Age-Related Characterization of Atrial Adenosine A1 Receptor Activation: Direct Effects on Chronotropic and Inotropic Function in the Fischer 344 Rat

Stephen C. Montamat,1,2 Richard D. Olson,1,2,3 Ramagopal V. Mudumbi,1,2 and Robert E. Vestal1,2

1Clinical Pharmacology and Gerontology Research Unit, Department of Veterans Affairs Medical Center, Boise, Idaho.
2Mountain States Medical Research Institute, Boise, Idaho.
3Departments of Medicine and Pharmacology, University of Washington School of Medicine, Seattle.

Cardiac tissues from senescent rats exhibit age-related changes in calcium handling and contractile function when compared to cardiac tissue from adult rats (Lakatta and Yin, 1982). For example, in hearts from senescent rats the duration of contraction (Lakatta et al., 1975) and intracellular calcium signals (Orchard and Lakatta, 1985) are prolonged, and the response to the positive inotropic effect of β-adrenergic stimulation is attenuated (Guarnieri et al., 1980). In humans, responsiveness to the positive chronotropic effect of cardiac tissue from adult rats (ECS0: 4.8 ± 0.7 vs 10.8 ± 1.5 nM). However, senescent left atrium (LA) were 15.4% less responsive to the maximal negative inotropic effects of R-PIA than adult LA. R-PIA did not significantly change resting force from basal values in either age group, but 90% relaxation time was prolonged threefold in senescent LA compared with adults. Radioligand binding experiments with 1,3-dipropyl-8-cyclopentylxanthine, a selective adenosine A1 receptor antagonist, showed a 56% greater density (Bmax) of adenosine A1 receptor in senescent than adult without differences in affinities (Kd). The increased sensitivity of senescent RA to the negative chronotropic effects of adenosine A1 receptor stimulation suggests a role for adenosine in abnormal sinus node function that occurs more frequently with age. Adenosine A1 receptor stimulation has more effect on relaxation than contraction in senescent LA compared with LA from adult F344 rats. However, the increase in density of adenosine A1 receptors suggests a functional dissociation between the availability of binding sites and receptor activation.

Adenosine, an endogenously produced nucleoside, has direct negative chronotropic and inotropic effects on right and left atrial tissues, respectively. Age-related differences in the effects of A1 adenosine receptor activation on atrial rhythmic and contractile function were investigated in adult (6–8 months) and senescent (23–24 months) Fischer 344 (F344) rats. Senescent right atria (RA) were more sensitive to the negative chronotropic effects of R-phenylisopropyladenosine (R-PIA), a selective A1 receptor agonist, than adult RA (ECS0: 4.8 ± 0.7 vs 10.8 ± 1.5 nM). However, senescent left atria (LA) were 15.4% less responsive to the maximal negative inotropic effects of R-PIA than adult LA. R-PIA did not significantly change resting force from basal values in either age group, but 90% relaxation time was prolonged threefold in senescent LA compared with adults. Radioligand binding experiments with 1,3-dipropyl-8-cyclopentylxanthine, a selective adenosine A1 receptor antagonist, showed a 56% greater density (Bmax) of adenosine A1 receptor in senescent than adult without differences in affinities (Kd). The increased sensitivity of senescent RA to the negative chronotropic effects of adenosine A1 receptor stimulation suggests a role for adenosine in abnormal sinus node function that occurs more frequently with age. Adenosine A1 receptor stimulation has more effect on relaxation than contraction in senescent LA compared with LA from adult F344 rats. However, the increase in density of adenosine A1 receptors suggests a functional dissociation between the availability of binding sites and receptor activation.
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age-related impairment of lipolysis by ADA is therefore suggestive of a greater role for local adenosine regulation of lipolysis in fat cells from older animals. Furthermore, Hoffman et al. (1984) also found that inhibition of isoproterenol-stimulated lipolysis by R-phenylisopropyladenosine (R-PIA), an analogue of adenosine selective for the adenosine A1 receptor, was greater in fat cells from older rats than from younger rats.

