Brief Definitive Communication

Ca²⁺ Preconditioning Elicits a Unique Protection Against the Ca²⁺ Paradox Injury in Rat Heart
Role of Adenosine

M. Ashraf, J. Suleiman, M. Ahmad

Abstract  Repeated Ca²⁺ depletion and repletion of short duration, termed Ca²⁺ preconditioning (CPC), is hypothesized to protect the heart from lethal injury after exposing it to the Ca²⁺ paradox (Ca²⁺ PD). Hearts were preconditioned with five cycles of Ca²⁺ depletion (1 minute) and Ca²⁺ repletion (5 minutes). These hearts were then subjected to Ca²⁺ PD, ie, one cycle of Ca²⁺ depletion (10 minutes) and Ca²⁺ repletion (10 minutes). Hearts subject to the Ca²⁺ PD underwent rapid necrosis, and myocytes were severely injured. CPC hearts showed a remarkable preservation of cell structure; ie, 65% of the cells were normal in CPC hearts compared with 0% in the Ca²⁺ PD hearts. LDH release was significantly reduced in CPC hearts compared with Ca²⁺ PD hearts (2.45±0.18 and 8.02±0.7 U·min⁻¹·g⁻¹, respectively). ATP contents of CPC hearts were less depleted compared with the Ca²⁺ PD hearts (5.9±0.8 and 3.0±0.16 μmol/g dry weight, respectively). Addition of the adenosine A₁ receptor agonist R-phenylisopropyl adenosine before and during Ca²⁺ PD provided protection similar to that in CPC hearts, whereas the nonselective adenosine A₁ receptor antagonist, 8-(p-sulfophenyl)-theophylline, blocked the beneficial effects of CPC. CPC-mediated protection was aborted when hearts subjected to CPC were treated with pertussis toxin (the guanine nucleotide or G-protein inhibitor). The present study suggests that Ca²⁺ preconditioning confers significant protection against the lethal injury of Ca²⁺ PD in rat hearts. Cardioprotection appears to result from adenosine release during preconditioning and by G,protein-modulated mechanisms. (Circ Res. 1994;74:360-367.)

Key Words  * preconditioning * adenosine * adenosine A₁ receptor * Ca²⁺ paradox * G proteins

In the heart, massive cell damage occurs within seconds when perfusate devoid of Ca²⁺ is followed by perfusion with solution containing Ca²⁺. This phenomenon has been called the Ca²⁺ paradox (Ca²⁺ PD). Loss of intracellular enzymes, Ca²⁺ overload and necrosis of the cell, and depletion of high-energy phosphates are common features of the Ca²⁺ PD. Mechanisms by which Ca²⁺ PD induces cell injury include altered Ca²⁺ channels, disrupted cell membrane, impaired Na⁺-[Ca²⁺] exchange, disruptive mechanical forces, and oxidative stress.

Recently, preconditioning of heart, ie, brief intermittent periods of ischemia, has been shown to reduce injury associated with longer periods of ischemia. Adenosine (ADO), an endogenous metabolite of cardiac tissue, is released as ATP is metabolized in ischemia and is implicated in ischemic heart protection. However, in the rat heart, ADO does not appear to play a central role in ischemic preconditioning (IPC). ADO is produced by myocytes under normal and stress conditions. During ischemia, ADO, a recyclable building block of ATP, accumulates as ATP synthesis is impaired. We reasoned that we would observe a similar preservation after Ca²⁺ PD if the heart were exposed to repeated stress by brief cycles of Ca²⁺ depletion and repletion; we have termed this phenomenon “calcium preconditioning” (CPC). Moreover, we wished to determine whether ADO, produced during CPC, activates ADO A₁ receptors and is responsible for preventing the regular Ca²⁺ PD. The present study tested the hypothesis that it is ADO-related mechanisms acting in concert with CPC that alleviate the lethal injury typically associated with the Ca²⁺ PD.

The results of the present study present a novel and unique mechanism for the pathogenesis of Ca²⁺ PD injury involving a potential role of ADO receptors acting via inhibitory GTP-binding protein (G, protein) in the reduction of Ca²⁺ PD damage.

