Tissue-specific Pattern of Stress Kinase Activation in Ischemic/Reperfused Heart and Kidney*

In this report we investigate the molecular mechanisms that contribute to tissue damage following ischemia and ischemia coupled with reperfusion (ischemia/reperfusion) in the rat heart and kidney. We observe the activation of three stress-inducible mitogen-activated protein (MAP) kinases in these tissues: p38 MAP kinase and the 46- and 55-kDa isoforms of Jun N-terminal kinase (JNK46 and JNK55). The heart and kidney show distinct time courses in the activation of p38 MAP kinase during ischemia but no activation of either JNK46 or JNK55. These two tissues also respond differently to ischemia/reperfusion. In the heart we observe activation of JNK55 and p38 MAP kinase, whereas in the kidney all three kinases are active. We also examined the expression pattern of two stress-responsive genes, c-Jun and ATF3. Our results indicate that in the heart both genes are induced by ischemia and ischemia/reperfusion. However, in the kidney c-Jun and ATF3 expression is induced only by ischemia/reperfusion. To correlate these molecular events with tissue damage we examined DNA laddering, a common marker of apoptosis. A significant increase in DNA laddering was evident in both heart and kidney following ischemia/reperfusion and correlated with the pattern of kinase activation, supporting a link between stress kinase activation and apoptotic cell death in these tissues.

Ischemia and ischemia coupled with reperfusion (ischemia/reperfusion) in the heart and kidney result in cell death and scar formation in these tissues, which can ultimately lead to congestive heart failure or renal failure and death. Recent studies in heart tissue, both in vitro and in vivo, suggest that cell death upon reperfusion is largely apoptotic in nature (1–4). In vivo studies demonstrated that apoptosis following occlusion of the coronary artery in the rat is the major contributing factor to myocardial damage after the insult. Subsequently, necrosis occurs and contributes to the progressive loss of cardiomyocytes with time after infarction (5). Apoptotic cell death also appears to play a role in hypertrophy of cardiac tissue in response to pressure overload (6, 7). The occurrence of apoptotic cell death in renal tissue has also been demonstrated following brief periods of ischemia and subsequent reperfusion (8).

The molecular mechanisms by which ischemia and ischemia/reperfusion lead to cell death and eventually to tissue damage have not yet been defined. Recent studies suggest that members of the mitogen-activated protein (MAP) kinase family, in particular Jun N-terminal kinase (JNK), are activated in the heart and kidney following ischemia and reperfusion of these tissues (9, 10). Therefore, this kinase signaling pathway may be an important molecular component responsible for tissue injury following ischemia and ischemia/reperfusion in the heart and kidney. However, the role of individual isoforms of JNK or the role of p38 MAP kinase has not been addressed.

The MAP kinase family, members of which are characterized as proline-directed serine/threonine-protein kinases, can be divided into three subgroups: the extracellular signal-regulated kinases, JNKs (also referred to as stress-activated protein kinases), and p38 MAP kinases. These kinase pathways are distinguished by activating signals, substrate specificity, and cellular responses (for review, see Refs. 11–13). While the extracellular signal-regulated kinases are predominantly activated by growth factors, the JNKs and p38 MAP kinases are activated by stress signals such as inflammatory cytokines, heat shock, ultraviolet light, and ischemia (9, 10, 14–16). Because JNKs and p38 MAP kinases are generally activated by the same stress signals, they have been collectively referred to as the stress kinases.

Two members of the JNK family (JNK1 (46 kDa) and JNK2 (55 kDa)) were initially identified as kinases with high affinity for the transcription factor c-Jun. JNKs phosphorylate c-Jun on specific N-terminal serine residues (Ser-63 and Ser-73) and enhance the ability of c-Jun to activate expression of genes containing c-Jun-responsive promoter elements (14, 17–20). Additional substrates for JNKs have been defined since their first identification; the substrates include ATF2 and Elk-1 (21, 22). Recently, at least 10 isoforms of JNK have been identified, which correspond to alternatively spliced variants derived from three genes. These JNK isoforms differ in their interaction with substrate proteins in vitro, suggesting that these isoforms may play specific roles in vivo (23). However, specific activation of individual isoforms in vivo has not been demonstrated to date. Multiple isoforms of p38 MAP kinase have also been identified (24–27). Although p38 MAP kinases and JNKs are activated by similar stress signals, the kinase cascade leading to the activation of p38 MAP kinases is distinct from the kinase cascade leading to the activation of JNKs (for review, see Ref. 28). Furthermore, the substrate specificity of p38 MAP kinase, although not well defined, is distinct from that of JNKs: p38 MAP kinase phosphorylates mitogen-activated protein kinase-activated protein kinase-2 and -3 and CHOP, which are not substrates for JNKs (29–31).

