Increased Myocardial Adenosine Production and Reduction of $\beta$-Adrenergic Contractile Response in Aged Hearts

James G. Dobson Jr., Richard A. Fenton, and Fred D. Romano

The contractile response of the aged adult heart to $\beta$-adrenergic stimulation is known to be reduced compared with the young adult heart. Since endogenous adenosine exerts an antiadrenergic action in the heart, this study was undertaken to determine if the basal endogenous level of myocardial adenosine increases with age and whether this increase mediates the reduced responsiveness of aged heart to $\beta$-adrenergic stimulation. Young (3–5 months) and aged (12–22 months) Sprague-Dawley adult rat hearts of CD and SD stock were perfused at constant pressure and paced at 270 contractions/min. The two age groups had a similar level of $+dP/dt_{\text{max}}$ (index of contractility) under control conditions. Adenosine release into the coronary effluent was $30\pm3$ nmol/min/g d.wt from young and $54\pm9$ nmol/min/g dry wt from aged hearts. Inosine release was also greater from the aged hearts. Isoproterenol ($10^{-8}$ M) stimulation increased contractile state by 113% in young hearts and only 69% in aged hearts. Isoproterenol further increased the adenosine and inosine release from both age groups. Theophylline ($5\times10^{-4}$ M), an adenosine antagonist, prevented the difference in the contractile response to isoproterenol stimulation between the young and aged hearts. Elevation of external calcium from 2 to 4 mM increased contractility equally in both age groups without influencing adenosine release. Myocardial oxygen consumption, coronary effluent $P_{O_2}$, oxygen supply-demand ratio, and lactate release were similar for both age groups, indicating that under the conditions studied the elevated release of adenosine by the aged hearts was not due to hypoxia. Aged (10–14 months) adult guinea pig hearts also displayed a reduced responsiveness to the isoproterenol stimulation and released more adenosine compared with young (3–4 months) adult guinea pig hearts. These findings suggest that enhanced adenosine levels that are present in the aged myocardium are responsible, in part, for the reduced contractile responsiveness of the older adult heart to $\beta$-adrenergic stimulation. (Circulation Research 1990;66:1381–1390)

As adult mammals age, profound changes occur in cardiovascular function. For example, augmentation of cardiac contractile performance during stress is diminished in rats\(^1\)\(^2\) and humans.\(^3\)\(^4\) Normally, during stress the contractile performance of the heart is increased as a result of sympathetic nervous system–mediated release of the neurotransmitter norepinephrine and increased levels of circulating epinephrine. These catecholamines interact with myocardial $\beta$-adrenoceptors, thereby activating adenylate cyclase. This results in an increased adenosine 3',5'-cyclic monophosphate (cAMP) production and cAMP-dependent protein kinase–catalyzed phosphorylation of cardiac proteins.\(^5\)\(^6\) The phosphorylation of various cellular proteins involved in contractile and metabolic function is thought to play a role in the increased contractile performance observed with $\beta$-adrenergic stimulation.\(^6\)\(^–\)\(^8\)

Adenosine, an endogenous metabolite of the heart, is thought to exist primarily in the myocardial interstitial fluid.\(^9\)\(^–\)\(^11\) Adenosine has an antiadrenergic action: it reduces the $\beta$-adrenoceptor–induced activation of adenylate cyclase and the subsequent increases in myocardial cAMP formation, cAMP-dependent protein kinase and glycogen phosphorylase activities, protein phosphorylation, and contractile state.\(^12\)\(^–\)\(^17\) Since $\beta$-adrenergic stimulation of the heart increases the release of adenosine into the coronary circulation,\(^18\)\(^–\)\(^20\) adenosine has been postulated to serve as a negative-feedback modulator of

---

From the Department of Physiology, University of Massachusetts Medical School, Worcester, Massachusetts.


Supported primarily by HL-36964 and in part by HL-22828 Public Health Service grants from the National Institutes of Health.

Address for reprints: James G. Dobson Jr., PhD, Department of Physiology, University of Massachusetts Medical School, 55 Lake Avenue North, Worcester, MA 01655.

Received November 25, 1988; accepted January 9, 1990.
β-adrenoceptor–mediated elevation of cardiac contractile and metabolic function.10,13,16 The attenuation of β-adrenergic–induced activation of adenylate cyclase appears to be via an A1 adenosine receptor, which mediates a decrease in ability of β-adrenergic agonists to promote the formation of a high-affinity complex composed of the agonist, β-adrenoceptor, and stimulatory guanine nucleotide binding protein.21

Since the aged adult rat heart has reduced adenylate cyclase activity and contractile responsiveness to β-adrenergic stimulation when compared with the young adult heart,1,2,22,23 it was hypothesized that an elevated level of adenosine in the aged adult myocardium might explain this phenomenon. An elevation of adenosine levels would augment the anti-adrenergic action of the nucleoside and render the older heart less responsive to β-adrenoceptor stimulation. The present study was undertaken to determine the release of adenosine and inosine from young and aged adult rat and guinea pig hearts in the absence and presence of β-adrenergic stimulation. Values of oxygen consumption, partial pressure of oxygen in the coronary effluents, oxygen supply-demand ratio, and lactate release were determined to assess the metabolic status of the hearts. To further investigate the possible role of adenosine in the reduced β-adrenergic responsiveness in aged adult hearts, theophylline, an adenosine receptor antagonist, was used. In addition, an increase in external calcium was employed as an inotropic intervention independent of β-adrenoceptor activation.

