Myocardial Protective Effects of Adenosine
Infarct Size Reduction With Pretreatment and Continued Receptor Stimulation During Ischemia

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Background. We hypothesized that 1) endogenous adenosine released during ischemia conferred an inherent cardioprotection, and 2) a pretreatment dose of adenosine before ischemia would provide additional protection independent of hemodynamic effects.

Methods and Results. Thirty-six anesthetized New Zealand White rabbits underwent 30 minutes of regional ischemia produced by coronary occlusion followed by 2 hours of reperfusion. The adenosine group (ADO, n=9) received a 5-minute pretreatment infusion of 140 μg/kg/min of adenosine before ischemia. A control group (SAL, n=9) received saline before ischemia. To separate the effects of adenosine used as a pretreatment versus the effects during ischemia, a third group (ADO+SPT, n=9) received adenosine as pretreatment followed by 10 mg/kg 8-p-sulphophenyl theophylline (8-SPT), an A1/A2-receptor antagonist given before ischemia, thus allowing pretreatment with adenosine but antagonizing its effects during ischemia. To preclude any protection from endogenous adenosine released during ischemia, the fourth group (SAL+SPT, n=9) received saline as pretreatment and 8-SPT before ischemia. Area of necrosis within the area at risk (infarct size) was determined with tetrazolium and Evans blue stains, and transmural blood flow was measured using radioactive microspheres. Collateral blood flow in the area at risk was similar in all groups, as was the size of the area at risk. Infarct size was reduced by adenosine pretreatment (ADO, 8.4±7.2%) in contrast to saline vehicle (SAL, 27.8±6.3%; p<0.05 versus ADO). α1/α2-Receptor blockade after adenosine pretreatment abolished the ischemic protection provided by pretreatment adenosine (ADO+SPT, 42.7±8.3%; p<0.05 versus ADO). Finally, receptor blockade of endogenously released adenosine without adenosine pretreatment increased infarct size by 24% over the nonpretreated saline group (SAL+SPT, 51.5±9.0%; p<0.05 versus SAL).

Conclusions. We conclude that 1) endogenous adenosine building up during ischemia is cardioprotective, and 2) pretreatment with adenosine confers cardioprotection independent of hemodynamic effects. Whether pretreatment effects of adenosine subsequently modulate the effects of endogenous adenosine (through alterations in receptor population or sensitivity) or endogenous and exogenous adenosine represent additive compartments is unclear. (Circulation 1992;86:986–994)

Key Words • preconditioning • reperfusion injury • myocardial blood flow • sulphinyl theophylline

Myocardial infarct size can be reduced by prior brief periods of coronary artery occlusion, an intervention that has been termed “ischemic preconditioning.”1-3 Ischemic preconditioning reduces the extent of necrosis resulting from a subsequent, more prolonged coronary occlusion in comparison to the infarct produced by prolonged occlusion only. Studies have investigated several potential mechanisms of preconditioning, which include high-energy phosphate depletion and washout of metabolites4,5 as well as the synthesis of heat shock protein. However, it has been found recently that the stimulation of adenosine receptors during preconditioning before ischemia may be the mechanism responsible for infarct size reduction.6 Furthermore, pharmacological stimulation of adenosine A1-receptors using selective adenosine analogues has produced infarct size reduction analogous to that seen with ischemic preconditioning7,8; therefore, adenosine may be initiating a protective mechanism before the ischemic insult that is propagated throughout ischemia and reperfusion.

Alternatively, the myoprotective effect of adenosine pretreatment may occur during two phases: 1) via receptor stimulation initiated with adenosine pretreatment and propagated throughout ischemia or additive effects of receptor stimulation during pretreatment and 2) during ischemia when adenosine is released endogenously. We hypothesized first that pretreatment with intravenous adenosine before ischemia could limit myocardial infarct size by receptor-mediated events and...
thus replicate the benefits found with ischemic preconditioning. Second, we hypothesized that endogenous adenosine conferred an inherent receptor-mediated protection that would be additive to any pretreatment adenosine. We used an in vivo rabbit model of 30 minutes of regional ischemia and 2 hours of reperfusion to study infarct size. To partition the potential actions of adenosine into a pretreatment interval (exogenously added) and an ischemic interval (endogenously released), we used the adenosine receptor antagonist 8-p-sulphophenyl theophylline (8-SPT) to antagonize adenosine receptors or displace adenosine from receptors during the ischemic period.