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for the stress kinases (JNKs and p38 MAP kinase) in stress-induced apoptosis (32–35). Furthermore, one of the major JNK substrates, c-Jun, has been implicated to be important in neuronal apoptosis (36, 37). In light of these observations, we examined the patterns of stress kinase activation in ischemic and ischemic/reperfused heart and kidney. Our results demonstrate specific patterns of stress kinase activation in these injured tissues. Furthermore, we observe a strong correlation between stress kinase activation, increased expression of stress kinase responsive genes, and initiation of apoptotic cell death. These results provide insight into the molecular events leading to tissue injury following ischemia/reperfusion. Their implications with respect to the tissue-specific patterns of injury progression are discussed.

EXPERIMENTAL PROCEDURES

Reagents—Antibodies specific for phosphorylated p38 MAP kinase (catalog number 9211S) were purchased from New England Biolabs and used at 1 μg/ml for Western blot analysis. Anti-p38 MAP kinase C-terminal antibodies (catalog number sc-553) and anti-JNK antibodies (catalog number sc-571) were purchased from Santa Cruz Biotechnolog Inc., and 0.5 μg/ml was used for Western blot analysis. The ECL kit for Western blot analysis was obtained from Amersham Life Science. GST-T–Jun (amino acids 1–223) protein was produced in Escherichia coli and purified using glutathione-Sepharose-4B (Pharmacia Biotech Inc.).

Ischemia and Ischemia/Reperfusion of Isolated Rat Heart—Adult male Sprague-Dawley rats were injected with heparin (5000 IU/kg, body weight intraperitoneally) and then were anesthetized with pentobarbital sodium (75 mg/kg, body weight, intraperitoneally). The hearts were excised and were placed in ice-cold Krebs-Henseleit buffer (containing 118 mM NaCl, 4.7 mM KCl, 2.5 mM CaCl2, 1.2 mM MgSO4, 1.2 mM KH2PO4, 25 mM NaHCO3, 0.5 mM EDTA, and 5 mM glucose). Each heart was immediately cannulated at the aorta, and perfusion was initiated with oxygenated (85% O2, 5% CO2) Krebs-Henseleit buffer (37°C) at a perfusion pressure of 75 mm Hg. Hearts were perfused for 30 min with Krebs-Henseleit buffer for equilibration. At this point hearts were subjected to global ischemia for 20 or 45 min and then were perfused for an additional 60 min. The presence of an ischemic insult was verified by a discernible darkening of the myocardial surface circumscribed by the epicardial coronary vasculature distal to the occlusion site.

In gel Kinase Assay—In gel kinase assays were performed as described previously (38) with some modifications. Proteins (80 μg/sample) extracted from heart and kidney tissues were separated on a 10% SDS-polyacrylamide gel that had been polymerized in the presence of 50 μg/ml GST-T–Jun (amino acids 1–223). After electrophoresis, the gel was washed five times for 10 min with 60 μl of buffer A (20 mM β-glycerophosphate, 20 mM sodium pyrophosphate, 0.2 mM sodium vanadate, 2 mM EDTA, 20 mM sodium fluoride, 10 mM benzamidine, 1 mM DTT, 20 μg/ml leupeptin, and 0.2 mM pefabloc SC) containing a Donice homogenizer. Cell debris was removed by centrifugation (20,000 × g for 10 min at 4 °C), and the supernatant was stored in aliquots at −70 °C.

Tissue Homogenization—A powder of frozen heart and kidney tissues was prepared using a mortar and pestle. For homogenization, 100 mg of tissue powder was weighed out and was homogenized in 1 ml of buffer B (20 mM HEPES (pH 7.5), 20 mM β-glycerophosphate, 20 mM sodium pyrophosphate, 0.2 mM sodium vanadate, 2 mM EDTA, 20 mM sodium fluoride, 10 mM benzamidine, 1 mM DTT, 20 μg/ml leupeptin, and 0.2 mM pefabloc SC) using a Dounce homogenizer. Cell debris was removed by centrifugation (20,000 × g for 10 min at 4 °C), and the supernatant was stored in aliquots at −70 °C.
RESULTS

Pattern of Stress Kinase Activation in Ischemic and Ischemic/Reperfused Heart—Isolated rat hearts were subject to global ischemia for either 20 or 45 min prior to reperfusion. Individual hearts were reperfused for various periods ranging from 1 min to 3 h. Extracts from the hearts were then prepared, and stress kinase activity was measured by either in gel kinase assay using c-Jun (amino acids 1–223) as a substrate, or Western blotting using phospho-specific antibodies.

