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Induction of nuclear factor κB and activation protein 1 in postischemic myocardium

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Abstract Ischemia/reperfusion induces nuclear factor κB (NF-κB) and AP-1 in rat hearts after 15 min of ischemia followed by reperfusion (R) for various periods of time (15 and 30 min, 1, 2, 3, 6, 12, and 24 h). Low levels of NF-κB and no signal for AP-1 were detected in shams and in non-ischemic tissue distant from the ischemic zone. In postischemic tissue, NF-κB levels increased biphasically with peak levels at 15 min and again at 3 h R. Immunoblotting showed minimal NF-κB p50 subunit at all times, with changes in p65 similar to EMSA results. Northern blots showed low p50 and increased p65 expression levels at both 2 and 3 h R. By contrast, AP-1 increased monophasically, with peak levels at 15 min R, which dropped steadily thereafter. These results indicate that NF-κB and AP-1 are differentially regulated during reperfusion, which may be a control mechanism for gene expression in reperfused myocardium.

Key words: Reperfusion injury; Activation protein 1; Nuclear factor κB; NF-κB p50–p65 subunit; Transcription factor

1. Introduction

In the past decade it has been established that free radicals — reactive oxygen intermediates (ROI) and NO — play a causal role in myocardial reperfusion injury, resulting in a condition characterized by myocardial dysfunction [1,2]. ROI have been shown in various in vitro systems to be important second messengers in the activation of nuclear factor κB (NF-κB) [3–5]. NF-κB, detected ubiquitously, is an inducible multisubunit DNA-binding protein that has been implicated in the transcriptional regulation of many genes involved in inflammation, immune responses, and acute phase reactions [3–5]. It consists of a DNA-binding subunit of 50 kDa that usually is found complexed with a second subunit of 65 kDa. In the cytosol, NF-κB exists as an inactive form because of its association with an inhibitory molecule, I-κB. Upon activation NF-κB dissociates from I-κB, and the p50–p65 heterodimer translocates to the nucleus, binds to decameric consensus sequences in the promoter/enhancer regions of the target genes, and induces their expression.

AP-1 is another well-characterized eukaryotic transcription factor that is highly regulated by the redox status of the cell [6,7]. It is composed of various subunits (jun, fos, and Fra) as dimers, which recognize with different affinities the AP-1 DNA-binding site. Though the mechanism of ROI induction of AP-1 is not clear, alteration of cell thiol redox status has been shown to induce c-fos and c-jun expression, and phosphorylation of jun proteins. Many factors including ionizing radiation, cytokines, and growth factors lead to AP-1 activation. Similar to NF-κB, activation of AP-1 induces expression of a variety of genes whose protein products may either protect the cells from, or make the cells more susceptible to, oxidative stress. Although we and others have shown increased proinflammatory cytokine (IL-1β, IL-6, and TNF-α) as well as iNOS expression in postischemic myocardium [8,9], the mechanisms by which these inflammatory molecules are up-regulated is not known. Since ROI are known to activate NF-κB and AP-1, and because these cytokines contain AP-1 and NF-κB response elements [10,11], it is reasonable to postulate that in ischemia/reperfusion (I/R), where ROI are known to be elevated [1,2], AP-1 and NF-κB may play a role. The purpose of this study was to examine the levels of AP-1 and NF-κB, and the expression of the major subunits of NF-κB (p50 and p65) at various times after a brief ischemic episode in reperfused myocardium.

2. Materials and methods

2.1. Animals

WKY rats (200 g) obtained from Charles River Laboratories were used in the present studies. Before their use in the experiments, animals were housed at least for 4 days in a climate- and light-controlled environment at the Laboratory Animal Care facility at the University of Texas Health Science Center, San Antonio. The animals were anesthetized with a rat cocktail, containing 44 mg/kg ketamine, 1 mg/kg acepromazine, and 8.5 mg/kg xylazine, intubated, and placed on a small animal ventilator. The chest was opened by midline sternotomy, and a 6-0 silk ligature was passed around the LAD coronary artery and through a snare. In the I/R group the snare was tightened until coronary occlusion occurred. This was readily discernible by the development of a dusky, bulging region of myocardium. Careful note was made of anatomic landmarks of this region. After 15 min the snare was released, allowing reperfusion of the ischemic zone. Animals (4/group) were randomly assigned to different durations of reperfusion (R), either 15 min, 30 min, 1, 2, 3, 6, 12, or 24 h. In sham-operated animals the same procedure was followed, but the snare was not tightened. After the experimental period the heart was rapidly excised and rinsed in ice-cold physiological saline. The right ventricle and atria were trimmed away, and the LV divided into ischemic and non-ischemic zones on the basis of the previously defined landmarks. The tissue was snap frozen in liquid N_2, and stored for not more than 3 days at −82°C before extracting total RNA and protein.

