Activation of Mitogen-Activated Protein Kinases in Human Heart During Cardiopulmonary Bypass

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Abstract—Mitogen-activated protein kinases (MAPKs) have been shown to be activated in both in vitro and in vivo models of cardiac tissue in response to ischemia/reperfusion injury. We investigated whether MAPKs are activated in human heart during coronary artery bypass grafting (CABG) surgery. During elective CABG surgery of 8 patients, 3 right atrial appendage biopsies were obtained at baseline, at the end of cross-clamping, and after coronary reperfusion. The expression of the p38-MAPK, c-Jun N-terminal kinase (JNK), and extracellular signal–regulated kinases (ERK1/2) MAPKs was not altered during CABG. The phosphorylation and activation of both ERK1/2 and p38-MAPK were increased ≈2-fold by ischemia and even more (8- and 4-fold, respectively) by reperfusion. Although the ischemic period did not result in a significant activation of JNK, an ≈6-fold increase in JNK activity could be observed after reperfusion. In conclusion, distinct activation patterns of ERK1/2, p38, and JNK MAPKs can be observed in human heart during CABG. (Circ Res. 2000;86:1004-1007.)

Key Words: myocardium ■ ischemia/reperfusion ■ stress-activated protein kinases

The mitogen-activated protein kinases (MAPKs) are activated by diverse stimuli and appear to mediate cellular responses including proliferation, differentiation, and adaptation to stress. Three major subfamilies have been characterized, including the extracellular signal–regulated kinases (ERK1/2), the c-Jun N-terminal kinases (JNK1/2), and the p38-MAPKs. It has been previously reported that although the ERKs are mainly involved in mediating anabolic processes such as cell division, growth, and differentiation, the JNKs and the p38-MAPKs are generally associated with cellular response to diverse stresses (reviewed in References 3 and 4).

In recent years, the possible importance of MAPK activation in the heart during ischemia/anoxia and reoxygenation has been raised. The JNKs and the p38-MAPKs have been consistently shown to be activated by myocardial ischemia/reperfusion in both animal models and in cardiomyocyte cell lines. The ERKs, however, have been shown to be activated in isolated animal hearts by ischemia/reperfusion by some, although not all investigators, possibly in response to oxidative stress. The exact role of the MAPKs in cardiac pathophysiology in humans has not been fully elucidated, partly because of limited information regarding the activation pattern of the MAPKs in the human heart. A recent study demonstrated the expression of JNK, p38-MAPK, and ERK1/2 in the human heart. A potential clinical relevance for their action was demonstrated by an increased activity of JNK and p38-MAPK in heart failure secondary to ischemic heart disease.

In the present study, we aimed at evaluating both the expression and activation of the different MAPKs in nonfailing human hearts by cardiopulmonary bypass during the course of coronary artery bypass grafting (CABG) surgery.

Materials and Methods
The study was performed on right atrial appendage samples obtained from patients during elective CABG surgery who were admitted to the Department of Cardiothoracic Surgery at Soroka Medical Center. Biopsies from 5 men and 3 women (mean age 68±12 years) were obtained. The protocol for the study was approved by the institutional ethics committee, and informed consent was obtained from all patients. Patients excluded from the study were those with left ventricular ejection fraction of <0.4, those requiring preoperative inotropic or intra-aortic balloon pump support, and those with uncontrolled systemic diseases (diabetes, hypertension, or renal failure). All patients received antianginotic and antihypertensive medications up until the day of surgery. Routine anesthetic and surgical protocols were used in all patients enrolled, and the same two surgeons used a similar technique to perform all operations. Briefly, blood cardioplegia was administered to all patients after cross-clamping of the aorta. Cardioplegia was given first antegrade, through the aortic root, and then retrograde through the coronary sinus with boluses of cold cardioplegia every 20 minutes. Patients were maintained at mild hypothermia (32°C rectal temperature), and pump flows were adjusted to a nonpulsatile perfusion pressure of 70 to 90 mm Hg. Before removal of the cross-clamp, an additional warm cardioplegia was given, followed by warm blood.

Heart Biopsies and Preparation
Biopsies (40 to 60 mg) were taken into liquid nitrogen at 3 time points during the operation: (1) during cannulation of the right atrium

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1004
The duration of the ischemia and reperfusion periods for each patient is presented in the Table. Samples were powdered under liquid nitrogen and homogenized in lysis buffer that contained (in mmol/L) Tris-HCl 50 (pH 7.5), EDTA 1, EGTA 1, NaF 50, sodium β-glycerophosphate 10, sodium pyrophosphate 5, activated sodium orthovanadate 1, 0.1% Triton X-100, and 0.1% 2-mercaptoethanol in the presence of protease inhibitors. The homogenates were further processed as previously described. Proteins (30 μg) were separated by SDS–polyacrylamide gel electrophoresis and subjected to Western blot analysis, using the following antibodies: anti-JNK1/2, anti–total p38, anti–dual-phosphorylated p38-MAPK (Sigma, Rehovot, Israel), anti-ERK2 (Santa Cruz Biotechnology, Inc), and anti–phospho ERK1/2 (Promega). Quantification was made by video densitometry analysis, as previously described.

**Immunoprecipitation and Kinase Assays**

ERK was immunoprecipitated using the above-mentioned antibody, from aliquots of 400-μg protein using standard methods. ERK activity was assayed using myelin basic protein and 32 Pγ-ATP as substrates. JNK activity was assayed using the SAP/JNK assay kit (New England Biolabs Inc). Briefly, JNK was precipitated by c-Jun fusion protein beads, and kinase reaction was carried out in the presence of nonradio-labeled ATP. In vitro c-Jun phosphorylation was selectively detected by Western blot using a phospho-Ser63 c-Jun antibody.

