Adrenergic Blockade Blunts Adenosine Concentration and Coronary Vasodilation During Hypoxia

Steven C. Herrmann and Eric O. Feigl

Myocardial hypoxia is thought to be an important stimulus for increasing interstitial adenosine concentration. The adenosine hypothesis of coronary control was investigated during steady-state hypoxia by making measurements of coronary venous and epicardial well adenosine concentrations in adrenergically intact dogs and in animals with α- and β-receptor blockade. In the adrenergically intact group, hypoxia sufficient to lower coronary venous oxygen tension to 8 mm Hg increased coronary blood flow 243% from normoxic values. Both coronary venous and epicardial well adenosine concentrations were increased throughout the hypoxic period. In the adrenergically blocked group, hypoxia to a similar level of coronary venous oxygen tension produced an increase in coronary blood flow of only 75%, which was significantly less than in the adrenergically intact group (p<0.01). Coronary venous adenosine was only transiently elevated, and epicardial well adenosine was unchanged from control levels. In a separate group of α- and β-receptor-blocked animals that received an infusion of L-homocysteine thiolactone during hypoxia, there was no difference in tissue S-adenosylhomocysteine levels compared with those of normoxic controls. It is concluded that much of the coronary vasodilation associated with systemic hypoxia is dependent on adrenergic activation and that adenosine may only play a role in sustained hypoxic vasodilation when adrenergic receptors are intact. (Circulation Research 1992;70:1203–1216)

KEY WORDS • S-adenosylhomocysteine • β-receptor • α-receptor

Coronary venous oxygen tension is relatively constant throughout the physiological range of myocardial oxygen consumption, indicating a remarkable match between coronary blood flow and myocardial metabolism.1 Berne2 and Gerlach et al3 first proposed that the nucleoside adenosine is the physiological transmitter between the cardiomyocytes and the vascular smooth muscle that produces the match between flow and metabolism. In the usual substrate form of the adenosine hypothesis,1 adenosine functions in a feedback loop, whereby a decrease in myocardial oxygen tension leads to an increase in the interstitial concentration of adenosine. The increase in adenosine concentration at the vascular smooth muscle adenosine receptor produces arteriolar vasodilation and an increase in coronary blood flow, thus correcting the fall in myocardial oxygen tension.

According to the adenosine hypothesis, cardiac hypoxia should be a potent stimulus for increased adenosine concentration in the interstitium. The adenosine hypothesis was tested by making measurements of coronary venous plasma adenosine concentration, epicardial well adenosine concentration, and intracellular S-adenosylhomocysteine (SAH) concentration during sustained hypoxia in chloralose-anesthetized dogs with

and without α- and β-receptor blockade. A sustained increase in adenosine concentration above normoxic control values was measured in both coronary venous blood and epicardial well fluid during hypoxia with intact adrenergic receptors. With α- and β-receptor blockade, a transient increase in plasma adenosine concentration and no change in epicardial well adenosine concentration was observed during hypoxia. These results indicate that adenosine may be important in hypoxic hyperemia only when adrenergic receptors are intact and that there may be an adenosine-adrenergic interaction in the control of coronary blood flow.

Materials and Methods

General Preparation

Twenty-four mongrel dogs (25–36 kg) of either sex were studied. Dogs were sedated with morphine sulfate (2.5 mg/kg s.c.) and anesthetized with α-chloralose (100 mg/kg i.v.). An adequate level of anesthesia was maintained by 500-mg supplements of α-chloralose as needed. Metabolic acidosis secondary to α-chloralose anesthesia was corrected by intravenous infusion of 1.5% sodium bicarbonate solution.

The dogs were intubated and mechanically ventilated with a positive-pressure respirator (model 607, Harvard Apparatus, South Natick, Mass.) with end-expiratory pressure between 0 and 5 cm H2O. End-expiratory CO2 was measured (model LB-2, Beckman Instruments, Fullerton, Calif.) and held between 4.5% and 5.5% by adjustment of tidal volume or respiratory frequency. A servo-control device (73A, Yellow Springs Instrument

From the Department of Physiology and Biophysics, University of Washington School of Medicine, Seattle, Wash.

Address for correspondence: Eric O. Feigl, MD, Department of Physiology and Biophysics SJ-40, University of Washington Medical School, Seattle, WA 98195.

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Coronary Sinus Cannulation

Under fluoroscopic control, a Sones catheter (model 5423, USCI, Billerica, Mass.) was placed down the jugular vein and positioned into the coronary sinus. The placement of the catheter was confirmed by palpating its position in the coronary sinus. Right atrial admixture was prevented by placing the catheter at least 15 mm into the coronary sinus and limiting the withdrawal rate of samples to 5–15 ml/min.4

Coronary Blood Flow

A cannula-tip Doppler flowmeter5 was placed inside the circumflex coronary artery via the right carotid artery (Figure 1, inset). The seal between the flowmeter tip and the circumflex coronary artery was tested before each experiment. Nitroglycerin (10 µg i.c.) was injected into a side tube of the flowmeter that opened proximal to the tip. The seal was considered adequate if no increase in coronary blood flow was observed. The same dose of nitroglycerin injected down the circumflex region caused a doubling of coronary flow. To normalize circumflex blood flow per gram of tissue, the perfused myocardium was demarcated by injecting crystal violet dye at the end of the experiment. In the homocysteine experiments in which the heart was rapidly frozen, the circumflex region was estimated to be 39% of the whole heart mass.6 The flowmeter was calibrated at the end of experiment by timed collection of blood at several flow rates.

