Inhibition of protein kinase Cα improves myocardial β-adrenergic receptor signaling and ventricular function in a model of myocardial preservation

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Supplemental material is available online.

Objective: The specific effect of protein kinase Cα, the primary ventricular calcium-dependent protein kinase C isoform, on myocardial protection is unclear. The objective of this study was to determine the role of protein kinase Cα in myocardial protection and recovery of function after cardioplegic arrest, cold preservation, and normothermic reperfusion, as relevant to cardiac transplantation.

Methods: We used an ex vivo murine model, and hearts were arrested with cold crystalloid cardioplegia or saline as a control and maintained at 4°C for 4 hours. This was followed by normothermic reperfusion for 90 minutes. Transgenic hearts with cardiac-specific activation or inhibition of protein kinase Cα were then studied to specifically examine the effects of protein kinase Cα on myocardial preservation in this model.

Results: Cardioplegic arrest with University of Wisconsin solution led to significantly improved postreperfusion hemodynamics and inhibition of myocardial protein kinase Cα activity relative to that seen in saline-treated control hearts. β-Adrenergic receptor signaling was also preserved with University of Wisconsin solution. Transgenic hearts with enhanced protein kinase Cα activity had poor postreperfusion hemodynamics, impaired β-adrenergic receptor signaling, and increased G protein–coupled receptor kinase 2 activity compared with those seen in nontransgenic control hearts. In contrast, transgenic hearts with inhibited protein kinase Cα activity had even better myocardial protection relative to control hearts and preserved β-adrenergic receptor signaling.

Conclusions: Current techniques of myocardial preservation are associated with inhibition of protein kinase Cα activity and maintenance of intact β-adrenergic receptor signaling. Activation of protein kinase Cα leads to enhanced β-adrenergic receptor desensitization and impaired signaling and ventricular function as a result of increased G protein–coupled receptor kinase 2 activity. This is a novel in vivo mechanism of G protein–coupled receptor kinase 2 activation. Strategies to specifically inhibit these kinases might improve long-term myocardial protection.

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The protein kinase C (PKC) family of serine–threonine kinases functions downstream of several membrane-associated signal transduction pathways.[E1] There are approximately 10 different isozymes that make up the PKC family, and they are broadly classified by their activation characteristics. The conventional PKC isozymes (α, βI, βII, and γ) are Ca^{2+} and lipid activated, whereas the novel isozymes (ϵ, θ, η, and δ) and the atypical isozymes (ζ and λ) are Ca^{2+} independent but activated by distinct lipids.[E2] PKCα is the predominant Ca^{2+}-dependent PKC isoform expressed in murine and human hearts.[E3]

Several reports have associated PKC activation with hypertrophy, dilated cardiomyopathy, ischemic injury, and mitogen stimulation.[E1] There is also some evidence implicating PKC isozymes as potential regulators of Ca^{2+} handling and

Abbreviations and Acronyms
ACT = specific activation
ATP = Adenosine triphosphate
βAR = β-Adrenergic receptor
CK = creatine kinase
GRK = G protein–coupled receptor kinase
INH = specific inhibition
PKC = protein kinase C
UW = University of Wisconsin

cardiomyocyte contractility. Stimulation of PKC activity by phorbol ester has been shown to decrease cardiac contractility in isolated rat hearts and isolated cultured cells, and this effect was abrogated with PKC inhibitors.[E4,E5] Previous studies have also shown that PKCα functions as a fundamental regulator of cardiac contractility and Ca^{2+} handling in myocytes.[E6,E7] For example, PKCα gene-deleted mice were shown to be hypercontractile, whereas transgenic mice overexpressing PKCα were hypocontractile.

The effects of cardioplegic arrest and hypothermic preservation on myocardial PKCα activity have not been specifically investigated, and that is the primary objective of this study. We used an ex vivo murine model of cardioplegic arrest, followed by cold preservation and subsequent normothermic reperfusion as relevant to heart transplantation, to determine whether PKCα activity might be an important factor in myocardial protection in this setting. In addition, a novel in vivo mechanism by which PKCα can regulate myocardial β-adrenergic receptor (βAR) signaling and ventricular function was investigated.

