Formation of S-Adenosylhomocysteine in the Heart. II: A Sensitive Index For Regional Myocardial Underperfusion

Andreas Deussen, Mathias Borst, Keith Kroll, and Jürgen Schrader

Rate of accumulation of myocardial S-adenosylhomocysteine (SAH) was used in an open-chest dog preparation as an index of free cytosolic adenosine levels. Following 30 minutes of coronary artery ligation and infusion of L-homocysteine thiolactone (10 μmol/kg/min i.v.) SAH levels increased from 1.3 (control) to 3.3 nmoles/g in the nonischemic and to values over 100 nmoles/g in the ischemic region. Compared with regional myocardial blood flow the enhanced rate of SAH accumulation was strictly confined to the ischemic area. As long as blood flow was 0.6–1.2 ml/min/g, SAH levels remained unchanged. However, they steeply increased when regional myocardial blood flow decreased below 60% of control. Tissue levels of adenine nucleotides, adenosine, and lactate were not significantly affected in the flow range of 0.4–0.6 ml/min/g but rate of SAH accumulation was enhanced by 400%. In the nonischemic myocardium, SAH accumulation was 60% higher in the subendocardium than in the subepicardium. Decreasing coronary perfusion pressure from 110 to 60, 45, and 35 mm Hg was associated with an exponential increase in coronary venous adenosine release only when perfusion pressure was below 60 mm Hg. Transmural mapping of SAH revealed that at 110 mm Hg SAH was homogeneously distributed, while at a perfusion pressure of 60 mm Hg SAH accumulation was enhanced only in the subendocardial layers. Decreasing perfusion pressure further to 40 and 30 mm Hg not only enhanced subendocardial SAH levels to 120 and 170 nmoles/g, respectively, but also considerably steepened the transmural gradient of SAH. SAH-hydrolase exhibited a broad pH-optimum and its activity in different parts of ventricular myocardium was identical. Our findings provide evidence that 1) measurement of SAH accumulation is a sensitive metabolic index for the assessment of regional myocardial ischemia, 2) significant formation of SAH occurs only when regional myocardial blood flow is less than 0.6 ml/min/g, and 3) transmural SAH gradient, a measure of free cytosolic adenosine, and coronary venous adenosine release significantly increase only when the autoregulatory reserve is exhausted. (Circulation Research 1988;63:250–261)
to S-adenosylhomocysteine (SAH) via the action of SAH-hydrolase. In the presence of elevated plasma levels of homocysteine, cystolic SAH-hydrolase preferentially converts adenosine into SAH (adenosine + homocysteine → SAH + H₂O). The SAH formed is not further metabolized, nor does it permeate cardiac cell membranes. As shown in the preceding study, the rate of SAH accumulation closely reflects steady-state changes in the level of free cytosolic adenosine. Perfusion of isolated guinea pig hearts with homocysteine (≤1 mM), which raised tissue levels of SAH as high as 3.5 μmoles/g, did not affect heart rate and myocardial contractility or impede hypoxia-induced vasodilation.

The aim of the present study was 1) to investigate the effects of regional cardiac ischemia on myocardial SAH formation in the presence of homocysteine and to determine the relation between regional myocardial blood flow and SAH accumulation, 2) to compare the rate of SAH formation in tissue levels of adenine nucleotides and lactate, 3) to elucidate the relation between SAH accumulation and tissue content of its precursor adenosine during myocardial ischemia, and 4) to utilize measurement of SAH-accumulation for defining the transmural site of adenosine formation during reduction of coronary perfusion pressure (autoregulation).

Materials and Methods

Animal Experiments

Mongrel dogs with a body mass of 20–30 kg were initially anesthetized with 0.5 g thiobutabarbitral (Inaktin-Byk®, Byk-Gulden, Konstanz, FRG) followed by administration of α-chloralose (50 mg/kg i.v.) and urethane (250 mg/kg i.v.). After intubation, the dogs were artificially ventilated by a respirator (Logik B 2, Assistance Technique Medicale—S.A.). For administration of supplementary anesthetic (approximately 10 mg/kg/hr chloralose and 50 mg/kg/hr urethane) and volume substitution, a catheter was placed into the right femoral vein. To obtain arterial blood samples and to measure arterial blood pressure, an 8F Goodale-Lubin catheter was advanced via the right brachial artery into the aortic arch and connected to a pressure transducer (P 23 ID, Statham, Gould, Oxnard, California). Arterial blood samples were frequently analyzed for PO₂, PCO₂, and pH (BMS 2 MK 2, blood micro system, Radiometer, Copenhagen) and if necessary ventilation was adjusted or bicarbonate solution was infused to keep these parameters within the normal range. Furthermore, a 5F catheter-tip manometer (PC 350, Millar, Houston, Texas) was advanced into the left ventricle via the right femoral artery under fluoroscopy. Left ventricular dP/dt and heart rate were derived from the left ventricular pressure signal. All hemodynamic parameters were continuously recorded on a polygraph (R 612, Beckman, München, FRG).

A thoracotomy was performed through the fifth intercostal space, and after opening of the pericardium, the heart was suspended in a pericardial cradle. Two experimental protocols were followed:

Regional ischemia. In nine dogs, one or two side branches of either the left circumflex coronary artery or the left anterior descending coronary artery were dissected free from the surrounding tissue, and a snare was placed around them for later occlusion. The perfusion area of these side branches approximated 10–30% of the free wall of the left ventricular myocardium. A polyethylene catheter (1 mm o.d.) was advanced into the left atrium via the appendage for infusion of L-homocysteine thiolactone dissolved in 0.9% NaCl solution (10 μmoles homocysteine/kg/min). Homocysteine infusion was started 1 minute prior to occlusion of the coronary vessels.