Neither JNK46 nor JNK55 activity, measured by in gel kinase assay, was detectable in control hearts or in hearts subjected to ischemia for either 20 min (data not shown) or 45 min (Fig. 1A). However, JNK55 activity was detectable within 1 min following reperfusion; it continued to increase, reaching maximal activity at approximately 1 h postreperfusion. Significant levels of JNK46 activity were still present at 3 h postreperfusion. Interestingly, no significant levels of JNK46 activity were detectable upon reperfusion of the heart until 1 h postreperfusion, when some weak JNK46 activity was evident. The lack of significant JNK46 activation in the heart was not a consequence of the absence of JNK46 expression, because JNK46 protein was readily detectable by Western analysis using an antibody recognizing both isoforms (Fig. 1B). Furthermore, the Western analysis confirmed that similar levels of JNK55 were present in all samples. This result indicates that there is a preferential activation of JNK55 in the isolated heart following reperfusion. The preferential activation of JNK55 was confirmed in vivo by examining JNK activation in dog heart tissue following ischemia/reperfusion (Fig. 1C). Small biopsies of cardiac tissue were removed from the exposed dog heart in vivo prior to ischemia and at the indicated time points following ischemia and reperfusion. The profile of JNK activation is essentially identical to that observed in the isolated rat heart. However, the peak in JNK55 activity appears to be reached sooner in vivo. Preferential activation of a JNK isoform in tissues has not been reported previously, and these observations may suggest a specific role for a JNK isoform in the heart.

p38 MAP kinase activity was measured by Western analysis using anti-phospho-p38 antibodies (Fig. 2A). In a parallel experiment, p38 MAP kinase protein levels were measured by Western analysis using an anti-p38 antibody to ensure that similar levels of p38 MAP kinase protein were present in all samples (Fig. 2B). No detectable p38 MAP kinase activity was present in control hearts. However, consistent with the recent report by Bogoyevitch et al. (41), p38 MAP kinase was activated in the heart subjected to 20 min of ischemia (Fig. 2B, right part). This activation was transient, because p38 MAP kinase activity was not detectable after 45 min of ischemia (Fig. 2A, left part). Significantly, upon reperfusion following 45 min of ischemia, p38 MAP kinase was rapidly reactivated: the kinase activity was detectable within 1 min postreperfusion, reached a maximal level at approximately 15 min, and returned to a low level within 3 h. We conclude that in the ischemic heart, p38 MAP kinase is transiently activated. Independent of the time of ischemia, p38 MAP kinase is active upon reperfusion.

Pattern of Stress Kinase Activation in Ischemic and Ischemic/Reperfused Rat Kidney—Rat kidneys, in vivo, were made ischemic by ligation of the renal artery for 1 h. Kidneys were reperfused for various periods ranging from 5 min to 3 h. Extracts were then prepared from the treated kidney or control (i.e., contralateral) kidney.

JNK activity was measured by in gel kinase analysis. As observed in the heart, no JNK activity was detectable in the control kidneys or in the ischemic kidney. Upon reperfusion, however, both JNK46 and JNK55 were activated within 10 min, unlike in the heart where JNK55 was preferentially activated. The activities of both kinases were high for approximately 90
min and returned to near basal level by 2 h (Fig. 3A). Therefore, the pattern of JNK isoform activation differs significantly in the kidney and heart. As a control, we showed that similar levels of JNK46 protein were present in all samples (Fig. 3B). Interestingly, the levels of JNK55 appeared to be lower in the kidney than in the heart, because the JNK antibody, which cross-reacts with both JNK46 and JNK55, failed to detect JNK55 in the kidney (compare Figs. 1B and 3B). In each case, the same amount of total cellular protein was loaded on the gels for Western analysis.

