Aged Rat Myocardium Exhibits Normal Adenosine Receptor-Mediated Bradycardia and Coronary Vasodilation But Increased Adenosine Agonist-Mediated Cardioprotection

Gentian Kristo, Yukihiro Yoshimura, Byron J. Keith, Robert M. Mentzer, Jr., and Robert D. Lasley

Department of Surgery, Division of Cardiothoracic Surgery, University of Kentucky College of Medicine, Lexington.

The purpose of this study was to determine whether aged myocardium exhibits decreased responsiveness to adenosine A1 and A2a receptor activation. Studies were conducted in adult (4–6 months) and aged (24–26 months) Fischer 344 Brown Norway hybrid (F344 × BN) rats. Effects of the adenosine A1/A2a agonist AMP579 were measured in isolated hearts and in rats submitted to in vivo regional myocardial ischemia. Aged isolated hearts exhibited lower spontaneous heart rates and higher coronary resistance, as well as normal A1- and A2a-mediated responses. There was no difference in control infarct size between adult and aged rats; however, AMP579 treatment resulted in a 50% greater infarct size reduction in aged rats (18 ± 4% of risk area) compared to adult rats (37 ± 3%). These findings suggest that adenosine A1 and A2a receptor-mediated effects are not diminished in normal aged myocardium, and that aged hearts exhibit increased adenosine agonist-induced infarct reduction.

Myocardial aging is associated with several changes including reduction in β-adrenergic receptor-mediated effects (1,2), reduced endothelium-dependent coronary dilation (3,4), and impaired relaxation and diastolic dysfunction (5). Mammalian myocardium expresses multiple adenosine receptor subtypes, the most well-known being the A1 and A2a receptor subtypes. Adenosine A1 receptors, located primarily on the cardiac myocytes, mediate adenosine’s effects in atrial and ventricular myocardium, including slowing cardiac conduction and antagonizing the effects of β-adrenergic stimulation. Adenosine A2a receptors, located primarily on vascular tissue, mediate adenosine’s potent coronary vasodilator effects (6). The results of adenosine A1 and A2a receptor signaling in aged myocardium have yielded conflicting findings. There are reports that signaling via at least one of these receptors is reduced (4,7–10), increased (11,12), or unaltered (13) in aged myocardium.

Aged myocardium has also been reported to be associated with increased oxidative stress (14,15), reduced tolerance to Ca2+ overload (16,17), and mitochondrial dysfunction (17,18). These observations have led to the general hypothesis that aged myocardium exhibits decreased tolerance to ischemia–reperfusion injury. However, a review of the literature indicates that these discrepant findings in rats may be due to the use of varying ages (16 to 26 months old) and strains (Wistar, Sprague-Dawley, Fischer 344) of aged rats. A recent study also indicates that these conflicting results may be due, in part, to the use of young rodents, which are not fully mature adults (27). Another significant factor may be the paucity of in vivo studies. To address these deficiencies in the present study we examined cardiac adenosine A1 and A2a receptor effects in aged F344 × Brown Norway hybrid (F344 × BN F1) rats. The F344 × BN F1 strain of rat, maintained at the National Institute on Aging (NIA), has been shown to exhibit aging effects without pathologies that may be present in aged inbred rats (31–33). Studies of adenosine A1 receptor-mediated bradycardia and A2a receptor-mediated coronary dilation were conducted in isolated perfused hearts. Adenosine receptor-mediated cardioprotection in aged myocardium was tested in an in vivo model of regional myocardial ischemia.

METHODS

All animals in this study received humane care according to the guidelines set forth in The Principles of Laboratory Animal Care formulated by The National Society for Medical Research and the National Institutes of Health Guide for the Care and Use of Laboratory Animals (National Institutes of Health Publication No. 86-23, Revised 1996). Animals were also used in accordance with the guidelines of the University of Kentucky Institutional Animal Care and Use Protocol. The F344 × BN rats were
obtained from the NIA rodent colony. These colonies are barrier-maintained and specific pathogen-free.