Materials and Methods

Male Sprague-Dawley rats weighing 220 to 250 g were anesthetized by 30 mg/kg pentobarbital. After intraperitoneal injection of 500 U/kg heparin sodium, hearts were removed and retrogradely perfused through the aorta in a noncirculating Langendorff apparatus with Krebs-Henseleit (KH) buffer as previously described. Briefly, hearts were perfused at a constant pressure of 80 mm Hg. After perfusion with the oxygenated KH buffer at 37°C for an equilibration period of 10 minutes, the perfusate was changed to KH buffer devoid of Ca²⁺ for 10 minutes, followed by Ca²⁺-containing KH buffer for 10 minutes to produce a typical Ca²⁺ PD. The temperature of the buffer perfusate was controlled by Thermomix and was measured continuously using a YSI model 43 telethermometer and a YSI 413 probe (Yellow Springs Instrument Co, Yellow Springs, Ohio) at the aortic cannula. The coronary effluent was collected in a beaker, and flow was determined volumetrically at the end of equilibration, during the Ca²⁺ depletion period, and at 30, 90, 180, 300, and 600 seconds during Ca²⁺ repletion.

Received May 28, 1993; accepted November 22, 1993.

From the Department of Pathology and Laboratory Medicine, University of Cincinnati Medical Center (Ohio).

Correspondence to Muhammad Ashraf, PhD, Department of Pathology and Laboratory Medicine, University of Cincinnati, College of Medicine, 231 Bethesda Ave, Cincinnati, OH 45267-0529.
Perfusion Medium

KH buffer consisted of (mmol/L) NaCl 118, KCl 4.7, MgSO4 1.2, KH2PO4 1.2, CaCl2 2.5, NaHCO3 25, and glucose 5.5. In the Ca2+-free medium, Ca2+ was omitted, and measurement with an atomic absorption spectrometer confirmed its absence. The buffer was continuously bubbled with 95% O2/5% CO2 beginning 50 minutes before heart perfusion to ensure a minimum PO2 of 350 mm Hg.

Experimental Groups

 Hearts were randomly assigned to the experimental groups.

Group 1: Normal Control

 Hearts were perfused for 30 to 65 minutes with KH buffer as a control for different experimental groups. Hearts perfused for 65 minutes did not differ from those perfused for 30 minutes.

Group 2: Ca2+ PD

 Hearts (n=5) were perfused with KH buffer lacking Ca2+ for 10 minutes, followed by KH buffer containing Ca2+ for 10 minutes.

Group 3: CPC

 Hearts (n=9) were perfused for five cycles for 1 minute in Ca2+-free KH buffer. Each cycle was followed by 5 minutes of Ca2+-containing KH buffer, and then the hearts were subjected to the Ca2+ PD perfusion sequence as in group 2.

Group 4: Ca2+ PD and ADO Agonist

 The protocol was similar to that for group 2, except R-phenylisopropyl adenosine (R-PIA, 10, 20, 50, and 100 μmol/L), an ADO1 receptor agonist, was added to KH buffer beginning 10 minutes before and continuing during the Ca2+ PD (n=3 to 8 for each group).

Group 5: CPC and ADO Antagonist

 The protocol was similar to that for group 3, except 8-(p-sulfophenyl)-theophylline (8SPT, 5, 10, and 20 μmol/L), an ADO1 receptor antagonist, was added during CPC (n=3 to 7 for each group).

Measurement of LDH

 LDH, an indicator of myocardial tissue injury, was determined in coronary effluent. This was assayed by a coupled-enzyme spectrophotometric technique using a Sigma assay kit. Measurement of enzyme activity was based on the oxidation of lactate and the rate of increase in absorbance at 340 nm. LDH measurements were obtained at the end of equilibration, during the Ca2+-free period, during CPC, and at 30, 90, 180, 300, and 600 seconds during Ca2+ repletion.

