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 Left ventricular tissue samples frozen in situ also were obtained after the infusate was withdrawn and analyzed for adenosine and lactate.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. Circ Res 45: 708-718, 1979

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 MVO₂ 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 MVO₂ 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 (P0₂ 90–130; Pco₂ 25–40; 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 P233 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 left and right sympathetic chains (T₁-T₆) 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.
Adequacy of the seal of the coronary catheter within the vessel was tested at the beginning of the experiment and periodically throughout each experiment by complete occlusion of the inflow tubing. If coronary perfusion pressure, as monitored in the extracorporeal circuit, dropped to below 25 mmHg during the occlusion, and if reactive hyperemia was demonstrated upon release of the occlusion, then no perfusion around the catheter was considered to occur. Distal (retrograde) coronary pressures following abrupt occlusion of a main coronary artery have been reported to range between extremes of 6 and 42 mmHg (Corday et al., 1974; Pasyk et al., 1971; Khouri et al., 1971; Fam and McGregor, 1969). Therefore, a drop in perfusion pressure to 25 mmHg or below was considered an acceptable pressure level with occlusion of a coronary artery to substantiate the development of a tight seal around the coronary catheter. Peak reactive hyperemic flows ranged between 100% and 150% above control for 15-second occlusions. Also, postmortem examination after perfusion of the coronary artery with India ink revealed no staining of the aorta or coronary vessel proximal to the catheter position. Data from the individual preparations were not accepted unless these criteria were met. The pressure gradient across the coronary catheter was approximately 20 mmHg for control flows of 35–40 ml/min; however, since the pressure gradient varies with flow, the flow-pressure relationship was determined for the coronary catheter used over the range of flow values encountered in this study, and each coronary perfusion pressure and diastolic resistance value was corrected for the gradient existing at the observed flow. No adjustments were made for variations in hematocrit.

Also, since in most cases the LC artery was cannulated (18/25), an additional test was performed at the completion of five randomly selected experiments to determine whether cannula position in the LC artery interfered with perfusion of the LAD artery. The LAD artery was cannulated distally with a 20 gauge 1½-inch longdwell catheter and perfusion pressure monitored. Perfusion pressure was found 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): methanol (10 parts). Silicic acid column chromatography was used to isolate the PGE fraction by using different concentrations of methanol in benzene and ethyl acetate to elute the major PG groups (A, E, F) serially. The PGE fraction was evaporated to dryness under nitrogen and reconstituted for radioimmunoassay.

Evaluation of Adenosine Loss from Pericardial Infusate

Studies using radiolabeled adenosine ([2,3-3H] adenosine, New England Nuclear) were conducted to evaluate possible turnover of adenosine in PCI under control conditions and with SGS. Labeled sucrose ([1-3H]sucrose, New England Nuclear) was used as a comparison marker. After being mixed with 40 ml of Krebs-Henseleit solution, the labeled adenosine PCI or labeled sucrose PCI was infused onto the epicardial surface of the heart. The infusate remained in contact with the epicardium for periods of 0.5 to 18 minutes and then was withdrawn completely. Duplicate 1-ml aliquots of the PCI were added to 5 ml of scintillation fluid (Beckman Ready-Solv HP) and counted for 3H activity in a Beckman liquid scintillation counter (LS8000). Counted radioactivity was corrected for quenching.

Enzymatic activity of PCI also was determined to evaluate adenosine breakdown occurring prior to inactivation by the boiling procedure. Forty milliliters of Krebs-Henseleit solution were introduced into the pericardial sac and allowed to remain for 4.5 minutes. The 40 ml were removed and a 10-ml aliquot was placed immediately in a boiling water bath for 10 minutes. Adenosine standard sufficient to achieve a final concentration of 100 pmol/ml 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
FIGURE 1 Left coronary artery blood flow responses (mean flow) to graded bilateral stellate ganglion stimulation.

derived from data sets containing all control and stimulation group values taken from each dog. The statistical significance of the difference observed for a variable before and after SGS was determined by a paired t-test. The least-squares method was used to calculate regression equation parameters and correlation coefficients. The standard error (s_v*) of the equation for the regression line is presented in Figures 2, 3, and 4 to indicate ± 1 SD from the regression line (Croxton, 1953).