**Isolated Perfused Rat Heart Preparation**

Adult (4–6 month) and aged (24–26 month) male F344 × BN rats were heparinized and then anesthetized with ketamine/xylazine (60 mg/kg + 6–9 mg/kg, i.p.). The heart was rapidly excised and the aorta was cannulated, followed by perfusion with Krebs-Henseleit buffer (KHB). The hearts were allowed to beat spontaneously and were perfused at a coronary perfusion pressure of 70 mmHg with KHB consisting of (in mM) 118 NaCl, 4.7 KCl, 1.2 MgSO₄, 1.2 KH₂PO₄, 1.5 CaCl₂, 25.0 NaHCO₃, 11.0 glucose, 1.0 pyruvate, and 0.005 EDTA. The perfusate also contained dextran at 15 g/L (average molecular weight 68,800). The perfusate was maintained at 37°C in a constant-temperature reservoir and was bubbled with 95% O₂/5% CO₂ resulting in pH 7.4. Myocardial temperature was maintained at 37°C by partially immersing the heart in a water-jacketed chamber filled with KHB. A fluid-filled latex balloon was inserted into the left ventricle (LV) to monitor LV function and heart rate.

Isolated hearts were allowed 15 minutes of equilibration, after which coronary flow was maintained constant during the generation of dose–response curves with AMP579 in adult (n = 5) and aged F344 × BN hearts (n = 4). Hearts were exposed to 1, 5, 10, 50, and 100 nM AMP579. Coronary perfusion pressure and heart rate responses were recorded 3 minutes after each dose, and 5 minutes after the washout of the final dose. The AMP579 stock solution was diluted in phosphate-buffered saline. This agonist has high affinity for both A₁ (Kᵢ = 5 nM) and A₂A (Kᵢ = 56 nM) receptor subtypes (34).

**In Vivo Animal Preparation**

Adult (4–6 month) and aged (24–26 month) male F344 × BN rats were anesthetized with ketamine/xylazine (60 mg/kg + 6–9 mg/kg, i.p.) with supplemental doses of ketamine as needed. A tracheotomy was performed, and the trachea was intubated with a cannula connected to a rodent ventilator (model 683; Harvard Apparatus, South Natick, MA). The rats were ventilated with room air supplemented with 100% O₂. The right femoral artery was cannulated to measure blood pressure and heart rate via a pressure transducer connected to a polygraph (model WindoGraf 900; Gould Instrument Systems, Valley View, OH). The right jugular vein was cannulated for fluid and drug administration. Arterial pH, partial pressure of carbon dioxide (PCO₂), and partial pressure of oxygen (PO₂) were determined regularly with the use of a pH/blood gas analyzer (model 1640; Instrumentation Laboratories, Lexington, MA), and were maintained within a normal physiological range by adjusting the respiratory rate and/or tidal volume. Body temperature was monitored with a rectal temperature probe and maintained at 38°C using heating pads.

A median thoracotomy was performed and, after removal of the pericardium, a 6-0 prolene suture was passed below the left descending vein and left coronary artery from the area immediately below the left atrial appendage to the right portion of the LV. The ends of the suture were then fed through a short length of propylene tubing to form a snare. After a 30-minute recovery from the surgical procedures, regional ischemia was induced by pulling up on the snare and clamping it onto the epicardial surface using a small hemostat. Coronary artery occlusion was confirmed by epicardial cyanosis and a decrease in blood pressure. Reperfusion, which was initiated by unclamping the hemostat and relieving tension from the snare, was confirmed by visualizing a marked epicardial hyperemic response.

For determination of infarct size, all rats underwent 25 minutes of regional ischemia and 2 hours of reperfusion. Vehicle-treated 4- to 6-month-old (n = 5) and 24- to 26-month-old (n = 6) rats were compared with adult and aged rats that received injections of the adenosine receptor agonist AMP579 (50 μg/kg, i.v. bolus, 30 minutes before ischemia; n = 5/group).

**Determination of Myocardial Infarct Size**

After 2 hours of reperfusion, the ligature at the coronary occlusion site was permanently tied off, and Evans blue solution (1%) was administered via the jugular vein to demarcate the LV area at risk (AAR). The animals were then killed with a pentobarbital overdose, and the hearts were excised. The LV was removed from the remaining tissue and cut into four cross-sectional pieces (≥2-mm thickness), which were incubated for 15 minutes in a 1% triphenyltetrazolium chloride (TTC) solution at 37°C. The AAR was marked by the absence of blue dye and infarcted tissue determined by the TTC negative stained region within AAR. Areas were then quantified by computerized planimetry. Infarct size was expressed as a percentage of the AAR.

**Statistical Analysis**

Results are expressed as mean ± standard error of the mean (SEM). Infarct size data were analyzed by one-way analysis of variance (ANOVA) followed by Tukey’s post test. Inter-group differences in hemodynamics were analyzed by two-way ANOVA for treatment and time, followed by post-test analysis with the Bonferroni method. Intragroup hemodynamic differences were analyzed by one-way ANOVA with repeated measures. A p value <.05 was considered statistically significant.

