Age-related changes in adenosine-mediated relaxation of coronary and aortic smooth muscle

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Hinschen, Andrea K., Roselyn B. Rose'meyer, and John P. Headrick. Age-related changes in adenosine-mediated relaxation of coronary and aortic smooth muscle. Am J Physiol Heart Circ Physiol 280: H2380–H2389, 2001.—We tested whether adenosine mediates nitric oxide (NO)-dependent and NO-independent dilation in coronary and aortic smooth muscle and whether age selectively impairs NO-dependent adenosine relaxation. Responses to adenosine and the relatively nonselective analog 5'-N-ethylcarboxamidoadenosine (NECA) were studied in coronary vessels and aortas from immature (1–2 mo), mature (3–4 mo), and moderately aged (12–18 mo) Wistar and Sprague-Dawley rats. Adenosine and NECA induced biphasic concentration-dependent coronary vasodilation, with data supporting high-sensitivity (pEC50 = 5.2–5.8) and low-sensitivity (pEC50 = 2.3–2.4) adenosine sites. Although sensitivity to adenosine and NECA was unaltered by age, response magnitude declined significantly. Treatment with 50 μM Nω-nitro-L-arginine methyl ester (L-NAME) markedly inhibited the high-sensitivity site, although response magnitude still declined with age. Aortic sensitivity to adenosine declined with age (pEC50 = 4.7 ± 0.2, 3.5 ± 0.2, and 2.9 ± 0.1 in immature, mature, and moderately aged aortas, respectively), and the adenosine receptor transduction maximum also decreased (16.1 ± 0.8, 12.9 ± 0.7, and 9.6 ± 0.7 mN/mm² in immature, mature, and moderately aged aortas, respectively). L-NAME decreased aortic sensitivity to adenosine in immature and mature tissues but was ineffective in the moderately aged aorta. Data collectively indicate that 1) adenosine mediates NO-dependent and NO-independent coronary and aortic relaxation, 2) maturation and aging reduce NO-independent and NO-dependent adenosine responses, and 3) the age-related decline in aortic response also involves a reduction in the adenosine receptor transduction maximum.

adenosine receptors; aging; coronary vasculature; endothelium; maturation; nitric oxide; rat heart

ADENOSINE MAY BE AN IMPORTANT regulator of coronary blood flow (6) and mediates dilation in a variety of vascular beds. Dilatory responses to adenosine show considerable tissue and species heterogeneity, suggesting roles for different receptor subtypes and/or effector mechanisms. Vascular adenosine receptors include the A₁ (4), A₂A (9, 10), A₂B (17, 33), and A₃ subtypes (12).

Although an intact endothelium is not obligatory for the vasodilator action of adenosine (46), these cells may contribute to adenosine responses in coronary, aortic, and pulmonary vessels (14, 40, 45, 50, 51). Thus, within a tissue, adenosine may mediate vascular responses via endothelium-dependent and endothelium-independent mechanisms (20, 52) and via different receptor subtypes. The relative roles of these different components remain poorly understood. Indeed, there is evidence for (22, 28, 52) and against (26, 30, 31) a role for the endothelium [or nitric oxide (NO)] in coronary adenosine responses.

In addition to an incomplete understanding of the receptors and mechanisms involved in adenosine-mediated dilatory responses, contradictory observations exist regarding the impact of age on adenosine responses (7, 34, 35, 38, 48). Age-related changes in adenosine responses may occur as a result of alterations in the contributions of NO-dependent vs. NO-independent responses, altered receptor expression and coupling, or alterations in the ability of the tissue itself to respond to receptor stimulation. In the present study, we tested the hypothesis that NO-dependent and NO-independent mechanisms contribute to adenosine-mediated coronary and aortic dilation and that age selectively reduces the NO-dependent adenosine response. Previous studies provide evidence of age-related reductions in NO-mediated vascular responses (3, 24). Specifically, we 1) characterized maturational and aging-related changes in adenosine responses in the coronary vasculature and aorta, 2) assessed the NO dependence of these responses, and 3) tested whether age impairs the ability of adenosine receptors to induce vascular relaxation (i.e., reduces the efficiency of adenosine receptor-effector transduction).

