Acadesine and Myocardial Protection

Studies of Time of Administration and Dose–Response Relations in the Rat

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Background. Although there are many factors that might contribute to tissue injury during ischemia and reperfusion, the loss of adenine nucleotides has long been considered to be of importance. This has led to the study of interventions designed to limit the loss of nucleotides or to enhance the rate of nucleotide resynthesis during reperfusion. Alternatively, the breakdown of adenosine triphosphate to adenosine might represent a protective response of the ischemic heart because adenosine is considered an anti-injury autacoid. Augmentation of endogenous adenosine levels might be beneficial. For these reasons, the protective properties of acadesine (AICAr: 5-amino-4-imidazole carboxamide riboside) were assessed in a rat model of myocardial ischemia and reperfusion.

Methods and Results. The protective properties of acadesine were studied in the isolated, perfused rat heart subjected to global hypothermic (20°C) ischemia and reperfusion. When acadesine was given as an in vivo pretreatment (100 mg/kg i.v. 15 minutes before study) followed by being administered as an additive (20 μmol/l) to the St. Thomas’ Hospital cardioplegic solution (single dose) and then as an additive (20 μmol/l) to the initial reperfusion (15 minutes) solution, the recovery of aortic flow after 2.5 hours of ischemia was improved from its control value of 16.5±3.9 ml/min to 28.9±4.1 ml/min (n=8 per group; p<0.05). Similar protection was seen with other indexes of cardiac function. Analysis of hearts obtained at the end of 2.5 hours of ischemia and 35 minutes of reperfusion revealed no significant differences in metabolite content between control and drug-treated hearts with the exception of inosine monophosphate, which was increased from its drug-free control value of 0.10±0.01 μmol/g dry wt to 0.86±0.06 μmol/g dry wt (p<0.05). In further studies (n=8 per group), with multidose (every 30 minutes) cardioplegia and extended periods (6 hours) of hypothermic ischemia, acadesine consistently led to higher mean recoveries of function and lower levels of creatine kinase leakage. Again, the only significant metabolic effect was an increase in tissue inosine monophosphate content. In studies (n=12 per group) to determine whether acadesine was acting before, during, or after ischemia, the drug was given 1) only as pretreatment (100 mg/kg i.v.), 2) only during single-dose cardioplegia (20 μmol/l), or 3) only during reperfusion (20 μmol/l). Significant protection was observed in the first two groups (recovery of aortic flow increased from 10.6±2.6 ml/min in the acadesine-free control to 22.6±2.8 and 23.6±3.1 ml/min, respectively; p<0.05). No significant protection was observed when acadesine was given only during reperfusion. In dose–response studies, acadesine (0, 5, 20, 50, 200, and 1,000 μmol/l; n=12 per group) was given only as a cardioplegic additive; the postischemic recoveries of aortic flow were 15.4±2.8, 16.9±3.6, 29.5±3.8, 27.4±3.8, 26.7±4.2, and 27.1±2.7 ml/min, respectively.

Conclusions. Acadesine improves the ability of the heart to recover from ischemia and reperfusion when administered before ischemia or with cardioplegia. The mechanism underlying the protection remains to be resolved. (Circulation 1992;86:598–608)

Key Words • myocardial preservation • rats • acadesine • ischemia • reperfusion • cardioplegia

The realization that reperfusion as well as ischemia might contribute to tissue injury has stimulated considerable interest in the possibility that certain drugs (administered during ischemia or at the time of reperfusion) might be used to enhance the postischemic recovery of the heart over and above that achieved by reperfusion alone. The advent of thrombolysis has given impetus to the investigation of such a possibility.

Although many factors undoubtedly contribute to tissue injury during ischemia and reperfusion, the importance of the loss of adenine nucleotides has long been debated. This has stimulated the study of interventions that may limit the loss of nucleotides (and their purine degradation products) or enhance the rate of nucleotide resynthesis during reperfusion. In this connection, improvement of the postischemic function has been observed as a consequence of the administration of exogenous adenosine triphosphate (ATP),1,2 adenosine,3–5 inhibitors of the degradation of adenosine,6–10 nucleoside transport inhibitors,9,11 inosine,12,13 and
acadesine (AICAr: 5-amino-4-imidazole carboxamide riboside). However, these and other studies have questioned the role of nucleotide depletion and repletion as a determinant of the postischemic recovery of contractile function. It could be argued that enhanced ATP repletion may be a biochemical marker of improved recovery or cardioprotection rather than the mechanism underlying any functional benefit.