In a first group (n = 5), myocardial biopsies were taken under control conditions (prior to homocysteine infusion) and after onset of ischemia from the ischemic and from a nonischemic region using a high-speed drill. The drill head was connected to a pressure unit (pressure: 0.5–1.0 bar), and biopsied tissue was blown directly into liquid nitrogen. Time required for this procedure was less than 2 seconds. The average sample weight was 25 mg (range 15–30 mg). In three of these five experiments, the free left ventricular wall was rapidly excised after 30 minutes of regional ischemia and frozen in liquid nitrogen for later measurements of subendocardial and subepicardial tissue levels of adenosine and SAH.

In a second group of experiments (n = 4), SAH accumulation following 30 minutes of ischemia was studied. In three experiments within this group, a coronary artery was occluded, and the relation between regional SAH accumulation and regional myocardial blood flow was determined with tracer microspheres using the reference flow technique. After 25 minutes of regional ischemia, approximately 20×10⁶ carbonized plastic microspheres (11.3 ± 0.1 μm diameter) labeled with ¹⁴¹Ce (NEN, Dreieich, FRG) were infused via the left atrial catheter, while a reference sample was withdrawn at a constant rate of 20 ml/min from the aorta at the level of the diaphragm. After 30 minutes of ischemia, the left ventricular wall containing the ischemic area was rapidly excised and frozen in liquid nitrogen and lyophylized and sectioned for measurement of metabolites and radioactivity. The basic principles underlying the tracer microsphere method required a sample size of at least 250 mg in our experiments, but the sensitivity of the analytical methods for measurement of adenosine and SAH permitted a higher spatial resolution. Therefore, in one experiment, only metabolite levels were measured for epicardial mapping without regional blood flow measurements.
Coronary underperfusion. In 11 experiments, the proximal end of the left circumflex coronary artery was dissected free from the surrounding tissue for cannulation with a steel cannula of 2.5 mm internal diameter. Perfusion of the artery was provided from a windkessel filled with the dog's own blood from the left femoral artery. Coronary perfusion pressure was measured at the tip of the cannula via a small internal tube using a pressure transducer (P23 ID, Statham). Coronary blood flow was measured by means of an electromagnetic flowmeter (SP2202, Statham) using a flow probe (SP 7515, Statham, 3 mm diameter) incorporated in the bypass perfusion line.

In five experiments (group 3), regional myocardial adenosine release, myocardial oxygen consumption, and myocardial lactate consumption were determined during graded coronary underperfusion. Coronary venous blood samples were collected via a catheter advanced into a coronary vein that drained the circumflex-perfused myocardium. Blood was sampled under control conditions (perfusion pressure of 110 mm Hg) as well as 2 and 5 minutes after coronary perfusion pressure was adjusted to either 150, 60, 40, or 30 mm Hg. To prevent uptake of plasma adenosine into red blood cells during the sampling process, 1 ml blood was withdrawn into a syringe containing 1 ml of an ice-cold "stopping solution" described recently.6

In six experiments (group 4), coronary perfusion pressure was adjusted to 110, 60, 40, or 30 mm Hg, respectively, for 30 minutes. L-Homocysteine thiolactone was continuously administered at a dose of 10 μmoles/kg/min i.v. started 10 minutes prior to the respective changes in perfusion pressure. At the end of the experiment, the free wall of the left ventricle containing the bypass-perfused area was rapidly removed and frozen in liquid nitrogen.

Analytical Procedures

The excised myocardium was freeze dried, and connective tissue was removed. For mapping of metabolites, the epicardial side of the myocardium was sectioned into tissue blocks with side lengths of 10 mm (tracer microsphere experiments) or 5-7 mm (other experiments). Unless otherwise indicated, only the subepicardial and subendocardial 3 mm of the tissue blocks were used for measurement of regional myocardial blood flow and metabolite levels. For measurement of the transmural gradient of SAH, the wall of the left ventricle was sliced into seven layers (thickness, 1.7-2.0 mm). For the determination of regional myocardial blood flow, tissue samples were weighed and radioactivity was measured in a gamma counter (Packard, Multi-Priias Downer's Grove, Illinois) together with reference blood samples and standards. For measurement of regional metabolite levels, samples were homogenized (Ultraturrax, Janke & Kunkel, Staufen, FRG) in ice-cold 0.5 M perchloric acid (10:1 vol/tissue wt) and after centrifugation at 20,000g for 20 minutes, the supernatant was neutralized with KOH (1 M), freeze dried, and redissolved in 0.5-1.5 ml distilled water.

Tissue adenosine. Two pumps (M-45 and M-6000 A, Waters, Eschborn, FRG) were programmed for gradient elution of samples (50-200 μl) injected (WISP 710B, Waters) directly onto a reversed phase column (8-C-18, 5 μm, Waters). A concave gradient was used at a flow rate of 1.5 ml/min over 8 minutes (effluent samples) or 12 minutes (myocardial samples). Initial conditions were 100% solvent A (ammonium acetate, 0.026 M, pH 5), final conditions were 100% solvent B (70:30 methanol/water [vol/vol]). Absorbance of column eluate was measured at λ = 254 nm and continuously recorded (model 441, Waters). Compounds were quantified by measuring peak height and comparing it to standards. Peaks were identified by comparison with retention times of external standards. In a number of tissue samples, adenosine was measured using a radioimmunoassay.17 Results obtained with both methods were identical.

