Anti-Stunning and Anti-Infarct Effects of Adenosine-Enhanced Ischemic Preconditioning

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Background—Adenosine-enhanced ischemic preconditioning (APC) extends the protection afforded by ischemic preconditioning (IPC) by both significantly decreasing infarct size and significantly enhancing post-ischemic functional recovery. In this study, the anti-infarct effects and the anti-stunning effects of APC in contributing to enhanced post-ischemic functional recovery were determined and compared with IPC.

Methods and Results—Sheep (n = 96) were subjected to 15, 30, 45, or 60 minutes of regional ischemia and 120 minutes of reperfusion. IPC hearts received 5 minutes of regional ischemia and 5 minutes of reperfusion before ischemia/reperfusion. APC hearts received a bolus injection of adenosine coincident with IPC. Adenosine hearts (ADO) received a bolus injection of adenosine before ischemia/reperfusion. Regional ischemia (RI) hearts received no pretreatment. Infarct size/area at risk was determined by tetrazolium staining. Regional myocardial function was determined by sonomicrometry. Segment shortening after 15 minutes of ischemia in which no infarct was incurred was 32.1 ± 10.6% in RI, 70.6 ± 8.5% in IPC, and 77.4 ± 6.0% in APC hearts. Segment shortening after 30 minutes of ischemia was 60.7 ± 6.3% in APC hearts (P < 0.05 versus RI, ADO, IPC) but was < 37% in all other groups. Infarct size/area at risk after 30 and 60 minutes of ischemia was, respectively, 25.8 ± 5.7% and 49.8 ± 6.0% in RI, 12.9 ± 3.0% and 29.2 ± 5.0% in ADO, 11.6 ± 2.4% and 24.6 ± 2.7% in IPC, and 5.1 ± 1.6% and 12.4 ± 2.0% in APC hearts (P < 0.05 versus RI, ADO, IPC).

Conclusions—APC and IPC exhibit anti-infarct and anti-stunning effects in the ovine heart, but these effects are rapidly diminished with IPC. APC significantly extends these effects, providing for significantly enhanced infarct size reduction and post-ischemic functional recovery (P < 0.05 versus IPC). (Circulation. 2000;102[Suppl III]:III-326-III-331.)

Key Words: adenosine • myocardial infarction • stunning, myocardial • ischemia • reperfusion

Recently, we have reported a myoprotective protocol by using a bolus injection of adenosine coincident with ischemic preconditioning (IPC), a protocol that we have termed adenosine-enhanced ischemic preconditioning (APC), 1–4 which extends and amends the protection afforded by IPC. In a series of reports, we have shown that APC both significantly decreases myocardial infarct size (P < 0.05) and significantly enhances post-ischemic functional recovery (P < 0.05) as compared with IPC and that APC is an effective cardioprotective protocol in both the isolated perfused rabbit heart 1–2 and in situ blood-perfused sheep heart. 3,4

The mechanism(s) by which APC affords enhanced cardioprotection remains to be elucidated; however, one of the major issues raised by APC-enhanced cardioprotection is whether the enhanced post-ischemic functional recovery obtained with APC as compared with IPC is due solely to the superior “anti-infarct” effects or whether it is also associated with the “anti-stunning” effects. To investigate this relation, we have used the in situ blood-perfused regional myocardial sheep heart model. Sheep were chosen to elucidate the effects of APC because the ovine heart is known to be free of cardiac diseases, including hypertrophy, dilation, fibrosis, parasites, cardiac storage diseases, atherosclerotic plaque, and infarction in species commercially available in the United States. 5 In addition, the ovine model has limited native collateral coronary circulation to allow for amelioration of infarct size. 6,7 The sheep model thus allows mechanico-functional and cellular analyses to be performed without the presence of intervening confounding pathological processes. To determine the effects of APC on myocardial stunning, sheep hearts were subjected to 15 minutes of left anterior descending coronary artery (LAD) occlusion and 120 minutes of reperfusion in which no infarct was incurred. A time course study with 30, 45, and 60 minutes of LAD occlusion was used to determine the cardioprotective limits of APC.

