Adenosine preconditions against endothelin-induced constriction of coronary arterioles

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Merkus, Daphne, David W. Stepp, Deron W. Jones, Yasuhiro Nishikawa, and William M. Chilian. Adenosine preconditions against endothelin-induced constriction of coronary arterioles. Am J Physiol Heart Circ Physiol 279: H2593–H2597, 2000.—Myocardial hypoperfusion is accompanied by concomitant increases in adenosine and endothelin-1 (ET-1) production, but the vasodilatory effect of adenosine prevails over that of ET-1. Therefore, we hypothesized that adenosine-induced or ischemic preconditioning reduces the vasoconstrictive effect of ET-1. Coronary arteriolar diameter in vivo was measured using fluorescence microangiography in anesthetized open-thorax dogs. ET-1 (5 ng·kg⁻₁·min⁻¹ administered intracoronary, n = 10) induced progressive constriction over 45 min [25 ± 6% (SE)]. The constriction was blocked by preconditioning with adenosine (25 μg·kg⁻¹·min⁻¹ administered intracoronary) for 20 min and 10 min of washout (n = 10) or attenuated by ischemic preconditioning (four 5-min periods of ischemia, 9 ± 5% at 45 min). To investigate the receptor involved in this process, coronary arterioles (50–150 μm) were isolated and pressurized at 60 mmHg in vitro. The ET-1 dose-response curve (1 pM–5 nM) was rightward shifted without preconditioning with adenosine (1 μM) for 20 min and 10 min of washout (n = 11). Blockade of A₂ receptors [8-(3-chlorostyryl)caffeine, 1 μM, n = 9] but not A₁ receptors [8-cyclopentyl-1,3-dipropylxanthine, 100 nM, n = 7] prevented this shift. These results suggest that adenosine confers a vascular preconditioning effect, mediated via the A₂ receptor, against endothelin-induced constriction. This effect may offer a new protective function of adenosine in preventing excessive coronary constriction.

 METHODS

In Vivo Coronary Microvascular Preparation

Twenty-six adult mongrel dogs (5–15 kg) were anesthetized with pentobarbital sodium (Nembutal, 30 mg/kg iv). The animals were placed on a homeothermic blanket to maintain a core body temperature of 37°C. The right femoral artery and vein were cannulated for measurements of aortic pressure and arterial blood gases and the administration of drugs, respectively. A 5-F fluid-filled catheter was advanced into the left ventricle from the left carotid artery to measure left ventricular pressure. The first derivative of the left ventricular pressure signal (LV dP/dt) was obtained by an online differentiator. A snare was placed around the vena cava to maintain aortic pressure constant by regulating venous return. Hemodynamic data were acquired continuously using ACODAS data acquisition software (DATAQ, Akron, OH). A tracheotomy was performed, and high-frequency jet ventilation was used. With the use of the maximum LV dP/dt as a timing reference, a solenoid connected to a pressure source (100% O₂, 6–12 psi) was triggered to open for 20–35 ms at the same time in each cardiac cycle. The small tidal volume minimizes respiratory movement, which occurs at the same frequency as the heartbeat. Arterial pH and blood gases were monitored frequently and maintained within the following ranges by adjustment of the tracheal catheter or by administration of sodium bicarbonate: 25–40 mmHg Pco₂, 100–200 mmHg PaO₂, 7.35–7.45.
mmHg $P_{O_2}$, pH 7.35–7.45. All animals routinely received propranolol (1 mg/kg), indomethacin (10 mg/kg), and the H$_2$-histamine receptor antagonist diphenhydramine (1 mg/kg) to limit tissue motion, reduce inflammatory reactions, and prevent anaphylactic reactions to the high-molecular-weight dextrans, respectively.

To visualize the epicardial surface, the heart was exposed by a left thoracotomy at the fifth intercostal space and stabilized in a partial pericardial cradle. A large coronary artery (left anterior descending coronary (LAD) or circumflex) was exposed, and a 24-gauge cannula was inserted to allow the measurement of coronary arterial pressure and the administration of intracoronary drugs and fluorochromes. For ischemic preconditioning, a snare was placed around a marginal or diagonal branch, which then was occluded to obtain ischemic preconditioning (see Protocols). After an area of easily visible epicardial microvessels was identified, four 22-gauge pins were passed horizontally through the left ventricle to minimize vertical cardiac motion. Neither maneuver appears to compromise coronary tone, because resting blood flow and vasodilator reserve are unaffected in each case (2).

