Age-related changes in A1-adenosine receptor-mediated bradycardia

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Hinschen, Andrea K., Roselyn B. Rose'Meyer, and John P. Headrick. Age-related changes in A1-adenosine receptor-mediated bradycardia. Am. J. Physiol. Heart Circ. Physiol. 278: H789–H795, 2000.—The impact of age on functional sensitivity to A1-adenosine receptor activation was studied in Langendorff-perfused hearts from young (1–2 mo) and old (12–18 mo) male Wistar rats. Adenosine mediated bradycardia in young and old hearts, with sensitivity enhanced --10-fold in old (negative logarithm of EC50 (pEC50) = 4.56 ± 0.11) versus young hearts (pEC50 = 3.70 ± 0.09). Alternatively, the nonmetabolized A1 agonists N4-cylohexyladenosine and (R)-N4-phenylisopropyladenosine were equipotent in young (pEC50 = 7.43 ± 0.12 and 6.61 ± 0.19, respectively) and old hearts (pEC50 = 7.07 ± 0.10 and 6.80 ± 0.11, respectively), suggesting a role for uptake and/or catabolism in age-related changes in adenosine sensitivity. In support of this suggestion, [3H]-adenosine uptake was approximately twofold greater in young than in old hearts (from 3–100 µM adenosine). However, although inhibition of adenosine deaminase and adenosine transport with 10 µM erythro-9-(2-hydroxy-3-nonyl)adenine hydrochloride and 10 µM S-(4-nitrobenzyl)-6-thioinosine increased adenosine sensitivity three- to fourfold, it failed to abolish the sensitivity difference in old (pEC50 = 4.95 ± 0.08) versus young (pEC50 = 4.29 ± 0.13) hearts. Data indicate that 1) age increases functional A1 receptor sensitivity to adenosine without altering the sensitivity of the A1 receptor itself, and 2) age impairs adenosine transport and/or catabolism, but this does not explain differing functional sensitivity to adenosine. This increased functional sensitivity to adenosine may have physiological significance in the older heart.

Increased metabolic demand or impaired O2 delivery (36). Alterations in this feedback loop may lead to age-related changes in local adenosine release, membrane receptor sensitivity, or cardiac responses to adenosine (32). During aging, myocardial contractile responses decline (9, 12), coronary dilator reserve decreases (12, 24), β-adrenergic responsiveness declines (9, 21, 35, 36), and sensitivity to ischemic injury increases (12, 18, 30). Interestingly, almost all of these age-related changes could result from alterations in responses to adenosine.

Recent evidence indicates that such age-related alterations in adenosine responses may occur. Adenosine has been shown to more effectively impair β-adrenergic responsiveness in old than in young hearts (8–11, 13, 17, 40, 42). The age-related change in this “indirect” A1-mediated action of adenosine could be due to the elevation of adenosine levels with age (9, 10, 17, 18, 28), which may result from impaired transport and catabolism (28) and/or increased intracellular substrate levels for adenosine formation (17). Altered sensitivity could also result from alterations in A1 receptor function. Conflicting data exist regarding the impact of age on A1-adenosine receptors. Some investigators have reported increased A1 density with aging (32, 40) and during development and maturation (31). Other investigators have reported no changes in A1 receptor density with aging and an age-related decline in coupling between A1 receptors and Gα proteins (4).

Because considerable controversy exists regarding age-related changes in A1 receptor-mediated responses, and because most studies to date have focused on indirect antiadrenergic responses, the primary aim of this study was to characterize age-related changes in “direct” A1-mediated bradycardia. We also tested whether any observed changes in functional sensitivity to adenosine were caused by changes at the level of the A1 receptor itself and/or changes in adenosine transport and metabolism.

