Canine Myocardial Adenosine and Lactate Production, Oxygen Consumption, and Coronary Blood Flow during Stellate Ganglia Stimulation

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SUMMARY The vasodilator adenosine is produced continuously by the normal dog myocardium. However, the relation of adenosine production to increased cardiac metabolism requires clarification. This study tested the hypothesis that increased myocardial O₂ demand promotes increased adenosine production to match coronary blood flow (CBF) to metabolic requirements. Increased adenosine production, therefore, should correlate with increased CBF and myocardial oxygen consumption (MVO₂). Graded changes in CBF were induced through bilateral stellate ganglia stimulation (SGS) in open-chest dogs. Left CBF, MVO₂, and the adenosine and lactate contents of pericardial infusate (Krebs-Henseleit solution) were measured. Pericardial infusate was placed in contact with the epicardium through a cannula introduced through the otherwise intact pericardium. Left ventricular tissue samples frozen in situ also were obtained after the infusate was withdrawn and analyzed for adenosine and lactate. The correlation of infusate adenosine concentration with CBF was found to be significant, as was the correlation of adenosine concentration with MVO₂. Tissue adenosine levels increased significantly with SGS and were reflected in significant increases in pericardial infusate adenosine content. Adenosine concentration of the infusate was also measured after contact for different times with the epicardium (0.5–18 minutes) and found to increase continually throughout the times studied, but it did not reach equilibrium with tissue adenosine levels. Loss of adenosine from the infusate also was found to be rapid, indicating a high turnover of pericardial fluid adenosine. Results support adenosine as a coupler of myocardial metabolism to CBF and the use of pericardial infusate adenosine content as an index of tissue adenosine production.

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THE ADJUSTMENT of coronary blood flow (CBF) is the principal means whereby myocardial oxygen supply and oxygen demand are matched (von Restorff et al., 1977; Katz and Feinberg, 1958; Alella, et al., 1955; Eckenhoff, et al., 1947). The high oxygen extraction from coronary blood under basal conditions and the dependence of the heart on aerobic metabolism require that oxygen delivery, and thus CBF, be the parameter adjusted to ensure oxygen supply-demand equilibrium. Increases in myocardial oxygen consumption (MVO₂) have been correlated with CBF increases (Khouri et al., 1965; Alella et al., 1955; Eckenhoff et al., 1947), and this parallel relation supports the suggestion that a product (or products) of myocardial metabolism may function as a mediator between metabolism and flow. However, the exact manner by which cardiac metabolism is coupled to CBF regulation is yet unknown.

The purine nucleoside, adenosine, is formed from AMP by the action of 5'-nucleotidase, and when released into the interstitial fluid, adenosine elicits a substantial vasodilator response (Rubio and Berne, 1964; Rubio et al., 1973; Wiedmeier et al., 1972). Adenosine was proposed as a key factor in the metabolic regulation of CBF (Berne, 1963) and has been demonstrated to be released from the normally oxygenated heart (Olsson, 1970; Rubio and Berne, 1969). Evidence supporting an adenosine hypothesis of CBF regulation has accumulated. However, most of these studies involve a decreased oxygen supply as the stimulus for an adenosine response (Schrader et al., 1977; Rubio et al., 1969, 1974; Snow et al., 1973; Olsson, 1970; Rubio and Berne, 1969; Katori and Berne, 1966). A significant parallelism has been demonstrated between coronary flow and tissue adenosine content as well as the rate of adenosine release from isolated guinea pig hearts subjected to varying degrees of hypoxia (Rubio et al., 1974). Also, in situ experiments revealed that adenosine is present in coronary sinus blood during reactive hyperemia following brief periods of coronary occlusion (Rubio et al., 1969) and...
that tissue adenosine levels also were shown to increase with coronary occlusion (Olsson, 1970). Changes in tissue adenosine concentration following 5- and 15-second coronary occlusions show time constants comparable to those of the associated reactive hyperemic flows (Olsson et al., 1978), indicating that adenosine may be mediating the hyperemic response.