**Methods**

**Surgical Preparation**

Male New Zealand White rabbits (4–5 kg) were anesthetized with an intramuscular injection of ketamine HCl (35 mg/kg) and xylazine (6 mg/kg). Adequate depth of anesthesia was ensured before any surgical procedures by the absence of pedal and palpebral reflexes. Subsequent doses of ketamine-xylazine (12 mg/kg and 2 mg/kg, respectively) were administered as necessary to maintain surgical anesthesia. Positive-pressure ventilation was established via a tracheostomy with a 3.0-mm internal diameter tracheal tube connected to a volume-cycled respirator (Edco Scientific; Chapel Hill, N.C.), which was supplied with oxygen-enriched room air. The tidal volume was set for approximately 10 ml, and the respiratory rate was adjusted between 30 and 40 cycles per minute to maintain the carbon dioxide tension between 35 and 40 mm Hg. Positive end-expiratory pressure (3 cm H2O) was applied to the expiratory limb of the respirator to reduce atelectasis. The right femoral artery and vein were cannulated for arterial blood pressure monitoring (Gould Instruments; Oxnard, Calif.) and fluid administration. ECG leads were attached to subcutaneous electrodes to monitor limb lead II.

The chest was opened by median sternotomy, and the pericardial sac was incised to expose the heart. A high-fidelity transducer-tipped catheter (Millar Instruments; Houston, Tex.) was introduced into the left ventricular (LV) cavity through a small puncture wound in the apex. The position of the catheter was confirmed by transducing a LV pressure waveform and secured in place by a purse-string suture. A small polyethylene catheter was inserted into the left atrium for administration of radiolabeled microspheres. A prominent marginal branch of the left circumflex coronary artery was identified, and a 4-0 silk suture on a tapered needle was passed around the vessel twice to allow reversible occlusion of the coronary artery. The animals were then systemically heparinized with 1,500 units of sodium heparin. A small temperature probe (Shiley; Irvine, Calif.) was positioned between the posterior wall of the heart and the pericardium to ensure that thoracic temperature remained between 38.0° and 39.0°C.

**Experimental Protocol**

After all surgical procedures had been performed, the animals were randomized into one of four experimental protocols, which are schematically represented in Figure 1. Animals in the adenosine group (ADO group, n=9) received adenosine (Sigma Chemical; St. Louis, Mo.) as a pretreatment before ischemia. Animals in the saline group (SAL group, n=9) received saline infusion before ischemia, thus lacking pretreatment receptor stimulation but allowing for adenosine to act during ischemia. To evaluate the effect of endogenously released adenosine during the ischemic period, two additional groups received 8-SPT (Research Biochemicals; Natick, Mass.), which was administered after pretreatment with either adenosine (ADO+SPT group, n=9) or saline (SAL+SPT group, n=9) but before ischemia, thus allowing pretreatment stimulation of adenosine receptors but antagonizing the effects of adenosine released during ischemia.

Microspheres were administered into the left atrium, and baseline hemodynamic measurements were taken before any experimental manipulations and before the infusion of either adenosine or saline. Next, the animals received a 3-minute intravenous infusion of either 140 μg/kg/min adenosine (ADO and ADO+SPT groups) or 0.9% normal saline (SAL and SAL+SPT groups) at an infusion rate of 2.25 ml/min. At 4 minutes into the infusion period, microspheres were administered, and hemodynamic measurements were taken while the infusion continued. At 5 minutes, the infusion pump was turned off. Animals in all groups were then allowed to stabilize for 10 minutes, at which point repeat measurements were taken after the infusion period. In the two study groups receiving the adenosine receptor antagonist during ischemia (ADO+SPT and SAL+SPT groups), 10 mg/kg of 8-SPT was delivered as a bolus and allowed to circulate for approximately 2 minutes before ischemia. In all four groups, the suture around the coronary artery was then tightened for 30 minutes to produce a zone of regional LV ischemia, which was confirmed by regional cyanosis, dyskinetic movement, and prominent ST segment elevation. At 28 minutes of ischemia, microspheres were administered, and hemodynamic measurements taken. The snare was then released after 30 minutes of ischemia to begin 120 minutes of reperfusion. Reperfusion was readily detected as a rapid reversal of cyanosis in the ischemic segment. Repeat hemodynamic measurements were taken at 15, 60, and 120 minutes of reperfusion. Microspheres, however, were only administered after 120 minutes of reperfusion. After all experimental measurements, animals were given an overdose of pentobarbital.

