Consistent Parallel Relationships among Myocardial Oxygen Consumption, Coronary Blood Flow, and Pericardial Infusate Adenosine Concentration with Various Interventions and β-Blockade in the Dog

Robert M. Knabb, Stephen W. Ely, Alban N. Bacchus, Rafael Rubio, and Robert M. Berne

SUMMARY. Coronary blood flow responds uniquely to changes in myocardial oxygen demand, regardless of the stimulus. If adenosine mediates this response, interstitial fluid adenosine concentration should also change in parallel with myocardial oxygen consumption and coronary blood flow during alterations of cardiac work. We tested this hypothesis by measuring coronary blood flow, myocardial oxygen consumption, and the concentration of adenosine in pericardial infusates, an index of interstitial fluid adenosine concentration, during six experimental conditions and control states in anesthetized, open-chest dogs. Significant alterations of myocardial oxygen consumption and coronary blood flow during aortic constriction, vagal stimulation, atrial pacing, or intravenous infusion of calcium chloride, norepinephrine, or isoproterenol were accompanied by significant alterations in pericardial infusate adenosine concentration. Significant linear relationships were determined among myocardial oxygen consumption, coronary blood flow, and pericardial infusate adenosine concentration for each of the experimental stimuli and their paired control values. There were no significant differences among the six different conditions for any of these relationships. In addition, these relationships were not altered by β-blockade in five dogs subjected to aortic constriction and calcium infusion. Although β-blockade may alter the effects of a stimulus, myocardial oxygen consumption, coronary blood flow, and adenosine all are affected proportionately. The results suggest that adenosine production responds to alterations of myocardial oxygen consumption independently of the stimulus which produces the change in oxygen demand, and the resultant change in interstitial fluid adenosine concentration may initiate the change in coronary blood flow to maintain the balance between oxygen supply and demand.

For many years, the fact that there is a close correlation between myocardial oxygen consumption (MVO₂) and coronary blood flow (CBF) has been recognized (Eckenhoff et al., 1947; Allela et al., 1955; Khouri et al., 1965). Increases in the oxygen demand of the heart must be matched by increases in oxygen supply, primarily via increases in coronary blood flow, since the heart is dependent on aerobic metabolism and extracts most of the oxygen from the blood perfusing it. This relationship has supported hypotheses which attribute regulation of coronary blood flow to vasoactive products of myocardial metabolism (Berne, 1963; Haddy and Scott, 1968).

A variety of mechanisms or vasoactive substances may contribute to the matching of oxygen supply to oxygen demand under different circumstances. However, the consistent parallelism observed between CBF and MVO₂ during a wide variety of experimental conditions may be indicative that a single mechanism or metabolite predominates in the adjustment of coronary vascular resistance.

Considerable evidence has accumulated to support the hypothesis (Berne, 1963) that alterations in the oxygen supply to demand ratio of the heart are compensated for by altered myocardial production of adenosine, which changes the diameter of the coronary resistance vessels and thereby restores the balance between oxygen supply and demand. This evidence, summarized in a recent review (Berne, 1980), includes studies which demonstrated increased production of adenosine during conditions of reduced oxygen supply (Katori and Berne, 1966; Rubio and Berne, 1969; Schrader et al., 1977a), as well as during increased oxygen demand (Wiedmeier and Spell, 1977; Foley et al., 1978; Miller et al., 1979; Watkinson et al., 1979; Saito et al., 1980; McKenzie et al., 1980, 1982; Bacchus et al., 1982).

If adenosine participates in the matching of CBF to MVO₂ under all circumstances then adenosine production must be linked to the resultant oxygen usage from a stimulus, and thus there should be consistent parallel relationships among MVO₂, CBF, and adenosine production, which are independent of the experimental conditions. We tested this
by inducing changes in MVO2 by a variety of experimental stimuli and in the presence of β-receptor blockade, and observed relationships among MVO2, CBF, and the concentration of adenosine in pericardial infusates (an index of interstitial fluid adenosine concentration).

Methods

General

Experiments were performed on adult, mongrel dogs of either sex, weighing between 18 and 32 kg. Animals were anesthesized with sodium pentobarbital (30 mg/kg, intravenously) with additional anesthetic given throughout the experiment as necessary. A cuffed endotracheal tube was inserted and positive pressure ventilation with room air enriched with 100% oxygen was initiated with a Harvard Apparatus model 607 respirator. Arterial blood gases and pH were maintained within the following ranges: PO2 90–150 mm Hg; PCO2 25–40 mm Hg; pH 7.35–7.45.