METHODS

Isolated perfused rat hearts. The following studies conform with the Guide for the Care and Use of Laboratory Animals [DHHS Publication No. (NIH) 85-23, Revised 1996]. Coronary studies were performed in hearts isolated from immature (1–2 mo old), mature (3–4 mo old), and moderately aged (12–18 mo old) male Wistar rats. Mean wet heart weights

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were 0.63 ± 0.03, 1.26 ± 0.04, and 1.57 ± 0.05 g for immature, mature, and moderately aged age groups, respectively. Rats were anesthetized with pentobarbital sodium (50 mg/kg ip). A thoracotomy was performed, and hearts were rapidly excised and immersed in ice-cold perfusion fluid. The aorta was immediately cannulated, and the hearts were perfused in a retrograde fashion at a pressure of 100 mmHg with a 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. A small polyethylene tube was inserted through the apex of the left ventricle to drain the cavity prevented intraventricular pressure development. Coronary perfusion pressure was measured using a Gould Statham P23XL pressure transducer (Viggo Spectramed, Oxnard, CA) connected to a sidearm of the aortic cannula and was continuously monitored and recorded on a Maclab data acquisition unit (AD Instruments, Castle Hill, Australia). Hearts were continuously bathed in buffer maintained at 37°C. Blood gas values were regularly monitored using a pH/blood gases analyzer (model 238, Ciba Corning Diagnostics, Essex, UK) to ensure an arterial pH of 7.40, Po₂ of ≈550 mmHg, and PCO₂ of 35 mmHg. Coronary flow rate was determined gravimetrically using a four-place balance. Perfusate was delivered to the heart using a peristaltic pump (Minipuls 2, Gilson, Middleton, WI). Before each individual experiment, the pump flow was calibrated to ensure accurate flow rate determination.

Functional responses in beating hearts. To examine functional characteristics of the coronary vasculature in beating hearts from the three age groups, hearts were isolated as described above (n = 6/group), perfused at a constant pressure of 100 mmHg, and electrically paced at 5 Hz for the duration of the experiment. Baseline measurements were made after a 60-min stabilization period. Maximal vasodilator responses to adenosine and reactive hyperemic responses were then determined randomly in each heart. Hyperemic flows were measured in hearts subjected to 60 s of total coronary occlusion followed by reperfusion. Maximal adenosine responses were acquired during infusion of 3 mM adenosine until stable maximal dilation was achieved. The order of treatment was randomized, and a 15-min recovery period was allowed between stimuli. To examine the role of endogenously released adenosine in reactive hyperemia, a second series of hearts (n = 7/group) was stabilized for 50 min and then switched to perfusion with buffer containing 100 mM 8-p-sulfophenyltheophylline and 10 IU/ml adenosine deaminase. After an additional 10 min of stabilization, responses to transient occlusion were acquired as described above.

Concentration-response curves for KCl and vasodilator drugs in perfused hearts. Cumulative concentration-response curves for KCl were acquired in immature (n = 3), mature (n = 6), and moderately aged hearts (n = 5) to determine contractile sensitivity and concentrations giving 85% or 95% of maximal response (EC₈₅ or EC₉₅) for the constrictor in each age group and to determine the minimal KCl concentration required to induce complete cardiac arrest (no detectable cardiac contractions). After a 30-min stabilization period, hearts were treated with cumulative concentrations of KCl ranging from 5 to 200 mM. Coronary vasoconstriction was allowed to plateau at each concentration before the level of KCl was further increased.

For vasodilator responses, hearts were stabilized for 30 min and then arrested by switching to a potassium-modified Krebs-Henseleit solution containing (in mM) 119 NaCl, 25 NaHCO₃, 100 KCl, 1.2 KH₂PO₄, 2.55 CaCl₂, 1.2 MgSO₄, 15 glucose, and 0.05 EDTA. The 100 mM KCl concentration was obtained from the concentration-response relationships acquired as described above and corresponds to the EC₉₅ for KCl in all age groups, determined as described above. An EC₉₅ (50–60 mM) was not used, since a significant percentage of hearts displayed sporadic contractions at these levels (i.e., were not fully arrested). During KCl preconstriction, coronary perfusion pressure was held constant at 160 mmHg in all three age groups to normalize coronary perfusion pressures between the different age groups and also to ensure adequate myocardial perfusion and oxygenation during constriction. After 20 min of stabilization, concentration-response curves were obtained for adenosine (0.1–300 μM) in immature (n = 7), mature (n = 6), and moderately aged hearts (n = 7). Concentration-response curves were also obtained for 1 mM–6 μM 5'-N-ethylcarboxamidoadenosine (NECA), the nonselective analog, in the absence (n = 12 for immature hearts and n = 11 for moderately aged hearts) and presence (n = 8 for immature hearts and n = 6 for moderately aged hearts) of 50 μM N⁶-nitro-l-arginine methyl ester (L-NAME). This concentration was shown to effectively abolish NO-dependent ACh responses in isolated vessels (see RESULTS). In all studies, only one concentration-response curve was obtained per heart; the hearts were then blotted, and wet weights were determined.