The role of adenosine in relation to age also has been investigated in the heart. Isoproterenol stimulation of contractile function in isolated perfused hearts from 18-month-old Sprague-Dawley rats was diminished by almost 40% when compared to the response in hearts from 4-month-old animals (Dobson et al., 1990). This decrement in older rats was associated with greater adenosine release in the perfusate. The age-related difference was abolished by the administration of 50 μM theophylline, a nonselective adenosine receptor antagonist. The results of similar experiments in older guinea pig hearts were comparable to the results in rats (Dobson et al., 1990). A subsequent investigation also found similar results in young adult and older adult Fischer 344 (F344) rats (Dobson and Fenton, 1993). Compared with young adult F344 rats, infusion of 10 nM isoproterenol resulted in almost 60% less increase in contractility (+ dP/dt) in isolated perfused hearts from older F344 rats. This age-related response was abolished by addition of 8-sulfophenylethanolamine (8-SPT, a nonselective adenosine receptor antagonist) or ADA. In addition to contractile function, age-related decrements in β-adrenergic stimulation of glycogen phosphorylase and cyclic AMP were also abolished by the addition of 8-SPT. The findings from these two studies suggest that interstitial concentrations of adenosine are greater in senescent myocardium, and the increase in adenosine concentration results in diminished β-adrenergic sensitivity in senescent heart. An increased sensitivity to the negative inotropic and chronotropic effects of adenosine in vivo is found in older compared with younger guinea pigs (Valentine et al., 1989). In contrast, the direct negative chronotropic response to adenosine was significantly less in sinus node preparations from senescent than from adult Wistar rats (DiGennaro et al., 1987).

Although these studies showed age-related alterations in endogenous adenosine availability and cardiovascular sensitivity to adenosine, the direct responsiveness to adenosine in isolated cardiac muscle in senescent animals has not been examined. Prior studies of age-related adenosine effect in heart have used older adult animals but not "senescent" animals. Animals are usually considered senescent when they live beyond the age at which 50% mortality occurs. For male F344 rats, median life span is 22–23 months, and rats over this age would be considered senescent (National Research Council, 1981). In addition, some studies have compared older animals to younger animals that are not fully mature, leading to ambiguity in the interpretation of the findings as indicative of maturation or aging. The present study was designed to investigate effects of R-PIA on the chronotropic response of isolated right atria (RA) and on contractile function of isolated left atria (LA) from senescent and adult male Fischer 344 rats, an established model for aging research.

METHODS

Animals. — Male Fischer 344 rats of 6–8 and 23–24 months of age were obtained from Harlan Industries (Indianapolis, IN) under contract with the National Institute on Aging. Upon arrival, rats were examined and housed in groups of two to four in 18" × 10" clear polycarbonate cages. All animals were maintained on Wayne’s Lab BLOX F-4 ad libitum with a 12-h light/dark cycle. Rats were used within four weeks of their arrival and after one week of observation. No animals exhibiting signs or symptoms of illness were used. Care and use of the animals was in accordance with the recommendations of the National Institutes of Health (1985) and the guidelines of the Animal Studies Subcommittee of the Boise Department of Veterans Affairs Medical Center.