**Measurement of Coronary Microvascular Diameter**

To measure coronary microvascular diameter, the cardiac surface was illuminated by a stroboscope (100-W Xen arc, Chadwick-Helmuth, El Monte, CA) that was triggered by the maximum LV dP/dt signal to flash once for 20–30 µs at the same point during each cardiac cycle. The strobe trigger signal was monitored in relation to left ventricular pressure for a precise determination of the strobe's position in the cardiac cycle. The combined use of low tidal volume jet ventilation and brief epicardial illumination both synchronized to the cardiac cycle causes the surface coronary microvessels to appear virtually motionless when viewed through an intravital microscope (Leitz Plomopak, Wild Leitz (2). The microscope objectives used were the Leitz EF4 ($\times 4$, NA 0.22) and the Leitz L10 ($\times 10$, NA 0.22).

To illuminate the inner diameters of the microvessels, 50- to 100-µl aliquots of FITC-dextran (25 mg/ml, mol wt 500,000) in 0.9% saline were injected through the coronary cannula. A Leitz H2 excitation-barrier filter was used to activate the fluorescein and receive the emitted light. Each injection causes arterial and venous vessels to fluoresce sequentially for 5–10 s. The anatomic landmarks of a particular vessel were identified, and five to eight images were obtained over a period of <1 min by using a Cohu silicone-intensified tube videocamera. The images were digitized directly from the camera by a frame digitizer (Scion Image) and transferred to a Macintosh computer (Apple Computer, Cupertino, CA) for diameter measurements using appropriate software (Image 2.18, National Institutes of Health Research Services Branch) (2). Diameters were measured by aligning cursors at the vessel edges. Measurements in pixels were converted to micrometers using a conversion factor determined from a micrometer grid. Typically, microvascular measurements over each image acquisition period vary by less than ±3% from the average value. Vessels were excluded from analysis if control microvascular diameters after interventions varied from the prior baseline by >10%.

**Protocols**

**Endothelin alone.** After acquisition of baseline data, endothelin (5 ng/kg/min administered into the heart) was infused for 45 min. Diameter measurements (10 vessels in 5 dogs, average diameter $112 \pm 13$ µm) and hemodynamic data were obtained at 15, 30, and 45 min.

**Endothelin with adenosine.** After acquisition of baseline measurements, adenosine (25 µg·kg$^{-1}$·min$^{-1}$) was infused for 20 min and then washed out for 10 min, during which diameter returned to control (10 vessels in 4 dogs, average baseline diameter $111 \pm 20$ µm). Vessels were excluded from analysis if diameter did not return to within 10% of the control diameter ($n = 4$). Endothelin-1 was then infused, and diameter measurements and hemodynamic data were obtained at 15, 30, and 45 min.

**Endothelin after ischemic preconditioning.** After acquisition of baseline measurements, a marginal or diagonal branch was occluded for 5 min and then reperfused for 5 min. This occlusion–reperfusion sequence was repeated three more times, then the occlusion was released and reperfusion was allowed for 20 min, during which diameter returned to baseline (10 vessels in 5 dogs). Vessels were excluded from analysis if diameter did not return to within 10% of the control diameter ($n = 2$). Endothelin-1 was then infused, and diameter measurements and hemodynamic data were obtained at 15, 30, and 45 min.

In preliminary experiments we found that endothelin-1 infusion caused a 20- to 30-mmHg increase in left ventricular and systemic pressure. Since changes in these pressures affect the vasculature, we maintained systemic pressure at baseline values by constriction of the inferior vena cava during administration of endothelin-1.

**Isolated Arterioles**

Coronary arterioles were isolated as previously described (11) and placed in ice-cold physiological saline solution composed of (in mM) 145.0 NaCl, 4.7 KCl, 2.0 CaCl$_2$, 1.17 MgSO$_4$, 1.2 Na$_2$PO$_4$, 5.0 glucose, 2.0 pyruvate, 0.02 EDTA, and 3.0 MOPS, buffered to pH 7.4 at 4°C and filtered (dissection buffer). A section of the myocardium was placed under a dissection microscope in a 4°C chamber, and vessels were carefully dissected free from the surrounding myocardial tissue and placed in dissection buffer containing 1% BSA. The vessels were cannulated on both ends with micropipettes (~50 µm OD) connected to pressurized reservoirs filled with physiological saline solution buffered at pH 7.4 at 37°C. The height of these reservoirs was varied to obtain the desired intraluminal pressure (60 mmHg). Vessels that failed to maintain pressure were excluded from analysis. Internal diameter of coronary microvessels was measured using a charge-coupled device camera (Sony CCD-IRIS) using a video caliper system. The vessel was slowly warmed to 37°C and allowed to develop spontaneous tone.