METHODS

Isolated, perfused rat hearts. All experiments were performed in hearts isolated from young (1–2 mo old) and old (12–18 mo old) male Wistar rats. Rats were anesthetized with 50 mg/kg pentobarbitone sodium administered intraperitoneally. A thoracotomy was performed, and hearts were rapidly excised and immersed in ice-cold perfusion fluid. The aorta was immediately cannulated, and hearts were perfused in a...
retrograde fashion at a pressure of 100 mmHg with a modified Krebs-Henseleit solution containing (in mM) 119 NaCl, 25 NaHCO₃, 4.7 KCl, 1.2 KH₂PO₄, 2.55 CaCl₂, 1.2 MgSO₄, 15 glucose, and 0.05 EDTA. Perfusate was equilibrated with 95% O₂-5% CO₂ at 37°C, giving a pH of 7.4. Intraventricular pressure development was prevented by inserting a small polyethylene tube through the apex of the left ventricle to drain the cavity. Coronary perfusion pressure was measured using a Gould Statham P23 XL pressure transducer (Viggo Spectramed, Oxnard, CA) connected to a side arm of the aortic cannula and was continuously monitored and recorded on a MacLab data acquisition unit (AD Instruments, Castle Hill, Australia). The hearts were continuously bathed in buffer maintained at 37°C. Blood gas values were regularly monitored using a Ciba Corning 238 pH/Blood Gas Analyzer (Ciba Corning Diagnostics, Halstead, UK) to ensure a pH of 7.40, a P O₂ of ≥ 550 mmHg, and a P CO₂ of 35 mmHg. Coronary flow rate was determined gravimetrically using a four-place balance. Perfusate was switched to constant flow perfusion for examination of A₁-adenosine receptor-mediated bradycardia. The nonspecific endogenous ligand adenosine and the A₁-specific analogs N⁶-cyclohexyladenosine (CHA) and (R)-N⁶-phenylisopropyladenosine (R-PIA) were infused to achieve concentrations ranging from 0.1 µM to 0.6 mM for adenosine, 1 nM to 1 µM for CHA, and 1 nM to 0.1 µM for R-PIA.

For the transport and metabolism blockade studies, erythro-9-(2-hydroxy-3-nonyl)adenine hydrochloride (EHNA) was added directly to perfusion fluid to a final concentration of 10 µM, whereas S-(4-nitrobenzyl)-6-thioinosine (NBTI) was infused into the aortic cannula to give a final concentration of 10 µM in 0.1% DMSO. Control experiments were performed in which vehicle alone was infused into the perfusate. When concentration-response curves were complete, hearts were removed, blotted, and weighed.

[³H]adenosine uptake. Hearts were equilibrated for 20 min at their intrinsic rate and then electrically paced at 300 beats/min with a Grass model SD9 stimulator (Grass Instruments, Quincy, MA) and perfused at a constant pressure of 100 mmHg. After 10 min, hearts were switched to constant flow for [³H]adenosine uptake studies. The adenosine stocks (containing both radiolabeled and unlabeled agonist) were infused at 5% of flow for 4 min per concentration. Effluent was collected after 3 min at each concentration, and flow was measured. At the end of the experiment, hearts were removed, blotted, and weighed. Aliquots of coronary effluent and infused adenosine stocks were placed in polyethylene scintillation vials and vortexed with 10 ml of biodegradable counting scintillant (BCS). These samples were left to incubate overnight. Aliquots were then analyzed in duplicate using a Tri-Carb 2000 CA Liquid Scintillation Analyzer (Packard Instruments, Downers Grove, IL). Uptake of [³H]adenosine was calculated according to the following equations:

\[
\text{uptake} = \frac{\text{(%uptake) \times [infused Ado](mol \cdot min^{-1} \cdot g^{-1})}}{(\text{infused Ado cpm}) - (\text{effluent Ado cpm}) \times 100\%}
\]

The concentration of adenosine in the coronary effluent was determined using a Tri-Carb 2000 CA Liquid Scintillation Analyzer (Packard Instruments, Downers Grove, IL). Uptake of [³H]adenosine was calculated according to the following equation:

\[
\text{uptake} = \frac{\text{(%uptake) \times [infused Ado](mol \cdot min^{-1} \cdot g^{-1})}}{(\text{infused Ado cpm}) - (\text{effluent Ado cpm}) \times 100\%}
\]

where Ado is adenosine and cpm is counts per minute.

Data analysis. Data are reported as means ± SE. All data were analyzed with the use of a multiway ANOVA followed by the Newman-Keuls post hoc test for individual comparisons when significant effects were detected. In all tests significance was accepted at the 95% confidence level (P < 0.05). A three-parameter logistic equation was used to fit data from concentration-response experiments

\[
\text{response} = \frac{A}{1 + \left(\frac{[\text{agonist}]}{EC_{50}}\right)^{\text{slope factor}}}
\]

where A is the response at infinite dose and [agonist] is the adenosine PIA or CHA concentration. Curves were fit to individual experiments with reported EC₅₀ values and maximum responses representing the means of individual determinations.