Aortic ring experiments. To obtain additional information regarding the impact of age on vascular sensitivity and responses to adenosine, studies were performed in aortic rings obtained from immature (n = 34), mature (n = 34), and moderately aged (n = 33) male Sprague-Dawley rats. Rings were prepared as described by us in detail for rats and other species (18, 20, 34, 35). Specifically, arterial segments were placed in the perfusion fluid used for perfused hearts and flushed gently to remove adherent blood cells. Segments were cleaned of connective tissue and cut into 2- to 4-mm transverse rings. Two ring segments from each animal were examined. Rings were vertically mounted on stainless steel wires passed through the lumen; one wire was attached to a Grass FT03C strain gauge and the other was fixed in place. The rings were placed in 20-ml tissue chambers and bathed in a modified Krebs-Henseleit solution containing (in mM) 118 NaCl, 4.7 KCl, 2.5 CaCl₂, 1.2 MgCl₂, 1.2 KH₉PO₄, 25.0 NaHCO₃, 11.0 glucose, and 0.03 EDTA. The solution was maintained at 37°C and constantly bubbled with 95% O₂-5% CO₂, giving a pH of 7.4. Rings were equilibrated for 60 min and then stretched to optimal tensions, determined to be 0.8 g in immature, 1.0 g in mature, and 1.5 g in moderately aged rings. These resting tension values were obtained in preliminary experiments (n = 6/group) by progressive stretch of rings in 0.1-g increments until maximal contractions to 65 mM KCl were observed. After 60 min of stabilization at optimal tensions, rings were maximally contracted with 65 mM KCl, washed three times, and allowed to recover for 30 min. In the first series of experiments, concentration-response curves for norepinephrine-induced contractions were acquired in quiescent rings from immature (n = 7), mature (n = 7), and moderately aged rats (n = 8). Concentration-response curves for adenosine were subsequently acquired in one of two ways. In one group, responses were obtained via the conventional cumulative method: adenosine concentration-response curves were acquired in vessels precontracted with a single EC₉₅ of norepinephrine (n = 7 for immature, n = 7 for mature, and n = 8 for moderately aged groups). This conventional cumulative method yields information regarding tissue sensitivity to adenosine but constrains response magnitude to the degree of precontraction. Thus it does not provide information regarding unconstrained receptor trans-
duction maxima. Therefore, we also acquired extended concentration-response curves for adenosine using functional antagonism, as described in detail by Lew (29). This method generates concentration-response curves unconstrained by tissue maximum response and permits comparison of occupancy-response coupling ranges for receptor systems (such as adenosine receptors) that maximally activate the tissue under study (29). Specifically, norepinephrine was applied cumulatively until tension increased to >50% of the tissue maximum response to 65 mM KCl. Adenosine was then applied cumulatively until vessels relaxed to <50% of maximal KCl tone (n = 7 for immature, n = 7 for mature, and n = 6 for moderately aged groups). This process was repeated until no further response to adenosine was observed or the adenosine concentration approached 10^n M. Data analysis. All aortic responses were normalized to vascular cross-sectional area using the following equation:

$$\text{cross-sectional area (mm}^2) = \frac{2 \times \text{blotted weight (mg)}}{1.06 \times \text{circumference (mm)}}$$

where 1.06 is vascular tissue density (mg/mm^3). At the end of each experiment, aortic rings were cut open, and ring circumference and blotted weight were measured.

