Differential regulation of activator protein-1 and heat shock factor-1 in myocardial ischemia and reperfusion injury: role of poly(ADP-ribose) polymerase-1

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Differential regulation of activator protein-1 and heat shock factor-1 in myocardial ischemia and reperfusion injury: role of poly(ADP-ribose) polymerase-1. Am J Physiol Heart Circ Physiol 286: H1408–H1415, 2004; 10.1152/ajpheart.00953.2003.—Poly(ADP-ribose) polymerase-1 (PARP-1), a nuclear enzyme activated in response to DNA strand breaks, has been implicated in cell dysfunction in myocardial reperfusion injury. PARP-1 has also been shown to participate in transcription and regulation of gene expression. In this study, we investigated the role of PARP-1 on the signal transduction pathway of activator protein-1 (AP-1) and heat shock factor-1 (HSF-1) in myocardial reperfusion injury. Mice genetically deficient of PARP-1 (PARP-1−/− mice) exhibited a significant reduction of myocardial damage after occlusion and reperfusion of the left anterior descending branch of the coronary artery compared with their wild-type littersmates. This cardioprotection was associated with a reduction of the phosphorylative activity of JNK and, subsequently, reduction of the DNA binding of the signal transduction factor AP-1. On the contrary, in PARP-1−/− mice, DNA binding of HSF-1 was enhanced and was associated with a significant increase of the cardioprotective heat shock protein (HSP)70 compared with wild-type mice. Microarray analysis revealed that expression of several AP-1-dependent genes of proinflammatory mediators and HSPs was altered in PARP-1−/− mice. The data indicate that PARP-1 may exert a pathological role in reperfusion injury by functioning as an enhancing factor of AP-1 activation and as a repressing factor of HSF-1 activation and HSP70 expression.

heat shock proteins; c-Jun NH2-terminal kinase; c-Jun; c-Fos; microarray analysis

THE MAIN THERAPEUTIC INTERVENTION after myocardial infarction is to reestablish the coronary blood flow. However, restoration of flow is accompanied by detrimental manifestations known as reperfusion injury (37). Despite the prevalence of reperfusion injury, the molecular mechanisms associated with this phenomenon remain undetermined. The reperfusion injury appears to be triggered by a large burst of oxidant molecules and amplified by accumulation into injured tissue of neutrophils (8). Current evidence suggests that the mechanism by which oxidants trigger an inflammatory process is related to their ability to activate a complex cascade of kinases through phosphorylative events. A downstream kinase of this cascade is JNK, which phosphorylates c-Jun, allowing its heterodimerization or homodimerization with c-Fos to form the active transcription factor activator protein-1 (AP-1) (6, 9). Activation of AP-1 induces a coordinated expression of genes of several proinflammatory mediators, including cytokines and adhesion molecules, thus leading to cell dysfunction and death (16). However, oxidative stress may also initiate a counterregulatory pathway through the activation of cytoprotective mediators. Specifically, endogenous myocardial protection may be mediated by the cardioprotective heat shock protein (HSP)70, which is rapidly induced in response to ischemia and is primarily regulated by heat shock transcription factor-1 (HSF-1) (7, 20).

Poly(ADP-ribose) polymerase (PARP) is a chromatin-associated nuclear enzyme that is activated by stranded DNA nicks and breaks in damaged cells, and it modifies nuclear proteins through the attachment of poly(ADP-ribose) units (18). Among the PARP family of enzymes, activation of PARP-1 has been shown to be a major cytotoxic pathway of tissue injury in different pathologies associated with inflammation, such as streptozocin-induced diabetes, cerebral ischemia, endotoxic and hemorrhagic shock, and localized colon inflammation (38). In previous in vivo studies, we have demonstrated that during myocardial reperfusion injury, activation of PARP-1 results in severe depletion of the cellular pools of high-energy phosphates, such as ATP and NAD+, leading to cell dysfunction and death (40, 42, 43). On the contrary, several studies from our laboratory and others have reported that pharmacological inhibition of PARP or genetic ablation of PARP-1 ameliorates the pathophysiologic changes of myocardial reperfusion injury (26, 34, 42).