The hearts were quickly excised and perfused with 300 ml of isomolar phosphate-buffered 1.0% 3,5,5-triphenyl-tetrazolium chloride (TTC) at 80 mm Hg and 37°C through the aortic root to demarcate viable tissue
(brick red stain) and necrotic tissue (pale yellow stain). After TTC infusion, the coronary artery was reoccluded, and the hearts were then perfused through the aortic root with 20 ml of 0.5% Evans blue dye to stain tissue in the normally perfused region. The hearts were then fixed in phosphate-buffered formalin overnight.

All animals were handled in compliance with the "Guide for the Care and Use of Laboratory Animals" published by the National Institutes of Health (NIH No. 80-23, revised 1985). All experiments were performed in an American Association for Accreditation of Laboratory Animal Care–approved laboratory with a protocol that was reviewed and approved by the Institutional Animal Care and Use Committee.

Hemodynamic Measurements

Hemodynamic measurements included mean arterial pressure, heart rate, LV peak pressure, and the maximum of the first derivative of the LV pressure waveform (dP/dt\textsubscript{max}). Rate–pressure product was calculated as the product of heart rate and LV peak pressure.

Myocardial Blood Flow

Transmural myocardial blood flow determination was made in risk (necrotic and ischemic tissue) and nonrisk (nonischemic tissue) zones using 15-\mu m tracer microspheres (3M; Minneapolis, Minn.) and the reference sampling technique as previously described by Heymann et al.\textsuperscript{8} Approximately 300,000 microspheres labeled with \textsuperscript{51}Cr, \textsuperscript{85}Sr, \textsuperscript{141}Ce, \textsuperscript{\textsuperscript{95}}Nb, and \textsuperscript{\textsuperscript{119}}Sn were withdrawn from the source vial, mixed with 1 ml of saline, and injected into the left atrium. Reference samples were withdrawn from the right femoral artery at a constant rate of 3.25 ml/min for a total of 2 minutes. After each reference sampling collection, the rabbits received an equal replacement volume of 6.0% hetastarch in 0.9% sodium chloride (DuPont Pharmaceuticals; Wilmington, Del.).

The whole heart was sliced transversely into five to seven slices of approximately 3 mm in thickness. After the right ventricle was removed, each LV slice was then carefully dissected into risk (including TTC red and pale tissue) and nonrisk (blue tissue) regions by an investigator who was unaware of the particular treatment group. Myocardial tissue in the risk region was further dissected into zones that became necrotic (TTC pale tissue) and zones that were nonnecrotic (TTC red tissue). Very small sections of myocardium that necrosed due to placement of the intraventricular catheter and coronary snare were discarded. The entire regions of interest were weighed and used to determine myocardial blood flow. The tissue and blood reference samples were analyzed for radioactivity in a well-type counter with a 3-in. sodium iodide crystal (LKB Wallac, Turku, Finland). True counts were obtained by correcting total counts for background radiation, spillover counts from overlapping channels, and energy loss caused by sample height. Blood flow was calculated as

\[
\text{tissue flow (in ml/min/100 g) = } \frac{[F] \cdot (C_t/C_b)}{(100/\text{sample weight})}
\]

where \(C_t\) and \(C_b\) are radioactive counts in the 2-minute reference sample and the tissue sample, respectively, and \(F\) is reference sample flow (milliliters per minute).

Tissue blood flow data were analyzed selectively by anatomical location and staining morphology using an interactive computer program developed in our laboratory. To minimize error in calculating collateral blood flow during ischemia in samples containing less than 400 microspheres,\textsuperscript{9} total counting time was extended to 2 minutes, and each sample was counted twice and averaged.

Determination of Infarct Size

The mass of nonrisk, nonnecrotic, and necrotic myocardial tissues was determined gravimetrically in tared vials. Area of necrosis (An) was determined to be the weight of necrotic (TTC pale) tissue. Area at risk (Ar) was calculated as the sum of the weights of necrotic and nonnecrotic (TTC red) tissue in the risk region. Nonischemic region was determined to be the weight of nonrisk (blue) tissue. Total LV weight was calculated as the sum of all LV tissue samples. Area at risk expressed as a percent of the entire left ventricle (Ar:LV) was calculated as (weight of nonnecrotic and necrotic tissue in the risk region divided by weight of entire LV) \times 100.

