Influence of β-Adrenergic Stimulation and Contraction Frequency on Rat Heart Interstitial Adenosine

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Adenosine (ADO) has an antiadrenergic action in the heart that causes an attenuation of contractile and metabolic responses elicited by β-adrenergic stimulation. The effect of an increase in oxygen consumption elicited by either β-adrenergic stimulation or an increase in contraction frequency on interstitial fluid and coronary effluent ADO levels was investigated in isolatedperfused isovolumically contracting rat hearts. ADO in left ventricular surface transudates and coronary effluents was rendered fluorescent with chloroacetaldehyde, and the formed ethenoadenosine derivative was quantitated with high-performance liquid chromatography fluorescence detection. Heart preparation integrity was verified by determining the activities of lactate dehydrogenase and ADO deaminase in the transudates. Isoproterenol (10^{-6} M) elicited a 45% increase in oxygen consumption and a 54% increase in developed left ventricular pressure in hearts paced at 240 beats/min. With isoproterenol the control transudate ADO concentration (304 pmol/ml) increased 493%, and the control effluent ADO concentration (48 pmol/ml) increased 259%. Increasing the contraction frequency from 180 to 300 beats/min in the presence of 10^{-6} M propranolol increased oxygen consumption by 45% and decreased left ventricular pressure by 29%. With the increase in contraction frequency, the transudate ADO concentration did not increase significantly. However, the ADO concentration in the effluent was an average of 269% greater in hearts contracting at the higher frequency. Increasing the contraction frequency of hearts treated with both 10^{-6} M propranolol and 10^{-5} M atropine also had no significant effect on the level of transudate ADO. The effluent level of ADO increased only 78%. Levels of ADO in transudates were not significantly affected by mesothelial cell metabolism. These results suggest that with β-adrenergic stimulation the interstitial level of ADO in the heart increases to levels that are sufficient to manifest its antiadrenergic effects. Furthermore, there is not always a correlation between the levels of ADO found in the interstitial and effluent fluid compartments. (Circulation Research 1990;66:457–468)

Adenosine limits the contractile and metabolic responsiveness of the mammalian heart to β-adrenergic stimulation.1–4 The manifestation of this antiadrenergic action in the well-oxygenated heart is suggested by the augmentation of isoproterenol (ISO)-induced positive inotropic responses when endogenous interstitial adenosine is metabolized by exogenously administered adenosine deaminase (ADA).5,5 During episodes of hypoxia or ischemia, enhanced levels of adenosine in the myocardium6 have been postulated to serve a negative-feedback role that limits heart responsiveness to adrenergic stimulation.1,7 Catecholamines are thought to initiate positive inotropic and glycogenolytic responses in the heart by interacting with β-adrenoceptors, which promote the activation of sarcolemmal adenylate cyclase.8 The resulting enhancement of cellular adenosine 3′,5′-cyclic monophosphate (cAMP) levels leads to the activation of cAMP-dependent protein kinase9,10 and, ultimately, the phosphorylation of myocardial proteins thought to be involved in contractile and metabolic responses.9–11 Adenosine elicits its antiadrenergic effects primarily by attenuating catecholamine-induced activation of adenylate cyclase via A1 adenosine receptors,12,13 thereby reducing cAMP-dependent
incorporation of phosphate into myocardial proteins.\textsuperscript{14} In this manner adenosine may protect the O\textsubscript{2}-deprived myocardium from catecholamine-induced necrosis.

While adenosine is continually released into the interstitial and vascular spaces of the normoxic myocardium as determined directly from left ventricular surface transudates and coronary effluent,\textsuperscript{6} the negative feedback inhibition of \(\beta\)-adrenergic stimulation by adenosine requires that interstitial levels of the nucleoside increase on appearance of \(\beta\)-adrenergic-elicited contractile and metabolic responses. Although evidence indicates an enhanced release of adenosine into the coronary effluent of adrenergically stimulated hearts,\textsuperscript{15} effluent levels of adenosine are influenced by endothelial cells lining the coronary vessels.\textsuperscript{16,17} Since the quantitative contribution of endothelial cells to the interstitial adenosine level remains to be determined, changes in coronary effluent levels of adenosine cannot be presumed to reflect changes in interstitial levels of this nucleotide.\textsuperscript{6,16}

This study was undertaken to determine the influence of adrenergic stimulation on interstitial levels of adenosine in the left ventricular myocardium by analyzing epicardial transudate fluid obtained from the surface of the left ventricle. Since adrenergic stimulation of the myocardium is known to increase myocardial oxygen consumption (MVO\textsubscript{2}), the importance of such an increase in MVO\textsubscript{2} on interstitial adenosine was also determined in atropine- and/or propranolol-treated hearts paced at different contraction frequencies to increase MVO\textsubscript{2} independent of neurohumoral stimulation.

