Activation of p38 mitogen-activated protein kinase abolishes insulin-mediated myocardial protection against ischemia-reperfusion injury

Weidong Chai,1,* Yangsong Wu,1,* Guolian Li,1 Wenhong Cao,2 Zequan Yang,3 and Zhenqi Liu1

1Division of Endocrinology and Metabolism, Department of Medicine, University of Virginia Health System; 2Endocrine Biology Program, the Hamner Institutes for Health Sciences; and 3Department of Surgery, University of Virginia Health System, Charlottesville, Virginia

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Insulin is a major pro-survival hormone that has been demonstrated to reduce ischemic/hypoxic injuries in the myocardium or cultured cardiomyocytes. Insulin, as part of the glucose-insulin-potassium (GIK) combination, has long been used as a therapy for acute myocardial infarction and has been shown to significantly decrease in-hospital mortality (8) and overall mortality (24). In isolated cardiac myocytes subjected to ischemia and reoxygenation, administration of insulin reduces apoptosis and protects cells against irreversible injury (12). Preischemic treatment with insulin triggers an infarct-limiting cardioprotective response in rabbit myocardium (3). This insulin-mediated cardioprotection is independent of the presence of glucose and involves the phosphatidylinositol 3-kinase (PI 3-kinase)/protein kinase B (Akt)/p70S6K signaling mechanism, as pharmacological inhibition of PI 3-kinase with wortmannin and the mammalian target of rapamycin (mTOR) with rapamycin abolishes insulin-mediated cardioprotection (3, 13).

In ex vivo experiments, insulin is capable of diminishing myocardial infarct size only when given before the onset of reperfusion (12, 13). The mechanisms underlying this phenomenon remain unclear. During the ischemia-reperfusion process, many kinases within the ischemic area are activated, including extracellular signal-regulated kinase (ERK)1/2, c-Jun NH2-terminal kinase (JNK)1/2, and p38 mitogen-activated protein kinase (p38 MAPK) (2, 22, 35). p38 MAPK, a stress-activated serine/threonine protein kinase belonging to the MAPK superfamily, plays a major role in regulating apoptosis, cytokine production, transcriptional regulation, and cytoskeletal reorganization (36). Many stimuli, including ultraviolet light, irradiation, heat shock, ischemia, hypoxia, osmotic stress, proinflammatory cytokines, and certain mitogens can activate this kinase. Recent evidence suggests that p38 MAPK plays a major role in the myocardial ischemia-reperfusion injury as its targeted inhibition reduces the cardiac injury and cell death following ischemia-reperfusion in vivo (14, 18, 22). On the other hand, prior activation of this kinase, similar to recurrent, short-duration ischemia, preconditions the myocardium against subsequent ischemia-reperfusion injury (20, 27, 37).

We have recently demonstrated that p38 MAPK plays a central role in palmitate-induced apoptosis (6) and tumor necrosis factor-α-induced insulin resistance (17) in endothelial cells, and in fatty acid-induced insulin resistance in hepatocytes (19). Since ischemia-reperfusion, especially reperfusion, potently activates p38 MAPK (2, 22, 35), we examined in the present study whether p38 MAPK modulates insulin-mediated...
cardioprotection against ischemia-reperfusion injury in vivo. Here, we provide strong evidence that activation of p38 MAPK abolishes insulin-mediated cardioprotection, whereas inhibition of p38 MAPK enables insulin’s cardioprotection even 30 min after the onset of reperfusion.

RESEARCH DESIGN AND METHODS

Experimental Protocols

Adult male Sprague-Dawley rats weighing 250–300 g were studied after an overnight fast. All rats were on a 12:12-h light-dark cycle and fed standard lab chow and water ad libitum prior to experimentation. Rats were anesthetized with pentobarbital sodium (50 mg/kg ip; Abbott Laboratories, North Chicago, IL), placed in a supine position on a heating pad (Cole-Parmer Instrument, Vernon Hill, IL), and intubated with PE-90 tubing to ensure a patent airway. After cannulation of carotid artery and external jugular vein through a midline neck incision and a 30-min baseline period to ensure hemodynamic stability and a stable level of anesthesia, rats were studied using one of the following four protocols. During the study, mechanical ventilation was maintained with room air with the use of a Harvard rodent ventilator at a frequency of 47–50 strokes/min and a tidal volume of 1.5–2.5 ml. The arterial catheter was connected through a three-way stopcock to a pressure probe. The heart rate and mean arterial pressure were monitored throughout the study (Transonic Systems, Ithaca, NY). Pentobarbital sodium was infused at a variable rate to maintain steady levels of anesthesia and blood pressure throughout the study. At the end of the study, hearts were excised and processed according to the procedures described below for evaluation of myocardial infarct size or protein phosphorylation. The investigation conforms with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996) and the study protocols were approved by the Animal Care and Use Committee at the University of Virginia.