2.2. Electrophoretic mobility shift assay

Protein extracts were prepared from frozen hearts using a buffer containing 20 mM Hepes, pH 7.8, 300 mM NaCl, 0.4 mM EDTA, 0.5 mM DTT, 25% glycerol, and 0.5 mM PMSF, spun at 14000 rpm for 10 min at 4°C. Protein concentration was determined by BCA protein assay reagent (Pierce, Rockford, IL). The electrophoretic mobility shift assay (EMSA) was performed essentially as described by Dent and Latchman [12] with some minor modifications. Briefly, a double-stranded oligonucleotide (NF-κB, 5′-AGT TGA GGG GAC TTT CCC AGG C-3′; AP-1, 5′-CGC TTG ATG ACT CAG CCG GAA-3′; Santa Cruz Biotechnology, Inc., Santa Cruz, CA) containing a tandem repeat of the consensus sequences of -GGGG-ACTTTCC- (NF-κB) or -TGACTCA- (AP-1) was end-labeled with

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[γ-^{32}P]ATP (Amersham, Arlington Heights, IL) and T4 polynucleotide kinase (Promega, Madison, WI). The binding reaction was performed by mixing 20 μg of protein extract, 2 μg of poly (dI-dC) (Pharmacia, Nutley, NJ) and 111 TBq/mmol [γ-^{32}P]ATP-labeled oligo probe in binding buffer (20 mM Hepes (pH 7.9), 1 mM $MgCl_2$, 4% Ficoll, 0.5 mM DTT, KCl to a final concentration of 50 mM) and then incubated for 40 min on ice. For the competition assay the protein extract (20 μg) was preincubated with homologous unlabeled oligonucleotide for 5 min on ice, followed by the addition of labeled probe. All the samples were then electrophoresed at 150 V through 4% polyacrylamide gel (0.25×TBE). The gels were dried and autoradiographed with intensifying screens, and the autoradiographic bands were semi-quantified by videoimage analysis. A corresponding mutant oligo (NF-κB, 5′-AGT TGA GGC GAC TTT CCC AGG C-3′; AP-1, 5′-CGC TTG ATG ACT TGG CCG GAA-3′; Santa Cruz Biotechnology, Inc.) was used as a control.

Antibodies and Western blot analysis: NF-κB subunit-specific polyclonal antibodies (p50 and p65) were from Santa Cruz Biotechnology, Inc. Specificity of these antibodies was verified by incubating them with their respective antigens for 1 h at 37°C followed by 14 h at 4°C.

Equal amounts of homogenates from ischemic and non-ischemic heart tissue were subjected to Tris-glycine 16.5% SDS-PAGE, and electroblotted onto nitrocellulose membrane (S&S, Inc., Keene, NH) [13,14]. The membranes were then incubated with 10% normal goat serum (pre-immune) to block for non-specificity followed by incubation at 23°C for 1 h and 18 h at 4°C with respective primary antibody (after determining the optimal concentrations of antibodies). The membranes were then washed with a buffer containing 20 mM Tris (pH 7.5), 500 mM NaCl, and 0.05% (v/v) Tween-20, incubated with