A catheter was inserted in the right femoral vein for the administration of drugs. A catheter was inserted in the right femoral artery and advanced to the arch of the aorta for monitoring aortic pressure and obtaining arterial blood samples. All pressure measurements were accomplished with Statham P23 low volume displacement transducers and a Gould 200 Brush recorder. Mean pressures were obtained by electronic filtering. A metal cannula was inserted in the left common carotid artery to serve as the source of blood for perfusing the left coronary artery. The cervical vagi were partially isolated from the carotid arteries and surrounding connective tissue. In all experiments, the vagi were cut following control periods.

A small thoracotomy was performed in the 5th right intercostal space to permit manual guidance of a USCI Sones Positrol catheter (7.5 french) into the coronary sinus via the right external jugular vein. The catheter tip was advanced at least 2 cm past the ostium of the coronary sinus to ensure that blood samples withdrawn through the catheter did not contain right atrial blood. The catheter was sutured in place at the exposure of the jugular vein, and the position of the catheter tip was confirmed manually from the left side of the chest prior to sampling. The 5th and 6th ribs were juxtaposed and the overlying muscle layers sutured together.

A thoracotomy was performed in the 4th left intercostal space to allow access to the left coronary artery. Heparin (750 U/kg) was administered intravenously before cannulation of the coronary artery to prevent clotting. Using the method of Miller et al. (1979), the circumflex branch or anterior descending branch of the left coronary artery was perfused via an extracorporeal circuit with blood from the dog's left common carotid artery. This circuit contained a cannulating, electromagnetic flow probe (Biotronex, 3.5 mm) which was calibrated each day prior to cannulation of the coronary artery. A bypass segment around the flow probe permitted zero flow settings without interrupting flow to the coronary artery. Pulsatile and mean coronary perfusion pressures were monitored via a side port in the circuit.

A metal guide cannula was introduced into the subclavian artery and advanced retrograde down the ascending aorta to the left coronary ostium. At this point, an inner polyethylene catheter (i.d. 2 mm) was advanced 1–2 cm to wedge in the circumflex or anterior descending branch of the left coronary artery. To measure blood flows accurately, it was essential that all flow through the vessel cannulated passed through the extracorporeal circuit and not around the catheter tip. Adequate seal of the catheter inside the vessel was verified by occluding the circuit upstream from the pressure port. Distal coronary pressures were always below 25 mm Hg, which is within the reported range of distal coronary pressures after abrupt occlusion of a major artery (Corday et al., 1974; Pasyk et al., 1971; Khouri et al., 1971). In addition, release of a 15-second occlusion always resulted in peak reactive hyperemic flows more than twice the preocclusion flow. At the end of each experiment, the area of myocardium perfused was stained via the coronary catheter with trypan blue. No portion of the coronary artery proximal to the position of the catheter tip was stained, again demonstrating the seal of the catheter inside the vessel. The stained myocardium was excised and weighed, and all coronary blood flows were normalized per 100 g left ventricle, using these weights. The average weight perfused was 57 g, with a range of 42–73 g.

For introduction and withdrawal of pericardial infusate, a flexible Silastic catheter (i.d. 3 mm) was inserted into the pericardial space through a small puncture hole in the pericardium and sutured in place to make a fluid-tight seal. The fluid used as pericardial infusates was an isosmotic Krebs-Henseleit solution (pH 7.4, 37°C, equilibrated with 95%, O2, 5% CO2) with the following millimolar composition: NaCl, 121.4; KCl, 4.7; CaCl2, 2.5; NaHCO3, 21.9; MgSO4, 1.2; KH2PO4, 1.2; glucose, 11.1.

Experimental Protocol

After cannulation of the left coronary artery, at least 45 minutes were allowed for the animal to stabilize before samples were taken. All samples were taken during steady state conditions of cardiac work, as evidenced by stable mean and pulsatile arterial pressure and mean coronary blood flow. A period of at least 5 minutes was allowed for the effects of any stimulus to reach a steady state before the sampling period was begun. At this time, the pericardial space was flushed 5–7 times with 25-ml aliquots of the Krebs-Henseleit solution. After the final rinse, another 25-ml aliquot was infused and left in contact with the epicardial surface of the heart for 4.5 minutes, after which time it was removed and placed in a 125-ml Erlenmeyer flask immersed in a boiling water bath for 10 minutes. At the halfway point during the 4.5-minute sampling period, paired arterial and coronary sinus blood samples were withdrawn anaerobically into heparinized glass syringes, capped, and kept on ice until analyzed for oxygen content (Lex-O2-con, Lexington Instruments). Samples were not acceptable if the pericardial infusates were contaminated by erythrocytes, since degradation of adenosine nucleotides from the cells could alter measured values of adenosine.