Methods

Animals

Animals were housed individually and provided with laboratory chow and water ad libitum. All experiments were approved by the Beth Israel Deaconess Medical Center Animal Care and Use Committee and the Harvard Medical Area Standing Committee on Animals (Institutional Animal Care and Use Committee) and conformed to the US National Institutes of Health guidelines regulating...
the care and use of laboratory animals (NIH publication 577-3, 1996).

**Surgical Preparation**

Dorset or Suffolk sheep of either sex (35 to 45 kg, n=96) were sedated with ketamine hydrochloride (20 mg/kg IM, Abbott Laboratories) and anesthetized with sodium pentobarbital (25 mg/kg IV, Abbott Laboratories). General anesthesia was maintained throughout the experiment with sodium pentobarbital. A tracheotomy was performed through a midline cervical incision (36F, Argyle), and was ventilation begun with a volume-cycled ventilator (North American Drager, model Narkomed II, Telford; oxygen, 40%; Tidal volume, 1000 mL; ventilation rate, 12 breaths/min; positive end-expiratory pressure, 3 cm H2O; inspiratory to expiratory time ratio, 1/2). The right internal jugular vein was cannulated for intravenous access and the right common carotid artery was cannulated for arterial blood sampling and intra-arterial blood pressure monitoring (Millar Instruments). Heparin sodium (Elkins-Sinn Inc; 5000 IU IV) and 1% lidocaine (Elkins-Sinn Inc; 5 mL IV) were given before thoracotomy. Heparin was administered at the same dose every 30 minutes to the end of the experiment. The pericardial sac was exposed through a median sternotomy and was opened to form a pericardial cradle. A catheter-tipped manometer (Millar Instruments) was introduced through the apex into the left ventricle (LV) to record LV pressure. A silk thread (0 silk, KR34H, Ethicon, Inc) was passed around the distal third of the LAD coronary artery or its large diagonal branch with a taper needle, and both ends of the silk tie were threaded through a small vinyl tube to form a snare. The coronary artery was occluded by pulling the snare, which was then secured by clamping the tube with a mosquito clamp. Myocardial ischemia was confirmed visually by regional cyanosis of the myocardial surface.

**Experimental Protocol**

Sheep (n=96) were randomly divided and subjected to 15, 30, 45, or 60 minutes of regional ischemia followed by 120 minutes of reperfusion. IPC hearts (n=25) received a 10-mL saline bolus injection (vehicle control) at the immediate start of ischemic preconditioning, coincident with the tightening the snare (5 minutes of zero-flow regional ischemia followed by 5 minutes of reperfusion) before regional ischemia. APC hearts (n=25) received a 10-mL bolus injection of 10 mmol/L adenosine (Adenoscan; Medico Inc) at the immediate start of ischemic preconditioning, coincident with the tightening of the snare (5 minutes of zero-flow regional ischemia followed by 5 minutes of reperfusion) before regional ischemia. APC hearts (n=25) received a 10-mL bolus injection of 10 mmol/L adenosine (Adenoscan; Medico Inc) at the immediate start of ischemic preconditioning, coincident with the tightening of the snare (5 minutes of zero-flow regional ischemia followed by 5 minutes of reperfusion) before regional ischemia. APC hearts (n=25) received a 10-mL bolus injection of 10 mmol/L adenosine 10 minutes before regional ischemia. Regional ischemia hearts (RI; n=24) received no pretreatment. The bolus was injected into the left atrial appendage with a 19-gauge needle. The concentration of adenosine was determined from preliminary investigations, which indicated that a bolus injection of 1 mmol/L adenosine per 10 g LV mass was required to allow for enhanced post-ischemic functional recovery and reduced infarct size in the rabbit and sheep heart. Hemodynamic variables were continuously acquired throughout the experiment with the use of a PO-NE-MAH digital data acquisition system (Gould), with an Acquire Plus processor board, LV pressure analysis software, and a Gould ECG/Biotech.