Materials and Methods

Animals 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, US Department of Health, Education, and Welfare, National Institutes of Health Publication No. 85-23, 1985, and the guidelines of the Animal Care Advisory Committee of the University of Massachusetts Medical School.

Perfused Heart Preparations

Male adult Sprague-Dawley rats of CD and SD stock were used primarily in these experiments; they were divided into two groups: young (3–5 months; mean, 4 months) and aged (12–22 months; mean, 18 months). In several studies, male adult Hartley guinea pigs were used; they were also divided into two groups: young (3–4 months; mean, 4 months) and aged (10–14 months; mean, 12 months). Since the aged adult rats had a broad age range and did not exceed 22 months of age, none of the animals was considered to be senescent. All animals were anesthetized and heparinized intraperitoneally with 40 mg/kg pentobarbital and 500 units heparin. The hearts were excised, rinsed briefly (~5 seconds) in ice-chilled physiological saline (PS), and immediately perfused at a constant perfusion pressure of 70 cm H2O with nonrecirculated PS at 37°C via an aortic cannula. During the course of the perfusion all hearts were freely suspended from the aortic cannula at room temperature (20°–22°C). The PS contained (mM) NaCl 119.4, KCl 4.7, CaCl2 2.0, NaHCO3 25.0, MgSO4 1.2, KH2PO4 1.2, glucose 10, and ascorbate 0.6. The pH was maintained at 7.4 by gassing the PS with 95% O2-5% CO2. The hearts were paced at 270 contractions/min with a voltage 10% above threshold and a pulse of 3–5 msec. Pacing was accomplished with a stimulator (model SD9, Grass Instrument, Quincy, Massachusetts) via platinum wire electrodes inserted into the right and left atria.

A latex balloon-tipped cannula (polyethylene; 1.5 mm i.d., 15 cm long) filled with degassed H2O was attached to a strain-gauge manometer (model P23Dd, Statham, Hato Rey, Puerto Rico) with a frequency response of 375 Hz and inserted via the left atrium into the lumen of the left ventricle for obtaining systolic left ventricular pressure development (LVP). LVP was assessed by a carrier preamplifier (model 8805B, Hewlett-Packard, Waltham, Massachusetts). The intraventricular balloon was initially inflated with the degassed H2O until LVP was maximal. The balloon volume was held constant during the course of the experiment to maintain a diastolic pressure of 5 mm Hg. Since the left ventricular lumen size was greater in aged hearts, the balloons employed for each age group were formed on brass molds of the lumens of the appropriately aged heart in diastole. The maximum rates of left ventricular pressure development (+dP/dtmax) and relaxation (–dP/dtmax) were derived from the LVP signal by resistance-capacitance differentiation (derivative preamplifier, model 8814A, Hewlett-Packard). All LVP, +dP/dtmax and –dP/dtmax data were recorded with a multichannel polygraph (model 7700, Hewlett-Packard).

Coronary flow was determined either gravimetrically by collecting the coronary effluent into a collecting tube or instantaneously by recording it with an electromagnetic flowmeter (model SP2201, Statham). For the latter, a cannulating flow probe (1.5 mm i.d.) was used and inserted into the aortic cannula just above the heart. The O2 tension of the coronary effluent was continuously monitored by directing the pulmonary artery outflow through a polyethylene cannula (1.5 mm i.d., 8 cm long) to a Clark-type polarographic electrode (model 53, Yellow Springs Instrument, Yellow Springs, Ohio) inserted into a low-volume fluid chamber. The dead space of the entire unit was 0.5 ml. The O2 content of the fluid was calculated using 0.0321 μl O2/mm Hg/ml PS as the solubility of O2. The myocardial oxygen consumption, expressed as milliliters of O2 consumed per minute per 100 g wet weight of ventricle, was obtained as the product of the coronary flow and the difference between the O2 contents of the aortic PS inflow and pulmonary artery outflow. The oxygen supply-demand ratio was calculated by dividing the rate of O2 delivered to each heart in milliliters of O2 per minute by its O2 consumption (in milliliters of O2 per minute).
TABLE 1. Body and Ventricular Weights, Ventricular Water, Ventricular Weight/Body Weight Ratios, Coronary Flow, and Coronary Flow/Ventricular Weight Ratios of Young and Aged Rats and Guinea Pigs