Data analysis. Values are means ± SE. Statistical comparisons between groups were made using a multway 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). One-site (3 parameter) and two-site (4 parameter) concentration-response relationships were fit to coronary or aortic data using the following equations:

$$\text{response} = A \frac{A}{1 + [(\text{agonist})/\text{pEC}_{50}]\text{slope factor}}$$

for one-site relationships and

$$\text{response} = A - \left\{ \frac{(A)}{100} \times \left( \frac{B}{1 + [(\text{agonist})/\text{pEC}_{50}]} \right) + \left( \frac{100 - B}{1 + [(\text{agonist})/\text{pEC}_{50}]} \right) \right\}$$

for two-site relationships, where A is the response at infinite dose, (agonist) is the adenosine or NECA concentration, B is the percent contribution from the first site, and pEC_{50} and pEC_{50}'s are apparent pEC_{50} values for the first and second sites, respectively. To determine whether the two-site model provided a statistically improved description of the data relative to the one-site model, an F-test was employed to compare regressions:

$$F = \frac{(SS_1 - SS_2)/(df_1 - df_2)}{SS_2/df_2}$$

where SS is the sum of squares, df is degrees of freedom for each model, and the subscripts 1 and 2 refer to the one- and two-site models, respectively. P < 0.05 was considered evidence of a statistically improved fit. Reported pEC_{50} or values and maximum responses represent means of individual determinations ± SE. For coronary responses not reaching a clear maximal relaxation (response plateau), data were constrained to the KCl-induced tone to extrapolate curves to reasonable limits. For extended aortic concentration-response curves, the maximal observed tissue response and the maximal response extrapolated from individual curve fits are reported.

Materials. Adenosine, ACh, KCl, norepinephrine, sodium nitroprusside, and adenosine deaminase were purchased from Sigma Chemical (Castle Hill, Australia). NECA, l-NAME, and 8-p-sulfophenylthioephyllyamine were purchased from Research Biochemicals (Natick, MA).

RESULTS

Effects of maturation and aging on basal and stimulated coronary flow in beating hearts. Resting (or basal) coronary flow was highest in hearts from immature animals and lowest in hearts from moderately aged animals (Table 1). The age-related decline in coronary flow was associated with reduced peak dilatation in response to adenosine and a parallel decline in the peak reactive hyperemic response to 60 s of occlusion. Vasodilatory reserve (maximal/basal flow), therefore, declines significantly with age (Table 1). Treatment with 8-p-sulfophenylthioephyllyamine and adenosine deaminase reduced basal coronary flow in immature (but not mature and moderately aged) hearts and significantly reduced reactive hyperemia in all age groups (Table 1). An age-related decline in hyperemic flow was still evident in the presence of adenosine inhibition.

Table 1. Basal and hyperemic coronary flows in Langendorff-perfused hearts from immature, mature, and moderately aged rats

<table>
<thead>
<tr>
<th></th>
<th>Immature</th>
<th>Mature</th>
<th>Moderately Aged</th>
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<tbody>
<tr>
<td>Basal flow, ml·min⁻¹·g⁻¹</td>
<td>16.8 ± 1.0</td>
<td>12.5 ± 0.9*</td>
<td>11.8 ± 0.9*</td>
</tr>
<tr>
<td>Peak adenosine flow, ml·min⁻¹·g⁻¹</td>
<td>28.2 ± 1.0</td>
<td>21.5 ± 0.8*</td>
<td>18.5 ± 0.6†</td>
</tr>
<tr>
<td>Peak hyperemic flow, ml·min⁻¹·g⁻¹</td>
<td>30.6 ± 1.1</td>
<td>22.3 ± 0.7*</td>
<td>18.0 ± 0.8†</td>
</tr>
<tr>
<td>Peak adenosine flow/peak hyperemic flow</td>
<td>0.95 ± 0.12</td>
<td>0.96 ± 0.09</td>
<td>1.02 ± 0.10</td>
</tr>
<tr>
<td>Vasodilatory reserve</td>
<td>1.83 ± 0.13</td>
<td>1.74 ± 0.11</td>
<td>1.51 ± 0.10††</td>
</tr>
<tr>
<td>Basal flow+8-SPT/Ado, ml·min⁻¹·g⁻¹</td>
<td>12.5 ± 1.0</td>
<td>11.6 ± 1.1</td>
<td>11.0 ± 0.8</td>
</tr>
<tr>
<td>Hyperemic flow+8-SPT/Ado, ml·min⁻¹·g⁻¹</td>
<td>22.3 ± 1.4‡</td>
<td>18.7 ± 1.1‡</td>
<td>16.0 ± 0.9†‡</td>
</tr>
</tbody>
</table>

Values are means ± SE of 6 rats in each group. SPT, 8-p-sulfophenylthioephyllyamine; Ado, adenosine. *P < 0.05 vs. immature hearts; †P < 0.05 vs. mature hearts; ‡P < 0.05 vs. untreated hearts.
Fig. 1. Effect of KCl on coronary resistance in perfused hearts obtained from immature (n = 6), mature (n = 6), and moderately aged (n = 6) rats. Values are means ± SE. *P < 0.05 vs. immature hearts; †P < 0.05 vs. mature hearts.