**Materials and Methods**

**Heart Preparation**

Animals used in this study were maintained and used in accordance with recommendations in the Guide for the Care and Use of Laboratory Animals prepared by the Institute of Laboratory Animal Resources, National Research Council, Department of Health and Human Services (National Institutes of Health publication No. 85-23, revised in 1985) and guidelines of the Animal Care Advisory Committee of the University of Massachusetts Medical School, Worcester. Male Sprague-Dawley rats (250–400 g) were anesthetized and heparinized intraperitoneally with pentobarbital sodium (40 mg/kg) and heparin sodium (500 units), respectively. After excision, each heart was immediately perfused at the constant rate of 8.0 ml/min with physiological saline (PS) at 37°C via an aortic cannula. The perfusion pressures ranged from 60 to 75 mm Hg. The PS contained (mM) NaCl 118, KCl 4.7, CaCl\textsubscript{2} 2.5, NaHCO\textsubscript{3} 25, KH\textsubscript{2}PO\textsubscript{4} 1.2, MgSO\textsubscript{4} 1.2, and glucose 10. The pH was maintained at 7.4 by gassing the PS with 95% O\textsubscript{2}-5% CO\textsubscript{2}. The great veins were ligated, and each heart was inverted and instrumented with modifications as illustrated in Figure 1 by techniques previously described.\textsuperscript{6} Briefly, developed left ventricular pressure was measured with a water-filled latex balloon inserted into the left ventricular cavity via the left atrium and attached by a polyethylene cannula to a Statham P23Dd pressure transducer (Gould, Cleveland, Ohio). The left ventricular pressure signal was electronically differentiated to obtain the maximum rate of left ventricular pressure development (+dP/dt\textsubscript{max}). All contractile data were recorded on a multichannel polygraph (model 2600, Gould). The optimal preload was determined by varying balloon volume until the maximal obtainable dP/dt was attained. The balloon volume was thereafter maintained constant permitting isovolumic contraction of the heart. The hearts were paced at the rates of 180, 240, or 300 beats/min. The temperatures of the aortic perfusate and the epicardial transudate droplets were determined with a Thermalert (model TH-8, Sensotek, Clifton, New Jersey).

A polyethylene cannula was placed in the pulmonary artery for sampling the coronary effluent. The coronary effluent O\textsubscript{2} tension was continuously monitored by directing the pulmonary artery fluid to a Clark-type polarographic electrode (model 53, Yellow Springs Instrument, Yellow Springs, Ohio). The dead space from the pulmonary artery to the point of coronary effluent collection distal to the O\textsubscript{2} electrode was approximately 0.9 ml. The O\textsubscript{2} content of the coronary effluent was calculated by using 0.0321 \(\mu\)l O\textsubscript{2}/mm Hg/ml as the solubility of O\textsubscript{2}. MVO\textsubscript{2}, expressed as milliliters of O\textsubscript{2} consumed per minute per 100 g dry weight of ventricular mass, was obtained as the product of the coronary flow and the difference between the O\textsubscript{2} contents of the aortic PS inflow and pulmonary artery outflow. The ventricular myocardium was dried overnight at 80°C, and the dry weight was determined.

**Experimental Protocols**

The effect of \(\beta\)-adrenocceptor stimulation on interstitial adenosine was ascertained by determining the levels of adenosine in left ventricular surface transudates from ISO-treated hearts and comparing them with the levels of the nucleoside present in coronary venous effluents that were collected simultaneously. At least 15 minutes after the hearts were inverted and instrumented, control ventricular transudate and coronary venous effluent samples were collected while pacing at 240 beats/min. ISO dissolved in 0.1% sodium metabisulfite was infused at 0.052 ml/min into the aortic cannula to achieve a PS ISO concentration of 10\textsuperscript{-8} M. At 1-minute intervals after the first evidence of an ISO-induced increase in contractile function, left ventricular transudate and coronary effluent samples were simultaneously collected from the hearts and retained on ice. After 5 minutes, the ISO infusion was terminated. Transudate and effluent samples were also collected 15 minutes after termination of the ISO infusion.