Sample Processing and Analysis

After immersion in the boiling water bath for 10 minutes, pericardial infusate (PCI) samples were kept on ice until the end of the experiment, when further processing was completed as previously described (Watkinson et al., 1979). Briefly, the samples were first centrifuged to remove calcium precipitates and proteinaceous material. The supernatant fractions were shaken with activated charcoal to adsorb the purines, which were subsequently eluted with a solution containing 10% pyridine, 50% ethanol, and 40% distilled water. The elution process was repeated...
Experimental Stimuli for Changing Cardiac Work

These experiments used six different experimental conditions to alter CBF and MVO₂ from control levels. Not all interventions could be done in all dogs, and therefore, presentation of results contains different control means for each intervention.

Aortic constriction was accomplished with a snare around the descending thoracic aorta. Gradual constriction was induced to elevate aortic blood pressure by 25–40 mm Hg. Calcium was infused intravenously as calcium chloride at a rate of 0.25 g/min. Vagal stimulation was by square wave electrical stimulation of the distal end of the cut right cervical vagus nerve. Stimulus intensity and frequency were adjusted to achieve bradycardia without causing abnormal rhythms. Atrial pacing was by square wave electrical stimulation of the left atrial appendage. Stimulus parameters were adjusted to achieve maximal heart rate attainable without producing arrhythmias. Norepinephrine was infused intravenously as norepinephrine bitartrate at a rate of 0.1–0.2 μg/kg per min. Isoproterenol was infused intravenously at rates of 0.1–0.2 μg/kg per min.

β-Blockade Experiments

To evaluate further the possibility of stimulus specific effects, we conducted a paired study in five animals in which the effects of aortic constriction and calcium infusion were examined before and after treatment with the β-receptor-blocking agent, propranolol. After control, aortic constriction, and calcium infusion samples were obtained, as described above, the animal received 2 mg/kg propranolol, intravenously. A adequacy of β-receptor blockade was affirmed by the absence of any hemodynamic effect to a bolus dose of isoproterenol (0.1 μg/kg, i.v.). A second set of control, aortic constriction, and calcium infusion samples then were obtained, using identical stimuli in the presence of propranolol. Values obtained before propranolol was administered were included as part of the basic relationships for aortic constriction and calcium.

Statistical Analysis

All experiments were designed so that paired comparisons could be applied. Thus, whereas not all interventions were performed in each dog, each experimental measurement could be compared with control values from the same dog. A paired t-test was used to compare the group of values from each intervention with its appropriate group of control values. For the β-blockade study, comparisons were desired between the experimental measurements and the control values, as well as for the same condition before and after β-blockade. For this purpose a two-way analysis of variance was used to detect significant differences among means, and Duncan’s test was used to determine which means were different.

Linear regression analysis was used to determine the relationship between two variables. So that relationships obtained under different experimental conditions could be compared, an analysis of variance of the interaction between the two variables was conducted.

Differences were considered significant at the 5% confidence level. All results are expressed as mean ± SE.

Results

Relationships among MVO₂, CBF, and PCI

Adenosine Concentration

Each of the six experimental stimuli induced significant alterations of CBF, MVO₂, and PCI adenosine concentration from control levels. The number of samples that could be obtained from a single dog varied, due to stability of the preparation as based on arterial pressure, reactivity of the coronary vascular bed, and the absence of erythrocytes in the pericardial infusates. Thus, in addition to a single control measurement, two to five interventions were applied in an individual experiment. Since not all interventions were performed in each dog, the effects of a given stimulus were compared with the appropriate group of paired control values, as shown in Table 1. A total of 87 observations were made as follows: control 26; aortic constriction, 16; calcium chloride infusion, 13; vagal stimulation, 10; atrial pacing, 8; norepinephrine infusion, 7; isoproterenol infusion, 7.

Neither arterial nor coronary sinus blood oxygen contents were significantly changed by any of the stimuli. Arterial oxygen content for the pooled controls was 19.5 ± 0.5 ml/100 ml. This relatively high arterial oxygen content resulted in large oxygen extractions and, thus, relatively low CBF for a given level of MVO₂.

CBF and MVO₂ for all experimental conditions were linearly related, as shown in Figure 1. Individual regressions for each stimulus and its paired controls, as well as for all conditions together, are described by the parameters for the first equation in Table 2. All seven regressions are highly significant, and are not statistically different from one another.

