ATP-sensitive potassium channel mediates delayed ischemic protection by heat stress in rabbit heart

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Hoag, Jeffrey B., Yong-Zhen Qian, Mohammed A. Nayeem, Michael D’Angelo, and Rakesh C. Kukreja. ATP-sensitive potassium channel mediates delayed ischemic protection by heat stress in rabbit heart. Am. J. Physiol. 273 (Heart Circ. Physiol. 42): H2458–H2464, 1997.—Heat shock protects against myocardial ischemia-reperfusion injury possibly via increased expression of heat shock proteins. The direct evidence of heat shock protein protection in vivo remains circumstantial, and no other new mechanism of protection has been proposed. Recent studies suggest that opening of ATP-sensitive K\(^+\) channels (K\(_{ATP}\) channels) plays an important role in ischemic preconditioning; however, it is not known whether this channel is also important in delayed protection conferred by heat shock. Anesthetized rabbits underwent heat shock treatment by raising core temperature to 42°C for 15 min. Twenty-four hours later, the animals were reassessed and subjected to regional ischemia-reperfusion. The specific K\(_{ATP}\) channel blockers glibenclamide (0.3 mg/kg ip) and sodium 5-hydroxydecanoate (5HD; 5 mg/kg iv) were used to block the channel function. The drugs were administered at two different times, either pre-heat stress or preischemia. Infarct size was determined by triphenyltetrazolium chloride staining. The 72-kDa heat shock protein (HSP 72) was measured by Western blots. Our results show that heat shock produced a marked reduction in infarct size (39.4 ± 8.1 to 14.3 ± 2.5% of risk area, P < 0.05). Glibenclamide and 5HD completely abolished heat shock-induced reduction in infarct size (42.3 ± 0.32 and 33.7 ± 4.8%) when given before ischemia-reperfusion; however, these antagonists failed to block protection when administered before the onset of heat shock. Furthermore, the enhanced expression of HSP 72 in heat shock groups was not diminished by glibenclamide or 5HD, suggesting a lack of a direct role of this protein in conferring cardiac protection by heat shock. The complete blockade of cardiac protection by glibenclamide and 5HD strongly suggests that opening of this channel is a very important component of heat shock-induced ischemic protection in rabbit hearts.

heat stress; adenosine 5′-triphosphate-sensitive potassium channel; heat shock proteins; ischemia

HEAT SHOCK, OR HYPERTERMIA, is an endogenous means of myocardial protection in which whole body hyperthermia protects the myocardium against ischemia-reperfusion injury 24 h after heat exposure (4–6). During heat shock, certain proteins known as heat shock proteins are synthesized. The major heat shock proteins are a set of highly conserved proteins having molecular masses of ~28, 70, 82, and 90 kDa. The most abundant and best studied subset of these is the 70-kDa protein family. It is now well established that multiple genes encode several distinct “70-kDa” heat shock protein members that have slightly different molecular weights and/or charges but are structurally and immunologically related (17). Some of these forms are typically heat inducible [the 72-kDa heat shock protein (HSP 72)], and their expression is stimulated severalfold after heat shock treatment. These proteins have subsequently been shown to protect the cells from further metabolic insults, including ischemia-reperfusion (4, 5, 13). Recent studies also suggest that opening of the ATP-sensitive K\(^+\) (K\(_{ATP}\) channel plays an important role in myocardial protection after acute ischemic preconditioning (9). This protection has been attributed to an increase in the outward K\(^+\) current, resulting in the shortening of the action potential (25), which in turn may spare ATP, thereby restricting entry of calcium into the myocyte through the voltage-sensitive calcium channels. Decreased intracellular calcium overload then reduces the ischemic injury and therefore enhances the preservation of myocytes. It has been shown that ischemic preconditioning is mimicked by acetylcholine, and this effect was completely abolished by the simultaneous administration of K\(_{ATP}\) channel antagonists in dogs (35) and rats (25). Recently, a preliminary study by Bernardo et al. (2) showed that the second window of ischemic preconditioning is also mediated by the opening of the K\(_{ATP}\) channel in rabbit heart. Grover et al. (10) showed that reduction in infarct size induced by the A\(_1\)-adenosine agonist (−)-N\(^6\)-(2-phenylisopropyl)-adenosine[(R)-N\(^6\)-(1-methyl-2-phenylethyl)-adenosine] was abolished by glyburide. Opening of K\(_{ATP}\) channels also appears to play a role after pharmacological preconditioning with monophosphoryl lipid A (MLA), a nontoxic derivative of endotoxin. Mei et al. (21) suggested that the endotoxin derivative MLA protects myocardium via activation of K\(_{ATP}\) channels. These results suggest that the K\(_{ATP}\) channel plays a major role in acute preconditioning and protection mediated by pharmacological agents such as adenosine, acetylcholine, and MLA. However, studies linking the K\(_{ATP}\) channel with heat shock-induced myocardial protection are currently lacking. Accordingly, the purpose of this investigation was to test 1) whether inhibition of K\(_{ATP}\) channels by glibenclamide or sodium 5-hydroxydecanoate (5HD) blocks heat shock-induced reduction in infarct size in vivo and 2) whether the inhibitors of K\(_{ATP}\) channels block the expression of HSP 72 in the heat-stressed rabbit hearts. We used an in vivo model of rabbit myocardial ischemia-reperfusion to answer these questions.
MATERIALS AND METHODS