In the case of acadesine, administration before and during regional ischemia in dog hearts has been reported to enhance the adenosine content of blood draining the ischemic myocardium. This effect may be expected to be advantageous, because adenosine has been shown to cause vasodilatation and improve postischemic coronary flow, to decrease neutrophil infiltration into ischemic tissue, and to inhibit free radical production in activated granulocytes, and to inhibit platelet aggregation.

In the present study, we used the isolated rat heart to 1) ascertain whether acadesine could afford protection against the injury sustained during global ischemia and reperfusion, 2) determine whether the drug is most effective when given before, during, or after ischemia, 3) define the dose–response characteristics of any observed protection, and 4) characterize the effect of the drug on purine metabolism.

Methods

Animals and Perfusion Procedures

Male Wistar rats weighing 300–350 g were used. All animals received humane care in compliance with the “Principles of Laboratory Animal Care” formulated by the National Society for Medical Research and the “Guide for the Care and Use of Laboratory Animals” prepared by the National Academy of Sciences and published by the National Institutes of Health (NIH No. 80–23, revised 1978).

Animals were anesthetized with pentobarbital sodium (60 mg/kg i.p.), and acadesine or saline was then administered via the femoral vein. After 15 minutes, sodium heparin (1,000 IU/kg) was administered also through the femoral vein, the chest was opened, and the heart was excised and placed in cold (4°C) saline. The aorta was cannulated and immediately infused for 2 minutes at a constant pressure of 45 mm Hg with the St. Thomas’ Hospital cardioplegic solution (containing in mmol/l: NaCl 110.0, KCl 16.0, MgCl₂ 16.0, CaCl₂ 1.2, NaHCO₃ 10.0; pH 7.8) at 20°C with or without the addition of acadesine. Hearts were then subjected to predetermined periods of hypothermic (20°C) global ischemia, during which they were immersed in the same cardioplegic solution. At the end of each ischemic period, the hearts were reperfused normothermically in the Langendorff mode for 15 minutes with crystalloid perfusion fluid (containing in mmol/l: glucose 11.1, NaCl 118.5, KCl 4.8, MgSO₄ 1.2, KH₂PO₄ 1.2, NaHCO₃ 25.0, CaCl₂ 1.4 at pH 7.4 when gassed with 95% O₂ plus 5% CO₂) at a constant pressure of 75 mm Hg. Before use, all perfusion fluids were filtered through a 5.0-µm porosity membrane to remove any particulate matter. In some studies, hearts also were perfused with acadesine added to the crystalloid solution during the first 15 minutes of reperfusion. During that time, the coronary effluent was collected for the determination of total creatine kinase (CK) leakage. This was followed by a 20-minute period of working (ejecting) perfusion, during which hearts were perfused at a constant filling pressure of 15 mm Hg and the left ventricle was ejected against a hydrostatic pressure equivalent to 75 mm Hg. At the end of the reperfusion period, various indexes of cardiac function (coronary flow, aortic flow, cardiac output, heart rate, peak aortic pressure, stroke volume, and stroke work) were measured. At the end of each experiment, the hearts were freeze-clamped and taken for extraction and metabolite analysis.

Experimental Protocols

Study 1: Effect of acadesine on postischemic functional recovery and metabolite content when administered before, during, and after ischemia. Rats received a bolus (100 mg/kg i.v.) of acadesine (100 mg/ml dissolved in saline) or the same volume of saline 15 minutes before the experiment. Hearts (n=8 per group) from animals pretreated with acadesine also received the drug (20 µmol/l) added to the cardioplegic solution (single dose), which was given immediately before 2.5 hours of hypothermic (20°C) global ischemia. In this study, acadesine (20 µmol/l) was included in the perfusion fluid during the first 15 minutes of the 35-minute reperfusion period.

In further experiments, hearts (n=8 per group) were subjected to an identical protocol, with the exception that the period of ischemia was extended to 6 hours, and multidose cardioplegia, with or without the addition of acadesine (20 µmol/l), was reinfused every 30 minutes.

Study 2: Effect of acadesine on postischemic functional recovery and metabolite content when administered before, during, or after ischemia. The experimental protocol for these studies was similar to that used in study 1. All hearts were subjected to single-dose cardioplegia and 2.5 hours of hypothermic (20°C) ischemia. Four groups of hearts (n=12 per group) were investigated: group 1, pretreated with saline; group 2, pretreated with acadesine (100 mg/kg i.v.) 15 minutes before the experiment; group 3, acadesine (20 µmol/l) added only to the cardioplegic solution; group 4, acadesine (20 µmol/l) added during the first 15 minutes of the 35-minute reperfusion period. Animals in groups 3 and 4 that did not receive acadesine as pretreatment received an equivalent volume of saline.