Measurement of Tissue ATP

 The heart was immediately frozen between aluminum plates cooled in liquid nitrogen and was freeze-dried for 24 hours.
FIG 4. a, Light micrograph of control myocardium shows well-preserved myofibers and nuclei (arrow). Original magnification ×200. b, Transmission electron micrograph of control heart shows well-preserved cell membrane, mitochondria (M), and nuclei (N). Glycogen is abundant (arrow). Original magnification ×10 700. c, Light micrograph shows regular Ca^{2+} paradox. Myocytes are totally destroyed. Mitochondria (M) are squeezed out of the contracted cells. Original magnification ×200. d, Transmission electron micrograph of same heart shown in c shows broken cell membranes (arrow) and calcified mitochondria (M). Myofibrils are transformed into indistinguishable fiber mass (F). Original magnification ×16 800. e and f, Light micrograph and transmission electron micrograph, respectively, of hearts subjected to Ca^{2+} preconditioning show morphology similar to that of control hearts (a and b). M indicates mitochondria; N, nucleus; and arrow, glycogen. Original magnifications ×200 (e) and ×11 000 (f).
ATP was determined as previously described. Freeze-dried tissue (50 to 100 mg) was weighed and crushed in a precooled glass tube. Five milliliters of cold (4°C) 6% trichloroacetic acid was used to extract ATP, and hearts were then homogenized for 2 minutes and centrifuged at 25 000g for 10 minutes. The pellet was resuspended in trichloroacetic acid and centrifuged. Supernatant was filtered, and the pH was adjusted to 7.0 by addition of 5 mol/L potassium carbonate. ATP was analyzed by absorbance at 340 nm in a Gilford spectrophotometer. The results were expressed in micromoles per gram dry weight.

Measurement of ADO
ADO was measured in the coronary effluent. One milliliter of effluent was collected at the end of the equilibration period and at the end of the CPC and treatment phases. Samples were frozen immediately and kept in the freezer at −4°C until further use. ADO was extracted from thawed samples at room temperature (25°C) in 6% trichloroacetic acid and analyzed by high-performance liquid chromatography as described.

Morphological Examination
Tissue from the midventricular wall was cut into 1.0-mm pieces, immersed in 2.5% buffered glutaraldehyde for 4 hours, rinsed in 0.1 mol/L sodium cacodylate buffer (pH 7.3), and postfixed in 1% buffered osmium tetroxide for 1 hour. The tissue pieces were dehydrated in ethanol and propylene oxide and embedded in Spurr medium. One-micrometer-thick sections were cut with a Sorvall MTB ultramicrotome, mounted on a glass slide, and stained with 1% toluidine blue. The representative blocks from each group were thin sectioned for transmission electron microscopy. These sections were stained with uranyl acetate and lead citrate and examined with a Hitachi H-600 electron microscope.

A semiquantitative estimate of cell damage was carried out on 1-µm-thick sections with light microscopy at ×200. Four to six randomly chosen blocks from each heart were examined for quantification of cell damage without prior knowledge of the treatment. Approximately 500 cells were analyzed in each heart, and one of four degrees of cell morphology was assigned to each cell. Cell morphology was assessed according to the following classification: (1) normal: compact myofibers with uniform staining of nucleoplasm, well-defined rows of mitochondria between the myofibrils, and no separations of opposing intercalated disks; (2) mild damage: same as above, except some vacuoles were present adjacent to the mitochondria; (3) moderate damage: reduced staining of cytoplasmic organelles, clumped chromatin material, wavy myofibers, and granularity of nucleoplasm; and (4) severe damage: similar to moderate damage except the cells with contraction band necrosis were added to this category. Ultrastructural features of this classification system were identified by transmission electron microscopy.

Statistical Analysis
LDH, ATP, coronary flow, and percent of cells in each morphological category were expressed as mean±SEM for each group. Group comparisons were done by ANOVA with multiple comparisons or t test when appropriate. An intergroup difference for any variable of P<.05 was considered significant.

Results
Ca²⁺ PD
During the Ca²⁺ PD, hearts underwent complete bleaching, indicating a loss of myoglobin and intracellular LDH. At the end of the Ca²⁺-free period, the enzyme release was not different from the control values. ADO measured from the coronary effluent at the end of Ca²⁺-free perfusion (1.8±0.8 nmol/g) was similar to the value obtained at the end of the equilibration period (1.9±1.1 nmol/g). Immediately after Ca²⁺ repletion, a ninefold increase in LDH release occurred (Fig 1). Coronary flow decreased by 18% during Ca²⁺ depletion and was further significantly reduced during Ca²⁺ repletion (Fig 2). In Ca²⁺ PD, ATP levels were reduced to 3.0±0.16 µmol/g dry weight as compared with the control value of 23.2±0.5 µmol/g dry weight (Fig 3).

