Cardiac ischemia-reperfusion injury induces matrix metalloproteinase-2 expression through the AP-1 components FosB and JunB

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synthesized by cardiomyocytes, fibroblasts, and endothelial cells (2, 8, 37). After 20 min of global ischemia, significant amounts of MMP-2 are found in the coronary effluent as early as 1 min following reperfusion, which represents release of stored ventricular MMP-2 protein (6). Coronary perfusate concentrations of MMP-2 increase during reperfusion in relation to increasing duration of ischemia (6). MMP-2 released as a consequence of ischemia-reperfusion injury has been corre-
lated with the acute declines in ventricular function observed in this model, and this effect has been attributed to MMP-2-
mediated cleavage of sarcomeric troponin I (6, 41). Short periods of ischemia-reperfusion injury (ischemic preconditioning) protect against ventricular dysfunction following pro-
longed ischemia, and this finding has been related to a dimin-
ished release of MMP-2 (21).

To date, all studies of MMP-2 within the setting of ische-
mia-reperfusion injury have focused on the release of pre-
formed MMP-2 protein stored within the ventricles, with the assumption that the limited time period of the isolated ischemia-
reperfused heart model would preclude activation of tran-
scriptional programs. In this report we detail studies using transgen-
MMP-2 promoter reporter mice and a series of functional assays that demonstrate a rapid induction of cardiac MMP-2 gene transcription and translation mediated by specific components of the AP-1 transcription factor com-
p. Furthermore, we provide evidence that the AP-1 components FosB and JunB regulate the intrinsic MMP-2 promoter in vivo following ischemia-reperfusion injury.

MATERIALS AND METHODS

MMP-2 reporter mice. The starting materials were plasmid p41 containing a 5-kb genomic fragment of the rat MMP-2 gene extending from –1686 bp relative to the translational start site to the middle of the second exon and plasmid p1.2 containing a polylinker 5’ to a SV40 polyadenylation signal and paired NotI sites flanking the entire insert. The MMP-2 coding sequence start codon was first mutated to TAG, and the entire 5-kb promoter region was then recovered by PCR, thereby adding 5’ MluI and 3’ KpnI sites and cloned into pl1.2 upstream from the SV40 polyadenylation site. An Escherichia coli β-galactosidase gene (lacZ) was then isolated by PCR with addition of a 5’ KpnI site (and Kozak consensus sequence) and a 3’ XhoI site. This product was ligated between the MMP-2 promoter and the SV40 polyadenylation sequence to generate the construct F8-β-gal. Trans-
genic mice in the CD-1 background strain were generated using standard protocols and characterized by PCR of tail-clipping DNA and by

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Southern blot analysis. F1 × F1 crosses were performed to generate the homozygous F8-β-gal mice (a total of eight integrated transgenes) used in this study.

Langendorff isolated perfused heart preparation. Adult male F8-β-gal transgenic and wild-type adult CD-1 controls (age 4 mo) were fed standard rodent chow and water ad libitum. The animals were acclimated in a quiet quarantine room for at least 3 days before experiments were started. The investigation was approved by the Animal Care Subcommittee of the San Francisco Veterans Affairs Medical Center and conformed with the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health (NIH Publication No. 85-23, Revised 1996). Mice were anesthetized with pentobarbital sodium (60 mg/kg ip) and administered the anticoagulant heparin sodium (5,000 USP U/kg ip). Isolated hearts were rapidly excised, washed in ice-cold arresting solution (120 mmol/l NaCl, 30 mmol/l KCl), and canulated via the aorta on a 20-gauge stainless steel blunt needle. Hearts were perfused at 70 mmHg on a modified Langendorff apparatus using Krebs-Henseleit solution containing (in mmol/l): 118 NaCl, 4.7 KCl, 2.5 CaCl2, 1.2 MgSO4, 1.2 KH2PO4, 24 NaHCO3, 5.5 glucose, 5.0 Na pyruvate, and 0.5 EDTA (pH 7.4). Hearts were perfused at 360 beats/min. Each heart was subjected to 20 min of reperfusion period, the ventricles were excised and rinsed with 4% buffered paraformaldehyde, followed by dehydration and embedded in paraffin for immunohistochemical analysis. In a separate experiment in 4% buffered paraformaldehyde, followed by dehydration and embedding in paraffin for immunohistochemical analysis. In a separate series of studies, the free radical scavenger N-(2-mercaptoethyl)-polyethylene glycol (1 mM, Ref. 38) was dissolved in the identical buffer used for perfusion (Krebs-Henseleit solution) and added during the last 10 min of the equilibration period, followed by progressive periods of ischemia-reperfusion as detailed in RESULTS.