The relationship between PCI adenosine concentration and MVO₂ is similarly shown in Figure 2, and by the parameters given for the second equation in Table 2. There is no significant difference between any of these individual regressions. Thus, for a given change in MVO₂, the amount of adenosine entering the PCI is the same, regardless of what stimulus initiated the change in MVO₂.

CBF and PCI adenosine concentration were also linearly related. Figure 3 demonstrates this relationship for all 87 observations, and the third set of
TABLE 1
Summary of Six Experimental Conditions and Their Respective Controls

<table>
<thead>
<tr>
<th>Condition</th>
<th>CBF (ml/min per 100 g)</th>
<th>MVO₂ (ml/min per 100 g)</th>
<th>PCI adenosine (pmol/ml)</th>
<th>MAP (mm Hg)</th>
<th>CVR (pμl/100 g)</th>
<th>O₂ extraction (ml/100 ml)</th>
<th>CS O₂ (ml/100 ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aortic constriction Controls</td>
<td>64.6 ± 3.4*</td>
<td>73.3 ± 5.8*</td>
<td>133 ± 3*</td>
<td>14.3 ± 0.4</td>
<td>4.3 ± 0.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>55.9 ± 3.5</td>
<td>45.9 ± 4.7</td>
<td>102 ± 3</td>
<td>14.4 ± 0.4</td>
<td>4.0 ± 0.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Calcium           Controls</td>
<td>76.3 ± 4.2*</td>
<td>82.3 ± 4.6*</td>
<td>110 ± 5*</td>
<td>15.2 ± 0.5</td>
<td>3.8 ± 0.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>47.6 ± 3.2</td>
<td>42.2 ± 3.3</td>
<td>100 ± 3</td>
<td>14.6 ± 0.5</td>
<td>4.1 ± 0.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vagal stimulation Controls</td>
<td>36.2 ± 5.3*</td>
<td>38.9 ± 5.5*</td>
<td>96 ± 4†</td>
<td>15.8 ± 0.9</td>
<td>4.6 ± 0.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>49.2 ± 5.7</td>
<td>56.2 ± 6.2</td>
<td>105 ± 4</td>
<td>16.0 ± 0.8</td>
<td>4.2 ± 0.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Atrial pacing Controls</td>
<td>52.1 ± 5.1*</td>
<td>66.1 ± 6.1†</td>
<td>107 ± 5</td>
<td>16.7 ± 1.0</td>
<td>4.4 ± 0.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>41.7 ± 3.2</td>
<td>49.2 ± 4.1</td>
<td>106 ± 6</td>
<td>16.4 ± 0.9</td>
<td>4.1 ± 0.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Norepinephrine Controls</td>
<td>58.2 ± 6.1†</td>
<td>70.6 ± 6.9†</td>
<td>126 ± 9†</td>
<td>15.5 ± 0.7†</td>
<td>4.0 ± 0.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>46.8 ± 6.0</td>
<td>41.0 ± 6.3</td>
<td>101 ± 5</td>
<td>14.4 ± 0.5</td>
<td>4.3 ± 0.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Isoproterenol Controls</td>
<td>62.6 ± 5.7*</td>
<td>75.6 ± 7.5*</td>
<td>92 ± 6</td>
<td>16.1 ± 1.1</td>
<td>4.6 ± 0.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>39.5 ± 2.8</td>
<td>45.6 ± 4.6</td>
<td>100 ± 4</td>
<td>16.5 ± 1.1</td>
<td>3.8 ± 0.2</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SE. MAP = mean arterial pressure; CVR = mean coronary vascular resistance; CS O₂ = coronary sinus oxygen content.
* Significantly different from respective control, P < 0.01.
† Significantly different from respective control, P < 0.05.

parameters in Table 2 describe significant, individual regressions, which, as for the relationships described above, are not significantly different from one another.

β-Blockade Experiments

Aortic constriction and infusion of calcium chloride in five animals produced significant elevations of CBF, MVO₂, and PCI adenosine concentration before and after β-receptor blockade with propranolol, as determined by analysis of variance. As shown in Figure 4, there was no significant difference between pre- and post-β-blockade CBF, MVO₂, and PCI adenosine during control and aortic constriction. β-Blockade significantly attenuated the response to identical doses of CaCl₂ compared with that before β-blockade, but CBF, MVO₂, and PCI adenosine were all affected similarly.