Examination of 1-µm-thick sections showed that the ultrastructure of the control heart was well maintained. Glycogen was abundant, and nuclear chromatin material was uniformly dispersed. The cell membrane was intact. At the end of Ca²⁺ depletion, all myocytes were relaxed and separated from each other at the intercalated disk. On Ca²⁺ repletion, most of the myocytes (99%) underwent severe contractures and were necrotic, extruding mitochondria at the periphery of cells (Fig 4c and 4d). At the ultrastructural level, the subcellular organelles were distorted in severely damaged cells. These cells were characterized by calcified mitochondria and ruptured sarcolemma (Fig 4d).

CPC
There was a dramatic decrease in LDH release in CPC hearts compared with the Ca²⁺ PD hearts (Fig 1). Maximum LDH release of 2.45±0.18 U·min⁻¹·g⁻¹ was observed at 1.5 minutes during Ca²⁺ repletion as compared with a value of 8.02±0.7 U·min⁻¹·g⁻¹ in control Ca²⁺ PD. Similarly, ATP contents were significantly preserved (Fig 3). CPC hearts maintained the flow during Ca²⁺ repletion at 56% of the normal flow rate, which was significantly higher than the control Ca²⁺ PD rate (Fig 2). We also assessed the effect of CPC with 2 minutes of Ca²⁺ depletion. Maximum LDH release and ATP contents were much less preserved compared with CPC at 1 minute of Ca²⁺ depletion (5.2±0.3 U·min⁻¹·g⁻¹ and 4.2±0.3 µmol/g dry weight, respectively).

The cellular structures were extremely well preserved in the CPC hearts subjected to the Ca²⁺ PD and were similar to structures in the control hearts (Fig 4e): 65% of cells were normal; 11.5%, 15.5%, and 7.6% were mildly, moderately, and severely damaged, respectively; and the damage was significantly less than that in the non-CPC hearts subjected to the Ca²⁺ PD (Table 1). At the electron microscopic level, the myocytes maintained their contacts at the intercalated disks and had abundant glycogen stores. Myofibrils were relaxed, and mitochondria were usually elongated. Nuclear chromatin material was uniformly dispersed. Cells that were severely injured were not different from the non-CPC hearts.

The release of ADO was monitored in the coronary effluent. At the end of the equilibration period, ADO release was 1.9±1.1 nmol/g (n=5), and it increased 11-fold at the end of the CPC phase (20.7±0.7 nmol/g) (n=5).

PD and ADO A₁ Agonist
To determine whether the beneficial effects of CPC were mediated by ADO or ADO-related compounds, we assessed the effect of R-PIA, an ADO A₁ receptor agonist, on myocardial ATP levels and LDH release during and after the Ca²⁺ PD. R-PIA acted in a...
dose-dependent manner (data not shown) (100 μmol/L conferred maximum protection), and 10 minutes of treatment before and during the Ca\(^{2+}\) PD was most effective. Significant reduction in LDH release (Fig 1) and preservation of ATP (Fig 3) were observed in hearts treated with R-PIA before and during Ca\(^{2+}\) PD as compared with control Ca\(^{2+}\) PD. LDH, ATP contents, and cell morphology were similar in control and CPC hearts. R-PIA-treated hearts maintained coronary flow during Ca\(^{2+}\) depletion at 57% of the normal flow rate, which was significantly higher than the control Ca\(^{2+}\) PD rate (Fig 2).

**CPC and ADO Antagonist**

If ADO were essential to the protection, then 8-SPT, a nonselective A\(_1\) receptor antagonist,\(^{13}\) should block the effects of CPC. The results support this rationale, as shown in Fig 1. Dose-response studies (5, 10, and 20 μmol/L; n=3 to 7 for each) showed that 10 μmol/L was the most effective concentration that had no effect on heart rate or coronary flow in normal hearts (Table 2). ATP loss and cell morphology in 8-SPT–treated hearts were similar to those observed in control Ca\(^{2+}\) PD, and LDH release was also higher (Figs 1 through 3, Table 1). In these hearts, ADO release at the end of the CPC period was drastically reduced to 3.4±0.6 nmol/g as compared with 20.7±0.7 nmol/g in nontreated CPC hearts. Similarly, coronary flow was similar to the control Ca\(^{2+}\) PD (Fig 2). To determine whether 8-SPT had any effect on Ca\(^{2+}\) PD, we added 10 μmol/L of 8-SPT during Ca\(^{2+}\) depletion and repletion (n=3). Maximum LDH release was 7.8±0.2 U \(\cdot\) min\(^{-1}\) \(\cdot\) g\(^{-1}\) and ATP content was 3.3±0.1 μmol/g dry weight, and these values were comparable to control Ca\(^{2+}\) PD.