The effect of contraction frequency on adenosine concentrations in left ventricular transudates and coronary effluents was determined. The hearts were continually perfused with 10\textsuperscript{-8} M propranolol to
block the effects of endogenously released catecholamines. This concentration of propranolol was found to completely inhibit the contractile response of the heart to $10^{-7}$ M ISO (data not shown). After the hearts were inverted and instrumented, pacing began at either 300 or 180 beats/min for 15 minutes. Transudate accumulating on the surface of the heart during this time period was aspirated and discarded. After the 15-minute period, transudate and coronary effluent samples were simultaneously collected at 1-minute intervals for 5 minutes. The atrial pacing rate was then either lowered to 180 or raised to 300 beats/min for hearts that were contracting initially at rates of 300 or 180 beats/min, respectively. Immediately after the change in rate, transudate and coronary effluent samples were again simultaneously collected at 1-minute intervals for 5 minutes. Data obtained from all hearts at each time point before and after the change in contraction frequency were pooled with those data obtained at corresponding time points for each of the two contraction frequencies. Additional experiments were conducted with hearts continuously exposed to $10^{-6}$ M propranolol as well as $10^{-5}$ M atropine to block endogenously released acetylcholine. Instrumented hearts were paced at 180 beats/min for 15 minutes, whereupon transudate and effluent samples were simultaneously collected, and the pacing rate was increased to 300 beats/min. Sample collection was continued at 1-minute intervals for 5 minutes. The pacing rate was subsequently reduced to 180 beats/min, and samples were again obtained at 5 minutes.

The presence of lactate dehydrogenase (LDH) and ADA in ventricular transudates and coronary effluents was determined and compared with the myocardial levels of the enzymes to evaluate tissue integrity after heart inversion and ventricular balloon inflation. Transudate and coronary effluent samples were collected before and after both heart inversion and balloon inflation. Additional samples were obtained before, during, and after $\beta$-adrenergic stimulation with $10^{-8}$ M ISO. Samples were stored at
−20°C until assayed for enzyme activity. At the termination of the experiment, some hearts were rapidly blotted, frozen in liquid nitrogen, pulverized with a Waring blender containing liquid nitrogen, and homogenized in 15 vol ice-cold PS with a Polytron generator (model PT-10, Brinkmann Instruments, Inc, Westbury, New York). The homogenate was centrifuged (0°C) at 15,000g for 20 minutes, and the supernatant was frozen until assayed for enzyme activity.

**Enzyme Assay Procedures**

Determination of enzymatic activities in transudate, effluent, and myocardial extract samples were conducted by use of a split beam Cary spectrophotometer (model 210, Varian Associates, Inc, Palo Alto, California) with a 1.0-cm path-length cell at 37°C. Assay solutions were allowed to equilibrate for 5 minutes before addition of samples to the incubation mixture. ADA activity was determined by adding 5–15 μl sample to 600 μl incubation mixture containing 50 mM potassium phosphate buffer (pH 7.4) and 0.23 mM adenosine and by recording the rate of decrease in absorbance at 265 nm for 5 minutes. The decrease in absorbance was linear for at least 10 minutes after the addition of commercially available ADA at levels in excess of those observed in the samples. The activity of LDH was determined by measuring the rate of decrease in absorbance at 340 nm for 5 minutes after addition of 5–20 μl sample to 590 μl incubation reagent containing 94 mM potassium phosphate buffer (pH 7.0), 0.78 mM sodium pyruvate, and 0.41 mM β-nicotinamide adenine dinucleotide. The decrease in absorbance was linear for 5 minutes after the addition of commercially available LDH at levels in excess of those observed in the samples. The volume activities for the enzymes were calculated by using the following relation: volume activity (units/ml) = (assay vol) (ΔA/min)/(ε)(sample vol) (1 cm) where ΔA is the change in absorbance and ε is the molar extinction coefficient. For the ADA and LDH assays, ε was 8.1 and 6.22, respectively. A unit is defined as a micromole of substrate transformed per minute at 37°C.

**Mesothelial Cell Metabolism and Adenosine**

Pericardium is composed of connective tissue covered by a layer of mesothelium continuous with that of the epicardium of the heart.18 Pericardia of rats and guinea pigs were obtained, dissected free of adhering fat, placed in incubation flasks containing PS maintained at 30°C, and gassed with 95% CO₂-5% O₂. After an equilibration period of 30 minutes, tissues were transferred to flasks containing fresh PS containing adenosine (10⁻⁶ M) and allowed to incubate with gentle agitation and gassing at 30°C for 30 minutes. At the end of the incubation period, tissues were removed, gently blotted, and weighed. Medium volumes were recorded, and aliquots were obtained for analysis of contained adenosine. Adenosine was found to be metabolized in PS after the pericardia were removed, possibly as a result of ADA released from damaged cells. This contribution to the loss of adenosine from medium in the presence of pericardium was assessed by incubating tissue-free media for an additional 30 minutes and obtaining additional aliquots for adenosine analysis. For guinea pig medium, non–tissue-related adenosine breakdown occurred at 0.31–3.78 pmol/min and accounted for 1.6–38.9% of the consumed adenosine. For rat medium, these values were 3.7–17.8 pmol/min and 9.7–39.1%, respectively. The metabolism of adenosine by the pericardia during the tissue incubation period could then be determined assuming the released ADA activities in the medium during and after the pericardium incubation were identical. Erythro-9-(2-hydroxy-3-nonyl)adenine (EHNA; 10⁻⁵ M), an inhibitor of ADA, was added to all media samples immediately after the above incubations. Adenosine was determined by standard HPLC separation and absorbance detection techniques.5 Surface
areas of pericardia were determined as follows. Selected pericardia were extended flat on aluminum foil, and the perimeters were traced. After trimming and weighing the foil pieces, the pericardial surface areas were calculated from the known ratio of foil area to foil weight. The ratio of pericardial area to wet weight of the traced tissue was used to determine areas of weighed tissues. Because of the fine nature of the rat pericardium and the number of rats needed to obtain an adequate amount of fat-free pericardia, experiments were also conducted using guinea pigs, which were found to possess pericardia that were easier to manipulate. After correction for evaporative changes in volume and degradation of adenosine during incubation by ADA, adenosine consumed per minute per square centimeter of pericardium was determined. Additional experiments were conducted with adenosine-free PS to assess net adenosine release by guinea pig pericardia.