Measurement of β-galactosidase activity. After the defined periods of ischemia-reperfusion, harvested ventricles from wild-type CD-1, F8-β-gal controls, or F8-β-gal mice were homogenized 2.5× in Passive Lysis Buffer (Promega) and incubated on ice for 20 min. The tissue homogenates were snap frozen in liquid nitrogen, thawed, and cleared by centrifugation at 12,000 g for 10 min at 4°C. Protein concentrations of the supernates were determined by the BCA protein assay (Pierce), using BSA as standard. The samples were diluted to 10 μg protein/μl DNAse free water and assayed for β-galactosidase activity using the Luminescent β-galactosidase Reporter System (BD Biosciences).

Immunodetection of β-galactosidase. Five-micron ventricular sections were deparaffinized and hydrated through graded alcohol series. Immunodetection of the β-galactosidase antigen was performed using the Mouse on Mouse Immunodetection Kit (M.O.M; Vector Laboratories, Burlingame, CA). Briefly, the sections were blocked with Avidin-Biotin Blocking Kit (Vector Laboratories), incubated for 1 h in M.O.M Mouse Blocking Reagent, washed, and incubated overnight at 4°C with murine monoclonal anti-β-galactosidase (Cortex Biochemical, San Leandro, CA) at 10 μg/ml in M.O.M diluent. Subsequently, the sections were washed and incubated with the secondary antibody M.O.M biotinylated Anti-mouse IgG for 10 min at room temperature. The sections were washed, exposed to M.O.M Vectastain Elite ABC reagent for 5 min, washed, and stained with nickel and 5% DAB (3,3′-diaminobenzidine tetrahydrochloride) (Ajinomoto, San Leandro, CA). The sections were incubated in peroxidase substrate solution (Vector Purple) for 8 min, washed, and lightly counterstained with methyl green.

Quantitative RT-PCR. PCR (Agilent 9800) for MMP-2 transcripts was performed using SYBR Green incorporation (Applied Biosystems, Foster City, CA) with the following primer pair: 5′-ATGCCACCGGACTGTCAGTCC3′ and 5′-TCCGACCACTACACCGAACCCTGATTGG-3′. Results were normalized to GAPDH: 5′-TGACATCAAAGGTGTTAGACGC-3′ and 5′-CACCCCTGTGCTGGCCGATCAT-3′.

Reactions were performed in quadruplicate; quantitation of mRNA expression was performed by the comparative Ct method (22). Results are expressed as the fold change in the respective treatment groups compared with perfused controls.

Quantitative MMP-2 Western blots. Cardiac extracts (200 μg sample) were prepared as detailed above were incubated overnight at 4°C with 100-μl gelatin-Sepharose beads in 500 μl 50 mM Tris–HCl (pH 7.4) to affinity absorb MMP-2 and eluted into SDS-PAGE sample buffer. Western blots were blocked with 4% BSA, 1× Tris-buffered saline (TBS), and 0.1% Tween 20. The blots were incubated overnight at 4°C with murine monoclonal anti-MMP-2 antibody, 1 μg/ml in PBS-0.1% BSA (Chemicon), followed by a 1-h incubation with a 1:50,000 dilution in PBS-0.1% BSA of horseradish peroxidase-conjugated goat anti-mouse IgG (Zymed, South San Francisco, CA) and detection with ECL reagent (Amersham Biosciences, Piscataway, NJ). Films were exposed in the linear range and MMP-2 protein bands quantified by laser-based densitometry (Typhoon, Amersham Biosciences).