<table>
<thead>
<tr>
<th></th>
<th>Young (3–5 mo)</th>
<th>Aged (12–22 mo)</th>
<th>Young (3–4 mo)</th>
<th>Aged (10–14 mo)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body wt (g)</td>
<td>277±15</td>
<td>479±23*</td>
<td>347±13</td>
<td>890±63*</td>
</tr>
<tr>
<td>Wet ventricular wt (mg)</td>
<td>1,179±56</td>
<td>1,643±64*</td>
<td>1,490±39</td>
<td>2,855±144*</td>
</tr>
<tr>
<td>Dry ventricular wt (mg)</td>
<td>165±9</td>
<td>240±10*</td>
<td>200±8</td>
<td>432±31*</td>
</tr>
<tr>
<td>Ventricular water (%)</td>
<td>86.0</td>
<td>85.4</td>
<td>86.6</td>
<td>84.9</td>
</tr>
<tr>
<td>Wet ventricular wt/body wt (mg/g)</td>
<td>4.26±0.16</td>
<td>3.44±0.12*</td>
<td>4.29±0.18</td>
<td>3.21±0.31*</td>
</tr>
<tr>
<td>Dry ventricular wt/body wt (mg/g)</td>
<td>0.603±0.014</td>
<td>0.507±0.011*</td>
<td>0.576±0.013</td>
<td>0.485±0.040*</td>
</tr>
<tr>
<td>Coronary flow (ml/min)</td>
<td>7.06±0.50</td>
<td>10.22±0.73*</td>
<td>7.25±0.41</td>
<td>14.34±1.00*</td>
</tr>
<tr>
<td>Coronary flow/wet ventricular wt (ml/min/g)</td>
<td>5.99±0.36</td>
<td>6.22±0.31</td>
<td>4.87±0.57</td>
<td>5.02±0.72</td>
</tr>
<tr>
<td>Coronary flow/dry ventricular wt (ml/min/g)</td>
<td>42.8±1.63</td>
<td>42.6±1.28</td>
<td>36.25±2.13</td>
<td>33.19±3.43</td>
</tr>
</tbody>
</table>

Values are mean±1 SEM for either animals or hearts (21 for rat and four for guinea pig). The ventricular weight represents the combined weight of both left and right ventricles.

*Significant difference from the corresponding value for the younger animal or heart.

Experimental Protocols and Techniques

Generally, young and aged hearts were perfused simultaneously on the same perfusion apparatus containing two aortic cannulas. However, when single young or aged hearts were used, they each were perfused in random order. After an equilibration period of 30 minutes, the hearts were perfused with oxygenated 37°C PS containing 10⁻⁸ M isoproterenol, 4 mM calcium, 10⁻⁶ M propranolol, 5 × 10⁻³ M theophylline, or a combination of two of these agents. Agents were administered from separate reservoirs by switching the appropriate valves just proximal to the aortic cannula. Protocols for various agents were as follows: Isoproterenol was administered for 1 minute. Calcium at 4 mM was administered for 1–2 minutes. Theophylline was administered 5 minutes before and during the isoproterenol administration. Before and during agent administration, coronary effluents were collected into ice-chilled test tubes for 1-minute intervals as the PS perfusate dripped from the apex of the heart and the distal outflow port of the oxygen electrode chamber. The effluent samples were immediately weighed to determine the coronary flow rate and placed for 5 minutes in a 95°–100°C water bath for denaturation of adenosine deaminase that might be present. After boiling, the samples were centrifuged at 1,500g for 5 minutes to remove denatured particulate matter. The supernatant was dehydrated overnight with a gentle stream of air at 35°–40°C. At the end of the perfusion period, the hearts were removed from the perfusion apparatus, gently blotted, and weighed to determine wet weight. Dry weight was determined after placing the hearts in a 60°C oven for 24 hours.

Biochemical Determinations

The dehydrated effluent samples were reconstituted with water to 20% of the original volume and filtered (0.45 μm). The samples were analyzed isocratically for adenosine and inosine on a high-performance liquid chromatograph (Waters Chromatography, Milford, Massachusetts) using a 5 μm C-18 Resolve column (Waters Chromatography) and a mobile phase of 10 mM KH₂PO₄ and 5% methanol (pH 4, 1.0–1.5 ml/min) with ultraviolet absorption measured at 254 nm by methods described previously. Peak verification was accomplished both enzymatically (adenosine deaminase or nucleoside phosphorylase) and by coelution of standards. The adenosine and inosine data are expressed as coronary effluent concentration in nanomoles per milliliter or as apparent release into the effluent in nanomoles per minute per gram dry heart weight.

Lactate in the coronary effluent was analyzed enzymatically using lactate dehydrogenase to catalyze the stoichiometric reduction of nicotinamide adenine dinucleotide. The release of lactate into the effluents is expressed as micromoles per minute per gram dry heart weight.