Protocol 1. Four groups of rats were studied under this protocol (Fig. 1A). Each rat received the ischemia-reperfusion procedure (see below for details). Ten minutes prior to the ligation of the left anterior descending (LAD) coronary artery (time = 0 min), an intravenous infusion of either normal saline and/or insulin (3 mU⋅min⁻¹⋅kg⁻¹) was begun, which lasted until the end of the reperfusion period. The insulin infusion rate was selected to increase the steady-state plasma insulin concentrations to high physiological range, ~500 pM (21). Insulin infusion was started either 10 min before the onset of ischemia (InsulinBI group), 5 min before the onset of reperfusion (InsulinAR group), or 30 min after the onset of reperfusion (InsulinAR group). During the insulin infusion, whole blood glucose was monitored every 10 min (Accu-Chek; Roche Diagnostics, Indianapolis, IN), and 30% dextrose was infused at a variable rate to maintain blood glucose within 10% of basal (21, 34). Control rats received only saline during the entire study period.

Protocol 2. Three groups of rats were studied under this protocol (Fig. 2). Each received an ischemia-reperfusion procedure and an intravenous injection of anisomycin (ASO, 2 mg/kg; Sigma, St. Louis, MO) 10 min prior to LAD ligation. Rats then received an intravenous infusion of either normal saline (Control + ASO) or a hyperinsulinemic euglycemic clamp (3 mU⋅min⁻¹⋅kg⁻¹) until the end of study. Insulin infusion was started either immediately after anisomycin injection, i.e., 10 min before the onset of ischemia (InsulinBI + ASO), or 5 min before the onset of reperfusion (InsulinAR + ASO).

Protocol 3. One group of rats was studied under this protocol (Fig. 3). After intravenous injection of anisomycin (ASO, 2 mg/kg; catalog no. A9789, Sigma), hearts were quickly excised at time 0, 10, 20, 30, 40, 70, or 210 min, freeze-clamped in liquid nitrogen, and stored at −70°C until analysis.

Protocol 4. Two groups of rats were studied under this protocol (Fig. 4). Each rat received the ischemia-reperfusion procedure and an intravenous injection of SB-239063 [trans-1-(4-hydroxycyclohexyl)-4-(4-fluorophenyl)-5-(2-methoxypyrimidin-4-yl) imidazole] (30 mg/kg; catalog no. S0560, Sigma) 10 min before the onset of reperfusion. Rats then received an intravenous infusion of saline (Control + SB-239063) or insulin, which began 30 min after the onset of reperfusion (InsulinAR + SB-239063). SB-239063 is a highly specific, second-generation p38 MAPK inhibitor (IC50 for p38 MAPK 0.044 μM, for MEK1/2 ERK1/2, and JNK >10 μM) (4). At the dose used, it completely inhibits p38 MAPK kinase activity (4, 10).

Ischemia-Reperfusion Procedure

A parasternal incision was made to open the left pleural cavity by cutting the left third and fourth ribs and intercostal muscle. A 6-0 polypropylene suture was passed underneath the LAD artery at a level
just below the left atrium. Myocardial ischemia was induced by tying the suture over two 2-0 silk sutures for a 30-min period. Significant electrocardiogram changes, including widening of QRS and elevation of S-T segment (monitored with a MacLab/8S Bridge/Bio Amplifier; AD Instruments), and color change (whitening) in the area at risk were used to confirm successful occlusion of the LAD artery. Reperfusion was initiated by untying the suture and lasted for 150 min.