**CPC and Pertussis Toxin**

ADO receptors are known to act via G proteins. To evaluate the involvement of G protein in ADO-mediated protection, we used pertussis toxin to uncouple this G protein. Different doses of pertussis toxin were used (2, 4, and 8 μg/L; n=3 to 6), and it was found that 4 μg was the dose that aborted CPC-mediated protection without altering baseline coronary flow (Table 2). Results were similar to those in 8-SPT–treated hearts. LDH release was higher (6.8±0.45 U \(\cdot\) min\(^{-1}\) \(\cdot\) g\(^{-1}\)) and similar to that in Ca\(^{2+}\) PD hearts. The cell damage was similar to control Ca\(^{2+}\) PD (Table 1). The release of ADO at the end of pertussis toxin treatment was almost reduced to zero (0.77±0.1 nmol/g). Addition of pertussis toxin to the Ca\(^{2+}\) depletion and repletion phases of a control Ca\(^{2+}\) PD had no effect on maximum LDH release and ATP contents when compared with results in the Ca\(^{2+}\) PD hearts.

**Discussion**

The major aims of the present study were to determine whether CPC prevents the lethal damage of the Ca\(^{2+}\) PD and to determine whether ADO might mediate preconditioning. The results reported here clearly demonstrate that CPC provides a significant protection against the deleterious effects of Ca\(^{2+}\) PD damage and that, under these conditions, cardioprotection is mediated by ADO.

The cellular injury induced by the Ca\(^{2+}\) PD is so abrupt and lethal that, so far, no therapeutic intervention has been effective against the damage.\(^{18}\) In our preconditioning protocol, the heart is subjected to multiple sequences of 1 minute of Ca\(^{2+}\) depletion followed by Ca\(^{2+}\) repletion for 5 minutes; after these sequences, we instituted the Ca\(^{2+}\) PD (ie, 10 minutes of Ca\(^{2+}\) depletion and 10 minutes of Ca\(^{2+}\) repletion). We refer to this phenomenon as CPC. During this preconditioning process, the heart resumes normal beating, and no structural or biochemical alterations are evident. If

---

**Table 1.** Semiquantitative Estimate of Cell Damage in Hearts Subjected to Ca\(^{2+}\) Paradox After Ca\(^{2+}\) Preconditioning

<table>
<thead>
<tr>
<th>Group</th>
<th>Normal</th>
<th>Mild</th>
<th>Moderate</th>
<th>Severe</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (n=4)</td>
<td>99±0.1</td>
<td>1±0.1</td>
<td>. . .</td>
<td>. . .</td>
</tr>
<tr>
<td>Ca(^{2+}) PD (n=4)</td>
<td>. . .</td>
<td>1±0.1</td>
<td>. . .</td>
<td>99±0.1</td>
</tr>
<tr>
<td>CPC+Ca(^{2+}) PD (n=4)</td>
<td>65.4±19.5*</td>
<td>11.5±7.8*</td>
<td>15.5±15.5*</td>
<td>7.6±7.6*</td>
</tr>
<tr>
<td>Ca(^{2+}) PD+R-PIA (n=4)</td>
<td>36.7±11.9*</td>
<td>1.7±1.4*</td>
<td>8.9±2.9*</td>
<td>49.76±14.3*</td>
</tr>
<tr>
<td>CPC+8-SPT+Ca(^{2+}) PD (n=3)</td>
<td>2.8±1.4</td>
<td>0</td>
<td>29.3±16.8</td>
<td>67.8±17.9</td>
</tr>
<tr>
<td>CPC+PTX+Ca(^{2+}) PD (n=3)</td>
<td>0</td>
<td>0</td>
<td>5.5±3.0</td>
<td>94.4±3</td>
</tr>
</tbody>
</table>

PD indicates paradox; CPC, Ca\(^{2+}\) preconditioning; R-PIA, R-phenylisopropyl adenosine; 8-SPT, 8-(p-sulfophenyl)-theophylline; and PTX, pertussis toxin. Values are mean±SEM. *P<.05 vs Ca\(^{2+}\) PD.