Oxygen Measurements

Arterial and venous oxygen tension, carbon dioxide tension, and pH were measured with an IL 1302 blood gas analyzer (Instrumentation Laboratories, Lexington, Mass.). Blood oxygen content was measured by the fuel cell method from blood samples drawn into chilled glass syringes (Lex-O2-Con, Waltham, Mass.).7,8 For normoxia samples, 20-µl aliquots of blood were analyzed for oxygen content. During hypoxia, two to five aliquots of blood were injected to increase the resolution of the Lex-O2-Con instrument. Myocardial oxygen consumption (microliters of oxygen per minute per gram) was calculated by multiplying the coronary blood flow by the arterial-venous oxygen content difference.

Lactate Measurement

Arterial and coronary venous lactate samples were drawn into vials containing NaF to prevent glycolysis and placed on ice. Lactate concentration was measured by an automated method by injecting blood into a model 23A lactate analyzer (Yellow Springs).9 The machine was calibrated with external lactate standards before and after analysis.

Plasma Adenosine Measurements

The adenosine assay is described in detail elsewhere.10 Briefly, blood for plasma adenosine samples was collected in chilled syringes11 that mixed the blood with an ice-cold enzymatic stop solution containing diprydamole and erythro-9-(2-hydroxy-3-nonyl) adenine (EHNA). After acidification, neutralization, and purification, samples were divided and one aliquot was treated with adenosine deaminase, forming a blank. The other adenosine was separated from other components by using an ion-pairing (tetraethylammonium hydrogen sulfate/potassium phosphate) solution and acetonitrile with a 220×2.1 mm C18 column (Applied Biosystems, Santa Clara, Calif.) on a 1090M chromatograph (Hewlett-Packard, Avondale, Pa.). Adenosine content was determined by subtracting the blank chromatogram from its paired unknown chromatogram and comparing
the difference with adenosine standards. Adenosine recovery by this method is 89% with an average coefficient of variation of 12% for physiological concentrations of adenosine.

Epicardial Well Adenosine Measurements

A 1-cm² plastic well with a ridge fashioned on the bottom was used to measure epicardial adenosine concentration. A foam mounting square (Scotch 3M, St. Paul, Minn.) glued to the base of the well outside the ridge was used as a flexible gasket to attach the well to the heart. Cyanacrylate was used to attach the foam base of the well to the myocardium. The ridge on the bottom of the well prevented glue from entering the sample chamber of the well. In the experiments in which adrenergic receptors were intact, cardiac contractions became very forceful, so the well was further supported by elastic sutures sewn to distal parts of the epicardium.12

The well solution was a modified Krebs buffer consisting of 140 mM NaCl, 4.7 mM KCl, 2.5 mM CaCl₂, 0.7 mM MgCl₂, and 1.7 mM HEPES buffer. This isotonic solution was titrated to pH 7.4 with NaOH before use. When the well was in place, several flushes of well solution over a 30-minute period were done to obtain stable conditions. During the experiment, 100 µL well solution was placed in the well and allowed to equilibrate for 5.5 minutes.12 At the end of the equilibration period, the well fluid was removed and transferred to a 300-µL glass vial, which was then capped and placed in boiling water to inactivate enzymes that may have diffused into the well solution. After a 10-minute boiling period, well samples were stored at room temperature and analyzed by high-performance liquid chromatography (HPLC) within 4 hours of the experiment. Just before analysis, the samples were centrifuged (18,500g for 10 minutes) and then injected onto the HPLC column with and without further treatment. Adenosine was separated with the plasma samples. Adenosine concentration was determined by integrating the area under the curve at the adenosine retention time and comparing the result with a regression line of area versus concentration for adenosine standards.

Tissue S-Adenosylhomocysteine Measurements

Transmural SAH content in the left ventricular free wall was measured in both normoxic and hypoxic adrenergically blocked animals after l-homocysteine thiocyanate was infused for 20 minutes (14 mg/kg i.v.; two thirds of the dose delivered in the first 5 minutes).13 Because a large portion of the homocysteine dose was administered in the first 5 minutes, plasma homocysteine concentration increased rapidly, thereby favoring uptake into the myocardial tissue. An infusion of homocysteine vehicle was used to match the infusion of homocysteine in the experiments in which SAH was not measured. At the end of the 20-minute infusion period, the heart was rapidly removed from the chest, and a section of left ventricular free wall was excised and frozen in liquid heptane cooled to the temperature of dry ice (−70°C). The frozen myocardium was lyophilized at −20°C for several days until the mass of the tissue ceased to change. After the samples were dry, the tissue was stored at −70°C until it was analyzed. The inner and outer 2 mm of the dried ventricular free wall were removed and not analyzed. Approximately 60 mg dried tissue was added to 1 ml of 1N perchloric acid and homogenized (Biospec Products, Bartlesville, Okla.) for 3 minutes. After homogenization, the samples were centrifuged for 20 minutes (18,500g) and the supernatant was removed and neutralized with 200 µL of 1 M HEPES buffer and 1N KOH.

