Hypoxia Enhances Isoproterenol-Induced Increase in Heart Interstitial Adenosine, Depressing β-Adrenergic Contractile Responses

R.A. Fenton and J.G. Dobson Jr.

Endogenous interstitial adenosine may protect the hypoxic heart by attenuating β-adrenergic–induced contractile and metabolic responses, thereby reducing energy utilization. Constant-flow perfused rat hearts were used to study 1) the effect of hypoxia on isoproterenol (ISO)–induced increase in interstitial adenosine, as estimated with epicardial surface transudates, and 2) the role of endogenous adenosine in hypoxic depression of ISO-induced contractile responses. ISO (1 nM for 10 minutes) in the normoxic heart increased transudate adenosine 114% from a pre-ISO normoxic value of 343 pmol/ml. ISO administered to the hypoxic heart increased transudate adenosine 357% from a pre-ISO hypoxic value of 797 pmol/ml. The absolute magnitude of the ISO-induced increase in transudate adenosine was 625% greater during hypoxia than during normoxia. This was associated with a reduction in the ISO-induced contractile response during hypoxia. In other experiments, with normoxia ISO (10 nM for 10 seconds) increased developed left ventricular pressure by 140 mm Hg, and the maximum rates of left ventricular pressure development and relaxation by 5,860 and 2,771 mm Hg/sec, respectively, above control values of 90 mm Hg, 2,250 mm Hg/sec, and 1,875 mm Hg/sec. Hypoxia reduced the three ISO-induced contractile responses by 50%, 56%, and 36%. However, 1,3-dipropyl-8-cyclopentylxanthine (5 × 10^-7 M), an adenosine A1-receptor antagonist, added to the hypoxic hearts resulted in only a 31%, 39%, and 9% reduction in the ISO-induced responses in developed left ventricular pressure and the maximum rates of left ventricular pressure development and relaxation, respectively. These findings indicate that the effectiveness of ISO as a stimulus for augmenting interstitial adenosine is increased with hypoxia. The resulting enhanced expression of the antiadrenergic action of adenosine attenuates the ISO-induced contractile response during hypoxia. (Circulation Research 1993;72:571–578)

KEY WORDS • myocardium • O2 supply-to-demand ratio • O2 consumption • contractility • dipropylcyclopentylxanthine

The level of adenosine in the heart increases with β-adrenergic catecholamine stimulation.1-3 The nucleoside has been reported to antagonize, in turn, the stimulatory effects of β-adrenergic agonists on contractile and metabolic function4-8 of the myocardium in both in vivo9,10 and in vitro5,7,11 studies. This antiadrenergic action, manifested as an attenuation of adenylyl cyclase activation12,13 and myocardial protein phosphorylation,14 is therefore a negative feedback mechanism8,7,11 mediated by adenosine-specific A1-receptors.12,15,16 Hypoxia is also a potent stimulus for the myocardial formation of adenosine.3,17 Thus, expression of the antiadrenergic action of adenosine may be of particular importance in the β-adrenergic–stimulated hypoxic heart. By reducing the adrenergic-elicited increase in contractile function, adenosine would promote a decrease in energy consumption at a time when energy production is limited because of a reduced supply of O2.

Adenosine is thought to derive, in part, from cardiomycocytes18 on dephosphorylation of AMP18,19 by cytosolic20 and/or sarcolemmal-bound21,22 5'-nucleotidase activity. The appearance of adenosine in the coronary effluent has been reported to increase exponentially with a rise in cytosolic AMP concentration of the intact heart, possibly as a result of the allosteric activation of 5'-nucleotidase.3 However, the level of adenosine in the coronary effluent is influenced by endothelial cells of the coronary vasculature.22 Changes in vascular adenosine levels may be different from those occurring in levels of interstitial adenosine.1,17 It is believed that only interstitial adenosine interacts with adenosine A1-receptors on cardiomyocytes, thereby allowing expression of its antiadrenergic action.1,16,17 To investigate the importance of adenosine as a negative feedback regulator in the hypoxic heart, it is necessary to determine the effect of hypoxia on the β-adrenergic–induced accumulation of adenosine in the interstitial compartment.