**Protocol**

Diameter changes in response to endothelin-1 (10$^{-12}$–10$^{-8}$ M) were measured in one group of vessels ($n = 9$, average diameter 111 ± 9 µm). The second group of vessels ($n = 11$, average diameter 94 ± 9 µm) was preconditioned with adenosine (1 µM) for 20 min and then subjected to 10 min of washout, during which the vessel diameter returned to control. Subsequently, the response to endothelin-1 (10$^{-12}$–10$^{-8}$ M) was measured. To identify the adenosine receptor involved in the effect of adenosine, vessels were incubated with the A$_1$-receptor antagonist 8-cyclopentyl-1,3-dipropylxanthine (DPCPX, 10$^{-7}$ M, $n = 7$, average diameter 81 ± 8 µm) and the A$_2$-receptor antagonist 8-(3-chlorostyryl)caffeine (CSC, 10$^{-6}$ M, $n = 9$, average diameter 98 ± 11 µm) together with adenosine, DPCPX and CSC were washed out, and endothelin-1 was administered. Responses of the arterioles to the various treatments are expressed as percentage of resting diameter.
Drugs

All drugs except indomethacin were dissolved in 0.9% saline solution. Indomethacin was dissolved in 95% ethanol and raised to pH 8.5 with 1 NaOH. The final concentration was diluted to 10 mg/ml with 0.9% saline. Propranolol, indomethacin, endothelin-1, DPCPX, CSC, and all buffer components were purchased from Sigma Chemical. Adenosine was purchased from Research Biochemicals.

Statistics

All statistics were performed on StatView software for the Macintosh (Abacus Concepts, Berkeley, CA). Differences in vascular diameter were evaluated by ANOVA with Fisher’s test as the post hoc multiple comparison test. Values are means ± SE. Significance was accepted at $P < 0.05$ in all experiments.

RESULTS

Effect of Endothelin-1 on Hemodynamics

To eliminate any complicating consequences of acute endothelin-induced hypertension in our study, we maintained aortic pressure constant by reducing venous return with a snare around the vena cava during endothelin-1 administration (Table 1).

Effect of Adenosine-Induced and Ischemic Preconditioning on Endothelin-1-Induced Constriction In Vivo

Administration of endothelin-1 resulted in progressive constriction of the arterioles over the duration of the experiment (25.1 ± 6.2% after 45 min of infusion; Fig. 1). Adenosine induced dilation of the arterioles (11 ± 4%), but after washout, diameter returned to its baseline value (diameter within 2% of first measurement). This “adenosine” preconditioning eliminated the constriction to endothelin-1 over the entire time course (1.1 ± 5.7% constriction after 45 min of infusion). Because adenosine is released during myocardial ischemia, we investigated whether ischemic preconditioning would mimic the effects of adenosine. Indeed, ischemic preconditioning significantly reduced the constriction to endothelin-1 (8.7 ± 4.8% constriction after 45 min of infusion).

Effect of Adenosine on Endothelin-1-Induced Constriction In Vitro

Endothelin-1 dose-response curves are shown in Fig. 2. Endothelin-1 caused dose-dependent constriction at >100 pM. Administration of adenosine caused dilation of the arterioles, but after washout, basal tone returned within 10 min. After this treatment, subsequent constriction to endothelin-1 was attenuated ($P < 0.05$), as illustrated by the rightward shift of the endothelin-1 dose-response curve. Since coronary microvessels possess $A_1$ and $A_2$ receptors, we investigated which receptor was responsible for this effect. Incubation of the vessels with an $A_2$-receptor antagonist, but not with an $A_1$-receptor antagonist, prevented the adenosine-induced shift of the endothelin-1 dose-response curve. Since coronary microvessels possess $A_1$ and $A_2$ receptors, we investigated which receptor was responsible for this effect. Incubation of the vessels with an $A_2$-receptor antagonist, but not with an $A_1$-receptor antagonist, prevented the adenosine-induced shift of the endothelin-1 dose-response curve.

Table 1. Hemodynamic data

<table>
<thead>
<tr>
<th></th>
<th>Aortic Pressure, mmHg</th>
<th>Heart Rate, beats/min</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>0 min</td>
<td>45 min of ET-1</td>
</tr>
<tr>
<td>Control</td>
<td>119 ± 4</td>
<td>114 ± 5</td>
</tr>
<tr>
<td>Adenosine</td>
<td>107 ± 8</td>
<td>112 ± 13</td>
</tr>
<tr>
<td>Ischemic preconditioning</td>
<td>90 ± 6</td>
<td>86 ± 7</td>
</tr>
</tbody>
</table>

Values are means ± SE. ET-1, endothelin-1.
curve. This indicated that the preconditioning effect of adenosine was mediated by $A_2$ receptors.