Materials. DMSO, adenosine, CHA, R-PIA, NBTI, and EHNA were all purchased from Sigma Chemical (Castle Hill, Australia). [³H]-adenosine (23.0 Ci/mmol) and BCS were purchased from Amersham Pharmacia Biotech (Castle Hill, Australia).

RESULTS

A₁ PIA receptor-mediated bradycardia with adenosine and CHA. Intrinsic heart rate was reduced with age, consistent with previous observations by Headrick (17, 18). The resting intrinsic heart rate for untreated young hearts was 316 ± 5 beats/min (n = 25), which was significantly higher (P < 0.05) than the intrinsic rate of 239 ± 7 beats/min for old hearts (n = 28). During examination of A₁-adenosine receptor-mediated bradycardia, it was found that old hearts displayed a significantly greater sensitivity to the endogenous signal (adenosine) than young hearts. EC₅₀ values obtained were almost an order of magnitude higher than those in the young group (Table 1 and Fig. 2A). In contrast, responses to the specific A₁-receptor agonists CHA and R-PIA were comparable in both young and old hearts (Table 1 and Fig. 2A and B). Dose-response curves acquired for adenosine in the absence of vehicle (DMSO) were almost identical to those obtained in its presence [negative logarithm of concentration inducing half-maximal contractile activity (pEC₅₀) = 3.79 ± 0.08 for young hearts (n = 11) and 4.33 ± 0.09 for old hearts (n = 12)].

Effect of nucleoside transport and metabolism inhibition on A₁ receptor-mediated bradycardia. Treatment of hearts with EHNA (a potent adenosine deaminase inhibitor) and NBTI (a purine transport inhibitor) resulted in insignificant reductions in intrinsic heart rate to 296 ± 10 beats/min in young hearts (n = 7) and 229 ± 6 beats/min in old hearts (n = 11). Intrinsic rate remained significantly higher in the young group (P < 0.05). Dose-response curves for adenosine in young and old hearts were repeated in the presence of EHNA (a
potent adenosine deaminase inhibitor) and NBTI (a purine transport inhibitor). The chronotropic sensitivity to infused adenosine was significantly enhanced three- to fourfold in both young and old hearts in the presence of these inhibitors (Table 1 and Fig. 1B). However, the relative shifts in sensitivity were similar for both age groups. Thus the difference in sensitivity between the young and old hearts was not abolished in the presence of the transport and catabolism blockers (Table 1). On the basis of relative $EC_{50}$ values, old hearts are approximately seven times more sensitive to adenosine than young hearts in the absence of the catabolism and/or transport inhibitors and approximately five times more sensitive in the presence of the inhibitors.

[3H]-adenosine uptake. To examine potential differences in adenosine transport in young versus old hearts, we studied the uptake of radiolabeled adenosine. Uptake of [3H]-adenosine in the young hearts was consistently higher across the entire concentration range examined, although this did not achieve statistical significance until infused adenosine concentrations exceeded 3 µM. Uptake was approximately two times greater in young than in old hearts at infused adenosine concentrations >3 µM (Fig. 3).

**DISCUSSION**

In this study, concentration-response relationships were obtained for adenosine-, CHA-, and R-PIA-mediated bradycardia in intact hearts from young and old animals. The older hearts displayed a significantly enhanced functional sensitivity to the nonspecific endogenous signal (adenosine) compared with the young hearts. Conversely, responses to the nonmetabolized $A_2$-specific agonists (CHA and R-PIA) were not altered significantly with age. Although these observations are suggestive of a role for adenosine catabolism in age-related changes in functional $A_1$ sensitivity, experiments performed in the presence of potent adenosine transport and catabolism inhibitors failed to normalize functional responses to adenosine. These findings indicate that there is a paradoxical increase in functional sensitivity to adenosine in the absence of changes in $A_1$ receptor sensitivity.

$A_1$ Receptor-mediated bradycardia in young and old hearts. A number of previous studies have demonstrated age-related reductions in functional sensitivity to adenosine $A_1$ agonists. These studies generally examine the indirect antiadrenergic effects of adenosine, with most studies focusing on inotropic (8–11, 40, 43) rather than chronotropic (46) effects of adenosine. Importantly, direct and indirect effects of adenosine occur via different mechanisms. The direct chronotropic effects of $A_1$ receptors are $cAMP$ dependent, involving $G$ protein activation of $K^+$ conductance and hyperpolarization of nodal tissue (1, 2). Indirect effects involve inhibition of $Ca^{2+}$ current activation and hyperpolarization-activated current (1). These latter effects may be $cAMP$ dependent, although this is controversial (15, 34). Thus recently documented age-related changes in antiadrenergic effects of adenosine (8–11, 40, 42, 43) may stem from alterations in one or more of these multiple paths. Only a small number of studies have