SAH was measured with HPLC. Quadruplicate samples of each layer were analyzed on a 3.9×30 cm C18 Micro-Bondapak column (Waters Chromatography Div., Milford, Mass.). A 150-µL sample was injected onto the column. Flow rate for the analysis was 800 µL/min, and temperature was maintained at 23°C. The separation consisted of a 13-minute isocratic period with an aqueous 20 mM KH₂PO₄ buffer followed by a linear gradient of 30% CH₃OH:H₂O (vol/vol) for 27 minutes to a final organic concentration of 65%. SAH concentration was calculated by comparing the area of SAH unknowns with a regression line obtained from the area of external standards injected during the analysis. SAH tissue values are expressed in wet weight concentration by correcting for the average wet-to-dry ratio of all tissue samples analyzed.

Drugs

All animals received atropine (0.5 mg/kg i.v.) to prevent bradycardia associated with hypoxia14 and reflex coronary vasodilation from chemoreceptor activation.15 After additional anesthetic, all animals received the neuromuscular blocking agent decamethonium (0.3 mg/kg i.v.) before the control and hypoxic periods to prevent respiratory movements. For the adrenergically blocked experiments, phenoxybenzamine (0.5 mg/kg i.v.; Dibenzyline, Smith Kline & French Laboratories, Philadelphia, Pa.) was given 2 hours before the experimental period and propranolol (2 mg/kg i.v. plus 0.2 mg/kg/hr i.v.) was given 1 hour before the experimental period.

Experimental Protocol

The experiment was designed to test the role of adenosine in sustained hypoxic hyperemia with and without adrenergic blockade. During the control period, arterial oxygen tension was maintained between 85 and 150 mm Hg by supplementing inspired air with oxygen. A timed sampling protocol, beginning with filling of the epicardial well, was used to obtain three sets of control measurements (see Figures 3 and 4).

Hypoxia was induced by decreasing the fraction of inspired oxygen to between 7% and 10% by adding nitrogen to produce a coronary sinus Po₂ of about 8 mm Hg. Steady-state hemodynamic and coronary blood flow responses to hypoxia occurred within 5–10 minutes of the onset of hypoxic ventilation. When a steady state was obtained, the epicardial well was filled with fresh buffer and a sampling procedure identical to that of the control period was initiated. End-expiratory CO₂ was maintained constant by manipulation of respiratory tidal volume or frequency throughout the hypoxia period.
Data Analysis and Experimental Criteria

Both mean coronary blood flow and arterial pressure were determined with averaging circuits using 2-second time constants. Myocardial oxygen consumption, lactate concentration and extraction, acid-base status, and adenosine concentration were expressed as the average of three control points and compared with the individual time measurements during hypoxia.

Unpaired Student's t tests were used to compare average coronary blood flow change (from normoxic to hypoxic) for the blocked and unblocked experiments. This procedure is equivalent to testing the interaction between dog and hypoxic effects in a repeated-measures analysis of variance. Paired t tests were used to compare average hypoxic venous minus arterial (v–a) and well adenosine values at each of three time points to an overall control value based on three time points on a within-dog basis (see Figure 4). The probability values reported are uncorrected for the three dependent comparisons. To make the Bonferroni adjustment for multiple comparisons, multiply the probability values in Figure 4 by 3. Multiple regression techniques were used to quantify relations between two variables on a within-dog basis (see Figures 5 and 6). A separate straight line with common slope was fit to the data for each of the eight dogs. The R² value reported is the squared multiple correlation coefficient from the analysis of variance table.

Based on findings from pilot experiments, two prospective experimental criteria were set. First, since steady-state coronary control was being studied, the elevation in coronary blood flow needed to be reasonably steady during hypoxia. The extremes of coronary blood flow varied by no more than 35% from the mean during hypoxia (average, 13.84±1.89%). Second, a cardiac lactate extraction of less than 10% was considered to be an indication of pathological hypoxia and anaerobic metabolism beyond the physiological response to hypoxia.16–18 One set of adenosine samples (at 16.5 minutes) in the adrenergically unblocked group was rejected for this reason.

Results

Unblocked Experiments

A recording from an experiment with adrenergic receptors intact is shown in Figure 2. The composite of the eight experiments in this group is shown in Figures 3 and 4. Lowering arterial oxygen tension from 116 to 22 mm Hg increased coronary blood flow from 0.68 to 2.36 ml/min/g. The average increase in coronary blood flow was 243%. Myocardial oxygen consumption during hypoxia was not different from normoxic values. Coronary venous oxygen tension decreased from 23 to 9 mm Hg during hypoxia. The v–a plasma adenosine concentration averaged 17 nM during the control period and increased to 161 nM during hypoxia. Epicardial well adenosine concentration increased from 221 nM in the control period to 358 nM during hypoxia. Values during control and hypoxia for the unblocked group are given in Table 1.