Of more physiological importance is the relationship between CBF and increases in myocardial metabolism under conditions in which blood flow and oxygen availability are not limiting. Evidence supporting a role for adenosine in coronary dilation associated with enhanced myocardial metabolic activity previously has been obtained from isolated heart preparations, in situ preparations, and in vivo experiments in which indirect hemodynamic indicators of changes in myocardial metabolism (e.g., heart rate, product of heart rate and mean arterial pressure, and maximum dP/dt) were related to adenosine release (Watkinson et al., 1979; Foley et al., 1978; Wiedmeier and Spell, 1977). Increased myocardial oxygen demand induced by catecholamines and histamine was shown to be correlated with increased adenosine levels in the coronary effluent of isolated guinea pig hearts (Wiedmeier and Spell, 1977), and increased external cardiac work produced by aortic constriction in the open-chest rat has been shown to elevate myocardial tissue levels of adenosine (Foley et al., 1978). Increased cardiac activity produced by stellate ganglion stimulation (SGS) in the open-chest dog and treadmill exercise in the intact dog also were shown to increase adenosine release from the epicardial surface of the heart (Watkinson et al., 1979). These latter studies suggest that adenosine release from the myocardium is increased under physiological conditions of increased metabolic activity.

Further experimentation is required to define the relationship proposed to exist among cardiac metabolism, adenosine release, and CBF regulation in terms of blood flow and oxygen consumption. Therefore, the objective of the present study was to test the hypothesis that, if CBF and \( \text{MVO}_2 \) reflect oxygen-supplying and oxygen-consuming processes for which a significant correlation has been established, and if adenosine functions as a mediator in the coupling of these processes, then a parallelism should be demonstrable between adenosine release and \( \text{MVO}_2 \) and adenosine release and CBF. The experimental approach adopted for this study was to determine if increases in metabolic activity caused by graded SGS would result in parallel increases in CBF and adenosine release.

**Methods**

**General**

Twenty-five mongrel dogs weighing 19–34 kg were studied. Each dog was anesthetized with an initial injection of sodium pentobarbital (30 mg/kg, iv), with additional anesthetic given as needed to maintain a constant level of anesthesia. The development of metabolic acidosis during anesthesia was prevented by a continuous drip infusion of 4% sodium bicarbonate (2 ml/kg per hour, iv). Rectal temperature was maintained at 37°C with a heating pad. Pulmonary ventilation was accomplished by a positive-pressure respirator (Harvard model 607D) with room air enriched with 100% O2. Blood gases and pH were monitored and maintained within acceptable ranges (\( \text{PO}_2 90–130; \text{PCO}_2 25–40; \text{pH} 7.36–7.44 \)). Respiratory rate and tidal volume were adjusted to dog body weight.

Blood pressure was measured at the level of the thoracic aorta with a polyethylene catheter and a Statham Model P23 pressure transducer. Pulsatile and mean arterial pressure, coronary perfusion pressure, coronary flow, and the electrocardiograph were recorded by a Gould Brush 200 recorder.

The heart was approached through a midsternal incision. The left and right stellate ganglia were isolated centrally, and the right and left sympathetic chains \((T_1-T_6)\) were removed; cardiac branches from the ganglia remained intact. Shielded palladium electrodes (Harvard Apparatus) were placed on the isolated stellate ganglia and wrapped with gauze. Electrode cables were connected to Grass SD5 stimulators. Bilateral cervical vagotomy was carried out to prevent reflex vagal effects secondary to SGS.

**Perfusion of Left Coronary Arteries**

Our experimental design required an intact pericardium. Therefore, to avoid opening the pericardial sac, an extracorporeal circuit was designed to permit free-flow perfusion of the left anterior descending (LAD) coronary artery or the left circumflex (LC) coronary artery via the coronary ostium. Blood coagulation in the extracorporeal circuit was prevented by the infusion of sodium heparin intravenously (1000 U/kg initially, plus 400 U/kg per hour). Blood was supplied to the circuit from the cannulated right common carotid artery and passed an electromagnetic flow probe (Biotronex) and a port through which coronary perfusion pressure was monitored, before entering the coronary catheter. The flow probe was calibrated in each experiment prior to coronary artery cannulation with blood from the experimental dog perfusing the circuit from the right carotid artery. The circuit also contained a flow probe bypass which permitted zero flow determinations throughout the course of the experiment without interrupting flow to the coronary vessels. A special metal guide cannula was used to position the coronary artery catheter at the left coronary ostium. The metal guide cannula was introduced through the left subclavian artery and advanced to the coronary ostium. By rotating the tip of the metal cannula, the coronary catheter (i.d.
of the left ventricle and weighed at the end of each experiment was stained with India ink, which was infused at pressures comparable with a 20-gauge 1/4-inch longdwell catheter and perfusion pressure monitored. Perfusion pressure was measured in the LAD artery to be within 5 mmHg of that measured in the extracorporeal circuit and did not change with complete occlusion of the left circumflex inflow. In addition, no electrocardiographic abnormalities suggesting ischemia were observed.