**Materials**

All salts, reagents, solvents, and glucose were of certified or HPLC grade from Fisher, Medford, Massachusetts. L-Isoproterenol HCl, atropine, and Dl-propranolol were obtained from Sigma, St. Louis, Missouri. Adenosine and all enzymes and substrates used for assay procedures were obtained from Boehringer-Mannheim, Indianapolis, Indiana.

**Statistical Treatment**

Data analysis for adenosine concentration, MVO$_2$, and contractile function was based on Student's $t$ distribution for paired or unpaired data.$^{19}$ Enzymatic activity data were analyzed by Student's $t$ test for unpaired data. A probability of $p<0.05$ indicated a statistically significant difference.

**Results**

**$\beta$-Adrenergic Stimulation**

The administration of ISO to the isolated inverted heart resulted in an increase in contractile function and MVO$_2$ (Figure 2). One minute after the initial appearance of ISO-induced contractile responses, left ventricular pressure significantly increased 54% above the control value of 65±6 mm Hg and remained significantly elevated throughout the 5-minute period of ISO administration. At the same time +dP/dt$_{\text{max}}$ increased 59% from a control value of 1,758±204 to 2,785±330 mm Hg/sec. The +dP/dt$_{\text{max}}$ remained significantly elevated throughout the ISO administration. Values for the two contractile indexes returned toward control levels after 10 minutes subsequent to cessation of ISO stimulation. Concomitant with increased contractile function, MVO$_2$ significantly increased 45% from an initial control rate of 50.7±3.2 ml O$_2$/min/100 g dry wt (Figure 2). As with contractile function, MVO$_2$ remained significantly elevated until after ISO administration was terminated. The coronary effluent O$_2$ tensions before ISO stimulation and at 3 minutes into the stimulation period were 289±26 and 115±19 mm Hg, respectively. Ratios of O$_2$ supply/O$_2$ demand at these points were 1.81±0.12 and 1.21±0.04, respectively.

ISO elicited an elevation of adenosine levels in both the left ventricular epicardial transudates and coronary effluents (Figure 3). Levels of transudate adenosine increased 299% from a control value of 304±44 pmol/ml at 1 minute of ISO stimulation. A further rise to 455% of control values occurred at 2 minutes, whereupon the adenosine concentration in the transudates remained constant for the remainder of the ISO administration. A significant difference from control adenosine was not reached in transudates until after 3 minutes of ISO stimulation. Adenosine levels in the transudates returned to control within 10 minutes after cessation of ISO stimulation. Values of adenosine in the coronary effluent significantly increased 108% from a control value of 48±9 pmol/ml at 1 minute. Sequential samples at 1-minute intervals revealed that the effluent adenosine concen-
increase in transudate adenosine with ISO stimulation for a given increase in $\text{MV}_2$ was 12-fold greater than that of the coronary effluent adenosine.

**Changes in Contraction Frequency**

The contractile function and $\text{MV}_2$ were determined in hearts paced at two different contraction frequencies (Figure 4). Propranolol, a $\beta$-adrenergic antagonist, was present to inhibit the effects of endogenously released catecholamines. At each time point after initiation of the designated contraction frequency, the left ventricular pressure was significantly lower in hearts contracting at the frequency of 300 beats/min when compared with hearts with a contraction frequency of 180 beats/min. Over the entire 5-minute period, left ventricular pressure was 36% lower in hearts contracting at the higher frequency. With a contraction frequency of 300 beats/min, $+dP/dt_{\text{max}}$ was $1,862 \pm 148 \text{ mm Hg/sec}$, a value significantly lower than that recorded with hearts contracting at 180 beats/min ($2,493 \pm 171 \text{ mm Hg/sec}$). The reduced magnitude of left ventricular pressure and $+dP/dt_{\text{max}}$ found at the higher contraction frequency occurred concomitantly with a significantly higher $\text{MV}_2$. Over the entire 5-minute period, $\text{MV}_2$ at 300 beats/min was an average of 27% greater than those values for $\text{MV}_2$ observed with hearts contracting at the rate of 180 beats/min. After 5 minutes of beating at 180 or 300 beats/min, the coronary effluent $\text{O}_2$ tensions were $174 \pm 21$ and $114 \pm 12 \text{ mm Hg}$. Ratios of $\text{O}_2$ supply/$\text{O}_2$ demand at these time points were $1.41 \pm 0.05$ and $1.25 \pm 0.03$, respectively.

