Cardioprotection by multiple preconditioning cycles does not require mitochondrial K\textsubscript{ATP} channels in pigs

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Schwartz, Lisa M., Timothy S. Welch, and Mark S. Crago. Cardioprotection by multiple preconditioning cycles does not require mitochondrial K\textsubscript{ATP} channels in pigs. Am J Physiol Heart Circ Physiol 283: H1538–H1544, 2002. First published June 27, 2002; 10.1152/ajpheart.00040.2002.—To test whether cardioprotection induced by ischemic preconditioning depends on the opening of mitochondrial ATP-sensitive K\textsuperscript{+} (K\textsubscript{ATP}) channels, the effect of channel blockade was studied in barbital-anesthetized open-chest pigs subjected to 30 min of complete occlusion of the left anterior descending coronary artery and 3 h of reflow. Preconditioning was elicited by two cycles of 5-min occlusion plus 10-min reperfusion before the 30-min occlusion period. 5-Hydroxydecanoate (5 mg/kg iv) was injected 15 min before preconditioning or pharmacological preconditioning induced by diazoxide (3.5 mg/kg, 1 ml/min iv). Infarct size (percentage of the area at risk) after 30 min of ischemia was 35.1 ± 9.9% (n = 7). Preconditioning markedly limited myocardial infarct size (2.7 ± 1.6%, n = 7), and 5-hydroxydecanoate did not abolish protection (2.4 ± 0.9%, n = 8). Diazoxide infusion also significantly limited infarct size (14.6 ± 7.4%, n = 7), and 5-hydroxydecanoate blocked this effect (30.8 ± 8.0%, n = 7). Thus the opening of mitochondrial K\textsubscript{ATP} channels is cardioprotective in pigs, but these data do not support the hypothesis that opening of mitochondrial K\textsubscript{ATP} channels is required for the endogenous protection afforded by preconditioning.

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MYOCARDIAL ISCHEMIC PRECONDITIONING (PC) is a powerful endogenous protective phenomenon whereby ischemic tissue is rendered resistant to cell death by one or a series of prior episodes of brief, nonlethal ischemia (24). Understanding the mechanism of this protection should provide valuable insight into the process of lethal ischemic injury and provide important clues for development of effective therapy for ischemic injury. Agents that mimic PC and limit myocardial infarction would allow the window of therapy to be extended until other treatments could be instituted.

Studies have implicated a cascade of cellular events initiated by PC. Activation of triggers of cardioprotection, including adenosine A\textsubscript{1}/A\textsubscript{3} (18, 19, 35, 41), bradykinin (9, 27), and opioid receptors (31, 32), subsequently activate downstream pathways involving PKC (23, 40, 45), tyrosine kinases (40) and mitogen-activated protein kinase (4, 5). These pathways may be linked, and opening of ATP-sensitive K\textsuperscript{+} (K\textsubscript{ATP}) channels (2, 34) may be the final step in the signal transduction process.

It has been originally proposed (25) that opening of K\textsubscript{ATP} channels causes shortening of the myocardial action potential duration, which in turn would decrease contraction and prevent further depletion of ATP and irreversible impairment of its energy metabolism. However, recent studies have demonstrated that the K\textsubscript{ATP} channel involved in the cardioprotection observed with channel openers is distinct from that located on the sarcolemma. Yao and Gross (44) demonstrated that biamalim reduced infarct size at a dose that did not affect action potential duration. Additionally, the protective qualities of BMS-180448 (12) and cromakalim (13) were dissociated from enhanced shortening of the action potential duration. Finally, opening of K\textsubscript{ATP} channels protected quiescent isolated cardiomyocytes independent of any action potential generation (1).

Prime candidates as an alternate cellular site for channel-mediated protection are mitochondria, which also possess K\textsubscript{ATP} channels (17) located on the inner mitochondrial membrane. The biochemical properties of the mitochondrial K\textsubscript{ATP} channel are very similar to those of the sarcolemmal K\textsubscript{ATP} channels, and they are both sensitive to many of the openers and blockers previously used in studies investigating the mechanism of PC. However, Garlid et al. (8) demonstrated that diazoxide was 2,000-fold more selective in opening mitochondrial channels than sarcolemmal K\textsubscript{ATP} channels in the heart. 5-Hydroxydecanoate (5-HD) can reverse diazoxide-induced mitochondrial K\textsuperscript{+} flux (8), yet has little effect on cardiac sarcolemmal channels (22). Therefore, diazoxide and 5-HD are unique tools for selective manipulation of mitochondrial K\textsubscript{ATP} channels.