The basal level of cytosolic AMP is greater in the hypoxic heart than in the normoxic heart.3 If 5'-nucleotidase is allosterically regulated,3 an equivalent β-adrenergic stimulation of hypoxic and normoxic hearts
may be expected to elicit a greater level of adenosine in the interstitial fluid of the oxygen-deficient heart than in the well-oxygenated heart. Since the antiadrenergic action of adenosine is dose dependent,6,7 an augmented interstitial level of adenosine in response to \( \beta \)-adrenergic stimulation of the hypoxic heart would induce a greater attenuation of the enhanced contractile function.

The goals of the present study were to 1) determine the effect of hypoxia on the elevation of interstitial adenosine in the presence of \( \beta \)-adrenergic stimulation with isoproterenol (ISO), a \( \beta \)-receptor agonist, and 2) determine if endogenous adenosine reduces the contractile responsiveness of the hypoxic heart to \( \beta \)-adrenergic stimulation. To achieve these goals, adenosine levels in epicardial surface transudate and coronary effluent were determined in ISO-stimulated normoxic or hypoxic isovolumetric contracting hearts. Contractile function was assessed before and after adenosine A\(_1\)-receptor antagonism. It was found that 1) hypoxia enhances the \( \beta \)-adrenergic–induced elevation of heart interstitial adenosine, and 2) inhibition of the antiadrenergic action of endogenous adenosine in the hypoxic heart partially prevents the depressed adrenergic–induced contractile increases.

**Materials and Methods**

*Isolated 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.

Male Sprague-Dawley rats (250–350 g) were anesthetized with pentobarbital sodium (40 mg/kg i.p.) and treated with heparin sodium (500 units i.p.) for at least 15 minutes. Hearts were removed, rinsed in iced physiological saline (PS), and perfused with 35.5°C PS via an aortic cannula at a constant rate of 10.8±0.3 ml/min. Perfusion pressures ranged from 55 to 75 mm Hg. The PS contained (mM) NaCl 118, KCl 4.7, CaCl\(_2\) 2.5, NaHCO\(_3\) 25, KH\(_2\)PO\(_4\) 1.2, MgSO\(_4\) 1.2, glucose 10, and pyruvate 1.8. The pH was maintained at 7.4 by gassing the PS with a 95% O\(_2\)-5% CO\(_2\) gas mixture. After the great veins were ligated, hearts were inverted and instrumented as previously described.1 Briefly, platinum electrodes were inserted into the right atrium to allow pacing of the heart at 270 contractions per minute. To measure developed left ventricular pressure (LVP), a water-filled latex balloon was inserted into the lumen of the left ventricle via the left atrium and attached by a polyethylene cannula to a Statham P23Dd pressure transducer (Gould, Cleveland, Ohio). The pressure signal was electronically differentiated (Gould differentiator) to obtain the maximum rates of left ventricular pressure development (+dP/dt\(_{max}\)) and relaxation (−dP/dt\(_{max}\)). Balloon volume was varied until the maximal obtainable dP/dt was attained. This optimal preload, ranging from 5 to 10 mm Hg, was maintained constant, permitting isovolumic contraction of the heart. Aortic perfusion pressure was monitored with a Statham P23Dd pressure transducer. At termination of the experiment, the ventricular myocardium was dried overnight at 80°C, and the dry weight in grams was determined.

The O\(_2\) tension of the coronary effluent was continuously monitored by directing the pulmonary artery fluid via a polyethylene cannula to a Clark-type polarographic electrode (model 53, Yellow Springs Instrument Co., Yellow Springs, Ohio). The temperatures of the aortic perfusate and the O\(_2\) electrode chamber used for calibrating the O\(_2\) electrode were determined by a Thermalert (model TH-8, Physitemp Instruments, Inc., Clifton, N.J.) with a microprobe (model MT-29/1, Physitemp) inserted into the perfusion line and the O\(_2\) electrode chamber. The O\(_2\) content of the aortic PS inflow and coronary effluent was calculated using 0.0321 \( \mu \)l O\(_2\)/mm Hg as milliliters as the solubility of O\(_2\) in PS. Myocardial oxygen consumption (MVO\(_2\)), expressed as milliliters O\(_2\) consumed per minute per 100 g dry weight, was obtained as the product of the coronary flow and the difference in O\(_2\) contents of the aortic PS inflow and pulmonary artery outflow. The ratio of O\(_2\) supply to O\(_2\) demand was calculated by dividing milliliters O\(_2\) supplied to the heart per minute by milliliters O\(_2\) consumed by the heart per minute.