Experimental protocol. — Adult and senescent male F344 rats were killed by decapitation and the hearts rapidly removed. Right atrium (RA) and left atrium (LA) were dissected from the other cardiac chambers and placed in a 15 ml muscle bath (30 °C) containing Krebs-bicarbonate buffer (pH 7.4) of the following composition: 127 mM NaCl, 2.3 mM KCl, 2.5 mM CaCl2, 24 mM NaHCO3, 1.3 mM KH2PO4, 0.6 mM MgSO4, and 5.5 mM glucose. The buffer was continuously bubbled with a mixture of 95% O2 and 5% CO2. Each muscle was affixed to a force transducer. LA were allowed to beat spontaneously. RA were stimulated electrically via punctate platinum electrodes to contract isometrically using square wave pulses (3 msec duration) 20% higher than threshold voltage at a stimulus rate of 30 contractions per minute. High-speed (100 mm/sec) oscillographic recordings were obtained as a permanent experimental record, and data were analyzed on line by a Buxco 4-channel Data Logger System (Buxco Electronics, Troy, NY) interfaced to an IBM-compatible microcomputer. The variables examined for each muscle included: (1) maximal rate of rise of force (+ dF/dt) as a measure of contractility, (2) resting force (RF) as a reflection of muscle stiffness, and (3) 90% relaxation time (90% RT, time required for peak developed force to decrease by 90%) as a measure of cardiac relaxation.

Muscles were stabilized for 120 minutes before beginning the R-PIA concentration-response studies. In all experiments, adenosine deaminase (ADA) was added to the muscle baths during the final 15 min of stabilization at a concentration of 3 units/ml. At this concentration, ADA had no effect on + dF/dt, RF, 90% RT, or RA contraction rate (CR). ADA was purified by dialyzing in phosphate buffer at pH 7.0 for 12–16 hours prior to use (Dobson, 1983), and ADA activity was quantified by spectrophotometric determination (Bergmeyer, 1983). R-PIA was added to the muscle bath until a complete cumulative concentration-response curve was obtained (.03 nM to 30 μM). Parameters of contraction and relaxation were measured following incubation (10 min) at each concentration of R-PIA. A stock solution of R-PIA was prepared in 0.1 M HCl to enhance solubility, and appropriate dilutions were made prior to administration. Vehicle control experiments were also performed. In these experiments, separate muscle preparations...
were treated identically to those that received R-PIA with the exception that only R-PIA vehicle was administered. These experiments allowed for correction of possible age-related differences in response to the vehicle and for assessment of the stability of the preparation over time. 8-SPT was prepared in 2 mM NaOH to enhance solubility, and appropriate dilutions were made prior to administration. 8-SPT or its vehicle was added to the muscle bath 10 minutes after ADA and left in contact with tissues for 15 min before construction of R-PIA concentration-response curve.

Adenosine A<sub>1</sub> receptor radioligand binding. — Saturation isotherms were obtained with 1,3-[^3H]dipropyl-8-cyclopentyl-xanthine ([^3H]DPCPX) as the adenosine A<sub>1</sub> receptor radioligand in crude atrial membrane preparations, as described previously (Musser et al., 1993). Briefly, atrial tissue (obtained from 3–4 pooled atria) was homogenized (Polytron homogenizer, setting 6, 10 sec) in ice-cold 25 mM imidazole buffer containing 0.32 M sucrose, 1 mM NaEDTA, 10 μM PMSF, 0.7 μg/ml pepstatin, and 0.5 μg/ml leupeptin. The homogenate was centrifuged at 1000 x g, the supernatant was filtered through gauze, diluted with an equal volume of the same buffer used for homogenization but without sucrose, and centrifuged at 48,000 x g for 30 min. The pellet was used immediately for binding studies or stored at -70°C until use within 48 h.

The crude atrial membrane pellet was resuspended in 25 mM imidazole buffer containing 10 units/ml ADA, preincubated for 30 min at 22°C, and used directly for radioligand binding assays. Saturation binding isotherms utilizing [^3H]DPCPX were conducted with a radioligand concentration range of 0.1 to 5 nM. Nonspecific binding was defined as that occurring in the presence of 3 mM theophylline. All binding experiments were carried out in a total volume of 250 μl containing 5 mM MgCl<sub>2</sub> and 1–2 mg/ml protein. Incubations with [^3H]DPCPX were performed at 22°C for 90 min and terminated by the addition of 3–4 ml of ice-cold buffer and filtration over GF/C filters using a Brandel Cell Harvester (Gaithersburg, MD). Filters were presoaked in 0.5% polyethyleneimine to reduce nonspecific binding. Samples were eluted overnight in scintillation cocktail and counted for radioactivity. Protein concentrations were determined by the method of Bradford (1976).