The area of the myocardium perfused during the experiment was stained with India ink, which was infused into the coronary cannula at the same site in the circuit from which perfusion pressure was measured. The ink was infused at pressures comparable to the experimental autoperfusion pressures. The stained area was cut from the remainder of the left ventricle and weighed at the end of each experiment. The average weight of perfused tissue was 64.2 ± 14.8 (SD) g in the 25 dogs studied.

PO2, PCO2, pH, and Lactate Measurements of Arterial and Coronary Sinus Blood

A polyethylene catheter (i.d. 2.0 mm) was advanced into the coronary sinus via the right external jugular vein. The catheter tip was positioned a minimum of 20 mm past the coronary sinus ostium. Blood withdrawn from the coronary sinus catheter was analyzed for oxygen content (Lexington Instruments Lex-O2-Con), PO2, PCO2, pH (Instrumentation Laboratories Model 113), and lactic acid (Gutmann and Wahlefeld, 1974). Arterial blood samples were taken simultaneously with coronary sinus blood samples and analyzed identically. The hematocrit ratio also was measured. Blood samples for lactic acid analysis were collected in ice-cold glass syringes and immediately mixed vigorously with two volumes of cold (0°C) 6% perchloric acid and centrifuged. The pH of the supernatant fraction was adjusted to 6.0 with KOH, decanted after salt precipitation, and stored at −20°C; lactic acid determinations were completed within 3 days of each experiment.

Pericardial Infusate and Tissue Sample Processing and Analysis

A flexible, Silastic cannula (i.d. 3.0 mm) was introduced into the pericardial space through a small puncture hole made in the pericardium. The cannula was held in position by a purse-string suture that was fluid-tight. Modified Krebs-Henseleit solution (37°C, pH 7.4, gassed with 95% O2-5% CO2) was infused and withdrawn through the cannula. The pericardial infusate (PCI) samples (40 ml), once withdrawn from the pericardial sac, were transferred immediately to 125-ml Erlenmeyer flasks. In 18 preparations, the Erlenmeyer flask was immersed in boiling water for 10 minutes, and in 7 preparations, 20 ml of the infusate were taken prior to boiling for prostaglandin E determination. The boiled perfusates were used for purine determinations and processed as previously described (Watkinson et al., 1979). One-milliliter aliquots also were taken from the 40-ml sample and analyzed for lactic acid. Since adenosine is degraded readily in the blood due to the presence of deaminating enzymes, the technique of sampling PCI provides the advantages of relative ease of sample handling and protection from loss of metabolite.

Tissue samples were obtained in 16 dogs from the free wall of the left ventricle midway between apex and base, avoiding large epicardial vessels. The transmural tissue sample was obtained by a stab incision with cutting-edged clamps precooled in liquid nitrogen. The in situ frozen sample was transferred immediately to a flask containing liquid nitrogen and the sample was dislodged from the clamps. Average wet weight of the tissue samples...
was 388 ± 41 (SD) mg. All samples were stored in liquid nitrogen until final processing. The frozen tissue samples were homogenized with a Polytron homogenizer in 10 volumes of cold (0°C) 1 n perchloric acid and then centrifuged. The supernatant fractions were neutralized with KOH and stored at −20°C. Both PCI and neutralized tissue extracts were analyzed for adenosine in a Perkin-Elmer 356 dual wavelength spectrophotometer as described previously (Dobson et al., 1971). Tissue lactate levels also were determined (Gutmann and Wahlefeld, 1974).

Prostaglandin E Assay of Pericardial Infusate

Radioimmunoassay (Clinical Assays) was used to assay for prostaglandin E (PGE) content of PCI collected under control conditions and during SGS. Infusate samples were acidified to pH 3.5–3.7 with 1 n HCl and extracted twice with ethyl acetate. After separation, the organic phase was evaporated to dryness under nitrogen and then redissolved in 0.2 ml of benzene (60 parts):ethyl acetate (40 parts):sucrose ([l-3H]sucrose, New England Nuclear) was added to the remaining 30 ml. After immediate mixing, another 10-ml aliquot was placed in the boiling water bath. This concentration of exogenous adenosine was sufficiently larger than the endogenous adenosine levels to permit changes in concentration due to enzymatic degradation to be readily measurable. The remaining 20 ml were divided into 10-ml aliquots, and any enzymatic activity was destroyed by heating in the boiling water bath at 1, 3, and 5 minutes after the addition of the adenosine standard.