Plasma adenosine. Blood samples mixed with stopping solution were centrifuged immediately at 8,000g for 2 minutes. One milliliter of the supernatant was removed and deproteinized with 100 μl 60% perchloric acid. After removal of the protein precipitate by centrifugation at 8,000g for 2 minutes and neutralization with 900 μl K 3PO 4 (0.5 M), the supernatant was passed over SEP-PAK C-18 cartridges (Waters). Adsorbed purines were eluted with 2 ml of a mixture of 70:30 methanol/water (vol/vol) and evaporated (Vortex Evaporator, Buchler, Fort Lee, New Jersey) to dryness. The residue was redissolved in 200 μl distilled water, of which 175 μl were assayed for adenosine by high-performance liquid chromatography as described above. Since adenosine cochromatographed with other unknown compounds, the adenosine containing fraction was rechromatographed for inosine after deamination of adenosine by adenosine deaminase.6 Recovery of adenosine added as an internal standard was greater than 95%.

Adenine nucleotides. Twenty microliters of the neutralized acid extract were injected onto a reversed phase column (8-C-18, 4 μm, Waters). Initial elution condition was 95% buffer (0.011 mM tetrabutylammonium hydrogensulfate, 0.061 mM KH 2PO 4, pH 5.8) and 5% acetonitrile for 5 minutes, followed by a 20-minute isocratic elution with 88% buffer and 12% acetonitrile at a flow rate of 1.5 ml/min. Absorbance of column eluate was continuously recorded at λ = 254 nm. Compounds were quantified by determination of peak area using external standards. Peaks were identified by comparison with retention times of external standards.

In addition to measurements of blood oxygen content (Lex-O2-Con, Lexington Instruments, Waltham, Massachusetts), plasma lactate was deter-
determined with an enzymatic test (Monotest Lactat, Boehringer, Mannheim, FRG).

SAH-Hydrolase Activity

Cardiac tissue was homogenized (Ultraturrax, Janke & Kunkel) for 1 minute in ice-cold 10 mM HEPES-KOH buffer (pH 7.4, 10:1 vol/tissue wt) which contained sucrose (0.25 M), MgCl₂ (1 mM), and mercaptoethanol (1 mM), strained through a double-layered gauze and homogenized for another minute (Potter homogenizer, Teflon pestle, Brann, Melsungen, FRG). For preparation of the cytosolic fraction the homogenate was centrifuged at 220,000g for 1 hour (Sorvall OTD 55B, Norwalk, Connecticut). The cytosolic fraction was then passed over a PD 10 gel filtration column (Pharmacia, Uppsala, Sweden), and the protein fraction containing SAH-hydrolase was eluted with 10 mM HEPES buffer.

The activity of SAH-hydrolase in the direction of hydrolysis was determined by a spectrophotometric assay described previously. In brief, 2 ml of the reaction mixture (HEPES 25 mM, MgCl₂ 2 mM, NaHPO₄ 0.1 mM, pH 7.4, 37°C) contained 20 units adenosine deaminase, 0.2 units purinenucleoside phosphorylase, 0.2 units xanthine oxidase, and 50 μM SAH. Reaction was started by adding an aliquot of the cytosolic fraction (200 μg protein). Changes in concentration of uric acid, reflecting hydrolysis rate of SAH, were monitored by measuring absorbance at 295 nm, reference beam set at 325 nm, using a dual wavelength, double beam spectrophotometer (model 557, Perkin-Elmer Uberlingen, FRG).

The activity of SAH-hydrolase in direction of synthesis was measured at 37°C, pH 7.4 in 1 ml of the reaction mixture described above. Erythro-9-(2-hydroxynon-3-yl)adenine (EHNA; 20 μM) was added to inhibit adenosine deaminase activity. Concentrations of adenosine and DL-homocysteine were 50 μM and 4 mM, respectively. The reaction was started with an aliquot of the cytosolic fraction (100 μg protein) and stopped after 20 minutes by addition of 100 μl HClO₄ (1 M). After neutralization with K₂PO₄ (0.5 M) samples were evaporated to dryness (Vortex Evaporator, Buchler), redissolved in 300 μl H₂O, of which 200 μl were assayed for SAH using high-performance liquid chromatography techniques described above. Protein was measured according to the method of Bradford.

Chemicals

Adenosine, inosine, SAH, ATP, ADP, AMP, adenosine deaminase, purinenucleoside phosphorylase, and xanthine oxidase were obtained from Boehringer, Mannheim, FRG. L-Homocysteine thiolactone and DL-homocysteine were purchased from Sigma, München, FRG. EHNA was obtained from Borroughs Wellcome, Research Triangle Park, North Carolina. All other reagents were obtained from Merck, Darmstadt, FRG.

Statistics

Data reported are mean ± SEM. To compare control values with those obtained during the different interventions Student’s t test for either paired or unpaired data was used. Differences were considered significant when p<0.05. In addition, regression analyses were performed based on the individual determinations.

Results

Regional Ischemia

Control values of hemodynamic parameters prior to regional ischemia for the nine dogs studied were peak left ventricular pressure, 128±5 mm Hg; left ventricular dP/dt max, 2,290±120 mm Hg/sec; heart rate, 151±6 beats/min; and diastolic aortic pres-

![FIGURE 1. Mapping of local values of subepicardial SAH content in the left ventricular wall following occlusion of a side branch of the circumflex coronary artery for 30 minutes. Infusion of L-homocysteine thiolactone (10 μmoles/kg/min) via the left atrium was started 1 minute before coronary occlusion. The excised free wall of the left ventricle (dotted line) was subdivided into 83 tissue blocks with a mean surface area of 35 mm² which were analyzed for SAH levels. For further details, see "Materials and Methods."](image-url)
sure, 100±5 mm Hg. Neither ligation of a small coronary artery nor infusion of L-homocysteine thiolactone (10 μmoles/kg/min) and tracer microspheres significantly affected any of these parameters. They remained unchanged until the end of the experiment (data not shown), which was after 30 minutes of regional ischemia.