Adenosine levels in left ventricular epicardial transudates from hearts contracting at 180 beats/min were not significantly different from those obtained from hearts contracting at 300 beats/min (Figure 5). Adenosine levels ranged from $207 \pm 37$ to $299 \pm 50 \text{ pmol/ml}$ at 180 beats/min and $234 \pm 43$ and $386 \pm 73 \text{ pmol/ml}$ at 300 beats/min. The largest difference in mean adenosine concentrations in hearts contracting at the rate of 180 or 300 beats/min occurred at 3 and 4 minutes into the sampling period, when the higher contraction frequency elicited a nonsignificant 36% increase in adenosine levels. Adenosine levels in the coronary effluent did not replicate the pattern observed with left ventricular epicardial transudates. All values for adenosine were significantly greater in

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**TABLE 1. Relation Between the Increase in Transudate or Effluent Adenosine Concentration and Increase in $\text{O}_2$ Consumption Occurring in the Heart With Isoproterenol Stimulation or an Increase in Contraction Frequency**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>$\Delta T$ (pmol/ml)</th>
<th>$\Delta E$ (pmol/ml)</th>
<th>$\Delta \text{MV}_2$ (ml $\text{O}_2$/min/100 g dry wt)</th>
<th>$\Delta T/\Delta \text{MV}_2$</th>
<th>$\Delta E/\Delta \text{MV}_2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isoproterenol</td>
<td>1,423</td>
<td>114</td>
<td>23.5</td>
<td>60.6</td>
<td>4.9</td>
</tr>
<tr>
<td>Frequency</td>
<td>70</td>
<td>389</td>
<td>11.8</td>
<td>5.9</td>
<td>33.0</td>
</tr>
</tbody>
</table>

$\Delta T$, increase in transudate adenosine concentration; $\Delta E$, increase in effluent adenosine concentration; $\Delta \text{MV}_2$, increase in $\text{O}_2$ consumption. Values for $\Delta T$, $\Delta E$, and $\Delta \text{MV}_2$ are differences between the average values determined between the control value obtained before stimulation with isoproterenol and the average value obtained for the latter 3–5 minutes of isoproterenol stimulation, or for the latter 2–5 minutes of each contraction frequency (180 or 300 beats/min).
effluents from hearts contracting at the higher frequency of 300 beats/min. The adenosine concentration in the effluent from hearts contracting at the frequency of 180 beats/min ranged from 129±30 to 159±50 pmol/ml. Adenosine levels in the effluent from hearts contracting at 300 beats/min were an average of 269% above those levels present in effluent from hearts contracting at the lower frequency. When comparing ratios of changes in transudate or effluent adenosine to MVO₂ for hearts contracting at 180 or 300 beats/min, the increase of the adenosine level for a given increase in MVO₂ was 5.6-fold greater for effluent than for transudate (Table 1).

A similar pattern of results was observed in hearts treated with propranolol and atropine for adrenergic and cholinergic blockade (Figure 6). The adenosine concentration in transudates obtained from hearts contracting at 180 beats/min was 221.9±22.4 pmol/ml. This value did not change substantially with an increase in the pacing rate to 300 beats/min until 5 minutes after the increase was initiated, when it was elevated 125%. Since the variability between samples was observed to be high, at 5 minutes the increase was not found to differ significantly from the control value. At 5 minutes after the return to pacing at 180 beats/min the transudate adenosine concentration was not different from that determined before the elevated pacing rate. The adenosine level in the effluents of hearts paced at 180 beats/min was 101.1±15.5 pmol/ml. This value increased significantly by 38% at 1 minute after the pacing rate was changed to 300 beats/min and reached a maximum 78% increase at 4 minutes. The adenosine concentration returned to control levels by 5 minutes after a return to a pacing rate of 180 beats/min.

Mesothelial Cell Metabolism of Adenosine

Adenosine levels found in epicardial surface transudates may be modified by the single layer of
were tissue preparations.

Samples incubated in hearts treated with Adenosine by the guinea pig pericardium was 4.7-fold greater than that of the rat pericardium. To determine if mesothelial cells stimulated by ISO contribute to the net levels of adenosine in transudates, guinea pig pericardia were incubated at 37°C in adenosine-free PS. After a 45-minute incubation period, no adenosine was detected in the bathing medium of each of four pericardia ranging in weight from 14.4 to 30.4 mg in the presence or absence of ISO (10^{-7} M).