All pressure and O\(_2\) tension data were recorded on a multichannel polygraph (model 2600, Gould) or a Zeos 386SX PC with a DAS-8 AD converter (MetraByte, Taunton, Mass.) and Snapshot Storage Scope software (HEM Data Corp., Southfield, Mich.) for data analysis.

**Protocols**

To investigate the effect of reduced O\(_2\) on \( \beta \)-adrenergic–induced adenosine formation, hearts were instrumented as described, and a period of 15–30 minutes was allowed to elapse. Hearts were then randomly assigned to one of four experimental groups (Figure 1).
Group A1: Normoxia without ISO. Transudate and effluent samples were collected approximately every minute for a total of 10 minutes. It has been found that the level of interstitial adenosine in this preparation remains constant for at least 1 hour.

Group A2: Normoxia with ISO. After collection of fluid samples as described for group A1, a 10-minute infusion of ISO was begun to achieve a final PS ISO concentration of 1 nM. Transudate and effluent samples were collected every minute for a total of 10 minutes. It has been found that the contractile responsiveness of this preparation to a 10-minute stimulation with 1 nM ISO is maintained for at least 1 hour.

Group A3: Hypoxia without ISO. After collection of fluid samples as described for group A1, the normoxic PS was rapidly changed to PS gassed with 50% O2-45% N2-5% CO2. Transudate and effluent samples were collected every 5 minutes for a total hypoxic perfusion period of 30 minutes.

Group A4: Hypoxia with ISO. After collection of fluid samples as described for group A1, normoxic PS was changed to PS gassed with 50% O2-45% N2-5% CO2, and transudate and effluent samples were collected every 5 minutes for a total of 20 minutes. At this time, a 10-minute infusion of ISO was begun for an aortic PS ISO concentration of 1 nM. Transudate and effluent samples were then collected every minute for a total of 10 minutes. Thus, the total duration of these experiments was 30 minutes.

To investigate whether endogenous adenosine limits the contractile responsiveness of the hypoxic heart to β-adrenergic stimulation, hearts were isolated and instrumented as described above. Hearts were randomly assigned to one of two experimental groups (Figure 1).

Group B1: ISO and hypoxia. The maximum contractile response to a 10-second infusion of 10^-8 M ISO was determined when hearts were perfused with normoxic PS (gassed with 95% O2-5% CO2). A second response was obtained after contractile parameters returned to the pre-ISO control level (<5 minutes). Because the second response was nearly identical to the first in this and succeeding trials, the two responses were averaged. Normoxic perfusion was then rapidly switched to perfusion with hypoxic PS (gassed with 50% O2-45% N2-5% CO2). ISO infusions were repeated, in duplicate, at 15 and 30 minutes of hypoxia.

Group B2: ISO and hypoxia with DPCPX. The above protocol was repeated with the exception that, at 20 minutes of hypoxia, the infusion of 1,3-dipropyl-8-cyclopentylxanthine (DPCPX), an adenosine A1-receptor antagonist, was begun with a final PS concentration of 5×10^-7 M for the remaining 10 minutes.