In the first experimental series (group 1), the time course of SAH accumulation during regional myocardial ischemia was studied. Local adenosine formation was accelerated by occluding a small coronary artery while homocysteine was simultaneously infused (10 μmoles/kg/min) to convert adenosine to SAH. Myocardial drill biopsies were taken from the subepicardial myocardium of the nonischemic as well as the ischemic area. Prior to homocysteine infusion and ischemia, epicardial tissue adenosine and SAH averaged 1.9 ±0.3 and 1.3 ±0.3 nmoles/g (n = 5), respectively. Following coronary artery ligation during infusion of homocysteine, SAH slightly increased to 1.7 ±0.2 nmoles/g after 15 minutes (n = 4) and to 2.3 ±0.2 nmoles/g after 30 minutes (n = 3) in nonischemic tissue. Prior to ischemia, however, SAH content increased to a value of 39.7 ±7.9 nmoles/g after 15 minutes and to 83.1 ±4.9 nmoles/g after 30 minutes, which was 36 times larger than the value in the nonischemic region. Linear regression analysis of the pooled data indicated that within the ischemic region, SAH linearly accumulated at a rate of 2.9 nmoles/g/min (r = 0.923, n = 28 samples in five different experiments).

In a separate experimental series (group 2), regional accumulation of SAH during myocardial ischemia was studied. After 30 minutes of ischemia the free wall of the left ventricle was rapidly removed for biochemical analysis. After freeze drying, the excised tissue was subdivided into epicardial samples with side lengths of 5–7 mm (no tracer microspheres) or 10 mm (tracer microspheres) for measurement of regional distribution of metabolites. Experimental conditions were otherwise as described above. Epicardial mapping of SAH is given in Figure 1. It revealed that elevated levels of SAH were restricted to an area which corresponded to the perfusion area of the occluded vessel. Within a narrow border zone, SAH levels increased from a mean value of 4.4 ±0.4 nmoles/g in the nonischemic region to values well above 100 nmoles/g in the ischemic area.
In three experiments of group 2, the relation between regional myocardial blood flow (tracer microsphere technique), adenosine and SAH levels across the ischemic zone was determined. In the representative subepicardial tissue slice shown in Figure 2, regional myocardial blood flow was greatly reduced in the ischemic area but total tissue adenosine was only slightly increased. SAH levels, however, were greatly elevated and closely reflected the decrease in myocardial flow. Note that the increase in SAH accumulation was particularly steep when regional myocardial flow decreased to values between 0.40 and 0.15 ml/min/g (Figure 2). The relation between regional myocardial blood flow and SAH accumulation for all subepicardial and subendocardial samples from the tracer microsphere experiments is shown in Figure 3. It is evident that SAH levels remained relatively unchanged as long as myocardial perfusion did not decrease below 0.6 ml/min/g. Any further decrease in regional flow, however, was associated with a pronounced increase in the myocardial SAH level.

In samples taken from the ischemic border zone (crosses in Figure 3), SAH was significantly elevated although flow values were not significantly decreased. All these samples were located adjacent to severely ischemic tissue samples (regional myocardial blood flow <0.25 ml/min/g; SAH >60 nmoles/g). This observation is presumably best explained by the much lower resolution of the tracer microsphere measurements when compared with metabolic measurements. As data in Figures 1 and 2 demonstrate, steep SAH gradients exist over only a few millimeters of myocardial tissue.

The relation between SAH accumulation and adenosine levels shown in Figure 4 was determined in the experiments of group 2. The relation was linear below adenosine levels of approximately 15 nmoles/g and demonstrated that in this range the increase in SAH was 5.4 times greater than the increase in total tissue adenosine. Half-maximal rate of SAH accumulation was associated with an adenosine level of about 20 nmoles/g. Above this value SAH-accumulation showed leveling off.

The tissue samples from experiments of group 2 were also analyzed for adenine nucleotides (ATP, ADP, AMP) as well as for lactate. As shown in Table 1, ATP remained essentially unchanged as long as myocardial blood flow was in the range of 1.22-0.4 ml/min/g. Below 0.4 ml/min/g perfusion impairment caused ATP to decrease and ADP and AMP to increase. Similarly, significant changes in tissue lactate and adenosine were only observed at a flow rate below 0.4 ml/min/g. In contrast, SAH increased by almost 400% when myocardial blood flow decreased below 0.6 ml/min/g.

In three experiments of group 1 and all experiments of group 2, total tissue adenosine and SAH content were measured in subendocardial and subepicardial tissue samples from the nonischemic region after 30 minutes of homocysteine infusion (n = 7). In three of the experiments of group 2 regional myocardial blood flow was measured with tracer microspheres. As summarized in Figure 5, subendocardial blood flow was slightly greater than that in the subepicardium. There was no statistically significant difference in total tissue adenosine content between the two regions. However, a small, but statistically significant, difference in total tissue SAH content was observed (p < 0.05 vs. control).