Effects of KCl and adenosine in the coronary vasculature. KCl elevated coronary vascular resistance in a concentration-dependent manner (Fig. 1). The pEC$_{50}$ for KCl declined modestly with maturation (from 1.98 ± 0.07 to 1.73 ± 0.06, P < 0.05) and did not change further with aging (1.64 ± 0.08). The magnitude of the KCl response increased significantly with maturation and aging (Fig. 1).

In hearts preconstricted with 100 mM KCl (the EC$_{95}$ for all age groups), adenosine induced biphasic concentration-dependent dilation (Fig. 2). A two-site model yielded a statistically superior fit compared with a one-site model. Data obtained from two-site curve fits are provided in Table 2. There was no age-related difference in the pEC$_{50}$ for adenosine at the low-sensitivity site. However, at the high-sensitivity site, there was a slight (2-fold) increase in sensitivity to adenosine with maturation (but not aging). Maturation and aging significantly reduced the magnitude of the high-sensitivity response (from >3 to ~1.5 mmHg·ml⁻¹·g⁻¹) and reduced its overall contribution to adenosine-mediated dilation (Table 2). The magnitude of responses to $10^{-5}$–$10^{-4}$ M adenosine was significantly reduced with maturation but was not substantially altered by further aging (Fig. 2). Responses and age-related differences were qualitatively similar when data were expressed as absolute units or relative to KCl-induced tone.

Effects of NECA ± L-NAME in the coronary vasculature. Results for NECA were qualitatively similar to those for adenosine, although NECA was substantially more potent. NECA induced a biphasic concentration-dependent dilation in immature and moderately aged hearts, with a two-site model providing a statistically superior fit to the data (Fig. 3). There were no significant age-related differences in pEC$_{50}$ values for NECA at the high- or low-sensitivity sites. However, dilatory responses for $10^{-7}$–$10^{-6}$ M NECA were significantly reduced with age (Fig. 3), and the magnitude of the high-sensitivity response was reduced by ~50% with aging (Table 2).

L-NAME inhibited NECA-mediated dilations, resulting in a one-site concentration-response curve in immature and moderately aged hearts (Fig. 3). Only a
single apparent site was present in L-NAME-treated hearts, with a pEC50 between those for the high- and low-sensitivity sites in untreated hearts. L-NAME treatment did not eliminate age-related reductions in response magnitude for 10^{-2}–10^{-6} M NECA. Again, responses and age differences were qualitatively similar whether expressed in terms of absolute resistance or relative to KCl-induced tone.

Responses to adenosine in aortic rings. Aortic rings from the three age groups responded differently to KCl. Constrictions to 65 mM KCl, normalized to tissue cross-sectional area, were significantly greater in mature rings (10.5 ± 0.3 mN/mm²) than in rings from immature (8.0 ± 0.3 mN/mm²) and moderately aged (7.5 ± 0.8 mN/mm²) rats. Additionally, aortic sensitivity to norepinephrine declined with maturation and aging, with pEC50 values of 8.1 ± 0.1 in immature rings (n = 6) vs. 7.3 ± 0.1 in mature rings (n = 7) and 7.2 ± 0.1 in moderately aged rings (n = 6, P < 0.05).

Conventional concentration-response curves for adenosine show that vascular sensitivity decreases significantly with maturation and aging (Fig. 4A, Table 3). Extended concentration-response curves verify this observation, demonstrating a 16-fold decline in sensitivity with maturation and a further 4-fold reduction with aging. Additionally, extended concentration-response curves show that the receptor transduction maximum (observed and extrapolated) declines significantly by up to 40–50% with maturation and aging (Fig. 4B, Table 3). This significant decline is evident when data are expressed as absolute units or relative to the contractile response to KCl (Table 3).