Area of necrosis expressed as a percent of risk region (An:Ar) was calculated as (weight of necrotic tissue in the risk region divided by weight of risk region) \times 100.

As a validation of the gravimetric determination of infarct size relative to planimetric determination of infarct size, we performed additional experiments (\(n=5\)) where infarct size was quantified using both gravimetric and planimetric analysis. Before heart samples were dissected for gravimetric analysis, each LV heart slice was measured for thickness and photographed in color on both sides before tissue dissection. Each slice was photographed next to an image of known area as a reference, thus allowing precise determination of actual area regardless of the magnification of the photograph. The photographs were then enlarged, and the nonischemic, nonnecrotic, and necrotic regions on the top and bottom surface of each slice were analyzed with a computer-driven digitizing tablet (Jandel Scientific; Sausalito, Calif.). The summed areas of each slice (top and bottom) by region were multiplied by slice thickness to estimate nonischemic, ischemic, and necrotic regions in three dimensions. The sums of all slices (per experiment) were used to calculate area of risk as a percentage of the LV (Ar:LV) and area of necrosis normalized for the area at risk (An:Ar).

Criteria for Inclusion

Animals were included for further data analysis when 1) coronary occlusion produced severe ischemia, which we defined as >90% reduction in coronary blood flow in the risk region, and 2) severe hypotension or ventricular fibrillation was not encountered.

Statistical Analysis

All data were analyzed using Statistical Analysis Software for the personal computer (SAS Institute; Cary, N.C.). Comparison of infarct size data, including Ar, An, and An:Ar group means were made by one-way ANOVA. Statistical significance for myocardial blood flow data in the risk and nonrisk zones was detected using two-way ANOVA corrected for multiple comparisons across time.\textsuperscript{10} Hemodynamic measurements were
compared using two-way ANOVA corrected for multiple comparisons across time. All data are reported as group means and standard deviations and were considered statistically significant at a probability value less than or equal to 0.05.

Results

The results reported represent data for 36 of a total of 46 completed experiments. Six experiments were excluded because of incomplete ischemia (blood flow in the area at risk was reduced by <90%). Furthermore, four additional experiments failed due to ventricular fibrillation during ischemia or upon reperfusion; however, the incidence of fibrillation was not related to a specific experimental group (incidence: SAL+SPT group, 2; SAL group, 1; and ADO group, 1).

Hemodynamic Data

Hemodynamic data for the four experimental groups are shown in Figure 2. Mean arterial pressure (Figure 2A) did not significantly differ among groups at the preinfusion time point. However, during infusion, ADO and ADO+SPT groups did show a significant reduction in mean arterial pressure compared with saline control groups (SAL and SAL+SPT groups). Hypotension associated with the 5-minute adenosine infusion was transient, as mean arterial pressure in both ADO and ADO+SPT groups had returned to preinfusion values 10 minutes later at the postinfusion time point. Mean arterial pressure was comparable between all groups during ischemia as well as during reperfusion (15, 60, and 120 minutes of reperfusion).

Heart rate (Figure 2B) was not different among groups at the preinfusion time point. During the infusion period, both ADO and ADO+SPT groups experienced a significant reflex elevation in heart rate in comparison with their saline controls. However, no group differences were detected at the postinfusion time point with values that were not significantly different from the preinfusion values. Group means for heart rate during both ischemia and throughout the reperfusion period (15, 60, and 120 minutes of reperfusion) were not significantly different from each other. We did not detect any significant group differences in LV peak pressure (Figure 2C), dP/dtMAX (Figure 2D), or Rate-pressure product (Figure 2E) during the entire study.

Myocardial Blood Flow Data

Transmural myocardial blood flow values for the four groups are presented in Figure 3 for nonrisk (panel A) and risk (panel B) regions. In the nonrisk region, there were no significant differences among groups at the preinfusion measurement. However, during the infusion period, myocardial blood flow increased significantly in those groups receiving adenosine infusion (ADO and ADO+SPT groups) relative to animals that received saline (SAL and SAL+SPT groups). At the postinfusion time point, transmural myocardial blood flow in the ADO and ADO+SPT groups had returned to levels that were not different from the preinfusion values. Although there were no significant differences among the groups during ischemia, myocardial blood flow had increased slightly over postinfusion values, but this was not statistically significant. At 120 minutes of reperfusion, no significant differences in nonrisk region blood flow were found among the groups.