There is strong evidence that PARP-1, by direct protein interaction and/or by poly(ADP-ribosylation), alters the function of a variety of transcription factors, including YY-1, AP-2, OCT-1, and NF-κB (10, 12, 25). These findings have suggested that PARP-1 may regulate the inflammatory response through modulation of signal transduction pathways and gene expression.

In this study, we provide insight into the mechanism by which PARP-1 deficiency affords protection in myocardial reperfusion injury. We demonstrated that the signal transduction profile of PARP-1-deficient mice (PARP-1−/− mice) subjected to experimental myocardial ischemia and reperfusion is altered. With the use of cDNA microarrays, we also identified gene expression profiles associated with reperfusion injury that may be regulated by PARP-1. Our results indicate that activa-

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tion of PARP-1 affords an additional level of regulation of the signaling pathway of myocardial reperfusion injury by serving as an enhancer modulator of AP-1 and a repressor modulator of HSF-1.

**MATERIALS AND METHODS**

Myocardial ischemia and reperfusion. This investigation conformed with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Pub. No. 85-23, Revised 1986) and commenced with the approval of the Institutional Animal Care and Use Committee. PARP-1−/− mice and their wild-type littermates (PARP-1+/+ mice; 129/SV × C57BL/6, 20–22 g) were anesthetized with thiopentone sodium (40 μg/g). Coronary occlusion and reperfusion was performed as previously described (40). The left anterior descending branch of the left coronary artery was occluded by ligation with a 7-0 silk suture over a 1-mm section of polyethylene-10 tubing, which was placed on top of the vessel. After 30 min of occlusion, reperfusion occurred by cutting the suture. Different groups of mice were killed at the end of the ischemia (30 min) or at various time points after reperfusion (5, 15, 30, 45, and 60 min and 6 and 24 h). Blood samples were collected. Hearts were rapidly harvested, and the left ventricles were used for histological and biochemical studies.

**Histopathological analysis.** Tissues were fixed in 4% paraformaldehyde and embedded in paraffin. Sections were stained with hematoxylin and eosin and evaluated by a pathologist blinded to the experimental protocol.

**Subcellular fractionation and nuclear protein extraction.** Heats were homogenized in a buffer containing 0.32 M sucrose, 10 mM Tris-HCl (pH 7.4), 1 mM EGTA, 2 mM EDTA, 5 mM Na$_2$HPO$_4$, 10 mM β-mercaptoethanol, 20 μM leupeptin, 0.15 μM pepstatin A, 0.2 mM PMSE, 50 mM NaF, 1 mM sodium orthovanadate, and 0.4 mM microcystin. The homogenates were centrifuged (1,000 g, 10 min), and the supernatant (cytosol + membrane extract) was collected to evaluate HSP70 content as described below. The pellets were solubilized in Triton buffer [1% Triton X-100, 150 mM NaCl, 10 mM Tris-HCl (pH 7.4), 1 mM EGTA, 1 mM EDTA, 0.2 mM sodium orthovanadate, 20 μM leupeptin A, and 0.2 mM PMSE]. The lysates were centrifuged (15,000 g, 30 min, 4°C), and the supernatant (nuclear extract) was collected to evaluate the content of HSP70, c-Fos, and phosphorylated c-Jun and activity of JNK, AP-1, and HSF-1.

**Western blot analyses.** The nuclear content of c-Fos and phosphorylated c-Jun and the cytosol and nuclear content of HSP70 were determined by immunoblot analyses. Cytosol and nuclear extracts were boiled in loading buffer [125 mM Tris-HCl (pH 6.8), 4% SDS, 20% glycerol, and 10% 2-mercaptoethanol], and 50 μg of protein were loaded per lane on an 8–16% Tris-glycine gradient gel. Proteins were separated electrophoretically and transferred to nitrocellulose membranes. For immunoblotting, membranes were blocked with 5% nonfat dried milk in Tris-buffered saline (TBS) for 1 h and then incubated with primary antibodies against c-Fos, phosphorylated c-Jun, or HSP70 for 1 h. Membranes were washed in TBS with 0.1% Tween 20 and incubated with secondary peroxidase-conjugated antibody. Immunoreaction was visualized by chemiluminescence. Densitometric analysis was performed using ImageQuant (Molecular Dynamics; Sunnyvale, CA).