Isolation of cardiac nuclear extracts. Ventricles from controls or after 30 min of ischemia and 30 min of reperfusion were rinsed with calcium-, magnesium-free PBS at 4°C containing 0.5 mM dithiothreitol, 0.2 mM phenylmethylsulfonyl fluoride, and 1× Complete Mini-protease inhibitor cocktail (Roche). Fifty milligrams of finely diced ventricle were homogenized in 0.75 ml hypotonic buffer [10 mM HEPES (pH 7.9), 1.5 mM MgCl2, 10 mM KCl, 0.5 mM dithiothreitol, 0.2 mM phenylmethylsulfonyl fluoride, and 1× Complete Mini-protease inhibitor cocktail] with 25 strokes using a loose-fitting Dounce pestle. The homogenate was incubated on ice for 15 min and pelleted at 700 g for 5 min at 4°C. Pelleted nuclei were washed twice in the hypotonic buffer, and the purity of the preparation was confirmed by inspection under phase-contrast microscopy. Nuclear extracts were prepared according to Dignam et al. (9). KCl (1 M) buffer was used for extraction, followed by dialysis overnight at 4°C in 20 mM HEPES (pH 7.9), 20% glycerol, 100 mM KCl, 0.2 mM EDTA, 0.5 mM dithiothreitol, and 0.2 mM phenylmethylsulfonyl fluoride. Protein concentrations were determined by the BCA assay.

Electrophoretic mobility shift assays. Synthetic complementary nucleotides (Oeron Technologies) corresponding to base pairs −1410 to −1362 bp relative to the MMP-2 translational start site were annealed and end-labeled using [γ-32P]ATP and purified on acrylamide gels. Gel shift reactions were performed as detailed (1). For antibody supershift assays, 2 μg of the appropriate Fos and Jun family member rabbit polyclonal antibodies (c-Fos, sc-52x; FosB, sc-7203x; Fra1, sc-605x; Fra2, sc-13017x; c-Jun, sc-45x; JunB, sc-8051x and JunD,sc-74x, Santa Cruz Biotechnology, Santa Cruz, CA) were added to the reactions and incubated for 45 min at room temperature before gel loading.

Immunodetection of transcription factors JunB and FosB. The ventricular sections were blocked with 5% goat serum in PBS for 30 min and incubated with either rabbit anti-JunB or anti-FosB IgG (Santa Cruz) at 20 μg/ml in PBS containing 0.1% BSA for 1 h at room temperature. The sections were washed three times with PBS containing 0.05% Tween-20. Subsequently, the sections were incubated with goat anti-rabbit IgG-alkaline phosphatase polyconjugates (Zymed) for 30 min at room temperature. Washed sections were incubated with Fast-Red chromogen substrate for 20 min, washed, and lightly counterstained with methyl green.

Chromatin immunoprecipitation. Chromatin immunoprecipitation (ChIP) from isolated ventricular nuclei was performed as given in detail (1). Antibodies for the immunoprecipitation step included rabbit polyclonal anti-FosB (sc48x) and anti-JunB (sc46x, Santa Cruz Bio-
of ischemia, followed by 30 min of reperfusion increased MMP-2 transcriptional activity to 62,350 ± 2,900 LEU, whereas 30 min of ischemia followed by 90 min of reperfusion increased MMP-2 transcriptional activity to 107,500 ± 3,200 LEU (P < 0.05 for both time points). There were no further increases in β-galactosidase activity seen in extracts from hearts subjected to longer periods of reperfusion (not shown). Ischemia-reperfusion injury had no significant effect on β-galactosidase activities in the wild-type hearts. In these experiments the left ventricular (LV) developed pressure measured at the end of the 90-min reperfusion period declined to 57 ± 7% of baseline at the end of reperfusion, whereas the LV end-diastolic pressure increased 2.5-fold from a baseline of 5 mmHg. Baseline measurements were obtained at the end of the 20-min equilibration period.