In the risk region, we did not observe a significant difference among groups at the preinfusion time point. During the infusion of adenosine, we found a significant increase in myocardial blood flow (ADO and ADO+SPT groups) relative to those receiving saline (SAL and SAL+SPT groups). These effects were transient in the risk region as well, because at the postinfusion measurement, myocardial blood flow had returned to values comparable to those at the preinfusion period. During ischemia, transmural blood flow in all groups (n=36) was reduced by an average of 93.2% (from 106.60±33.69 after infusion to 7.15±6.21 during ischemia, p<0.05) without any significant difference among groups. Therefore, collateral blood flow was similar among all four groups. However, at 120 minutes of reperfusion, transmural blood flow in the ADO+SPT and SAL+SPT groups was significantly reduced in comparison with groups that did not receive 8-SPT; these data correspond to the groups with the largest infarcts and are most likely related to the reduction in flow in necrotic tissue.

Average myocardial blood flow during ischemia in nonrisk regions and necrotic and necrotic tissue in the risk region are reported in Table 1 and show the distribution of blood flow during ischemia within each staining region. Regional myocardial blood flow was significantly higher in the nonrisk region (not involved in coronary occlusion) compared with the area at risk. Blood flow in the TTC-red risk region was higher than necrotic area at risk, with marginal significance (p=0.096). Transmural blood flows were calculated from an average of 4,263±3,114 microspheres in the nonischemic zone and 234±384 microspheres in the area at risk.

Validation of Gravimetric Versus Planimetric Infarct Sizing

Data from additional experiments (n=5) were used to validate gravimetric determination of area-at-risk infarct size. Regression analysis of gravimetric Ar:LV versus planimetric Ar:LV resulted in a correlation coefficient of 0.826. Similarly, regression analysis of gravimetric An:Ar against planimetric An:Ar yielded a correlation coefficient of 0.874. The accuracy of infarct sizing by the gravimetric technique can subsequently be corroborated by the sharp differentiation of regional myocardial blood flow in each of the various tissue regions (Table 1).

Infarct Size

Myocardial tissue weights for the entire LV, the area at risk, and necrotic myocardium are presented in Table 2. Expressed as a percentage of the LV, myocardium in the area at risk was not different among groups and averaged 27.0±9.9% in the ADO group, 27.2±5.6% in the SAL group, 29.1±8.9% in the ADO+SPT group, and 25.4±6.9% in the SAL+SPT group (p=0.80).

Myocardial necrosis expressed as a percentage of the area at risk (An:Ar) for each group is shown in Figure 4. Pretreatment with adenosine reduced necrosis compared with saline control animals (ADO versus SAL group, p<0.05). When pretreatment with adenosine was followed with adenosine receptor antagonism during
ischemia, the resulting infarct was 32% greater than the ADO group and 12% greater than the SAL group (p<0.05 for both comparisons). The greatest infarct size was observed when pretreatment adenosine was absent and endogenous adenosine receptor stimulation was blocked (SAL+SPT). Blockade of endogenous adenosine receptor activation in the group increased infarct size by 24% compared with the SAL group in which endogenous adenosine was allowed to activate receptors.

**Discussion**

In the present study, we hypothesized that the cardioprotective effects of adenosine could be divided into two temporal compartments: 1) the pretreatment phase and 2) the ischemia–reperfusion phase when endogenous adenosine is presumably released by the myocardium. We found that a brief pretreatment with adenosine preceding the ischemic event significantly reduced infarct size compared with the vehicle group in which endogenous adenosine during ischemia was unblocked. Pretreatment with adenosine and subsequent blockade of the adenosine receptors during ischemia–reperfusion (ADO+SPT group) increased infarct size by 32% compared with the unblocked pretreatment group. The receptor-mediated cardioprotection of pretreatment adenosine may have been initiated during the pretreatment period, possibly by stimulation of \( \alpha_1 \)-receptor subtypes\(^7,8\) setting in motion events that were then propagated throughout ischemia, the propagation being interrupted by displacement of adenosine from receptors by SPT during the ischemia–reperfusion phase. We cannot, however, rule out that receptors were incompletely blocked by SPT. Alternatively, endogenous adenosine elaborated during ischemia–reperfusion may have contributed cardioprotection independent of and in addition to that conferred by pretreatment. The observation that adenosine receptor blockade in the nonpretreated group (SAL+SPT) significantly increased infarct size by 24% over that in the untreated (SAL) group strongly suggests that endogenous adenosine accumulated during ischemia exerts receptor-me-
diated protection independent of a pretreatment effect. However, the data do not discern whether the protective mechanisms of endogenous adenosine are independent of or modulated by pretreatment receptor activation. Such modulatory interaction could occur via alterations in receptor population, receptor sensitivity, or signal transduction by pretreatment adenosine. Therefore, the data suggest that 1) pretreatment with adenosine before the ischemic event reduces infarct size independent of any hemodynamic effects, 2) the protective mechanisms of pretreatment adenosine are active before ischemia is imposed, and 3) endogenous adenosine accumulating during ischemia confers significant cardioprotection leading to reduction of infarct size.