**Assay of JNK activity.** The activity of JNK was determined by immune complex kinase assay and was estimated as the ability to phosphorylate glutathione-S-transferase (GST)-c-Jun (21, 44). After immunoprecipitation of lysate with specific antibody directed to JNK1, the immunoprecipitate was incubated for 30 min at 30°C in 40 μl of reaction buffer [25 mM HEPES (pH 7.6), 20 mM MgCl$_2$, 20 mM glycerolphosphate, 0.1 mM sodium orthovanadate, 2 mM dithiothreitol, 25 μM ATP, and 5 μCi of [γ-32P]ATP]. GST-c-Jun (1–79) (4 μg) was used as the substrate for JNK. Reaction products were separated by SDS-PAGE and visualized by autoradiography. Densitometric analysis was performed using ImageQuant (Molecular Dynamics).

EMSAs. EMSAs were performed as previously described (44). Oligonucleotide probes corresponding to AP-1 or HSF-1 consensus sequences were labeled with [γ-32P]ATP using T4 polynucleotide kinase and purified in Bio-Spin chromatography columns (Bio-Rad; Hercules, CA). Ten micrometers of nuclear protein were incubated with EMSA buffer (12 mM HEPES (pH 7.9), 4 mM Tris-HCl (pH 7.9), 25 mM KCl, 5 mM MgCl$_2$, 1 mM EDTA, 1 mM dithiothreitol, 50 ng/mL poly(dI-C), 12% (vol/vol) glycerol, and 0.2 mM PMSF) and radiolabeled oligonucleotide. Excess of unlabeled oligonucleotide was added in some samples for competition to verify the specificity of AP-1 and HSF-1 binding (data not shown). Protein-nucleic acid complexes were resolved using a nondenaturing polyacrylamide gel and run in 0.5 × TBE (45 mM Tris-HCl, 45 mM boric acid, and 1 mM EDTA) for 1 h at constant current (30 mA). Gels were transferred to Whatman 3M paper, dried under a vacuum at 80°C for 1 h, and exposed to photographic film at −70°C with an intensifying screen. Densitometric analysis was performed using ImageQuant (Molecular Dynamics).

**DNA microarray analysis.** Total RNAs were isolated from hearts, and 10 μg of total RNA were quantitatively amplified and biotin labeled as previously described (41). Briefly, RNA was converted to double-stranded cDNA with an oligo(dT) primer that has a T7 RNA polymerase site at the 5′ end. The cDNA was used directly in an in vitro transcription reaction in the presence of biotinylated nucleotides to produce antisense RNA, which was hybridized overnight to Genechips (Affymetrix U74A; San Jose, CA) displaying probes for 15,100 genes and expressed sequence tags. Chips (n = 2 for each group) were washed, stained with phycoerythrin-streptavidin, and read with an Affymetrix Genechip scanner and accompanying gene expression software. DNA microarray data were filtered for genes with RNA expression changes of twofold lower or greater at 1, 6, and 24 h after reperfusion in PARP-1−/− mice compared with wild-type mice. This screen resulted in 705 genes. With the K-means algorithm in GeneSpring software (Silicon Genetics; Redwood City, CA), clustering approaches with hierarchical tree analysis applied to the expression ratio measurements resulted in the selection of several genes with known functions as proinflammatory regulators or HSPs.

**Materials.** The primary antibody directed at c-Jun phosphorylated at serine 63 and 73 was obtained from New England Biolabs (Beverly, MA). The primary antibodies directed at c-Fos, HSP70, JNK1, and the oligonucleotide for AP-1 were obtained from Santa Cruz Biotechnology (Santa Cruz, CA). The oligonucleotide probe for HSF-1 corresponded to a previously published heat shock element consensus sequence (7) and was synthesized by the University of Cincinnati DNA Core Facility. All other chemicals were from Sigma (St. Louis, MO).

