An essential role of NFκB in tyrosine kinase signaling of p38 MAP kinase regulation of myocardial adaptation to ischemia

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Abstract We have recently demonstrated that myocardial adaptation to ischemia triggers a tyrosine kinase regulated signaling pathway leading to the translocation and activation of p38 MAP kinase and MAPKAP kinase 2. Since oxidative stress is developed during ischemic adaptation and since free radicals have recently been shown to function as an intracellular signaling agent leading to the activation of nuclear transcription factor, NFκB, we examined whether NFκB was involved in the ischemic adaptation process. Isolated perfused rat hearts were adapted to ischemic stress by repeated ischemia and reperfusion. Hearts were pretreated with genistein to block tyrosine kinase while SB 203580 was used to inhibit p38 MAP kinases. Ischemic adaptation was associated with the nuclear translocation and activation of NFκB which was significantly blocked by both genistein and SB 203580. The ischemically adapted hearts were more resistant to ischemic reperfusion injury as evidenced by better function recovery and less tissue injury during post-ischemic reperfusion. Ischemic adaptation developed oxidative stress which was reflected by increased malonaldehyde formation. A synthetic peptide containing a cell membrane-permeable motif and nuclear sequence, SN 50, which blocked nuclear translocation of NFκB during ischemic adaptation, significantly inhibited the beneficial effects of adaptation on functional recovery and tissue injury. In concert, SN 50 reduced the oxidative stress developed in the adapted myocardium. These results demonstrate that p38 MAP kinase might be upstream of NFκB which plays a role in ischemic preconditioning of heart.

Key words: Nuclear factor κB; Mitogen associated protein kinase; Tyrosine kinase; Ischemia; Heart; Adaptation; Oxidative stress; Signal transduction

1. Introduction

Mammalian heart can be adapted to ischemia by repeatedly subjecting it to short-term reversible ischemia each followed by another short duration of reperfusion [1,2]. This phenomenon, known as ischemic adaptation, causes the production of oxidative stress leading to the induction of gene expression which is subsequently translated into the development of several stress-related proteins responsible for the heart's defense [3]. Although the beneficial effects of ischemic stress adaptation are well recognized, controversies exist regarding the mechanism of signal transduction by which ischemic stress builds up the heart's defense. Myocardial adaptation to ischemia has recently been shown to be mediated through the activation of tyrosine kinase receptor protein. The signal transduction process appears to involve tyrosine kinase-coupled phospholipase D and MAP kinases which lead to the activation of MAPKAP kinase 2 [4,5]. Our recent study demonstrated that the ischemic stress specifically translocates and activates p38 MAP kinases which directly activate MAPKAP kinase 2 [6].

The nuclear factor κB (NFκB) appears to be a critical regulator for gene expression induced by diverse stress signals including mutagenic, oxidative and hypoxic stresses. NFκB is a ubiquitous transcription factor which is translocated in response to oxidative stress from its inactive cytoplasmic form by releasing the inhibitory subunit IκB from NFκB [7,8]. Activation of NFκB is likely to be involved in the induction of gene expression associated with the ischemic adaptation, since this transcription factor has recently been found to play a crucial role in the regulation of ischemia/reperfusion-mediated gene expression [9].

Substantial evidence exists to support the notion that oxygen-derived free radicals are generated during the reperfusion of ischemic myocardium resulting in the development of oxidative stress [10,11]. We speculated that ischemia/reperfusion-induced oxidative stress could cause translocation of NFκB which might be involved in the regulation of the activation of MAP kinases observed in conjunction with myocardial stress adaptation. To examine the role of NFκB in tyrosine kinase signaling during myocardial adaptation to ischemia, isolated rat hearts were adapted to ischemic stress by repeated ischemia and reperfusion [12]. The adapted heart resulted in the nuclear translocation and activation of NFκB which was completely blocked either by inhibiting tyrosine kinase phosphorylation or by inhibiting p38 MAP kinase activation. Beneficial effects of ischemic adaptation were blocked by pretreating the hearts with a NFκB inhibitor, SN 50 peptide. These results suggest that NFκB, situated downstream of p38 MAP kinase, plays a crucial role in myocardial adaptation to ischemia.