Data analysis. — Results of R-PIA concentration-response experiments were analyzed both as percent change from baseline measurement and as percent of maximal effect. Mean vehicle control values for each concentration in each age group were subtracted from individual responses to vehicle and differences in EC<sub>50</sub> (effective concentration producing 50% of the maximum response) for R-PIA in the absence and presence of 8-SPT in each age group. EC<sub>50</sub> values were calculated from the linear portion of the log concentration-response curves. The saturation experiments were analyzed using ReceptorFit Saturation Two-Site Analysis (Lundon Software, Inc., Chagrin Falls, OH). Data are reported as mean ± SEM.

Chemicals. — The following chemicals were used: R-PIA and 8-SPT (Research Biochemicals Inc., Natick, MA), and calf intestine ADA in glycerol solution (Boehringer Mannheim, Indianapolis, IN). R-PIA was dissolved in 0.1 M HCl, and 8-SPT was dissolved in 2 mM NaOH. Appropriate dilutions of R-PIA and 8-SPT were made from these stock solutions for use in concentration-response experiments. All drugs were prepared fresh daily, and all concentrations are expressed as molar concentrations.

RESULTS
Cumulative administration of R-PIA to electrically stimulated isolated LA from both adult and senescent rats resulted in a concentration-dependent decrease in contractility, +dF/dt [F(10,130) = 68.08, p < .001, two-way ANOVA; Figure 1]. Responses were calculated as a percentage of basal +dF/dt prior to R-PIA administration and were corrected for response to vehicle at each concentration of R-PIA. Responses to vehicle were not significantly different in LA from adult and senescent rats (~19.4 ± 4.4% in adult LA vs. ~17.6 ± 4.1% in senescent), indicating similar stability between the two atrial preparations over the course of the experiment (data not shown). Basal +dF/dt was not different between adult and senescent rats prior to administration of R-PIA (26.6 ± 2.4 and 27.1 ± 2.2 grams/sec in adult and senescent LA, respectively; Table 1). The concentration response to R-PIA differed significantly between adult and senescent LA preparations [F(10,130) = 3.04, p < .01, two-way ANOVA].
two-way ANOVA). Furthermore, R-PIA administration resulted in a greater maximum reduction of +dF/dt in adult LA than senescent LA (−43.1 ± 6.5%, adult vs −27.7 ± 4.1%, senescent; p < .05).

Although maximal reduction of +dF/dt was significantly greater in adult LA than senescent LA (−43.1 ± 6.5%, adult vs −27.7 ± 4.1%, senescent), the EC₅₀ for the negative inotropic effect of R-PIA was not statistically different between adult and senescent LA. The EC₅₀ was 9.7 ± 1.9 nM for adult and 9.5 ± 1.6 nM for senescent LA (Table 2).

Basal RF was not different between adult and senescent LA, but basal 90% RT was 49% greater (p < .001) in senescent LA than in adult LA (Table 1). Administration of R-PIA did not affect RF in either group (Figure 3A). In contrast, there was a significant concentration-dependent prolongation of 90% RT in both groups [F(12,204) = 16.25, p < .001, two-way ANOVA; Figure 3B] with greater prolongation in the senescent LA [F(12,204) = 1.83, p < .05, two-way ANOVA; Figure 3B].