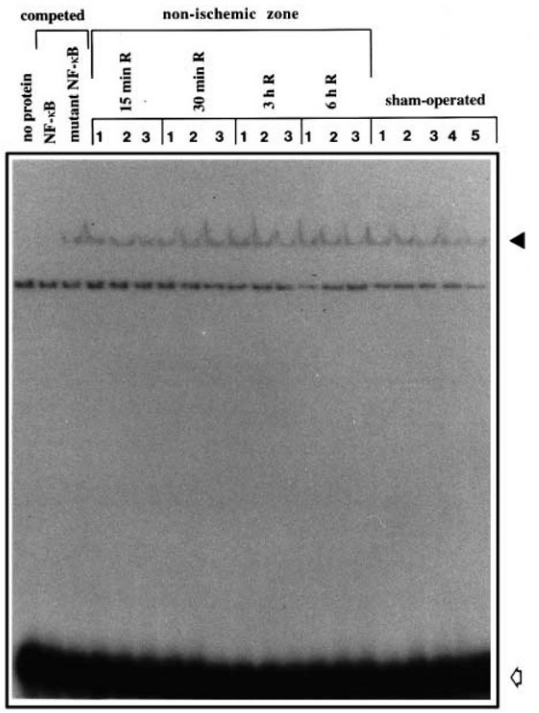

Fig. 1. Analysis of NF-κB levels by EMSA. EMSA was performed with 20 μg of heart homogenate and ^{32}P-labeled double-stranded NF-κB consensus sequence and electrophoresed through 4% polyacrylamide gel. The autoradiographic exposure time was 8 h at −80°C with intensifying screens, and the autoradiogram of the native gel is shown. Each lane represents protein homogenate from an individual animal. ◄, Complexes tested for specific binding; ⇐, unincorporated labeled probe. Non-ischemic zone: myocardium adjacent to ischemic zone at the indicated periods of reperfusion. Sham-operated: control. Competed: NF-κB: labeled NF-κB+250-fold excess unlabeled NF-κB (consensus sequence) oligo+20 μg of homogenate from postischemic portion of myocardium at 15 min R. mutant NF-κB: labeled NF-κB+250-fold excess unlabeled mutant NF-κB oligo+20 μg of homogenate from postischemic portion of myocardium at 15 min R.

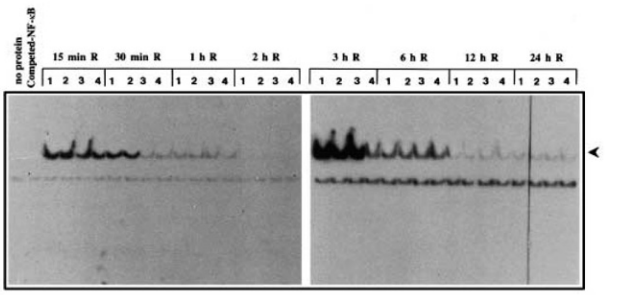

Fig. 2. Effect of various periods of reperfusion following a single episode of ischemia on NF-κB levels. EMSA was performed essentially as described in Section 2. Each lane represents protein homogenate from an individual animal. The autoradiographic exposure time was 8 h at −80°C with intensifying screens, and the autoradiogram of the native gel is shown. No protein: all the components in the reaction mixture including labeled NF-κB are present except the protein homogenate. Competed NF-κB: Competed with 250-fold excess unlabeled NF-κB and protein homogenate from sample 1 of 15 min R.

the second antibody (goat anti-rabbit) for 2 h at 23°C, washed, and incubated at 23°C for 2 h with [^{125}I]protein A (0.33 μCi/ml; Amersham). Autoradiography was performed by exposing the blots to Kodak XAR-5 film at −80°C with intensifying screens. The intensity of autoradiographic bands was semi-quantified as described above.

RNA extraction and Northern blot analysis. Total RNA was extracted from frozen heart tissue using acid-guanidium isothiocyanate-phenol-chloroform [15], and equal amounts (30 μg) of RNA were denatured in 2.2 M formaldehyde, size fractionated on 0.8% agarose gels containing 0.5 μg/ml ethidium bromide to check RNA integrity and loading equivalency, and electroblotted at 4°C onto a nitrocellulose membrane (Schleicher and Schuell, Inc., Keene, NH) in 0.025 M phosphate buffer, pH 6.5 [13,14]. RNA was UV cross-linked (Stratalinker 2400, Stratagene, La Jolla, CA) to the membrane. The blot was prehybridized for 4 h at 42°C in a prehybridization buffer that contained 50% formamide, 0.1% SDS, 5×SSC, 2.5×Denhardt's, 250 μg/ml denatured sonicated salmon sperm DNA (Stratagene), 50 mM Na_2PO_4, pH 6.5. The blots were then hybridized at 42°C for 16 h with ^{32}P-labeled probes (6×10^5 cpm/ml), washed twice at 23°C in 6×SSC/0.5% SDS, twice at 37°C in 1×SSC/0.5% SDS, and once at 57°C in 0.1×SSC/0.5% SDS. All blots were then exposed at −80°C to Kodak XAR-5 film with Kodak intensifying screens, and the intensity of the autoradiographic bands was semi-quantified by videoimage analysis [13,14]. The same membrane was reprobed after stripping off its previous label.