2. Materials and methods

2.1. Chemicals

Genistein and SN 50 peptide were purchased from Research Biochemicals, Natick, MA and Calbiochem, CA, respectively. SB 203580 was a gift from the SmithKline and French Laboratories, Philadelphia, PA. P65 antibody was purchased from Santa Cruz Biotechnological Co., CA. All other chemicals were of high purity and obtained from Sigma Chemical Co., St. Louis, MO.

2.2. Isolated perfused heart preparation

Fifty-four Sprague Dawley rats weighing about 300 g were anesthetized with pentobarbital (80 mg/kg, i.p.). After intravenous administration of heparin (500 IU/kg), the chests were opened, the hearts were rapidly excised and mounted on a non-recirculating Langendorff perfusion apparatus [13]. Retrograde perfusion was established at a pressure of 100 cm H_2O with an oxygenated normothermic Krebs-

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Henseleit bicarbonate (KHB) buffer with the following ion concentrations (in mM): 118.0 NaCl, 24.0 $NaHCO_3$, 4.7 KCl, 1.2 KH_2PO_4, 1.2 $MgSO_4$, 1.7 $CaCl_2$, and 10.0 glucose. The KHB buffer had been previously equilibrated with 95% O_2/5% CO_2, pH 7.4 at 37°C. To examine nuclear translocation of NFκB, the hearts were randomly divided into five groups. Isolated hearts were perfused with: KHB buffer for 75 min (group I); KHB buffer for 15 min for stabilization followed by 5 min ischemia and 10 min reperfusion (group II); KHB buffer for 15 min in the absence (group III) or presence of 5 μM SB 203580 (group IV) or 100 μM genistein (group V) and then subjected to ischemic stress adaptation by repeated ischemia and reperfusion by inducing global ischemia for 5 min followed by 10 min of reperfusion, repeating the process four times as described previously [12,13]. A schematic diagram of the protocol is shown in Fig. 1. Another three groups of hearts were studied to examine the effects on NFκB inhibition on post-ischemic myocardial functions. For this, isolated perfused rat hearts were perfused with KHB buffer for 75 min followed by 15 min of ischemia and 2 h of reperfusion (group VI). Another two groups of hearts were perfused for 15 min with buffer in the absence (group VII) or presence (group VIII) of 18 μM SN 50 peptide, and then subjected to ischemic stress adaptation as described for group III. Hearts were then made globally ischemic for 15 min followed by 2 h of reperfusion. A group of four hearts were perfused with 18 μM SN 50 peptide only for 3.5 h to examine whether SN 50 peptide by itself had any effects on myocardial function. For NFκB binding activity (groups I–V), left ventricles from the control and experimental hearts were kept frozen at liquid nitrogen temperature. Myocardial functions, creatine kinase (CK) release and malonaldehyde (MDA) formation were determined in the hearts from groups VI–VIII as described below.

2.3. Evaluation of myocardial functions

To evaluate myocardial performance, the Langendorff preparation was switched to the working mode following the preconditioning protocol [13]. Control experiments were performed without subjecting the hearts to preconditioning. Aortic flow was measured by a calibrated rotameter. Coronary flow rate was measured by a timed collection of the coronary perfusate that dripped from the heart. After a 10-min aerobic perfusion of the heart, the aortic inflow line was clamped at a point close to the origin of the aortic cannula. Reperfusion was initiated by unclamping the aortic line. Before ischemia and during reperfusion, heart rate (H), coronary flow (CF) and aortic flow (AF) rates were registered. Left ventricular developed pressure (LVDP), which was defined as the difference between LV systolic and end-diastolic pressure (EDP) and the first derivative of LVDP (LVdp/dt), were also recorded.