To determine the precise cellular sources of MMP-2 transgene transcription, we performed immunohistochemical staining for the *E. coli* β-galactosidase antigen on paraffin-embedded ventricular sections from controls and from ventricles subjected to 30 min of ischemia-90 min of reperfusion. We chose to perform immunohistochemical detection of the β-galactosidase antigen due to the superior sensitivity of this method, as opposed to β-galactosidase-mediated cleavage of the chromogenic X-gal substrate. As depicted in Fig. 2, there was minimal staining for the β-galactosidase antigen in the wild-type, nontransgenic heart (Fig. 2A). Consistent with the basal level of MMP-2 transcription assessed by the quantitative β-galactosidase assay (Fig. 1), there was a low level of patchy immunohistochemical staining in the ventricles of the control, noninjured F8-β-gal mice (Fig. 2B). This staining is primarily confined to myocytes. Ischemia-reperfusion injury resulted in major increases in β-galactosidase antigen staining in all cardiac cell types. As shown in Fig. 2C, there is intense, patchy staining of the myocytes seen in a cross-sectional aspect, as well as staining of the more filamentous cardiac fibroblasts seen in juxtaposition to the larger myocytes (Fig. 2, C and D). In addition, the endothelial cells of small capillaries and in the coronary artery demonstrate substantial β-galactosidase antigen immunohistochemical staining (Fig. 2, E and F). Thus ischemia-reperfusion injury results in significant increases in MMP-2 transcription in all major cardiac cell types.

**Ischemia-reperfusion injury mediates intrinsic MMP-2 transcription and translation inhibition by the free radical scavenger MPG.** The above studies demonstrated increased transcription of the β-galactosidase reporter cassette driven by the transgenic MMP-2 promoter. To validate these observations within the context of the intrinsic MMP-2 gene, we performed real-time quantitative PCR for MMP-2 transcript abundance in ventricular extracts following ischemia-reperfusion injury, in the presence or absence of the free radical scavenger MPG. MPG is a hydroxyl radical scavenger previously shown at concentrations of 1 mM to reduce cardiac ischemia-reperfusion-mediated injury (38). MMP-2 transcript levels were not significantly affected by perfusion with 1 mM MPG in the absence of ischemia-reperfusion injury (Fig. 3A). After 30 min of ischemia and 30 min of reperfusion, there was a moderate 1.6-fold increase in MMP-2 transcript abundance, which was statistically significant. After 30 min of ischemia and 90 min of reperfusion, there was a larger 2.8-fold increase in MMP-2 transcript abundance, which was blocked by perfusion with MPG. MPG perfusion did not signif-

![Graph](http://ajpheart.physiology.org/DownloadedFrom/)
significantly affect MMP-2 transcript abundance in the absence of ischemia-reperfusion injury.

As detailed in Fig. 3, there was a trend for decreased MMP-2 content in cardiac lysates in hearts maintained with perfusate alone. MMP-2 protein content was not significantly increased following 30 min of ischemia and 30 min of reperfusion but was significantly increased 2.4-fold following 30 min of ischemia and 90 min of reperfusion, and this increase was blocked by inclusion of MPG in the perfusate. Thus the temporal patterns of intrinsic MMP-2 transcription and translation closely parallel the patterns obtained with the F8-H9252-gal transgenic mice.

Ischemia-reperfusion injury mediates MMP-2 transcription through the AP-1 binding site. We previously demonstrated with cultured cardiac fibroblasts that defined AP-1 complex components bind the sequence \(-1394\)CCTGACCTCC present in the rat MMP-2 promoter (bold letters denote the core AP-1 binding matrix sequence, Ref. 3). To determine whether enhanced MMP-2 transcription following ischemia-reperfusion injury utilized a similar mechanism of action, a series of studies was performed to determine whether occupancy of this site was affected by cardiac ischemia-reperfusion injury. The first set of experiments employed electrophoretic mobility shift assays using a radiolabeled oligonucleotide encompassing the AP-1 binding site and nuclear extracts isolated from either control hearts or following 30 min ischemia and 90 min of reperfusion. The shorter period of reperfusion, as opposed to the 90 min used in the \(\beta\)-galactosidase studies detailed above, was chosen due to the relative short half-life of the AP-1 transcription factors following induction of injury. As shown in Fig. 4A, nuclear extracts from control hearts yield clear-cut mobility shifts with the radiolabeled AP-1 oligonucleotide that could be competed by inclusion of cold, unlabeled oligonucleotide. A similar pattern of shifted bands was observed with the nuclear extracts from hearts subjected to ischemia-reperfusion injury; however, by densitometry there was a greater than threefold in the amount of shifted oligonucleotide, consistent with an increased abundance of the cognate nuclear binding proteins. The AP-1 sequence specificity of the nuclear protein-oligonucleotide interaction was confirmed using an oligonucleotide in which the core TGAC sequence required for AP-1 complex binding was mutated to ACAC. As shown in Fig. 4B, competition with unlabeled mutated oligonucleotide failed to eliminate nuclear protein binding to the AP-1 oligonucleotide sequence, demonstrating the sequence specificity of nuclear protein-DNA interaction.