**Determination of Myocardial Infarct Size**

At the end of each study, the heart was quickly excised, and the myocardial infarct size was determined. Briefly, the heart was cannulated through the ascending aorta and slowly flushed first with 3–5 ml of normal saline containing 5 U/ml heparin and then 10 ml of 1.0% triphenyltetrazolium chloride (TTC) in phosphate buffer (pH 7.4, 37°C). After TTC staining, the LAD was reoccluded by tightening the ligating suture left in the myocardium after ischemia-reperfusion procedure. The heart was then perfused with 5–8 ml of 5% phthalo blue to delineate the nonischemic tissue. After rinsing out of the excessive dye with ice-cold saline, the heart was then frozen at −10°C for 1 h and trimmed free of right ventricle and atria, and the left ventricle was cut into 5–7 transverse slices perpendicular to the long axis of the heart. Each slice was then fixed in 10% neutral buffered formalin solution. Each slice was weighed and photographed from both sides under a low-magnification microscope (Olympus SZX12; Olympus Optical) mounted with a high-resolution digital camera (DVC-1300 RGB Color; DVC, Austin, TX). The imaging system was controlled by a computer running Image Pro-Plus software (version 4.0; Media Cybernetics, Silver Spring, MD). The images were then transferred to PhotoShop (Adobe), and the borders of the infarction (TTC unstained area), ischemic risk area (TTC unstained and stained area) were traced for both sides of each tissue slice. The infarct size was determined using the following formula and was expressed as percentage of ischemic risk area:

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\text{infarct size (\%)} = \frac{\text{TTC unstained area}}{\text{TTC unstained area + TTC stained area}} \times 100.
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**Quantitation of ERK1/2, p38 MAPK, and JNK1/2 Phosphorylation**

Pieces (~30 mg) of frozen myocardium were weighed, powdered in ice-cold cell lysis buffer (20 mM Tris, pH 7.5, 150 mM NaCl, 1 mM EDTA, 1 mM EGTA, 1% Triton X-100, 2.5 mM sodium pyrophosphate, 1 mM β-glycerophosphate, 1 mM Na3VO4, 1 mg/ml leupeptin, 1 mM PMSF) and then disrupted by sonication using a microtip probe (0.5 s on and 0.5 s off for 45 s total) at a 3.0 power setting on the Fisher XL2020 sonicator (Fisher Scientific, Pittsburgh, PA). The homogenate was centrifuged at 15,000 g for 10 min at 4°C. Aliquots of the supernatant containing ~100 μg of protein were diluted with an equal volume of SDS sample buffer and electrophoresed on a 10% polyacrylamide gel. Proteins were then electrophoretically transferred to nitrocellulose membranes. After being blocked...
with 5% low-fat milk in Tris-buffered saline plus Tween 20, membranes were incubated with antibodies against phospho-ERK1/2 (Thr<sup>202</sup>/Tyr<sup>204</sup>), phospho-p38 MAPK (Thr<sup>180</sup>/Tyr<sup>182</sup>), or phospho-SAPK/JNK (Thr<sup>183</sup>/Tyr<sup>185</sup>) (Cell Signaling, Beverly, MA), respectively, overnight at 4–8°C according to manufacturer’s recommendations. After incubation with a second antibody coupled to horse-radish peroxidase, the blots were developed using an enhanced chemiluminescence Western blotting kit (Amersham Life Sciences, Piscataway, NJ). Autoradiographic films were scanned densitometrically (Molecular Dynamics, Piscataway, NJ) and quantitated using ImageQuant 3.3 software. To ensure equal loading of proteins, all membranes were stripped and reprobed using antibodies against p38 MAPK, JNK, or ERK1/2, respectively, and the ratios of phosphospecific to total density were calculated.

Quantitation of IRS-1 Serine Phosphorylation

After the tissue homogenization and centrifugation described above, aliquots of supernatant containing 500 μg of protein in 1,000 μl of lysis buffer were incubated with 4 μg of primary antibody against IRS-1 (catalog no. 06-248; Upstate Cell Signaling, Lake Placid, NY) overnight at 4°C. Protein G-agarose was then added, and the mixture was kept at 4°C for 1 h with gentle rocking. After being washed six times with lysis buffer, the beads were spun down (1,000 g for 30 s), resuspended in 25 μl of 2 × sample buffer (375 mM Tris-HCl, pH 6.8, 12% SDS, 60% glycerol, 300 mM dithiothreitol, and 0.06% bromophenol blue), and boiled for 5 min. IRS-1 serine phosphorylation was then determined as stated above by using antibodies against phosphoserine (catalog no. AB 1603; Millipore, Billerica, MA) and IRS-1.

Statistic Analysis

All data are presented as means ± SE. Statistical analyses were performed with SigmaStat 3.1.1 software (Systat Software), using one-way repeated-measures ANOVA with post hoc Bonferroni testing or two-tailed t-test where appropriate. A P value of <0.05 was considered statistically significant.