Since β-blockade tended to decrease CBF, MVO₂,

![Figure 1. Relationship between myocardial oxygen consumption and mean coronary blood flow in 26 anesthetized dogs during six experimental stimuli and control states. Equation for regression line given in Table 2, column 1.](http://circres.ahajournals.org/)

TABLE 2
Slopes, Intercepts, and Correlation Coefficients for Individual Relationships among MVO₂, CBF, and PCI Adenosine Concentration

<table>
<thead>
<tr>
<th>Relationship</th>
<th>CBF = a × MVO₂ + b</th>
<th>PCI ADO = a × MVO₂ + b</th>
<th>CBF = a × PCI ADO + b</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>a</td>
<td>b</td>
<td>r</td>
</tr>
<tr>
<td>Aortic constriction</td>
<td>6.18 ± 0.9*</td>
<td>6.91 ± 0.3*</td>
<td>0.93*</td>
</tr>
<tr>
<td>Calcium</td>
<td>6.09 ± 0.9*</td>
<td>6.16 ± 0.3*</td>
<td>0.93*</td>
</tr>
<tr>
<td>Vagal stimulation</td>
<td>7.03 ± 3.7*</td>
<td>7.37 ± 0.8*</td>
<td>0.95*</td>
</tr>
<tr>
<td>Atrial pacing</td>
<td>5.18 ± 3.8*</td>
<td>7.85 ± 0.8*</td>
<td>0.83*</td>
</tr>
<tr>
<td>Norepinephrine</td>
<td>6.70 ± 0.53</td>
<td>0.94*</td>
<td>1.65 ± 8.07</td>
</tr>
<tr>
<td>Isoproterenol</td>
<td>5.11 ± 8.71</td>
<td>0.87*</td>
<td>6.96 ± 2.94</td>
</tr>
<tr>
<td>All conditions</td>
<td>6.23 ± 3.20</td>
<td>0.92*</td>
<td>6.99 ± 2.84</td>
</tr>
</tbody>
</table>

n = number of observations for each relationship; a = slope; b = intercept; r = correlation coefficient.
* Significant relationship, P < 0.01.
† Significant relationship, P < 0.05.
and PCI adenosine, the effects of $\beta$-receptor blockade were evaluated by comparing the relationships among CBF, $\text{MVO}_2$, and PCI adenosine obtained after $\beta$-blockade with the same relationships before propranolol was administered (Table 3). Analysis of variance determined no significant difference between any of the relationships before and after propranolol.

**Discussion**

These experiments support the hypothesis that adenosine mediates changes in CBF which maintain the balance between myocardial oxygen supply and the oxygen demands of the heart (Berne, 1963). This critical balance can be perturbed by either decreasing the oxygen supply to the heart or by increasing the work and, therefore, the oxygen demands, of the heart. Until a few years ago, most investigations of the adenosine hypothesis examined the former of these two cases, and considerable evidence supports a role for adenosine in the maintenance of normal oxygen supply during hypoxic perfusion (Rubio and Berne, 1969; Rubio et al., 1974; Schrader et al., 1977).

**Table 3**

Slopes, Intercepts, and Correlation Coefficients for Relationships among $\text{MVO}_2$, CBF, and PCI Adenosine Concentration before and after $\beta$-Blockade with Propranolol

<table>
<thead>
<tr>
<th></th>
<th>CBF = a \times \text{MVO}_2 + b</th>
<th>PCI ADO = a \times \text{MVO}_2 + b</th>
<th>CBF = a \times \text{PCI ADO} + b</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$a$</td>
<td>$b$</td>
<td>$r$</td>
</tr>
<tr>
<td>Before $\beta$-block</td>
<td>5.9</td>
<td>10.8</td>
<td>0.89*</td>
</tr>
<tr>
<td>After $\beta$-block</td>
<td>8.2</td>
<td>-9.8</td>
<td>0.86*</td>
</tr>
</tbody>
</table>

$a$ = slope; $b$ = intercept; $r$ = correlation coefficient.

* Significant relationship, $P < 0.01$. 

---

**FIGURE 2.** Relationship between myocardial oxygen consumption and pericardial infusate adenosine concentration. Symbols represent same conditions as defined in Figure 1. Equation for regression line given in Table 1.

**FIGURE 3.** Relationship between pericardial infusate adenosine concentration and mean coronary blood flow. Symbols represent same conditions as defined in Figure 1. Equation for regression line given in Table 2.