**Cellular Integrity of Heart Preparation**

The activities of two cytosolic enzymes, LDH and ADA, were determined in the transudates and effluents to assess the effect of mechanical manipulation or adrenergic stimulation on the cellular integrity of the hearts (Table 3). Analysis of effluent collected immediately before and after heart inversion indicated that no significant change in LDH or ADA activities occurred with inversion. Transudates were not collected from noninverted hearts since the absence of contamination by coronary effluent could not be assured. Repetitive alternating 5-minute episodes of inflation and deflation of the intraventricular balloon to 5 mm Hg and 0 mm Hg end-diastolic pressure, respectively, did not alter LDH and ADA activities in transudates or effluents (data not shown). Hearts were stimulated with ISO 15 minutes after inversion. The LDH activities in the transude on inversion and 15 minutes after ISO stimulation were an average of 102-fold and 84-fold greater, respectively, than corresponding values in the coronary effluent. With ISO, LDH activity was 549-fold greater in the transudate than in the corresponding coronary effluent. Although a similar overall trend was seen with ADA, transudate activities of this enzyme were significantly different from the corresponding coronary effluent values only immediately after inversion of the heart and inflation of the intraventricular balloon. The activities for LDH and ADA in whole heart homogenates were 529.692±55.718 and 547±4 units/g wet wt, respectively. Thus, assuming an extracellular space of 36.5 ml/100 g wet wt, at steady state 30 minutes subsequent to heart inversion, LDH and ADA activities in the interstitial space represented 0.0004% and 0.0028%, respectively, of the total activities for these enzymes present in the whole heart.

**Discussion**

**β-Adrenergic Stimulation**

The concentration of adenosine in transudate obtained from hearts isovolumically contracting at 240 beats/min was 304 pmol/ml before adrenergic stimulation. Previously we have reported transudate levels of adenosine to range from 30 to 67 pmol/ml in the paced (270 beats/min) nonworking heart.6,7 This suggests that interstitial levels of adenosine in the oxygenated heart become enhanced with an increase in work load. It is significant to note that the trans-
date concentration of the nucleoside exceeded that of the coronary effluent by approximately sixfold. Thus, it is apparent that the projected physiological effect of adenosine on heart function is potentially greater than the nucleoside concentration in the coronary effluent would indicate. Although ISO transudate and effluent levels of adenosine increased concomitantly with MVO₂, at steady state (3–5 minutes) the ratio of increase in adenosine concentration per unit increase in MVO₂ was 12-fold greater for the transudate than the similar ratio for the increase in coronary effluent adenosine (Table 1). This suggests that interstitial adenosine originates primarily from cardiomyocytes and that changes cannot be predicted easily from values of effluent adenosine. Moreover, the results clearly suggest that the interstitial levels of adenosine are sufficient to manifest an antiadrenergic action in the heart.¹,³,⁵

Catecholamine stimulation of the inverted hearts for 5 minutes elicited an increase in transudate adenosine concentration from 300 to 1,800 pmol/ml. The increased level of adenosine did not result from the ISO stimulation of the mesothelial cell layer. It may be thought that these values are an underestimate as a result of ADA activity in the transudate. However, ADA activity normally works in concert with other mechanisms to determine the net level of adenosine in the interstitial fluid. If deaminase and adenosine are distributed equally throughout the transudate droplets and the interstitial fluid, the adenosine present in the former most likely is representative of the net adenosine in the latter. Any effect of deaminase on the transudate level of adenosine once the transudate droplet is formed is thought to be negligible because the diffusion distance for adenosine from interstitial space to transudate droplet is small, the transudate is collected within 15 seconds, the temperature of the transudate on the surface of the heart is approximately 30.5°C, and the transudate is chilled immediately on collection. The activity of ADA is of greater concern in studies using pericardial infusates and epicardial surface wells in which a large sample volume provides a “sink” into which adenosine diffuses and at least 5 minutes are required for sample collection. Under these conditions, the adenosine that is deaminated is only slowly replenished by nucleoside diffusing from the interstitium.

Interstitial levels of adenosine may be underestimated as a result of mesothelial cell metabolism of adenosine in surface transudates. If it is assumed that a 2-μl transudate droplet containing 0.4 pmol adenosine (200 pmol/ml) is a hemisphere, the cross-sectional area in contact with the heart would be 4.47×10⁻² cm². About 2% of the total adenosine present in the droplet (7.6×10⁻³ pmol) would be consumed every 15 seconds. Thus, while the transudate values for adenosine underestimate the interstitial values, the differences resulting from mesothelial cell metabolism are thought to be minimal.