**DISCUSSION**

We observed in this study that ischemic preconditioning and preconditioning with adenosine reduced the vasoconstrictive effects of endothelin-1. This effect of adenosine was mediated through the $A_2$ receptor. As such, adenosine may act to mitigate the very potent constriction of endothelin-1 during hypoperfusion, when both substances are released. Before discussing the implications of our findings, we first describe some limitations that bear on the results and conclusions.

**Methodological Limitations**

The interstitial adenosine concentration in our ischemic preconditioning experiments is unknown. Measurements of interstitial adenosine during preconditioning yield concentrations in the micromolar range (5). In our experiments, in which we exogenously administered adenosine, we used concentrations in this range. We used an adenosine concentration of 1 $\mu$M in vitro, and we administered 25 $\mu$g·kg$^{-1}$·min$^{-1}$ adenosine in the in vivo experiments. This dose corresponds to a dose of 50–100 $\mu$M (on the basis of an estimated LAD flow of 10–20 ml/min in a 10-kg dog). Although adenosine is rapidly metabolized in vivo, this dose should be sufficient to mimic the concentration of interstitial adenosine during ischemia, as is corroborated by the observation that ischemic preconditioning and adenosine preconditioning yield similar results.

In the in vivo experiments, we administered only one dose of endothelin-1. The in vivo dose of 5 ng·kg$^{-1}$·min$^{-1}$ gives an estimated intracoronary concentration of $\sim 1$–2 nM (on the basis of an estimated LAD flow of 10–20 ml/min in a 10-kg dog). This dose also induces robust constriction in vitro, whereas preconditioning with adenosine almost completely blocked constriction to this dose of endothelin-1 in vitro and in vivo. Although we acknowledge the constraints of drawing conclusions on the basis of a single dose of endothelin-1, our data show that constriction to this potent in vivo dose was completely abolished by preconditioning with adenosine. Furthermore, the seriousness of this in vivo limitation is lessened by the corroborating results of the complete dose-response relationships to endothelin-1 in vitro.

Another limitation of our in vivo results is that we can only measure diameter of epicardial arterioles. Differences between endocardial and epicardial arterioles have been reported but consist mainly of differences in sensitivity rather than different pathways. Studies by other laboratories have shown that the dose of endothelin-1 that we administered decreased coronary blood flow significantly (3); thus it is likely that endocardial and epicardial vessels constrict to endothelin-1 (9). Adenosine and endothelin-1 are agonists that appear to act in coronary resistance vessels (12); thus it is likely that the effect we observed in epicardial vessels can be extrapolated to endocardial vessels as well.

**Implications of the Study**

The exogenous administration of adenosine clearly shows that adenosine plays a role in preventing endothelin-induced constriction. The results of the ischemic preconditioning are more difficult to interpret, because ischemia stimulates the production of many substances, including endothelin-1 and adenosine (10, 15, 22). Endothelin-1 in itself is also able to induce preconditioning against ischemia-induced myocardial injury (21). However, we do not know its role in our experiments. Nevertheless, ischemic preconditioning clearly decreases the sensitivity of the coronary vasculature to endothelin. Adenosine-induced protection against endothelin-1-induced constriction may be an explanation for the observation that endothelin receptor antagonism reduced infarct size in control, but not in preconditioned, hearts (10). We speculate that the reduced susceptibility to endothelin-induced constriction may be beneficial to coronary blood flow. Indeed, several studies have shown that ischemic preconditioning is capable of improving coronary flow to the area at risk during recovery from a prolonged period of ischemia (7, 16), although others fail to find such an effect (4). The different findings may be attributed to different preconditioning and reperfusion protocols.

In cardiac myocytes the preconditioning effect of adenosine appears to be primarily mediated by $A_1$ receptors (14, 17). However, the adenosine $A_2$ receptor predominates in the coronary vasculature (8, 19). Preconditioning of endothelial cells against anoxia and reoxygenation injury in vitro is mediated by activation of this receptor (24). Furthermore, activation of this receptor protects the endothelium against neutrophil adherence during ischemia-reperfusion and may, therefore, protect the coronary vasculature against neutrophil-related damage (6, 20, 23). It is therefore not surprising that the adenosine-induced protection against endothelin-1-induced constriction is also mediated through the $A_2$ receptor. In accordance with our findings, a study by Peng et al. (18) showed that ischemic preconditioning in isolated, perfused hearts is capable of reducing endothelin-1-induced cardiac injury and that this was mediated through calcitonin gene-related peptide and protein kinase C. Since adenosine $A_2$ receptors also activate protein kinase C (13, 24), modulation of protein kinase C may be a uniform way of protecting against endothelin-1-induced constriction.

Taken together, activation of $A_2$ receptors via adenosine protects the vasculature from excessive constriction produced by endothelin-1. We speculate that this preconditioning against constriction may facilitate or restore flow to areas of compromised perfusion.

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REFERENCES