Study 3: Dose–response studies of acadesine as an additive to the St. Thomas' Hospital cardioplegic solution. The experimental protocol for these studies was identical to that used in study 2, with the exception that neither acadesine nor saline was administered as pretreatment or during reperfusion; instead, acadesine was added to the single-dose cardioplegic solution at different concentrations (0, 5, 20, 50, 200, or 1,000 µmol/l).

Study 4: Metabolic consequences during ischemia of the administration of acadesine. The experimental protocol for these studies was again identical to that used in study 2, with the exception that hearts were not reperfused. Instead, they were freeze-clamped at the end of the ischemic period and taken for the determination of
nucleoside and nucleotide contents. The following groups \((n=6\) per group\) were studied: group A, hearts not subjected to ischemia; instead, they were immediately excised and taken for metabolite analysis without any ex vivo perfusion; group B, hearts subjected to ischemia but without being given acadesine at any stage; group C, animals that received acadesine \((100\ \text{mg/kg i.v.})\) as a pretreatment 15 minutes before study; group D, acadesine \((20\ \mu\text{mol/l})\) added only to a single dose of cardioplegic solution; group E, animals that received acadesine as pretreatment \((100\ \text{mg/kg i.v.})\) and acadesine \((20\ \mu\text{mol/l})\) added to the cardioplegic solution.

**Metabolic Analysis**

The frozen hearts were lyophilized, weighed, and homogenized in 3 ml of perchloric acid \((0.6\ \text{mol/l})\) at 4°C. Each homogenate was then centrifuged at 2,000g for 10 minutes at 4°C. A portion \((0.6\ \text{ml})\) of each supernatant was neutralized by the addition of 0.045 ml potassium carbonate \((5\ \text{mol/l})\), left on ice for 5 minutes, and centrifuged. A sample of the supernatant was passed through a 0.45-μm mesh nylon filter and stored at \(-20°C\) before high-performance liquid chromatography (HPLC) analysis.

Nucleosides and nucleotides were analyzed using a Waters HPLC system comprising two 510 pumps, a 1-ml static mixing chamber, a WISP 712 automated sample injector with refrigeration unit, and a 441 detector set at 254 nmol. Adenine nucleotides (ATP, adenosine triphosphate; ADP, adenosine diphosphate; AMP, adenosine monophosphate) and their degradation products were determined, and the values obtained were used to calculate the following indexes of myocardial energy status:

\[
\text{Adenylate pool} = [\text{ATP} + \text{ADP} + \text{AMP}] \\
\text{Energy charge potential (ECP)} = [0.5 \text{ ADP} + \text{ATP}] / [\text{ATP} + \text{ADP} + \text{AMP}] 
\]

The limits of detection for quantification of spectrally matched metabolites in 50-μl aliquots taken from the 3-ml samples were 5 nmol/g tissue for the nucleotides and 10 nmol/g tissue for the nucleosides.

**Expression of Results and Statistical Analysis**

Coronary flow \((\text{ml/min})\), aortic flow \((\text{ml/min})\), peak aortic systolic pressure \((\text{mm Hg})\), and heart rate \((\text{beats per minute})\) were recorded as previously described;26; cardiac output \((\text{ml/min})\) was calculated by adding aortic flow to coronary flow, stroke volume \((\text{ml per beat})\) was determined by dividing cardiac output by heart rate, and stroke work \((10^5\ \text{dyne} \cdot \text{mm Hg/beat})\) was calculated from stroke volume by peak aortic systolic pressure. CK leakage was expressed as IU/15 min/g dry wt, and tissue metabolites as micromoles per gram of dry weight.

All results are expressed as mean±SEM. The two-tailed, unpaired Student’s \(t\) test was used for comparison between two means. ANOVA was used for comparison of more than two means by using the Tukey test. A difference was considered statistically significant at a value of \(p<0.05\).

**Results**

**Study 1: Effect of Acadesine on Postischemic Functional Recovery and Metabolite Content When Administered Before, During, and After Ischemia**

**Contractile function:** Single-dose cardioplegia study. The in vivo pretreatment of the rats with acadesine or saline had no effect on blood pressure or heart rate measured during the 15 minutes before excision of the heart. The administration of acadesine before, during, and after ischemia resulted in a statistically significant \((p<0.05)\) improvement in the recovery of various indexes of cardiac function after 2.5 hours of hypothermic ischemia (Figure 1, panels A–E). Thus, aortic flow recovered to \(28.9±4.1\ \text{ml/min}\) in the acadesine group compared with only \(16.5±3.9\ \text{ml/min}\) \((p<0.05)\) in the acadesine-free group. These absolute recoveries equate to approximately 47% and 27% of the aortic flow recorded in aerobic hearts that had not been subjected to ischemia (Table 1). Other indexes of cardiac function, such as coronary flow (Figure 1A), cardiac output (Figure 1C), and stroke volume (Figure 1D), were also improved as a consequence of acadesine treatment. Heart rate recovered fully in the acadesine-free and acadesine-treated groups \((254±16\) and \(265±9\) beats per minute, respectively).

