Introduction

Heart disease remains the leading cause of death in adults in the United States and worldwide, with coronary artery disease being the most common form that often leads to a myocardial infarction. While rapid restoration of coronary blood flow is crucial to preserving cardiac tissue function, it also results in an additional insult known as NOX-2 mediated ROS during reperfusion via cytokine receptor activation (Figure 1) (1). Activated PKCβII via cytoskeletal disorganization (DAG). Actinomycin PKCβII increases ROS and O2− release from damaged mitochondria and NADPH oxidase, respectively, and increases dNOS activity. It also stimulates mitochondrial p66Shc protein, a component in the pathway resulting in opening of the mitochondrial permeability transition pore (mPTP), which in turn leads to release of proapoptotic factors into the cytoplasm to further promote tissue injury during reperfusion.

Hypothesis

Of the many proteins that PKCβII phosphorylates, for the purposes of this study, we believe NOX-2 phosphorylation is a key pathway in the ROS mediated damage in MI/R injury. Thus, we hypothesize that myr-PKCβII+ will reduce infarct size and improve post-reperfusion cardiac function as compared to non-drug treated controls, whereas myr-PKCβII− treated hearts will not improve these parameters.

Research Design

Male Sprague-Dawley rats (~300g, Charles River, Springfield, MA) were anesthetized with IP. pentobarbital (60mg/kg) and anticoagulated with 100U of heparin. The heart was then removed and placed on the perfusion needle of the Langendorff apparatus. A pressure transducer was placed into the left ventricle to measure cardiac function, as previously described (6, 7).

A schematic of the MI/R protocol is depicted below:

Baseline (15min.)

Infarct Size (%)

Reperfusion (5min.)

TTC staining

At the end of the reperfusion period, all hearts were frozen at -20 °C for 30 min, sectioned into 2mm slices and incubated at 37°C in 1% triphenyltetrazolium chloride (TTC). The percentage between dead heart tissue (i.e., unstained) to total heart tissue weight was calculated for each infarct size.

Statistical Analysis

All data in the text, figures, and table are presented as means ± S.E.M. The data were analyzed by ANOVA using the Fisher’s PLSD test. Probability values of <0.05 are considered to be statistically significant.

Results

Table 1. Initial and final cardiac function values and infarct size for control, myr-PKCβII−, PKCβII+ and myr-PKCβII−/− in 55 male Sprague-Dawley rats. 

<table>
<thead>
<tr>
<th>Group</th>
<th>Initial Left Ventricular Developed Pressure (LVDP) (mmHg)</th>
<th>Final Left Ventricular Developed Pressure (LVDP) (mmHg)</th>
<th>Infarct Size (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>10±1</td>
<td>5±1</td>
<td>25±6</td>
</tr>
<tr>
<td>myr-PKCβII−</td>
<td>208±8</td>
<td>104±7</td>
<td>10±1</td>
</tr>
<tr>
<td>PKCβII+</td>
<td>58±4</td>
<td>58±4</td>
<td>1±1</td>
</tr>
<tr>
<td>myr-PKCβII−/−</td>
<td>58±5</td>
<td>58±5</td>
<td>1±1</td>
</tr>
</tbody>
</table>

The significant improvement in final post-reperfusion LVDP in myr-PKCβII−/− treated hearts is attributed to the significant reduction in final LVDP values (i.e. ~37mmHg) compared to control and myr-PKCβII+ hearts (i.e., ~58mmHg), and is reflected in the significant restoration of the final maximal rate of contractility (+dP/dtmax) and relaxation (-dP/dtmin).

These results suggest that: 1) Inhibition of myocardial NOX-2 activity may be the principal pathway through which myr-PKCβII− mediates its cardioprotective effects. In MI/R injury. 2) Treatment with myr-PKCβII− would be an effective strategy to limit MI/R injury in heart attack patients upon reperfusion via fibronectin therapy, angioplasty or coronary artery bypass surgery.

References