Experimental Protocol

A stabilization period of 1 hour was permitted between completion of coronary artery cannulation and the beginning of the experimental protocol. The pericardial space was flushed a minimum of 4 times with 40 ml of Krebs-Henseleit solution initially, and between experimental manipulations. For control samples, the Krebs-Henseleit solution was introduced into the pericardial space and allowed to remain in contact with the epicardium for 4.5 minutes. The fluid then was withdrawn rapidly (15–20 seconds) using a 50-ml glass syringe and transferred immediately to an Erlenmeyer flask for processing as described above. During the period in which the solution remained in the pericardial sac, arterial and coronary sinus blood samples were collected in glass syringes for blood gas, pH, and lactic acid determinations.

Samples collected during bilateral SGS were handled in the same manner as control samples. Krebs-Henseleit solution was infused into the pericardial space. Graded stimulation (2-10 Hz, 5–7 V, 5-msec duration) was begun 1 minute after infusion of the solution was completed and was continued for 2.5 minutes. Blood samples were collected at 2.0 minutes of stimulation. The Krebs-Henseleit solution was withdrawn 1 minute after the completion of stimulation and processed as described above. A 15-minute recovery period was permitted between stimulations.

Tissue samples collected under conditions of SGS were obtained immediately after Krebs-Henseleit solution was removed and the pericardial sac opened. In experiments in which tissue samples were obtained, stimulation was continued through the time of tissue sampling (total stimulation time of 3.5 minutes). PCI samples collected under these conditions were used for comparison with tissue levels and were not included with data for 2.5-minute stimulations.

Data Analysis

Hemodynamic data were analyzed at points before (control) and during each response to SGS. Control values were taken from the 1-minute period immediately before the stimulation was begun. Steady state stimulation values were taken between 1.5 and 2.0 minutes of stimulation when the total stimulation period was 2.5 minutes. Standard errors reported reflect variability between dogs and were...
Results

\[ \text{MVO}_2, \text{CBF, and Adenosine} \]

An example of the CBF response (mean flow) to graded bilateral SGS is shown in Figure 1. Typically, an initial brief decrease in CBF was followed by a substantial increase in flow, which reached a plateau and was sustained for the duration of the stimulation period.

The relationship of MVO\(_2\) to CBF is shown in Figure 2 for experiments completed on 13 dogs. Graded increases in CBF are shown to be correlated with increased in MVO\(_2\) (\(r = 0.894; P < 0.001\)). Data plotted include control and stimulation measurements for each dog with each symbol representing a single dog. MVO\(_2\) values ranged from a low control of 4.5 to a maximum with stimulation of 21.6 ml O\(_2\)/min per 100 g left ventricle. CBF values ranged from the lowest control of 28 to a high of 152 ml/min per 100 g with SGS.

The relationship between adenosine and MVO\(_2\) is shown in Figure 3. Increases in MVO\(_2\) are paralleled by increases in adenosine concentration (pmol/ml) of PCI (\(r = 0.724; p < 0.001\)). Adenosine values ranged from 4.5 (lowest control) to 64.9 pmol/ml PCI with SGS. MVO\(_2\) values ranged from 4.5 to 21.6 ml O\(_2\)/min per 100 g left ventricle. Figure 4 shows the relation of PCI adenosine concentration to CBF. The correlation (\(r = 0.634\)) is lower than

\[ Y = 10.8 + 14.9X \]
\[ r = 0.894 \quad p < 0.001 \quad n=50 \]
\[ S_{yx} = 2.05 \]

\[ Y = 6.52 + 0.316X \]
\[ r = 0.634 \quad p < 0.001 \quad n=50 \]
\[ S_{yx} = 7.46 \]
that found for \( \text{MVO}_2 \) and adenosine, but is statistically significant \((P < 0.001)\).

Data (mean ± SE) are presented in Table 1 for \( \text{MVO}_2 \), CBF, PCI adenosine concentration, mean coronary perfusion pressure, heart rate, and diastolic resistance obtained under control conditions and at the maximum \( \text{MVO}_2 \) response with SGS. Statistical comparisons (paired t-test) showed significant differences \((P < 0.001)\) between control and stimulation responses for each of these parameters.