Materials

L-Isoproterenol hydrochloride (Sigma Chemical, St. Louis, Missouri) was prepared in 0.1% (wt/vol) sodium metabisulfite (Fisher, Boston, Massachusetts) and diluted in PS containing ascorbate (Fisher) at its final concentration. Adenosine, inosine, adenosine deaminase, nucleoside phosphorylase, and lactate dehydrogenase were obtained from Boehringer Mannheim, Indianapolis, Indiana. Theophylline and DL-propranolol chloride were purchased from Sigma Chemical. Pentobarbital was obtained from Abbott Laboratories (North Chicago, Illinois). All salts, acids, bases, dextrose, sodium heparin, and solvents were reagent, certified, or high-performance liquid chromatography grade from Fisher or Baker (Phillipsburg, New Jersey).

Statistical Methods

Analysis of variance (ANOVA) was performed using single-classification ANOVA with two or multiple groups of paired or unpaired observations. A probability of p<0.05 was accepted as statistically significant.
Results

General Characteristics of Preparations From Young and Aged Rats

The aged rats in this study weighed 73% more than the young rats (Table 1). After the perfusion period of approximately 40 minutes, the wet and dry ventricular weights of the aged hearts were 39% and 45% greater, respectively, than the weights of the young hearts. However, the percent water in the ventricular myocardium was similar for both age groups. The wet and dry ventricular weight/body weight ratios were greater for the young rats. While the hearts were paced (270 contractions/min) and perfused at constant pressure (70 cm H2O), coronary flow was 45% higher in aged hearts than in young hearts. However, the coronary flow/ventricular weight (wet or dry) ratios were similar for both age groups.

Contractile Response of Rat Hearts

The aged rat hearts were less mechanically responsive to β-adrenergic stimulation with isoproterenol than the young hearts (Figures 1 and 2). Both young and aged hearts displayed similar control levels of developed LVP and +dP/dt max and –dP/dt max. Isoproterenol at 10^-8 M elicited a peak increase of 176%, 189%, and 167% within 30 seconds and a sustained increase of 109%, 111%, and 134% after 1 minute in LVP, +dP/dt max, and –dP/dt max, respectively, in young hearts. In aged hearts the isoproterenol only elicited a peak increase of 114%, 136%, and 139% within 30 seconds and a sustained increase of 59%, 68%, and 98% after 1 minute in LVP, +dP/dt max, and –dP/dt max, respectively. With the exception of the peak increase in –dP/dt max, all of the isoproterenol-elicited contractile responses were significantly reduced in the aged compared with the young hearts.

Rat Heart Coronary Effluent Adenosine and Inosine

The concentrations of adenosine and inosine in the coronary venous effluent as well as the rates of adenosine and inosine release into the effluent were elevated in the aged rat heart (Figure 3). Adenosine concentration was 0.84±0.23 nmol/ml in the coronary effluent of young hearts and 106% greater in the effluent of aged hearts (Figure 3A). One minute of isoproterenol stimulation (10^-8 M) tended to elevate the concentration of adenosine in the effluent from both young and aged hearts. Adenosine release was 29.8±2.2 nmol/min/g dry wt in the coronary effluent of young hearts and 82% greater in the effluent of aged hearts (Figure 3B). The isoproterenol stimulation tended to increase adenosine release from young and aged hearts.

The inosine concentration was 4.0±0.9 nmol/ml in the coronary effluent of young hearts and 132% greater in the effluent of aged hearts (Figure 3C). One minute of isoproterenol stimulation (10^-8 M) elevated the concentration of inosine in the effluent from young hearts by 72% but only minimally increased inosine concentration in the aged heart effluent. Inosine release was 135±14 nmol/min/g dry wt in the coronary effluent of young hearts and 76% greater in the effluent of aged hearts (Figure 3D). The isoproterenol stimulation increased inosine release from the young hearts by 38% and tended to elevate the release from aged hearts.

Myocardial Oxygen Consumption

The partial pressures of oxygen (Po2s) in the control coronary effluents of both young and aged hearts ranged from 382 to 408 mm Hg and were not
**TABLE 2. Effect of Isoproterenol on Coronary Venous Effluent PO₂, Myocardial Oxygen Consumption, and Oxygen Supply-Demand Ratios of Young and Aged Rat Hearts**

<table>
<thead>
<tr>
<th>Rat hearts</th>
<th>ISO</th>
<th>Young (3–5 mo)</th>
<th>Aged (12–22 mo)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coronary effluent PO₂ (mm Hg)</td>
<td>–</td>
<td>382±34</td>
<td>408±23</td>
</tr>
<tr>
<td>Myocardial oxygen consumption (ml O₂/min/100 g wet wt)</td>
<td>–</td>
<td>9.18±0.51</td>
<td>9.57±0.67</td>
</tr>
<tr>
<td>Oxygen supply-demand ratio</td>
<td>–</td>
<td>1.65±0.13</td>
<td>1.82±0.13</td>
</tr>
</tbody>
</table>

Values are mean±1 SEM for 20–22 hearts. Coronary venous effluent PO₂, myocardial oxygen consumption, and oxygen supply-demand ratio were determined as described in “Materials and Methods.” All values were obtained either immediately before (−) or at the end (+) of a 1-minute 10⁻⁸ M isoproterenol (ISO) administration period.