---

**Table 2.** Dose-Dependent Effects of 8-(p-Sulfophenyl)-theophylline and Pertussis Toxin on Tissue ATP and LDH Release During Ca\(^{2+}\) Preconditioning Followed by Ca\(^{2+}\) Paradox

<table>
<thead>
<tr>
<th>Tissue ATP, μmol/g dry wt</th>
<th>Maximum LDH Release, U (\cdot) min(^{-1}) (\cdot) g(^{-1})</th>
</tr>
</thead>
<tbody>
<tr>
<td>8-SPT</td>
<td></td>
</tr>
<tr>
<td>5 μmol/L</td>
<td>3.9±0.2 (n=3)</td>
</tr>
<tr>
<td>10 μmol/L</td>
<td>2.8±0.4 (n=7)</td>
</tr>
<tr>
<td>20 μmol/L</td>
<td>2.3±0.11 (n=3)</td>
</tr>
<tr>
<td>PTX</td>
<td></td>
</tr>
<tr>
<td>2 μg/L</td>
<td>4.2±0.2 (n=3)</td>
</tr>
<tr>
<td>4 μg/L</td>
<td>3.4±0.2 (n=6)</td>
</tr>
<tr>
<td>8 μg/L</td>
<td>2.8±0.17 (n=3)</td>
</tr>
</tbody>
</table>

8-SPT indicates 8-(p-sulfophenyl)-theophylline; PTX, pertussis toxin. Values are mean±SEM.
these hearts are then exposed to Ca\(^{2+}\) PD, the expected injury is markedly reduced. Although CPC is different from IPC,\(^{10}\) it appears that both phenomena share the same factors. In both CPC and IPC, hearts produce significant quantities of ADO. It is well known that ADO formation and release is increased in the ischemic heart,\(^{14,16}\) which provides potentially beneficial effects by activating the ADO \(A_1\) receptor\(^{13}\) or through anti-adrenergic effects.\(^{19}\) In the rat, ADO does not appear to be involved in IPC.\(^{13}\) However, Olsson\(^{20}\) reported an increase of ADO from 0.32 to 1.82 nmol/g after a 15-second occlusion produced by coronary ligation in the dog; similarly, Berne and Rubio\(^{12}\) reported an increase in ADO within 25 seconds of hypoxia in the rat from 3.2 to 17.00 nmol/g. The reduction in coronary flow after CPC is an interesting observation, which perhaps suggests that IPC is being induced by CPC. At present, it is not known whether this is true. However, the decrease in coronary flow after IPC is much less than that seen in CPC.\(^{21}\)

ADO exerts its protective effects both by extracellular and intracellular mechanisms. To further investigate the role of ADO in CPC, we reasoned that, if ADO were involved and if ADO action is mediated by an extracellular mechanism (ie, binding to the \(A_1\) receptor), then ADO itself or an ADO agonist should mimic the beneficial effects of CPC. Hearts treated with the ADO \(A_1\) receptor agonist R-PIA during the Ca\(^{2+}\) PD exhibited protection almost similar to that in CPC hearts. Ten minutes of pretreatment was more protective (as shown above) than adding R-PIA during the Ca\(^{2+}\) PD only (data not shown). It has been reported previously that infarct size is reduced in hearts pretreated with ADO or ADO \(A_1\) receptor agonist;\(^{21}\) ionic changes and cardiac function are also improved.\(^{19}\) It is well known that activation of \(A_1\) receptors opens K\(^{+}\) channels in the myocardium.\(^{22}\) Since opening the K\(^{+}\) channels would hyperpolarize myocardial cells and subsequently reduce Ca\(^{2+}\) influx via voltage-regulated Ca\(^{2+}\) channels, it is possible that ADO could ameliorate Ca\(^{2+}\) PD damage via this mechanism. Adenosine also attenuates the contractile response elicited by \(\beta\)-adrenergic stimulation\(^{23}\) and reduces Ca\(^{2+}\) transients via reduced activity of cAMP-dependent protein kinase.\(^{24}\) The link between ADO and CPC is further substantiated by the fact that protection afforded by CPC can be mimicked by replacing the CPC period with administration of R-PIA.