The NF-κB p50 and p65 cDNA probes (a kind gift from Dr. Martin L. Scott, MIT, Cambridge, MA) [16,17] were labeled with [α-^{32}P]dCTP (3000 Ci/mmol; Amersham) to a specific activity of 0.6–1.0×10^9 cpm/μg, using random hexanucleotide primers (Boehringer Mannheim, Indianapolis, IN). In order to assess loading equivalency and RNA integrity, 28S rRNA (h28S rRNA; 40mer single-stranded synthetic oligonucleotide; Oncogene Science, Inc., Uniondale, NY) was used as an internal control, and was 5′-end labeled with [γ-^{32}P]ATP (Amersham, Arlington Heights, IL) and T_4 polynucleotide kinase (Gibco BRL, Grand Island, NY). mRNA sizes were determined in relation to the relative mobility of 28S and 18S rRNA, and an mRNA ladder (0.24–9.5 kb; Gibco BRL).

Statistical analysis. The data (mean ± SEM) for levels of expression of individual protein (EMSA and Western blot) and mRNA were subjected to analysis of variance with post hoc Dunnett's 1-tailed *t*-tests (NF-κB EMSA, Immunoblotting and Northern), and 1-sample Student's *t*-test for significance (AP-1 EMSA, as AP-1 was not detected in the controls). $P<0.05$ was considered significant.

3. Results

3.1. NF-κB levels

Fig. 1 shows NF-κB levels in the myocardium from controls (sham-operated and non-ischemic portion adjacent to is-

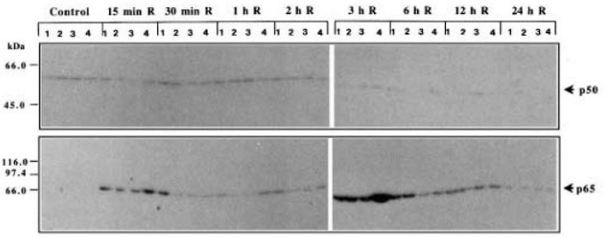

Fig. 3. NF-κB p50 and p65 subunit protein levels in control (sham-operated) and postischemic myocardium for (n=4). Equal amounts of protein per lane were electrophoresed using Tris-glycine 16.5% SDS-PAGE. The separated proteins were electroblotted onto nitrocellulose. After blocking with 10% normal goat serum, the membrane was sequentially incubated with the primary, secondary and ^{125}I-Protein A. Autoradiographic exposure time was 4 days for both the subunits. Pre-stained protein molecular mass standards (Bio-Rad) were shown on the left. Arrow indicates the relative position of protein detected by the respective antibody.

chemic zone). Low and consistent levels of NF-κB were detected in normal myocardium (sham-operated) at steady state, and no detectable change was observed in the non-ischemic tissue. Specificity of the signal was verified in a competition assay wherein the signal detected by labeled NF-κB was abolished when the protein homogenate was preincubated with excess unlabeled NF-κB oligo before the addition of labeled NF-κB. When a mutant oligo was used for the competition, there was no interference with the signal generated by labeled NF-κB. Furthermore, when labeled mutant NF-κB was used in the EMSA, no signal was detected (data not shown).

Fig. 2 shows changes in NF-κB levels in the postischemic myocardium. Significantly higher levels of NF-κB were detected at 15 min R in all 4 animals (densitometry: 3-fold; $P<0.0001$), remained high in two out of four animals at 30 min R (2-fold, not significant), and then fell to steady state at 1 h R. Again at 3 h R, the levels rose significantly higher (4.56-fold; $P<0.0001$), and were 1.5-fold higher than the levels at 15 min R ($P<0.005$). The levels remained high at 6 h R (3-fold; $P<0.005$), and fell thereafter and remained low until 24 h R, indicating a biphasic regulation of NF-κB in postischemic myocardium.

3.2. NF-κB p50 and p65 protein levels

Because variation in NF-κB levels was not detected in either sham-operated animals or in non-ischemic tissue adjacent to the ischemic zone, and between these two groups (Fig. 3), we used myocardium from sham-operated animals as controls for Western blotting experiments. Low and consistent levels of NF-κB p50 subunit were observed in both the controls and in postischemic myocardium at all time periods tested (Fig. 3). In the case of p65 subunit, the levels were higher at 15 min R (densitometry: 5-fold; $P<0.0001$), and decreased gradually thereafter (30 min R, 3-fold; $P<0.025$; 1 h R, 2-fold, $P<0.001$; 2 h R, 2-fold, $P<0.005$) (Fig. 3). Again at 3 h R, the levels raised significantly higher (7-fold: $P<0.0001$; 1-fold: $P<0.01$ vs. levels at 15 min R) and remained high ($P<0.0001$) at 6 h (5-fold), 12 h (3-fold), and fell thereafter at 24 h R (2-fold: $P<0.001$).