Materials and Methods

All animals received humane care in compliance with the "Principles of laboratory animal care" formulated by the National Society for Medical Research and the "Guide for the care and use of laboratory animals" prepared by the National Academy of Sciences and published by the National Institutes of Health. All experimental protocols were approved by the Institutional Animal Care and Use Committee of the University of Cincinnati.

Ex Vivo Cardiac Physiology

The mice used in this study were 3 months of age, and all were male. All mice in this study were of the FVB/n background. The mice were anesthetized intraperitoneally with pentobarbital solution. After thoracotomy, the hearts were rapidly excised and placed on a Langendorff apparatus. The hearts were perfused in a retrograde aortic fashion at a constant mean pressure of 50 mm Hg with Krebs–Henseleit bicarbonate buffer solution (118 mmol/L NaCl, 4.7 mmol/L KCl.2, 25 mmol/L $MgSO_4$, 1.2 mmol/L KH_2PO_4, 2.5 mmol/L $CaCl_2$, and 11 mmol/L glucose, pH 7.4) at 37°C and equilibrated with a mixture of 95% oxygen and 5% carbon dioxide gas. The preload was also held constant at a perfusate flow rate of 5 mL/min. During the stabilization period, a polyethylene catheter P-50 was inserted into the left ventricle through the left atrium and connected to a pressure transducer. The left ventricular pressure signals were digitized at 1 kHz and analyzed by using the computer software Biobench (National Instruments). The first positive and negative derivatives of the left intraventricular pressure curve (+dP/dt and −dP/dt) and the duration of contraction and relaxation (time to peak pressure) and time to half relaxation were calculated. Pacing was used to maintain a heart rate of 400 beats/min in all studies.

Research Design

All hearts were perfused for 30 minutes to achieve stable hemodynamics. This was followed by antegrade delivery of 3 mL of cold University of Wisconsin (UW) preservation solution or 3 mL of cold saline in the control group. After arrest, hearts were stored in UW solution at 4°C for 4 hours. The cold preservation period was then followed by 90 minutes of normothermic reperfusion with loading conditions identical to the initial basal period of perfusion. After hemodynamic measurements, all hearts were snap-frozen in liquid nitrogen for later preparation for biochemical studies.

Transgenic Mice

The transgenic mice used in this study have been previously described.[E7] There is an approximately 1.5-fold increase in myocardial PKCα activity driven by cardiac-specific expression of a specific receptor for activated C kinase peptide using the α-myosin heavy chain promoter. The transgenic animals at 3 months of age showed no differences in cardiac morphology or histology compared with the nontransgenic control animals, and baseline systolic and diastolic cardiac function was also not different from that seen in control animals.[E7] The transgenic mice with cardiac-specific inhibition of PKCα activity showed a 25% decrease in PKCα activity compared with that seen in nontransgenic control animals.[E7]

Experimental Groups

In the first set of experiments, the 2 groups consisted of hearts receiving 3 mL of cold UW solution (cardioplegia [CP] group) and hearts receiving 3 mL of cold saline (SA group) before hypothermic preservation in UW solution. The second set of experiments involved comparing transgenic hearts with activation or inhibition of PKCα with nontransgenic control hearts to further delineate the effects of this PKC isoform on myocardial function after cardioplegic arrest and preservation.

Creatine Kinase Activity

An assay kit (Sigma) was used to quantitate total creatine kinase (CK) activity in 1 mL of myocardial perfusion effluent at the end of the reperfusion period to determine the degree of cardiac myocyte damage.

Protein Immunoblotting

After the perfusion protocol, atrial tissue was removed, and the ventricles were snap-frozen in liquid nitrogen. Ventricles were then homogenized with a Polytron (Brinkman) at 10,000 rpm in ice-cold lysis buffer, including protease inhibitors. PKC isoform partitioning in subcellular fractions was assayed in cytosolic