**Contractile function:** Multidose cardioplegia study. In these experiments in which multidose cardioplegia was used and the period of ischemia was extended to 6 hours (Figure 2, panels A–E), further evidence of a protective effect was obtained. Thus, aortic flow (Figure 2B) was \(32.3±2.7\ \text{ml/min}\) in the acadesine-free control group but \(42.6±2.5\ \text{ml/min}\) in the acadesine-treated group \((p<0.05)\). It is interesting to note that because of the multiple infusion of cardioplegia, the recovery of function in the control group of the 6-hour ischemia protocol tended to be greater than in the control group of the 2.5-hour ischemia protocol in which only a single dose of cardioplegia was used.

**Enzyme leakage.** In studies with both single-dose and multidose cardioplegia, the leakage of CK tended to be lower in the acadesine-treated groups \((33±5\) and \(18±3\) IU/15 min/g dry wt, respectively) than in the control groups \((48±8\) and \(26±4\) IU/15 min/g dry wt, respectively); however, these improvements did not achieve a level of statistical significance.

**Metabolic studies.** Table 2 shows that, in comparison with aerobic controls, ischemia and reperfusion in the acadesine-free hearts resulted in significant decreases in tissue ADP and ATP contents and in the adenylate pool (AP) and increases in AMP. Acadesine had no effect on the tissue content of any metabolite except that of inosine monophosphate (IMP), which increased to values greater than those seen in aerobic control tissue. ECP at the end of reperfusion was normal in the acadesine-free control and was unaffected by the administration of acadesine.

**Study 2: Effect of Acadesine on Postischemic Functional Recovery and Metabolite Content When Administered Before, During, or After Ischemia**

**Contractile function.** The administration of acadesine, as pretreatment alone or as an additive to single-dose cardioplegia alone, before a 2.5-hour period of ischemia, resulted in comparable and signifi-
cant improvements in postischemic cardiac function (Figure 3, panels A–E). Thus, aortic flow was improved from 10.6±2.6 ml/min in group 1 (acadesine-free) to 22.6±2.8 ml/min in group 2 (acadesine as pretreatment alone) and 23.6±3.1 ml/min in group 3 (acadesine in the cardioplegic solution alone). However, aacadesine given during reperfusion alone (group 4) failed to improve the recovery of aortic flow (10.8±2.6 ml/min; NS). As in the previous study, heart rate was unaffected by the aacadesine treatment (recovering to 244±8, 263±6, 255±9, and 244±10 beats per minute in groups 1, 2, 3, and 4, respectively).

In seeking to assess whether treatment with aacadesine before, during, and after ischemia affords better protection than pretreatment alone or cardio-

plegia alone, it is necessary to compare the results of this study with those of study 1 with single-dose cardioplegia. It should be stressed that these studies were carried out at different times; however, other than the time of drug administration, the protocols were identical. The functional recoveries were similar in all groups. Thus, for example, aortic flow recovered to 22.6±2.8 ml/min in the group with aacadesine administered as pretreatment and to 23.6±3.1 ml/min when aacadesine was given as an additive to single-dose cardioplegia. When aacadesine was given as a pretreatment plus as an additive to single-dose cardioplegia and also during reperfusion (study 1), the recovery was 28.9±4.1 ml/min (NS when compared with the other groups).

**Table 1. Cardiac Function in Aerobic Control Hearts**

<table>
<thead>
<tr>
<th>Study</th>
<th>n</th>
<th>Coronary flow (ml/min)</th>
<th>Aortic flow (ml/min)</th>
<th>Cardiac output (ml/min)</th>
<th>Heart rate (beats per minute)</th>
<th>Stroke volume (ml/beat)</th>
<th>Stroke work (10^6 dynes · mm Hg/beat)</th>
</tr>
</thead>
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<tr>
<td>1</td>
<td>8</td>
<td>22.4±0.9</td>
<td>61.3±1.9</td>
<td>83.7±2.3</td>
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<td>0.32±0.01</td>
<td>45.8±1.7</td>
</tr>
<tr>
<td>3</td>
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<td>85.6±2.3</td>
<td>274±7</td>
<td>0.31±0.01</td>
<td>44.3±2.1</td>
</tr>
</tbody>
</table>