Collection and Analysis of Epicardial Transudates and Coronary Effluents

Fluid sample collection and analysis were conducted as described previously with modification. Transudate samples were collected within 15-30 seconds from the entire epicardial surface of the left ventricle using disposable 5-μl microsampling glass pipettes (Fisher, Springfield, N.J.). Coronary venous effluent samples were collected simultaneously from the outflow port of the O2 electrode chamber with disposable 10-μl microsampling glass pipettes (Fisher). Immediately after collection, sampling pipettes were placed on an ice-cold aluminum block until termination of the experiment, whereupon they were immediately transferred to a -20°C freezer until analysis the next day. Adenosine was measured in 3–6 μl transudate or 10 μl coronary effluent with 20 μl of 1.5 M chloroacetalddehyde and 10 μl water to form fluorescent 1,N6-ethenoadenosine as described previously. Ethenoadenosine was analyzed by high-performance liquid chromatography (HPLC) (Waters Chromatography Division, Milford, Mass.) using fluorometric detection (model 749 with high sensitivity attachment, McPherson Instruments, Acton, Mass.). Isocratic elution was accomplished with a flow rate of 0.4 ml/min and a mobile phase consisting of 5% methanol in 10 mM KH2PO4, pH 3.5. Chromatographic data were collected and analyzed using a Maxima 820 chromatographic workstation (Waters). Fluorometer output was recorded in microvolts, and the chromatographic responses were integrated and reported as peak areas (microvolt-seconds). Derivatization efficiency was determined as follows: The first two samples of transudate from each heart were pooled and then equally divided. After addition of the known amount of adenosine to one sample, analysis of both samples allowed determination of derivatization efficiency for adenosine contained in the transudate from each heart. This procedure was repeated for coronary effluent. Values for derivatization efficiency were assumed to remain constant for all the remaining fluid samples obtained from a particular heart. The adenosine concentration in the transudates is given in picomoles per milliliter. Adenosine in the effluents is given in either picomoles per milliliter or nanomoles released per minute per gram dry weight.

Materials

All reagents, solvents, salts, glucose, and pyruvate were of certified or HPLC grade from Fisher. 1-ISO and DPCPX were obtained from Sigma and Research Biochemicals, Inc., Natick, Mass., respectively. Water used for heart perfusion and assays was purified with a Milli-Q water purification system (Millipore Corp., Bedford, Mass.). Chloroacetalddehyde and adenosine used for assay procedures were obtained from Aldrich Chemical Co., Milwaukee, Wis., and Boehringer-Mannheim, Indianapolis, Ind., respectively.

Statistical Treatment

Data analysis was conducted by analysis of variance and the Newman-Keuls test or Student’s t test for paired samples. A probability of p<0.05 was chosen as indicating a statistically significant difference.

Results

Adenosine in Epicardial Transudates and Coronary Effluents

The time courses for the appearance of adenosine in transudate and effluent fluid during hypoxic and then hypoxic+ISO episodes are depicted in Figures 2 and 3. In normoxic hearts free of ISO stimulation, the level of adenosine in transudates was 288±67 nM. In the same hearts, coronary effluent adenosine was 5.4-fold less at 53±8 nM. During hypoxia, the adenosine content of transudate and effluents in the same hearts rose 128% and 100%, respectively, within 10 minutes to a level that...
was maintained throughout the 20-minute hypoxic period. These maintained elevations also have been observed in hearts that were exposed to 30 minutes of hypoxia (data not shown). When normoxic and hypoxic hearts were stimulated with ISO, the transudate and effluent adenosine levels increased to a maximal value and then began to descend toward the levels present in the heart immediately before the administration of ISO; i.e., the elevation in adenosine was transient and of variable duration. The phasic appearance of adenosine in coronary effluent with ISO administration has been reported previously.25

The appearance of adenosine in the transudate with ISO stimulation was greater in hypoxic than normoxic hearts (Table 1). Only samples obtained at 3–8 minutes were used to calculate mean values of adenosine appearing in the transudate during the 10-minute period of ISO stimulation of normoxic or hypoxic hearts. The administration of 1 nM ISO significantly increased transudate adenosine 114% in the normoxic hearts. Hypoxia resulted in a significant 132% elevation of the adenosine level compared with the levels present in normoxic hearts. Mean values were determined from 5 to 30 minutes after initiation of hypoxia. Administration of ISO to hypoxic hearts resulted in a further 357% increase of adenosine in the epicardial transudate above those levels present in the hypoxic heart. The actual increase in the transudate adenosine level elicited by ISO stimulation was 7.3-fold greater when hearts were perfused with hypoxic PS (2,843 pmol/ml) than with normoxic PS (392 pmol/ml).

The release of adenosine into the coronary effluent increased 238% with hypoxia (Table 1). Whereas the administration of 1 nM ISO was not found to significantly change adenosine release in the normoxic heart, a similar adrenergic stimulation elicited a significant 80% increase in adenosine release into the coronary effluent of the hypoxic heart.