**Data analysis.** All values are expressed as means ± SE of n observations (n = 3–12 animals/group). The results were examined...
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analysis using the default statistical comparison provided by Gene-
was considered signifi-
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lished studies demonstrating that, although both genotypes
(Fig. 1). These data are consistent with our previously pub-
architectural alterations characterized by interstitial edema
histological features of myocardial injury were typical of mild
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ment of contraction bands and interstitial edema. In addition,
myofibrils showed thinning and wavy patterns, consistent with
reperfusion injury. On the contrary, in PARP-1
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exhibit a similar drop in mean arterial blood pressure during
the ischemic period and early reperfusion, PARP-1
mice
by ANOVA with individual comparisons performed by t-test. For
microarray analysis (n = 2), P values were derived from t-test
analysis using the default statistical comparison provided by Gene-
Spring (Silicon Genetics; Redwood City, CA). A P value of <0.05
was considered significant.

RESULTS

Severity of myocardial damage. In wild-type PARP-1
mice, ischemia (30 min) and reperfusion (24 h) resulted in a
marked tissue injury, which was characterized by the develop-
ment of contraction bands and interstitial edema. In addition,
myofibrils showed thinning and wavy patterns, consistent with
reperfusion injury. On the contrary, in PARP-1
mice, the
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the ischemic period and early reperfusion, PARP-1
mice
exhibit a significant reduction of infarct size (38 ± 4% of the
area at risk) compared with wild-type mice (60.5 ± 6% of the
area at risk) at 24 h after reperfusion (40).

Activation of AP-1, phosphorylation of c-Jun, and activity of
JNK. Because the activation of AP-1 has been implicated in
myocardial reperfusion injury (16, 21, 44), we determined the

Table 1. GenBank accession numbers, symbols, and
descriptions of 31 genes differentially regulated in PARP-1
and PARP-1
mice after ischemia and reperfusion

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<th>GenBank Accession Nos.</th>
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<td>PARP, poly(ADP-ribose) polymerase.</td>
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nuclear activation of this factor. In PARP-1\(^{+/+}\) mice, DNA binding of AP-1 steadily increased after reperfusion. In PARP-1\(^{+/−}\) mice, activity of AP-1 exhibited similar kinetics, increasing in a time-dependent fashion after reperfusion. However, the degree of activation was significantly reduced compared with wild-type mice (Fig. 2). At later stages of reperfusion (24 h), AP-1 activation was similar in both experimental groups; the fold increase of AP-1 activity was 2.39 ± 0.52- and 2.20 ± 0.47-fold versus basal levels at time 0 (set to 1), respectively, in PARP-1\(^{+/+}\) and PARP-1\(^{+/−}\) mice.

Because the activity of AP-1 may be regulated by the availability of its subunits (16), we determined the content of c-Fos and phosphorylated c-Jun in nuclear extracts by Western blotting. Under basal condition, PARP-1\(^{+/+}\) and PARP-1\(^{+/−}\) mice exhibited no content of c-Fos, which was expressed after ischemia and reperfusion in a time dependent-manner similarly in both genotypes, thus suggesting that PARP-1 activation is not required for c-Fos expression. The content of c-Jun, with phosphorylated sites at serine 63 and 73, increased after early reperfusion in wild-type mice. In contrast, PARP-1\(^{+/−}\) mice
exhibited a lower content of phosphorylated c-Jun compared with wild-type mice, thus suggesting that PARP-1 activation is required for the phosphorylation of this subunit (Fig. 3).

Because the phosphorylation of c-Jun is mediated by JNK (16, 21), we further determined the nuclear activity of JNK. A time-course analysis showed that JNK activity increased after ischemia and early reperfusion and declined thereafter in wild-type mice. In PARP-1−/− mice, the degree of activity of JNK was significantly reduced compared with wild-type mice (Fig. 4). At later stages of reperfusion (24 h), JNK was similarly activated in both experimental groups; the fold increase of JNK activity was $1.27 \pm 0.06$- and $1.09 \pm 0.14$-fold versus basal levels at time 0 (set to 1), respectively, in PARP-1+/− and PARP-1−/− mice.