To test the hypothesis that cardioprotection induced by PC depends on opening of mitochondrial K\textsubscript{ATP} channels (8), we investigated the effect of channel blockade...
by 5-HD on PC and diazoxide-induced protection in an in vivo pig model of ischemia and reperfusion.

**METHODS**

Surgical preparation. All experiments performed in this report conformed with the standards in the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publication No. 85-23, Revised 1996). Yorkshire pigs of either sex, weighing between 18 and 22 kg and free of clinically evident disease, were entered into this study.

Pigs were sedated with an intramuscular injection of 15 mg/kg ketamine hydrochloride (Vetalar, Fort Dodge Laboratories) and anesthetized with pentobarbital sodium (30 mg/kg, Sigma; St. Louis, MO). Pigs were placed on a fluid-filled heating pad (Automatic K-module model 220, American Hamilton; Cincinnati, OH) designed to maintain a core body temperature at least 37°C, as measured by a thermister probe placed in the rectum. Interanimal variation in temperature was minimized with careful monitoring and the use of ice packs or warming blankets placed on the animal as needed.

A tracheotomy was performed, and pigs were mechanically ventilated (Fraser/Harlake anesthesia ventilator model 701; Orchard Park, NY) using room air supplemented with oxygen. A saline-filled catheter was placed in the right external jugular vein for drug administration and fluid infusion. A 9-Fr catheter introducer (Catheter Sheath Introducer System, Cordis; Miami, FL) was placed in the right carotid artery. Through this introducer catheter, a Mikro-Tip dual optical pressure transducer catheter (model SPC-780C, Millar) was inserted to measure aortic and ventricular pressure and permit simultaneous electronic differentiation to yield the change in pressure over change in time (dP/dt). End-tidal CO2 was monitored continuously (Hewlett-Packard model 78356A; Palo Alto, CA), arterial blood gases were measured periodically, and ventilatory parameters were adjusted as needed to maintain blood gases within physiological ranges. Slow intravenous infusion of normal saline maintained hydration throughout the surgery, and additional anesthetic was administered as needed.

A left thoracotomy was performed in the fourth intercostal space. The heart was suspended in a pericardial cradle, and the left anterior descending (LAD) coronary artery was isolated distal to the first or second diagonal branch. A strip of moistened umbilical tape was passed around the vessel for later coronary occlusion, which was accomplished by snaring it into a small plastic tube that allowed visualization of the occluded artery within it. Ischemia was verified by the development of a sharply defined region of cyanosis and electrocardiographic (ECG) changes. Reperfusion was verified by the appearance of reactive hyperemia in the previously occluded region. The chest incision was covered with moistened gauze to prevent desiccation and to provide thermal insulation.

Aortic and left ventricular blood pressure, left ventricular dP/dt, lead I of the ECG, and core body temperature were measured throughout the experiment and recorded using a Gould RS3800 Recorder (Gould; Cleveland, OH) and MacLab System (Apple Computer; Cupertino, CA). The pigs were allowed at least 20 min after surgical preparation to return to a steady state before experimentation.

Experimental design. Pigs were randomly assigned to one of five groups (Fig. 1). After various treatment protocols (see below), all animals were subjected to a 30-min test period of regional ischemia, followed by 3 h of reperfusion. After the surgical preparation was completed, baseline blood gases, hematocrit, hemodynamics, ECG, and temperature were measured. These measurements were repeated midway (15 min) into the test episode of ischemia. Any pig that developed ventricular fibrillation (VF) was resuscitated, if possible, using direct current low-energy (5–10 J) countershock applied through internal paddles within 10 s of onset. Intractable VF animals, defined as animals requiring more than three attempts to defibrillate during one episode of VF, were excluded from the study.