Care of animals. All procedures involving animals were conducted in conformity with the guidelines of the Committee on Animals of Virginia Commonwealth University and the National Institutes of Health for the Care and Use of Laboratory Animals.

Whole body hyperthermia. Male New Zealand White rabbits weighing between 2.9 and 3.5 kg were anesthetized with intramuscular ketamine-HCl (35 mg/kg) and xylazine (5 mg/kg). A rectal thermometer was placed in each rabbit, and core temperature was raised to 42°C. The animals were then allowed to recover over the next 24 h at room temperature.

Surgical preparation. Rabbits were reanesthetized with ketamine-HCl and xylazine, and additional anesthetic was administered during the experiment as needed. A tracheotomy was performed, and the animals were ventilated by positive pressure with room air, which was supplemented with 100% oxygen if needed to maintain blood gases in the physiological range. The rate and volume of respiration were also adjusted during the experiment to maintain pH, PCO2, and PO2 within normal limits (pH between 7.3 and 7.5, PCO2 between 18 and 45 mmHg, and PO2 between 60 and 115 mmHg). Catheters were placed in the left carotid artery for blood pressure (Digi-Med Blood Pressure Analyzer model 200 with a TXD-310 pressure transducer) and arterial blood gas measurements (238 pH/Blood Gas Analyzer; Ciba Corning, Essex, UK) and in the jugular vein for drug and fluid administration. Leads were placed in all four limbs and attached to a Grass polygraph (model 7D; Quincy, MA) to record the animal’s electrocardiogram. Also, body temperature was maintained at 37°C during the surgical procedures and ischemia-reperfusion (I/R) experiments. A left thoracotomy was performed between the fourth and fifth ribs, and the pericardium was incised to expose the heart. A 5-0 silk thread was passed around the left anterior descending (LAD) coronary artery with an atraumatic needle and threaded through a small vinyl tube to form a snare. The coronary artery was occluded by pulling the snare tight and securing it with a hemostat. Myocardial ischemia was determined by S-T segment elevation and the appearance of regional cyanosis. Reperfusion was documented by observation of hyperemia and resumption of contractions in the area below the snare at the time of release.

Measurement of infarction and risk areas. After completion of the experimental protocol, the ligature around the LAD coronary artery was retightened, and ~4 ml of 10% Evans blue dye was injected into the jugular vein until the eyes turned blue. The rabbits were killed, and their hearts were harvested and cut into six transverse slices of equal thickness. The slices were stained by incubation for 15 min in 1% triphenyltetrazolium chloride (TTC) in an isotonic phosphate buffer (pH 7.4). Tetrazolium reacts with NADH in the presence of dehydrogenase enzymes, causing the viable tissue (area at risk) to stain a deep red color. The necrotic tissue does not react with TTC and remains pale yellow. After staining, the sections were placed in Formalin for preservation, and measurements of the risk area, the infarct area, and the area of the left ventricle (LV) were made using Bioquant imaging software for computer-aided morphometry. From each section, the ischemic risk area (unstained by blue dye) and the infarcted area (unstained by TTC) were outlined and measured by planimetry. The area from each region was averaged from the slices. Infarct size was expressed both as a percentage of total LV and as a percentage of the ischemic risk area.