Gene expression of inflammatory mediators. To further examine the role of PARP-1 on gene expression during reperfusion, microarray analysis was performed with heart-derived mRNA from PARP-1−/− and wild-type mice subjected to myocardial ischemia (30 min) and reperfusion (1, 6, and 24 h). Only genes with known function as inflammatory mediators whose expression has been shown to be AP-1 dependent were selected for the analysis. Large increases of the AP-1 dependent genes for IL-6, IL-1β, IL-1α, cyclooxygenase-2 (COX-2), and metalloproteinase 3 and the adhesion molecules P-selectin, ICAM-1, and VCAM-1 were observed in wild-type mice after reperfusion. The genes for chemokine ligands 5 and 2, chemokine receptor-1, and the small inducible cytokine B member 9 were also elevated in wild-type mice after reperfusion. On the contrary, PARP-1−/− mice exhibited a significant lower expression of these genes at 1 or 6 h after reperfusion. At the later stage of reperfusion (24 h), the genes of most of these mediators were similarly expressed in both wild-type and PARP-1−/− mice (Table 1 and Fig. 5). Interestingly, PARP-1−/− mice exhibited a significant increase in the gene expression of IL-1 receptor antagonist and IL-7 receptor (Fig. 5, cluster B). A similar increase of gene expression of other IL receptors and receptor antagonists (Fig. 5, cluster C), E-selectin and E-selectin ligand, the tissue inhibitor of metalloproteinase 1 (Fig. 5, cluster F), and other chemokines (Fig. 5, cluster H) was observed in both genotypes.

Expression of HSP70 and activation of HSF-1. To further establish the mechanisms of cardioprotection in the absence of PARP-1, we determined the myocardial expression of HSP70, a stress-induced protein with putative antiapoptotic effects (13, 20). Western blot analyses indicated that HSP70 expression was modestly increased by ischemia and reperfusion injury in the nuclear compartment but decreased in the cytosol in PARP-1−/− mice. In contrast, a significant increase above basal levels was observed in ischemic or reperfusion conditions in cytosolic and nuclear extracts of PARP-1−/− mice (Fig. 6).

Because the HSP70 expression is regulated at the genetic level by HSF-1 (7), we next determined the DNA binding activity of this transcription factor. A time-course analysis showed that DNA binding activity of HSF-1 slightly increased after ischemia, whereas it rapidly declined after reperfusion in wild-type mice. In contrast, in PARP-1−/− mice, DNA binding of HSF-1 significantly increased after ischemia and reperfusion compared with wild-type mice (Fig. 7). At later stages of reperfusion (24 h), HSF-1 activation was similar in both experimental groups; the fold increase of HSF-1 activity was $1.46 \pm 0.16$- and $1.17 \pm 0.07$-fold versus basal levels at time 0 (set to 1), respectively, in PARP-1+/− and PARP-1−/− mice.

Microarray analysis demonstrated that Hspa4, the murine gene of HSP70, was significantly increased over basal levels in PARP-1−/− mice, whereas it was downregulated in wild-type mice after reperfusion, thus confirming the results of Western blotting analysis. Dnajb1, an Hsp40 homolog gene, was also significantly increased in PARP-1−/− mice compared with wild-type mice (Table 1 and Fig. 8, cluster A). A similar increase of the gene Hmox1, for heme oxygenase-1 (HSP32), and Dnajc7, another Hsp40 homolog gene, was observed in both genotypes (Fig. 8, cluster B). Other genes for components of the HSP family were similarly downregulated in both genotypes (Fig. 8, cluster C).