**FIGURE 4.** Effects of aortic constriction and calcium infusion before and after $\beta$-blockade with propranolol. Solid stars represent significantly different values from respective control value. Open stars represent significantly different values from same condition before $\beta$-blockade.
or after interruption of flow (Rubio et al., 1969; Olsson, 1970; Olsson et al., 1978; Saito et al., 1981). A growing body of evidence also supports a role for adenosine in mediating changes in CBF under more physiological conditions, when oxygen demand is augmented during conditions of normal oxygen supply (Wiedmeier and Spell, 1977; Foley et al., 1978; Miller et al., 1979; McKenzie et al., 1980, 1982; Saito et al., 1980; Bacchus et al., 1982).

Of particular relevance to this study are two previous studies in which MVO$_2$, CBF, and PCI adenosine concentration were measured. Miller et al. (1979) increased cardiac work with bilateral stellate ganglion stimulation, and, in addition to finding significant correlations among MVO$_2$, CBF, and PCI adenosine, showed that changes in PCI adenosine were proportional to changes in tissue adenosine. Bacchus et al., (1982) used physiological stimuli (exercise, excitement, feeding) in conscious dogs and found linear relationships among MVO$_2$, CBF, and PCI adenosine concentration. In both these studies, cardiac work was elevated via the sympathetic nervous system; thus it was not determined whether adenosine production responded to the change in oxygen usage or, directly, to the stimulus. The current study utilized various interventions to alter cardiac work to identify further the factors which change myocardial adenosine production.

Critical to the conclusions of this study is the validity of using the amount of adenosine accumulating in pericardial infusates as an index of interstitial fluid adenosine concentration. Various investigators have used tissue measurements, venous washout, and pericardial infusates as indexes of the concentration of adenosine in the interstitium, and each of these methods has advantages and disadvantages, as summarized in a recent report from this laboratory (Bacchus et al., 1982). An index of the interstitial concentration of a metabolite need not determine the actual concentration of that metabolite, but, to be a reliable index, changes in the index should occur only in response to changes in the interstitial fluid concentration of that metabolite. The reliability of using pericardial infusates is supported by evidence that adenosine accumulates in the pericardial infusates in an exponential fashion which may approach equilibrium with interstitial fluid adenosine concentration (Miller et al., 1979; Olsson et al., 1982). This suggests that transfer into pericardial infusates takes place by a process of simple diffusion, in which case the interstitial fluid concentration of adenosine is the factor that determines the amount of adenosine accumulating over a fixed period of time.

This assumption, and, thus, the interpretations of pericardial infusate data, have however been questioned in a recent paper by Kusachi and Olsson, (1983). Examining the time course of adenosine accumulation in pericardial superfusates, they found that, under control conditions, a steady state adenosine concentration (average 68 nM) was usually attained within 4.5 minutes, in sharp contrast to an earlier finding that a steady state (average 236 nM) was not reached until 20–30 minutes (Olsson et al., 1982). Furthermore, they found that the rate of adenosine equilibration varied substantially among different animals, between successive trials in the same animal, and for different experimental conditions. Interpretation of these findings are difficult in view of the conflicting results obtained in two studies from the same laboratory. Furthermore, it is possible that the different rates of equilibration that they observed were the result of the large variability present in the data.

Whereas many details of their experimental protocol were different from those used in our study, we must consider the effects of possible differences in the rate of adenosine equilibration on our results. Three cases are possible: (1) the rate of adenosine equilibration in pericardial infusates is the same for control conditions as for the experimental interventions; (2) the rate is faster during control conditions; (3) the rate is faster during experimental interventions. The first case will be true if transfer into pericardial infusates is via diffusion. If this is the case, then the concentration in pericardial infusates after a fixed time of equilibration is a true quantitative index of the concentration when a steady state is reached. The second case could lead to an underestimation of the steady state amount of adenosine during the experimental interventions as compared with controls. Thus, the concentration at 4.5 minute may not be a quantitative index of interstitial adenosine concentration, but increases above control levels would be underestimated, not overestimated. This could conceivably be the cause of a decrease in pericardial infusate adenosine concentration with vagal stimulation. The third case could lead to an overestimation of the steady state amount of adenosine during experimental conditions, compared with controls, if sampling occurs before a steady state is reached during the control periods. If this is indeed 4.5 minutes, as found by Kusachi and Olsson (1983), then this case cannot affect our data.

Thus, if any of these limitations are a factor, it would seem that our index of interstitial adenosine concentration may underestimate the changes that occur with changes in cardiac work. It must be stressed that we use pericardial infusate adenosine concentration as an index, not a direct measure of interstitial adenosine concentration. Since the pericardial space borders not only the left ventricle, but also the atria, right ventricle, and the parietal pericardium, the concentration of adenosine in pericardial infusates is not entirely the result of diffusion from the left ventricle, and uptake or production by any of these other structures will alter the absolute levels of adenosine. Thus, even the steady state pericardial adenosine concentration may not be in equilibrium with left ventricular interstitial fluid. Since these other structures are, however, less influenced by changes in cardiac work, it is reasonable...
to assume that changes in pericardial infusate adenosine levels, as compared with control levels, are due predominantly to changes in left ventricular adenosine concentration.