3.3. NF-κB p50, and p65 mRNA levels

Fig. 4 shows changes in NF-κB subunit mRNA levels in control (sham-operated) and in the postischemic myocardium. In control animals, both p50 and p65 mRNA was detected but at low levels, with levels of p65 which were little higher than p50. In postischemic myocardium, low and consistent levels of p50 were detected at all times tested. In contrast, p65 mRNA levels increased at 1 h R (densitometry: 1.4-fold, $P<0.05$), peaked at 2 h R (2.9-fold: $P<0.001$), remained elevated at 3 h R (1.3-fold: $P<0.05$) and declined gradually thereafter, and remained low until 24 h R (Fig. 4).

3.4. AP-1 levels

Fig. 5 shows changes in AP-1 levels in control (non-ischemic tissue adjacent to ischemic zone and sham-operated) and in the postischemic myocardium. No signal was detected in either control. However, signal was readily detected in postischemic myocardium, with peak levels at 15 min R ($P<0.001$; Fig. 5B). Though the levels declined gradually, they were still high at 30 min, 1 h, 2 h, and 3 h R, and by 24 h R very weak signal for AP-1 was detected. Preincubation of protein homogenate with excess unlabeled AP-1 consensus oligo abolished specific signals obtained by labeled AP-1, demonstrating the specificity of signals (Fig. 5A). Further, when competed with mutant AP-1 oligo, there was no interference with the signal generated by labeled AP-1. Furthermore, when labeled mutant AP-1 was used in the EMSA, no signal was detected (data not shown).

4. Discussion

The results of our experiments show for the first time that there is a biphasic regulation of NF-κB in postischemic myocardium. Immunoblotting and mRNA analyses revealed increased levels of NF-κB p65 subunit, but low and consistent levels of p50 subunit. In addition, increased levels of AP-1 were detected, but in a monophasic manner. These results

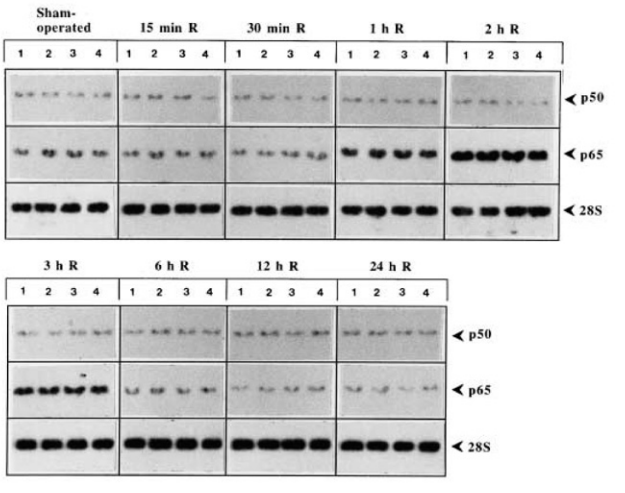

Fig. 4. Expression of NF-κB p50 and p65 subunit mRNA in the control and postischemic myocardium. Thirty micrograms of total RNA per lane was electrophoresed, electroblotted onto nitrocellulose, and fixed by UV irradiation. The blot was reprobed after stripping off the previous probe. mRNA size was determined in comparison to the relative mobility of 28S and 18S rRNA and to that of the mRNA ladder (0.2–9.5 kb). 28S rRNA was used as an internal control and indicates equal levels of RNA loading in all lanes of the gel. The autoradiographic time was 4 days for p50 and p65, and 14 h for 28S rRNA. Even after 8 days of autoradiographic exposure, no signal was detected in lanes that did not have detectable levels of mRNA.