**Figure 1.** Bar graphs show effect of aacadesine on postischemic recovery of function after 35 minutes of reperfusion in hearts subjected to single-dose cardioplegia (2 minutes) followed by 2.5 hours of hypothermic (20°C) global ischemia. Aacadesine was administered as pretreatment (100 mg/kg i.v.) 15 minutes before the experiment, as an additive (20 μmol/l) to the St. Thomas' Hospital cardioplegic solution, and during the first 15 minutes of reperfusion (20 μmol/l). Panel A: Coronary flow; panel B: aortic flow; panel C: cardiac output; panel D: stroke volume; panel E: stroke work. Each column represents the mean of eight hearts; bars indicate SEM. *p<0.05 compared with aacadesine-free control group.
Enzyme leakage. As in study 1, mean CK leakage was reduced when acadesine was used as a pretreatment (group 2, 42±6 IU/15 min/g dry wt) or when acadesine was added to the cardioplegic solution (group 3, 44±9 IU/15 min/g dry wt); however, this reduction was not statistically significant when compared with that in the acadesine-free group (59±7 IU/15 min/g dry wt). CK leakage, when acadesine was present only during reperfusion, was 75±12 IU/15 min/g dry wt, a value that did not differ significantly from that of the acadesine-free controls.

Metabolic studies. As in study 1, the tissue contents of adenine nucleotides, nucleosides, and purines were similar in the acadesine-free group and in the various acadesine-treated groups. However, acadesine caused a significant increase of IMP content when administered as a pretreatment (0.56±0.03 μmol/g dry wt versus 0.16±0.03 μmol/g dry wt in controls; p<0.05) but not when given as an additive to the cardioplegic solution (0.17±0.04 μmol/g dry wt) or during reperfusion alone (0.16±0.03 μmol/g dry wt). ECP recovered to the same extent in all study groups.

Study 3: Dose–Response Studies of Acadesine as an Additive to the St. Thomas' Hospital Cardioplegic Solution

Contractile function. The results in Figure 4 show the effect of the addition of different concentrations of acadesine (0, 5, 20, 50, 200, or 1,000 μmol/l) to the single-dose cardioplegic solution. Taking cardiac output (Figure 4C) as illustrative of the indexes of cardiac function, the addition of 20 μmol/l acadesine resulted in a significant improvement in posts ischemic recovery (46.8±4.1 ml/min versus 30.2±3.1 ml/min in the acadesine-free group). Doses of acadesine lower than 20 μmol/l did not improve recovery, and doses greater than 20 μmol/l (e.g., 50, 200, or 1,000 μmol/l) produced no further improvements in protection.

Enzyme leakage. CK leakage reflected the results for functional recovery, although the improvement failed to achieve a level of statistical significance (51±9, 34±3, 45±6, 41±3, and 31±4 IU/15 min/g dry wt in the 5, 20, 50, 200, and 1,000 μmol/l acadesine groups, respectively, versus 51±7 IU/15 min/g dry wt in the acadesine-free control group).
**Metabolic studies.** The profiles for the tissue contents of adenine nucleotides, nucleosides, and purines were similar to those observed in the preceding two studies, with acadesine exerting no significant effect. There was, however, a trend toward an increased content of IMP. Independent of the dose of acadesine, the mean IMP content was two- to threefold higher in the acadesine-treated groups (0.08±0.03, 0.10±0.04, 0.09±0.04, 0.07±0.01, and 0.12±0.03 μmol/g dry wt with 5, 20, 50, 200, and 1,000 μmol/l of acadesine, respectively) than in the acadesine-free control group (0.04±0.01 μmol/g dry wt). These differences failed to achieve a level of statistical significance. As in the previous studies, ECP recovered to its aerobic control value in each instance, with or without acadesine.

**Study 4: Metabolic Consequences During Ischemia of the Administration of Acadesine**

In these studies, hearts were not reperfused but instead were freeze-clamped at the end of ischemia and taken for metabolite analysis. The results in Table 3 show that ischemia reduced ATP and ADP contents (18% and 66%, respectively) and significantly increased nucleoside and purine contents. There was a significant increase in IMP content in the acadesine-treated hearts; this increase was greatest in the group receiving acadesine during both pretreatment and cardioplegia (p<0.05 when compared with hearts receiving acadesine in cardioplegia alone). The tissue content of acadesine and its monophosphate derivative (ZMP) were also increased in the acadesine-treated groups,
especially in those receiving the drug as a pretreatment. Acadesine had no effect on any of the other metabolites under any of the conditions studied. As expected, the ECP fell to below 50% of the aerobic control value in both acadesine-free and acadesine-treated groups, but there were no significant differences between these groups.