The relation between coronary blood flow and v–a or epicardial well adenosine concentration in the unblocked animals is shown in the left panels of Figure 5. There was a positive and significant correlation between coronary blood flow and both v–a and epicardial well adenosine concentrations during hypoxia with α- and β-receptors intact.

The left panels of Figure 6 show the relation between v–a and epicardial well adenosine concentration and coronary sinus oxygen tension. As coronary sinus oxygen tension decreased, v–a adenosine concentration significantly increased (Figure 6A). Epicardial well adenosine concentration also significantly increased as coronary sinus oxygen tension decreased during hypoxia with α- and β-receptors intact (Figure 6B).

α-Receptor- and β-Receptor-Blocked Experiments

A recording from an α- and β-receptor–blocked experiment is shown in Figure 7. The composite of the eight animals in this group is shown in Figures 3 and 4. Decreasing arterial oxygen tension from 108 to 28 mm Hg increased coronary blood flow from 0.56 to 0.97 ml/min/g. The average increase in coronary blood flow was 75%, which was significantly less than in the adrenergically intact group (p<0.01). Myocardial oxygen consumption during hypoxia was not different from normoxic values. Coronary sinus oxygen tension decreased from 22 to 8 mm Hg during hypoxia. The v–a plasma adenosine concentration increased from 14 nM during normoxia to 30 nM during hypoxia, most of the increase being due to the transient increase at 4.5 minutes. Paired analysis of control and hypoxic adenosine measurements revealed that v–a adenosine concentration was increased significantly only at the 4.5-minute sampling period, despite a sustained steady-state increase in coronary blood flow during hypoxia. The epicardial well adenosine concentration was 251 nM during the control period and 204 nM during the hypoxic period. Values during control and hypoxia for the α- and β-receptor–blocked group are given in Table 2.

The relations between coronary blood flow and v–a adenosine or epicardial well adenosine concentration in the α- and β-receptor–blocked experiments are shown in the right panels of Figure 5. Considering all samples taken throughout the hypoxic period, there was a significant correlation between coronary blood flow and v–a adenosine concentration during hypoxia with adrenergic receptor blockade (Figure 5C). Paradoxically, there was a significant inverse correlation between coronary blood flow and epicardial well adenosine concentration with α- and β-receptor blockade during hypoxia (Figure 5D).

The right panels of Figure 6 show the relation between v–a or epicardial well adenosine concentration and coronary sinus oxygen tension during hypoxia with α- and β-receptor blockade. There was a shallow but significant inverse correlation between v–a adenosine and coronary venous oxygen tension (Figure 6C), much of this being due to the transient elevation after 4 minutes of hypoxia (Figure 4). Epicardial well adenosine concentration was not correlated with coronary sinus oxygen tension during α- and β-receptor blockade (Figure 6D).

α-Receptor- and β-Receptor–Blocked Homocysteine Experiments

The composite of the eight animals for the experimental period in which homocysteine was infused is
FIGURE 2. Recordings from an adrenergic unblocked experiment. In this experiment, coronary venous Po2 averaged 9 mm Hg during hypoxia. Coronary blood flow increased 290% from normoxic values. Mean aortic pressure increased from 86 to 143 mm Hg at the start of the hypoxic sampling period and declined to 120 mm Hg at the end of the hypoxic period. Heart rate increased from 201 beats per minute in normoxia to 215 beats per minute during hypoxia.
shown in Figure 8. Arterial oxygen tension averaged 126 mm Hg in the normoxic animals during homocystine infusion and 29 mm Hg in the animals made hypoxic during homocystine infusion. The average coronary blood flow was 0.47 ml/min/g in the normoxic group and 0.72 ml/min/g in the hypoxic group. Myocardial oxygen consumption was similar in both groups during homocystine infusion. Average coronary sinus oxygen tension was 21 mm Hg in the normoxic animals and 7 mm Hg in the hypoxic group. Blood metabolite values for both the normoxic and hypoxic experiments are given in Table 3.

The transmural distribution of tissue SAH in the normoxic and hypoxic groups is given in Figure 9. There...
was no difference in the SAH concentration in any layer between the two groups.

**Discussion**

The adenosine hypothesis, as originally described by Berne\(^2\) and Gerlach et al.\(^3\) suggests that adenosine is the physiological transmitter between the cardiomyocytes and the vascular smooth muscle in a feedback system that maintains myocardial oxygen tension constant. A necessary prediction of the adenosine hypothesis is that sustained myocardial hypoxia will cause a sustained increase in interstitial adenosine concentration. The present experiments were designed to test the adenosine hypothesis during physiological levels of hypoxia by