The work of Thornton et al. first demonstrated that adenosine receptors were involved in mediating the myocardial protection of ischemic preconditioning. Subsequently, studies from the same laboratory documented that infarct size reduction can be gained with pharmacological stimulation of adenosine \( \alpha_1 \)-receptors before ischemia. However, receptor stimulation before ischemia was accomplished with \( \text{N}^\text{6}-\text{1}-(\text{phenyl}-2\text{R}-\text{isopropyl})\text{adenosine} \) (R-PIA), an \( \alpha_1 \)-selective analogue that possesses a long biological half-life. Because R-PIA has a long half-life, it is possible that their studies produced infarct size reduction as a result of \( \alpha_1 \)-receptor stimulation before ischemia as well as ongoing receptor stimulation during ischemia and reperfusion; however, we have produced infarct size reduction using a brief pretreatment with adenosine, which is rapidly degraded.

This suggests that only transient receptor stimulation is required to initiate a more sustained protective mechanism by adenosine pretreatment, or, alternatively, that a more prolonged stimulation of the nonvasodilating \( \alpha_1 \)-receptor subtype is operative.

Interestingly, the study by Liu et al. did not find infarct size reduction in one study group in which intravenous adenosine was used as pretreatment in contrast to the present study. Their suggestion was that adenosine, delivered intravenously, was too dilute in the coronary circulation to stimulate cardiac adenosine \( \alpha_1 \)-receptors, although intravenous adenosine did produce significant \( \alpha_2 \)-mediated hypotension in their study. Although Liu et al. did not measure coronary blood flow in their in situ model, our intravenous infusion of 140 \( \mu \text{g/kg/minute} \) adenosine, which is considerably lower than the dosage used by Liu et al., produced transient but significant decreases in blood pressure (Figure 2A) and large increases in myocardial blood flow (Figures 3A and 3B), thus substantiating adenosine’s distribution to peripheral as well as cardiac sites. Under these conditions, intravenous adenosine infusion produced a significant reduction of infarct size in contrast to the in

**TABLE 1. Myocardial Blood Flow During Ischemia for Each Staining Pattern in all Animals Combined**

<table>
<thead>
<tr>
<th>Myocardial region</th>
<th>Flow (ml/min/100 g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blue (nonrisk)</td>
<td>131.55 ± 40.62*</td>
</tr>
<tr>
<td>Red (risk, nonnecrotic)</td>
<td>8.24 ± 8.13†</td>
</tr>
<tr>
<td>Pale (risk, necrotic)</td>
<td>5.42 ± 6.81†</td>
</tr>
</tbody>
</table>

* \( p < 0.050 \), nonischemic zone vs. ischemic and necrotic zones.
† \( p = 0.076 \), ischemic zone vs. necrotic zone.

**TABLE 2. Myocardial Tissue Weights: Left Ventricle, Area at Risk, and Area of Necrosis**

<table>
<thead>
<tr>
<th>Group</th>
<th>LV (g)</th>
<th>Ar (g)</th>
<th>An (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ADO</td>
<td>4.43 ± 0.78</td>
<td>1.15 ± 0.35</td>
<td>0.09 ± 0.09*</td>
</tr>
<tr>
<td>SAL</td>
<td>4.70 ± 0.52</td>
<td>1.27 ± 0.23</td>
<td>0.42 ± 0.24</td>
</tr>
<tr>
<td>ADO+SPT</td>
<td>4.34 ± 1.19</td>
<td>1.27 ± 0.52</td>
<td>0.55 ± 0.33†</td>
</tr>
<tr>
<td>SAL+SPT</td>
<td>4.63 ± 0.74</td>
<td>1.15 ± 0.30</td>
<td>0.59 ± 0.23†</td>
</tr>
</tbody>
</table>

LV, mass of entire left ventricle; Ar, mass of myocardium in risk region; An, mass of necrotic myocardium.