### Table 1. Concentration-response data for bradycardia mediated by adenosine (in absence or presence of EHNA and NBTI) and CHA in young and old hearts

<table>
<thead>
<tr>
<th>Treatment</th>
<th>n</th>
<th>pEC50</th>
<th>Maximum Response, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adenosine</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Young</td>
<td>7</td>
<td>3.70±0.09*</td>
<td>97±1</td>
</tr>
<tr>
<td>Old</td>
<td>7</td>
<td>4.56±0.11</td>
<td>101±1</td>
</tr>
<tr>
<td>Adenosine+ EHNA/NBTI</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Young</td>
<td>7</td>
<td>4.29±0.13†</td>
<td>98±1</td>
</tr>
<tr>
<td>Old</td>
<td>11</td>
<td>4.95±0.08†</td>
<td>103±3</td>
</tr>
<tr>
<td>CHA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Young</td>
<td>7</td>
<td>7.43±0.12</td>
<td>86±6</td>
</tr>
<tr>
<td>Old</td>
<td>8</td>
<td>7.07±0.10</td>
<td>100±4</td>
</tr>
<tr>
<td>R-PIA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Young</td>
<td>7</td>
<td>6.61±0.19</td>
<td>103±2</td>
</tr>
<tr>
<td>Old</td>
<td>8</td>
<td>6.80±0.11</td>
<td>103±3</td>
</tr>
</tbody>
</table>

Values are means ± SE and were obtained from concentration-response curves shown in Figs. 1 and 2. Maximum response, mean maximal response determined from individual dose-response curves, expressed as a percentage of preinfusion heart rate. EHNA, erythro-9-(2-hydroxy-3-nonyl)adenine hydrochloride; NBTI, S-(4-nitrobenzyl)-6-thioinosine; CHA, N''-cyclohexyladenosine; R-PIA, (R)-N''-phenylisopropyladenosine. *P < 0.05 vs. old hearts. †P < 0.05 vs. adenosine alone.

![Fig. 1. Concentration-response curves for bradycardia in constant flow perfused hearts in response to adenosine alone [A; n = 7 for young (1–2 mo) hearts, n = 7 for old (12–18 mo) hearts] and adenosine in presence of the adenosine deaminase inhibitor erythro-9-(2-hydroxy-3-nonyl)adenine hydrochloride (EHNA; 10 µM) or the adenosine transport inhibitor S-(4-nitrobenzyl)-6-thioinosine (NBTI; 10 µM) (n = 7 for young hearts, n = 11 for old hearts). Heart rate response is expressed as a percentage of preinfusion heart rate (%baseline). Values are means ± SE. *P < 0.05 vs. old hearts. †P < 0.05 vs. values in absence of EHNA and NBTI.](http://ajpheart.physiology.org/)
examined age-related changes in the direct chrono- 
tropic response (7, 32, 33).

Headrick (17) previously established an age-related 
increase in sensitivity to the direct negative chrono-
tropic effects of adenosine in rat, consistent with obser-
vations in anesthetized guinea pigs (6, 47). Similarly, 
Mudumbi et al. (33) observed increased inotropic sensi-
tivity to \( R \)-PIA in senescent myocardium. However, 
these investigators found no age-related change in 
chronotropic sensitivity in the right atrium, an observa-
tion supported by de Garavilla et al. (6). A subsequent 
study by Montamat et al. (32) revealed a very modest 
(2-fold) age-related increase in chronotropic sensitivity 
to \( R \)-PIA. In direct contrast, Di Gennaro et al. (7) 
reported an age-related decline in chronotropic sensitiv-
ity of rat sinus node to adenosine. Thus variable and 
even opposing observations have been made regarding 
the impact of age on direct \( A_1 \) receptor-mediated re-
sponses.