**Contractile Function and Oxygen Consumption**

Hypoxia depressed contractile function in the absence and presence of ISO (Table 1). Stimulation of the normoxic heart with 1 nM ISO increased +dP/dt max by 53%. This increase resulted in a 34% increase in MVo2, which was reflected in a 21% reduction in the O2 supply-to-demand ratio (OSDR). Hypoxia resulted in a reduction in contractile function, as indicated by a 22% reduction in +dP/dt max when compared with normoxic perfusion. This decrease in contractile function was accompanied by a 15% decrease in MVo2 and a 37% decrease in OSDR. Stimulation of the hypoxic heart with 1 nM ISO resulted in a 29% increase in +dP/dt max.

Despite a significant 19% increase in MVo2 with ISO stimulation, the mean OSDR was calculated to decrease only 4%.

**Effect of DPCPX on the Contractile Response of Hypoxic Hearts to ISO**

Values for LVP, +dP/dt max, and −dP/dt max before and during ISO stimulation of hearts successively exposed to normoxia, hypoxia, and hypoxia with DPCPX are reported in Table 2. In the normoxic heart, 10−6 M ISO administered as a 10-second infusion elicited significant 156%, 260%, and 148% increases in LVP, +dP/dt max, and −dP/dt max, respectively. In the absence of β-adrenergic stimulation, hypoxia reduced these respective contractile variables 36%, 29%, and 56% less than in normoxic control hearts. Although contractile function with hypoxia was increased by ISO stimulation, the values of the respective contractile variables for the ISO-stimulated hypoxic hearts were 44%, 48%, and 44% less than those values recorded in the ISO-stimulated normoxic hearts. DPCPX in hypoxic hearts had no effect on LVP, +dP/dt max, and −dP/dt max before β-adrenergic stimulation. However, in ISO-stimulated hypoxic hearts, the respective increases observed for these contractile variables were 21%, 24%, and 28% greater than those obtained before DPCPX administration.

To better understand the effect of DPCPX on the contractile responsiveness of the hypoxic heart to ISO stimulation, the ISO-induced increases in LVP, +dP/dt max, and −dP/dt max in hearts successively exposed to normoxia and hypoxia without or with
DPCPX are depicted in Figure 4. Hypoxia alone significantly reduced the responses of these contractile variables by 50%, 56%, and 36%, respectively. Addition of DPCPX to the hypoxic PS resulted in responses significantly greater than those observed in the absence of DPCPX. LVP, +dP/dt max, and −dP/dt max were increased by 37%, 38%, and 41%, respectively. However, whereas the ISO responses for LVP and +dP/dt max remained significantly below those of the normoxic values, the ISO response value for −dP/dt max returned to the ISO response observed when hearts were perfused with normoxic PS.

**Discussion**

The major findings of this study are that 1) the β-adrenergic–induced increase in interstitial adenosine observed in the normoxic perfused heart is enhanced with hypoxia, and 2) reduced contractile responsiveness of the hypoxic heart to β-adrenergic stimulation is partially mediated by adenosine. These considerations are important for hearts that are incapable of maintaining an optimal phosphorylation potential as a result of reduced O2 delivery to the myocardium. Endogenous adenosine previously has been found to limit the β-adrenergic–induced increase in contractile activity of the well-oxygenated heart. A similar limitation of β-adrenergic stimulation would prove beneficial to the myocardium during a period of reduced energy production capacity. Because manifestation of the antiadrenergic effect of adenosine is dose dependent, a greater presence of interstitial adenosine in response to β-adrenergic stimulation of the hypoxic heart would enhance the negative feedback action of the nucleoside.

ISO stimulation of the hypoxic heart resulted in a 7.3-fold greater increase in the transudate adenosine level when compared with the ISO-stimulated normoxic heart. Several possible mechanisms that may explain this observation can be considered. Enhanced myocardial adenosine release into the coronary circulation has been observed previously with an increase in MVO2. Although adenosine release is increased concomitantly with an increase in MVO2 in the present study, as in others, effluent and transudate adenosine levels, with hypoxia, were found to rise while MVO2 fell. With ISO stimulation, the increase in MVO2 was greater in normoxic than hypoxic hearts. Yet the presence of adenosine in the transudate of the hypoxic hearts was significantly greater than in ISO-stimulated normoxic hearts. These data do not support a role for MVO2 per se in the enhancement of ISO-induced adenosine release into the interstitium by hypoxia. Other studies support this contention. Increases in heart rate significantly increase MVO2 without increasing interstitial adenosine.