**RESULTS**

**Isolated Perfused Heart Results**

The dose–response curves for the effects of AMP579 on heart rate and coronary perfusion pressure are shown in Figures 1 and 2, respectively. Baseline heart rate in aged rats (215 ± 2 beats/min) was significantly lower than in adult animals (279 ± 8 beats/min). AMP579 administration induced dose-dependent reductions in heart rate in both groups. The changes in heart rate with each dose of AMP579 and 5 minutes after washout were similar between groups. The changes in heart rate with each dose of AMP579 and 5 minutes after washout were similar between groups. Baseline coronary flow, normalized to heart weight, was lower in aged (8.4 ± 0.5 ml/min/g) versus adult (10.9 ± 0.8 ml/min/g) rats. Treatment with AMP579 decreased coronary perfusion pressure in both groups, with perfusion pressures at each dose being significantly lower in the 24- to 26-month-old hearts.
In Vivo Myocardial Infarct Protocol

Table 1 summarizes systemic hemodynamic data and heart-to-body weight ratios. Baseline heart rate and mean arterial pressure were similar between groups. In the vehicle-treated 24- to 26-month-old rats, heart rate was significantly lower than in 4- to 6-month-old groups at 2 hours of reperfusion. There were no other significant differences in hemodynamics between adult and aged rats. There were no differences in heart-to-body weight ratios.

Figure 3 illustrates the decrease in heart rate and mean arterial pressure that occurred after the administration of AMP579. Both groups showed similar heart rate responses to AMP579, as heart rate decreased from 283 ± 9 to 170 ± 9 beats/min after 5 minutes in adults, and from 252 ± 7 to 147 ± 9 beats/min in aged rats. As shown in Figure 2B during the first 5 minutes after AMP579 administration, the mean arterial pressure in the 24- to 26-month-old rats was significantly higher than in 4- to 6-month-old animals. There were no other significant differences between groups.

Figure 4 summarizes the region at risk and infarct size results in the presence and absence of AMP579 treatment. The ischemic areas at risk were similar in all groups (41 ± 2% in both the 4- to 6-month-old and 24- to 26-month-old vehicle-treated rats, 40 ± 2% in AMP579 4- to 6-month-old animals, and 39 ± 5% in AMP579 24- to 26-month-old group). In vehicle-treated controls, the infarct size was 55 ± 2% in adult animals, and 59 ± 2% in aged rats. Pre-ischemic administration of AMP579 significantly decreased infarct size in both adult and aged rats. Infarct size was reduced to 37 ± 3% in the 4- to 6-month-old rats and to 18 ± 4% in the 24- to 26-month-old animals. The difference in infarct size between the adult and aged animals with AMP579 preconditioning was statistically significant.

DISCUSSION

The results of the present study indicate that aging in F344 × BN rats is associated with: (a) normal A1 and A2a receptor-mediated modulation of heart rate and coronary flow, (b) no increase in necrosis following in vivo coronary artery occlusion, and (c) a preserved ability to be protected against in vivo myocardial necrosis by the adenosine receptor agonist AMP579. These findings indicate the importance of using established models of aging and the relevance of in vivo models when studying myocardial ischemia–reperfusion injury in aged myocardium.

There have been numerous reports examining whether adenosine receptor signaling is altered in aged myocardium; however, these studies have yielded disparate findings (4,7–13). Studies examining adenosine A1 signaling in rat heart ventricular membranes have reported both decreased (8) and increased A1 signaling (11,12). Studies examining adenosine A1 receptor signaling in normal intact myocardium

Table 1. Hemodynamics During In Vivo Myocardial Infarction Protocol

<table>
<thead>
<tr>
<th>Hemodynamics</th>
<th>Vehicle/Adults</th>
<th>Vehicle/Aged</th>
<th>AMP579/Adults</th>
<th>AMP579/Aged</th>
</tr>
</thead>
<tbody>
<tr>
<td>HR, bpm</td>
<td>276 ± 4</td>
<td>230 ± 5</td>
<td>223 ± 13</td>
<td>217 ± 10</td>
</tr>
<tr>
<td>MAP, mmHg</td>
<td>277 ± 11</td>
<td>226 ± 24</td>
<td>243 ± 20</td>
<td>228 ± 15</td>
</tr>
<tr>
<td>Heart/BW ratio, mg/g</td>
<td>2.4 ± 0.2</td>
<td>2.7 ± 0.3</td>
<td>3.2 ± 0.4</td>
<td>2.7 ± 0.4</td>
</tr>
</tbody>
</table>

Notes: Values are expressed as mean ± standard error of the mean; n > 5/group. Baseline values were taken before the coronary artery occlusion; Vehicle/AMP579 values were taken 30 minutes after administration; ischemia values were taken at 15-minute occlusion.