Discussion

The present studies demonstrate that 1) the administration of acadesine (100 mg/kg) as a pretreatment, as an additive (20 μmol/l) to the St. Thomas’ Hospital cardioplegic solution, and during early reperfusion (20 μmol/l) improves substantially the posts ischemic recovery of function of the rat heart after hypothermic ischemia; 2) a similar beneficial effect can be achieved when acadesine is given as a pretreatment alone, as an additive to the cardioplegic solution alone, or throughout the experiment (i.e., given as a pretreatment plus in cardioplegia and during reperfusion); 3) the minimal dose of acadesine that provided maximum protection as an additive to the cardioplegic solution was 20 μmol/l; 4) the mechanism of the protection remains to be resolved, although these studies suggest that the conservation (or enhanced repletion) of the adenine nucleotide pool is not a significant factor. A number of aspects of our results warrant further discussion.

Studies of Acadesine and Myocardial Protection

There have been a number of studies \(^{14,15,28-33}\) in which the protective effects of acadesine have been assessed in terms of function and/or metabolism. In some of these, protection was observed, others reported no effect, and one reported increased injury. Thus, Mitsos et al.,\(^ {14}\) using the isolated blood-perfused cat heart subjected to 60 minutes of global ischemia, found that acadesine (administered before ischemia and during reperfusion) improved the recovery of left ventricular developed pressure (LVDP) from 62% to 93%. However, Ambrosio et al.,\(^ {28}\) using the isolated, crystalloid, perfused rabbit heart subjected to 20 minutes of global ischemia, with acadesine administered only during reperfusion, saw no effect on the recovery of function. The findings of the present study are in full agreement with these two studies.\(^ {14,28}\) Hoffmeister et al.,\(^ {29}\) with 45 minutes of regional ischemia in the dog heart in vivo and a continuous infusion of acadesine during 3 hours of reperfusion, reported a progressive deterioration of segment shortening in the drug-treated group. It is worth emphasizing that in these experiments, the drug was given only at reperfusion and at a high dose directly into the coronary artery (9 mmol/l). Furthermore, myocardial function was assessed by ultrasonic crystals implanted in the subendocardium, and, unfortunately, the recovery in the midmyocardium and epicardium was not studied.

The ability of acadesine to afford protection when given before ischemia is also controversial. Thus, in contrast to our studies and those of Mitsos et al.,\(^ {14}\) Mentzer et al.,\(^ {30}\) using the isolated rat heart treated with acadesine before ischemia (10 minutes) and during reperfusion (60 minutes), observed an 83% recovery of LVDP in the controls versus 76% in the acadesine-treated hearts. A possible reason for the lack of protection in this study might be the short duration of ischemia. Ischemic durations as short as 10 minutes result in high recoveries of function (83% in Mentzer’s study); this leaves little scope for demonstrating any protection—especially if corrections are made for the time-matched deterioration of the preparation.\(^ {34,35}\) In this connection, it has been our experience with acadesine, with the St. Thomas’ Hospital cardioplegic solution and with preconditioning,\(^ {37}\) that protective interventions are often unable to exert a major effect when used with very short or very long periods of ischemia. The time window of protection is such that there is little scope for protection with short durations, and after long durations, the injury is so great that the tissue is no longer responsive to protective interventions.

The present study indicates that acadesine tended to improve the posts ischemic recovery of coronary flow. However, the extent of this improvement varied considerably among the various treatment groups. Greatest benefit was observed when acadesine was administered as pretreatment, as an additive to single-dose cardioplegia, and as an additive during reperfusion. As with studies of contractile function, the literature is characterized by controversy over the effects of acadesine on the recovery of coronary flow. Swain et al.,\(^ {31}\) using 12 minutes of regional ischemia in the dog heart, showed that the administration of acadesine at the onset of
TABLE 2. (Continued)

<table>
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<th>ZTP</th>
<th>ATP</th>
<th>AP</th>
<th>ECP</th>
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ischemia and throughout 24 hours of reperfusion has no significant effect on the postischemic recovery of flow. However, it should be noted that in this study, the coronary flow in the untreated hearts recovered to control levels, leaving little room for the demonstration of drug-induced protection. The same high recovery of coronary flow was obtained in control hearts in the study of Mitsos et al.,14 and they, too, were unable to demonstrate a significant improvement in flow after the administration of acadesine. In contrast, Gruber et al.,15 in a similar experimental model but with a longer period of ischemia (60 minutes), reported that acadesine, given 45 minutes before and during ischemia, increased collateral flow to the ischemic zone. These authors also showed that acadesine increased the concentration of adenosine in the venous blood draining from the ischemic tissue and decreased granulocyte accumulation. In attempting to reconcile various studies of acadesine and coronary flow, it appears that the experimental conditions (e.g., duration of ischemia), the time of drug administration, the time at which coronary flow is measured, together with a variety of other factors, might influence the outcome.