Results

MVO₂, 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 MVO₂ to CBF is shown in Figure 2 for experiments completed on 13 dogs. Graded increases in CBF are shown to be correlated with increased MVO₂ (r = 0.894; P < 0.001). Data plotted include control and stimulation measurements for each dog with each symbol representing a single dog. MVO₂ values ranged from a low control of 4.5 to a maximum with stimulation of 21.6 ml O₂/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 MVO₂ is shown in Figure 3. Increases in MVO₂ 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. MVO₂ values ranged from 4.5 to 21.6 ml O₂/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...
TABLE 1 Changes in Hemodynamic and Metabolite Parameters with Stellate Ganglia Stimulation

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control (n = 15)</th>
<th>Response at maximum MVO₂ (n = 15)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MVO₂ (ml O₂/min per 100 g)</td>
<td>7.0 ± 0.47</td>
<td>16.0 ± 0.98</td>
</tr>
<tr>
<td>CBF (ml/min per 100 g)</td>
<td>46 ± 3</td>
<td>96 ± 6</td>
</tr>
<tr>
<td>Adenosine (pmol/ml*)</td>
<td>18.8 ± 1.7</td>
<td>40.3 ± 3.2</td>
</tr>
<tr>
<td>Coronary perfusion pressure (mm Hg)</td>
<td>86 ± 4</td>
<td>110 ± 7</td>
</tr>
<tr>
<td>Heart rate (beats/min)</td>
<td>120 ± 4</td>
<td>205 ± 2</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</td>
</tr>
</tbody>
</table>

Values are mean ± SE.

Data (mean ± SE) are presented in Table 1 for MVO₂, CBF, PCI adenosine concentration, mean coronary perfusion pressure, heart rate, and diastolic resistance obtained under control conditions and at the maximum MVO₂ 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 MVO₂ in control and stimulation groups. The arterial-coronary sinus blood oxygen difference (A-CSO₂) showed no significant increase above control at maximum MVO₂ response with SGS (Table 2). Coronary sinus blood PO₂ was found not to change significantly from control (20 mmHg) with maximum MVO₂ response (19 mmHg). Oxygen extraction, which also reflects changes in A-CSO₂, was

TABLE 2 Changes in Blood O₂, CO₂, pH, and Lactate Levels with Stellate Ganglia Stimulation

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>Response at maximum MVO₂</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arterial-coronary sinus blood O₂ difference (vol%) (n = 15)</td>
<td>15.1 ± 0.7</td>
<td>17.0 ± 0.7 NS</td>
</tr>
<tr>
<td>Arterial blood (n = 15)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pco₂</td>
<td>34.8 ± 0.9</td>
<td>42.3 ± 1.5 P &lt; 0.05</td>
</tr>
<tr>
<td>pH</td>
<td>7.41 ± 0.01</td>
<td>7.37 ± 0.02 NS</td>
</tr>
<tr>
<td>Coronary sinus blood (n = 15)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pco₂</td>
<td>48.9 ± 1.3</td>
<td>65.9 ± 2.6 P &lt; 0.05</td>
</tr>
<tr>
<td>pH</td>
<td>7.35 ± 0.01</td>
<td>7.28 ± 0.02 P &lt; 0.05</td>
</tr>
<tr>
<td>Po₂</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 × 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.
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 MVO&lt;sub&gt;2&lt;/sub&gt;</th>
<th>Ischemic Time</th>
<th>Adenosine Pericardial Infusate</th>
<th>Lactate Myocardial Tissue</th>
<th>Lactate Pericardial Infusate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>7.9 ± 0.8 (n = 8)</td>
<td></td>
<td>0.85 ± 0.08 (n = 8)</td>
<td>20.8 ± 1.18 (n = 8)</td>
<td>0.22 ± 0.02 (n = 12)</td>
</tr>
<tr>
<td>Stellate ganglia</td>
<td>15.4 ± 1.3 (n = 8)</td>
<td>0.19 ± 3.0</td>
<td>38.0 ± 3.0 (n = 7)</td>
<td>2.13 ± 0.23 (n = 8)</td>
<td>0.33 ± 0.03 (n = 8)</td>
</tr>
<tr>
<td>(3.5 min)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tbody>
</table>

Values are mean ± SE.