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**FIGURE 4.** Graphs showing oxygen and adenosine values from the eight adrenergically unblocked experiments (closed circles) and the eight \(\alpha\)- and \(\beta\)-blocked experiments (open squares). Arterial \(P_0\), during hypoxia was higher in the adrenergically blocked experiments; however, coronary venous \(P_v\), was the same in two groups. Both venous minus arterial (v–a) and epicardial well adenosine concentrations were significantly elevated during hypoxia vs. paired normoxic values with adrenergic receptors intact. With \(\alpha\)- and \(\beta\)-blockade, hypoxia resulted in a transient increase in v–a adenosine concentration, while epicardial well adenosine concentration was unchanged. Stars indicate p<0.05 vs. the average of the three paired control values. Values are mean±1 SEM.
making measurements of coronary \( v \)-\( a \) plasma adenosine concentration, epicardial well adenosine concentration, and myocardial tissue SAH levels in animals with and without adrenergic blockade. With adrenergic receptors intact, hypoxia increased coronary blood flow 243% from control levels and both \( v \)-\( a \) plasma and epicardial well adenosine concentrations were significantly increased from normoxic values throughout the hypoxic period. With \( \alpha \)- and \( \beta \)-receptor blockade, hypoxia increased coronary blood flow only 75% from control levels, and \( v \)-\( a \) plasma adenosine concentration was only transiently elevated, while epicardial well adenosine concentration was unchanged from control levels. The present results indicate that much of the coronary vasodilation during hypoxia is due to adrenergic receptor activation and that adenosine may have a role in sustained hypoxic hyperemia only when adrenergic receptors are intact.

In comparing the results from the two groups in this study, it was necessary to standardize the hypoxic stimulus to the myocardium. The choice of matching coronary venous oxygen tension between the adrenergically intact and blocked groups stems from previous experiments of the coronary circulation during hypoxia. Berne et al.\(^{19} \) demonstrated that only modest changes in coronary blood flow during hypoxia were observed in open-chest dogs when coronary venous oxygen content was above 5.5 vol%. However, a coronary venous oxy-

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### TABLE 1. Adrenergically Intact Experiments

<table>
<thead>
<tr>
<th></th>
<th>Normoxic control (average)</th>
<th>Hypoxia (4 min)</th>
<th>Hypoxia (10 min)</th>
<th>Hypoxia (16 min)</th>
</tr>
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<tbody>
<tr>
<td>Coronary flow (ml/min/g)</td>
<td>0.68±0.003</td>
<td>2.4±0.43</td>
<td>2.4±0.44</td>
<td>2.3±0.38</td>
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<td>Coronary sinus Po₂ (mm Hg)</td>
<td>23.2±0.1</td>
<td>9.4±0.3</td>
<td>8.6±0.5</td>
<td>7.7±0.6</td>
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<td>Arterial O₂ content (vol%)</td>
<td>21.6±1.0</td>
<td>7.2±0.95</td>
<td>6.7±0.96</td>
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<td>Venous O₂ content (vol%)</td>
<td>6.0±0.45</td>
<td>1.3±0.21</td>
<td>1.2±0.17</td>
<td>1.0±0.16</td>
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<tr>
<td>MVo₂ (( \mu )l/min/g)</td>
<td>101.9±0.5</td>
<td>119.8±11.7</td>
<td>111.1±10.0</td>
<td>105.9±9.1</td>
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<td>Arterial adenosine (nM)</td>
<td>16.6±3.4</td>
<td>28.7±3.9</td>
<td>31.6±6.0</td>
<td>27.5±5.6</td>
</tr>
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<td>Venous adenosine (nM)</td>
<td>33.6±3.0</td>
<td>193.1±59.0</td>
<td>186.1±65.8</td>
<td>128.5±23.0</td>
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<tr>
<td>( v )-( a ) adenosine (nM)</td>
<td>16.9±1.8</td>
<td>164.5±56.8</td>
<td>154.1±67.0</td>
<td>102.6±23.3</td>
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<td>Well adenosine (nM)</td>
<td>220.8±21.2</td>
<td>350.9±62.1</td>
<td>348.6±53.3</td>
<td>351.7±57.9</td>
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<td>Arterial PCO₂ (mm Hg)</td>
<td>37.1±0.5</td>
<td>38.4±1.4</td>
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<td>40.0±1.9</td>
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<tr>
<td>Arterial [H⁺] (nM)</td>
<td>38.9±0.5</td>
<td>40.7±1.7</td>
<td>41.7±1.5</td>
<td>43.6±2.1</td>
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<td>Base excess (mM)</td>
<td>0.7±0.3</td>
<td>0.3±0.5</td>
<td>0.3±0.4</td>
<td>-0.9±0.7</td>
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<tr>
<td>Arterial lactate (nM)</td>
<td>1.03±0.12</td>
<td>2.12±0.29</td>
<td>2.53±0.34</td>
<td>3.10±0.45</td>
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<tr>
<td>Lactate extraction (%)</td>
<td>53.3±1.92</td>
<td>26.7±4.89</td>
<td>24.1±5.90</td>
<td>23.3±5.61</td>
</tr>
</tbody>
</table>

Values are mean±SEM. At 16 minutes of hypoxia, \( n=6 \) for arterial adenosine, venous adenosine, \( v \)-\( a \) adenosine, and well adenosine; \( n=8 \) for all other variables. MVo₂, myocardial oxygen consumption; \( v \)-\( a \), venous minus arterial.