A decrease in the OSDR has been proposed as a stimulus for enhanced adenosine release. The present observations can be explained only partially by changes in the OSDR. Hypoxia perfusion elicited a significant increase in transudate adenosine levels that was concomitant with a fall in OSDR. The levels of adenosine in transudates of well-oxygenated hearts were significantly elevated with ISO stimulation; this elevation was concomitant with a significant reduction in the calculated OSDR. However, despite only a nonsignificant 4% decrease in the calculated OSDR between the hypoxic hearts and ISO-stimulated hypoxic hearts, the increase in transudate adenosine was 6.3-fold the increase observed in ISO-stimulated normoxic hearts, in which the OSDR decreased a significant 21%.

Other more plausible mechanisms may be suggested. Nucleoside release into coronary effluent from ISO-stimulated or hypoxic hearts is exponentially related to the cytosolic free AMP level, as determined with 31P nuclear magnetic resonance spectroscopy. It has been suggested that this relation reflects an allosteric activation of ecto-5′-nucleotidase. In the present study, adenosine concentrations in the interstitial fluid were determined, since it is this adenosine that directly influences cardiomyocyte function. If 5′-nucleotidase is

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**TABLE 1. Effect of Hypoxia on Epicardial Transudate Adenosine Level, Release of Adenosine Into the Coronary Effluent, Maximum Rate of Left Ventricular Pressure Development, O2 Consumption, and O2 Supply-to-Demand Ratio in the Presence and Absence of Isoproterenol in Isolated Rat Hearts**

<table>
<thead>
<tr>
<th></th>
<th>Normoxic</th>
<th>Normoxic+ISO</th>
<th>Hypoxic</th>
<th>Hypoxic+ISO</th>
</tr>
</thead>
<tbody>
<tr>
<td>Transudate adenosine level (pmol/ml)</td>
<td>343±51</td>
<td>735±94*</td>
<td>797±151†</td>
<td>3,640±406*</td>
</tr>
<tr>
<td>Effluent adenosine release (nmol/min per gram dry wt)</td>
<td>2.07±0.50</td>
<td>2.51±0.37</td>
<td>6.99±0.67†</td>
<td>12.61±1.07*</td>
</tr>
<tr>
<td>+dP/dt max (mm Hg/sec)</td>
<td>1,864±178</td>
<td>2,853±235*</td>
<td>1,461±71†</td>
<td>1,883±137*</td>
</tr>
<tr>
<td>O2 consumption (ml O2/min per 100 g dry wt)</td>
<td>56.2±3.1</td>
<td>75.4±2.2*</td>
<td>47.6±2.9†</td>
<td>56.6±5.9*</td>
</tr>
<tr>
<td>O2 supply/demand ratio</td>
<td>1.88±0.12</td>
<td>1.49±0.05*</td>
<td>1.19±0.04†</td>
<td>1.14±0.05</td>
</tr>
</tbody>
</table>

ISO, 1 nM isoproterenol; +dP/dt max, maximum rate of left ventricular pressure development. Data represent the mean of values obtained during normoxia, 5–30 minutes of hypoxia, or 3–8 minutes of ISO administration to normoxic or hypoxic hearts. Values are mean±1 SEM for data collected from 21 hearts. Normoxic and hypoxic hearts were perfused with physiological saline equilibrated with 95% or 50% O2 in 5% CO2 (balance, N2), respectively. See “Materials and Methods” and Figure 1 for protocol and details.

*p<0.05 vs. corresponding value without ISO.

†p<0.05 vs. corresponding value with normoxic perfusion.
allosterically regulated, an ISO-elicited increase in the cellular free AMP level in the normoxic heart might be expected to result in a smaller increase in the interstitial adenosine level than a similar ISO-induced increase in free AMP in the hypoxic heart, when the basal cellular AMP would be higher. This is, in fact, what was observed. A second possible mechanism may involve adenosine nucleotides that are released presynaptically or from cardiomyocytes. These nucleotides are subsequently hydrolyzed to adenosine by cell-surface nucleotidases. The nucleotide concentration in coronary effluent has been found to increase in response to β-adrenergic stimulation. Hypoxia may enhance the release of nucleotides into the interstitial fluid in response to ISO stimulation, resulting in a greater increase in interstitial adenosine with hypoxia compared with normoxia.