Acadesine has been reported to enhance adenylate repletion31 or increase the rate of ATP synthesis32 in the reperfused myocardium. Other studies,14,28–30,33 like the present, report relatively little effect on tissue purines or high-energy phosphates. In seeking to explain the conflicting findings with acadesine, a number of possibilities can be identified. First, the choice of species varies considerably between investigators, with studies being carried out in rats, cats, and dogs. Other variables include the duration of ischemia, the temperature during ischemia (normothermia or hypothermia), the

**FIGURE 4.** Bar graphs show effect of various concentrations of acadesine (0, 5, 20, 50, 200, or 1,000 µmol/l) as an additive to the St. Thomas' Hospital cardioplegic solution on the postischemic recovery of function measured after 35 minutes of reperfusion in hearts subjected to single-dose cardioplegia (2 minutes) followed by 2.5 hours of hypothermic (20°C) global ischemia. Panel A: Coronary flow; panel B: aortic flow; panel C: cardiac output; panel D: stroke volume; panel E: stroke work. Each column represents the mean of 12 hearts; bars indicate SEM. *p<0.05 compared with acadesine-free control group.
choice of dose, the timing (before, during, and/or after ischemia), and the frequency of administration. The duration of reperfusion may be another important variable, as may be the choice of blood or crystalloid perfusates. Because it is well established that the post-ischemic replenishment of adenosine nucleotides is a slow process, it may require longer periods of reperfusion to be studied before an effect of the drug on the recovery of nucleotides can be demonstrated. Such a possibility might explain the results of Swain et al. who, in experiments with 12 minutes of ischemia and 24 hours of reperfusion, reported an enhancement of tissue ATP and guanosine triphosphate (GTP) contents. In other studies, including the present one, which are characterized by much shorter periods of reperfusion, no such effect was observed. Indeed, in a follow-up study by Glower et al., 12 hours of reperfusion was not associated with an increase in adenine nucleotides by adacrine. Any argument concerning the importance of prolonged reperfusion does not, of course, help to explain the immediate protective effects of adacrine in terms of recovery of cardiac function.

Possible Mechanisms

Depletion of the adenine nucleotide and purine pools has been postulated to play a role in the cardiac dysfunction that persists after ischemia. Because the phosphorylated metabolite of adacrine (ZMP) is an intermediary metabolite, it has been proposed that it might increase the rate of ATP resynthesis after ischemia and reperfusion. A number of studies, including the present one, have failed to demonstrate such an effect; however, in only one of these was the reperfusion period extended beyond 3 hours. If the precursors of the nucleotide salvage pathway are rapidly washed out by reperfusion and are therefore unavailable, the only other ways to restore the nucleotide pool are the de novo synthesis pathway (which is a slow process that has been estimated to replace only 0.4% of the pool per hour) or the salvage of purine precursors carried in the blood. Adacrine treatment increases IMP levels (an intermediate in de novo synthesis) several-fold in comparison with control hearts. However, ZMP inhibits adenylsuccinate lyase, the enzyme that catalyzes the transformation of IMP to AMP, thereby preventing repletion of high-energy adenylates. Adacrine does not influence the rate of degradation of ATP during ischemia, a finding that is supported by others. One potential explanation for the reported augmented repletion of ATP is that adacrine-induced cardioprotection allowed for a more rapid and/or complete recovery of myocardial integrity, a biochemical marker of which is the restoration of nucleotide levels.

Recent studies by Gruber et al. have shown that the administration of adacrine for 45 minutes before and also during, a 60-minute occlusion of the left anterior descending coronary artery in the dog heart resulted in substantial increases in the concentrations of adenosine in the coronary venous effluent during ischemia and immediately after reperfusion. The authors suggested that the beneficial effect of adacrine might therefore be mediated through the ability of adenosine to increase collateral blood flow to the ischemic region, to inhibit superoxide generation by neutrophils, or to prevent platelet aggregation. However, in the present studies, adacrine caused no detectable increase in tissue adenosine content either at the end of ischemia or after reperfusion. Admittedly, venous adenosine levels were not measured in the present study; therefore, an increase cannot be ruled out. Several experimental differences between our studies and those of Gruber et al. could also account for the variation in results; these include species differences, normothermic versus hypothermic ischemia, global versus regional ischemia, in vitro crystalloid versus in vivo blood-perfused preparations, and intermittent versus continuous administration of the drug.

Indirect evidence for possible mechanisms or sites of action of adacrine can be derived from the different regimens of administration and doses of drug used in the present studies. For example, the fact that the administration of low doses of adacrine, whether as a pretreatment (with long periods of exposure of tissue to the drug) or as an additive to the cardioplegic solution (with short periods of exposure) was equally effective, could suggest that adacrine acted at some inhibitory or regulatory site rather than simply at a substrate level.