### Critique of Experimental Protocol

Inherent in the hypothesis being tested is the premise that blood flow and oxygen availability are not compromised and, therefore, that the changes in adenosine release result solely from increased oxygen demand and not from conditions of inadequate blood supply to the myocardium. Therefore, to estimate the possible contribution of ischemia as a determining factor in the adenosine response to SGS, additional variables considered to be indexes of tissue oxygenation were evaluated in relation to \( \text{MVO}_2 \) in control and stimulation groups. The arterial-coronary sinus blood oxygen difference (A-CSO\(_2\)) showed no significant increase above control at maximum \( \text{MVO}_2 \) response with SGS (Table 2). Coronary sinus blood Po\(_2\) was found not to change significantly from control (20 mmHg) with maximum \( \text{MVO}_2 \) response (19 mmHg). Oxygen extraction, which also reflects changes in A-CSO\(_2\), was

### Table 1  Changes in Hemodynamic and Metabolite Parameters with Stellate Ganglia Stimulation

<table>
<thead>
<tr>
<th></th>
<th>Control ((n = 15))</th>
<th>Response at maximum ( \text{MVO}_2 ) ((n = 15))</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \text{MVO}_2 ) (ml O(_2)/min per 100 g)</td>
<td>7.0 ± 0.47</td>
<td>16.0 ± 0.98 (P &lt; 0.001)</td>
</tr>
<tr>
<td>CBF (ml/min per 100 g)</td>
<td>46 ± 3</td>
<td>96 ± 6 (P &lt; 0.001)</td>
</tr>
<tr>
<td>Adenosine (pmol/ml*)</td>
<td>18.8 ± 1.7</td>
<td>40.3 ± 3.2 (P &lt; 0.001)</td>
</tr>
<tr>
<td>Coronary perfusion pressure (mm Hg)</td>
<td>86 ± 4</td>
<td>110 ± 7 (P &lt; 0.01)</td>
</tr>
<tr>
<td>Heart rate (beats/min)</td>
<td>120 ± 4</td>
<td>205 ± 2 (P &lt; 0.001)</td>
</tr>
<tr>
<td>Diastolic resistance, (mm Hg/ml per min per 100 g)</td>
<td>1.93 ± 0.14</td>
<td>1.09 ± 0.14 (P &lt; 0.005)</td>
</tr>
</tbody>
</table>

Values are mean ± SE.
* Calculated per milliliter of pericardial infusate.

### Table 2  Changes in Blood O\(_2\), CO\(_2\), pH, and Lactate Levels with Stellate Ganglia Stimulation

<table>
<thead>
<tr>
<th></th>
<th>Control ((n = 15))</th>
<th>Response at maximum ( \text{MVO}_2 ) ((n = 15))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arterial-coronary sinus blood O(_2) difference (vol%) ((n = 15))</td>
<td>15.1 ± 0.7</td>
<td>17.0 ± 0.7 (P &lt; 0.05)</td>
</tr>
<tr>
<td>Arterial blood PCO(_2) ((n = 15))</td>
<td>34.8 ± 0.9</td>
<td>42.3 ± 1.5 (P &lt; 0.05)</td>
</tr>
<tr>
<td>pH ((n = 15))</td>
<td>7.41 ± 0.01</td>
<td>7.37 ± 0.02 (NS)</td>
</tr>
<tr>
<td>Coronary sinus blood PCO(_2) ((n = 15))</td>
<td>48.9 ± 1.3</td>
<td>65.9 ± 2.6 (P &lt; 0.05)</td>
</tr>
<tr>
<td>pH ((n = 15))</td>
<td>7.35 ± 0.01</td>
<td>7.28 ± 0.02 (P &lt; 0.05)</td>
</tr>
<tr>
<td>Po(_2) ((n = 15))</td>
<td>20.4 ± 1.1</td>
<td>19.0 ± 1.0 (NS)</td>
</tr>
<tr>
<td>Arterial-coronary sinus blood lactate difference (mm(^+)) ((n = 15))</td>
<td>0.80 ± 0.11</td>
<td>0.51 ± 0.25 (NS)</td>
</tr>
<tr>
<td>Lactate extraction ((A-CS/A \times 100)) ((n = 15))</td>
<td>38.0 ± 4.5</td>
<td>21.7 ± 12.4 (NS)</td>
</tr>
</tbody>
</table>

Values are mean ± SE. NS = not significant.
* Calculated per liter of plasma.
73% for control and increased to 83% with maximum O₂ consumption. Coronary sinus blood PCO₂ was significantly increased, and sinus blood pH was significantly decreased with SGS (Table 2). Arterial blood PCO₂ also was increased with stimulation, whereas arterial blood pH was not significantly decreased.

Arterial and coronary sinus blood, PCI, and myocardial tissue lactate levels were determined as a measure of the relative metabolic status of the myocardium during SGS and showed no significant A-CS difference (Table 2). Lactate extraction from arterial blood was not significantly decreased at maximum MVO₂ response (Table 2) and remained within an acceptable range for normal values. Coronary sinus blood lactate increased from 1.41 ± 0.19 (control) to 2.26 ± 0.45 mM with maximum MVO₂ response; however, the increase was not statistically significant. Similarly, arterial blood lactate levels increased above control (2.21 ± 0.20 mM) with maximum O₂ consumption (2.76 ± 0.46 mM); however, the increase was not significant. In contrast to changes in arterial and coronary sinus blood lactate levels, the PCI and myocardial tissue lactate levels were significantly increased with SGS (Table 3). However, there was no significant correlation between tissue or PCI lactate and MVO₂.