TABLE 1. Effects of Reduced Myocardial Blood Flow on Tissue Metabolite Levels

<table>
<thead>
<tr>
<th>Regional myocardial blood flow (ml/min/g)</th>
<th>Range (mean ± SEM)</th>
<th>n</th>
<th>ATP (μmoles/g)</th>
<th>ADP (μmoles/g)</th>
<th>AMP (μmoles/g)</th>
<th>Adenosine (nmoles/g)</th>
<th>S-Adenosylhomocysteine (nmoles/g)</th>
<th>Lactate (μmoles/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.22-0.90</td>
<td>1.02 ± 0.03</td>
<td>17</td>
<td>5.33 ± 0.17</td>
<td>1.05 ± 0.05</td>
<td>0.14 ± 0.01</td>
<td>2.0 ± 0.3</td>
<td>3.9 ± 0.3</td>
<td>0.67 ± 0.12</td>
</tr>
<tr>
<td>0.89-0.60</td>
<td>0.77 ± 0.02</td>
<td>7</td>
<td>5.41 ± 0.39</td>
<td>1.07 ± 0.09</td>
<td>0.14 ± 0.02</td>
<td>2.2 ± 0.2</td>
<td>5.4 ± 1.6</td>
<td>0.83 ± 0.18</td>
</tr>
<tr>
<td>0.59-0.40</td>
<td>0.50 ± 0.02</td>
<td>9</td>
<td>4.88 ± 0.21</td>
<td>1.19 ± 0.09</td>
<td>0.16 ± 0.01</td>
<td>2.9 ± 0.8</td>
<td>21.0 ± 3.1</td>
<td>0.97 ± 0.19</td>
</tr>
<tr>
<td>0.39-0.20</td>
<td>0.28 ± 0.02</td>
<td>16</td>
<td>4.49 ± 0.21</td>
<td>1.26 ± 0.06</td>
<td>0.17 ± 0.1</td>
<td>22.1 ± 6.6</td>
<td>57.4 ± 8.8</td>
<td>3.85 ± 0.85</td>
</tr>
<tr>
<td>0.19-0.02</td>
<td>0.28 ± 0.02</td>
<td>17</td>
<td>3.50 ± 0.31</td>
<td>1.45 ± 0.07</td>
<td>0.25 ± 0.2</td>
<td>46.9 ± 9.1</td>
<td>69.5 ± 5.6</td>
<td>7.02 ± 1.16</td>
</tr>
<tr>
<td>0.09-0.01</td>
<td>0.09 ± 0.01</td>
<td>17</td>
<td>2.50 ± 0.31</td>
<td>1.45 ± 0.07</td>
<td>0.25 ± 0.2</td>
<td>46.9 ± 9.1</td>
<td>69.5 ± 5.6</td>
<td>7.02 ± 1.16</td>
</tr>
</tbody>
</table>

Data are from a total of 66 samples obtained in three different experiments.

*p < 0.01, t < 0.001, p < 0.05 vs. control (flow 1.02 ± 0.03 ml/min/g).
level between the subendocardial and the subepicardial samples. SAH content, however, was significantly elevated (60%) in the subendocardial layers (Figure 5).

Coronary Underperfusion

Following protocol for group 3 under control conditions, at a perfusion pressure of 110 mm Hg in the cannulated coronary artery, arterial and coronary venous plasma levels of adenosine were 75.4±14.0 and 132.8±26.7 pmol/ml plasma, respectively (p<0.02, n = 5). Arterial and coronary venous plasma levels of lactate were 2.6±0.6 and 2.4±0.4 μmol/ml, respectively (NS, n = 5). Increasing coronary perfusion pressure to 150 mm Hg did not significantly affect the hemodynamic and metabolic parameters (Table 2, Figure 6). However, upon decreasing coronary perfusion pressure to 60, 45, and 35 mm Hg, circumflex coronary blood flow, coronary venous oxygen content, and regional myocardial oxygen consumption decreased considerably although global hemodynamic parameters were not significantly affected (Table 2). Adenosine release from the circumflex-perfused myocardium progressively increased with decreasing perfusion pressure while lactate consumption was reversed to a net lactate production although it did not reach the level of significance (n = 5; Figure 6). When coronary perfusion pressure was decreased from 45 to 35 mm Hg the release of adenosine increased by 109±35% (p<0.05; n = 5), whereas mean arteriovenous difference of lactate at both perfusion pressures was identical (-1.2±0.6 vs. -1.2±0.4; n = 5).

In separate experiments, coronary perfusion pressure was adjusted from 110 to 40 mm Hg for 20 minutes, and the time course of adenosine release was determined. Mean arteriovenous difference under control conditions was 36 pmol/ml plasma, and after 1, 5, 10, and 20 minutes of underperfusion, the difference increased to 109, 373, 334, and 415 pmol/ml plasma, respectively. (Mean values from two experiments.)

To determine the site of the enhanced adenosine formation within the myocardium that resulted in increased coronary venous release (Figure 6), coronary perfusion pressure was adjusted to 110, 60, 40, and 30 mm Hg during homocysteine infusion and the resulting transmural distribution of SAH was determined. Following this protocol (group 4) the results given in Figure 7 were derived. As shown in this figure, at a perfusion pressure of 110 mm Hg myocardial SAH levels were nearly homogeneously distributed across the left ventricular wall. At a perfusion pressure of 60 mm Hg, SAH levels were slightly increased in the subendocardial layers only. Decreasing pressure further to 40 and 30 mm Hg not only enhanced subendocardial SAH levels to values of 120 and 170 nmoles/g but also steepened the transmural gradient of SAH considerably. In addition, the bound-

**TABLE 2. Effects of Graded Coronary Under-Perfusion on Global Cardiovascular Hemodynamics and on Regional Coronary Blood Flow, Coronary Venous Oxygen Content, and Myocardial Oxygen Consumption**

<table>
<thead>
<tr>
<th>Coronary perfusion pressure (mm Hg)</th>
<th>150 (Control)</th>
<th>110 (Control)</th>
<th>60</th>
<th>45</th>
<th>35</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peak left ventricular pressure (mm Hg)</td>
<td>137±9</td>
<td>136±8</td>
<td>132±8</td>
<td>132±7</td>
<td>132±10</td>
</tr>
<tr>
<td>Left ventricular dP/dt max (mm Hg/sec)</td>
<td>2,390±305</td>
<td>2,280±240</td>
<td>2,310±290</td>
<td>2,340±260</td>
<td>2,300±360</td>
</tr>
<tr>
<td>Heart rate (beats/min)</td>
<td>150±11</td>
<td>150±8</td>
<td>153±9</td>
<td>156±9</td>
<td>156±9</td>
</tr>
<tr>
<td>Diastolic aortic pressure (mm Hg)</td>
<td>109±9</td>
<td>108±6</td>
<td>104±6</td>
<td>102±6</td>
<td>104±7</td>
</tr>
<tr>
<td>Coronary blood flow (ml/min)</td>
<td>55±12</td>
<td>45±9</td>
<td>31±5*</td>
<td>23±6*</td>
<td>15±11*</td>
</tr>
<tr>
<td>Coronary venous oxygen content (ml/100 ml)</td>
<td>8.6±1.3</td>
<td>9.1±0.7</td>
<td>6.2±0.9*</td>
<td>5.7±1.1*</td>
<td>5.0±0.6*</td>
</tr>
<tr>
<td>Regional myocardial oxygen consumption (ml/min)</td>
<td>5.9±1.1</td>
<td>5.5±0.8</td>
<td>4.2±0.4*</td>
<td>3.0±0.5*</td>
<td>2.2±0.5*</td>
</tr>
</tbody>
</table>