**DISCUSSION**

The present study provides the first evidence that PARP-1 is a major requisite for a selective regulation of the stress-induced signaling pathways of AP-1 and HSF-1 during the very early phase of myocardial reperfusion injury. Specifically, we have demonstrated that mice with genetic ablation of PARP-1 exhibit a significant cardioprotection, which is associated with downregulation of the proinflammatory pathway of AP-1, whereas the antiinflammatory pathway of HSF-1 is enhanced with consequent modification of gene expression of several inflammatory mediators.
Numerous experimental studies have proven that the activation of AP-1 is implicated in myocardial reperfusion injury. Enhancement of AP-1 DNA binding activity has been found in areas of infarction in rodents subjected to myocardial ischemia and reperfusion (4, 32, 44). These data have been confirmed in humans, because nuclear translocation of AP-1 has been found in cardiac biopsies of patients with unstable angina (36). In our study, we found that in the absence of PARP-1, the nuclear binding of AP-1 is depressed during reperfusion. At the examination of the upstream cascade that leads to the activation of this transcription factor, we found that the reduction of AP-1 activity in PARP-1−/− mice is a secondary event to the reduction of JNK activity. To our knowledge, this is the first report demonstrating that PARP-1 is requisite for a complete activation of the phosphorylative activity of this kinase during reperfusion injury in vivo. These data are in agreement with our previous reports demonstrating that AP-1 DNA binding is completely abolished in vitro PARP-1-deficient fibroblasts and that this event is consequent to alteration of JNK phosphorylative activity, which affects the AP-1 dimer composition (2).

To further understand the role of PARP-1 in the regulation of AP-1, we studied gene expression patterns of known inflammatory mediators. DNA microarray analysis showed that loss of the PARP-1 gene is associated with significantly reduced expression of AP-1-dependent mediators, such as COX-2, IL-6, IL-1β, and IL-1α and the adhesion molecules of ICAM-1, P-selectin, and ICAM-1 and several chemokine ligands and receptors. These findings are consistent with our previous data demonstrating that genetic or pharmacological inhibition of PARP-1 reduced adhesion molecule expression of P-selectin and ICAM-1 and neutrophil infiltration in vivo myocardial reperfusion injury (46) and reduced the plasma release of inflammatory cytokines (40). AP-1-dependent metalloproteinase 3 (19) plays a major role in the degradation of the extracellular matrix and myocyte misalignment, which contributes to the loss of heart function after myocardial infarction (5). Loss of the PARP-1 gene was also associated with a significant reduction of this matrix enzyme, which well correlated with the mild architectural damage and maintenance of myocyte alignment of the reperfused heart.

It has been shown that HSP70 directly protects against myocardial damage, improves metabolic recovery, and reduces infarct size in hearts of transgenic mice (13, 20). Synthesis of HSP70 requires HSF-1, the primary mediator of the heat shock response (7). Interestingly, we found that PARP-1 influenced the inflammatory response also by repressing DNA binding and transcriptional activation of HSF-1. Specifically, we observed impairment in the heat shock response during reperfusion in association with decreased DNA binding of HSF-1 in hearts of wild-type compared with PARP-1−/− mice. Thus PARP-1-induced changes of HSF-1 may contribute to an increased vulnerability to ischemic injury in wild-type mice. Of interest, gene analysis revealed that only Hsp4, the gene for HSP70, and Dnajb1, an Hsp40 homolog gene, were upregulated in PARP-1−/− mice, whereas the genes for other HSPs were expressed similarly to wild-type mice. These variable effects of PARP-1 inhibition on gene expression of HSPs may reflect influences of multiple regulatory systems. In the heart, for example, HSF-1 has been reported to regulate HSP25 and HSP70, whereas cytosolic HSP90 and mitochondrial HSP60 appear to require other signaling mechanisms (39). In right atrial biopsies taken from 15 patients during coronary artery bypass grafting, HSP70 increased in the nucleus as well as in the cytosol of myocytes and endothelial cells, whereas other members of the HSF family, like HSP27 and HSP90, showed no significant changes in protein levels during cardioplegia and reperfusion (30). Alternatively, it is also possible that expression of HSPs may be different according to the stage of myocardial injury. Thus the inability to confirm altered regulation of other HSPs (such as HSP25, -60, or -90) may be related to the time selected after reperfusion for our gene analysis. For example, mitochondrial HSP60 of patients undergoing cardiac operations is not increased after an obligatory period of ischemia, cardioplegic arrest, and reperfusion after extracorporeal circulation (29), whereas HSP60 production seems to be associated with the development of chronic heart failure (33). The PARP-1 regulation of HSF-1 observed in our in vivo study is in contrast with our previous findings demonstrating that HSF-1 activation is completely abolished in PARP-1-deficient fibroblasts stimulated with peroxynitrite (2). Furthermore, in Drosophila, chromosomal PARP seems to be required to produce normal amounts of HSP70 after heat exposure (35). Taken together, these data suggest that PARP-1 regulation of the heat shock response may be dependent on several variables, including cell types, inflammatory challenge, temporal stage of myocardial injury, and involvement of other signaling pathways.