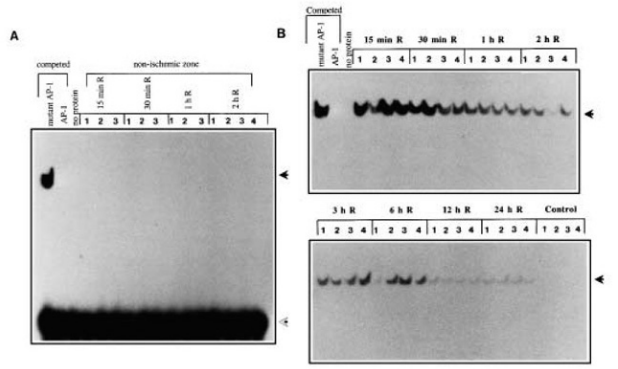

Fig. 5. (A) Levels of AP-1 in non-ischemic tissue adjacent to ischemic zone (internal control) detected by EMSA. EMSA was performed essentially as described in the legend to Fig. 1. After incubation of 20 μg of heart homogenate with ^{32}P-labeled double-stranded AP-1 consensus sequence, it was electrophoresed through 4% polyacrylamide gel. The autoradiographic exposure time was 8 h at −80°C with intensifying screens, and the autoradiogram of the native gel is shown. Each lane represents protein homogenate from an individual animal. ←, Complexes tested for specific binding; ⇐, unincorporated labeled probe. Competed: (1) AP-1: labeled AP-1+250-fold excess unlabeled AP-1 (consensus sequence) oligo+20 μg of homogenate from postischemic myocardium at 15 min R. (2) Mutant AP-1: Labeled AP-1+250-fold excess unlabeled mutant AP-1 oligo+20 μg of homogenate from postischemic myocardium at 15 min R. (B) Levels of AP-1 in postischemic myocardium at indicated periods of reperfusion. Control: sham-operated.

indicate that NF-κB p65 as opposed to p50 may play a predominant role, and AP-1 and NF-κB are differentially regulated in myocardium during ischemia/reperfusion.

We found a biphasic regulation of NF-κB, with increased levels at an early time point (15 min), and again at 3 h R. It is likely that increased levels of ROI present in the tissue immediately after reperfusion activated the preexisting NF-κB which was present in the cytoplasm, resulting in increased NF-κB at 15 min R. Given the transient nature of ROI elevation, however, it is difficult to attribute the secondary rise in NF-κB at 3 h R to this mechanism. The timing of this event suggests it may have resulted from synthesis of new protein, induced by the I/R sequence. In view of the known presence of proinflammatory cytokines in the tissue under these circumstances (possibly induced by activated NF-κB early in reperfusion), it is reasonable to speculate that they may have further induced ROI production, and provided the stimulus for increased NF-κB at 3 h R. Prior work has shown that many NF-κB activators induce oxidative stress: cells treated with TNF-α, IL-1, or γ-irradiation all had increased production of ROIs [18,19]. While the precise mechanisms by which these cytokines increase ROI production is not known, evidence suggests that in the case of TNF-α, mitochondrial production of ROI through an alteration in the mitochondrial electron flow occurs [20]. Regardless of their source, presence of ROI likely leads to activation of NF-κB.

A second key observation of our study is the detection of increased levels of p65, but low and consistent levels of p50 in the postischemic myocardium. Further, mRNA analysis revealed similar results with low and consistent levels of p50, and an increase in p65 with peak levels at 2 h reperfusion. The levels dropped steadily, and by 24 h reperfusion, the levels were similar to that observed at basal conditions. Induction of NF-κB at very early times is reported to be at the post-translational level, and this may underlie the initial peak we observed in NF-κB, resulting from activation of existing p50 and p65 or p65 homodimers. The subsequent increase observed at 3 h reperfusion, coincident with presence of mRNA, is suggestive of increased p65 transcription and translation. Prior studies have shown that p50 is a DNA-binding subunit, and p65 is an I-κB binding subunit. Following appropriate stimuli, I-κB dissociates from p65 and existing p50–p65 heterodimers translocate to the nucleus [3–5]. Recent studes have, however, shown that in some instances p65 can act as a potential transcriptional activator of κB enhancer-containing promoters [21,22] in the absence of association with p50. In addition, p65 has been shown to form heterodimers with c-Rel before binding to DNA [23]. Because the role of other subunits is not known, additional studies will be required to clearly establish the function and regulation of p65 in the pathophysiology of myocardial reperfusion injury.