**Regional Myocardial Function**

Regional myocardial function was assessed by sonomicrometry (Sonometrics Digital Ultrasonic Measurement System, Sonometrics Corp) with 5 digital piezoelectric ultrasonic probes (2.0 mm) implanted in the subendocardial layer ~10 mm apart within the ischemic area, with 2 pairs placed parallel to the minor axis of the heart and secured to the epicardium with polypropylene stitches (5-0 Prolene, 8580H, Ethicon Inc). The probes were left in place until the end of the experiment. Digital data were inspected for correct identification of end-diastolic and end-systolic points by means of post-processing software (SonoView, Sonometrics Corp). Measurements were made over at least 3 cardiac cycles in normal sinus rhythm and then averaged. The ventilator was stopped during data acquisition to eliminate the effects of respiration. The end-diastolic segment length was measured at the onset of positive dP/dt and the end-systolic segment length at peak negative dP/dt. Regional contractility was assessed as segment shortening (SS). Wall motion abnormalities were assessed as systolic bulging (SB), defined as the bulging of the myocardium after the end of diastole, and as post-systolic shortening (PSS) defined as the shortening after the end of systolic ejection. SS, SB, and PSS were calculated according to the equations. Time course changes in SS were expressed as a percentage of equilibrium values to minimize variability among individual animals.

**Measurement of Infarct Size**

Ischemic area at risk was delineated by monastryl blue pigment injection into the aorta after ligation of the involved artery at the end of the experiment. Infarct size was determined by triphenyl tetrazolium chloride staining (Sigma Chemical Co) and was expressed as a percentage of area at risk. The area at risk and the area of infarcted zone were measured by computerized planimetry (Scion Image, Scion Corp) as previously described.

**Statistical Analysis**

Statistical analysis was performed with the SAS (version 6.12) software package (SAS Institute, Inc). The mean±SEM value is shown for all variables. Statistical significance was determined by repeated-measures ANOVA, with the group as a “between-subjects” factor and time as a “within-subjects” factor. Post hoc comparisons between groups for both the average effect and at individual time points were made with the use of a Bonferroni correction to adjust for the multiplicity of tests. Statistical differences between groups in infarct size were evaluated by ANOVA. Linear regression analysis was performed to determine the relation between SS, infarct size, and regional ischemic time in each group. Differences in regression lines between groups were compared by means of the general linear model. The general linear model was also used to test for significant nonlinear (eg, quadratic) effects. Statistical significance was claimed at P<0.05.

**Results**

**Experimental Exclusions**

A total of 104 animals were randomly assigned to experimental protocols, with 8 animals excluded because of failure to complete the experimental protocol. One animal (RI) failed to resuscitate after 15 minutes of regional ischemia, 2 animals (1 RI and 1 ADO) failed to resuscitate after 30 minutes of regional ischemia, and 3 animals (1 RI, 1 IPC, and 1 APC) failed to resuscitate after 60 minutes of regional ischemia caused by sustained ventricular fibrillation and were killed humanely. Two animals (1 ADO in the 15-minute regional ischemia protocol and 1 APC in the 30-minute regional ischemia protocol) were killed humanely before experimental manipulation because of severe pneumonia and the presence of diffuse pericardial adhesion.