2.4. Estimation of CK release

CK was quantified from 0.5 ml of plasma obtained prior to ischemic adaptation and at 30 min, 60 min and 120 min during reperfusion. CK was analyzed by the enzymatic assay method using a CK assay kit (Sigma Diagnostics, St. Louis, MO). The absorbance was read at 340 nm using a Beckman DU-8 spectrophotometer.

2.5. Measurement of MDA formation in heart

MDA was estimated in heart muscle to determine the development of oxidative stress and free radical generation as described previously [14]. In short, weighed heart biopsies were homogenized in 2 ml of 20% trichloroacetic acid, 5.3 mM sodium bisulfite, kept on ice for 10 min, centrifuged at $3000\times g$ for 10 min, and then supernatants were collected, derivatized with 2,4-dinitrophenylhydrazine (DNPH), and extracted with pentane. Aliquots of 25 μl in acetonitrile were injected onto a Beckman Ultrasphere C_{18} (3 mm) column. The products were eluted isocratically with a mobile phase containing acetonitrile-water-acetic acid (40:60:0.1, v/v) and measured at three different wavelengths (307 nm, 325 nm and 356 nm) using a Waters M-490 multichannel UV detector. The peak for malonaldehyde was identified by co-chromatography with DNPH derivative of the authentic standard, peak addition, UV pattern of absorption at the three wavelengths, and by GC-MS.

2.6. Isolation of nuclear proteins

Nuclear proteins were isolated from the heart according to the method described previously by Schreiber et al. [15] with slight modification. In short, about 150 mg of left ventricle from heart tissue was homogenized in ice-cold Tris-buffered saline (TBS) and centrifuged at $3000\times g$ for 5 min at 4°C. The pellet was resuspended by gentle pipetting in 1.5 ml of ice-cold hypotonic buffer containing 10 mM HEPES, pH 7.9, 10 mM KCl, 0.1 mM EDTA, 0.1 mM EGTA, 1 mM DTT, 0.5 mM PMSF, and 1 μM each of aprotinin, pepstatin and leupeptin. The solution was allowed to swell on ice for 15 min. After addition of 100 μl of 10% Nonidet P-40, the tube was vigorously vortexed for 45 s. This homogenate was centrifuged for 30 s at 4°C in a microcentrifuge tube. The supernatant contained the cytoplasmic protein.

The nuclear pellet was resuspended in a solution containing 20 mM HEPES, pH 7.9, 0.4 M NaCl, 1 mM EDTA, 1 mM EGTA, 1 mM DTT, 1 mM PMSF and 1 μM each of aprotinin, pepstatin and leupeptin. The tubes were vigorously shaken at 4°C for 30 min on a shaking platform. The extracts were then centrifuged and the supernatants were stored at −70°C. Protein concentration was estimated using a Pierce protein assay kit (Pierce Chemical Co., Rockford, IL).

2.7. Electrophoretic mobility assay (EMSA)

NFκB oligonucleotide (AGTTGAGGGGACTTTCCCAGG) (2.5 μl, 20 ng/μl) was labeled using T4 polynucleotide kinase as previously described [16]. The binding reaction mixture contained in a total volume of 20.2 μl, 0.2 μl DTT (0.2 M), 1 μl BSA (20 mg/ml), 4 μl PdI-dC (0.5 μg/μl), 2 μl buffer D^+, 4 μl buffer F, 2 μl ^{32}P-oligo (0.5 ng/μl) and 7 μl extract containing 10 μg protein. Composition of buffer D^+ was 20 mM HEPES, pH 7.9, 20% glycerol, 100 mM KCl, 0.5 mM EDTA, 0.25% NP 40 while buffer F contained 20% Ficoll 400, 100 mM HEPES, pH 7.9, and 300 mM KCl. Incubation was carried out for 20 min at room temperature. 10 μl of the solution was loaded onto a 4% acrylamide gel and separated at 80 V until the dye hit the bottom. After electrophoresis, gels were dried and exposed to Kodak X-ray film at −70°C.