L-NAME significantly inhibited responses to adenosine in immature and mature, but not moderately aged, aortas (Fig. 4B, Table 3). Despite the pronounced reductions in response amplitude, only the pEC50 for immature hearts was reduced by L-NAME (Table 3). Although L-NAME did not abolish age-related reductions in sensitivity and response amplitude for adenosine, the inhibitory effects of L-NAME did decline with maturation and were negligible in aged tissue.
Responses to ACh and sodium nitroprusside in aortic rings. ACh potently relaxed norepinephrine-contracted rings from all age groups (Fig. 5A). Response magnitude and sensitivity to ACh were not markedly altered by maturation but were significantly reduced with aging. pEC50 values for ACh were 7.6 ± 0.2 and 7.4 ± 0.1 in immature and mature rings, respectively, and 7.1 ± 0.1 in moderately aged rings (P < 0.05). At 50 μM, l-NAME almost completely abolished responses to ACh in all age groups (Fig. 5A). Sodium nitroprusside concentration dependently relaxed aortic rings from all age groups, and sensitivity was independent of age (Fig. 5B). However, there was a modest but significant aging-related decline in response magnitude for the lowest sodium nitroprusside concentration examined (3 nM).

NO-dependent and NO-independent responses to adenosine. Assuming that vascular responses in the presence of l-NAME represent NO-independent relaxations (verified by abolition of ACh-mediated dilation with 50 μM l-NAME), we calculated the l-NAME-insensitive (NO-independent) response in coronary and aortic tissues by subtraction of the NO-independent response from control responses (Fig. 6). We previously applied this analysis in studies of vascular tissue. Here we show that adenosine and NECA induce biphasic concentration-dependent coronary dilation in immature, mature, and moderately aged hearts, supporting the existence of multiple adenosine “sites” or receptors. The coronary responses are partially NO dependent, and the age-related decline in response magnitude (but not sensitivity) involves reductions in NO-dependent and NO-independent coronary responses and sensitivity to NECA and adenosine involve reductions in the magnitude of the NO-dependent and NO-independent relaxations (Fig. 6). Figure 6A shows that the NO-dependent coronary response (occurring at 10^{-8}–3 \times 10^{-7} M NECA) is reduced by >70% with aging. Similarly, the NO-independent response (in the presence of l-NAME) is reduced by >70% with aging. Figure 6B depicts similar changes in aortic tissue responses with maturation and shows that there is no detectable NO-dependent response in moderately aged tissue.

Table 3. Comparison of pEC50 and maximal responses for adenosine in aortic rings from immature, mature, and moderately aged rats

<table>
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<tr>
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<th>Immature</th>
<th>Mature</th>
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<tr>
<td><strong>Cumulative concentration-response curve</strong></td>
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<tr>
<td>pEC50, M</td>
<td>5.4 ± 0.4</td>
<td>3.7 ± 0.2*</td>
<td>3.1 ± 0.1†‡</td>
</tr>
<tr>
<td>Maximum, mN/mm^2</td>
<td>7.7 ± 0.3</td>
<td>9.8 ± 0.4*</td>
<td>7.3 ± 0.4*</td>
</tr>
<tr>
<td><strong>Extended concentration-response curve (control)</strong></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>pEC50, M</td>
<td>4.7 ± 0.2</td>
<td>3.5 ± 0.2*</td>
<td>2.9 ± 0.1**†‡</td>
</tr>
<tr>
<td>Maximum, mN/mm^2</td>
<td>16.1 ± 0.3</td>
<td>12.9 ± 0.4*</td>
<td>9.6 ± 0.7**†‡</td>
</tr>
<tr>
<td>Extrapolated maximum, mN/mm^2</td>
<td>16.2 ± 0.2</td>
<td>14.6 ± 0.5*</td>
<td>10.5 ± 1.2**†‡</td>
</tr>
<tr>
<td>Maximum, relative to KCl</td>
<td>2.0</td>
<td>1.3</td>
<td>1.1</td>
</tr>
<tr>
<td><strong>Extended concentration-response curve (+l-NAME)</strong></td>
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<tr>
<td>pEC50, M</td>
<td>4.1 ± 0.1‡</td>
<td>3.2 ± 0.2*</td>
<td>2.8 ± 0.1**†‡</td>
</tr>
<tr>
<td>Maximum, mN/mm^2</td>
<td>13.1 ± 0.7‡</td>
<td>10.5 ± 0.5*‡</td>
<td>8.7 ± 0.7**†‡</td>
</tr>
<tr>
<td>Extrapolated maximum, mN/mm^2</td>
<td>13.8 ± 0.4‡</td>
<td>11.9 ± 0.4*‡</td>
<td>9.9 ± 0.2**†‡</td>
</tr>
<tr>
<td>Maximum, relative to KCl</td>
<td>1.6</td>
<td>1.1</td>
<td>1.0</td>
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Values are means ± SE. *P < 0.05 vs. immature; †P < 0.05 vs. mature; ‡P < 0.05 vs. untreated (control).