Coronary effluent levels of adenosine previously have been used to estimate the nucleoside concentration in the interstitial fluid. Adenosine in effluents from nonworking and working guinea pig hearts have been reported to increase 50-fold¹⁵ or 14-fold,²² respectively, with adrenergic stimulation. However, the adenosine content of the coronary perfusion fluid may be heavily influenced by the nucleoside metabolism of the endothelial cell layer.⁶,¹⁶ Others have attempted to circumvent this difficulty by obtaining pericardial fluid bathing the surface of the heart. An increase in canine heart MVO₂ with stellate ganglion stimulation elicited an increase in pericardial fluid adenosine from 18.8 to 40.3 pmol/ml. However, these values were dependent on equilibration time and the volume of the pericardial infusate. These values may

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**Table 3. Lactate Dehydrogenase and Adenosine Deaminase Activities in Fluid Samples Collected From the Left Ventricular Epicardial Surfaces and Coronary Effluents of Inverted Perfused Rat Hearts**

<table>
<thead>
<tr>
<th>Condition</th>
<th>Lactate dehydrogenase (10⁵ units/ml)</th>
<th>Adenosine deaminase (10⁵ units/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Transudate</td>
<td>Effluent</td>
</tr>
<tr>
<td>Before inversion</td>
<td>ND</td>
<td>14.9±6.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(8,5)</td>
</tr>
<tr>
<td>Inverted with isoproterenol</td>
<td>2,518±239*</td>
<td>24.6±10.2</td>
</tr>
<tr>
<td></td>
<td>(8,6)</td>
<td>(10,6)</td>
</tr>
<tr>
<td>15–25 min after inversion</td>
<td>1,068±340++</td>
<td>0.0</td>
</tr>
<tr>
<td>(No ISO)</td>
<td>(4,4)</td>
<td>(4,4)</td>
</tr>
<tr>
<td>15 min after inversion</td>
<td>2,911±819+</td>
<td>5.3±2.2</td>
</tr>
<tr>
<td>(10⁻⁵ ISO)</td>
<td>(4,3)</td>
<td>(4,3)</td>
</tr>
<tr>
<td>15 min after stimulation</td>
<td>530±192++</td>
<td>6.0±2.5</td>
</tr>
<tr>
<td>(10⁻⁵ ISO)</td>
<td>(3,3)</td>
<td>(4,3)</td>
</tr>
<tr>
<td>1–2 hours after inversion</td>
<td>191±39+++</td>
<td>0.0</td>
</tr>
<tr>
<td>(No ISO)</td>
<td>(5,4)</td>
<td>(5,4)</td>
</tr>
</tbody>
</table>

Values are the mean±1 SEM of enzyme activities determined as described in "Materials and Methods.” ND, not determined; ISO, isoproterenol. Numbers in parentheses indicate number of fluid samples (left) taken from number of hearts (right).

*Significant difference from the corresponding effluent value.†Statistically significant difference from the value obtained at inversion.
also be reduced by ADA activity and mesothelial cell metabolism. Most recently, Heller and Mohrmann24 found that epicardial surface transudate from noninverted isovolumic contracting rat hearts possessing a rubber seal around the ventricles contained 130 pmol adenosine/mL. Although this value is approximately half the present control value, concerns regarding the functional integrity of their preparation have been reported.25 The question must also be asked concerning whether transudate is representative of the myocardial tissue as a whole or just the uppermost layers of the epicardium. The present experiments cannot conclusively answer this question. Others have presented affirmative evidence using either the determination of permeability-surface area products for a number of molecules26 in an inverted heart preparation or intramyocardial injections of methylene blue dye and [14C]adenosine in dog hearts with epicardial wells.27 From these approaches, it would appear that transudates most likely represent an average of the adenosine concentration in the interstitial space.

Contraction Frequency

The negative staircase characteristic of rat heart muscle28 was observed. Transudate levels of adenosine in hearts treated with propranolol did not significantly increase with an elevation in contraction frequency despite a significant increase in MVO₂. There was a distinct, though nonsignificant, increase in these levels at 5 minutes with hearts treated with propranolol and atropine. Since the pacing rate was returned to 180 beats/min immediately after transudate was collected, it is not known if this elevation would have continued with time to become statistically significant. In contrast to data obtained from transudates, the adenosine concentration in effluent from hearts contracting at 300 beats/min was 1.5-fold greater than that observed from hearts contracting at 180 beats/min. It is interesting that for a given increase in MVO₂, a change in contraction frequency affected effluent levels of adenosine more than transudate levels. This was not the case in the ISO-stimulated heart in which the reverse was true. Thus, a change in contraction frequency appears to have had its greatest effect on adenosine release into the vascular compartment, perhaps by endothelial cells.17