p38 MAP kinase activity in the kidney was measured by Western analysis using anti-phospho-p38 MAP kinase antibodies. As observed in the heart, no p38 MAP kinase activity was detectable in control kidneys. However, in contrast to the heart, where p38 MAP kinase was transiently activated by short periods of ischemia (20 min), p38 MAP kinase was not activated by short periods of ischemia (15 min or 30 min) in the kidney (data not shown). Rather, p38 MAP kinase was activated only after prolonged ischemia for 60 min (Fig. 4A). After reperfusion, p38 MAP kinase activity was maintained for 5 min and then returned to basal level within 2 h postreperfusion (Fig. 4A). Similar levels of p38 MAP kinase protein were present in all samples as determined by Western analysis (Fig. 4B). Taken together, our results on the activation of stress kinases in the heart and kidney demonstrate very distinct tissue-specific patterns of stress kinase activation by ischemia and ischemia/reperfusion. These distinct patterns of kinase activation are likely to have important implications in the progression of tissue injury, as discussed below.

Increased Expression of Stress Kinase-responsive Genes in Ischemic and Ischemic/Reperfused Tissues—Stress stimuli, which result in stress kinase activation, have been shown to lead to increased expression of several immediate early genes, including c-Jun and ATF3 (40, 42). We have examined the expression levels of these immediate early genes in response to ischemia and ischemia/reperfusion in both the heart and kidney.

In the heart, c-Jun expression was measured by in situ hybridization. In the control hearts, the antisense c-Jun RNA probe failed to detect a signal, indicating undetectable levels of c-Jun expression (Fig. 5, left panels). Ischemia alone, induced by ligation of the left anterior descending coronary artery, resulted in localized expression of c-Jun on the endocardium side of the ventricle wall (Fig. 5, upper right panel). As noted above, we observed transient activation of p38 MAP kinase early in ischemia (Fig. 2A). It is likely that induced c-Jun expression in the endocardium following ischemia is mediated by ATF2, a transcription factor whose activity is enhanced by p38 MAP kinase phosphorylation and has been shown to regulate c-Jun transcription (43). Following reperfusion, c-Jun expression was induced throughout the endocardium and epicardium (Fig. 5, lower right panel). The broad pattern of c-Jun expression upon reperfusion is most likely mediated by the phosphorylation and activation of both c-Jun and ATF2 transcription factors by JNK and p38 MAP kinase. The role of the stress kinases in mediating induced c-Jun expression through activation of both c-Jun and ATF2 transcription factors is supported by the expression pattern of ATF3 following ischemia and ischemia/reperfusion. Previously, we examined the expression of ATF3 by in situ hybridization following ischemia and ischemia/reperfusion in the heart (40). The expression pattern of ATF3 is very similar to that of c-Jun. Like c-Jun transcription, ATF3 transcription is regulated by both c-Jun and ATF2 transcription factors (44). Activation of these transcription factors by both p38 MAP kinase and JNK most likely induces...
ATF3 expression in a pattern similar to that of c-Jun. Because the endocardium is more severely damaged than the epicardium in the ischemic heart, we speculate that the expression of both c-Jun and ATF3 in the endocardium following ischemia is linked to cellular damage in this region. Expression of c-Jun and ATF3 throughout the ventricular wall upon reperfusion may play a role in more widespread tissue damage.

The expression levels of c-Jun and ATF3 in the kidney were examined by Northern analysis (Fig. 6). No detectable levels of either c-Jun or ATF3 mRNA were observed in control kidneys or kidneys subjected to ischemia for 1 h. This correlates with our inability to detect p38 MAP kinase activity in the ischemic kidney until 1 h after ischemia. However, within 20 min following reperfusion, both c-Jun and ATF3 mRNA levels increased significantly, reached maximal levels by 1.5 h, were maintained at a high level for at least 3 h, and returned to the basal level by 24 h. We conclude that ischemia followed by reperfusion leads to a rapid and transient expression of these stress-responsive genes in the kidney as a consequence of stress kinase activation.

To examine the localization of c-Jun and ATF3 expression in the kidney, we performed in situ hybridization (Fig. 7). Confirming the results of the Northern analysis, no detectable levels of either c-Jun or ATF3 expression were observed in the control (data not shown) or ischemic kidneys (Fig. 7A). Upon reperfusion of the kidneys for 90 min, high levels of both c-Jun and ATF3 expression were observed (Fig. 7B). Interestingly, c-Jun and ATF3 were expressed at high levels within the medulla of the kidney, the region of the kidney most susceptible to damage following ischemia/reperfusion (45). This result is reminiscent of the observation that, in the ischemic heart, c-Jun and ATF3 were initially induced in the endocardium, the region of the heart most susceptible to damage. This observation further supports the hypothesis that the high level of both c-Jun and ATF3 expression may be linked to tissue damage.