*Significant difference from the corresponding value in the absence of isoproterenol.

**FIGURE 3. Effect of 10⁻⁸ M isoproterenol on the concentrations of adenosine and inosine (in nanomoles per milliliter) in the coronary effluents (panels A and C) and the net release of these nucleosides (in nanomoles per minute per gram dry weight) into the effluents (panels B and D) of perfused young and aged rat hearts.** Coronary effluent was collected 2 minutes before (CON) and during a 1-minute administration of isoproterenol (ISO). Values represent mean±1 SEM for 31 hearts. *Significant difference from the appropriate control value. †Significant difference from the corresponding young heart effluent value.

significantly different (Table 2). While isoproterenol stimulation for 1 minute lowered venous PO₂ by 120% and 97% in young and aged hearts, respectively, the lowered values were not significantly different from each other. The myocardial oxygen consumption for control young and aged hearts ranged from 9 to 10 ml O₂/min/100 g wet wt. The values for the two age groups were not significantly different from each other. Isoproterenol stimulation (10⁻⁸ M) for 1 minute increased myocardial oxygen consumption in both age groups by 19–25%, but there was no significant difference between the groups. The control oxygen supply-demand ratios were 1.65 and 1.82 for young and aged hearts, respectively, and were not significantly different. These ratios decreased with isoproterenol stimulation by 37–42%, but the lowered values for the young and aged hearts were not significantly different from each other.

**Antagonism of Reduced Contractile Responsiveness in Aged Hearts**

An adenosine receptor antagonist, theophylline (5×10⁻⁵ M), was used to assess the role of adenosine in the reduction of contractile responsiveness of the aged heart to isoproterenol. The methylxanthines are known to antagonize adenosine receptor-elicited responses.12,16,17 This concentration of theophylline was employed because we have previously reported that in perfused rat hearts it does not influence myocardial cAMP levels or cAMP-dependent protein kinase activity by inhibiting phosphodiesterase activity.16

Theophylline eliminated the reduced contractile responsiveness of the aged hearts to isoproterenol stimulation (Figure 4) but did not significantly influence control LVP, +dP/dt max and −dP/dt max for both young and aged hearts (compare Figures 2 and 4). In the presence of theophylline, isoproterenol stimulation (10⁻⁸ M) for 1 minute produced increases of approximately 165% in the peak LVP response and 125% in the 1-minute LVP response. There were no significant differences between the increases observed in the young versus aged hearts. The isoproterenol-induced increases in the peak and 1-minute sustained +dP/dt max responses ranged from 97% to 184% for both young and aged hearts. There was no significant difference between the increases for both age groups. With isoproterenol the increases in −dP/dt max ranged from 121% to 219% for both young and aged hearts. Once again, there was no significant difference between increases in both age groups. The theophylline also eliminated the enhanced coronary effluent adenosine and inosine concentrations and release observed in the aged hearts. Adenosine release from young and aged hearts was 28.9±8.5 and 22.5±5.4 nmol/min/g dry wt, respectively. Upon stimulation with 10⁻⁸ M isoproterenol, adenosine release was 22.7±5.4 nmol/min/g dry wt from young hearts and 22.5±3.5 nmol/min/g dry wt from aged hearts. Thus, theophylline appeared to prevent the differences in contractile responsiveness to isoproterenol stimulation and adenosine release between young and aged hearts observed in the absence of the methylxanthine.

**Elevated Calcium, Contractility, Adenosine, and Inosine**

An elevation of Ca²⁺ concentration is known to enhance the cardiac contractile state.14,27 It has been reported that, although the perfused senescent rat heart has a reduced contractile responsiveness to...
by guest on July 9, 2017 http://circres.ahajournals.org/ Downloaded from

FIGURE 4. Effect of 5×10−5 M theophylline on the isoproterenol-induced increase in left ventricular pressure and maximal rates of left ventricular pressure development (+dP/dt max) and relaxation (−dP/dt max) of perfused young and aged rat hearts. Theophylline was administered at least 5 minutes before and during the 1-minute period of 10−4 M isoproterenol stimulation. Isoproterenol administration for 1 minute elicited maximal responses within 30 seconds (PEAK ISO) and sustained responses at 1 minute (1 MIN ISO). Values represent mean±1 SEM of nine hearts. *Significant difference from the appropriate control value.

β-adrenoceptor stimulation, the young and senescent hearts are equally responsive mechanically to elevated Ca2+ levels.28 In the present studies, the PS Ca2+ concentration was increased from 2 to 4 mM to determine whether the young and aged hearts were equally responsive mechanically and whether the Ca2+ change influences adenosine and inosine release in the coronary effluent of both age groups. For these studies 10−6 M propranolol was included in the PS to prevent the action of endogenous catecholamines. This concentration of the β-adrenoceptor antagonist prevented the contractile response of these hearts to 10−7 M isoproterenol (data not shown).