DISCUSSION

Adenosine may play a key role in coronary vasoregulation during elevations in energy demand and during and after ischemia or hypoxia (6, 41). We previously showed that maturation is associated with alterations in vascular sensitivity to adenosine (35–37) and observed changes in adenosine levels during postnatal development (37). However, mechanisms underlying these age-related changes and their physiological relevance remain unclear. Moreover, no data exist regarding the relative roles of NO-dependent vs. NO-independent responses in different age groups or regarding the impact of age on the adenosine receptor transduction maxima in vascular tissue. Here we show that adenosine and NECA induce biphasic concentration-dependent coronary dilation in immature, mature, and moderately aged hearts, supporting the existence of multiple adenosine “sites” or receptors. The coronary responses are partially NO dependent, and the age-related decline in response magnitude (but not sensitivity) involves reductions in NO-dependent and NO-independent responses to adenosine.
responses are those acquired in the presence of 50 μM adenosine (A) in aortas from different age groups. NO-independent (L-NAME sensitive) responses for NECA in coronary vessels (B) demonstrate a single adenosine site of the functional response. The physiological significance of this response seems questionable, since the pEC50 corresponds to extracellular adenosine concentrations >0.5 mM, and interstitial or vascular adenosine levels rarely exceed 0.01 mM, except during global myocardial ischemia (19, 49). However, an intracellular receptor would explain low sensitivity to extracellular agonists and would also explain the continued responsiveness of coronary vessels at high agonist concentrations (i.e., nonsaturation of the functional response). The physiological significance of this response seems questionable, since the pEC50 corresponds to extracellular adenosine concentrations >0.5 mM, and interstitial or vascular adenosine levels rarely exceed 0.01 mM, except during global myocardial ischemia (19, 49). However, an intracellular receptor could respond effectively to cytosolic adenosine, potentially stimulating relaxation during periods of vascular deenergization.

**Multiple adenosine sites in coronary vessels.** Adenosine appears to mediate coronary dilation by interacting with two sites or mechanisms possessing quite different sensitivities (Fig. 2, Table 1). A straightforward explanation is that two receptor subtypes with different sensitivities exist in coronary vessels, an interpretation consistent with other recent studies (16, 43). All age groups displayed high- and low-sensitivity responses to adenosine, with pEC50 values of ~5.5 and 2.4, respectively. The pEC50 values for adenosine and NECA at the high-sensitivity site are compatible with adenosine A2 receptors. A2A and A2B subtypes have been localized to coronary vessels, with A2A receptors mediating coronary relaxation in the dog (13), pig (30), and guinea pig (5) and A2B receptors mediating coronary relaxation in humans (26) and in the rat (31). It is therefore likely that the high-sensitivity site reflects activation of adenosine A2B receptors. In contrast, the pEC50 values for adenosine and NECA at the low-sensitivity site are consistent with known affinities at A2 or A3 receptors. A low-affinity P3 receptor such as that reported in aortic smooth muscle (8) could contribute to this response, and there is also evidence supporting an intracellular adenosine receptor in vascular tissue (42). An intracellular receptor would explain low sensitivity to extracellular agonists and would also explain the continued responsiveness of coronary vessels at high agonist concentrations (i.e., nonsaturation of the functional response). The physiological significance of this response seems questionable, since the pEC50 corresponds to extracellular adenosine concentrations >0.5 mM, and interstitial or vascular adenosine levels rarely exceed 0.01 mM, except during global myocardial ischemia (19, 49). However, an intracellular receptor could respond effectively to cytosolic adenosine, potentially stimulating relaxation during periods of vascular deenergization.
bitary properties. Importantly, L-NAME inhibited adenosine responses in immature and moderately aged hearts, eliminating the biphasic nature of the concentration-response curves. However, the age-related decline in response magnitude was preserved. Conversion of the biphasic concentration-response curve to a monophasic curve, with a pEC50 between the pEC50 values for high- and low-sensitivity sites in untreated hearts, indicates that L-NAME inhibits or antagonizes the high-sensitivity site (since it is unlikely that the low-sensitivity site is sensitized by an inhibitor). Moreover, the absence of a detectable low-sensitivity response indicates antagonism by L-NAME, with a rightward shift to concentrations beyond the NECA range examined. The data shown in Fig. 3, coupled with the demonstrated ability of L-NAME to abolish NO-dependent vasodilation (Fig. 5), support mixed NO-dependent and NO-independent responses to adenosine in coronary vessels, with a major role for the NO-dependent response at lower agonist concentrations (Fig. 6). The NO-independent adenosine response is less sensitive than the NO-dependent response (by >10-fold).