The effects of 10 μM 8-SPT on contractile function are shown in Table 2. 8-SPT did not cause age-related differences in basal LA +dF/dt or RF. However, 90% RT was shortened (5 msecs; p < .05) in senescent LA but not in adult LA. In the presence of 10 μM 8-SPT, the negative inotropic response to R-PIA was shifted rightward for both adult and senescent LA (Figures 4A and 4B). In adult LA, the shift was almost 100-fold (EC₅₀: 911 ± 114 nM in presence of 8-SPT vs 9.7 ± 1.9 nM in the absence of 8-SPT; p < .05). The rightward shift (more than twofold) in response to 10 μM 8-SPT was also significant in senescent LA (EC₅₀: 24.1 ± 3.1 nM in the presence of 8-SPT vs 9.5 ± 1.6 nM in the absence of 8-SPT; p < .05), but much less than that seen in adults (p < .05). The maximum negative inotropic effect (decrease in +dF/dt) of R-PIA was enhanced significantly in the presence of 10 μM 8-SPT in senescent LA (−50.9 ± 5.4% vs −27.7 ± 4.1%, p < .05), but not in adult LA (−54.5% ± 4.8 vs −43.1 ± 6.5%).

Basal spontaneous CR in isolated adult and senescent RA were not significantly different (Table 1). There was no decrease in CR in spontaneously beating adult or senescent RA treated with vehicle, indicating that the preparations were stable in both groups for the duration of the experiment. Administration of R-PIA resulted in similar negative chronotropic effects in senescent and adult RA. The CR was completely abolished in most RA in both age groups (CR at maximal inhibition: 6 ± 16 beats/min, senescent RA vs 2 ± 6 beats/min, adult RA). However, R-PIA was twice as potent at inhibiting CR in senescent compared with adult RA. EC₅₀ values were statistically different between age groups (10.8 ± 1.5 nM, adult vs 4.8 ± 0.7 nM, senescent; p < .05). The positive chronotropic response to 10 μM 8-SPT was not different in the adult vs senescent RA (Table 2).

Saturation isotherms obtained with the adenosine A₁ receptor antagonist radioligand [1H]DPCPX in crude membrane preparations from adult and senescent atrial myocardium indicated that the binding was saturable and concentration dependent. Scatchard plots of the data were linear, suggesting a single homogeneous class of binding sites (Figure 5). Although the density of adenosine A₁ receptors (Bₛₒₜₐₜ, Bₛₒₜₐₜ) in atria of senescent rat (35.1 ± 2.5 fmol/mg protein) was 56% greater than adult (22.5 ± 2.6 fmol/mg protein; n = 4 per age group; p < .01), there was no difference in their affinities (Kᵢₐ) (adult: 0.75 ± 0.1 and senescent: 0.56 ± 0.1 nM; p > .1).

**DISCUSSION**

Left atrial contraction contributes to left ventricular filling, and this contribution becomes more important with age in humans. In healthy young volunteers, the fraction of left ventricular filling by the LA is approximately 20–25% but rises to 40–45% in healthy elderly volunteers (Miyatake et al., 1984; Sartori et al., 1987). With greater reliance on the LA for diastolic ventricular filling, contractile function of the LA becomes important as humans age. Thus, modulators of LA function, such as adenosine, may play a more important role in regulating cardiac function with increasing age.

**Table 1. Basal Values of Atrial Contractility, Resting Force, Relaxation, and Contraction Rate**

<table>
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<tr>
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<th>Adult</th>
<th>Senescent</th>
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<tr>
<td>Basal CR (beats/min)</td>
<td>180 ± 18</td>
<td>137 ± 14</td>
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<tr>
<td>Basal RF (grams)</td>
<td>0.43 ± 0.06</td>
<td>0.44 ± 0.02</td>
</tr>
<tr>
<td>Basal +dF/dt (grams/sec)</td>
<td>26.6 ± 2.4</td>
<td>27.1 ± 2.2</td>
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<tr>
<td>Basal 90% RT (msec)</td>
<td>49 ± 4</td>
<td>73 ± 4*</td>
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<tr>
<td>Basal EC₅₀ (nM)</td>
<td>9.5 ± 1.6</td>
<td>9.7 ± 1.9</td>
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**Notes:** Values are mean ± SEM. The number of atrial preparations used was 7–10 per age group. Basal values were obtained 15 min after addition of ADA, but prior to R-phenylisopropyladenosine (R-PIA) administration.