The intracellular events which lead to the activation of NF-κB (i.e. dissociation of I-κB) are complex and involve phosphorylation and proteolytic reactions that are in general controlled by the redox status of the cell [23]. Immediately after the initiation of reperfusion from ischemia, the myocardium is exposed to unphysiologically high levels of ROI [1,2], and consequent oxidative stress, which activates NF-κB. Prior studies have shown that activation of NF-κB in various immune cells results from the addition of oxidants such as H_2O_2, which leads to release of I-κB from NF-κB heterodimer in the cytoplasm leading to p50–p65 translocation to the nucleus [24].

We and others have shown that there is induction of proinflammatory cytokines such as IL-β, IL-6 and TNF-α in the postischemic myocardium [8,9]. Because these cytokines contain κB response elements in their promoter/enhancer regions, it is reasonable to speculate that increased ROI produced during I/R might have activated NF-κB, which in turn might have induced, through various signaling pathways, mRNA expression of these cytokines. It is now well established that in addition to ROI, cardiac tissue generates NO in response to soluble inflammatory mediators, through the delayed expression of iNOS [25]. The 5′-flanking region of iNOS is quite complex and contains consensus sequences for several transcription factors, some in multiple copies, including two copies of the NF-κB response element, and two copies of TNF response elements. This indicates that once it is activated, NF-κB can translocate to the nucleus, and bind to the iNOS promoter [26]. Of note, Goldring et al. have demonstrated that by reducing NF-κB/Rel activity through competitive binding (of phosphorothioate-modified oligo containing three copies of NF-κB consensus sequences), a 50% reduction in NO output and a reduction in the quantity of iNOS protein was achieved, implying that activated NF-κB plays a major role in the induction of iNOS [27]. Thus, NF-κB, which is involved in the regulation of IL-1, IL-6, and TNF-α could also represent a potential transcriptional modulator of iNOS in the myocardium.

The third key observation of our study is the detection of increased AP-1 levels in the postischemic myocardium. AP-1 was not detected in the controls (sham-operated) and its levels increased in a monophasic manner. In contrast to NF-κB where a second peak was observed at 3 h R, AP-1 levels declined gradually after an initial increase at 15 min of reper-

fusion. Though we did not measure the levels of AP-1 during the ischemic period, in various in vitro systems, substantial increase in AP-1 levels was demonstrated during hypoxic conditions. In a cancerous cell line (HeLa), Rupec and Baeuerle have shown increased NF-κB activity within 15 min after initiation of reperfusion, while increased AP-1 was detected during hypoxia itself [28]. Further, levels of AP-1 increased in a biphasic manner during reoxygenation. They argued that during reoxygenation, increased intracellular ROI activate existing NF-κB by dissociation from its inhibitor IκB, while low levels of free radicals during hypoxia, a condition similar to that observed during antioxidant treatment, induced AP-1. Though AP-1 is induced during both prooxidant and antioxidant conditions, they speculated that AP-1 induced during prooxidant status may have weak DNA binding and weak transactivating potential, and during antioxidant status, it will have full DNA-binding and full transactivating potential [28]. However, more studies are needed to elucidate the precise roles of these factors in postischemic myocardium.

Because both NF-κB and AP-1 are activated by cytokines such as IL-1 and TNF-α, the positive synergy between NF-κB and the subunits of AP-1 might have important implications for both immune and inflammatory responses. Stein et al. have shown functional cross-coupling of NF-κB p65 and AP-1 families of transcription factors, resulting in increased DNA binding activity of NF-κB [29]. Both c-fos and c-jun synergised with NF-κB by physically interacting with p65 subunit [29]. Whether such interactions exist in reperfused myocardium is not known. In conclusion, we have shown a biphasic increase in levels of NF-κB and a monophasic increase in AP-1 in the postischemic myocardium. Of the two subunits of NF-κB, p65 appears to play the dominant role, while p50 levels were consistent over time. This raises the interesting possibility that NF-κB may serve as an important regulator of transcriptional events during I/R. This factor may participate in the rapid production of inflammatory cytokines (IL-1, IL-6, TNF-α, iNOS) during reperfusion, and may contribute to the reversible loss in function and tissue damage during the postischemic period. While more studies are needed to clarify the role of AP-1 during ischemia/reperfusion, interference with the activation or the activity of NF-κB may prove to be beneficial in suppressing or minimizing some of the deleterious effects observed following reperfusion of ischemic tissue.