73% for control and increased to 83% with maximum O<sub>2</sub> consumption. Coronary sinus blood P<sub>CO</sub>2 was significantly increased, and sinus blood pH was significantly decreased with SGS (Table 2). Arterial blood P<sub>CO</sub>2 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 MVO<sub>2</sub> 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 MVO<sub>2</sub> response; however, the increase was not statistically significant. Similarly, arterial blood lactate levels increased above control (2.21 ± 0.20 mM) with maximum O<sub>2</sub> 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 MVO<sub>2</sub>.

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 MVO<sub>2</sub> 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 was found to be related inversely to the volume of fluid infused. 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 ± 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 ± 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 5** Determination of Adenosine Catabolism in Pericardial Infusate after 4.5 Minutes in Pericardial Sac

<table>
<thead>
<tr>
<th>Exp 1</th>
<th>Exp 2</th>
<th>Exp 3</th>
<th>Exp 4</th>
<th>Exp 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adenosine concentration before addition of adenosine standard (pmol/ml)</td>
<td>30.9</td>
<td>25.7</td>
<td>23.7</td>
<td>30.6</td>
</tr>
<tr>
<td>Quantity of adenosine added to infusate after removal from pericardial sac (pmol/ml)</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Adenosine concentration (pmol/ml) at 0 min in vitro incubation</td>
<td>120.5</td>
<td>120.5</td>
<td>120.5</td>
<td>125.6</td>
</tr>
<tr>
<td>5 min in vitro incubation</td>
<td>117.6</td>
<td>123.4</td>
<td>117.6</td>
<td>140.5</td>
</tr>
</tbody>
</table>
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 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, endogenous 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 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 PO2 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 was considered. 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 vasodilation. Also, the correlation obtained in the present study between coronary sinus blood PCO2 and CBF was low (r = 0.411, n = 50), indicating a minor contribution to the overall vasoregulatory process. Thus the role of increased CO2 or acidosis may be to influence myocardial oxygenation by promoting O2 dissociation from hemoglobin and to potentiate adenosine-induced hyperemia (Merrill et al., 1978; Raberger et al., 1975).

Substantial increases in lactate concentration of coronary venous blood and myocardial tissue have been employed as indicators of inadequate perfusion and the onset of anaerobic metabolism. Changes in lactate levels of the blood, PCI, and myocardial tissue were examined to determine whether perfusion of the myocardium was adequate under control and SGS conditions. The absence of significant changes in blood lactate levels indicates that tissue metabolism was not compromised. The decreased arterial-coronary sinus lactate difference with SGS suggests an increased lactate production by the heart and its washout by the blood (Table 2). Coronary sinus blood lactate increased with maximum MVO2 response; however, the changes were not statistically significant. Tissue lactate levels increased significantly above control with maximum O2 consumption (Table 3), but the increases obtained with stimulation were well below tissue values reported for ischemia (Kleitke et al., 1976; Griggs et al., 1972; Gudbjarnason et al., 1970; Braasch et al., 1968). Also, arterial blood lactate levels were elevated with SGS, which would contribute to the observed increase in tissue lactate. Therefore, the observations that coronary sinus blood PO2 was not changed, and that blood lactate levels were increased only slightly with SGS indicate that the increased tissue lactate levels are compatible with a physiological response to sympathetic stimulation and not the result of limited O2 availability. Any PGE involvement in a regulatory scheme was not supported by these experiments in that no change in PGE content of PCI was apparent with increased oxygen consumption.

In conclusion, this study shows that adenosine is released from the heart under conditions of increased myocardial oxygen demand in which blood flow and oxygen availability are not limiting. Also, the significant correlations among adenosine release, MVO2, and CBF provide additional support for the metabolic regulation of CBF, and particularly support a role for adenosine in the coupling of myocardial metabolism to CBF.

References


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

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