Antibody supershift experiments were performed to determine the components of the nuclear proteins that specifically interacted with the AP-1 oligonucleotide. These experiments are summarized in Fig. 5. Antibody supershift studies with nuclear extracts from control hearts detected low levels of several AP-1 components, including Fra2, c-Jun, JunB, and JunD (Fig. 5A). Antibody supershift studies with the nuclear extracts from the ischemia-reperfused hearts demonstrated major increases in the amount of shifted JunB protein, as well as the appearance in substantial amounts of FosB protein, which was not detected in the control nuclear extracts (Fig. 5B). There was no significant change in these extracts in the amount of shifted Fra2, c-Jun, or JunD proteins, which remained at barely detectable levels.
Immunohistochemical staining for the AP-1 complex components was used to confirm the results obtained with electrophoretic mobility shift assay on isolated nuclear extracts. As shown in Fig. 6, immunohistochemical staining of control ventricles for JunB did not reveal significant nuclear staining of any major cardiac cell type (Fig. 6A). Similar results were observed with staining for the FosB antigen (Fig. 6B). After 30 min of ischemia-30 min of reperfusion, there was a dramatic increase in nuclear staining for JunB (Fig. 6C) and for FosB (Fig. 6D). Staining for both antigens was evidenced in the large myocyte nuclei (arrows in Fig. 6, C and D). Higher magnification showed intense cardiomyocyte nuclear staining for FosB (Fig. 6E), as well as nuclear staining within fibroblast and capillary endothelial cell nuclei (Fig. 6F). Similar nuclear staining patterns were also observed for JunB (not shown). These studies confirm that a defined period of ischemia-reperfusion injury results in the induction and nuclear localization of the FosB and JunB antigens in each major cardiac cell type.

In a final series of experiments, using chromatin immunoprecipitation, we asked whether the intrinsic MMP-2 promoter, within the context of cardiac genomic DNA, was occupied by JunB or FosB proteins following ischemia-reperfusion injury. The orthologous murine MMP-2 promoter has an overall nucleotide identity of 87% to the rat MMP-2 promoter used to construct the transgene, including complete conservation of the AP-1 binding site.

**Fig. 4.** Electrophoretic mobility shift assay (EMSA) of ventricular nuclear extracts (NE). EMSA demonstrates specific nuclear protein-DNA interactions with the 1420- to 1362-bp oligonucleotide containing the activator proteins-1 (AP-1) site. A: protein-DNA binding is specifically inhibited by the inclusion of increasing concentrations of cold competitor oligonucleotide into the incubation mixtures. Note that ventricular extracts from hearts subjected to I/R injury contain more nuclear protein DNA binding activity than control extracts, consistent with an increase in AP-1 nuclear proteins. B: mutated cold competitor oligonucleotide (mut 1420-1362) fails to compete for nuclear protein-DNA binding, thereby confirming the specificity of nuclear protein-DNA interaction with the AP-1 site in the 1420- to 1362-bp oligonucleotide.

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Fig. 5. Identification of AP-1 site nuclear binding proteins as FosB and JunB. A: EMSA of ventricular nuclear proteins isolated from control hearts using antibodies to each Fos and Jun family member. Low amounts of Fra2, c-Jun, JunB, and JunD are supershifted under these conditions. B: with the use of nuclear extracts from ventricles subjected to I/R injury (30 min ischemia/30 min reperfusion), there are clear supershifts of nuclear protein-DNA complexes with antibodies to FosB and JunB.