Apoptotic Cell Death in Cardiac and Renal Tissue following Ischemia and Ischemia/Reperfusion—A number of recent studies have demonstrated a link between stress kinase activation and initiation of apoptotic cell death (32–35). To examine if there was evidence of apoptotic cell death in the rat heart and kidney following ischemia/reperfusion as indicated, glyceraldehyde-3-phosphate dehydrogenase (G3PDH) served as a control for RNA loading.

![Figure 4](https://example.com/fig4.png)  
**Figure 4.** Activation of p38 MAP kinase in ischemic and ischemia/reperfused rat kidney. A, Western blot analysis of phosphorylated p38 MAP kinase in kidneys subjected to ischemia (60 min) or ischemia/reperfusion for the indicated times. Aliquots of extracts (500 μg of protein) as described in the Fig. 3 legend, were immunoprecipitated with anti-p38 MAP kinase C-terminal antibodies. The immunoprecipitated p38 MAP kinase was resolved on a 10% SDS-polyacrylamide gel and analyzed by immunoblot using anti-phospho-p38 MAP kinase antibodies to examine the phosphorylation status of the kinase. B, the same membrane from A was stripped and rebotted with anti-p38 MAP kinase C-terminal antibodies to show equivalent amounts of p38 MAP kinase protein in each sample.

![Figure 5](https://example.com/fig5.png)  
**Figure 5.** Localized expression of c-Jun in the rat heart following ischemia and ischemia/reperfusion. Tissue sections from rats subjected to the indicated treatments were analyzed by in situ hybridization using c-Jun antisense RNA as a probe. The signals were detected by autoradiographic emulsion after a 10-day exposure for the ischemic hearts and 5-day exposure for the ischemia/reperfused hearts. The sections were counterstained with hematoxylin and eosin, and pictures were generated by dark field photography giving pinkish white signals on a green background. −, sham control hearts; +, treated hearts. Magnification, × 25.

![Figure 6](https://example.com/fig6.png)  
**Figure 6.** Increased expression of c-Jun and ATF3 following ischemia/reperfusion in rat kidney. Northern analysis of c-Jun and ATF3 mRNA levels in rat kidney following ischemia/reperfusion as indicated. Glyceraldehyde-3-phosphate dehydrogenase (G3PDH) served as a control for RNA loading.
However, DNA laddering was detectable within 30 min of reperfusion, and the maximal amount of DNA laddering was evident 1 h following reperfusion (Fig. 8A), indicating a rapid increase in apoptotic cell death following reperfusion and stress kinase activation in the heart.

Similar to the heart, in the kidney no obvious amount or only a trace amount of DNA laddering was observed in the control kidney and kidney subjected to ischemia alone. Upon reperfusion an increase in DNA laddering was observed, but it was not detectable until 2–3 h following reperfusion. The degree of DNA laddering observed at 24 h following reperfusion was significantly above that observed at 3 h following reperfusion (Fig. 8B). This observation suggests that the initiation of apoptotic cell death following reperfusion and stress kinase activation in the heart.

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**DISCUSSION**

In this study we have demonstrated both tissue-specific and isoform-specific patterns of stress kinase activation following ischemia/reperfusion in the heart and kidney. This stress kinase activation leads to an increase in the expression levels of both c-Jun and ATF3, which are believed to play a role in cell death (36, 37, 40). The pattern of increased expression of these transcription factors corresponds to regions within the heart and kidney most susceptible to damage following ischemia/reperfusion. The proposal that stress kinase activation is linked to apoptotic cell death is further supported by our observation of a strong correlation between the time course of stress kinase activation and the increase in DNA laddering observed in ischemic/reperfused heart and kidney tissue. These results provide insight into the potential molecular mechanisms that lead to damage of both cardiac and renal tissue following ischemia/reperfusion.

One of the most interesting observations in our study is the transient activation of p38 MAP kinase following a short period of ischemia (15 min) in the heart, whereas prolonged ischemia (60 min) was required for p38 MAP kinase activation in the kidney. It is interesting to note that the length of time of ischemia required to give severe loss of function differs significantly between the rat heart and kidney. In the rat heart, ischemia for longer than 20 min results in severe loss of function (47). However, in the rat kidney, ischemia for 60–90 min is required to observe severe loss of function (48). We propose that activation of p38 MAP kinase during ischemia may initiate events that ultimately contribute to cell death and tissue damage. A role for p38 MAP kinase in apoptotic cell death is supported by several recent studies (34, 49). The activation of p38 MAP kinase early during ischemia in the heart may, at least in part, be the mechanism responsible for the more rapid loss of function in the heart compared with the kidney, where p38 MAP kinase is activated only after prolonged ischemia. The studies performed to measure p38 MAP kinase activity do not distinguish between the multiple isoforms of p38 MAP kinase.
kinase identified to date (24–27). It is possible that individual isoforms of p38 MAP kinase are activated by specific conditions in the heart and kidney, leading to distinct patterns in the progression of tissue injury.