Experimental protocol. Eight groups of rabbits were subjected to I/R, using the following protocols (Fig. 1): group I (control), 30 min of ischemia followed by 3 h of reperfusion; group II, control rabbits treated with glibenclamide (0.3 mg/kg ip) 30 min before I/R; group III, rabbits subjected to heat shock by raising core body temperature to 42°C for 15 min followed by I/R 24 h later; group IV, heat-shocked rabbits treated with glibenclamide 30 min before I/R; group V, rabbits given glibenclamide 30 min before heat shock followed by I/R 24 h later; group VI, rabbits treated with 5HD (5 mg/kg) 15 min before I/R; group VII, heat-shocked rabbits treated with 5HD 30 min before I/R; and, finally, group VIII, animals treated with 5HD 15 min before heat shock followed by I/R 24 h later. For Western blot analysis of HSP 72, animals were treated identically to groups above, except the animals were killed and hearts were collected without carrying out I/R.

Exclusion criteria. Animals were omitted from the study if 1) coronary artery occlusion did not produce severe ischemia (>20% of the ventricle at risk), 2) ventricular fibrillation or electrical instability occurred, or 3) heart rate was <100 beats/min or mean arterial pressure was <50 mmHg during ischemia.

Fig. 1. Diagrammatic representation of experimental protocol. Control, 30 min of ischemia followed by 3 h of reperfusion; Glib, control rabbits were treated with glibenclamide (0.3 mg/kg ip) 30 min before ischemia-reperfusion (I/R); HS, rabbits were subjected to heat shock by raising core body temperature to 42°C for 15 min followed by I/R 24 h later; Glib + HS, heat-shocked rabbits were treated with glibenclamide 30 min before I/R; Glib + 5HD, rabbits were given 5HD 30 min before heat shock followed by I/R 24 h later; 5HD, rabbits were treated with sodium 5-hydroxydecanoate (5HD; 5 mg/kg) 15 min before I/R; 5HD + HS, heat-shocked rabbits were treated with 5HD before I/R; and 5HD + Glib, heat-shocked rabbits were treated with 5HD 15 min before heat shock followed by I/R 24 h later.
severe hypotension was observed during the period of ischemia or reperfusion, or 3) the animal died during the surgical procedure and did not finish the entire protocol.

Measurement of heat shock proteins. Three animals from each of the experimental groups were used separately for measurement of HSP 72 by Western blot analysis. The animals were treated with each of the preparation protocols up to the point of I/R, at which point the hearts were quickly removed and the LV was separated and frozen in liquid nitrogen for Western blot analysis of HSP 72. The frozen LV tissue was homogenized in 0.1 M phosphate buffer (pH 7.0) containing 5% sodium dodecyl sulfate (SDS), 1% mercaptoethanol, and 0.1 mM phenylmethylsulfonyl fluoride (PMSF) for 1 min with a Polytron tissue homogenizer, using a PT-10 probe (Brinkmann), and then strained through a 27-gauge needle, followed by centrifugation at 14,000 revolutions/min for 10 min. Protein concentration was measured, using the Bio-Rad Protein Assay, based on Bradford’s dye-binding procedure (16). After electrophoresis, the proteins on the gel were transferred to Western polyvinylidene fluoride membranes (Schleicher & Schuell, Keene, NH) by electroelution. Protein transfer was confirmed by employing prestained molecular weight markers (Bio-Rad, Hercules, CA). After transferring and blocking with nonfat dry milk, we incubated the polyvinylidene difluoride membranes with a mouse monoclonal antibody cross-reacting to the inducible form HSP 72 (Stressgen Biotechnologies, Victoria, BC, Canada) at a dilution of 1:2,000. The second antibody was a horseradish peroxidase-conjugated rabbit anti-mouse immunoglobulin G (IgG) used at a 1:2,000 dilution. The membranes were developed with the use of enhanced chemiluminescence (Amersham, Arlington Heights, IL) and exposed to X-ray film for the appropriate time.