*p<0.05 vs. control, f*p<0.02 vs. control; n = 5.
ary with normal tissue moved toward the subepicardium (Figure 7).

SAH-Hydrolase Activity

Regional differences of SAH-hydrolase activity in the heart may have influenced the measured levels of SAH in the experiments reported. Therefore, the activity of the enzyme was determined in different areas of the dog heart. From data given in Table 3, it is evident that there are no significant regional differences in the enzymatic activity between ventricular wall and septum. Furthermore, SAH-hydrolase activity in the subendocardium was similar to that measured in the subepicardium. Only the atria exhibited a slightly but significantly higher SAH-hydrolase activity ($p<0.01$).

The pH-dependency of dog heart SAH-hydrolase activity is shown in Figure 8. Hydrolytic activity was maximal at pH 7.0 and considerably decreased above pH 7.5 and below pH 6.5. When assayed in the synthetic direction, SAH-hydrolase activity was maximal at pH 8.0 and continuously decreased with reduced pH.

**Figure 6.** Relation between arteriovenous differences of adenosine and lactate and coronary perfusion pressure. Coronary perfusion pressure was raised from 110 to 150 mm Hg and then decreased to 60, 45, and 35 mm Hg. Blood samples were taken 5 minutes after each step in coronary perfusion pressure. Data are from five experiments.

![Figure 6](image6.png)

**Figure 7.** Transmural tissue levels of SAH in the left ventricular myocardium at different coronary perfusion pressures (110, 60, 40, and 30 mm Hg). SAH levels refer to 30 minutes of intravenous infusion of homocysteine (10 μmoles/kg/min). Thickness of each layer was between 1.7 and 2.0 mm.

![Figure 7](image7.png)

**Discussion**

Most adenosine in tissue is bound to intracellular proteins, presumably SAH-hydrolase. The half-life of this adenosine-SAH-hydrolase complex is several hours, and its pool size remains unchanged even when cell damage is induced. Because of a large protein-bound fraction, considerable changes in the concentration of free cytosolic adenosine may produce only small changes in total tissue content of adenosine. As shown in the preceding study, the rate of SAH accumulation in the presence of L-homocysteine is proportional to the concentration of free cytosolic adenosine. Together with measurement of global tissue adenosine levels, this permitted the calculation of free cytosolic aden-

**Table 3.** Specific Activities of SAH-Hydrolase in Tissue Homogenates From Different Parts of the Dog Heart

<table>
<thead>
<tr>
<th>Tissue Homogenate</th>
<th>SAH-Hydrolase activity (nmol/min/mg protein)</th>
<th>Synthesis</th>
<th>Hydrolysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Left ventricle</td>
<td></td>
<td>0.59 (2)</td>
<td>0.38±0.07</td>
</tr>
<tr>
<td>Epi</td>
<td></td>
<td>0.57 (2)</td>
<td>0.36±0.03</td>
</tr>
<tr>
<td>Right ventricle</td>
<td></td>
<td>0.38±0.10</td>
<td>(4)</td>
</tr>
<tr>
<td>Septum</td>
<td></td>
<td>0.35±0.03</td>
<td>(4)</td>
</tr>
<tr>
<td>Atria</td>
<td></td>
<td>0.70±0.12*</td>
<td>(7)</td>
</tr>
</tbody>
</table>

Numbers in parentheses are the number of observations from different hearts.
SAH, S-adenosylhomocysteine; Epi, epicardium; and Endo, endocardium.

*p<0.01 atrium vs. left ventricle.
tissue acidosis. In severely ischemic myocardium flow may impede substrate availability and cause adenosine. A profound reduction in coronary blood depends on the concentration of free cytosolic experimental conditions, the rate of SAH-formation concentration greater than 200 pM. Under these conditions, the concentration of SAH-hydrolase catalyzed reaction to SAH synthesis. 12 Like other enzymes, the activity SAH-hydrolase is saturated by homocysteine at a concentration as well as the intracellular concentration of hydrogen ions. The homocysteine concentration in plasma is normally approximately 10 μM and left ventricle or between subendocardium and subepicardium (Table 3). In contrast, the atria contained twice the activity of the enzyme compared with ventricular myocardium.