Contractile function was depressed by hypoxia in the absence of β-adrenergic stimulation. This depression with hypoxia may have resulted, in part, from a reduced responsiveness of contractile filaments to changes in cellular Ca2+. Perhaps by inhibition of ATP hydrolysis by ADP, Blockade of adenosine receptors with DPCPX did not alter this hypoxic-induced depression of contractile activity. This would be expected in that the mechanism by which adenosine modulates ventricular contractile function presupposes that the myocardium is being stimulated by β-adrenergic catecholamines.

The ISO-induced enhancement of contractile function was attenuated in the hypoxic heart. This effect was, in part, reversed by DPCPX, suggesting that the antiadrenergic action of endogenous adenosine played a role in the reduced responsiveness of the hypoxic heart to β-adrenergic stimulation. DPCPX is an adenosine antagonist with a Kd of 11 nM and a high specificity for adenosine A1-receptors. Similar restoration of hypoxic-depressed ISO responses has been reported for hypoxic rat atria treated with adenosine deaminase and the hypoperfused canine heart perfused with the relatively nonspecific adenosine antagonist 8-phenyltheophylline. It is interesting that with DPCPX the depressed value of ISO-induced change in −dP/dt max was fully restored to the magnitude observed in normoxic hearts (Figure 4). However, the magnitude of changes in LVP and +dP/dt max in response to ISO stimulation, although returning significantly toward those magnitudes found with normoxia, remained significantly depressed. It has been reported previously that adenosine does not uniformly inhibit all aspects of ISO-induced changes in cellular function.

The nucleotide release of nucleotides into the effluent has been found to increase in response to hypoxia. Hypoxic-depressed contractile function may be attenuated through stimulation of adenosine A1-receptors by adenosine and its phosphoribosyl derivatives, but not by cAMP or by isoproterenol. Hypoxic-induced changes in left ventricular pressure; ISO, isoproterenol; +dP/dt max and −dP/dt max, maximum rates of left ventricular pressure development and relaxation, respectively. Values are mean±1 SEM for data collected sequentially (left to right) from four hearts.

Paced normoxic and hypoxic hearts were perfused with physiological saline gassed with 95% or 50% O2; respectively, in 5% CO2 (balance, N2). ISO (10−6 M) was infused twice at 15 and 30 minutes of hypoxic perfusion. DPCPX (0.5 μM), an adenosine A1-receptor antagonist, was continuously administered during the final 15 minutes of hypoxia. See “Materials and Methods” and Figure 1 for further details regarding the protocol.

*Significantly different from the corresponding value obtained at 0 minutes of hypoxia. Significantly different from the corresponding value obtained at 15 minutes of hypoxia.

<table>
<thead>
<tr>
<th>Hypoxia</th>
<th>Normoxia</th>
<th>−DPCPX</th>
<th>+DPCPX</th>
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<tbody>
<tr>
<td>LVP (mm Hg)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>−ISO</td>
<td>90±8</td>
<td>58±4*</td>
<td>59±5*</td>
</tr>
<tr>
<td>+ISO</td>
<td>230±9†</td>
<td>128±9*†</td>
<td>155±5††</td>
</tr>
<tr>
<td>+dP/dt max (mm Hg/sec)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>−ISO</td>
<td>2,250±199</td>
<td>1,606±222*</td>
<td>1,632±182*</td>
</tr>
<tr>
<td>+ISO</td>
<td>8,110±320†</td>
<td>4,211±290†</td>
<td>5,212±164††</td>
</tr>
<tr>
<td>−dP/dt max (mm Hg/sec)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>−ISO</td>
<td>1,875±123</td>
<td>819±49*</td>
<td>815±48*</td>
</tr>
<tr>
<td>+ISO</td>
<td>4,646±295†</td>
<td>2,605±363†</td>
<td>3,327±389††</td>
</tr>
</tbody>
</table>

DPCPX, 1,3-dipropyl-8-cyclopentylxanthine; LVP, developed left ventricular pressure; ISO, isoproterenol; +dP/dt max and −dP/dt max, maximum rates of left ventricular pressure development and relaxation, respectively. Values are mean±1 SEM for data collected sequentially (left to right) from four hearts.