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**FIGURE 5.** Graphs showing regression of coronary blood flow and adenosine concentration. With adrenergic receptors unblocked (left panels), there is a positive correlation with both venous minus arterial and epicardial well adenosine concentrations. The positive correlation between coronary blood flow and adenosine suggests that adenosine may mediate the hypoxic coronary vasodilation with adrenergic receptors unblocked. With \( \alpha \)- and \( \beta \)-receptors blocked (right panels), coronary blood flow was positively correlated with venous minus arterial adenosine (panel C). Epicardial well adenosine concentration was inversely correlated with coronary flow (panel D). Note the difference in scales.
gen content below 5.5 vol% resulted in a marked coronary vasodilation. Berne suggested that the lack of coronary vasodilation until coronary venous content fell below 5.5 vol% was evidence that myocardial, and not intra-arterial, oxygen tension was critical for the control of coronary blood flow. The control of coronary blood flow by myocardial oxygen tension was used as indirect evidence for interstitial adenosine concentration being the physiological transmitter between the cardiomyocytes and the vascular smooth muscle during hypoxia. Coronary venous oxygen tension was used as an indicator of the level of tissue oxygen tension in the present experiments. Figure 4 shows that coronary venous oxygen tension was the same in the adrenergically blocked and intact groups, indicating that the putative myocardial stimulus for augmented interstitial adenosine and coronary blood flow was matched between the two groups.

Under conditions of aerobic metabolism in the heart, myocardial lactate extraction is 40–60%. However, severe myocardial hypoxia can result in anaerobic metabolism and a decrease in myocardial lactate extraction below 10% or net lactate production. Under such severe conditions, adenosine concentration would probably increase even in adrenergically blocked animals. To prevent the possibility of augmented adenosine levels secondary to anaerobic metabolism, a prospective experimental criterion based on cardiac lactate extraction was used in the present experiments. The experiment was terminated, and adenosine samples were not ana-

| Table 2. α-Receptor- and β-Receptor-Blocked Experiments |
|---------------------------------|-----------------|-----------------|-----------------|
|                                  | Normoxic control (average) | Hypoxia (4 min) | Hypoxia (10 Min) | Hypoxia (16 min) |
| Coronary flow (ml/min/g)        | 0.56±0.01         | 0.91±0.07       | 0.98±0.08       | 1.0±0.08         |
| Coronary sinus Po2 (mm Hg)      | 21.9±0.3          | 7.8±1.3         | 8.9±1.1         | 8.7±1.1          |
| Arterial O2 content (vol%)      | 18.6±0.42         | 8.7±0.04        | 8.9±0.45        | 8.7±0.54         |
| Venous O2 content (vol%)        | 4.7±0.47          | 0.9±0.20        | 1.2±0.18        | 1.2±0.14         |
| MVO2 (μl/min/g)                | 77.9±0.6          | 69.3±4.5        | 73.5±3.4        | 73.8±3.7         |
| Arterial adenosine (nM)         | 16.4±3.80         | 15.0±3.18       | 18.7±2.95       | 21.1±2.76        |
| Venous adenosine (nM)           | 30.4±6.1          | 63.1±11.2       | 39.6±5.5        | 42.0±6.4         |
| v—a adenosine (nM)             | 13.9±1.0          | 48.0±12.2       | 30.9±5.3        | 20.9±5.7         |
| Well adenosine (nM)             | 251.2±42.8        | 207.9±53.7      | 193.4±59.5      | 211.6±76.8       |
| Arterial Pco2 (mm Hg)           | 37.7±0.6          | 39.0±0.7        | 38.2±0.5        | 37.6±0.7         |
| Arterial [H+] (nM)              | 41.2±0.6          | 42.8±0.8        | 41.1±1.1        | 40.7±1.2         |
| Base excess (mM)                | ‐0.4±0.1          | ‐0.9±1.0        | ‐0.2±0.7        | ‐0.8±0.7         |
| Arterial lactate (mM)           | 1.48±0.19         | 4.06±0.59       | 4.21±0.69       | 4.48±0.77        |
| Lactate extraction (%)          | 56.2±1.45         | 28.4±2.85       | 26.6±2.10       | 24.6±2.48        |

Values are mean±SEM (n=8). MVO2, myocardial oxygen consumption; v—a, venous minus arterial.
Figure 7. Recordings from an α- and β-blocked experiment. Coronary venous PO₂ averaged 10 mm Hg in the hypoxic period. Coronary blood flow increased 87% from normoxic values. Aortic pressure averaged 71 mm Hg in both normoxic and hypoxic periods.
lyzed if myocardial lactate extraction fell below 10%. One set of adenosine samples in an adrenergically unblocked animal (at 16.5 minutes) was not analyzed because of the lactate extraction criterion.

Because direct sampling of the interstitial compartment is not possible, other indexes of interstitial adenosine must be used. The results of the paired v-a and epicardial well adenosine concentrations (Figure 4) during hypoxia, with adrenergic receptors intact, support a role for adenosine in hypoxic coronary vasodilation and confirm previous experiments demonstrating increased adenosine during hypoxia.2,13,21-24 Further, the significant positive correlation between coronary blood flow and both v-a and epicardial well adenosine concentrations with adrenergic receptors unblocked also supports the adenosine hypothesis (Figure 5, left panels). As Berne's original formulation of the adenosine hypothesis would predict, there was a significant inverse correlation with both v-a and epicardial well adenosine concentrations and coronary venous oxygen tension when adrenergic receptors were intact (Figure 6, left panels). The correlations of adenosine concentration with coronary blood flow and coronary sinus oxygen tension shown in Figures 5 and 6 exhibit considerable scatter. This scatter reflects the variability in the methods but also suggests that adenosine is not the sole controller of coronary blood flow during hypoxia. It must be stressed, however, that the correlations demonstrated between coronary blood flow, coronary oxygen tension, and adenosine concentration are not sufficient to prove causality.