Fig. 6. Immunohistochemical detection of JunB and FosB proteins in cardiac nuclei after I/R injury. A: control ventricle stained for JunB. B: control ventricle stained for FosB antigen. Abundant nuclear staining for JunB (C) and FosB (D) after I/R injury. E: higher power magnification shows intense cardiomyocyte nuclear staining for JunB (black arrows). F: nuclear staining for FosB in a fibroblast (blue arrow) nucleus and a capillary endothelial cell (black arrow). Final magnifications: A–D: ×200; E: ×400; F: ×600.
AP-1 binding site. Controls, or hearts subjected to 30 min of ischemia and 30 min of reperfusion, were prepared for chromatin immunoprecipitation using specific anti-JunB and anti-FosB antibodies, followed by PCR amplification under linear conditions. The results of these experiments are shown in Fig. 7. Figure 7, lanes B and C, represents positive murine genomic and chromatin controls, with the expected 683-bp amplicon product. Lanes D through G represent a series of negative controls as detailed in Fig. 7. As shown in lane H, incubation of isolated cardiac chromatin with an antibody to JunB, followed by PCR, yields the predicted 683-bp amplicon. Under the linear conditions used for amplification, there was no significant difference in the relative abundance of the 683-bp amplicon between control cardiac chromatin and the chromatin isolated from hearts subjected to ischemia-reperfusion (cf. lanes H and I). In contrast, there was no amplification of the 683-bp product using the control chromatin and the antibody to FosB (lane J), whereas a very substantial amount of amplicon was recovered using chromatin isolated from hearts subjected to ischemia-reperfusion (lane K). These studies demonstrate, within the context of cardiac genomic DNA and the intrinsic murine MMP-2 promoter, that the AP-1 binding site is occupied by JunB homodimers under control conditions, whereas ischemia-reperfusion injury results in the recruitment of FosB to the AP-1 binding site, presumably as a heterodimeric complex with JunB.

DISCUSSION

The early responses and regulation of the MMP-2 promoter during ischemia-reperfusion injury in intact organs, such as the heart, have not been previously studied. Whereas MMP-2 synthesis is frequently mischaracterized as constitutive in nature, there exists a complex regulatory network that drives transcription of this gene in development and disease processes (5, 16, 17, 25–28, 34). In contrast with many members of the MMP gene family, the synthesis of MMP-2 is widely considered to be independent of the AP-1 transcriptional complex. Using cultured cardiac fibroblasts and cardiomyocytes, we recently reported that hypoxia enhances the MMP-2 transcriptional response to angiotensin II and endothelin-1 (3). By using a series of deletion constructs, we found that the hypoxia-response element in the MMP-2 promoter consisted of a noncanonical AP-1 binding site located at −1392 bp relative to the translational start site. Analyses of nuclear extracts and antibody supershift experiments indicated that this site was primarily occupied by Fra1/JunB heterodimers under control conditions and that in vitro hypoxia resulted in a major induction of FosB, with resultant increases in transcription rates. The current in vivo study was designed to determine whether similar regulatory events control enhanced MMP-2 transcription within the intact heart under conditions of defined ischemia-reperfusion injury. As detailed, the current studies demonstrate a rapid induction of both MMP-2 gene transcription and translation in response to ischemia-reperfusion injury. We note that expression of the β-galactosidase reporter protein under the control of the MMP-2 promoter was more rapid than that observed with the intrinsic MMP-2 gene. This is likely due to intrinsic differences in β-galactosidase and MMP-2 mRNA processing or translation.

Ischemia-reperfusion injury induces synthesis and nuclear localization of two discrete members of the AP-1 transcription complex FosB and JunB and is associated with enhanced occupancy of the intrinsic MMP-2 promoter by FosB/JunB complexes. Thus, these in vivo studies are consistent, to a large extent, with the in vitro observations obtained with cultured cardiac fibroblasts subjected to hypoxia and indicate that all three major cardiac cell types participate in this process within the context of the intact heart.