Paced normoxic and hypoxic hearts were perfused with physiological saline gassed with 95% or 50% O2; respectively, in 5% CO2 (balance, N2). ISO (10−6 M) was infused twice at 15 and 30 minutes of hypoxic perfusion. DPCPX (0.5 μM), an adenosine A1-receptor antagonist, was continuously administered during the final 15 minutes of hypoxia. See “Materials and Methods” and Figure 1 for further details regarding the protocol.

*Significantly different from the corresponding value obtained at 0 minutes of hypoxia. Significantly different from the corresponding value obtained at 15 minutes of hypoxia.
ple, in a study of Ca\textsuperscript{2+} transients in electrically stimulated cardiomyocytes, adenosine was found to completely suppress an ISO-elicted increase in the rate of Ca\textsuperscript{2+} transient decline but only partially attenuate the accompanying increase in the transient magnitude.\textsuperscript{8} ISO modulates cardiomyocyte function by a sequence of events that is initiated by the activation of a stimulatory guanine nucleotide regulatory protein, G\textsubscript{s},\textsuperscript{28} and adenylyl cyclase\textsuperscript{12} and culminates in the phosphorylation of cellular regulatory proteins by cAMP-dependent protein kinase.\textsuperscript{14} In the hypoxic heart, adenosine, via adenosine A\textsubscript{1}-receptor inhibition of adenylyl cyclase,\textsuperscript{12,13} would attenuate ISO activation of cellular processes mediated by cAMP.\textsuperscript{39-41} These processes would be fully restored to normoxic activity levels with the inhibition of adenosine by DPCPX, as was observed by \(-dP/dt_{\text{max}}\), if adenosine were the only inhibitory factor involved. Incomplete return to normoxic values of ISO-sensitive changes in LVP and \(+dP/dt_{\text{max}}\) after adenosine receptor blockade would suggest that mechanisms governing ISO-induced changes in force development are influenced not only by adenosine but by other factors as well. ISO-sensitive mechanisms affected may include sarcoplasmic Ca\textsuperscript{2+} channel activation\textsuperscript{42} and sarcoplasmic reticular Ca\textsuperscript{2+} release,\textsuperscript{43} both of which may be directly activated by G\textsubscript{s} as well as indirectly by cAMP-dependent phosphorylation. The action of DPCPX is thought to be elicited only via adenosine A\textsubscript{1}-receptor antagonism and not by myocardial phosphodiesterase inhibition for the following reasons: 1) DPCPX alone had no effect on contractile function in the absence of ISO stimulation. 2) The concentration of DPCPX presently used was 100-fold less than that of theophylline, which previously (at 5 \times 10^{-5} M) was not found to elevate cAMP levels in the heart.\textsuperscript{9} 3) The IC\textsubscript{50} for the inhibition of phosphodiesterase I-III by DPCPX has been found to be >10^{-4} M.\textsuperscript{44}

The data presented herein do not support the contention that reduced contractile responsiveness to adrenergic stimulation of the hypoxic heart results from an induced decrease in β-adrenergic receptor number.\textsuperscript{45} Although the overall contractile function remains depressed in the DPCPX-treated hypoxic heart, restoration of the ISO-induced changes in \(-dP/dt_{\text{max}}\) to normoxic levels by DPCPX would indicate that adrenergic coupling to adenylyl cyclase was unhindered in the hypoxic heart except by the action of adenosine.\textsuperscript{15}

In summary, rat hearts perfused with either well-oxygenated PS or PS with reduced O\textsubscript{2} content (hypoxia) were stimulated with a low concentration of the β-adrenergic agonist ISO. As determined from analysis of epicardial surface transudates, ISO was found to induce an increase in interstitial adenosine levels that was greater with hypoxia than with normoxia. The contractile responsiveness of the heart to adrenergic stimulation was reduced with hypoxia. This reduction was reversed with adenosine A\textsubscript{1}-receptor blockade. It is concluded that the contractile responsiveness of the hypoxic heart to β-adrenergic stimulation is limited by adenosine, thus fostering reduced energy consumption by the hypoxic heart. Therefore, adenosine appears to play an important role in the survival of the hypoxic myocardium subjected to β-adrenergic stimulation.

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**References**

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Hypoxia enhances isoproterenol-induced increase in heart interstitial adenosine, 
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