**Figure 2. Contractility (+dF/dt) in electrically driven isolated LA from adult (○, n = 10) and senescent (●, n = 9) F344 rats shown as percent maximal inhibitory response. Responses are corrected for vehicle controls. ADA was present at a concentration of 3 units/ml. EC₅₀s were 9.7 ± 1.9 nM for adult and 9.5 ± 1.6 nM for senescent LA.**
During states of increased cardiac workload, the release of adenosine from local tissues is believed to be a negative feedback mechanism to enhance blood flow and diminish inotropy (Belardinelli et al., 1989). Several studies (Dobson et al., 1990; Dobson and Fenton, 1993; Hoffman et al., 1984) suggest that older tissues release more endogenous adenosine into the interstitium. For example, in isolated perfused hearts from older rats, there is a 65% higher concentration of adenosine in the coronary effluent during basal conditions and 50% greater concentration during stimulation with 10 nM isoproterenol than from adult rat hearts (Dobson et al., 1990). Although these studies did not utilize truly senescent animals, their results suggest that aging is associated with increased local adenosine concentrations in cardiac tissues. Such increases in adenosine levels in older hearts may be expected to play a greater regulatory role in determining LA performance and diastolic function than in younger hearts. A specific aim of the present study was to examine the direct age-related effects of adenosine A<sub>1</sub> receptor activation on cardiac function of LA from rats. Concentration-dependent negative inotropic effect of adenosine A<sub>1</sub> receptor stimulation by R-PIA, a non-hydrolyzable analogue of adenosine, and the antagonistic effect of 8-SPT on R-PIA stimulation suggest that in both adult and senescent LA, these negative inotropic effects were mediated by adenosine receptors. In addition, our results demonstrated that the maximum negative inotropic effect of R-PIA was significantly less in senescent LA than in adult LA (Figure 1). This might have resulted from a lower density of adenosine A<sub>1</sub> receptors in LA from senescent rats compared with LA from adult rats. However, radioligand binding data in the present study indicated that although affinities were similar in the two age groups \( [F(12,204) = 16.25, p < .001, \text{two-way ANOVA}] \) with greater prolongation in senescent LA \( [F(12,204) = 1.83, p < .05, \text{two-way ANOVA}] \).
Figure 4. Contractility (+dF/dt) in electrically driven isolated LA (A: adult; B: senescent) F344 rats shown as percent maximal inhibitory response in the absence (Control) and presence (SPT, 10 μM) of 10 μM 8-SPT. Responses are corrected for vehicle controls. ADA was present at a concentration of 3 units/ml. 8-SPT or vehicle was added 15 minutes after and 5 min prior to R-PIA administration. EC50 values: 9.7 ± 1.9 nM and 911 ± 114 nM for adult LA in the absence and presence of 8-SPT, respectively (p < .05), and 9.5 ± 1.6 nM and 24.1 ± 3.1 nM for senescent LA (p < .05).

Figure 5. Saturation isotherms from representative experiments showing the specific binding of [3H]DPCPX in crude membranes from adult (Panel A) and senescent (Panel B) rat atrial myocardium. Scatchard plots (insets) are consistent with the presence of a single population of homogeneous binding sites.

Experiments were conducted in the presence of 3 units/ml ADA to remove any influence of endogenous adenosine on adenosine A1 receptor function in senescent LA. In the present study, the shift in response to R-PIA produced by the antagonist 8-SPT at a concentration higher (10 μM) than the reported Kd (1 μM) value at the adenosine A1 receptor (Shamim et al., 1989) was significantly less in senescent LA (Figure 4), and the age-related difference in the maximum negative inotropic response to R-PIA was abolished in the presence of 8-SPT. These results also confirm the dissociation between adenosine receptor function and the density of binding sites.