Pigs undergoing PC were subjected to two 5-min periods of regional ischemia, each followed by 10 min of reperfusion before the 30-min test period of ischemia. Diazoxide-treated groups received a 10-min intravenous infusion of the drug (3.5 mg/kg in saline, 1 ml/min; Schering) using a Harvard infusion pump (Harvard Apparatus PHD2000, Harvard Ap-
paratus; Holliston, MA), which was followed by a 10-min equilibration period before the test ischemia. 5-HD (5 mg/kg in saline; Sigma) was injected intravenously 15 min before either the diazoxide pretreatment or PC. Pilot studies in an additional four pigs verified that increasing the dose of 5-HD to 10 mg/kg did not affect our results in the PC group (data not shown).

At the completion of the experiment, heparin (5,000 units) and an additional 10 mg/kg pentobarbital were administered intravenously. The hearts were excised rapidly and processed for postmortem analysis of the area at risk (AAR) and infarct size.

Postmortem analysis. To determine the anatomic boundaries of the previously ischemic and nonischemic tissues, catheters were placed in the unoccluded left main coronary artery, the right coronary artery, and the LAD coronary artery at the level of occlusion. Triphenyltetrazolium chloride (TTC, 1%, Sigma) and monastral blue (4%, Sigma) were simultaneously infused at 37°C and 100–120 mmHg of perfusion pressure into the previously occluded LAD coronary artery, and the left main coronary artery plus right coronary artery, respectively. Both TTC and monastral blue were added to 90 mmol/l sodium phosphate buffer (pH 7.4), to which 1 mM dextran (mol wt 77,800, Sigma) was added to maintain physiological intravascular oncotic pressure. The heart then was fixed by coronary perfusion with, and subsequent immersion in, phosphate-buffered (pH 7.4) 3.7% formalin. At least 48 h postfixation, the left ventricle was removed and sliced into eight transverse slices, which were weighed and had their apical surfaces photographed. The color slides were digitized, the nonischemic area (stained blue), AAR (stained brick red), and area of infarction (nonstained) were identified and traced from ×15 magnified images, and infarct size was calculated using Sigma Scan Pro (Jandel Scientific; San Rafael, CA) on a personal computer-based computer. The infarct size was expressed as a fraction of the area of the occluded bed at risk of infarction (%AAR).

Data analysis. Data are expressed as group means ± SE. Differences between groups were compared using analysis of variance (ANOVA) using the Student-Newman-Keuls multiple-comparison posttest analysis. The size of the region of the left ventricle supplied by the occluded artery (AAR), collateral flow, and the animal’s core temperature (37°C) are significant independent predictors of infarct size. Thus our data analysis controlled for both the AAR and the animal’s temperature [innate collateral flow is absent in these species (29) and thus does not influence infarct size]. To control for variation in the AAR, the size of the infarction was expressed as a percentage of this area. To control for variation in temperature, differences in the relationships between infarct size and temperature were analyzed using analysis of covariance (ANCOVA) using infarct size as the dependent variable and temperature as the independent covariate. Adjusted group means generated by the ANCOVA program (e.g., mean infarct size adjusted for any intergroup variation in temperature) were compared using Student’s t-test. For all analyses, a P value of ≤0.05 was considered to indicate statistical significance.

RESULTS

Group assignments and mortality. Forty-two pigs were assigned to one of five groups. The number of animals enrolled in each group, the number developing VF, and exclusions are summarized in Table 1. Thirty minutes of ischemia followed by reperfusion is associated with a high incidence of VF in all groups. Twenty-four pigs developed VF during occlusion, and this generally occurred between 15 and 20 min of test ischemia. VF during reflow occurred in 11 pigs, generally within the first 30 s of reperfusion. Cardioversion and immediate restoration of blood pressure was successful in all but three experiments. Thus three pigs were excluded due to intractable VF. Miscellaneous technical difficulties resulted in the exclusion of three additional pigs that failed to complete the protocol. The final data analysis is based on 36 pigs.

Baseline predictors of myocardial infarct size. Hemodynamic data obtained midway through the 30-min occlusion are listed in Table 2. Heart rate and systolic or diastolic blood pressure did not differ significantly between any two groups. Contractility (assessed by dP/dt) also did not differ among groups.

The rate-pressure product (an index of myocardial oxygen demand), AAR, and rectal temperature measured at the midpoint of the test period of occlusion are shown in Fig. 2. Thus the baseline predictors of myocardial infarct size in pigs were not different among treatment groups.