Increasing the PS Ca2+ from 2 to 4 mM significantly enhanced LVP, +dP/dt max, and −dP/dt max by 121%, 124%, and 128%, respectively, in young hearts (Figure 5). The increase in PS Ca2+ significantly increased these three variables by 141%, 163%, and 185% in aged hearts. However, there was no significant difference in these increases between the two age groups. Also, the control values for these mechanical variables obtained in the presence of 10−6 M propranolol were similar to the values observed in the absence of the β-adrenoceptor antagonist (compare with Figure 2).

The adenosine concentration in the effluent and release of the nucleoside into the effluent was not significantly altered when PS Ca2+ was elevated from 2 to 4 mM in either the young or aged hearts (Figure 6). However, in aged hearts, the adenosine concentration was greater by 44% (Figure 6A), and adenosine release was greater by 116% (Figure 6B); these differences are comparable with those presented in Figure 3. Inosine concentration in the effluent tended to decrease when PS Ca2+ was elevated (Figure 6C). The release of inosine into the effluent was significantly decreased by 29% in the aged hearts (Figure 6D). Inosine concentration and inosine release were greater by 62% and 106%, respectively, in the aged hearts compared with the young hearts.

General Characteristics of Young and Aged Guinea Pig Preparations

The above results indicate that with the reduced contractile responsiveness of the aged rat heart to β-adrenergic stimulation there is an increase in adenosine in the coronary effluent. To ascertain the universality of this age-related phenomenon, experiments were carried out using young and aged guinea pig hearts. The aged guinea pig weighed 157% more than the young guinea pig (Table 1). After a 40-minute perfusion period, the wet and dry ventricular weights of the aged hearts were 92% and 116% greater, respectively, than the young hearts. The percent water of the ventricular myocardium was similar for both age groups. The wet and dry ventricular weight/body weight ratios were greater for the young guinea pigs. Although the coronary flow was
FIGURE 6. Effect of increasing physiological saline (PS) calcium concentration from 2 to 4 mM on the concentrations of adenosine and inosine (in nanomoles per milliliter) in the coronary effluents (panels A and C) and net release of these nucleosides (in nanomoles per minute per gram dry weight) into the effluents (panels B and D) of perfused young and aged rat hearts. Coronary effluent was collected 2 minutes before (2 mM) and during the second minute of a 2-minute administration of elevated PS Ca\(^{2+}\) (4 mM). Values represent mean±1 SEM for 12 hearts. *Significant difference from the appropriate control value. †Significant difference from the corresponding young heart effluent value.

98% higher in the aged hearts, the coronary flow/ventricular weight (wet or dry) ratios were similar for both age groups.

Contractile Responsiveness of Guinea Pig Hearts

The aged guinea pig hearts were less mechanically responsive to isoproterenol stimulation compared with the young hearts (Figure 7). The control levels of LVP, +dP/dt_{max} and −dP/dt_{max} were similar for both age groups. Isoproterenol at 10\(^{-8}\) M in young adult hearts elicited peak increases of 230%, 471%, and 335% within 30 seconds and sustained increases of 158%, 324%, and 293% after 1 minute in LVP, +dP/dt_{max} and −dP/dt_{max}, respectively. However, in aged hearts the isoproterenol produced only peak increases of 126%, 180%, and 178% and sustained increases of 68%, 122%, and 130% in LVP, +dP/dt_{max} and −dP/dt_{max}, respectively. All the isoproterenol responses, with exception of the peak and sustained 1-minute −dP/dt_{max} values in the aged hearts, were significantly reduced compared with the increases observed in the young hearts.

Guinea Pig Heart Coronary Effluent Adenosine and Inosine

The concentrations of adenosine and inosine in the coronary venous effluent and the rates of adenosine and inosine release into the effluent were elevated in the aged guinea pig heart (Figure 8). The adenosine concentration was 0.027±0.004 nmol/ml in the coronary effluent of young hearts and 165% greater in the effluent of aged hearts (Figure 8A). One minute of isoproterenol stimulation (10\(^{-8}\) M) increased the concentration of adenosine in the effluent of young and aged hearts by 103% and 66%, respectively. Adenosine release was 0.94±0.12 nmol/min/g dry wt in the coronary effluent of young hearts and 42% greater in the effluent of aged hearts (Figure 8B). The isoproterenol stimulation increased adenosine release from young and aged hearts by 103% and 82%, respectively.

The inosine concentration was 0.83±0.10 nmol/ml in the coronary effluent of young hearts and 85% greater in the effluent of aged hearts (Figure 8C). One minute of isoproterenol stimulation (10\(^{-8}\) M) tended to decrease the inosine concentration in the young heart effluents and significantly decreased by 33% the concentration of the nucleoside in aged heart effluents. Inosine release was 23.8±2.2 nmol/min/g dry wt in the coronary effluent of young hearts and 31% greater in the effluents of aged hearts (Figure 8D). The isoproterenol stimulation had no influence on inosine release from young hearts but decreased by 59% the release from aged hearts.