**Table 3 Changes in Myocardial Tissue and Pericardial Infusate Levels of Adenosine and Lactate with Bilateral Stellate Ganglia Stimulation**

<table>
<thead>
<tr>
<th></th>
<th>Adenosine</th>
<th>Lactate</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>MVO₂</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>0.85 ± 0.08</td>
<td>20.8 ± 1.18</td>
</tr>
<tr>
<td>(n-8)</td>
<td>(n = 8)</td>
<td>(n = 8)</td>
</tr>
<tr>
<td>Stellate ganglia</td>
<td>1.96 ± 0.19</td>
<td>38.0 ± 3.0</td>
</tr>
<tr>
<td>stimulation (3.5 min)</td>
<td>(n = 7)</td>
<td>(n = 8)</td>
</tr>
<tr>
<td></td>
<td>1.59 ± 0.16</td>
<td>2.13 ± 0.23</td>
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<tr>
<td></td>
<td>(n = 8)</td>
<td>(n = 8)</td>
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<tr>
<td></td>
<td>0.22 ± 0.02</td>
<td>0.33 ± 0.03</td>
</tr>
<tr>
<td></td>
<td>(n = 7)</td>
<td>(n = 8)</td>
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<tr>
<td></td>
<td>P&lt; 0.005</td>
<td>P&lt; 0.005</td>
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<td></td>
<td>P&lt; 0.005</td>
<td>P&lt; 0.05</td>
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<td></td>
<td>P&lt; 0.05</td>
<td>P&lt; 0.05</td>
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</tbody>
</table>

Values are mean ± SE.

Relationship of Pericardial Infusate to Tissue Levels of Adenosine and Lactate

To characterize the relationship between PCI and tissue levels of adenosine and lactate and, therefore, to determine how well PCI levels serve as indexes of myocardial changes in these metabolites, a separate but identically performed series of experiments was conducted. Table 3 presents values for infusate samples collected under control conditions and with SGS. Tissue adenosine levels with SGS were increased 130%. This increase in tissue adenosine was reflected in a significant increase in PCI adenosine levels (83% above control). Tissue lactate levels were increased 34% with maximum MVO₂ response, and PCI lactate concentration increased 50% above control with SGS.

**Effect of Infusate Volume on Adenosine Concentration**

Additional information was obtained to evaluate PCI adenosine levels by defining the observed adenosine infusate concentration in terms of volume of infusate introduced into the pericardial sac and time (minutes) present in the sac (Table 4). Under control conditions, the volume of infusate was doubled randomly in separate measurements in the same preparation. Volumes selected were 10, 20, and 40 ml of infusate. These volumes remained in the pericardial sac 4.5 minutes. Adenosine concentration doubled as the volumes were reduced by one-half from 40 ml to 20 ml, and then to 10 ml. The reverse pattern was observed with increasing (doubling) infusate volumes. These results indicate that the amount of adenosine released within a given time period is independent of the volume infused.

**Effect of Duration of Infusate Contact with Epicardium on Adenosine Concentration**

Varying the time the infusate remained in the pericardial sac while holding volume constant also was evaluated. Times of contact of the infusate with the epicardial surface of the heart were increased from 0.5 to 18 minutes using a volume of 40 ml. Adenosine concentrations were found to increase with time (Fig. 5), showing a rapid initial washout of adenosine into the infusate followed by a slower but continuous washout. Figure 5 also shows that with SGS a similar washout curve to that of control occurred, but the quantity of adenosine released
was approximately double that measured in controls ($P < 0.005$).