Table 1. Animal mortality and exclusions

<table>
<thead>
<tr>
<th>Group</th>
<th>Number Entered</th>
<th>Intractable Oclusion</th>
<th>Technical Exclusions</th>
<th>Final Number Entered</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>8</td>
<td>6(1)</td>
<td>0(0)</td>
<td>0</td>
</tr>
<tr>
<td>PC</td>
<td>8</td>
<td>5(0)</td>
<td>3(0)</td>
<td>1</td>
</tr>
<tr>
<td>5-HD + PC</td>
<td>11</td>
<td>6(1)</td>
<td>4(1)</td>
<td>1</td>
</tr>
<tr>
<td>Diazoxide</td>
<td>8</td>
<td>5(0)</td>
<td>1(0)</td>
<td>1</td>
</tr>
<tr>
<td>5-HD + diazoxide</td>
<td>7</td>
<td>2(0)</td>
<td>3(0)</td>
<td>0</td>
</tr>
</tbody>
</table>

Incidence of ventricular fibrillation (VF) during either occlusion or reflow period is indicated for each group. Cardioversion and immediate restoration of blood pressure was generally successful (see RESULTS). Numbers within parentheses indicate the number of these animals excluded due to intractable VF. PC, myocardial ischemic preconditioning; 5-HD, 5-hydroxydecanoate.

Table 2. Hemodynamic variables at baseline and after 15 min of coronary artery occlusion

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Occlusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart Rate, beats/min</td>
<td>145 ± 10</td>
<td>157 ± 10</td>
</tr>
<tr>
<td>PC</td>
<td>127 ± 4</td>
<td>138 ± 5</td>
</tr>
<tr>
<td>5-HD + PC</td>
<td>133 ± 11</td>
<td>134 ± 9</td>
</tr>
<tr>
<td>Diazoxide</td>
<td>125 ± 7</td>
<td>137 ± 10</td>
</tr>
<tr>
<td>5-HD + diazoxide</td>
<td>130 ± 8</td>
<td>142 ± 9</td>
</tr>
<tr>
<td>Systolic blood pressure, mmHg</td>
<td>104 ± 3</td>
<td>98 ± 4</td>
</tr>
<tr>
<td>Control</td>
<td>108 ± 2</td>
<td>100 ± 4</td>
</tr>
<tr>
<td>PC</td>
<td>107 ± 3</td>
<td>104 ± 5</td>
</tr>
<tr>
<td>5-HD + PC</td>
<td>101 ± 1</td>
<td>95 ± 6</td>
</tr>
<tr>
<td>Diazoxide</td>
<td>109 ± 4</td>
<td>98 ± 3</td>
</tr>
<tr>
<td>5-HD + diazoxide</td>
<td>90 ± 4</td>
<td>81 ± 4</td>
</tr>
</tbody>
</table>

Values are means ± SE; data represent all surviving pigs (n = 36). There were no significant differences among groups in baseline or occlusion values.
Effect of 5-HD on PC. The regression of measured infarct size expressed as a percentage of the AAR versus temperature is shown in Fig. 3. As expected from previous experience with the canine model, there is a direct relationship between infarct size and temperature among untreated pigs. When limitation of infarct size occurs, the size of infarcts is less than that shown by the expected relationship. Two 5-min cycles of PC ischemia followed by 10 min of reperfusion markedly limited infarct size. However, pretreatment with the mitochondrial KATP channel blocker 5-HD did not block the cardioprotective effect of PC.

Effect of 5-HD on diazoxide-induced protection. The relationship between measured infarct size and temperature for the control group, the diazoxide-treated group, and the diazoxide-treated group that was also pretreated with 5-HD is illustrated in Fig. 4. Diazoxide markedly limited the infarct size in all pigs with the exception of one having a core temperature of 39.7°C. Pretreatment with 5-HD completely blocked this cardioprotective effect.

Adjusted mean infarct size for each group, derived from ANCOVA of these data, is shown in Fig. 5. Infarct size was limited markedly by both PC and diazoxide treatment. Pretreatment with 5-HD blocked the diazoxide-induced cardioprotection but did not prevent cardioprotection by PC.