RESULTS

Temporal Dependence of Insulin-Mediated Myocardial Protection Against Ischemia-Reperfusion Injury

We first examined the temporal dependence of the insulin effect on infarct size following the ischemia-reperfusion procedure. As shown in Fig. 1, 30 min of ischemia followed by 150 min of reperfusion resulted in an infarct size of 53 ± 2% in saline control rats. Insulin, when given prior to the onset of ischemia (Insulin<sub>BI</sub> group), reduced the infarct size by ~60% (to 21 ± 4%, P < 0.001). When given before the onset of reperfusion (Insulin<sub>BB</sub> group), insulin then significantly decreased the infarct size by 32% (to 36 ± 4%, P < 0.02). However, this effect was significantly less than that seen in the Insulin<sub>BI</sub> group (P < 0.05). This insulin-mediated decrease in the infarct size disappeared entirely if insulin was given 30 min after the onset of reperfusion (Insulin<sub>AR</sub> group 54 ± 4%, P = 0.101 vs. Control).

Ischemia-Reperfusion Activates MAPKs

As ischemia-reperfusion activates various MAPKs that are involved in cell growth, death and apoptosis, and insulin resistance, we next explored the impact of ischemia-reperfusion on three major MAPKs in the myocardium, including ERK1/2, JNK1/2, and p38 MAPK. After rats were subjected to the ischemia-reperfusion procedure with saline infusion during the entire study, hearts were excised, and the ischemic area was dissected from the healthy (nonischemic) area. Protein phosphorylations of ERK1/2, JNK1/2, and p38 MAPK between the ischemic area and nonischemic area were compared. As shown in Fig. 2, ischemia-reperfusion increased the phosphorylation of ERK1/2 3.5-fold (3.2 ± 0.4 vs. 0.9 ± 0.3, P < 0.0007), of JNK1/2 7.4-fold (0.6 ± 0.09 vs. 0.08 ± 0.05, P < 0.02), and of p38 MAPK 23-fold (2.3 ± 0.8 vs. 0.1 ± 0.08, P < 0.05).

Activation of p38 MAPK Abolishes Insulin-Mediated Cardioprotection Against Ischemia-Reperfusion Injury

We have previously shown (6, 17, 19) that p38 MAPK plays a central role in fatty acid and cytokine-induced apoptosis and...
insulin resistance in cultured endothelial cells and hepatocytes. Given the fact that we observed a 23-fold increase in p38 MAPK phosphorylation in the ischemic area, we next examined the impact of p38 MAPK activation on insulin-mediated myocardial protection during the ischemia-reperfusion procedure by intravenously injecting anisomycin (2 mg/kg) 10 min before the onset of ischemia. Anisomycin, a translational inhibitor secreted by streptomyces spp., strongly activates p38 MAPK and JNK in mammalian cells (11, 30, 39), thus mimicking the effect of ischemia-reperfusion. As shown in Fig. 3, intravenous injection of anisomycin did not change the infarct size in saline-infused animals (Control + ASO 54 ± 1%) but completely abolished the insulin-mediated decrease in infarct size in the rats that received insulin either before the onset of ischemia (InsulinAR + ASO 46 ± 3%) or before the onset of reperfusion (InsulinAR + ASO 48 ± 8%).

Activation of p38 MAPK is Associated with Increased IRS-1 Serine Phosphorylation in Rat Myocardium

To further define the relationship between p38 MAPK/JNK activation and insulin resistance in the rat myocardium, we examined the time course of p38 MAPK/JNK activation. After intravenous injection of anisomycin (2 mg/kg), hearts were excised at 0, 10, 20, 30, 40, 70, and 210 min, and the phosphorylation of p38 MAPK, and JNK1/2 was determined. As shown in Fig. 4, anisomycin significantly increased the phosphorylation of JNK1/2 (P = 0.003, ANOVA), with maximal effect at 20 min (3-fold increase, P < 0.02). JNK phosphorylation gradually returned to baseline after 40 min. On the other hand, the increase in the phosphorylation of p38 MAPK was greater (P < 0.001, ANOVA), with a nearly 8-fold increase at 10 min and a 12-fold increase at 20 min. Even at the end of the study (210 min), p38 MAPK phosphorylation remained significantly higher than the baseline (2-fold, P < 0.04). This was associated with a marked and persistent increase in IRS-1 serine phosphorylation (P < 0.001, ANOVA), which started at 20 min and persisted to the end of the study (210 min; Fig. 4C). Thus, it appears p38 MAPK activation is more likely the cause of persistent IRS-1 serine phosphorylation.