It appears that ADO-sensitive mechanisms are crucially involved in the pathogenesis of Ca\(^{2+}\) PD injury. Ca\(^{2+}\) overload is one of the major consequences of the Ca\(^{2+}\) PD,\(^{4}\) resulting in extreme myofilament contractures and rounding of myocytes. \(\beta\)-Adrenergic receptors couple with Ca\(^{2+}\) channels via a G protein,\(^{25}\) and in this way, their activation may result in the Ca\(^{2+}\) overload observed in the Ca\(^{2+}\) PD. ADO could act as an antiadrenergic agent, reducing \(\beta\)-adrenergic stimulation mediated by ADO \(A_1\) receptors. At present, it is not clear whether catecholamines have any role in the Ca\(^{2+}\) PD injury. The present data suggest that ADO is necessary for CPC, perhaps by reducing receptor-mediated Ca\(^{2+}\) overload involving G proteins.\(^{26}\)

The findings of the present study strongly implicate a role of ADO itself and ADO \(A_1\) receptor-mediated actions in CPC. ADO \(A_1\) receptors are coupled to Ca\(^{2+}\) channels via G proteins.\(^{26}\) Occupancy of the receptor is important in triggering the protection, and blockade of the receptor with a nonselective ADO \(A_1\) receptor antagonist (8-SPT) completely eliminates the protective effect. Similarly, these responses are also sensitive to treatment of hearts with pertussis toxin, suggesting that the receptors are coupled to G proteins. The concentration of ADO release from ATP\(^{13}\) during CPC may be sufficient to maintain activation of ADO receptors during the Ca\(^{2+}\) depletion phase. The present data are in agreement with this rationale. Biochemical and pharmacologic studies in heart cells indicate that ADO \(A_1\) receptors, which inhibit adenylate cyclase, are coupled to G proteins.\(^{27}\) Inhibition of adenylate cyclase results in less accumulation of cAMP and favors dephosphorylation of Ca\(^{2+}\) channels, thus causing a decrease in Ca\(^{2+}\) current.\(^{28}\) Therefore, it is likely that entry of Na\(^+\) through Ca\(^{2+}\) channels during Ca\(^{2+}\) depletion is blocked because of dephosphorylation of Ca\(^{2+}\) channels. Moreover, the rapid activation of Na\(^+\)-Ca\(^{2+}\) exchange, which occurs in Ca\(^{2+}\) PD on Ca\(^{2+}\) repletion, is also blocked. The results of the present study tend to support this hypothesis. Contraction bands, which are common in the Ca\(^{2+}\) PD because of Ca\(^{2+}\) overload,\(^{6}\) are rare, and the cell ultrastructure is well maintained in CPC hearts. ATP contents, however, do not recover fully but are significantly augmented in the CPC hearts.

In summary, the present study shows that lethal injury of the Ca\(^{2+}\) PD is reduced by ADO \(A_1\) receptor-mediated mechanisms. This effect of ADO \(A_1\) receptor in the Ca\(^{2+}\) PD is mediated through a pertussis toxin–sensitive G protein. This is the first study showing that CPC appears to be a promising intervention against Ca\(^{2+}\) PD damage.

Acknowledgment

This study was supported in part by National Institutes of Health research grant HL-41358 from the National Heart, Lung, and Blood Institute.

References

11. Liu GS, Thrton J, Van Winkle DM, Stanley AWH, Olsson RA, Downey JM. Protection against infarction afforded by precondi-
Ca2+ preconditioning elicits a unique protection against the Ca2+ paradox injury in rat heart. Role of adenosine. Fixed.
M Ashraf, J Suleiman and M Ahmad

Circ Res. 1994;74:360-367
doi: 10.1161/01.RES.74.2.360

Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1994 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circres.ahajournals.org/content/74/2/360

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation Research can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation Research is online at:
http://circres.ahajournals.org//subscriptions/