Up to the present, it has not been possible to measure the interstitial fluid concentration of adenosine directly, and thus it cannot be stated with certainty that any index currently in use is determined solely by interstitial fluid adenosine concentration. Therefore, interpretation of the results of these experiments, as for any experiments using one of these indirect indexes, involves the assumption that the index is reliable.

A simple linear relationship between CBF and MVO$_2$ has been demonstrated for a variety of experimental conditions since first reported by Eckenhoff et al. in 1947. In the present series of experiments, six different conditions were employed to alter MVO$_2$ and CBF from their control values. These maneuvers yielded a linear relationship between CBF and MVO$_2$ with a highly significant positive correlation, as shown in Figure 1. Furthermore, the slopes of the individual CBF vs. MVO$_2$ regression lines (Table 2) obtained for each experimental condition and the paired control values were not significantly different, indicating that the relationship between CBF and MVO$_2$ is unaltered by different interventions that alter MVO$_2$.

The consistent relationship between CBF and MVO$_2$ has suggested that the major factors controlling CBF are intrinsic to the heart, lending support to hypotheses that attribute regulation of CBF to metabolic substances produced by the parenchymal cells in response to their oxygen balance (Berne, 1963; Haddy and Scott, 1968; Berne and Rubio, 1979). Metabolic regulation of CBF may involve a combination of different substances as mediators. If, however, one particular vasoactive metabolite plays a major role in coupling CBF to MVO$_2$, then its production should be independent of the stimulus, and dependent on the resultant change in MVO$_2$, just as is true for CBF. These experiments examine this latter possibility for adenosine. It was the specific design of these experiments to utilize a variety of experimental conditions to examine the constancy of the relationships between MVO$_2$ and PCI adenosine and between PCI adenosine concentration and CBF.

As summarized in Table 2, significant positive correlations were found between MVO$_2$ and PCI adenosine concentration for each of the six experimental conditions and the paired control values. Like the individual relationships for MVO$_2$ and CBF, there is no significant difference among any of the control and experimental conditions, suggesting that altered ISF adenosine concentration, and, hence, altered adenosine production, occurred in response to the change in MVO$_2$ and was not specific to a particular stimulus.

The similar results regarding the relationship between PCI adenosine concentration and CBF (Table 2) demonstrate that the mean CBF at a given level of PCI adenosine concentration is the same for the six different stimuli which altered adenosine production. This suggests that a similar consistent relationship exists between interstitial fluid adenosine concentration and CBF.

Similar relationships were obtained by Saito et al. (1980), who found MVO$_2$, CVR, and tissue adenosine levels to be covariant with one another during various stimuli which changed cardiac work. In contrast to the current study, however, infusion of isoproterenol resulted in greater myocardial adenosine production than other stimuli which resulted in the same increase in MVO$_2$. A major difference between the two studies that may not allow direct comparisons is our use of an index of interstitial fluid adenosine concentration, and Saito's measurement of total tissue adenosine content, which, in addition to extracellular adenosine, also measures free or bound intracellular adenosine. Saito et al. (1980) concluded that the higher adenosine levels with isoproterenol supported the hypothesis (Olsson et al., 1973; Schrader and Gerlach, 1976) that adenosine production may preferentially occur from AMP which has been produced by the action of phosphodiesterase on cAMP.

A feature common to many of the experimental procedures used in this and other studies is the involvement of exogenous or endogenous catecholamines. Because of this, and the conflicting results obtained with isoproterenol, a paired study was conducted that was designed to elucidate the involvement of catecholamine-stimulated increases in cAMP, through the use of the $\beta$-receptor-blocking agent, propranolol. In order to study a range of oxygen consumptions both before and after $\beta$-blockade, we chose stimuli that would not depend solely on $\beta$-receptor stimulation to induce changes in MVO$_2$. Therefore, experiments were carried out with aortic constriction and calcium infusion as experimental stimuli before and after $\beta$-blockade.

