azaguanine the addition of epinephrine to oxygenated perfusion fluid resulted in the release of inosine, hypoxanthine and adenosine from the isolated cat heart (table 3). Only two experiments were performed with epinephrine. However, the quantities of the adenine nucleotide degra-
tive products appearing in the perfusate were several fold greater than was observed with the cat hearts subjected to severe hypoxia.

Maximal release of the compounds was achieved with epinephrine concentrations between 0.3 and 0.5 μg/ml of perfusion medium.

**Discussion**

It has been previously reported that anoxia and severe hypoxia result in the breakdown of myocardial nucleotides with the appearance of inosine and hypoxanthine in the car-

![Figure 2](image)

**FIGURE 2**

The relationship of oxygen tension in the perfusion fluid to coronary flow and the concentration of hypoxanthine, inosine and adenosine in the perfusates of isolated perfused cat hearts treated with 8-azaguanine. The numbers in parentheses indicate the number of cat hearts studied at the different oxygen tensions.

![Figure 3](image)

**FIGURE 3**

Relationship between coronary flow and the log of myocardial adenine nucleotide degradation products release during hypoxia of cat hearts treated with 8-azaguanine. Regression line, $y = 116.1x + 183.2$, $r = 0.73$. Dashed lines = 95% confidence limits.

**TABLE 3**

<table>
<thead>
<tr>
<th>Exp.</th>
<th>Epinephrine</th>
<th>Adenosine</th>
<th>Inosine</th>
<th>Hypoxanthine</th>
<th>Adenosine + Inosine + Hypoxanthine</th>
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</thead>
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<tr>
<td>1</td>
<td>0.1</td>
<td>0</td>
<td>0</td>
<td>18</td>
<td>18</td>
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<tr>
<td></td>
<td>0.5</td>
<td>149</td>
<td>484</td>
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<tr>
<td></td>
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<td>109</td>
<td>566</td>
<td>378</td>
<td>1053</td>
</tr>
<tr>
<td></td>
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<td>151</td>
<td>584</td>
<td>310</td>
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<td>0</td>
<td>37</td>
<td>52</td>
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<tr>
<td></td>
<td>0.2</td>
<td>106</td>
<td>126</td>
<td>328</td>
<td>560</td>
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<td></td>
<td>0.3</td>
<td>515</td>
<td>812</td>
<td>561</td>
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<td>681</td>
<td>403</td>
<td>1725</td>
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<tr>
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<td>414</td>
<td>343</td>
<td>1199</td>
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<tr>
<td></td>
<td>5.0</td>
<td>207</td>
<td>287</td>
<td>285</td>
<td>779</td>
</tr>
</tbody>
</table>

In each experimental period the hearts were perfused with 200 ml of oxygenated saline solution containing epinephrine. The quantities of adenosine, inosine and hypoxanthine are those which were recovered from the entire perfusate.

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ADENOSINE AND CORONARY FLOW

The present study shows that in the presence of an adenosine deaminase inhibitor, adenosine is also found in the perfusates of hypoxic hearts. Adenosine has been shown to enter myocardial cells where it is rapidly incorporated into ATP and ADP. Our findings and those of Richman and Wyborny indicate that under the influence of hypoxia, adenosine can also leave the myocardial cell prior to deamination. The amounts of adenosine recovered by Richman and Wyborny in the perfusates of the rabbit heart were greater than we observed in our experiment on cats and guinea pigs. This difference is undoubtedly due to the facts that (a) they achieved greater and probably complete adenosine deaminase inhibition by addition of the 8-azaguanine to the perfusion fluid whereas we added it as a brief infusion and (b) the perfusion fluid was recycled through the hearts in their experiments whereas it passed through the coronary vessels only once in our experiments. In the absence of an adenosine deaminase inhibitor only inosine and hypoxanthine are recoverable in the perfusate of the isolated heart subjected to anoxia. However, if the rate of degradation of the myocardial adenine nucleotides is accelerated by the use of an uncoupler of oxidative phosphorylation, adenosine appears in the perfusate without use of an adenosine deaminase inhibitor.

These observations are compatible with the concept that adenosine is formed during myocardial hypoxia and diffuses out of the myocardial cell to reach the resistance vessels and induce vasodilation. Also in support of this notion are the direct relationships between coronary flow, the concentration of the adenosine, inosine, and hypoxanthine in the perfusates and the degree of hypoxia.

The results obtained with epinephrine are in harmony with the adenosine hypothesis. Although myocardial oxygen tensions were not known, it is quite conceivable that they were markedly reduced due to the epinephrine-induced increase in tissue oxygen need and the low oxygen-carrying capacity of the perfusion medium.

Myocardial oxygen tension may well be the factor responsible for adenosine release, but it remains to be established that the adenosine released and the coronary dilation observed with hypoxia do indeed represent a cause and effect relationship. Adenosine is effective as a vasodilator at concentrations below those presently detectable in perfusates or blood. Therefore, it cannot be determined if it is the physiological mediator of metabolic vasodilation until more sensitive methods for its detection are available and less drastic stimuli elicit its release from the myocardium.

Acknowledgment

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References

Release of Adenosine from Anoxic Hearts: Relationship to Coronary Flow
MAKOTO KATORI and ROBERT M. BERNE

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