Table 1
Effects of SN 50 on myocardial functions

		Heart rate (beats/min)	Developed pressure (mm Hg)	dp/dt_{max} (mm Hg/s)	Aortic flow (ml/min)	Coronary flow (ml/min)	Infarct size (%)
Control	Baseline	305 ± 3.2	73 ± 1.5	2983 ± 74	43.5 ± 0.9	24.1 ± 0.6	–
	R30	312 ± 7.9	67 ± 6.5	2618 ± 261	30.3 ± 2.2†	23.2 ± 1.5	
	R60	304 ± 11.8	47.1 ± 2.0†	2018 ± 268	24.3 ± 3.2†	23.2 ± 1.4	
	R120	295 ± 4.7	35.4 ± 1.8†	1520 ± 63†	14.4 ± 0.7†	18.1 ± 0.4†	35 ± 3.2
Adapted	Baseline	294 ± 4.9	74 ± 3.1	3064 ± 111	44.0 ± 0.9	24.3 ± 0.4	–
	R30	299 ± 6.9	77.1 ± 2.0	2972 ± 125	34.8 ± 1.4	26.5 ± 1.3	
	R60	299 ± 7.5	74.6 ± 2.5*	2803 ± 99*	33.4 ± 1.2*†	27.1 ± 1.3	
	R120	285 ± 9.0	45.4 ± 1.4*†	2055 ± 89*†	24.3 ± 1.2*†	20.6 ± 0.3†	17.5 ± 1.7*
SN 50	Baseline	311 ± 8.3	78 ± 3.2	3297 ± 163	42.2 ± 0.6	24.7 ± 1.3	–
	R30	301 ± 4.7	55 ± 4.0**	2219 ± 135**	18.8 ± 2.8**†	24.5 ± 1.3	
	R60	304 ± 3.8	42 ± 3.6**†	1784 ± 156**†	13.3 ± 2.6**†	27.1 ± 1.3	
	R120	296 ± 7.4	17 ± 1.1**†	1015 ± 90**†	4.7 ± 0.2**†	25.32 ± 1.9	36.5 ± 4.2**

$^{\dagger}P < 0.05$ compared to baseline; $*P < 0.05$ compared to control; $**P < 0.05$ compared to adapted.
Results are shown as means ± S.E.M. of six rats per group.
R30, R60 and R120: hearts subjected to 30 min ischemia followed by 30, 60 and 120 min reperfusion.

2.8. Statistical analysis

For statistical analysis, a two-way analysis of variance (ANOVA) followed by Scheffé's test was first carried out using Primer Computer Program (McGraw-Hill, 1988) to test for any differences between groups. If differences were established, the values were compared using Student's *t*-test for paired data. The values were expressed as mean ± S.E.M. The results were considered significant if P was less than 0.05.

3. Results

3.1. Myocardial adaptation to ischemic stress by repeated ischemia and reperfusion and its inhibition by SN 50 peptide

As expected, hearts subjected to repeated ischemia and reperfusion demonstrated significantly improved post-ischemic ventricular recovery as compared to non-adapted hearts. Neither ischemic adaptation nor SN 50 peptide treatment altered heart rate during ischemia and reperfusion (Table 1). Aortic flow was drastically reduced during reperfusion in both non-adapted and adapted groups. However, the degree of reduction was significantly lower in the adapted group. Coronary flow was not affected by ischemic adaptation. For both groups, the developed pressure (DP) and the first derivative of developed pressure (dp/dt) were lower during reperfusion compared to baseline but these values were significantly higher in the adapted hearts.

Inhibition of NFκB translocation by SN 50 peptide tended to inhibit the improved myocardial performance afforded by myocardial adaptation to ischemic stress. As shown in Table 1, post-ischemic aortic flow, DP as well as dp/dt were significantly lower in the SN 50 peptide group as compared to those in the ischemically stress-adapted group suggesting that NFκB plays a significant role in myocardial adaptation to ischemia. SN 50 peptide by itself had no effects on myocardial performance or heart rates (results not shown).

