**Myocardial Genetics: Signal Transduction**

**Tuesday, March 09, 2004, Noon-2:00 p.m.**

**POSTER SESSION**

**Moriaw Convention Center, Hall G**

**Presentation Hour: 1:00 p.m.-2:00 p.m.**

### 294A Abstracts - Myocardial Ischemia and Infarction

**JACC March 3, 2004**

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<tr>
<th>Poster Number</th>
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<th>Authors</th>
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<td>1136</td>
<td>Postconditioning by Repetitive I/R Cycles Subsequent to Myocardial Ischemia Protects the Rat Heart From Infarction and Is Dependent on the Activation of PI 3-Kinase and mTOR</td>
<td>Christoph Weinbrecher, Claudia Reuflner, Susanne Sütterlin, László Sárvány, Ruth H. Strasser</td>
<td>University of Technology, Dresden, Germany</td>
<td>Background: Ischemic preconditioning (pre-C) is a powerful mechanism in reducing the infarct size of the heart. Recently it was found in an in situ dog model that postconditioning (post-C) by repetitive ischemia/reperfusion (I/R) cycles after infarction may be protective and may reduce the infarct size. Objectives and methods: To address the question, whether post-C may be as protective as pre-C, infarct size was determined in isolated perfused rat hearts. To test whether post-C is dependent on the signaling of PI 3-kinase/protein kinase B (Akt),wortmannin, LY294002 and rapamycin were used to inhibit PI 3-kinase or mTOR, a signaling molecule downstream of Akt, respectively. Results: Control hearts (60 min regional ischemia followed by 2 hours of reperfusion) had an infarct size of 64±6% whereas pre-C (three cycles 5 min I/R each preceding the 60 min of infarction) reduced it to 15±3% of the risk zone (p&lt;0.001). Post-C induced by three cycles of 30 sec I/R each starting immediately after the onset of reperfusion following the 60 min of infarction also reduced the infarct size significantly to 28±5% (p&lt;0.001 vs. control). Statistically this was not significantly different from pre-C. 0.1 µM wortmannin or LY294003, both PI 3-kinase inhibitors, which were given during the 60 min of infarction and the first 10 min of reperfusion, completely blocked the protection from post-C (61±7%, 67±5%, resp.: p&lt;0.001 vs. post-C). 0.1 µM rapamycin also blocked the protection of post-C (51±14%, p&lt;0.01 vs. post-C) albeit this inhibition was not as complete as with the PI 3-kinase inhibitors. Conclusion: Protection of the heart from infarction is possible either through preceding (pre-C) or subsequent (post-C) I/R stimuli. Post-C is nearly as protective as pre-C regarding the protection of infarct size and myocardium. PI 3-kinase plays a role in the activation of the PI 3-kinase/Akt pathway. The additional components involved in the signal transduction pathway of post-C, however, to have been determined. Post-C offers fascinating clinical possibilities for the therapy of myocardial infarction since the patient with infarction presents not prior but after the onset of infarction.</td>
<td>PI 3-Kinase, mTOR, Akt, wortmannin, LY294002, rapamycin, PI 3-kinase inhibitors</td>
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### 1136-81 Epsilon Protein Kinase C (PKC) Activation by Delta PKC in Ethanol-Induced Cardiac Protection From Ischemia

**Kejii Inagaki, Daria Mochly-Rosen**

**Stanford University School of Medicine, Stanford, CA**

**Background:** Previous studies have demonstrated that acute ethanol exposure induces activation of ε Protein Kinase C (PKC) and εPKC, and mimics ischemic preconditioning via εPKC activation. However, the role of εPKC isoform in ischemia/reperfusion is still controversial. Here, we investigated the role of δεPKC in ethanol-induced cardioprotection using a δεPKC-selective activator (δεPKC-RACK), a selective inhibitor (IVI-1), and a εPKC-selective inhibitor (IVI-2) in isolated mouse hearts.

**Methods:** Mice were injected intraperitoneally with 0.5g/kg of ethanol or δεPKC-RACK, and saline (IVI-1 or IVI-2) before ischemia. Mouse hearts were then subjected to a 30-minute global ischemia and a 120-minute reperfusion. Experimental treatment of ethanol 60 minutes, but not 10 minutes, before ischemia reduced infarct size and CPK release. Pretreatment with IVI-2 completely inhibited ethanol-induced cardioprotection. Pretreatment with IVI-1 induced cardioprotection when mice were injected with ethanol 10 minutes before ischemia, but IVI-1 partially inhibited ethanol-induced cardioprotection when mice were injected with ethanol 60 minutes before ischemia. δεPKC injection 60 minutes, but not 10 minutes, before ischemia induced cardioprotection and this cardioprotection was completely inhibited by pretreatment with IVI-2. Furthermore, δεPKC-RACK induced the translocation of δεPKC from the cytosol to the particulate fraction 60 minutes, but not 10 minutes, before ischemia, in vivo. However, in isolated cardiomyocytes, δεPKC treatment did not induce translocation of δεPKC. Conclusion: With treatment with (0.5g/kg) or δεPKC activator induces cardioprotection against ischemia/reperfusion one hour, but not immediately before ischemia. Ethanol-induced δεPKC activation confers cardioprotection via δεPKC activation one hour after treatment. δεPKC-induced δεPKC activation may be mediated by soluble factors released from non-cardiomyocytes.