A number of recent studies have also suggested an important role for JNK in apoptosis induced by various stress stimuli in vitro (32–35). Our results provide evidence to support a role for JNK in apoptosis in vivo by demonstrating a strong correlation between JNK activation and apoptosis in both the ischemic/reperfused heart and kidney. Reperfusion in both the heart and kidney results in rapid activation of JNK. The activation of JNK is followed by the occurrence of apoptotic cell death, as measured by DNA laddering. In the heart, apoptosis is evident within 30 min, whereas it is not until 2–3 h after reperfusion that apoptosis is evident in the kidney. We suggest that the early activation of p38 MAP kinase during ischemia in the heart may initiate events that allow for a more rapid acceleration of apoptosis upon JNK activation in the heart compared with the kidney. This hypothesis is supported by the recent observations of Fliss and Gattinger (50), published while this manuscript was under review. They observed that apoptosis was evident in rat heart tissue after 2.25 h of continuous ischemia. We speculate that this apoptotic cell death may be initiated by p38 MAP kinase activation. They also noted that the degree of apoptosis was accelerated by reperfusion after 45 min of ischemia. This, we believe, is likely to be mediated by JNK activation. Another recent observation is that prolonged activation of JNK is required to induce apoptosis in T-cells, while transient JNK activation (<1 h) led to T-cell activation (51). We observe prolonged JNK activity following reperfusion in both the heart and kidney, again supporting a link between JNK activity and apoptosis.

Of particular interest in this study is the preferential activation of the JNK55 isoform in the heart. It has been shown in vitro that JNK isoforms have different affinities for their substrates and hence are likely to have different activities with regard to substrate phosphorylation (19, 20, 23). A recent study demonstrated stronger activation of JNK46 compared with JNK55 following cytokine stimulation of chondrocytes, and it was suggested that the JNK46 isoform may play a more important role in chondrocytes (52). However, to date there has been no evidence demonstrating specific activation or a specific role for JNK isoforms in vivo. Our results demonstrate that there is preferential activation of a JNK55 isoform in the heart, both in vitro and in vivo, following ischemia/reperfusion. Therefore, a JNK55 isoform may play a specific role in the processes leading to damage of tissue following ischemia/reperfusion.

While this manuscript was in preparation, Bogoyevitch et al. (41) reported the activation of both JNK46 and JNK55 isoforms following ischemia/reperfusion in the isolated rat heart, which is inconsistent with our results. The reason for the discrepancy remains to be determined. However, we have observed the preferential activation of JNK55 in the heart upon reperfusion both in vitro and in vivo in two species, rat and dog.

It has been proposed that stress kinase activation has a positive rather than a negative effect following ischemia/reperfusion, being involved in ischemic “conditioning” rather than apoptosis (9, 41). Preconditioning is associated with induction of proteins thought to be cardioprotective, including heat shock proteins. Heat shock proteins are substrates for the kinase mitogen-activated protein kinase-activated protein kinase-2, which is activated by p38 MAP kinase (53). However, for the reasons discussed above, we currently favor a role for the stress kinases in tissue damage rather than protection following ischemia/reperfusion. Confirmation of the role of stress kinase activation in either apoptosis or cardioprotection awaits the availability of specific kinase inhibitors.

We have provided evidence for the occurrence of apoptotic cell death in both heart and kidney following ischemia/reperfusion, the progression of which appears to be linked to the tissue-specific pattern of stress kinase activation. The importance of apoptotic cell death in the in vivo evolution of infarct size has been demonstrated in a number of species from rat to human (2, 5, 54). A central role for apoptosis in tissue injury following ischemia/reperfusion suggests that inhibition of the apoptotic pathway may be a novel treatment for both acute myocardial infarction and acute renal damage. Because the activation of stress kinases may be an early event in the pathway leading to apoptosis, an ability to inhibit stress kinase activation may reduce apoptotic cell death and presumably the consequent tissue damage.

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