The altered responses to R-PIA in the presence of 8-SPT could be due to age-related changes in local adenosine concentrations, altered adenosine A1 receptor availability in the presence of 8-SPT, or the presence of age-dependent and 8-SPT insensitive adenosine A1 receptor subtypes. Our experiments were conducted in the presence of 3 units/ml ADA to remove any influence of endogenous adenosine on adenosine A1 receptors, but whether or not this concentration or even higher concentrations of ADA reduce extracellular adenosine concentrations to inactive levels is controversial (Linden, 1989; Parkinson and Fredholm, 1992). Thus, under these conditions the actual concentration of endogenous adenosine at the cell surface location of adenosine A1 receptors is not known. The direct positive inotropic effect of 8-SPT (Table 2) was observed even in the presence of 3 units/ml ADA, which was included in the bath prior to 8-SPT to remove endogenous adenosine. Also, it is known that 8-SPT, being a polar anionic compound, does not cross cell membranes (Heller and Olsson, 1985). Therefore, the positive inotropic effect of 8-SPT is most likely due to blockade of adenosine receptor stimulation by endogenous adenosine rather than phosphodiesterase inhibition. The fact that ADA preincubation does not significantly interfere with this positive inotropic effect of 8-SPT suggests that endogenous
Adenosine A1 receptor activation also caused age-related changes in cardiac relaxation. The prolongation of 90% RT by R-PIA was greater in senescent than adult LA. The prolongation of 90% RT by R-PIA in senescent and adult LA was antagonized by pretreatment with 8-SPT (data not shown). How adenosine A1 receptor activation may modify cardiac relaxation is unclear, however. Relaxation is determined by processes involved in calcium metabolism. In rat myocardium, calcium metabolism, including sequestration, storage, and release, is controlled primarily by the sarcoplasmic reticulum (Langer, 1992). Thus, it seems likely that age-dependent changes in relaxation and response to R-PIA may involve age-dependent changes in sarcoplasmic reticulum function and responsiveness to adenosine A1 receptor activation.

In contrast to inotropic responses, senescent RA were more sensitive to the negative chronotropic effect of the adenosine A1 receptor-selective agonist R-PIA than adult RA. Therefore, less endogenous adenosine should be required to produce negative effects on the spontaneous CR in senescent RA due to enhanced adenosine A1 receptor sensitivity. This finding may explain the age-related decline in basal heart rate (Cavato et al., 1974) and diminished heart rate response to β-adrenergic stimulation with age in the F344 rat heart (Schmidlin et al., 1992). Enhanced adenosine A1 receptor sensitivity in senescent RA tissue may result in greater negative chronotropic effects of endogenous adenosine on the sinus node with age. However, in contrast to LA, there were no significant age-related effects of the nonselective adenosine receptor antagonist 8-SPT on spontaneous CR in isolated RA.

In conclusion, divergent responses occur to adenosine A1 receptor stimulation in RA from senescent F344 rats compared with that in LA from adult animals. The impaired inotropic response to an adenosine analogue with age may be due to desensitization or downregulation of adenosine A1 receptors by the elevated concentration of extracellular adenosine that occurs with age. A greater slowing of cardiac relaxation to adenosine A1 receptor stimulation occurs in senescent LA compared with adult LA, suggesting that, with increased age, A1 receptor activation regulates calcium homeostasis to a greater extent. These results suggest that, relative to adults, senescent LA function may be insensitive to the negative inotropic effects of endogenous adenosine, preserving LA function during senescence when diastolic filling of the left ventricle is more dependent on LA contraction. Senescent RA were more sensitive to the negative chronotropic effect of adenosine A1 receptor stimulation than adult RA, possibly accounting, in part, for the age-related decrease in basal and isoproterenol-stimulated heart rate.

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Address correspondence to Dr. Robert E. Vestal, Research Service (151), VA Medical Center, 500 West Fort Street, Boise, ID 83702. E-mail: rvestal@euskadi.idbsu.edu

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