Drugs. Glibenclamide, Evans blue, mercaptoethanol, PMSF, and TTC were purchased from Sigma Chemical (St. Louis, MO). 5HD was purchased from Research Biochemicals International (Natick, MA). The vehicle for glibenclamide was 40% propylene glycol and 10% ethanol in deionized water mixed to a 1 mg/ml concentration. 5HD was dissolved in saline at a concentration of 15 mg/ml.

Statistics. All measurements of infarct size, area at risk, blood gases, and hemodynamic values are expressed as group means ± SE. Changes in blood gases, infarct size, and hemodynamic variables were analyzed by a two-way repeated-measures analysis of variance to determine the effects of time and group, using a SigmaStat software package. If the global tests showed major interactions, post hoc contrasts between different time points within the same group or among different groups were performed using Tukey’s test. Statistical differences were considered significant if P < 0.05.

RESULTS

Mortality and exclusion. Sixty-eight rabbits were initially entered into the infarct study. The number of rabbits assigned to each group, the number excluded, the survival percentage, and reasons for exclusion are summarized in Table 1.

<table>
<thead>
<tr>
<th>Group</th>
<th>Total No. of Animals</th>
<th>No. of Animals Excluded</th>
<th>Survival, %</th>
<th>Reason For Exclusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>9</td>
<td>2</td>
<td>78</td>
<td>Both animals developed severe arrhythmias during ischemia-reperfusion</td>
</tr>
<tr>
<td>HS</td>
<td>8</td>
<td>1</td>
<td>88</td>
<td>Animal died from prolonged ventricular tachycardia</td>
</tr>
<tr>
<td>Glib</td>
<td>10</td>
<td>3</td>
<td>70</td>
<td>Animals developed severe hypotension during reperfusion</td>
</tr>
<tr>
<td>Glib + HS</td>
<td>11</td>
<td>4</td>
<td>64</td>
<td>Four animals died: 1 from severe hypotension, 2 from thermometer malfunction, 1 from unknown causes 80 min into reperfusion</td>
</tr>
<tr>
<td>5HD</td>
<td>9</td>
<td>2</td>
<td>78</td>
<td>Animal died from prolonged ventricular tachycardia</td>
</tr>
<tr>
<td>HS + 5HD</td>
<td>8</td>
<td>1</td>
<td>88</td>
<td>Animal died from technical failure of respirator</td>
</tr>
<tr>
<td>5HD + HS</td>
<td>7</td>
<td>0</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>71</td>
<td>14</td>
<td>82</td>
<td></td>
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Blood gases. The blood gases and pH were measured to ensure consistent and sufficient respiration throughout the course of the experiments. The range of pH was between 7.3 and 7.5, PCO2 was between 19 and 43 mmHg, and PO2 was between 69 and 111 mmHg. The group means were within normal physiological limits throughout the experimental protocol.

Hemodynamic parameters. Heart rate, mean arterial blood pressure (MAP), and rate-pressure product are shown in Table 2. No significant differences in the baseline levels of these parameters were observed among groups. In addition, the heart rate and MAP remained relatively stable throughout the reperfusion period. Mean values were not significantly different among the groups at any time point for all the groups.

Infarct size and area at risk. Figure 2A shows the infarct size expressed as the percentage of anatomic area at risk in the eight groups. Infarct size was 39.4 ± 6.4% or 33.7 ± 4.8%, respectively (P < 0.01). Treatment of heat-shocked rabbits with glibenclamide or 5HD before I/R resulted in a significant increase in infarct size to 37.8 ± 6.4% or 33.7 ± 4.8%, respectively (P < 0.01). Glibenclamide and 5HD failed to block heat shock-induced reduction in infarct size when administered before the onset of whole body hyperthermia. Also, non-heat-shocked control rabbits treated with glibenclamide or 5HD had an infarct size of 42.3 ± 3.2% or 39.9 ± 2.7%, respectively. These values were not signifi-
Table 2. Hemodynamic values during ischemia and reperfusion