ADO, group that received adenosine pretreatment before ischemia; SAL, group that received saline before ischemia; ADO+SPT, group that received adenosine as pretreatment followed by SPT (8-SPT, 8-p-sulfophenyl theophylline); SAL+SPT, group that received saline as pretreatment and 8-SPT before ischemia.

* \( p < 0.05 \) vs. SAL, ADO+SPT, and SAL+SPT groups.
† \( p < 0.05 \) vs. ADO and SAL groups.
Figure 4. Bar graph of myocardial necrosis data showing area of necrosis expressed as percentage of the area at risk. ADO, group that received adenosine pretreatment before ischemia; SAL, group that received saline before ischemia; ADO+SPT, group that received adenosine as pretreatment followed by SPT (8-SPT, 8-p-sulfophenyl theophylline); SAL+SPT, group that received saline as pretreatment and 8-SPT before ischemia. *p<0.05 ADO group vs. SAL, ADO+SPT, and SAL+SPT groups. **p<0.05 ADO+SPT group vs. SAL and ADO groups. +p<0.05 SAL+SPT group vs. ADO+SPT, SAL, and ADO groups.

The in situ results of Liu et al. but was consistent with protection they observed in isolated hearts.

In the present study, the combined effect of pretreating the myocardium with adenosine and the unpaired receptor stimulation during ischemia (ADO group) by either propagated effects of pretreatment adenosine or additional effect of endogenous adenosine was most effective in preserving myocardium subjected to ischemia. Thornton et al. showed that adenosine receptor blockade with 8-SPT during an ischemic preconditioning occlusion eliminated infarct size reduction. However, in this study, 8-SPT was given before the ischemic preconditioning occlusion with the intention of blocking adenosine receptors during preconditioning. Used in this way, 8-SPT would have also have blocked receptors during ischemia, thereby suppressing any protective effect that might have been mediated by endogenous adenosine released during ischemia. Adenosine receptor blockade throughout the entirety of their study thereby limits the conclusions that could be made about time period during which the protective effect of adenosine was actually conferred. However, in the present study, we allowed pretreatment receptor stimulation to take place, thereby initiating protection but blocking effects of adenosine during ischemia (in the ADO+SPT group). Myocardial protection was lost with adenosine receptor antagonism during ischemia, thus substantiating that pretreatment adenosine activates cardioprotective mechanisms before ischemia is imposed. The interruption of receptor-mediated events during ischemia suggests that these events are initiated by pretreatment adenosine and propagated during ischemia–reperfusion, or that a second source of adenosine, i.e., endogenous adenosine, contributes an additional component of protection as suggested by Lasley et al. Our data support the significant contribution made by endogenous adenosine released during ischemia–reperfusion.

Potential Mechanisms

Apart from whether adenosine's actions were expressed predominantly during pretreatment or ischemia, adenosine may have had effects on several potential mechanisms of ischemia–reperfusion injury. One possibility is that adenosine infusion may have altered the infiltration or activation of neutrophils in the ischemic bed, thus limiting neutrophil-mediated injury in the groups that received adenosine. We did not evaluate neutrophil activity in the present study and therefore cannot comment on the contribution made by neutrophils in the effects of adenosine we observed. However, previous studies also established that neutrophil inactivation occurs through adenosine a2-receptors on the cell surface. If neutrophil inactivation via a2-receptor-mediated effects were responsible for the infarct size reduction that we saw, then the study by Thornton et al. using an intravenous pretreatment with CGS 21680 (an agonist specific for the a2-receptor), should have produced a protective effect as well. However, this degree of protection did not occur in their study. In addition, the observation that infarct size reduction can be accomplished with a2-selective analogues, which presumably does not affect neutrophils, further argues against the potential for neutrophil involvement in the pretreatment protocol.