A notable result in the present study is that the coronary venous adenosine concentration was a more sensitive measurement than epicardial well adenosine concentration. A significant transient elevation in v-a adenosine was observed at the beginning of the hypoxic periods in the α- and β-blocked group, but this was not observed in the corresponding epicardial well measurements (Figure 4). In the α- and β-blocked group, coronary flow was positively correlated with v-a adenosine concentration (Figure 5C), while blood flow was inversely correlated with epicardial well adenosine (Figure 5D). Also, in the α- and β-blocked group there was the expected inverse correlation between v-a adenosine and coronary sinus oxygen tension (Figure 6C), but this was not observed with the epicardial well adenosine concentration (Figure 6D). Thus, when physiological changes are examined, the v-a adenosine concentration is probably more useful than are epicardial well measurements.

Systemic hypoxia is a severe stress that results in elevated catecholamine levels.14,25,26 Because catecholamines have been shown to increase both coronary venous adenosine concentration27 and epicardial well adenosine concentration,12 it is unclear in the present experiments and in other whole-animal hypoxia exper-
the action of SAH hydrolase. However, the equilibrium constant of the hydrolase reaction favors SAH synthesis and will proceed in this direction in the presence of excess homocysteine. The accumulation of SAH during infusion of homocysteine is a measure of free intracellular adenosine concentration. In the present experiments, measurements of myocardial SAH levels were made during both normoxia and hypoxia in two groups of adrenergically blocked dogs. Figure 9 illustrates that myocardial SAH levels during hypoxia were not different in any layer of the ventricular wall from the normoxic controls, indicating that intracellular adenosine was not elevated during hypoxia with adrenergic blockade. The failure of intracellular adenosine to increase during hypoxia with adrenergic receptors blocked confirms the results of the plasma and epicardial well measurements in the adrenergically blocked group.

The magnitude of the hypoxic coronary vasodilation was markedly different between the adrenergically intact and blocked groups, despite similar levels of coronary venous oxygen tension (Figures 3 and 4). Folle and Aviado also demonstrated an attenuation of hypoxic coronary vasodilation with adrenergic blockade. The attenuation of coronary flow with adrenergic blockade suggests that much of the coronary vasodilation attributed to hypoxia is not dependent on myocardial oxygen tension but is mediated by catecholamines during hypoxia. Trivella et al. have recently demonstrated that coronary blood flow can be increased by β-activation in the absence of inotropic and chronotropic effects, so it is probable that catecholamines may have activated coronary β-receptors to cause vasodilation during hypoxia in the adrenergically intact group. Further, since adenosine was elevated throughout the hypoxic period with adrenergic receptors intact (Figure 4), it is possible that catecholamines potentiated adenosine-mediated vasodilation by activating α-receptors, as described by Hori and coworkers.

The mechanism for the adrenergic-adenosine interaction found in the present experiments is unknown. Adrenergic β-receptor agonists have been shown to increase adenosine concentration in both coronary venous plasma and epicardial well measurements. Adrenergic β-receptor activation in the myocardium is associated with increased levels of second messenger cyclic AMP. Cyclic AMP is converted to AMP by phosphodiesterase, and AMP is the substrate for 5'-nucleotidase, which converts AMP to adenosine. Manfredi and Sparks suggested that the augmented adenosine release observed during norepinephrine infusion could be due to increases in cyclic AMP mediated by adrenergic β-receptor activation. However, Barden-

![Graph showing myocardial tissue content of S-adenosylhomocysteine of eight α- and β-blocked experiments in which homocysteine was infused during normoxia (n=4) and during hypoxia (n=4). There was no difference in transmural S-adenosylhomocysteine concentration. Values are mean±1 SEM.](image-url)

**Table 3. Blood Metabolite Values for Homocysteine Experiments With α- and β-Blockade**

<table>
<thead>
<tr>
<th></th>
<th>Normoxia</th>
<th></th>
<th>Hypoxia</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>4 min</td>
<td>10 min</td>
<td>16 min</td>
<td>4 min</td>
</tr>
<tr>
<td>Arterial Pco₂ (mm Hg)</td>
<td>38.1±0.1</td>
<td>38.0±0.4</td>
<td>37.6±0.3</td>
<td>37.7±0.7</td>
</tr>
<tr>
<td>Arterial [H⁺] (nM)</td>
<td>41.0±0.2</td>
<td>41.0±0.5</td>
<td>40.5±1.0</td>
<td>43.9±1.0</td>
</tr>
<tr>
<td>Base excess (mM)</td>
<td>0.1±0.2</td>
<td>0.5±0.3</td>
<td>0.2±0.2</td>
<td>−2.0±0.3</td>
</tr>
<tr>
<td>Arterial lactate (mM)</td>
<td>1.58±0.16</td>
<td>1.55±0.21</td>
<td>1.45±0.23</td>
<td>3.19±0.5</td>
</tr>
<tr>
<td>Lactate extraction (%)</td>
<td>60.8±2.74</td>
<td>58.3±3.50</td>
<td>58.3±3.73</td>
<td>35.3±2.0</td>
</tr>
</tbody>
</table>

Values are mean±SEM (n=4 for both sets of experiments).
heuer and Schrader\textsuperscript{35} demonstrated that the contribution of the cyclic AMP pathway in the overall production of adenosine is small; therefore, it is unclear to what extent cyclic AMP levels contributed to the results of the present study.