**Adenosine Loss from Pericardial Infusate**

Another evaluation of the PCI model was carried out in association with the above group of experiments. The quantity of adenosine in the PCI should be a function of the tissue adenosine level. Complete equilibration of measured PCI with tissue adenosine levels was not attained at 18 minutes of epicardial contact with PCI and, therefore, another factor which would influence the adenosine concentration of the infusate, viz, uptake of adenosine from the infusate into the tissue, was investigated. Experiments were conducted in which labeled adenosine ([2,3-3H] adenosine) was mixed with infusate to achieve a final activity of 11.5 nCi/ml infusate (0.4 pmol/ml). The labeled solution was infused onto the epicardial surface of the heart as described previously and removed after various times of contact (0.5–18 minutes). The results are shown in Figure 6 in which the labeled adenosine was found to be removed rapidly from the infusate such that, at 4.5 minutes, $39.8 \pm 4.7\%$ ($n = 9$) of the initial activity was removed from the infusate. This is in contrast to labeled sucrose ([1-^3H]sucrose; 17 nCi/ml infusate; 0.1 nmol/ml) where $19.8 \pm 1.9\%$ ($n = 5$) of the initial activity was removed from the infusate at 4.5 min. Initial activity (counts/min per ml) was corrected for the unretrievable residual

<table>
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<tr>
<th>TABLE 5 Determination of Adenosine Catabolism in Pericardial Infusate after 4.5 Minutes in Pericardial Sac</th>
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<tr>
<td><strong>Exp</strong></td>
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<tr>
<td>Adenosine concentration before addition of adenosine standard (pmol/ml)</td>
</tr>
<tr>
<td>Quantity of adenosine added to infusate after removal from pericardial sac (pmol/ml)</td>
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<tr>
<td>Adenosine concentration (pmol/ml) at 0 min in vitro incubation</td>
</tr>
<tr>
<td>1 min in vitro incubation</td>
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<tr>
<td>3 min in vitro incubation</td>
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<td>5 min in vitro incubation</td>
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volume of infusate remaining in the pericardial sac after the sac was flushed (five times) between labeled samples. This residual volume was generally 10% (4 ml) of the infused volume. At 18 minutes more than 80% of the initial activity of the labeled adenosine was lost from the infusate. The same experiments were conducted with a higher initial infusate adenosine concentration (20 pmol/ml infusate); however, the rate of removal of labeled adenosine was found to be the same as that shown in Figure 6, in which the initial adenosine concentration was 0.4 pmol/ml infusate. Also, SGS was found not to alter the rate of loss of labeled adenosine from the infusate (Fig. 6).

**Enzyme Activity in Pericardial Infusate**

The capacity of the PCI in contact with the epicardium to alter adenosine concentration of the infusate due to the leakage of enzymes from the myocardium also was tested. The results are shown in Table 5 for five dogs. After in situ incubation for 4.5 minutes, the infusate was removed from the pericardial sac and a known quantity of standard adenosine (100 pmol/ml) was added to the infusate. Five minutes of in vitro incubation (37°C) with this perfusate containing standard adenosine showed no reduction in adenosine content.

**PGE Levels in Pericardial Infusate**

PGE concentration measured in PCI under control conditions averaged 25 ± 3.3 pg/ml infusate (n = 7). PCI adenosine concentration (control) in the same experimental series was 23 ± 2.4 pmol/ml infusate. SGS did not change the PGE concentration of the infusate (27 ± 6.1 pg/ml), whereas the adenosine concentration increased to 48 ± 13 pmol/ml infusate.

**Discussion**

The capacity of the heart to match oxygen delivery to increases in metabolic demands by affecting vascular resistance has been demonstrated amply (von Restorff et al., 1977; Khouri et al., 1965; Katz and Feinberg, 1958; Alella et al., 1955; Eckenhoff et al., 1947). However, the mechanism linking flow changes to changes in cardiac metabolism has remained an enigma. Adenosine production has been shown experimentally to increase with increased cardiac afterload (McKenzie et al., 1979; Foley et al., 1978), catecholamine stimulation (Wiedmeier and Spell, 1977; Katori and Berne, 1966), and treadmill exercise (Watkinson et al., 1979). Also, increased adenosine release has been reported to be correlated with decreases in coronary vascular resistance associated with aortic constriction or isoproterenol infusion (McKenzie et al., 1979). Therefore, if increased oxygen demand is a physiological stimulus for adenosine release and consequent increase in CBF, then adenosine production should parallel increases in CBF and MVO₂. The results of this study support the hypothesis that a parallelism exists between increases in MVO₂ and adenosine release and between increases in CBF and adenosine release. The significant correlations obtained indicate that adenosine is released with increased oxygen demand and can increase CBF by effecting a decrease in vascular resistance.