Inhibition of p38 MAPK Enables Insulin-Mediated Cardioprotection Even in InsulinAR Rats

We next examined whether inhibition of p38 MAPK would restore insulin sensitivity in protecting myocardium against the ischemia-reperfusion injury in InsulinAR rats. Rats were given SB-239063, a potent and specific p38 MAPK inhibitor, intravenously 10 min before reperfusion. SB-239063 treatment per se slightly but significantly decreased the infarct size in saline-infused rats (Control + SB-239063, 39.6 ± 10.5%, P < 0.03 vs. Control). This is consistent with prior reports of p38 MAPK inhibition protecting myocardium against ischemia-reperfusion injury (14, 22, 23). However, the infarct size in InsulinAR + SB-239063 rats decreased to 16.5 ± 4.5%, a level comparable to that seen in InsulinBI rats (P = 0.377). Thus, inhibition of p38 MAPK completely restored insulin’s cardioprotective effect, even when insulin was given 30 min after the onset of reperfusion.

DISCUSSION

Using a rodent ischemia-reperfusion model and the insulin clamp technique, the present study provides strong evidence that, although insulin potently limits myocardial infarct size when given before reperfusion, activation of p38 MAPK abolishes this insulin-mediated cardioprotection and inhibition of p38 MAPK restores insulin’s effect, even when insulin is given 30 min after reperfusion in vivo.

The finding of a temporal dependence of insulin’s cardioprotective effect is important. As shown in Fig. 1, when insulin was given prior to the onset of ischemia it reduced the infarct size by ~60%. However, 30 min of ischemia before giving insulin significantly blunted insulin’s effect, and an additional 30 min of reperfusion prior to giving insulin completely abolished its effect. This is consistent with a previous study in isolated,perfused rat hearts subjected to 35 min of regional myocardial ischemia and 2 h of reperfusion, in that insulin administered at the onset of reperfusion attenuated infarct size by 45%, but the protection was abrogated if insulin administration was delayed until 15 min into reperfusion (13). Thus, it is not surprising that in a large, randomized controlled trial conducted among patients with ST-segment elevation myocardial infarction who presented within 12 h of symptom onset intravenous GIK infusion for 24 h had a neutral effect on mortality, cardiac arrest, cardiogenic shock, and reinfarction (32). The prolonged delay after the onset of symptoms might have contributed to the lack of benefit of GIK infusion in that study. This underscores the importance of using insulin as early as possible after the onset of ischemic symptoms and prior to the reestablishment of coronary perfusion to maximally salvage myocardium.

We have in the current study observed a 23-fold increase in p38 MAPK phosphorylation in the ischemic area in saline control rats and inhibition of p38 MAPK prior to the onset of reperfusion moderately but significantly decreased the infarct size. This is consistent with previous reports that myocardial ischemia-reperfusion potently activates p38 MAPK (2), which functions as a pro-death signaling effector in cultured cardiomyocytes as well as the intact heart. In cultured neonatal rat
Cardiac myocytes, ischemia activated p38 MAPK and induced apoptosis, and inhibition of p38 MAPK with its specific inhibitor SB-203580 reduced the activation of caspase-3 and protected cardiac myocytes against extended ischemia in a dose-dependent manner (23). Similarly, inhibition of p38 MAPK decreased myocardial apoptosis and improved posts ischemic cardiac function in a rodent ischemia-reperfusion model (22).

Transgenic mice expressing a dominant-negative mitogen-activated protein kinase kinase (MKK6) mutant or a dominant-negative p38α mutant were each significantly protected from ischemia-reperfusion injury, as assessed by infarct area measurements, DNA laddering, terminal deoxynucleotidyltransferase-mediated dUTP nick end labeling (TUNEL), and functional assessment of ventricular performance (14). This effect likely occurred via upregulation of Bcl-2 protein levels (14). In vitro, adenoviral-mediated gene transfer of dominant-negative-encoding p38 vectors reduced apoptosis induced by 2-deoxyglucose treatment, whereas overexpression of wild-type p38α or an activated M KK6 mutant each enhanced cell death (14).