### Table 3. Tissue Metabolite Contents in Ischemic, Nonreperfused Rat Hearts With or Without Administration of Adacrine Before and/or During Ischemia

<table>
<thead>
<tr>
<th>Group</th>
<th>Acadesine</th>
<th>INO</th>
<th>ADO</th>
<th>ZMP</th>
<th>IMP</th>
<th>AMP</th>
<th>ADP</th>
</tr>
</thead>
<tbody>
<tr>
<td>A (aerobic control)</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>0.41±0.02</td>
<td>3.00±0.14</td>
<td>6.73±0.28</td>
</tr>
<tr>
<td>B (adacrine free)</td>
<td>...</td>
<td>3.85±0.14</td>
<td>2.08±0.08</td>
<td>...</td>
<td>0.88±0.19</td>
<td>13.19±0.52</td>
<td>4.43±0.36</td>
</tr>
<tr>
<td>C (adacrine as pretreatment)</td>
<td>1.42±0.05*</td>
<td>3.67±0.15</td>
<td>2.18±0.08</td>
<td>0.45±0.03*</td>
<td>1.94±0.08*</td>
<td>12.87±0.75</td>
<td>4.86±0.10</td>
</tr>
<tr>
<td>D (adacrine in cardioplegia)</td>
<td>1.01±0.03*</td>
<td>3.73±0.10</td>
<td>2.11±0.08</td>
<td>...</td>
<td>1.68±0.18*</td>
<td>12.74±0.74</td>
<td>4.40±0.16</td>
</tr>
<tr>
<td>E (Adacrine as pretreatment plus in cardioplegia)</td>
<td>1.84±0.07*t</td>
<td>3.84±0.18</td>
<td>2.00±0.14</td>
<td>0.62±0.04*</td>
<td>2.37±0.15*t</td>
<td>12.17±0.33</td>
<td>4.54±0.23</td>
</tr>
</tbody>
</table>

*n = 6 rat hearts per group.

INO, inosine; ADO, adenosine; ZMP, adacrine monophosphate; IMP, inosine monophosphate; AMP, adenosine monophosphate; ADP, adenosine diphosphate; ZTP, adacrine triphosphate; ATP, adenosine triphosphate; AP, adenylyl acid; and adacrine (all in μmol/g dry wt) and ECP, energy charge potential, were assessed at the end of 2.5 hours of hypothermic (20°C) global ischemia.

Group A, hearts not subjected to ischemia; group B, hearts subjected to ischemia but without adacrine at any stage; group C, adacrine (100 μg/kg i.v.) administered as a pretreatment 15 minutes before study; group D, adacrine (20 μmol/l) added only to the cardioplegic solution (single dose); group E, adacrine administered as pretreatment (100 μg/kg i.v.) and in the cardioplegic solution (20 μmol/l).

* * p<0.05 compared with group B.

** p<0.05 compared with group D.

*** p<0.05 compared with group C.
The plateau dose–response relation obtained with acadesine added to the cardioplegic solution also provides some valuable information. In this respect, the possibility that acadesine may act by changing the ionic composition of the cardioplegic solution (which can have a profound effect on the protective properties of the solution[40–42]) can be ruled out.

Clinical Relevance and Applicability of Acadesine

The ability of acadesine to improve the functional recovery after a period of global ischemia might find valuable application during routine cardiac surgery or heart transplantation (using the drug either as a pretreatment or as an additive to a cardioplegic solution). Nevertheless, it must be stressed that the present studies were carried out under conditions of moderate hypothermic ischemic arrest and that, if acadesine is to be used in cardiac transplantation, benefit should also be evaluated at lower temperatures (i.e., 4–6°C).

Conclusions

The present study demonstrates that acadesine, when administered either as a pretreatment or as an additive to the cardioplegic solution, protects the isolated, perfused rat heart against injury during ischemia and reperfusion. When added during reperfusion alone, no immediate benefit is apparent. The protective effects of acadesine are dose dependent, and we could find no evidence that the mechanism of action involved the conservation or enhanced repletion of the adenine nucleotide pool.

An important question that needs to be addressed is whether the protective effect of acadesine, as observed in the present study, represents a drug-induced acceleration in the rate of recovery (i.e., overcoming myocardial stunning) without any long-term effects on the extent of postischemic function or whether acadesine achieves an absolute reduction in the extent of the injury with a sustained improvement in contractile state. To resolve this issue, studies of long-term functional recovery (at least 24 hours) would be required. Such studies would also provide us with further information relating to the long-term effects of acadesine on the recovery of contractile function and adenine nucleotide metabolism.

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