A main finding of our study is that PARP-1-dependent regulation of the signal transduction of AP-1 and HSF-1 and gene expression appears to be a very early event occurring within the first hour of reperfusion. The degree of PARP-1 regulation of these transduction pathways and expression of most of the genes was, in fact, relatively modest or even absent at 24 h. Although the gene analysis in our study was limited only to a few time points after reperfusion, it is also likely that the ischemic period itself promotes upregulation of inflammatory mediators. It has been demonstrated that ischemia alone is
sufficient to induce myocardial TNF-α gene expression and peptide synthesis (31). Furthermore, studies with microarray analysis have shown that transcription responses and expression of several genes for enzymes and inflammatory mediators are altered in murine hearts subjected to ischemia alone (17). Nevertheless, our data are consistent with the assumption that therapeutic approaches targeting PARP-1 in the very early stage of reperfusion may exert beneficial effects in the salvage of the ischemic myocardium.

Our findings are in agreement with other in vivo reports demonstrating a role of PARP-1 in signal transduction. In a similar model of ischemia and reperfusion, we have demonstrated that genetic ablation of PARP-1 is associated with a reduction of NF-κB activation and reduction of gene expression of several apoptotic modulators (43). In experimental stage of reperfusion may exert therapeutic approaches targeting PARP-1 in the very early

protein interaction independent of its (ADP-ribosyl)ative function. Protein-protein interaction is recognized as a mechanism for PARP-1 to function as a specific transcriptional coactivator of NF-κB (12). Other studies have also proven that PARP-1 modulates transcription by direct interaction with AP-2 (15), Oct-1 (22), YY-1 (23), and TEF-1 (3). Other scientific reports suggest the hypothesis that PARP-1 may affect transcription through poly(ADP-ribosyl)ation. Amstad et al. (1) have demonstrated that poly(ADP-ribosyl)ation is an important event for the elongation and activation of protooncogenes. Several transcription factors such as TEF-1 (3), transcription factor IIF (27), YY-1, TATA-binding protein (24), and recombinant p50 and p65 NF-κB subunits (14) can be poly (ADP-ribosyl)ated by PARP-1 in vitro. Poly(ADP-ribosyl)ation has been demonstrated to suppress the ability of NF-κB to form a complex with its specific DNA probe in vitro nuclear extracts (14) and to enhance the activity of DNA-protein kinase (28). This dual regulatory role of PARP-1, i.e., dependent or independent of the (ADP-ribosyl)ative activity, is further supported by recent findings demonstrating that genetic deletion of PARP-1, but not pharmacological inhibition of its activity, reduced NF-κB binding in glial cells. However, pharmacological inhibition of the catalytic activity of PARP-1 was able to reduce gene expression of inducible nitric oxide synthase (10).

In conclusion, we suggest that PARP-1 participates in the regulation of transcription by functioning as an enhancing factor of the activation of AP-1 and as a repressing factor of the heat shock response. Our findings also raise the important prospect that the targeting of PARP-1 may ameliorate the myocardial dysfunction by interrupting the inflammatory process at the signal transduction level.

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REFERENCES