**Statistical Analysis**

Values are given as mean±SE. The Wilcoxon nonparametric test for paired values was used for comparisons between values obtained in control, ischemia, and reperfusion biopsies.

![Figure 1](http://circres.ahajournals.org/)

**Figure 1.** JNK activation in human heart during CABG. JNK activity was assessed as described in Materials and Methods. Representative phospho-c-Jun immunoblot demonstrates the in vitro activity of immunoprecipitated JNK and the densitometry values of samples from 5 patients. C and Cont indicate control; I, ischemia; and R, reperfusion.

**Figure 2.** Expression and activation of p38-MAPK in human heart during CABG. A, Representative total p38-MAPK immunoblot. B, Immunoblot of dual-phosphorylated p38-MAPK and the densitometry values of blots from 5 patients. IB indicates immunoblot.
control level (patient numbers 3 and 7). Regression analysis revealed a significant ($P<0.01$) inverse correlation ($r = -0.94$) between the reperfusion period and the degree of p38-MAPK phosphorylation over basal. These data may suggest that p38 phosphorylation is transient during the reperfusion state, reflecting either the transient presence of an activating factor and/or the existence of an activation-termination loop. Collectively, these data demonstrate that during the course of CABG procedure, activation of various members of the MAPK subfamily occurs in human heart. The pattern of activation may differ between the various MAPKs, with JNK being activated primarily by reperfusion (Figure 1), whereas p38 and ERK1/2 by ischemia and even more by reperfusion (Figures 2 and 3).

The experimental model used in the present study imposes strict intrinsic limitations, including access solely to right atrial appendage tissue and limited sample number and size available. In addition, during the course of the operation, the myocardium is exposed to multiple factors, which include not only ischemia and reperfusion but also alterations in temperature and electrolyte concentrations, as well as various pharmacological agents used during the surgical and anesthetic procedures.

Despite these obvious limitations, the finding of activation of the stress-responsive MAPKs during CABG is consistent with observations obtained in various experimental models for ischemia/reperfusion. In cell culture systems, as well as in ex vivo and in vivo models, JNK activation was primarily observed during reperfusion, whereas p38-MAPK was activated during hypoxia, and this activation was maintained during the reperfusion period. In the present study, we demonstrate that both short and prolonged ischemia periods do not result in significant JNK activation, whereas p38-MAPK is activated. During reperfusion, both MAPKs are activated, whereas JNK activation appears to outlive that of p38-MAPK. Despite the significant inverse correlation between the duration of the reperfusion period and the degree of JNK or p38-MAPK activation, a reperfusion period $>30$ minutes was still associated with a 4-fold increase in JNK activity compared with control, whereas p38-MAPK activation decayed to near-control values. Nevertheless, the possibility that factors other than hypoxia reperfusion contributed to the activation of JNK and p38-MAPKs during CABG cannot be ruled out. Specifically, cold exposure, alterations in the intracellular calcium concentration, catecholamines such as phenylephrine, norepinephrine, and isoproterenol have been shown to activate MAPKs. In particular, these factors may have contributed to the activation of ERK1/2, which we demonstrate to occur in human heart during both the ischemia and the reperfusion periods of CABG (Figure 3). ERK1/2 has been shown to be activated by

**Discussion**

In the present study, we report that during the course of CABG procedure, activation of various members of the MAPK subfamily occurs in human heart. The pattern of activation may differ between the various MAPKs, with JNK being activated primarily by reperfusion (Figure 1), whereas p38 and ERK1/2 by ischemia and even more by reperfusion (Figures 2 and 3).

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reactive oxygen species and ischemia/reperfusion in noncardiomyocytes, as well as in cultured rat cardiac myocytes, H9C2 cardiac muscle cells, rat primary cardiomyocytes, and in vivo animal models of cardiac ischemia/reperfusion. However, several studies indicate that ERK1/2 activation during ischemia/reperfusion in the heart is limited. Thus, the specific stimuli involved in ERK1/2 activation during CABG remain to be elucidated.

Various downstream effectors of the MAPK cascades and their implications to various cellular functions are now being discovered. The possibility that activation of MAPKs during CABG may play a role in clinically relevant outcomes of this common procedure is intriguing. For example, activation of p38-MAPK and heat shock protein 27 by oxidative stress has been demonstrated to result in cytoskeleton disarrangement, suggesting a cellular mechanism for reduced cardiac contractility during recovery from CABG. In addition, it has been shown that inhibition of p38-MAPK in perfused rabbit hearts decreases cardiomyocyte apoptosis, resulting in improvement of cardiac function after myocardial ischemia and reperfusion. In humans, a recent ex vivo study demonstrated in right atrial appendage biopsies that p38-MAPK is indeed being activated by ischemia/reperfusion. More importantly, preincubation of human samples with the p38-MAPK inhibitor SB203580 resulted in improved posts ischemic force development. These data in conjunction with the present study suggest that p38-MAPK activation occurs during CABG and may therefore play a causative role in the impaired contractile function observed after this common procedure. Consistent with this notion are the beneficial effects reported for angiotensin-converting enzyme (ACE) inhibitors administered either before or during cardiopulmonary bypass on various parameters of myocardial ischemic injury and on contractile dysfunction and metabolic derangement induced by ischemia and reperfusion. These may be mediated by the capacity of ACE inhibitors to prevent MAPK activation. Additional larger-scale clinical trials are needed to establish more definitively the role of the MAPKs as potential molecular targets for therapeutic intervention.

In conclusion, the present study demonstrates the activation of MAPKs in human heart during CABG surgery. The availability of right atrial appendage tissue may thus provide an easy and safe method to assess the relevance of MAPK activation to clinically important outcomes of CABG.

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References

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