Earlier studies of MMP-2 regulation by ischemia-reperfusion injury have primarily relied on gelatin zymography of ventricular extracts or coronary effluent (6, 32). Although valuable, this approach does not permit definition of the synthesizing cell types as was performed in the current study. Cardiomyocyte expression of MMP-2 has been associated with contractile dysfunction, possibly due to intracellular cleavage of sarcomeric troponin I (6, 42). Endothelial cell expression of MMP-2 as observed in this study may directly contribute to endothelial cell dysfunction through the MMP-2-mediated generation of vasoconstrictor endothelin peptides or promotion of neutrophil-endothelial cell adhesion (11, 12). In addition, MMP-2 directly induces platelet aggregation, which could further contribute to disruption of the cardiac microcirculation following ischemia-reperfusion injury (35). The short-term effects of MMP-2 expression on the cardiac fibroblast population are unclear at this time.

Small molecule mediators of ischemia-reperfusion injury include oxygen free radicals and peroxynitrite (10, 13, 24, 37, 40, 41). Peroxynitrite and related oxidants can directly activate MMP-2 via disruption of the cysteine link between the prodomain and Zn²⁺ in the catalytic domain (30, 41). Redox stresses also signal through the activation of specific transcriptional networks. Specifically, cardiac redox stress following ischemia-reperfusion injury has been linked to activation of the AP-1 and NF-κB transcription factors (10, 24, 33). AP-1
transcription factor activation has been linked to phosphorylation by extracellular signal-regulated kinase 1/2 (ERK) and is associated with nuclear accumulation of Fos and Jun proto-oncogenes in hypoxic cardiac myocytes (29, 43). Importantly, there is a link between the AP-1 and NF-κB pathways, because NF-κB regulates the transcription of several Fos and Jun proto-oncogene family members (14). The regulatory regions of both the FosB and JunB genes include NF-κB binding sites, and functional interaction of NF-κB with a 3′ JunB regulatory region has been demonstrated (23). Thus it is reasonable to speculate that the cardiac-specific induction of the FosB and JunB transcription factors observed following ischemia-reperfusion injury occurs through signaling via NF-κB activation as well as ERK phosphorylation. This possibility is supported experimentally by the suppression of MMP-2 transcription and translation when the free radical scavenger MPG was included in the perfusion solution. We note that NF-κB is unlikely to directly affect MMP-2 transcription, because the promoter lacks canonical binding sites.

Chromatin immunoprecipitation studies indicated that the AP-1 binding site of the intrinsic MMP-2 promoter was occupied by JunB homodimers under basal conditions. JunB homodimers have a lower binding affinity to DNA than FosB-JunB heterodimers and less transactivation capacity (19). This pattern is consistent with the levels of MMP-2 expression, which are low in the controls and greatly induced by ischemia-reperfusion injury and promoter occupancy by JunB-FosB heterodimers. Our observations are also consistent with the rapid appearance of mRNA transcripts for MMP-2 after focal cerebral ischemia in the baboon (4) and after short episodes of ischemia-reperfusion in the rat (36). The deleterious effects of MMP-2 activation (6, 42) on the extent of cardiac damage produced by acute ischemia-reperfusion injury can be prevented by MMP-2 inhibition. Thus Girič et al. (15) recently reported that pharmacological inhibition of MMP-2 in rats produced cardioprotection equivalent to ischemic preconditioning. Although hyperlipidemia prevented the beneficial effect of preconditioning, cardioprotection in the presence of hyperlipidemia was preserved during pharmacological inhibition of MMP-2 (15). Our data also help to provide a molecular basis for both the acute and more prolonged elevation of peripheral blood levels of MMP-2 reported in patients with acute coronary syndromes and myocardial infarction (20).

In summary, we have demonstrated that induction of MMP-2 transcription and translation following ischemia-reperfusion injury is part of a coordinated genetic response mediated by specific AP-1 transcription factor components. The observation that MMP-2 transcription is induced in all three major cardiac cell types suggests that MMP-2 may affect the final phenotypic manifestations of ischemia-reperfusion injury at multiple cellular levels, underscoring the significance of these observations. Future studies aimed at inhibition of JunB/FosB promoter binding, coupled with microarray analysis, may be expected to provide further mechanistic insights into ischemia-reperfusion injury.

GRANTS

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