Reperfusion appears more important in activating p38 MAPK in the myocardium than ischemia. In rat hearts subjected to 20 min of global ischemia, MAPK-activated protein kinase-2 (MAPKAPK2), a kinase immediately downstream from p38 MAPK, activity did not change (27). In rabbit or canine models, ischemia alone caused only a moderate but transient increase in p38 MAPK activity whereas ischemia followed by reperfusion caused a persistent elevation in p38 MAPK activity with maximal activation being reached 10–15 min after reperfusion (22, 35). As p38 MAPK is a very important player in mediating cell apoptosis, this difference in p38 MAPK activation may explain the finding that in anesthetized open-chest dogs permanent ischemia without reperfusion caused only necrosis with very few apoptotic cells, whereas ischemia followed by reperfusion caused both necrosis and apoptosis (38). This may also explain the cardioprotective effect of p38 MAPK inhibition that is also temporally dependent, as administration of SB-203580 before ischemia and during reperfusion completely inhibited p38 MAPK activation and exerted the most cardioprotective effects, whereas administering SB-203580 10 min after reperfusion (when p38 MAPK is maximally activated) failed to convey significant cardioprotection (22).

The observations that anisomycin treatment abolished insulin-mediated myocardial protection in insulin1R and insulin2R rats and that inhibition of p38 MAPK enabled insulin effect in insulin2R rats strongly suggest that activation of p38 MAPK plays a pivotal role in this temporal dependence of insulin’s infarct-limiting effect. Although the exact mechanisms underlying this p38 MAPK-mediated insulin resistance in the myocardium is unclear, our results show that activation of p38 MAPK with intravenous injection of anisomycin was associated with a persistent, twofold increase in IRS-1 serine phosphorylation. Serine phosphorylation of IRS-1 reduces insulin signaling through the PI 3-kinase/Akt pathway, and insulin exerts its many metabolic and pro-survival effects mainly via this pathway (3, 12, 13, 16, 28, 29, 31, 33). We have previously demonstrated in cultured endothelial cells and hepatocytes that p38 MAPK plays a critical role in free fatty acid and tumor necrosis factor-α-induced insulin resistance by increasing serine phosphorylation and decreasing tyrosine phosphorylation of IRS-1 (17, 19). As stated above, the effect of p38 MAPK may also be related to increased cell apoptosis as it mediates the apoptotic process (36), and we (6) have recently demonstrated that p38 MAPK plays pivotal role in palmitate-induced apoptosis in cultured endothelial cells. Taken together, p38 MAPK not only mediates reperfusion injury but also induces insulin resistance by increasing IRS-1 serine phosphorylation and abolishes insulin’s myocardial protective effects.

Inasmuch as ischemia-reperfusion also activates JNK (2, 9, 25, 35) which also mediates cellular apoptosis, contradictory reports have shown a pro-survival and apoptotic function in cultured cardiomyocytes and in vivo experiments. Although inhibition of JNK using a peptide inhibitor (25) or a nonpeptide ATP-competitive inhibitor (9) protected the myocardium against ischemia-reperfusion injury, both genetic inhibition and activation of JNK1/2 within the heart in mice protected the myocardium from ischemia-reperfusion-induced cell death in vivo (15). Moreover, activation of JNK has been shown to promote survival of cardiac myocytes after oxidative stress (7) and be cytoprotective during nitric oxide-induced cardiac myocyte apoptosis (1). These findings underscore the complexity associated with JNK signaling in the regulation of cellular apoptosis. In the current study, we also observed a sevenfold increase in JNK phosphorylation following ischemia-reperfusion. This increase in JNK phosphorylation appears to be mediated by reperfusion and not by ischemia, as a modest decline in JNK activity was observed after 30 min of global ischemia in rat hearts (27). Moreover, ischemia per se for 30 or 45 min failed to activate JNK, but reperfusion was associated with a gradual activation of JNK (35). Although JNK activation in the current study may have contributed to cell injury, it appears that JNK activation alone cannot account for ischemia-reperfusion-induced insulin resistance, as inhibition of p38 MAPK with SB-239063 completely restored insulin sensitivity in protecting myocardium even when insulin was given 30 min into reperfusion. At the dose selected, SB-239063 specifically inhibits p38 MAPK but not JNK (4, 10).

In summary, the present study demonstrated a temporal dependence of insulin-mediated myocardial protection against ischemia-reperfusion injury. We also showed that activation of p38 MAPK not only mediates ischemia-reperfusion injury, it also abolishes insulin’s cardioprotective effects. Our results suggest that early use of insulin and targeted inhibition of p38 MAPK in the myocardium may have therapeutic potential in patients with acute coronary syndrome.

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