**Substrate availability and pH.** Increased levels of L-homocysteine and adenosine convert the net direction of the SAH-hydrolase catalyzed reaction to SAH synthesis. 12 Like other enzymes, the activity of cytosolic SAH-hydrolase depends on substrate concentration as well as the intracellular concentration of hydrogen ions. The homocysteine concentration in plasma is normally approximately 10 μM and infusion of homocysteine in the dog at a rate of 10 μmoles/kg/min would be expected to increase this level to the millimolar range assuming homocysteine is distributed evenly throughout the different fluid spaces. As shown for the isolated guinea pig heart 12 and predicted from the kinetic parameters, 13 SAH-hydrolase is saturated by homocysteine at a concentration greater than 200 μM. Under these experimental conditions, the rate of SAH-formation depends on the concentration of free cytosolic adenosine. A profound reduction in coronary blood flow may impede substrate availability and cause tissue acidosis. In severely ischemic myocardium with blood flow less than 0.1 ml/min/g, SAH formation was found to be slightly depressed when compared with that of less ischemic samples (0.2–0.3 ml/min/g) (Figure 3). Local changes in tissue pH may have contributed to this effect. A reduction of intracellular pH from 7.4 to 6.0, as found in severe cardiac ischemia, 21 will reduce the activity of the enzyme in dog myocardium by 40% (Figure 8). Despite these unfavorable conditions, SAH levels in severely ischemic tissue samples were still 16-fold above respective control values.

**Hemodynamic effects.** The homocysteine concentration should not affect cardiovascular hemodynamics. Similar to findings obtained in the isolated perfused guinea pig heart, 12 homocysteine neither affected ventricular performance nor altered basal coronary blood flow in the present study.

**Adenosine, SAH, and Myocardial Oxygen Supply**

Two major hypotheses have been proposed to explain the relation between adenosine formation and myocardial energy metabolism (for review, see Feigl 20 and Sparks and Bardenheuer 22): 1) A decrease in the supply/demand ratio for oxygen may be the stimulus for an enhanced adenosine formation by the heart. 1, 2 Inadequate oxygen supply to the mitochondria reduces ATP synthesis, and the decrease in the cytoplasmic phosphorylation potential may finally mediate the enhanced adenosine formation. 23 In this view, the vasodilator adenosine is thought to act in a feedback controlled system adjusting oxygen supply to myocardial oxygen requirements. 1, 2) Adenosine is formed in direct proportion to cardiac ATP-utilization. 24 In this model, myocardial oxygen consumption as such determines the formation of adenosine which remains unaffected by secondary increases in coronary flow.

Both hypotheses have recently been evaluated in the isolated perfused guinea pig heart in which coronary flow and myocardial oxygen requirements have been systematically altered. 3, 4 It was found that the major stimulus of cardiac adenosine formation is the ratio of myocardial oxygen supply to myocardial oxygen demand and not the oxygen consumption as such. The present study permits a first evaluation of the oxygen supply/demand hypothesis in the heart in situ. As long as regional myocardial blood flow ranges between 1.2 and 0.6 ml/min/g, levels of free cytosolic adenosine as reflected by the rate of SAH accumulation were not significantly affected (Figure 3). However, curtailing oxygen delivery further (coronary blood flow below 0.6 ml/min/g) caused a steep increase in the rate of SAH accumulation. This relation was similar for samples derived from the subendocardium and the subepicardium. Apparently, the heart in situ can compensate for a certain reduction in oxygen supply without any increase in free cytosolic adenosine.

Although free cardiac adenosine also increased in the isolated perfused guinea pig heart when oxygen supply was reduced by lowering perfusate P O2, 12 there is an important difference as compared with the dog heart in situ. SAH in the isolated perfused
heart increased threefold when perfusate \( P_0 \) was decreased from 660 (normoxia, 95% oxygen) to only 495 mm Hg.\(^{12}\) This difference may be due to the possibility that oxygenation of isolated buffer-perfused heart preparations is borderline even under "normoxic" conditions. Any decrease in oxygen supply may therefore result in an enhanced rate of adenosine formation.

**SAH as a Sensitive Index of Myocardial Ischemia**

Accumulation of SAH was closely related to the underperfused myocardial area. Within a narrow border zone, SAH steeply increased to values approximately 30-fold above respective controls (Figures 1 and 2). Compared with the increase in SAH, the changes in tissue adenine nucleotides and adenosine were rather small. A decrease in regional myocardial blood flow from 0.77 to 0.50 ml/min/g changed ATP, ADP, and AMP levels by \(-9\%\), \(+11\%\), and \(+15\%\), respectively (changes are not significant), while SAH levels were increased by 400% at the same time. The marked increase in SAH with only a minor decrease in ATP level is not an unexpected finding. Tissue content of ATP is approximately 5,300 nmoles/g in the dog heart, while those of adenosine and SAH are only 2 and 1 nmoles/g, respectively (Table 1). Thus, decreases in ATP too small to measure could cause large changes in the dephosphorylated degradative products such as adenosine, which in the presence of homocysteine is in part converted into SAH.

Since at a given concentration of free adenosine the rate of SAH accumulation is constant over time,\(^{12}\) this will further augment the initial signal. As to the amount of ATP trapped as SAH, it cannot be calculated from our data that irrespective of the degree of myocardial ischemia, only 3–6% of the lost ATP was recovered in the SAH-fraction (Table 1). This finding probably explains why trapping of adenine compounds in the SAH pool during ischemia did not accelerate repletion of ATP stores during myocardial reperfusion.\(^{25}\)

Using SAH as a sensitive index of myocardial ischemia has several practical advantages. Labile metabolites such as ATP, phosphocreatine, and adenosine require very rapid tissue fixation procedures such as freeze clamping for accurate metabolite measurements. Freeze clamping, however, distorts the tissue and makes mapping of metabolites in different layers of the heart impossible. However, tissue SAH content is an integral, via free cytosolic adenosine, of ATP breakdown over time. Therefore, because tissue levels of SAH are not so labile, they are less influenced by the tissue fixation procedure and transmural gradients of SAH can be determined using conventional freezing techniques.