**Acute Effects of Adenosine**

Adenosine bolus injection transiently decreased heart rate from 117±8 to 82±5 bpm and mean arterial pressure from 113±5 to 65±2 mm Hg. Heart rate and the mean arterial pressure returned to equilibrium levels by 45±2 seconds and 99±3 seconds, respectively, after the bolus injection, in agreement with previous results.
**Left Ventricular Weight and Area at Risk**

<table>
<thead>
<tr>
<th>Group</th>
<th>LV wt, g</th>
<th>Area at Risk, %</th>
<th>n</th>
<th>LV wt, g</th>
<th>Area at Risk, %</th>
<th>n</th>
<th>LV wt, g</th>
<th>Area at Risk, %</th>
<th>n</th>
<th>LV wt, g</th>
<th>Area at Risk, %</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>RI</td>
<td>111.4±7.1</td>
<td>17.6±1.2</td>
<td>6</td>
<td>111.7±5.7</td>
<td>17.0±1.2</td>
<td>7</td>
<td>110.6±8.9</td>
<td>14.9±1.6</td>
<td>4</td>
<td>104.5±4.4</td>
<td>15.7±1.9</td>
<td>7</td>
</tr>
<tr>
<td>ADO</td>
<td>107.6±3.6</td>
<td>17.1±1.1</td>
<td>6</td>
<td>116.2±3.6</td>
<td>16.9±1.5</td>
<td>6</td>
<td>113.5±5.2</td>
<td>15.0±1.4</td>
<td>4</td>
<td>108.0±3.1</td>
<td>15.8±0.9</td>
<td>6</td>
</tr>
<tr>
<td>IPC</td>
<td>115.5±4.2</td>
<td>17.0±1.7</td>
<td>6</td>
<td>109.7±3.6</td>
<td>17.3±1.4</td>
<td>7</td>
<td>110.7±4.2</td>
<td>16.0±2.6</td>
<td>5</td>
<td>105.2±2.4</td>
<td>16.2±1.4</td>
<td>7</td>
</tr>
<tr>
<td>APC</td>
<td>116.1±5.1</td>
<td>16.7±1.3</td>
<td>6</td>
<td>108.4±4.0</td>
<td>16.6±1.6</td>
<td>7</td>
<td>108.5±7.5</td>
<td>16.3±2.1</td>
<td>5</td>
<td>103.3±2.9</td>
<td>161.1±2.4</td>
<td>7</td>
</tr>
</tbody>
</table>

*wt indicates weight.*

**Determination of the “Anti-Stunning” Effects of APC Cardioprotection**

To determine the “anti-stunning” effects of APC, sheep hearts (n=24, n=6 each for RI, ADO, IPC, and APC) were subjected to 15 minutes of regional ischemia and 120 minutes of reperfusion. No myocardial infarction was observed in any heart in any group with this protocol. No significant differences in LV weight and area at risk were observed within or between groups (Table).

**Hemodynamics (LV Global Function)**

No significant differences in heart rate, LV systolic pressure, LV end-diastolic pressure, LV peak developed pressure, +dP/dt, or mean arterial pressure were observed within or between groups after equilibrium and during ischemia and reperfusion.

**Regional Myocardial Function**

No significant differences in SS, SB, and PSS were observed between groups at the end of equilibrium. During 15 minutes of regional ischemia (30 to 45 minutes of perfusion), paradoxical bulging of the ischemic myocardium was observed in all groups, with SS decreasing to −27.4±7.8% in RI hearts, −19.6±6.6% in ADO hearts, −13.2±8.7% in IPC hearts, and −11.9±13.5% in APC hearts. After 120 minutes of reperfusion, SS was 32.1±10.6% in RI hearts, 35.7±10.9% in ADO hearts, 70.6±8.5% in IPC hearts (P<0.05 versus RI, ADO), and 77.4±6.0% in APC hearts (P<0.05 versus RI, ADO) (Figure 1).

No significant differences in SB or PSS were observed between groups during ischemia and reperfusion.

**Determination of Time Course of APC Cardioprotection**

To determine the time course of APC cardioprotection, sheep hearts (n=72, n=4 to 7 each for RI, ADO, IPC and APC) were subjected to 30, 45, or 60 minutes of regional ischemia and 120 minutes of reperfusion. No significant differences in LV weight and area at risk were observed within or between groups (Table).