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References

[1] Jabr, R.I. and Cole, W.C. (1995) Cir. Res. 76, 812–834.
[2] Hess, M.L. and Manson, N.H. (1984) J. Mol. Cell. Cardiol. 16, 969–985.
[3] Grilli, M., Chiu, J.J. and Lenardo, M.J. (1993) Int. Rev. Cytol. 148, 1–62.
[4] Baeuerle, P.A. and Baltimore, D. (1991) In The Molecular Aspects of Cellular Regulation, Hormonal Control, Regulation of Gene Transcription (Cohen, P. and Foulkes, J.G., eds.) pp. 409–432, Elsevier, Amsterdam.
[5] Baeuerle, P.A. (1991) Biochem. Biophys. Acta. 1072, 63–80.
[6] Angel, P. and Karin, M. (1991) Biochem. Biophys. Acta. 1072, 129–157.
[7] Sen, C.K. and Packer, L. (1996) FASEB J. 10, 709–720.
[8] Freeman, G.L. and Chandrasekar, B. (1996) FASEB J. 10, A275 (1583).
[9] Herskowitz, A., Choi, S., Ansari, A.A. and Wesselingh, S. (1995) Am. J. Pathol. 146, 419–428.
[10] Kuprash, D.V., Udalova, I.A., Turetskaya, R.L., Rice, N.R. and Nedospasov, S.A. (1995) Oncogene 11, 97–106.
[11] Lieberman, T.A. and Baltimore, D. (1990) Mol. Cell. Biol. 10, 2327–2334.
[12] Dent, C.L. and Latchman, D.S. (1993) In Transcription Factors: a Practical Approach. (Latchman, D.S., ed.) pp. 1–26, IRL Press, New York.
[13] Chandrasekar, B., Melby, P.C., Troyer, D.A. and Freeman, G.L. (1996) Biochem. Biophys. Res. Commun. 223, 365–371.
[14] Chandrasekar, B., McGuff, H.S., Aufdermorte, T.B., Troyer, D.A., Talal, N. and Fernandes, G. (1995) Clin. Immunol. Immunopathol. 3, 291–296.
[15] Chomczynski, P. and Sacchi, N. (1987) Anal. Biochem. 162, 156–159.
[16] Ghosh, S., Gifford, A.M., Riviere, L.R., Tempst, P., Nolan, G.P. and Baltimore, D. (1990) Cell. 62, 1019–1029.
[17] Nolan, G.P., Ghosh, S., Liou, H.-C., Tempst, P. and Baltimore, D. (1991) Cell. 64, 961–969.
[18] Osborn, L.S., Kunkel, S. and Nabel, G.J. (1989) Proc. Natl. Acad. Sci. USA 86, 2336–2340.
[19] Datta, R., Hallahan, D.E., Kharbanda, S.M., Rubin, E., Sherman, M.L., Huberman, E., Weichselbaum, R.R. and Kufe, D.W. (1992) Biochemistry 31, 8300–8306.
[20] Schulze-Osthoff, K., Beyaert, R., Vandevoorde, V., Haegeman, G. and Fiers, W. (1993) EMBO J. 12, 3095–3104.
[21] Ruben, S.M., Dillon, P.J., Schreck, R., Henkel, T., Chen, C.-H., Maher, M., Baeuerle, P.A. and Rosen, C.A. (1991) Science 251, 1490–1493.
[22] Ruben, S.M., Narayanan, R., Klement J.F., Chen, C.-H. and Rosen, C.A. (1992) Mol. Cell. Biol. 12, 444–454.
[23] Staal, F.J., Roederer, M., Herzenberg, L.A. and Herzenberg, L.A. (1990) Proc. Natl. Acad. Sci. USA 87, 9943–9947.
[24] Meyer, M., Schreck, R. and Baeuerle, P.A. (1993) EMBO J. 12, 2005–2015.
[25] Zweier, J.L., Wang, P. and Kuppusamy, P. (1995) J. Biol. Chem. 270, 304–307.
[26] Xie, Q.-W., Kashiwabara, Y. and Nathan, C. (1994) J. Biol. Chem. 269, 4705–4709.
[27] Goldring, C.E.P., Narayanan, R., Lagadec, P. and Jeannin, J.-F. (1995) Biochem. Biophys. Res. Commun. 209, 73–79.
[28] Rupec, R.A. and Baeuerle, P.A. (1995) Eur. J. Biochem. 234, 632–640.
[29] Stein, B., Baldwin, A.S. Jr., Ballard, D.W., Greene, W.C., Angel, P. and Herrlich, P. (1993) EMBO J. 12, 3879–3891.