Previous studies provide support for endothelium- or NO-dependent components to coronary adenosine responses in the guinea pig (28), dog (52), and pig (22). On the other hand, studies in human and porcine tissues support mixed NO-dependent and NO-independent mechanisms of adenosine-mediated coronary dilation. Contradictory observations within a single species (22, 26) demonstrate a need for further research. One complicating factor, demonstrated here, is that age markedly reduces the magnitude of the NO-dependent response (Fig. 6). Thus studies performed in tissue from older subjects may not reveal significant NO-mediated adenosine responses.

Mechanisms contributing to age-related reductions in the vascular adenosine response. There are three basic ways by which age might reduce the vascular response to adenosine. First, recruitment of different receptor subtypes may extend the response range if these receptors have different affinities. This first possibility is consistent with the apparent existence of two coronary adenosine receptors (a high-affinity A2A receptor and a low-affinity receptor of unknown identity), as discussed above, and with the observation that age significantly reduces the magnitude of the high-sensitivity response (Table 2). A second possible mechanism involves a change in the relative roles of different effector mechanisms. Effects of L-NAME in coronary and aortic tissues support mixed NO-dependent and NO-independent adenosine responses (Figs. 3 and 4) and demonstrate that the magnitude of both components declines with age (Fig. 6). A decline in NO-dependent relaxation is consistent with previous studies demonstrating reduced NO-dependent vasodilation with aging (3, 24) and is consistent with our observation of an age-related decline in the response to ACh (Fig. 5A). Thus NO-mediated vasodilation appears to be generally impaired with aging. The age-related decline in the L-NAME-insensitive (i.e., NO-independent) adenosine response contrasts with observations of unaltered or increased responses to endothelium-independent dilators (23, 43) and with our observation of an unaltered response to sodium nitroprusside (Fig. 5B). Our data therefore support a selective age-related inhibition of the NO-independent adenosine response together with an age-related decline in the NO-dependent response (Fig. 6).

The third way in which age might reduce the adenosine response is via a reduction in the ability of activated adenosine receptors to induce measurable tissue responses. Thus, as tissues age, vascular responses may only be evident at higher fractional receptor occupancies. To assess the potential role of a change in the efficiency of adenosine receptor transduction, we compared unconstrained adenosine response maxima in tissues from all age groups. Specifically, we employed functional antagonism to remove the constraint that the level of preconstriction normally imposes (i.e., 100% relaxation of the preimposed tone), as described in detail by Lew (29). The range of the resultant extended concentration-response curves reflects the agonist efficacy or receptor transduction maximum. Because we examine the same agonist in the same tissue at different ages, the response ranges reflect changes in receptor transduction maximum. Our data show that age consistently reduces aortic sensitivity and peak response magnitude for adenosine (Fig. 4, Table 3), indicating a decline in the ability of adenosine receptors to induce vascular relaxation in older tissue. Thus older tissues are not only less sensitive, but they also display a lower intrinsic ability to relax at peak levels of receptor activation. Adenosine receptors in immature vessels can therefore produce a greater vasodilatory stimulus at lower fractional occupancies than receptors in mature or moderately aged vessels.

Conclusions. The present study reveals the presence of multiple sites or receptors mediating vasodilation to adenosine in coronary vessels and demonstrates significant maturational and aging-related reductions in the adenosine response. The high-sensitivity adenosine response may be mediated by A2B receptors, whereas the identity of the low-sensitivity site remains obscure. Effects of L-NAME indicate that adenosine mediates vasodilation via NO-dependent and NO-independent mechanisms in coronary and aortic tissues. Age-related reductions in adenosine-mediated vasodilation involve changes in both of these responses and may also involve an age-related reduction in the intrinsic ability of vascular tissue to respond to adenosine receptor activation. These alterations in adenosine receptor-mediated responses may impact significantly on vascular function and coronary flow regulation with age.
REFERENCES

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