Aortic constriction and calcium infusion produced significant elevations in CBF, MVO$_2$, and PCI adenosine concentration before and after propranolol, compared with the appropriate control values, as shown in Figure 4. For each of the relationships among MVO$_2$, CBF, and PCI adenosine concentration described in Table 3, the slopes of the lines before and after $\beta$-blockade are not significantly different, indicating that, whereas propranolol may alter the absolute values of MVO$_2$, CBF, and PCI adenosine concentration, the relationships among these variables remained unchanged.

The constant relationship between MVO$_2$ and CBF suggests that the coupling between them is not affected by $\beta$-receptor blockade. Most important, the constant relationship between MVO$_2$ and PCI adenosine concentration illustrates that there is no alteration in adenosine production by $\beta$-receptor activation that cannot be accounted for by the resultant change in MVO$_2$. 
The finding that β-blockade did not reduce adenosine production at any level of MVO₂ is in agreement with the observation that increases in MVO₂ produced by the activation of β-receptors with isoproterenol did not cause greater adenosine production than any other stimulus at the same MVO₂. Thus, β-receptor activation is not a necessary intermediate in the stimulation of myocardial adenosine production, and changes in adenosine production with β-receptor activation occur secondary to β-receptor stimulation of MVO₂.

Whereas the lack of greater adenosine production for a given MVO₂ with isoproterenol differs from the findings of Saito et al. (1980), a possibility which remains unexplored is that isoproterenol may cause disproportionate increases in intracellular adenosine relative to the change in extracellular adenosine. It is interesting to note that administration of propranolol in their study did not result in a lower adenosine production for a given MVO₂ as in the present study. They did not follow propranolol administration with stimuli to elevate MVO₂ but rather used propranolol as a single stimulus. The observations of this study do not support the idea that adenosine is preferentially formed from AMP which arises as a result of accelerated cAMP formation (Olsson et al., 1973; Schrader and Gerlach, 1976). Although adenosine may function to limit the myocardial effects of catecholamines (Schrader et al., 1977b), these results do not suggest that catecholamines result in greater adenosine production than other stimuli which produce the same MVO₂. By effectively ruling out catecholamine-induced alterations in adenosine production, the results extend and support the constant parallelisms among CBF, MVO₂, and adenosine production.

In conclusion, the results of this study are consistent with and support the hypothesis that adenosine mediates the coupling between MVO₂ and CBF. We have demonstrated a constant relationship between MVO₂ and PCI adenosine, with or without β-receptor blockade, suggesting that adenosine production responds to the resultant level of MVO₂ and not directly to the stimulus that initiates the change in MVO₂. The proposed chain of events is initiated by an increased demand for oxygen by the myocardial cells, which in turn increase their production of adenosine according to that demand. The resultant increase in ISF adenosine concentration causes relaxation of the vascular smooth muscle cells of the resistance vessels and, hence, an elevated CBF. Although the results are consistent with and support this hypothesis, they can by no means be construed as proof thereof. It is possible that some other vasoactive substance(s) is also produced in a manner that is coupled to oxygen usage, or that other substances interact with adenosine to produce the resultant change in CBF. However, the known vasoactivity of adenosine and the consistency of these relationships suggests that adenosine plays a major role in mediating the changes in coronary blood flow.

We gratefully acknowledge the technical assistance of V. Teyal, C. Taylor, and L. Wigginton.

Preliminary reports of this work were presented in the 31st Annual Fall Meeting of the American Physiological Society in Toronto, Ontario, October 14, 1980, at the 32nd Annual Fall Meeting of the American Physiological Society in Cincinnati, Ohio, October 13, 1981, and at the 66th Annual Spring Meeting of the Federation of American Societies for Experimental Biology in New Orleans, Louisiana, April 22, 1982.

Supported by U.S. Public Health Service Grant HL03848.

Address for reprints: Robert M. Berne, M.D., Dept. of Physiology, Box 449, Univ. of Virginia School of Medicine, Charlottesville, Virginia 22908.

Received October 11, 1982; accepted for publication May 19, 1983.

References


References
Schrader J, Haddy FJ, Gerlach E (1977a) Release of adenosine, inosine and hypoxanthine from the isolated guinea pig heart during hypoxia, flow-autoregulation and reactive hyperemia. Pfluegers Arch 369: 1–6

INDEX TERMS: Active hyperemia · Local regulation · Metabolic regulation · Adenosine hypothesis
Consistent parallel relationships among myocardial oxygen consumption, coronary blood flow, and pericardial infusate adenosine concentration with various interventions and beta-blockade in the dog.

R M Knabb, S W Ely, A N Bacchus, R Rubio and R M Berne

doi: 10.1161/01.RES.53.1.33

*Circulation Research* is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1983 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circres.ahajournals.org/content/53/1/33