3.2. Effects of SN 50 peptide on CK release from heart

Release of CK is considered as a presumptive marker for tissue necrosis and cell death. As shown in Fig. 2, CK release increased in all the three groups of hearts. However, a slight increase was noticed in the control heart, and only after 1.5 h of perfusion with SN 50 peptide. The increase in CK release from the non-adapted hearts was significantly higher compared to the ischemically adapted group. Post-ischemic CK release was significantly elevated in both ischemically adapted and SN 50 peptide-treated hearts, but it was significantly higher for SN 50 peptide-treated hearts as compared to adapted hearts suggesting that SN 50 peptide blocked the beneficial effects of ischemic adaptation by increasing tissue injury. The amount of CK was not affected when hearts were perfused with SN 50 peptide only for 3.5 h (results not shown).

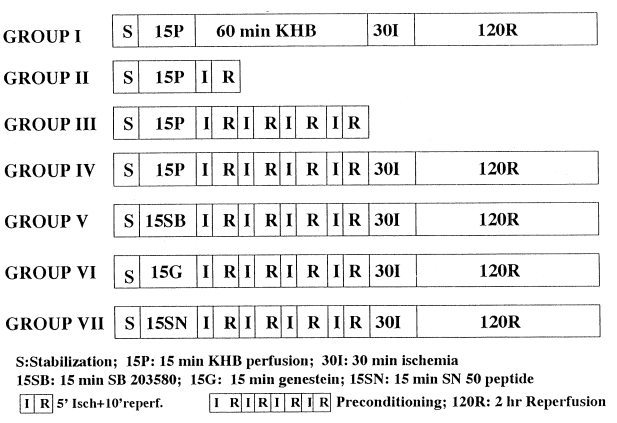

Fig. 1. Protocol of the experiment.

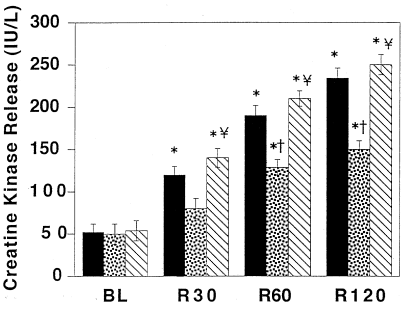

Fig. 2. Effects of ischemic adaptation and SN 50 peptide on CK release from the hearts during post-ischemic reperfusion. Isolated rat hearts were perfused for 15 min in the absence (stippled bars) or presence (hatched bars) of 18 μM SN 50 peptide followed by ischemic adaptation by repeated ischemia and reperfusion, 15 min global ischemia (15I) and 120 min of reperfusion (R120). Control experiments were perfusion by perfusing the isolated hearts for 75 min with KHB buffer only followed by 15 min ischemia (15I) and 120 min reperfusion (120R) (black bars). Results are expressed as means ± S.E.M. of six different rats per group. $^{*}P<0.05$ compared to baseline; $^{\dagger}P<0.05$ adapted vs. non-adapted control; $^{¥}P<0.05$ SN 50 peptide vs. adapted.

3.3. Effects of SN 50 peptide on MDA formation in heart

MDA formation truly reflects the development of oxidative stress in biological systems. MDA content of heart did not change even after 3.5 h of perfusion with SN 50 peptide only (results not shown). In all other groups, MDA increased steadily and progressively as a function of the reperfusion time ($P<0.5$ compared to baseline, Fig. 3). However, there were no significant differences in MDA content of the hearts between the groups.

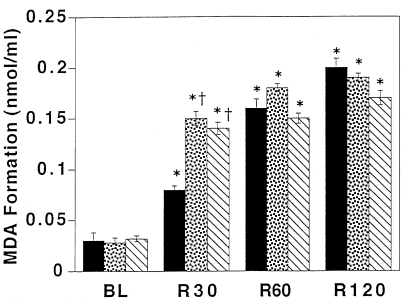

Fig. 3. Effects of ischemic adaptation and SN 50 peptide on MDA formation in the hearts during post-ischemic reperfusion. Isolated rat hearts were perfused for 15 min in the absence (stippled bars) or presence (hatched bars) of 18 μM SN 50 peptide followed by ischemic adaptation by repeated ischemia and reperfusion, 15 min global ischemia (15I) and 120 min of reperfusion (R120). Control experiments were perfusion by perfusing the isolated hearts for 75 min with KHB buffer only followed by 15 min ischemia (15I) and 120 min reperfusion (120R) (black bars). Results are expressed as means ± S.E.M. of six different rats per group. $^{*}P<0.05$ compared to baseline; $^{\dagger}P<0.05$ adapted vs. non-adapted control.