*p < .05 vs baseline.
*p < .05 vs matching controls.
*p < .05 vs vehicle 4-6 month (mo).

Bpm = beats per minute; RF = reperfusion; HR = heart rate; MAP = mean arterial pressure; BW = body weight.
have also yielded conflicting findings (4,7,9,10,13). The results of the present study indicate that both in vivo and isolated aged myocardium exhibit a normal A1 receptor-mediated negative chronotropic effect. Spontaneous heart rate was significantly lower in aged isolated hearts, consistent with several other reports on aged myocardium (9,25,28,35,36). In fact, there is evidence that the decreased spontaneous heart rate in aged rats may be due to increased adenosine release (35).

Although it is thought that aging is associated with decreased vascular reactivity, there have been very few studies of adenosine receptor-mediated coronary reactivity in intact hearts. Two such studies, conducted in aged (12 to 20 months old) isolated perfused rat (Wistar and Sprague-Dawley) hearts, indicated decreased coronary dilation with the adenosine receptor agonists NECA and CGS21680, but in both of these studies the hearts were arrested and the coronary vasculature was preconstricted with KCl (4,9). Our present findings suggest that adenosine receptor-mediated coronary vasodilation is not reduced in aged myocardium. In fact, at the lowest dose tested (1 nM), AMP579 exerted a greater decrease in coronary perfusion pressure in aged rat hearts compared to adult rat hearts. The presence of normal receptor-mediated coronary dilation occurred despite the fact that aged myocardium exhibited increased baseline coronary resistance, an observation previously reported in the hearts of aged F344 × BN rats (37).

The adenosine agonist that we used in the present study, AMP579, has a high affinity for both A1 and A2a receptors, with little (if any) activity or binding to adenosine A3 receptors (34). Binding of this agonist to adenosine A2b receptors has not yet been reported. We have previously reported that AMP579 exerted similar coronary vasodilation as the selective A2a agonist CGS21680 in isolated hearts from Sprague-Dawley rats (38). Because adenosine A1 receptor activation does not induce coronary vasodilation, and adenosine A2b receptors are low-affinity adenosine receptors (39), our findings are consistent with the hypothesis that hearts from aged F344 × BN rats exhibit normal responses to adenosine A2a receptor-mediated coronary vasodilation. Although the decrease in mean arterial blood pressure in the first 5 minutes after AMP579 administration was smaller in the aged rats, this response is a result of systemic, not cardiac, adenosine A2a receptor activation. It is possible that the systemic vasculature in aged rats exhibits decreased vasodilatory responses to adenosine receptor activation.

The results of our in vivo studies also address two controversial aspects regarding aged myocardium: (1) Does aged myocardium exhibit altered tolerance to ischemia–reperfusion? and (2) Can aged myocardium be protected or preconditioned against this injury to the same extent as adult myocardium?
myocardium? With respect to the first issue, reports that myocardial aging is associated with increased myocyte oxidative stress, altered intracellular Ca\(^{2+}\) handling, and mitochondrial dysfunction (14–18) have led to the hypothesis that aged hearts exhibit reduced tolerance to ischemia–reperfusion injury compared to normal adult myocardium. However, a review of the literature indicates that studies of myocardial ischemia–reperfusion in aged rodents yield inconsistent findings (19–27).

Another limitation in the cardiac aging literature is the paucity of in vivo studies, and even these exhibit contradictory findings (23,24,26,40). Leichtweis and colleagues (23) showed that 24-month-old F344 rats did not exhibit greater reductions of LV systolic pressure and dP/dt following 30 minutes of coronary artery occlusion, whereas Liu and colleagues (24) reported a greater decrease in cardiac index and stroke volume index in 19-month-old F344 × BN rats following a similar protocol. However, in neither of these studies was infarct size reported, and global ventricular function measurements cannot be used to quantify injury induced by regional ischemia. In a more recent study, Liu and colleagues (26) concluded that 20-month-old F344 × BN rats exhibited increased in vivo myocardial reperfusion injury. Although these authors reported increased creatine kinase release in the aged rats, they did not quantify the AAR or infarct size. In addition, leukocyte infiltration, myeloperoxidase activity, and tissue superoxide levels were more elevated in the young rats than in the aged rats.