<table>
<thead>
<tr>
<th>Group</th>
<th>Baseline</th>
<th>Preischemia</th>
<th>End ischemia</th>
<th>30 min</th>
<th>60 min</th>
<th>120 min</th>
<th>180 min</th>
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</thead>
<tbody>
<tr>
<td>Control</td>
<td>HR</td>
<td>175±14</td>
<td>173±14</td>
<td>178±16</td>
<td>172±13</td>
<td>172±13</td>
<td>166±13</td>
</tr>
<tr>
<td></td>
<td>MAP</td>
<td>115±4</td>
<td>120±5</td>
<td>105±5</td>
<td>110±4</td>
<td>112±4</td>
<td>99±5</td>
</tr>
<tr>
<td></td>
<td>RPP</td>
<td>22.0±1.8</td>
<td>23.0±2.4</td>
<td>19.9±2.2</td>
<td>20.7±2.3</td>
<td>20.6±1.9</td>
<td>17.7±1.4</td>
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<tr>
<td>Glib</td>
<td>HR</td>
<td>190±13</td>
<td>209±14</td>
<td>211±15</td>
<td>203±12</td>
<td>210±18</td>
<td>211±15</td>
</tr>
<tr>
<td></td>
<td>MAP</td>
<td>113±7</td>
<td>118±10</td>
<td>108±10</td>
<td>101±9</td>
<td>95±9</td>
<td>82±9</td>
</tr>
<tr>
<td></td>
<td>RPP</td>
<td>23.5±2.6</td>
<td>27.2±3.2</td>
<td>24.6±2.3</td>
<td>22.5±2.4</td>
<td>22.2±2.6</td>
<td>19.7±2.3</td>
</tr>
<tr>
<td>HS</td>
<td>HR</td>
<td>169±8</td>
<td>188±11</td>
<td>191±12</td>
<td>181±9</td>
<td>173±11</td>
<td>178±13</td>
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<tr>
<td></td>
<td>MAP</td>
<td>109±7</td>
<td>113±8</td>
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<td>101±4</td>
<td>105±4</td>
<td>99±5</td>
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<tr>
<td></td>
<td>RPP</td>
<td>20.1±1.4</td>
<td>23.3±2.1</td>
<td>21.1±1.6</td>
<td>19.9±1.1</td>
<td>19.6±1.2</td>
<td>19.1±1.4</td>
</tr>
<tr>
<td>HS + Glib</td>
<td>HR</td>
<td>183±18</td>
<td>197±19</td>
<td>198±20</td>
<td>186±17</td>
<td>181±15</td>
<td>178±16</td>
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<td></td>
<td>MAP</td>
<td>105±10</td>
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<td>95±8</td>
<td>96±7</td>
<td>94±7</td>
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<tr>
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<td>RPP</td>
<td>20.8±2.1</td>
<td>19.6±1.1</td>
<td>19.5±0.9</td>
<td>18.7±0.9</td>
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<td>17.5±1.2</td>
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<tr>
<td>Glib + HS</td>
<td>HR</td>
<td>184±11</td>
<td>204±11</td>
<td>200±13</td>
<td>191±13</td>
<td>185±14</td>
<td>192±19</td>
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<td></td>
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<td>22.4±1.5</td>
<td>26.4±1.7</td>
<td>23.1±2.0</td>
<td>21.8±2.4</td>
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<tr>
<td>5HD</td>
<td>HR</td>
<td>195±9</td>
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<td>205±20</td>
</tr>
<tr>
<td></td>
<td>MAP</td>
<td>103±5</td>
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<td>RPP</td>
<td>22.5±2.0</td>
<td>23.8±2.2</td>
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<td>19.5±2.1</td>
<td>20.9±2.7</td>
<td>20.6±3.3</td>
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<tr>
<td>HS + 5HD</td>
<td>HR</td>
<td>182±6</td>
<td>206±10</td>
<td>201±14</td>
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<td>205±14</td>
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<tr>
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<td>18.7±1.1</td>
<td>18.8±1.2</td>
<td>17.8±0.9</td>
</tr>
</tbody>
</table>

Values are means ± SE for heart rate (HR; in beats/min), mean arterial pressure (MAP; in mmHg), and rate-pressure product (RPP; in (mmHg/min) x 1,000). See Fig. 1 for diagrammatic explanation of groups and protocol.

cantly different compared with control ischemic-reperfused hearts (39.4 ± 8.1%, P > 0.05).