Since PCI adenosine concentration increased in parallel with MVO₂ when cardiac activity was increased by SGS (Fig. 3), changes in PCI adenosine should reflect changes in myocardial tissue adenosine production. Also, since vasoactive adenosine is considered to be restricted to the interstitial space, tissue adenosine should serve as a basis for describing the relationship between interstitial space, tissue adenosine should serve as a basis for describing the relationship between interstitial space and PCI adenosine. Therefore, the quantity of adenosine entering the infusate should be proportional to the tissue adenosine level. Results of experiments characterizing the relationship between tissue and PCI adenosine and lactate indicate that PCI serves as a virtual sink into which metabolites diffuse from the interstitium under the influence of a substantial gradient. Equilibrium between interstitial fluid and infusate would not be expected to occur because of the relatively short time of contact of infusate with the epicardium and the large portion of total cell membrane surface area not in contact with the infusate. Table 3 shows the quantitative relationship between myocardial tissue and PCI adenosine and lactate levels under control conditions and with bilateral SGS. Tissue adenosine and lactate levels were significantly increased by SGS, and these increases in tissue levels were reflected by significant increases in infusate adenosine and lactate concentrations. Also, when interstitial adenosine concentration was calculated from the measured tissue adenosine content (Table 3), assuming an interstitial volume of 0.35 ml/kg tissue, the PCI adenosine concentration under control conditions was 0.9% of the calculated interstitial adenosine concentration (2.4 nmol/ml). A similar quantitative relationship exists with SGS where PCI adenosine was 0.7% of the calculated interstitial adenosine concentration (5.6 nmol/ml). Thus, approximately the same fraction of interstitial adenosine enters the PCI under control conditions and with SGS, when contact time between epicardium and infusate is constant.

With different times of contact, as shown in Figure 5, a relationship suggestive of a diffusion mechanism for adenosine was obtained. The initial more rapid phase of adenosine washout from the epicardium is followed by slower rate which remained constant throughout the times monitored. The slower phase of washout may reflect the rate of adenosine replacement in the superficial epicardium. At 18 minutes, the control PCI adenosine concentration reflected approximately 2% of the calculated interstitial adenosine concentration. The data of Table 4 also provide evidence for the diffusion of adenosine. The quantity of adenosine enter-
ing the infusate is constant and independent of the volume of infusate.

PCI adenosine should equal interstitial adenosine levels at infinite time if uncomplicated diffusion from tissue to infusate is involved. However, reports of adenosine content of endogeneous pericardial fluid range from 0.38 to 1.1 nmol/ml (Snow et al., 1973; Rubio and Berne, 1969), and these values reflect distributions of adenosine into pericardial fluid which are between 26% and 70% of calculated interstitial adenosine levels, assuming an interstitial space of 0.35 ml/g tissue. Therefore, endogeneous pericardial fluid adenosine levels do not attain equilibrium with estimated interstitial fluid levels. This disparity may be due to the possibility that some fraction of the total tissue adenosine is not freely diffusible since it may be compartmentalized. Such restrictions of the diffusion of adenosine would mean that the PCI adenosine was actually closer to equilibrium with freely diffusible interstitial adenosine than would be apparent from whole tissue samples. Another factor that would influence adenosine levels in the PCI is the uptake of adenosine from the infusate. Figure 6 indicates that labeled adenosine is removed rapidly from PCI. Therefore, adenosine content of the PCI would be the net result of a significant turnover of adenosine in PCI.

The lack of significant changes in arterial-coronary sinus blood O2 differences and coronary sinus blood Po2 (Table 2) indicate that tissue oxygenation was not compromised, even with maximum MVO2 response. Von Restorff et al. (1977) and Khouri et al., (1965) have demonstrated that increases in O2 extraction ranging from 75% to 93% may occur under physiological conditions of increased cardiac work and, therefore, the moderate increases in O2 extraction observed in these experiments (73% to 83%) with maximum MVO2 response are not unphysiological. These changes in O2 extraction without a change in coronary sinus blood PO2 suggest a shift in the O2 dissociation curve. The increased coronary sinus blood PCO2 observed with maximum MVO2 response and the concomitant decrease in sinus blood pH would contribute to a mechanism for increasing O2 extraction. In addition, capillary recruitment (Henquell and Honig, 1976) in response to increased cardiac work with SGS also could contribute to the observed increase in O2 extraction under physiological conditions.

The contribution of CO2 to the vasoregulatory response of increased metabolic demand warrants consideration. Coronary sinus blood PCO2 was observed in this study to increase with SGS; however, the range of PCO2 values obtained was within generally accepted limits for control values. Also, the observations of Case et al. (1978) under conditions of constant flow indicate that CO2 is a relatively weak vasodilator, requiring twice the increase in sinus blood PCO2 to any decrease in sinus blood PO2 to effect the same magnitude of coronary vasodila-

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Canine myocardial adenosine and lactate production, oxygen consumption, and coronary flow during stellate ganglia stimulation.

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