DISCUSSION

The results of the present study indicate that, although opening of mitochondrial KATP channels with diazoxide infusion induces cardioprotection in pigs, cardioprotection against infarction, induced by a dual 5-min PC stimulus, does not require activation of these channels. 5-HD, a specific mitochondrial KATP channel blocker, prevented the cardioprotective effects of both PC and diazoxide.
blocker, prevented diazoxide-induced protection but had no effect on the endogenous protection induced by PC. Thus activation of the mitochondrial KATP channel is not a critical element in PC-induced protection.

KATP channels and cardioprotection. Identification of the end effector(s) ultimately activated by PC has been elusive, although some evidence suggests that opening of KATP channels represents a final step in this signal transduction process. Direct activation of KATP channels is cardioprotective. Administration of bimakalim (44) and cromakalim (13) before ischemia can limit infarct size in dogs. Armstrong et al. (1) showed that pinacidil could protect rabbit cardiomyocytes against simulated ischemia. In guinea pig hearts, postischemic recovery of function was improved greatly by either of the KATP channel openers BMS-180448 or cromakalim (12). Diazoxide preserves postischemic function as well as that observed with PC or cromakalim in isolated rat hearts (8). Similar results have been found in isolated rabbit cardiomyocytes (21) and anesthetized rabbits (3). Our results in pigs reported here provide further support that specific activation of mitochondrial KATP channels is protective against subsequent ischemia.

The means by which opening of mitochondrial KATP channels confers cardioprotection is unclear. In steady-state conditions, K⁺ uptake into the mitochondrial matrix is balanced by K⁺ efflux by a K⁺/H⁺ antiporter that likely maintains mitochondrial volume homeostasis. However, transient opening of mitochondrial KATP channels would cause a net influx of K⁺ and hence increase matrix volume, thereby altering mitochondrial volume regulation and control of cellular bioenergetics (7). This may be beneficial in ischemia as it may prevent wasteful ATP hydrolysis (8). Alternatively, because of the K⁺/H⁺ antiporter, K⁺ influx would tend to dissipate the potential across the inner membrane and uncouple electron transfer. This would reduce the driving force for Ca²⁺ accumulation into the mitochondria and thus prevent detrimental mitochondrial Ca²⁺ overload (16).

However, the mitochondrial KATP channel has not been established as an end effector in cardioprotection induced by endogenous PC. The mitochondrial KATP channel opening instead might be acting as a signal transduction element. Recent evidence obtained in isolated rabbit hearts suggests that KATP channel opening triggers protection through free radical generation and subsequent activation of kinases (26). The end effector modulated by this kinase pathway is unknown. In this in vivo preparation, 5-HD blockade would be effective both during the pretreatment phase as well as during test ischemia, and thus we do not explicitly distinguish between potential trigger and end effector roles of the channel. However, our data do not support the requirement of these channels as either a trigger or an end effector in PC.

The inability of channel blockade to prevent cardioprotection in myocardium preconditioned with ischemia despite blockade in myocardium preconditioned with diazoxide indicates that a fundamental difference exists in the mechanism initiated by these two stimuli. Much of the evidence in support of the KATP channel hypothesis is based on pharmacological blockade of the protective effects of PC by inhibitors of KATP channels (11). However, there have been discrepancies. Glibenclamide failed to block the protection of PC in isolated rat hearts (20), and 5-HD has been reported to either block (30) or have no effect (14) on PC in this species. Glibenclamide also did not prevent the anti-infarct effect of PC in rabbits (38), although 5-HD did block the cardioprotection of PC (15). PC in dogs could be abolished by both glibenclamide (10) and 5-HD (2). In pigs, KATP channel inhibition with glibenclamide blocked the reduction in infarct size by PC (34). 5-HD effectively blocked (-)-N⁶-(2-phenylisopropyl)-adenosine-induced cardioprotection (41), although we were unable to block endogenous protection by PC in the present study using a dose of 5-HD shown to effectively block the protection of PC in other protocols (15, 30) and diazoxide-induced protection.

Glibenclamide blocks both sarcolemmal and mitochondrial KATP channels, and it would be expected that a potential mechanism involving mitochondrial KATP channels would be susceptible to blockade by either glibenclamide or 5-HD. Disparate results among investigators may be attributed to differences in pharmacological profiles between these drugs. It has been shown that glibenclamide loses efficacy during ischemia (42), and thus PC may reduce its ability to block KATP channels. In contrast, the blocking activity of 5-HD appears to be seen only during ischemia by competing with the ATP binding site (22). Furthermore, it has been demonstrated that glibenclamide also blocks Na⁺/K⁺-ATPase (28), cardiac Cl⁻ channels (39), and the expression of inducible nitric oxide synthase (43). Thus 5-HD appears to be preferable to glibenclamide as a KATP channel antagonist when determining the involvement of mitochondrial KATP channels in PC.