Either the brief adenosine infusion or the adenosine released during ischemia could have altered the amount of collateral blood flow to the ischemic bed during coronary occlusion, thus limiting the severity of ischemia, particularly in those groups receiving adenosine as a pretreatment. Our studies were conducted in rabbits, which have been established as a collateral-deficient model. Although we found considerable variability in collateral blood flow, our criteria excluded animals with collateral blood flow exceeding 10% of control blood flow. Myocardial blood flow in the risk region indicates that there was no difference in transmural blood flow to the ischemic bed among groups, and that adenosine pretreatment did not alter the degree of collateral flow in the area at risk. The severity of ischemia and the small sample sizes obtained from the rabbit heart limited the number of microspheres present in the area at risk to less than 400 necessary for 90% accuracy. Therefore, error may have been introduced in measuring collateral blood flow despite efforts to optimize counting statistics. The possibility that infarct size limitation resulted from reduction of the severity of ischemia by recruitment of collaterals in response to a 5-minute infusion of adenosine is not likely.

The reduction of oxygen demand, associated with negative inotropic actions of adenosine, might have limited infarction in animals that received adenosine as pretreatment. We used the rate-pressure product as an index of myocardial oxygen demand, which has been correlated with oxygen demand in working cardiac muscle. Although this index is imprecise compared with directly measured oxygen consumption or to other indexes of oxygen demand (wall stress, pressure–volume area), we did not find any differences in rate–pressure product among groups, suggesting that decreasing global oxygen demand was not the mechanism for protection by adenosine pretreatment. Proper evaluation of such a hypothesis would require a different model, in which regional venous sampling could be used to calculate oxygen consumption directly.
Reduction of the extent of ischemia–reperfusion injury by adenosine may involve the activation of ATP-sensitive K⁺ channels on cardiac myocytes. Kirsch et al. has demonstrated this potential link in isolated cardiac tissue in which the ATP-sensitive K⁺ channel was found to be tightly coupled to the adenosine α₁-receptor by a GTP-binding protein. Their results show that the channel opens (increasing outward K⁺ conductance) when the receptor is stimulated. It has also been shown that anti-ischemic effects are observed with drugs that selectively open the ATP-sensitive K⁺ channel such as pinacidil and cromakalim. These effects were theorized to be related to hyperpolarization of ischemic myocytes. The possibility of an ATP-sensitive K⁺ channel being opened by adenosine receptor stimulation from either exogenous or endogenous sources might be one mechanism of adenosine-mediated protection. A recent report has indeed suggested that activation of K⁺ channels is an important mechanism behind the protective effect of ischemic preconditioning.

Exogenous adenosine administered in a pretreatment regimen and endogenous adenosine released from myocytes during ischemia may have exerted cardioprotection through different receptor subtype–mediated mechanisms. Several studies strongly suggest α₁-receptor activation in cardioprotective actions of pretreatment adenosine. As suggested by Liu et al. and Thornton et al., pretreatment adenosine—which parenterally administered or released secondary to a preconditioning coronary occlusion—may act through α₁-receptor–mediated mechanisms, possibly stimulation of Kₓ, ATP channels. The resulting hyperpolarization would potentially attenuate ischemic injury which would, in turn, attenuate the magnitude of the subsequent reperfusion injury. Antagonism of this process would be consistent with the intermediate infarct size observed in the ADO+SPT group. The prolonged expression of the effects of pretreatment adenosine independent of α₁-mediated hemodynamic (vasodilator) effects may be propagated by occupation of high-affinity α₁-receptors found in the heart. This prolonged receptor occupancy (and presumably stimulation) coupled with attenuation of ischemic severity through Kₓ, ATP channel stimulation would propagate the cardioprotection mechanism through pretreatment and ischemia–reperfusion phase. On the other hand, the additional cardioprotection that was unmasked in the SAL+SPT group may be exerted through α₂-receptor–mediated mechanisms, including inhibition of neutrophil activity, primarily during reperfusion. Direct substantiation of two pharmacologically separate roles of adenosine mediated by different receptor subtypes and working in tandem is currently lacking. However, our data do suggest the functional expression of two temporal compartments of receptor-mediated cardioprotection.

Summary

Our findings demonstrate that a brief period of adenosine infusion before ischemia can significantly reduce infarct size. More importantly, we have shown that this protection is initiated before the ischemic event, with additional protection conferred either by propagation of the pretreatment effect during ischemia–reperfusion or the additive protection of endogenous adenosine accumulated during ischemia. The degree of protection conferred by endogenous adenosine in the untreated and receptor-antagonized group was responsible for a 24% reduction in infarct size. However, whether the preischemic activation of adenosine receptors and subsequent cardioprotection of pretreatment adenosine modulates the effects of endogenous adenosine is not clear.

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