Lactate Release From Rat and Guinea Pig Hearts

The release of lactate into the coronary venous effluents was determined to indicate whether the aged
hearts were hypoperfused or whether differences in metabolism existed between the age groups of both rat and guinea pig hearts. Lactate release was similar for both young and aged hearts for each species (Table 3). However, the lactate release was fourfold to eightfold greater in the rat hearts. Isoproterenol (10^-8 M) stimulation for 1 minute significantly increased lactate release from only the rat hearts.

**TABLE 3. Lactate Release Into the Coronary Effluents From Young and Aged Rat and Guinea Pig Hearts**

<table>
<thead>
<tr>
<th></th>
<th>Lactate release (µmol/min/g dry wt)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
</tr>
<tr>
<td>Rat hearts</td>
<td></td>
</tr>
<tr>
<td>Young adult (3–5 mo)</td>
<td>4.54±1.00</td>
</tr>
<tr>
<td>Aged adult (12–22 mo)</td>
<td>5.59±1.19</td>
</tr>
<tr>
<td>Guinea pig hearts</td>
<td></td>
</tr>
<tr>
<td>Young adult (3–4 mo)</td>
<td>1.29±0.39</td>
</tr>
<tr>
<td>Aged adult (10–14 mo)</td>
<td>1.05±0.26</td>
</tr>
</tbody>
</table>

Values are mean±1 SEM for either 10 rat or four guinea pig hearts. Coronary effluents were collected for 1 minute before (Control) or during (Isoproterenol) a 1-minute 10^-8 M isoproterenol administration period.

*Significant difference from the corresponding control value.

**FIGURE 8. Effect of 10^-8 M isoproterenol on the concentration of adenosine and inosine (in nanomoles per millilitre) in the coronary effluents (panels A and C) and the net release of these nucleosides (in nanomoles per minute per gram dry weight) into the effluents (panels B and D) of perfused young and aged guinea pig hearts. Coronary effluent was collected 2 minutes before (CON) and during a 1-minute administration of isoproterenol (ISO). Values represent mean±1 SEM for four hearts. *Significant difference from the appropriate control value. †Significant difference from the corresponding young heart effluent value.**

**Discussion**

The results presented indicate that aged adult rat and guinea pig hearts release greater amounts of adenosine and have a reduced contractile response to β-adrenergic stimulation compared with young adult hearts. To the best of our knowledge, this is the first report revealing that older adult hearts from two mammalian species have higher concentrations of adenosine and inosine in their coronary venous effluents and a greater release of the nucleosides into their effluents.

The observation that older hearts have a reduced contractile responsiveness to β-adrenergic stimulation confirms previous findings by others. Since adenosine is known to have an antiadrenergic action in the heart, it is suggested that the nucleoside plays an important role in the reduced contractile responsiveness. This notion is supported by the following observations: 1) More adenosine is observed in the coronary effluent of aged hearts. This elevated level of adenosine is associated with the reduced contractile responsiveness of the aged heart to β-adrenergic stimulation. 2) The reduced contractile responsiveness in the aged heart is not observed when the adenosine antagonist theophylline is used. Presumably, like other methylxanthines, theophylline blocks the effects of adenosine. 3) Aging does not reduce the contractile responsiveness of the hearts to a doubling of external Ca^2+. This is in agreement with previous studies performed with perfused young and senescent rat hearts. Adenosine also does not influence Ca^2+-induced positive inotropic responsiveness in cardiac tissue. There is less of a reduction of the β-adrenergic-induced increase in -dP/dtmax compared with the marked reduction of LVP and +dP/dtmax with aging. Previously, it has been reported that adenosine exogenously added to perfused young adult rat hearts markedly attenuated β-adrenergic–induced increases in LVP and +dP/dtmax and has less influence on -dP/dtmax. The above evidence suggests that adenosine, as a result of its antiadrenergic action, is important in the reduced contractile responsiveness of the aged heart to β-adrenergic stimulation.

The reason for the enhanced effluent adenosine concentration and release of the nucleoside into the venous effluent observed in the aged heart is not readily apparent. However, several factors can be excluded: 1) Differences in coronary flow: Aged and young hearts were equally perfused because the coronary flow/ventricular weight (wt) ratios were similar for both age groups. Only the absolute levels of the coronary flow for both groups were different because the hearts were perfused at constant pressure much like the in situ heart. 2) O2 supply-demand imbalance: Aged hearts appeared neither to be hypoxic nor to have an imbalance between O2 delivery and O2 demand relative to young hearts. This is of concern because hypoxia and/or an imbalance between O2 supply and O2 demand...
are potent stimuli of myocardial adenosine formation and release. Myocardial oxygen consumption, venous effluent PO_2, and oxygen supply-demand ratio were all similar for both young and aged hearts. Isoproterenol stimulation changed the above parameters in a way that indicated an enhanced utilization of oxygen; the changes were similar in magnitude for both groups. Lactate release was also similar for both heart age groups. 3) Equilibration of hearts: The young and aged adult hearts appeared to be equally equilibrated after 30 minutes of perfusion at constant pressure. This conclusion was reached with the observations that coronary venous PO_2, oxygen consumption, O_2 supply-demand ratio, and lactate release were similar for both age groups. Moreover, these results indicate that the aged adult hearts were not globally hypoxic relative to young adult hearts. Whether there were small local areas in the aged heart myocardium that may have been hypoxic or ischemic cannot be ascertained by the above-mentioned measured variables.