**Hemodynamics (LV Global Function)**

No significant differences in heart rate, LV systolic pressure, LV end-diastolic pressure, LV peak developed pressure, +dP/dt, or mean arterial pressure were observed within or between groups before and during 30, 45, or 60 minutes of regional ischemia and after 90 minutes of reperfusion.

**Myocardial Infarct Size**

Infarct size expressed as a percentage of area at risk after 30, 45, and 60 minutes of regional ischemia and 120 minutes of reperfusion is shown for each group in Figure 2. Infarct size after 30 minutes of regional ischemia and 120 minutes of reperfusion was 25.8±5.7% in RI hearts, 12.9±3.0% in ADO hearts.
Infarct size was significantly increased ($P<0.05$) in all groups as the regional ischemic time was increased from 30 minutes to 45 or 60 minutes. Infarct size after 45 minutes of regional ischemia and 120 minutes of reperfusion was $31.1 \pm 2.1\%$ in RI hearts, $20.6 \pm 1.6\%$ in ADO hearts ($P<0.05$ versus RI), $17.6 \pm 1.3\%$ in IPC hearts ($P<0.05$ versus RI), and $10.2 \pm 1.3\%$ in APC hearts ($P<0.05$ versus RI, ADO, IPC). Infarct size after 60 minutes of regional ischemia and 120 minutes of reperfusion was $49.8 \pm 6.0\%$ in RI hearts, $29.2 \pm 5.0\%$ in ADO hearts ($P<0.05$ versus RI), $24.6 \pm 2.7\%$ in IPC hearts ($P<0.05$ versus RI), and $12.4 \pm 2.0\%$ in APC hearts ($P<0.05$ versus RI, ADO, IPC).

Regional Myocardial Function

Segment shortening (SS, % of equilibrium values) at the end of 120 minutes of reperfusion after 15, 30, 45, and 60 minutes of regional ischemia is shown for each group in Figure 1. No significant differences in SS, SB, and PSS were observed between groups at the end of equilibrium.

After 30 minutes of regional ischemia and 120 minutes of reperfusion, SS was $16.4 \pm 3.9\%$ in RI hearts, $29.2 \pm 10.6\%$ in ADO hearts, $30.7 \pm 8.3\%$ in IPC hearts, and $60.7 \pm 6.3\%$ in APC hearts ($P<0.05$ versus RI, ADO, IPC). SB was $3.0 \pm 0.3\%$ in RI hearts, $3.8 \pm 0.7\%$ in ADO hearts, $3.2 \pm 0.9\%$ in IPC hearts, and $1.3 \pm 0.2\%$ in APC hearts ($P<0.05$ versus RI, ADO, IPC).

After 45 minutes of regional ischemia and 120 minutes of reperfusion, SS was $8.1 \pm 4.3\%$ in RI hearts, $13.4 \pm 4.8\%$ in ADO hearts, $15.9 \pm 12.3\%$ in IPC hearts, and $36.4 \pm 7.7\%$ in APC hearts ($P<0.05$ versus RI, ADO).

No significant differences in SS, SB, or PSS were observed between groups after 60 minutes of regional ischemia and 120 minutes of reperfusion.

Relation Between Infarct Size, SS, and Regional Ischemic Time

The relation between infarct size (% of area at risk) and regional ischemic time (minutes) is shown for each group in Figure 3. Linear regression analysis indicated that there was a strong linear relation between infarct size and regional ischemic time within each group ($P<0.001$). The linear regression equations were $y = 1.090x - 13.63$ for RI hearts, $y = 0.635x - 8.13$ for ADO hearts, $y = 0.512x - 5.69$ for IPC hearts, and $y = 0.278x - 3.57$ for APC hearts. The correlation coefficient was $0.880$ for RI hearts, $0.843$ for ADO hearts, $0.847$ for IPC hearts, and $0.795$ for APC hearts. There was no evidence for a quadratic effect in any group. Group by time was a significant factor for infarct size for 30, 45, and 60 minutes of regional ischemia ($P<0.001$), showing that the slopes were different, and APC significantly decreased infarct size ($P<0.001$ versus RI, ADO, IPC) at these regional ischemic times.