Figure 2B shows the infarct size expressed as a percentage of the LV. A similar trend was observed. The mean infarct size value was 21.6 ± 4.4% in the control group; it was reduced significantly to 8.8 ± 1.4% in heat-shocked rabbits (P < 0.02). Again, both glibenclamide and 5HD significantly blocked heat shock-induced protection without having a significant effect in non-heat-shocked rabbits. Moreover, these drugs failed to block infarct size reduction when given before heat shock.

Figure 2C shows the area at risk in eight groups. These areas ranged from 50 to 64% with no significant differences among all the groups (P > 0.05). These data suggest that changes in the size of infarcts observed among various groups were not related to the percentage of area of LV occluded by our technique.

HSP 72. Because heat shock is known to increase the expression of HSP 72, we further investigated whether the blockade of heat shock-induced protection by glibenclamide or 5HD was accompanied by inhibition of this protein. Western blot analysis of cellular proteins was carried out to measure the synthesis of HSP 72, using monoclonal antibody directed against the inducible isoform of HSP 72.

Figure 3 shows the expression of HSP 72 in eight groups. A large increase in the expression of the inducible form of HSP 72 in the LV was observed in heat-shocked rabbits, whereas the non-heat-shocked control or the drug-treated control hearts demonstrated minimal expression of HSP 72. Furthermore, except for some variations, treatment with glibenclamide or 5HD either before or after heat shock did not decrease the expression of HSP 72.

DISCUSSION

The major findings of this investigation can be summarized as follows: 1) whole body hyperthermia, when given 24 h before I/R, resulted in a significant protection as indicated by reduction in infarct size; 2) specific KATP channel antagonists glibenclamide and 5HD significantly blocked the protective effect of whole body hyperthermia when given just before I/R, although they failed to block protection when administered before whole body hyperthermia; and, finally, 3) heat shock-induced expression of HSP 72 was not diminished by glibenclamide or 5HD. These data strongly suggest the involvement of KATP channels in heat shock-induced myocardial protection in the rabbit heart. Moreover, blockade of protection in heat-shocked hearts.
with a substantial concentration of HSP 72 present suggests that this protein may not have a direct role in protection; however, this protein could be linked to another effector of protection.

The mechanism of heat shock-induced myocardial protection continues to be enigmatic, although previous studies strongly suggest that one or more members of the heat shock protein family play an intimate role in protecting the myocyte from ischemic injury. Currie et al. (4) demonstrated that heat shock significantly improved contractile recovery, reduced creatine kinase efflux, and increased catalase activity during reperfusion in rat hearts. In vivo, a significant reduction in infarct size after heat shock in rat (6) and rabbit hearts (18) has also been reported. The amount of 70-kDa heat shock protein (HSP 70) induced was found to be correlated with reduction in infarct size (12). Moreover, transgenic mice overexpressing HSP 70 have been shown to be tolerant to the I/R injury (19, 26, 28). The increased levels of HSP 70 occurring after I/R have been associated with the preservation of tissue ATP (5), decreased production of oxygen-derived free radicals (23), and increased myocardial catalase levels (33).

Despite compelling circumstantial evidence for the role of HSP 72, direct proof of its protective role in vivo does not exist. Furthermore, a recent study from our laboratory demonstrated maximum production of the 27-kDa heat shock protein and HSP 72 by 4 h after whole body hyperthermia, whereas protection was not evident until after 12–24 h in the rat heart (32). These data suggest that the presence of heat shock proteins was not sufficient to explain the ischemic protection after heat shock. Accordingly, we hypothesized that either heat shock confers protection by an alternate mechanism or some unknown downstream event must be completed before heat shock or the heat shock proteins can provide protection in the ischemic heart. Therefore, in pursuit of this unknown effector of heat shock-induced protection, we considered the opening of the K\textsubscript{ATP} channels as a potential mediator of cardiac protection. Previous studies have demonstrated that K\textsubscript{ATP} channel blockers prevent the beneficial effects of preconditioning, whereas K\textsubscript{ATP} channel openers mimic the protective nature of ischemic and heat shock preconditioning (1, 3, 22). In the present investigation, we demonstrated that specific blockers of the K\textsubscript{ATP} channel completely abolished the delayed protective effect of heat shock. Both glibenclamide and 5HD were effective...
in attenuating heat shock protection only when administered just before the onset of ischemia but not when given before whole body hyperthermia 24 h earlier. Shigematsu et al. (31) reported that shortening of the action potential duration produced by no-flow ischemia persisted during the early phase of reperfusion and was preventable by glibenclamide when applied at the onset of reperfusion. Because the KATP channel blockers did not abolish preconditioning when administered before heat shock, we conclude that either the heat shock protein or the unknown effector activates the channel at least several hours after heat shock, after the drug has cleared the myocyte. This assumption mirrors our previous observations that heat shock preconditioning does not begin to appear until ∼12 h after whole body hyperthermia (32). Downey et al. (8) proposed that once the heart is subjected to a sublethal ischemia, the myocardium somehow remembers that it has been preconditioned. This memory most probably reflects the protein kinase C (PKC)-induced phosphorylation of an unknown protein that then activates the KATP channel in a functionally opened state for a certain period of time. On the basis of these findings, it has been postulated that the short-term-memory effect involved in ischemic preconditioning is related to persistent activation of the KATP channels caused by a preceding brief period of ischemia.