The enhanced concentration and release of adenosine into the coronary venous effluent is thought to reflect a greater level of the nucleoside in the extracellular or interstitial fluid compartment. It is from this compartment that adenosine is believed to exert its antiadrenergic action by attenuating β-adrenergic receptor–mediated activation of adenylate cyclase.16,17,21,33 With elevated adenosine levels in the aged heart, there would be a greater response to the nucleoside. Actually levels of adenosine in the interstitial space may be higher than those levels presently measured in the effluent. The effluent levels of inosine are also elevated. Although inosine has no antiadrenergic action,10,14 it is thought to represent that portion of released myocardial adenosine that is deaminated by the capillary endothelium as the adenosine passes into the coronary circulation.19,20,34 This would imply that the interstitial level of adenosine may approach the sum of both adenosine and inosine observed in the effluent. We have previously reported that assessment of interstitial adenosine levels by sampling epicardial surface transudates suggests that interstitial levels of adenosine are at least twofold higher than effluent levels.11

The adenosine concentration and release of the nucleoside into the guinea pig coronary venous effluents were approximately 1/20 and 1/30, respectively, those values of rat heart effluents. The perfused guinea pig heart is known to produce less adenosine compared with the rat heart.11,20,24 Since phenylisopropyladenosine, an adenosine analogue, at a concentration of 10 nM is known to attenuate isoproterenol-induced activation of cardiac membrane adenylate cyclase17 and contractility,35 the levels of adenosine observed in the guinea pig effluents should be of sufficient magnitude to exert an antiadrenergic action in these hearts.

Other possibilities could explain the difference in adenosine release between young and aged hearts. The aged hearts could have a greater level of 5′-nucleotidase activity, the enzyme response for adenosine formation from adenosine 5′-monophosphate. These hearts could have a reduced activity level of adenosine deaminase, the enzyme responsible for adenosine breakdown to inosine. In addition, there could be other undefined metabolic alterations in the aged hearts. Although the guinea pig hearts were not aged to the same extent as the rat hearts, the older guinea pig heart still released more adenosine than its younger counterpart. However, as the adult heart ages, it is still not clear whether there is a gradual augmentation of adenosine release with time or whether at some point in the adult aging process there is an abrupt increase in adenosine release.

Increasing perfusion fluid Ca^{2+} from 2 to 4 mM enhanced contractility but did not influence the differences in adenosine and inosine concentration and release into the coronary effluents of young and aged hearts. However, inosine release was decreased when Ca^{2+} was elevated in aged hearts. At present, the significance of the later observation is not known.

There are numerous reports in the literature dealing with the reduced contractile responsiveness of the aged heart to β-adrenergic stimulation. An alteration in excitation-contraction coupling,29 a reduction in the activation of adenylate cyclase by catecholamines,22 and an impairment of agonist β-receptor interaction21 are reported to be involved. The present findings implicate the antiadrenergic action of adenosine as a common mechanism underlying previously reported manifestations of aging that involve the coupling of the β-adrenergic receptors to adenylate cyclase. However, the present results would not explain an age-associated difference in mechanisms that may be distal to the receptor–cyclease complex. Increased antiadrenergic action of adenosine may serve as a convenient and uniform explanation for the many reports indicating a reduced responsiveness to catecholamines in aged adult and even senescent hearts, but caution should be exercised with this conclusion because the mechanisms involved in the differences to catecholamine stimulation may not be similar over the entire life span from neonate to senescence.

In summary, it is suggested that an increase in myocardial adenosine is in part responsible for the attenuation of β-adrenergic–elicited responses in the aged adult heart. Since an elevation of extracellular calcium enhances contractility equally in young and aged adult hearts, it appears that the decreased contractility of the aged heart is not due to a defect in the contractile apparatus per se but, rather, to a limitation by adenosine of the receptor signal transduction mechanism.

**Acknowledgments**

The authors wish to thank Vida Juodaitis, Lynne M. Shea, and Diane L. Waice for their excellent technical assistance.
References


Key Words: adenosine · isoproterenol · cardiac contractility · insulin · aging · lactate · myocardial oxygen consumption · theophylline
Increased myocardial adenosine production and reduction of beta-adrenergic contractile response in aged hearts.
J G Dobson, Jr, R A Fenton and F D Romano

Circ Res. 1990;66:1381-1390
doi: 10.1161/01.RES.66.5.1381

Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1990 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7330. Online ISSN: 1524-4571

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/66/5/1381

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/