If the enhanced functional sensitivity to adenosine 
observed here (Fig. 1A) and previously (17) is due to 
enhanced functional sensitivity of the \( A_1 \) receptor (i.e., 
altered \( A_1 \) density and/or receptor-effector coupling), 
then \( A_1 \)-mediated responses to nonmetabolized and 
\( A_1 \)-specific agonists should also be enhanced. We exam-
ined responses to CHA and \( R \)-PIA and found no differ-
ences in functional sensitivity to these agonists in the 
two age groups studied (Fig. 2). This contrasts with 
other studies utilizing the same agonists (32, 33), 
although we noted that the difference in sensitivity was 
quite modest in these studies. From our data we 
conclude that an increase in age from 1–2 to 12–18 mo 
increases the apparent functional sensitivity to \( A_1 \) 
activation by adenosine without directly altering \( A_1 \) 
receptor sensitivity itself.

The absence of any age-related differences in func-
tional responses to CHA and \( R \)-PIA clearly demon-
strates a lack of change in \( A_1 \) sensitivity, density, or 
coupling with aging. Other investigators have observed 
quite variable effects of age (if any) on \( A_1 \) density, 
receptor affinity, and/or receptor coupling. In terms of 
\( A_1 \)-receptor density, some groups have documented 
either an increase in aged myocardium (33, 40) or no 
change (4, 32). Some groups have reported an increased 
agonist \( A_1 \) receptor affinity (40) or an unchanged \( A_1 \) 
affinity (32, 33). \( A_1 \) receptor coupling has been reported 
to decline with aging (4). The weight of evidence from 
these admittedly few and contradictory studies sug-
gests that there may be minor changes, if any, in \( A_1 \) 
receptor density during aging and a decline in \( A_1 \) 
affinity and coupling. These changes are unlikely to 
account for the five- to sevenfold difference in sensitiv-
ity to adenosine in the presence and absence of trans-
port and/or catabolism inhibition. Moreover, irrespec-
tive of these previous findings, our data clearly 
demonstrate a lack of impact of age on functional 
sensitivity to two different but selective \( A_1 \)-receptor 
agonists (Fig. 2).

Another possible explanation for the age-related 
increase in adenosine sensitivity is that the nonselective 
endogenous agonist may activate multiple receptor 
subtypes (i.e., \( A_1, A_2, A_3 \)) with opposing actions, whereas 
CHA and \( R \)-PIA will specifically activate \( A_1 \) receptors. 
When examining \( A_1 \)-mediated responses, one cannot 
exclude or ignore the effects of endogenous adenosine 
on other receptor subtypes. For example, activation of 
\( A_1 \) receptors exerts an antiadrenergic action attenuat-
ing \( \beta \)-adrenoceptor-mediated responses, whereas activ-
ation of \( A_2 \) receptors can enhance the \( \beta \)-adrenergic 
response (42). In this respect, the chronic elevation in 
endogenous adenosine levels in older hearts could 
downregulate myocardial \( A_2 \) receptors, leading to im-
pairment of any potential inhibition of \( A_1 \)-mediated 
responses. However, we should note that although \( A_2 \) 
receptors have been shown to indirectly alter \( A_1 \)-
mediated inotropic responses, there is no evidence from in vitro or in vivo models that A2-receptor activation directly modifies heart rate or conduction or indirectly modifies chronotropic responses to A1 activation. Nonetheless, further studies may directly examine the impact of A2-receptor activation or inhibition on A1-adenosine receptor-mediated bradycardia in the heart.

Age-related changes in adenosine transport and catabolism. Our data indicate that changes in adenosine sensitivity with age cannot be caused by alterations in A1 receptor sensitivity. Alternatively, differences in the levels and/or myocardial transport and catabolism of the endogenous ligand could play a role. A number of studies documented elevated vascular and interstitial adenosine levels in aged hearts (8–11, 17). Headrick (17) documented increased intracellular substrate levels for adenosine in aged myocardium, and other studies showed reductions in adenosine transport in aged hearts (28). Increased endogenous adenosine levels might artificially enhance apparent sensitivity to infused adenosine, although all evidence indicates that resting levels are only modestly enhanced (<2-fold) (17, 28), and this cannot explain a five- to sevenfold shift in sensitivity to applied adenosine. Moreover, these small changes in endogenous adenosine would equally increase apparent sensitivity to other adenosine agonists. Because this did not occur for CHA or R-PIA, we conclude that increased functional sensitivity to adenosine is unrelated to endogenous adenosine levels. On the other hand, impaired adenosine uptake and/or catabolism (28) could increase functional sensitivity to adenosine.