We do not know the mechanism by which heat shock may have opened KATP channels, although we can speculate on several possibilities. Recent studies by Hu et al. (11) have demonstrated that PKC activates KATP channels in rabbit and human ventricular myocytes by reducing channel sensitivity to intracellular ATP concentration. Phorbol 12,13-didecanoate-induced activation of the KATP channel was blocked by a highly selective PKC inhibitor in these studies. Direct evidence of the activation of PKC with heat shock is currently lacking, although a preliminary study from our laboratory demonstrated blockade of heat shock-induced cardiac protection in rat hearts by chelerythrine, a specific PKC antagonist (14). The mechanism by which heat shock activates PKC remains unclear. One plausible hypothesis is that heat shock transiently increases intracellular calcium, which may trigger a signal transduction cascade leading to the activation of PKC, which leads to phosphorylation of the KATP channel directly or via another unknown effector protein. A recent study by Saad and Hahn (29) reported activation of voltage-dependent K+ channels after heating in a radiation-induced fibrosarcoma cell line. These currents were blocked by tetraethylammonium cations with modification of extracellular K+ currents. Negulaeva et al. (24) demonstrated that exogenous HSP 70 resulted in an activation of outward currents through K+-selective channels. Although these studies do not directly implicate a similar activation of KATP channels induced by heat shock or heat shock proteins, such a possibility cannot be ruled out; further studies showing the cause-and-effect relationship, if any, of HSP 72 with the activation of KATP channels in cardiac myocytes warrant future investigations.

Some controversy surrounds the effectiveness of glibenclamide in blocking preconditioning. Thornton et al. (34) failed to block preconditioning with glibenclamide in the rabbit. In addition, they observed a proischemic effect of glibenclamide with an increase in infarct area in nonpreconditioned hearts. These authors argued that the anesthetic pentobarbital sodium may have affected the ability of glibenclamide to block preconditioning. Switching the anesthetic agent from pentobarbital sodium to ketamine-xylazine resulted in blockade of preconditioning (7). In the present study, using ketamine-xylazine as the anesthetic agent, we did not observe the proischemic effect of glibenclamide, as demonstrated by nonsignificant differences in the infarct size between glibenclamide-treated hearts and the nontreated controls. Similarly, Gross and Auchampach (9) and Qian et al. (27) used pentobarbital sodium in their dog and rat studies and still were able to block the preconditioning. In addition, we used SHD, an ischemia-selective KATP channel antagonist that has no known systemic metabolic effects (30). SHD only blocks the K+ current in the KATP channels under ischemic conditions (20). Our results show that SHD has no proischemic effects, as judged by the infarct size after I/R.

In conclusion, for the first time, our results show that myocardial protection induced by whole body hyperthermia is completely blocked by specific blockers of the KATP channel. These blockers did not inhibit the expression of HSP 72 in the intact myocardium, suggesting that this protein may not be directly involved in ischemic protection after heat shock. Alternatively, HSP 72 may be involved in the opening of KATP channels leading to the protection of the ischemic myocardium, either directly after posttranslational modification and/or translocation or indirectly through as yet unknown effectors. Consequently, further rigorous studies are required to clearly define the role of HSP 72 in myocardial protection and elucidate its relationship with the opening of the KATP channel at the cellular level.

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