This notion is supported by the observations with hearts treated with atropine and propranolol. The much smaller effect of frequency on effluent adenosine in these hearts may find explanation in the work of Schrader et al.29 These investigators found that acetylcholine administered to isolated guinea pig hearts induced an atropine-sensitive adenosine release into the coronary effluent, perhaps as the result of the stimulation of cholinergic receptors on endothelial cells. It is suggested that in the present study the release of endogenous acetylcholine elicited by an increased pacing frequency may, in part, have caused a cholinergic-induced adenosine release from endothelial cells into the coronary effluent. However, since cholinergic blockade with atropine did not entirely attenuate the augmentation of effluent adenosine levels, it is possible that effluent adenosine may also have originated from the ecto-ATPase and ADPase mediated degradation of the ATP released from neural tissue.30

It has been reported for the conscious dog that a rise in pericardial infusate levels of adenosine is elicited by an increase in heart rate.20 This observation is not in conflict with the presently reported results because the increased adenosine level may have reflected stimulation by the intact sympathetic innervation. In the present experiments the effect of endogenous catecholamine was blocked with propranolol. Heller and Mohrmann24 also have reported that the adenosine level in epicardial transudate obtained from noninverted rat hearts did not change significantly with an increase in pacing frequency from 180 to 360 beats/min. However, these investigators have recently discussed properties of the heart model used in their studies28 that suggest that an enhanced level of interstitial adenosine in response to an increase in contraction frequency may have dissipated as a result of a loss in vascular endothelial cell integrity. This is not thought to be a problem in this study since the gradient of adenosine from interstitial to vascular fluid was maintained with ISO stimulation.

The present data do not provide a definitive explanation for the absence of effect of enhanced contraction frequency on interstitial adenosine levels. However, insight may be gained by comparing these data with those obtained from the adrenergically stimulated hearts. It has been suggested that the O₂ supply/demand ratio may play a role in the regulation of adenosine release from cardiomyocytes.31,32 These ratios for rapidly paced hearts were of a magnitude that previously had not been found to stimulate the release of adenosine from isolated hearts.31 However, the ratios were identical to those of ISO-stimulated hearts that did contain significantly elevated interstitial levels of adenosine. It previously has been suggested that the rate of energy utilization by the heart also modulates adenosine release.32 The turnover of cAMP is a component of energy utilization. Since cellular levels of cAMP are known to be elevated in response to β-adrenergic agents,11 it is conceivable that AMP derived from cAMP may be one precursor of interstitial adenosine in ISO-stimulated hearts. Transudate levels of adenosine in rapidly paced hearts remained unchanged because cAMP levels are not increased in cardiac muscle subject to an increase in contraction frequency.33 Obviously, additional experiments would be required to verify these possibilities.

Heart Preparation Integrity

Inversion of the heart ensures that the transudate is not contaminated by vascular effluent originating from the great veins and pulmonary artery. Activities of the cytosolic enzymes LDH and ADA were determined for coronary effluent to verify the absence of
cellular damage resulting from heart inversion. Heart inversion and balloon inflation did not alter the release of LDH into the coronary effluent. As reported by others for the isolated rat heart, effluent LDH activity decreased from an initial elevated level immediately after heart isolation to a significantly reduced level within 15 minutes and remained low up to 2 hours after inversion. ISO stimulation resulted in the augmentation of the level of LDH activity in transudates. Increased mechanical activity or adrenergic stimulation have been observed by others to elicit enhanced LDH activities in rat heart coronary effluent. However, in the present study increases in LDH activity were observed only in the transudate. The enzymatic activity of ADA in the transudate was higher than in the effluent immediately after inversion of the heart but declined thereafter to a low level and remained unaltered even by ISO stimulation. These data indicate that cellular integrity is not compromised by inversion of the heart and placement of the intraventricular balloon. Furthermore, the observation that the activity of LDH is 1,000-fold greater in transudate than in coronary effluent would lend further evidence to the notion that transudates collected from the epicardial surface of the heart are derived primarily from the interstitial space. A similar conclusion was advanced by Wienen and Kammermeier in a report documenting similar concentration differences for creatine kinase between epicardial transudates and coronary effluents obtained from isolated perfused rat hearts.

In summary, adenosine levels in the left ventricular epicardial transudate and coronary effluent, the contractile function, and the MVO₂ of the isolated perfused inverted rat heart increased significantly with β-adrenergic stimulation. In the presence of adrenergic and cholinergic blockade, an increase in the contraction frequency increased MVO₂ despite a fall in contractile function. Little effect on transudate adenosine levels was observed even though coronary effluent adenosine increased to a greater degree than with adrenergic stimulation for a similar increase in MVO₂. These data suggest that 1) the release of adenosine into the interstitial space of the heart in response to adrenergic stimulation is of sufficient magnitude to serve as a physiologically important negative-feedback agent and 2) MVO₂ and coronary effluent levels of adenosine are not good predictors of interstitial levels of adenosine.

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