The relation between SS (% of equilibrium values) and regional ischemic time (minutes) is shown for each group in Figure 4. Linear regression analysis indicated that there was a linear relation between SS and regional ischemic time within each group ($P<0.05$). The linear regression equations were $y = -0.82x + 47.20$ for RI hearts, $y = -0.89x + 59.65$ for ADO hearts, $y = -1.28x + 80.54$ for IPC hearts, and $y = -1.26x + 96.92$ for APC hearts. The correlation coefficient was $0.722$ for RI hearts, $0.739$ for ADO hearts, $0.729$ for IPC hearts, and $0.822$ for APC hearts. There was some evidence for a non-linear effect in the IPC group ($P<0.05$), but no non-linearity was found in the other three groups (all $P>0.20$). With the general linear model, there was both a significant group effect and a significant group by time effect (both $P<0.05$), showing that both the intercepts and the slopes differed between groups and that APC significantly preserved segment shortening ($P<0.05$ versus RI, ADO, IPC) at these regional ischemic times.

Discussion

Previous studies have shown that IPC attenuates stunning in the rat and perhaps the rabbit heart but not the canine or porcine heart. In this report, we show that IPC and APC attenuate stunning in the ovine heart. In our cardioprotective protocol (APC), we have used single-cycle IPC rather than multiple-cycle IPC, based on our previous reports indicating that in the in situ blood-perfused regional ischemic sheep heart model, single-cycle IPC provided greater infarct size reduction than 3-cycle IPC. To investigate the “anti-stunning” effects of APC in the sheep heart, we have used 15 minutes of regional ischemia, based on the report of Weisel, who has suggested that between

![Figure 3](https://example.com/figure3.png)

**Figure 3.** Relation between infarct size (% of area at risk) and RI time (minutes) for each group. Linear regression analysis shows that there is a strong linear relation between infarct size and RI time within each group ($P<0.001$).

![Figure 4](https://example.com/figure4.png)

**Figure 4.** Relation between SS (% of equilibrium values) and RI time (minutes) for each group. Linear regression analysis shows that there is a linear relation between SS and RI time within each group ($P<0.05$).
10 and 20 minutes of regional ischemia may be required to induce stunning in the sheep heart. In our experiment, we have used 15 minutes of regional ischemia, based on preliminary experiments indicating that there was no myocardial necrosis with this protocol. This regional ischemic time was chosen to allow for compliance with the strict definition of myocardial stunning in which mechanical dysfunction persists after reperfusion despite the absence of irreversible myocardial damage. 15,16 When the ischemic time is increased beyond 15 minutes, the effects of infarct size act to modulate post-ischemic functional recovery. Our data indicate that after 15 minutes of regional ischemia and 120 minutes of reperfusion, stunning was evident, with regional SS (% of equilibrium) returning to only 32.1 ± 10.6% in RI hearts. It is important to note that no myocardial infarction was observed in any heart in any group after 15 minutes of regional ischemia and 120 minutes of reperfusion. In addition, no apoptosis (TUNEL staining) was observed in any heart in any group (results not shown). Our results show that with the use of this model of stunning, IPC and APC significantly preserved SS as compared with RI. No significant difference in SS was observed between ADO and RI hearts, indicating that while both IPC and ADO have anti-stunning effects, there are no apparent anti-stunning effects with ADO.