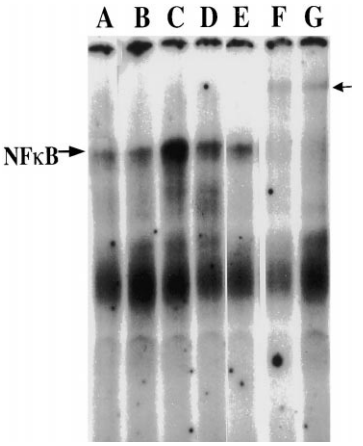

Fig. 4. Effects of ischemic adaptation on NFκB activity. In non-ischemic control hearts there was no translocation of NFκB as can be seen in lane A. In the ischemically adapted groups, translocations of NFκB were significantly increased as noted in lanes B and C. The binding activities were decreased when genistein or SB 203580 was used to block tyrosine kinase or p38 MAP kinase activity, respectively, as shown in lanes D and E. Supershift assay using polyclonal antibody (p65) was performed to verify that these bands were indeed due to NFκB translocation (lanes F and G).

3.4. *Effects of SB 203580 and genistein on the nuclear translocation of NFκB*

NFκB binding activity was found to be very low in non-ischemic control hearts (Fig. 4, lane A), but it was clearly detected in the adapted hearts. A significant increase in NFκB binding was found in the hearts subjected to once 5 min ischemia followed by 10 min reperfusion (Fig. 4, lane B). The binding was further increased in the hearts that were adapted by repeating this adaptation sequence four times (lane C). As shown in lanes D and E, NFκB binding activity was significantly decreased in the hearts pretreated with either genistein or SB 203580 suggesting that NFκB activation is regulated by both tyrosine kinase phosphorylation and p38 MAP kinase activity. To confirm NFκB binding activity with p65, we performed supershift assays with polyclonal antibodies recognizing NFκB p65 subunit proteins as shown in lanes F and G of Fig. 4.

4. Discussion

In this study, we have demonstrated that myocardial adaptation to ischemia is associated with the nuclear translocation and activation of NFκB. Activation of NFκB was blocked by genistein or SB 203580 indicating that nuclear translocation of NFκB is regulated by tyrosine kinase phosphorylation and p38 MAP kinase activation. Inhibition of nuclear translocation of NFκB by the SN 50 peptide blocked the beneficial effects of ischemic adaptation suggesting that NFκB plays a role in the intracellular signaling potentiated by ischemic adaptation.

We have used a synthetic peptide containing a cell membrane-permeable motif and nuclear localization sequence, SN 50, to block the nuclear translocation of NFκB. Such a cell-permeable peptide import approach was shown to be quite effective to block nuclear translocation of NFκB in cultured endothelial and monocyte cells [17]. The authors of this study clearly showed that cell-permeable SN 50 peptide controlled signal-transduction-dependent subcellular traffic of transcription factors leading to cellular responses to different agonists without exerting any cytotoxic effects. The results of our study also verified that SN 50 indeed blocked the nuclear translocation of NFκB in the hearts undergoing ischemic adaptation (results not shown).

Ischemic adaptation may be defined as a phenomenon in which interruption of brief reversible ischemia by brief reperfusion episodes improves myocardial tolerance to subsequent prolonged ischemia. In fact this has emerged as a state-of-the-art technique for cardioprotection [1,2,12,18]. Such adaptive protection is associated with the reduction of cellular energy demand and anaerobic metabolism during a subsequent period of sustained ischemia. This technique can delay the onset of further irreversible injury, reduce subsequent post-ischemic ventricular dysfunction, decrease the incidence of arrhythmias, and reduce infarct size which makes ischemic adaptation a potential tool for clinical application [1,2,12,18].