In the present study there was no difference in infarct size between vehicle-treated aged and adult F344 × BN rats, suggesting that in vivo myocardial tolerance to acute ischemia–reperfusion is not reduced with normal aging. This observation is consistent with that of Raya and colleagues (28), who reported that in vivo myocardial infarct size in 23-month-old F344 × BN rats was no different than that in 7-month-old rats. Our findings are also consistent with the results of isolated heart studies in which infarct size was measured (28,41,42).

Although there are several reports that isolated hearts from aged rats cannot be protected by standard ischemic and/or pharmacological preconditioning (22,29,30,41,42), there appear to be only two studies examining adenosine preconditioning in aged myocardium (25,28). In perfused hearts from 16- to 18-month-old mice, adenosine preconditioning did not improve postischemic function (24). Schulman and colleagues (28) reported that isolated hearts from aged (18- to 20-month-old) Sprague-Dawley rats could not be preconditioned with the adenosine A\(_1\) receptor agonist CCPA. To our knowledge, this is the only study to examine adenosine receptor preconditioning in in vivo aged myocardium. Our present findings indicate that aged F344 × BN rats exhibit preserved, and even augmented, ability to be protected against in vivo myocardial infarction with the adenosine agonist AMP579. The discrepancy between our present results and those of Schulman and colleagues (28) could be due to differences in strains of rats, age of both adult and aged rats, and type of preparation (in vivo vs in vitro).

Another explanation for this difference could be our use of the adenosine receptor agonist, AMP579, which activates both A\(_1\) and A\(_2a\) receptors, whereas Schulman and colleagues (28) used an adenosine A\(_1\) agonist. We have recently reported that the myocardial infarct-reducing effect of this mixed agonist is blocked by both an A\(_1\) and an A\(_2a\) adenosine receptor agonist in in vivo rat myocardium (43). Because A\(_2a\) selective adenosine agonist administration prior to ischemia does not reduce infarct size (44), the greater infarct reduction with AMP579 in aged rats in the present study may be due to the combined effects of A\(_1\) and A\(_2a\) receptor stimulation. The greater magnitude of infarct size reduction with AMP579 in the aged rats could have been due to the reported greater activation of ventricular A\(_1\) receptors in aged myocardium (11,12).

We used an in vivo preparation for the ischemia studies, because it is the more physiological preparation. Our observation that myocardial infarct size is not increased in the aged F344 × BN rat is consistent with a previous report by Raya and colleagues (40). Our results are also consistent with those of Przyklenk and colleagues (45), who reported that infarct size reduction with ischemic preconditioning is maintained in in vivo aged (4-year-old) rabbits, although a subsequent study by this same group indicated that the mechanism of ischemic preconditioning in aged animals may differ from that in normal adults (46).

Our in vivo findings are consistent with the results of two observations in human aged myocardium. An analysis of results from the Thrombolysis In Myocardial Infarction-4 (TIMI-4) study indicated that there was no difference in infarct size between patients >65 years of age and younger patients (47). Another report concluded that ischemic preconditioning elicited during preinfarction angina was protective in patients 60 years old or older (48).

**Summary**

The results of the present study indicate that hearts from F344 × BN aged rats exhibited normal adenosine responsiveness to cardiac adenosine A\(_1\) and A\(_2a\) receptor activation. Aged F344 × BN rats also show no change in tolerance to in vivo myocardial ischemia–reperfusion injury with aging or decreased ability to be preconditioned with an adenosine receptor agonist. These findings may have important clinical relevance, because a significant percentage of patients who undergo open heart surgery are elderly.

**Acknowledgments**

This work was supported by National Institutes of Health Grant R01HL66132 and National Institute on Aging Grant 1R03AG022121 (RDL).

Address correspondence to Robert D. Lasley, PhD, Department of Surgery, University of Kentucky College of Medicine, 800 Rose Street, Lexington, KY 40536-0298. E-mail: rlasley@uky.edu

**References**

10. Xu J, Gao F, Ma XL, et al. Effect of aging on the negative chrono-
11. Shryock JC, Belardelli L. Adenosine and adenosine receptors in the cardiovascular system; biochemistry, physiology, and pharmacology.
15. Lakatta EG, Sollott SJ. The “heartbreak” of older age.
34. Kloner RA, Poole K, Shook T, Przyklenk K, Perritt K, Cannon CP. Comparison of acute myocardial infarct size in patients > or = 65 years versus < 65 years in the prethrombolytic period versus the thrombolytic period. Am J Cardiol. 2002;89:1291–1293.

Received March 11, 2005
Accepted June 15, 2005
Decision Editor: James R. Smith, PhD