When the regional ischemic time was increased from 15 to 30, 45, or 60 minutes, infarct size was found to be significantly increased with time. Infarct size was increased ∼2-fold from 30 minutes to 60 minutes of regional ischemia in each experimental group. However, our results show that infarct size was significantly decreased at each regional ischemic time with APC (P < 0.05 versus RI, ADO, and IPC). Although ADO or IPC were able to decrease infarct size to a similar extent (P < 0.05 versus RI), the relative infarct levels in these groups were ∼2 times greater (P < 0.05 versus APC) than that in APC hearts at each regional ischemic time. Thus, the use of APC allowed for the significant extension of regional ischemic time, providing equal infarct size reduction at 60 minutes of regional ischemia as that afforded by ADO or IPC at 30 minutes of regional ischemia. These data suggest that APC extends the “anti-infarct” effects of both ADO and IPC.

Our data also show that after 30, 45, and 60 minutes of regional ischemia and 120 minutes of reperfusion, neither ADO nor IPC improved SS as compared with RI. In contrast, SS in APC hearts after 30 minutes of regional ischemia and 120 minutes of reperfusion was significantly increased as compared with all other groups. SS in APC hearts after 45 minutes of regional ischemia and 120 minutes of reperfusion was significantly increased as compared with RI and ADO hearts. These results indicate that APC significantly enhanced post-ischemic functional recovery after 30 minutes (P < 0.05 versus RI, ADO, IPC) and 45 minutes (P < 0.05 versus RI, ADO) of regional ischemia and 120 minutes of reperfusion.

To account for the effects of infarct size on post-ischemic regional functional recovery, we have compared all SS data at the end of 120 minutes of reperfusion with that found in RI hearts after 15 minutes of regional ischemia and 120 minutes of reperfusion in which no infarct was incurred, indicating the level of regional myocardial dysfunction caused by stunning alone (Figure 1). The anti-stunning effects in IPC hearts evident after 15 minutes of regional ischemia were rapidly lost and were obviated as the regional ischemic time was increased to 30 minutes, with no significant difference in SS observed between IPC hearts at 30 minutes of regional ischemia and RI hearts at 15 minutes of regional ischemia. In contrast, SS in APC hearts after 30 minutes of regional ischemia was significantly increased as compared with that in RI hearts after 15 minutes of regional ischemia, indicating that although the APC hearts at 30 minutes of regional ischemia included 5.1 ± 1.6% of infarct size, the heart treated with APC exhibited significantly better regional myocardial function than that found in the heart with pure stunning induced by 15 minutes of regional ischemia. These results indicate that the anti-stunning effects in APC hearts are maintained as the regional ischemic time is increased to 30 minutes. APC extends the “anti-stunning” effects of IPC, and the APC enhanced post-ischemic functional recovery observed after 30 minutes of regional ischemia occurs through the significant extension of “anti-stunning” effects (P < 0.05 versus IPC). However, the “anti-stunning” effects of APC were transient, being obviated as the regional ischemic time was increased to 45 minutes.

In previous reports, it has been shown that recovery from stunning may occur days to weeks after ischemia. 17,18 In our experiments, we have used 120 minutes of reperfusion. Owing to the limitations of our model, we were unable to investigate regional mechanical function at these extended time points. While the “anti-stunning” effects of APC are transient, being obviated as the regional ischemic time is increased beyond 30 minutes, the decreased regional myocardial function may recover to a greater extent in the heart treated with APC in which the significantly enhanced “anti-infarct” effects of APC allow for the significant preservation of viable myocardium (P < 0.05 versus ADO, IPC). The significantly greater reduction in myocyte necrosis provided by APC as compared with ADO and IPC would be of greater benefit to the myocardium.