Despite a great deal of research of the preconditioning phenomenon over the last decade, the pathophysiology and the mechanisms by which preconditioning exerts cardioprotection remain controversial. It is generally believed that one or more intracellular mediators rapidly released in response to stress are responsible for preconditioning. While there is a great deal of controversy regarding the exact pathway for signal transduction, protein kinase C (PKC) has been implicated as involved in signalling this adaptive response [19]. Recent findings indicate that multiple kinases including MAP kinases and MAPKAP kinase 2 are likely to be involved in the preconditioning signaling process [4,5,20]. Additionally, receptor tyrosine kinase appears to play a role in transmembrane signaling of the stress response. This adaptive signal is proposed to be mediated through a phospholipase D-dependent MAPKAP kinase 2 pathway [4].

The acutely developing adaptive effect is short-lived, lasting for only up to 2–3 h. Hearts can subsequently undergo a secondary and delayed adaptation to stress presumably through the induction of the expression of new genes and their subsequent translation into proteins. A number of genes and proteins have been identified as possibly involved in the development of *second window* or delay preconditioning including heat shock proteins, superoxide dismutase, catalase, nitric oxide synthase as well as ATPase 6 and cytochrome *b* subunits [21]. Such an adaptive response becomes evident only after approximately 24 h of stress treatment and may include stress induced by heat shock, oxidant or other stress-inducible agents. MAPKAP kinase 2 appears to link the early preconditioning effect to the delayed adaptive response [4,5].

Recently, oxygen-derived free radicals have been implicated in transmembrane signaling [22]. In this study, the authors provided evidence that a tyrosine kinase inhibitor, herbimycin A, and a free radical scavenger, *N*-acetylcysteine, inhibit free radical-induced activation of NFκB indicating that activation triggered by reactive oxygen species is dependent on tyrosine kinase activity. The results of our study support this report and provide further evidence that such signaling also involves p38 MAP kinase. Activation of MAP kinases by oxygen radicals receives further support from a recent observation where potent activation of extracellular signal-regulated ERK2 kinase occurred within 10 min of H_2O_2 treatment [23]. This study also showed that H_2O_2 also moderately activated other kinases including p38 MAP kinase. Lipopolysaccharides (LPS), which can adapt the heart to oxidative stress [24], have been

found to induce tyrosine phosphorylation of p38 protein [25]. Phosphorylation of p38 was blocked by treatment of cells with a protein tyrosine kinase inhibitor, herbimycin A, suggesting that tyrosine phosphorylation is involved in LPS-induced oxygen free radical signaling.

Evidence is rapidly accumulating to indicate that oxidative stress/free radicals lead to the activation of NFκB which in turn induces the expression of genes [26–28]. Interestingly, H_2O_2 was found to activate DNA binding of NFκB in vivo, but not in vitro [29], suggesting that a byproduct of H_2O_2 and not H_2O_2 by itself may be responsible for the activation of NFκB. Another related study using transient catalase overexpression in COS-1 cells showed that H_2O_2 may not serve as a messenger for tumor necrosis factor α- or phorbol ester-induced NFκB activation [30]. It is possible that $OH^{\bullet}$ radical formed by a transient metal-catalyzed Fenton reaction during the reperfusion of ischemic heart [31] can induce NFκB activation. Inhibition of NFkB induction by antioxidants further supports a role of free radicals in NFκB activation [32].

In summary, to the best of our knowledge, the results of our study provide evidence for the first time that the nuclear transcription factor NFκB is involved in the regulation of ischemic preconditioning of the heart. The finding that p38 MAP kinase might be upstream of NFκB further supports our previous reports that MAPKAP kinase 2 could be the most likely link between the preconditioning and adaptation mediated by gene expression [33]. p38 activation appears to be an important step in the translocation and activation of the nuclear transcription factor NFκB which in turn may be involved the induction of the expression of a variety of stress-inducible genes [3].

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