Adrenergic \( \alpha \)-receptor blockade blunts coronary vasodilation caused by infused adenosine.\textsuperscript{36,37} Hori and coworkers\textsuperscript{32,38} have demonstrated that \( \alpha_2 \)-receptor activation results in augmented adenosine release during graded ischemia, while \( \alpha_2 \) activation results in increased vasodilator sensitivity of coronary vessels to adenosine. The results of the present study are consistent with those of Hori et al.

Adrenergic activation during hypoxia might increase intracellular levels of AMP. An increase in AMP concentration would promote adenosine formation by mass action via the 5\textsuperscript{'}-nucleotidase reaction. Magnetic resonance measurements made during norepinephrine infusion in isolated hearts\textsuperscript{39,40} indicate a decreased phosphorylation potential and thus an increased level of intracellular AMP; however, this has not been observed in vivo.\textsuperscript{41,42}

Imai et al.\textsuperscript{43} have suggested that adenosine formation occurs via ecto-5\textsuperscript{'}-nucleotidase activity on ATP released from adrenergic nerves. It is possible that ectoenzyme conversion of ATP released from adrenergic nerves could account for the difference in the v \( \rightarrow \) a and epicardial well adenosine response during hypoxia between the two groups. However, two lines of evidence argue against this possibility. First, Schütz et al.\textsuperscript{42} showed that adenosine release into the coronary effluent during hypoxia was not affected by the ecto-5\textsuperscript{'}-nucleotidase inhibitor \( \alpha, \beta \)-methylene adenosine 5\textsuperscript{'}-diphosphate (AOPCP), suggesting that extracellular conversion of adenosine nucleotides to adenosine was not an important pathway in adenosine formation during hypoxia. Second, Borst and Schrader\textsuperscript{44} observed that, in the presence of AOPCP, adenosine nucleotide release from the myocardium during hypoxia was small compared with the release of adenosine. Because the release of the necessary precursors for ectoenzyme conversion to adenosine is minimal, it is unlikely that adenosine formation via ecto-5\textsuperscript{'}-nucleotidase was an important difference between the two groups in this study.

Another possibility for the adrenergic-adenosine interaction found in the present experiments involves increased activity of 5\textsuperscript{'}-nucleotidase. The activity of in vitro 5\textsuperscript{'}-nucleotidase is under allosteric control by inorganic phosphate, hydrogen ion, and the phosphate energy charge.\textsuperscript{45,46} He et al.\textsuperscript{46} suggest that the biphasic relation observed between adenosine release and phosphorylation potential is evidence that in vivo 5\textsuperscript{'}-nucleotidase is under allosteric control. It is possible that adrenergic activation, either directly or indirectly, increases 5\textsuperscript{'}-nucleotidase activity by an allosteric mechanism, which could explain the difference between the adrenergically intact and blocked groups in the present study.

Wangler et al.\textsuperscript{47} observed that the \( d \)-isomer of propranolol, a compound with membrane stabilizing effects but without \( \beta \)-blocking activity, blunted the increase in adenosine release observed with hypoperfusion. Wangler et al.\textsuperscript{48} later concluded that \( d \)-propranolol acted by preventing a fall in coronary venous oxygen tension during underperfusion. In the present experiments, coronary venous oxygen tension during hypoxia was similar in the adrenergically intact and blocked groups (Figure 4); therefore, it is unlikely that the membrane stabilizing effect of \( dl \)-propranolol on coronary venous oxygen tension was a factor in the difference between the two groups with and without adrenergic blockade.

In conclusion, the adenosine hypothesis was tested by making measurements of coronary v \( \rightarrow \) a plasma adenosine concentration, epicardial well adenosine concentration, and myocardial tissue SAH content during hypoxia in open-chest, anesthetized dogs. With adrenergic receptors intact, coronary flow increased 240\%, while v \( \rightarrow \) a and epicardial well adenosine concentrations were increased relative to normoxic control values throughout an 18-minute period of hypoxia. In adrenergically blocked animals subjected to hypoxia that produced similar levels of coronary venous oxygen tension, coronary flow increased only 75\%, which was significantly less than in the adrenergically intact group (\( p < 0.01 \)). The v \( \rightarrow \) a adenosine concentration was only transiently increased at the beginning of steady-state coronary hypoxia, and epicardial well adenosine concentration was unchanged from control. In the presence of adrenergic blockade, myocardial levels of SAH were not significantly higher during hypoxia than normoxic values. It is concluded that much of the coronary vasodilation associated with systemic hypoxia is dependent on adrenergic activation and that adenosine may play a role in sustained hypoxic coronary vasodilation only when adrenergic receptors are intact.

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