We report that [3H]-adenosine uptake is significantly greater (~2-fold) in young than in old hearts at adenosine concentrations exceeding 3 µM (Fig. 3). Moreover, uptake was consistently, albeit insignificantly, higher at lower adenosine concentrations in young hearts. These findings are consistent with recent reports of reduced transport in aged myocardium (11, 28), which might contribute to elevated adenosine levels and impaired β-adrenergic responsiveness, as suggested by Dobson and colleagues (8–11, 28). This might also explain the increase in functional sensitivity to adenosine observed (Fig. 1). It should be noted, however, that the change in adenosine transport will only lead to small age-related differences in extracellular adenosine levels (e.g., at 10 µM adenosine, myocardial transport will reduce extracellular adenosine by ~5% in old and ~10% in young hearts) (Fig. 3). Such differences are clearly insufficient to account for the shift in functional sensitivity observed (Fig. 1). Nonetheless, to further test the possibility that an age-related decline in adenosine uptake and/or catabolism might contribute, we confounded EHNA and NBTI to block both adenosine deaminase and adenosine transport, respectively. These drugs significantly shifted the concentration-response relationships for adenosine to lower concentrations in both young and old hearts, reflecting significant transport and catabolism of infused adenosine (Fig. 3). Importantly, the blockers failed to eradicate the difference in functional sensitivity to adenosine. Thus differences in adenosine transport and catabolism do not adequately explain the reported change in functional sensitivity to adenosine with aging.

Experimental limitations. One possible explanation for the inability of NBTI and EHNA to eliminate age-related changes in adenosine sensitivity is that the inhibitors might be ineffective at blocking transport and catabolism. Nucleoside transporters in mammalian cells are classified as NBTI sensitive and NBTI insensitive (14, 38). NBTI-sensitive transporters are inhibited only by nanomolar concentrations of NBTI (22, 39), whereas NBTI-insensitive transporters are inhibited by micromolar concentrations. The proportions of NBTI-sensitive and -insensitive transport are species and tissue dependent (38), and nucleoside transport in the rat heart occurs primarily via the NBTI-sensitive carrier (48). In any case, the 10 µM concentration of NBTI used here will effectively block both carriers. Moreover, it has been shown that 5 µM NBTI completely inhibits nucleoside transport in isolated hearts and erythrocytes (44), 10 µM NBTI almost completely blocks transport in cardiomycocytes within 30 s (41), and 12 µM NBTI completely inhibits adenosine transport in cardiac sarcolemmal vesicles (16). Endothelial adenosine transport is also almost totally blocked by 10 µM NBTI, with 94–99% inhibition of transport of 1–10 µM adenosine (41). These studies all indicate that 10 µM NBTI will effectively inhibit cardiovascular adenosine transport. In relation to adenosine deaminase blockade, it was recently shown that 5 µM EHNA infused into the coronary circulation maximally inhibits cardiovascular adenosine deaminase (23). We chose a twofold-higher concentration here to ensure effective blockade of the enzyme. We also undertook additional NBTI-EHNA experiments with the addition of 1.5 µM iodotubercidin, a maximally effective dose of this selective adenosine kinase inhibitor (23). It has been proposed that recycling of adenosine by adenosine kinase is reduced in the aged heart, allowing increased basal release from aged hearts (11). The addition of iodotubercidin failed to produce any additional shifts in the concentration-response relationships for adenosine in both young and old hearts (data not shown). This indicates that NBTI and EHNA eliminated adenosine transport and catabolism in perfused hearts and that changes in adenosine kinase activity are not likely to be involved in the differing sensitivity to adenosine.

Because EHNA and NBTI applied at the present 10 µM concentrations will completely or nearly completely block adenosine catabolism by adenosine deaminase, as well as adenosine transport, and because the magnitude of the difference in adenosine uptake between young and old hearts is insufficient to explain the five- to sevenfold difference in sensitivity, other factors must account for enhanced functional sensitivity to adenosine, but not CHA and R-PIA, in older hearts. Further studies are warranted to unravel the mechanisms underlying this change.

In summary, the results of the present study indicate that age increases cardiac functional sensitivity to adenosine, as assessed by the direct negative chrono-
tropic response of the perfused heart. Interestingly, the alteration in adenosine sensitivity is not attributable to a change in adenosine A₁ receptor sensitivity or to changes in cardiovascular handling of adenosine. Adenosine transport is shown to be reduced in old hearts, and this may account for elevated resting levels of adenosine. Reduced adenosine A₁ receptor-mediated relaxations to adenosine in guinea pig aorta. Am. J. Physiol. Heart Circ. Physiol. 259: H62–H67, 1990.


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