The individual contribution of “anti-stunning” or “anti-infarct” effects to overall cardioprotection remains to be elucidated. Our data would indicate that APC cardioprotection occurs through the additive actions of ADO and IPC. ADO exhibits no apparent “anti-stunning” effects, whereas IPC exhibits “anti-stunning” effects at 15 minutes of regional ischemia. The “anti-stunning” effects of IPC, however, are negligible as the regional ischemic time is increased beyond 15 minutes. In addition to these findings, we have also shown that both ADO and IPC exhibit similar “anti-infarct” effects at each regional ischemia time (P < 0.05 versus RI). These results suggest that the mechanism of ADO is exclusively due to “anti-infarct” effects, whereas the mechanism of IPC is due to both “anti-stunning” effects and “anti-infarct” effects. A bolus injection of adenosine coincident with IPC (APC) extends the cardioprotection of either ADO or IPC by significantly prolonging the “anti-stunning” effects of IPC from 15 minutes to 30 minutes and significantly increasing the “anti-infarct” effects of both ADO and IPC for at least 60 minutes of regional ischemia.

In earlier reports by others, a correlation between infarct size and SS has been observed, but no significant differences between RI and IPC could be determined because of limitations of study size. 19 In our study, we have examined 96 hearts in total: 22 to 25 hearts each in RI, ADO, IPC, and APC (for 15, 30, 45, and 60 minutes of regional ischemia combined). Analysis of the
relation between infarct size and regional ischemic time revealed that there was a strong linear effect between infarct size and regional ischemic time within each group (P<0.001), indicating that as the regional ischemic time was increased, infarct size was increased in each group, in agreement with earlier observations by Connelly et al.20 The general linear model indicated that group by time was a significant factor for infarct size at 30, 45, and 60 minutes of regional ischemia (P<0.0001) and that APC significantly decreased infarct size as compared with all other groups at these regional ischemic times (Figure 3).

Analysis of the relation between SS and regional ischemic time indicated that there was a linear relation between SS and regional ischemic time within each group (P<0.05) and that as the regional ischemic time was increased, SS was decreased in each group. The general linear model indicated that there was some evidence for a non-linear effect in the IPC group (P<0.05), but no non-linearity was found in the other 3 groups (all P>0.20), showing that the effects of IPC on SS were rapidly lost as the regional ischemic time was increased beyond 15 minutes. Although there is no significant difference in SS between groups at 60 minutes of regional ischemia, the general linear model analyzing the relation between overall regional ischemic time including 15, 30, 45, and 60 minutes and SS reveals that there is both a significant group effect and a significant group by time effect (both P<0.05), indicating that APC provides superior recovery of SS during these time points (Figure 4).

The mechanism by which APC cardioprotection is conferred remains to be elucidated. In previous reports, we speculated that APC cardioprotection occurs through the additive effects of adenosine and IPC. Recent investigations by us21,22 have shown that APC cardioprotection acts by activation of adenosine receptors and K<sub>ATP</sub> channels. The use of adenosine or IPC alone was not sufficient to allow for significant infarct size reduction and enhanced post-ischemic functional recovery. Our data herein would extend these observations by showing that enhanced cardioprotection afforded by APC occurs by extension of the “anti-infarct” effects of IPC and ADO (P<0.05) and the “anti-stunning” effects of IPC (P<0.05). The “anti-stunning” effects of APC are significantly better preserved (P<0.05) than in IPC but are transient and are obviated as the regional ischemic time is increased beyond 30 minutes. The “anti-infarct” effects of APC appear to be preserved through 60 minutes of regional ischemia.

The clinical application of APC remains to be resolved in the human model. However, with the increase in the number of CABG operations performed on the beating heart without cardiopulmonary bypass,23 the development of cardioprotective protocols such as APC is mandatory. Off-pump CABG procedures increase the risk of myocardial infarction or stunning caused by regional ischemia, which may be poorly tolerated in patients with poor collateralization or depressed ventricular function.24 Recent reports have suggested that IPC might be performed before surgical arteriotomy, allowing for noncardioprotective protection.25 Our results suggest that APC would be superior to IPC for use during off-pump CABG to ameliorate the surgically induced ischemia/reperfusion injury including both myocardial stunning and infarction.

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References
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