### 1136-84 Simulated Ischemia/Reperfusion Promotes Akt Phosphorylation Via Both Redox-Sensitive and Insensitive Mechanisms In H9c2 Cells

**Tae-Hwa M. Fyo, Jin Choo, Emory University/Atlanta VA Medical Center, Atlanta, GA**

**Background:** Akt is a serine/threonine kinase that has been shown to play key regulatory roles in many physiological processes including glucose metabolism, cell proliferation and migration, and cell survival against apoptosis. The paradigm for activation of Akt involves phosphatidylinositol 3-kinase (PI3K)-dependent membrane localization followed by phosphorylation of two critical residues, Ser473 near the carboxyl terminus and Thr380 in the activation loop. In this study, we examined the effects of simulated ischemia/reperfusion (I/R) on Akt in cardiac cells. We hypothesized that reactive oxygen species production during reperfusion is a prerequisite for phosphorylation to occur at each of the two phosphorylation sites. Methods: H9c2 cells, derived from embryonic heart tissue, were used for the study. Simulated ischemia was induced by incubating the cells in an “ischemic medium” placed in an air-tight anaerobic chamber purged with 95%N2/5%CO2. Following 3 hr of “ischemia,” cells were “reperfused” with maintenance culture medium for 1 hr. Site-specific Akt phosphorylation was evaluated by Western analysis using phospho-specific antibodies against Ser473 phospho-Akt and Thr380 phospho-Akt. Results: Ischemia alone induced a selective decrease in Ser473-phospho-Akt levels (58% reduction compared to normoxic controls, p<0.05) without affecting Thr380 phosphorylation. Reperfusion caused both Ser473 and Thr380-phospho-Akt levels to increase without affecting total Akt levels. The effect of I/R on Ser473 phosphorylation and Thr380 phosphorylation was completely blocked by pretreatment of the cells with the PI3K inhibitor wortmannin (100 nM). In contrast, pretreatment of the cells with the antioxidant N-acetyl-cysteine (2 mM) significantly blunted the effect of I/R on Thr380 phosphorylation without affecting Ser473 phosphorylation. Conclusion: In H9c2 cells, I/R pretreatment of Akt at both Ser473 and Thr380 is PI3K-dependent manner. While the phosphorylation of Thr380 (presumably by the action of PDK1, the upstream kinase of Akt) is redox-sensitive, autophosphorylation of Akt at Ser473 appears to be redox-insensitive.

### 1136-85 Time-Dependent Upregulation of Extracellular Matrix Proteins in Chronic Hibernating Myocardium: Lack of Progressive Degeneration

**Vijay S. Iyer, Gen Suzuki, Julieta M. Hardy, Brendan M. Heavey, James A. Failavollita, John M. Canty, Jr., University at Bufalo, Bufalo, NY, VA Medical Center, Bufalo, NY**

**Background:** Biopsies from hibernating myocardium have led to divergent conclusions regarding the role of adaptation vs. degeneration. The latter is inferred from upregulation of ECM protein functional recovery. We tested the hypothesis that ECM protein expression is an early event that normalizes in the adapted state. Methods: Swine (n=12) were chronically instrumented with 1.5 mm LAD stenoses to produce hibernating myocardium. RNA was isolated from flash frozen subendocardial tissue and hybridized for cdNA microarray analysis at 3 (n=6) or 5 months (n=6) after instrumentation. Results: Physiological features of hibernating myocardium were no different at 3 vs 5 months and there was no functional or pathological evidence of progressive deterioration. 1.2.1 mm of LAD stenosis, p<0.01) and wortmannin (LAD 2.4±0.5 vs. 5.9±0.5 mm, p<0.01) were reduced in comparison with normal regions. Expression of ECM proteins was time dependent (Table). ECM proteins including collagen, fibronectin, and vimentin were initially upregulated in hibernating LAD regions. For 5 months, the expression of ECM proteins decreased to levels that were similar to normal regions. Conclusion: The normalization of ECM genes after 5 months is consistent with the stability of connective tissue demonstrable by pathology. Thus, divergent clinical conclusions regarding adaptation vs. structural degeneration reflect time-dependent alterations in remodeling.

### BH4 content and eNOS activity at baseline and during ischemia (mean±SE of 5 experiments)

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<tr>
<th>BH4 (pmol/mg)</th>
<th>eNOS (pmol/min/mg)</th>
<th>eNOS after exogenous BH4 (pmol/min/mg)</th>
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<tr>
<td>2.5±0.51</td>
<td>0.45±0.09*</td>
<td>0.38±0.05*</td>
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<tr>
<td>0.16±0.02</td>
<td>0.08±0.01*</td>
<td>0.04±0.01*</td>
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<tr>
<td>0.27±0.02</td>
<td>0.24±0.01*</td>
<td>0.11±0.01*</td>
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**Conclusions:** Our data show that ischemia induces a major loss in cardiac BH4 content, and that eNOS activity is critically anchored to BH4 bioavailability in this setting. Supplementation with exogenous BH4 may partially restore eNOS function.