**Transmural Gradient of Adenosine Formation**

Measurement of rate of SAH formation permits the evaluation of even subtle transmural differences in the level of free cytosolic adenosine. In the unstressed heart, SAH formation in the subendocardium exceeded that in the subepicardium by 60% (Figure 5). Since specific activity of SAH-hydrolase is the same in subendocardial and subepicardial layers of left ventricular myocardium (Table 3), this finding clearly indicates that a higher free cytosolic adenosine level prevails in the subendocardium. Under these conditions total tissue content of adenosine was the same in the subendocardium as in the subepicardium, in agreement with results obtained by others.\(^{26,27}\) It is interesting to note that in subendocardial layers oxygen consumption is higher,\(^{28,29}\) while at the same time oxygen supply may be impeded because of extravascular compression during systole.\(^{30}\) Whether differences in tissue oxygenation are responsible for the increased free adenosine level or whether differences in adenosine forming and metabolizing enzymes between subendocardium and subepicardium can account for this finding remains to be elucidated.

Upon decreasing coronary perfusion pressure to the lower range of autoregulation (60 mm Hg) SAH levels increased first in the subendocardial layers. This result confirms the view that the intrinsic capacity of the heart to maintain constant blood flow despite changes in arterial perfusion pressure (autoregulation) is first exhausted in the subendocardial layers.\(^{31}\) In addition, this finding provides further evidence for the view that in the unstressed heart adenosine is only produced at an accelerated rate when the oxygen delivery to the heart relative to its demand for oxygen is compromised. Lowering coronary perfusion pressure below the range of autoregulation not only caused the subendocardial SAH level to increase steeply but also considerably augmented and steepened the transmural SAH gradient. The ischemia, as evidenced by the rate of SAH accumulation, was clearly a function of distance from the epicardium (Figure 7).

Transmural gradients have been measured in the past for other metabolites and enzymes of cardiac energy metabolism. Under control conditions, levels of adenine nucleotides in the subendocardium were reported to be identical to those in the subepicardium, and the ratio for lactate was also found to be close to unity.\(^{32}\) Upon decreasing coronary perfusion pressure in an in situ model to values of approximately 40 mm Hg, Griggs et al\(^{33}\) found tissue lactate to be four times greater in the subendocardium than in the subepicardium, while ATP in the subendocardium was decreased by 10%. In another study in which \(\beta\)-adrenergic stimulation in the presence of a proximal aortic stenosis caused a fall in systolic coronary perfusion pressure by 120 mm Hg below left ventricular systolic pressure, subendocardial lactate was 2.2-fold greater and ATP levels in the subendocardium were diminished by 3%.\(^{34}\) It should be noted that a higher glycogen content\(^{35}\) as well as a greater activity of glycogen phosphorylase and other glycolytic enzymes\(^{35,36}\) are present in the subendocardium. Thus, given the same degree of
Adenosine and Coronary Blood Flow Regulation

The adenosine hypothesis of metabolic control of coronary blood flow postulates that the adenosine formed by the heart is causally involved in the adjustment of coronary blood flow to changing metabolic demands of the myocardium. The implications of this hypothesis have been intensively studied during the last decade (for review, see Feigl and Olsson and Bürger). Using adenosine deaminase to degrade endogenously formed adenosine was shown to attenuate the reactive hyperemic response but not to alter basal coronary blood flow or pressure autoregulation. With a different experimental approach the present study demonstrates that upon decreasing coronary perfusion pressure to the lower range of autoregulation (60 mm Hg), the transmural gradient of the free cytosolic adenosine level remained largely unchanged and was slightly elevated only in the subendocardial layers. Given the sensitivity of SAH to detect even small changes in free cytosolic adenosine, this observation makes it rather unlikely that adenosine plays a role during pressure autoregulation, thus confirming and extending the functional studies of Dole et al.

It should be noted that it has previously been shown that adenosine deaminase attenuates coronary vasodilation in the canine heart during hypoxia. As shown in the preceding study, the isolated perfused guinea pig heart the amount of adenosine generated during hypoxic perfusion appears to be quantitatively sufficient to account for most of the observed increase in coronary flow. Similarly, the subendocardial free cytosolic adenosine levels in the dog were significantly higher than those in the subepicardium. Together with the findings of a higher subendocardial blood flow and oxygen consumption, this in agreement with the prediction made by the adenosine hypothesis. Whether this parallelism, however, indicates a causal relation remains to be elucidated.

Another important implication of the present study concerns the validity of measurements of pericardial fluid adenosine to reflect the concentration of interstitial adenosine. The transmural gradient of SAH strongly argues against the possibility that interstitial fluid adenosine from the subendocardium and midmyocardium can reach the subepicardial layers in significant amounts. Thus, it is most likely that pericardial fluid adenosine only reflects changes in the outermost layers of the heart and may miss changes originating from deeper layers. Conversely, we found a good agreement between coronary venous adenosine release and SAH accumulation (Figures 6 and 7). Thus, despite the known limitations of adenosine release data, for example, by rapid metabolism and preferential uptake by the coronary endothelium, this index apparently more closely reflects transmyocardial adenosine production rates than measurement of pericardial fluid adenosine.

Taken together our findings on SAH formation during myocardial ischemia and coronary underperfusion are in line with the view that also in the dog heart in vivo adenosine production critically depends on the supply/demand ratio for oxygen. In contrast to findings obtained in the isolated guinea pig heart, the heart in situ can tolerate a certain reduction in oxygen supply (approximately 40%) without concomitant changes in the formation of adenosine.

Acknowledgments

We thank Ms S. Aldus and Ms B. Patzer for their expert technical assistance during the experiments.

References


---

**Key Words** • adenosine • S-adenosylhomocysteine • SAH-hydrolase • myocardial underperfusion
Formation of S-adenosylhomocysteine in the heart. II: A sensitive index for regional myocardial underperfusion.
A Deussen, M Borst, K Kroll and J Schrader

Circ Res. 1988;63:250-261
doi: 10.1161/01.RES.63.1.250

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/63/1/250

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation Research can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation Research is online at:
http://circres.ahajournals.org/subscriptions/