Parallel pathways within the signal transduction response. Myocardial PC has been universally found to exert cardioprotective effects, although it is unclear if the mechanism of action is universally identical in all circumstances. More likely, redundant parallel signal
transduction pathways exist to confer the preconditioned state. For example, it appears that simultaneous activation of adenosine, bradykinin, and opioid receptors can all contribute to triggering PC. In contrast to results reported primarily in the rabbit that indicate that these triggers may converge on a common pathway involving G<sub>i</sub> protein and PKC activation, inhibition of PKC alone does not prevent PC in pigs (40). Combined inhibition of PKC and protein tyrosine kinase did effectively block the endogenous cardioprotection, suggesting a complex signal cascade involving both protein kinases acting through parallel pathways. Recently, it has been reported that 5-HD can completely block protection in isolated rabbit hearts triggered by bradykinin or opioids but not adenosine (6), indicating that all G<sub>i</sub>-coupled receptors may not use the same signal transduction pathway to trigger PC.

If redundant pathways subsequent to channel activation do exist, we cannot rule out the total possible contribution of K<sub>ATP</sub> channel activation as a trigger for the cardioprotective effect. For example, our data are consistent with a role for these channels as one of multiple activators of kinase pathways through free radical generation (26). Similarly, these data would be consistent with the contribution of K<sub>ATP</sub> channels as one of several end effectors in PC.

Severity of the stimulus may dictate the contribution of the signal transduction pathway. The degree of involvement of the multiple pathways may be dictated by the severity of the stimulus during the PC period. Although a threshold exists below which PC is ineffective, infarct size reduction is a graded phenomenon above this threshold (33) and may be dependent on recruitment of parallel mechanisms. 5-HD effectively prevented cardioprotection in anesthetized rats (30), rabbits (15), and dogs (2) in which the PC protocol utilized a single-cycle 5-min occlusion known to provide minimum-threshold levels of protection. However, in isolated rat hearts, protection induced by four cycles of PC could not be blocked by 5-HD (14). In the present study, two cycles of 5-min occlusions with intervening reperfusion periods would be expected to result in a maximal PC response based on results from our previous studies in dogs (36). Full activation of the cardioprotective response may recruit alternate pathways not involving mitochondrial K<sub>ATP</sub> channels that could mask any potential role of these channels in cardioprotection. Although we have established conditions of preconditioning that do not require mitochondrial K<sub>ATP</sub> channel activation, it is not known if a less severe preconditioning stimulus in this model can be blocked by mitochondrial K<sub>ATP</sub> channel inhibition.

Study limitations. We cannot measure the subcellular effects of 5-HD directly in these in vivo conditions. Because the time course of 5-HD administration was identical in both the diazoxide and PC groups, administered as a bolus 15 min before initiation of treatment, the diazoxide group serves as a surrogate control of the effectiveness of our 5-HD administration. This dose was sufficient to reach and inactivate the mitochondrial channels as evidenced by its effective blockade of diazoxide-induced protection. However, innate differences exist between these two treatment protocols subsequent to the 15-min 5-HD equilibration phase. In the diazoxide-treated group, the coronary artery supplying the AAR was open during the entire period before the test occlusion. The intermittent closed status of the artery during PC could have resulted in a somewhat different concentration of 5-HD within the myocardium during the PC cycles. Without a direct measurement of channel status at the mitochondrial level, we cannot be certain that all channels were completely inactivated by 5-HD treatment during both preconditioning cycles. However, even doubling the dose of 5-HD that was effective against diazoxide did not prevent cardioprotection by PC (see METHODS).

In summary, although opening these channels is cardioprotective, our data indicate that the pathway of endogenous protection afforded by PC is not dependent on mitochondrial K<sub>ATP</sub> channel activation but can be fully achieved through some as-yet-undefined end effector. Further studies need to be done to discern potential convergence of ischemic and diazoxide-induced preconditioning pathways on end effector(s) involved in this powerful cardioprotective mechanism.

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