Inhibition of protein kinase C α 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 C α , 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 C α 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 C α were then studied to specifically examine the effects of protein kinase C α 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 C α 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 C α 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 C α 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 C α activity and maintenance of intact β -adrenergic receptor signaling. Activation of protein kinase C α 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 isoforms that make up the PKC family, and they are broadly classified by their activation characteristics. The conventional PKC isoforms (α , β I, β II, and γ) are Ca²⁺ and lipid activated, whereas the novel isoforms (ϵ , θ , η , and δ) and the atypical isoforms (ζ and λ) are Ca²⁺ independent but activated by distinct lipids.^{E2} PKC α is the predominant Ca²⁺-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 isoforms as potential regulators of Ca²⁺ handling and

Abbreviations and Acronyms

ACT	= specific activation
ATP	= Adenosine triphosphate
β AR	= 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 PKC α functions as a fundamental regulator of cardiac contractility and Ca²⁺ handling in myocytes.^{E6,E7} For example, PKC α gene-deleted mice were shown to be hypercontractile, whereas transgenic mice overexpressing PKC α were hypocontractile.

The effects of cardioplegic arrest and hypothermic preservation on myocardial PKC α 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 PKC α activity might be an important factor in myocardial protection in this setting. In addition, a novel in vivo mechanism by which PKC α can regulate myocardial β -adrenergic receptor (β AR) 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, 25 mmol/L MgSO₄, 1.2 mmol/L KH₂PO₄, 2.5 mmol/L CaCl₂, 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 PKC α 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 PKC α activity showed a 25% decrease in PKC α 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 PKC α 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

(100,000g supernatant) and Triton X-100–extracted membrane (100,000g pellet) ventricular fractions. Sixty micrograms of protein for each cytosolic and membrane sample was electrophoresed through 12% Tris–glycine gels and then transferred to nitrocellulose membranes. The membranes were blocked for 1 hour in 5% nonfat milk and probed with commercially available primary polyclonal antibodies to PKC α , ϵ , and δ isoforms (Santa Cruz Biotechnology). The membranes were then washed in TBS-Tween solution and incubated with a goat anti-rabbit secondary antibody, followed by exposure to an ECL (Amersham) reagent. Densitometry was performed with a FluorChem 8800 with AlphaEaseFC FluorChem 8900 software (Alpha Innotech Corp).

Measurement of G Protein–coupled Receptor Kinase Activity

The membrane fractions of the myocardial extracts were used to determine G protein–coupled receptor kinase (GRK) activity. Extracts (100 μ g of protein) were incubated with rhodopsin-enriched rod outer-segment membranes in reaction buffer containing the following: MgCl₂, 10 mmol/L; Tris-HCl, 20 mmol/L; ethylenediamine tetraacetic acid, 2 mmol/L; ethyleneglycol-bis(β -aminoethylether)-N,N,N',N'-tetraacetic acid, 5 mmol/L; and adenosine triphosphate (ATP), 0.1 mmol/L (containing [γ -³²P]ATP). After incubating in white light for 15 minutes at room temperature, reactions were quenched with ice-cold lysis buffer and centrifuged for 15 minutes at 13,000g. Sedimented proteins were resuspended in 25 μ L of protein gel loading dye and treated with 12% SDS-PAGE. Phosphorylated rhodopsin was visualized by means of autoradiography of dried polyacrylamide gels and quantified by using a Molecular Dynamics PhosphorImager.

Radioligand Binding Assays

Total β AR density was determined by incubating 25 μ g of cardiac sarcolemmal membranes with a saturating concentration of iodine 125–labeled cyanopindolol and 20 μ mol/L alprenolol to define nonspecific binding. Sarcolemmal membrane samples were studied in triplicate with 80 pmol/L iodine 125–labeled cyanopindolol and 10^{-4} mol/L isoproterenol in 250 μ L of binding buffer (50 mmol/L HEPES [pH 7.3], 5 mmol/L MgCl₂, and 0.1 mmol/L ascorbic acid). Assays were performed at 37°C for 1 hour and then filtered over GF/C glass fiber filters (Whatman) that were washed twice and counted in a gamma counter. Data were analyzed by using the nonlinear least-square curve fit (GraphPad Prism).

Sarcolemmal Membrane Adenylyl Cyclase Activity

Cardiac sarcolemmal membranes (20 μ g of protein) were incubated for 15 minutes at 37°C with (α -³²P)ATP under basal conditions, 10^{-4} mol/L isoproterenol, or 10 mmol/L NaF. Cyclic adenosine monophosphate production was quantified by using standard methods described previously.¹⁸

Statistical Analysis

Results are presented as means $\pm$ standard error of the mean (SEM). Experimental groups were compared by using the Student *t* test or 1-way analysis of variance, as appropriate. The Bonferroni test was applied to all significant analysis of variance results by using SigmaStat software.

TABLE 1. Hemodynamic measurements of left ventricular function before and after cold preservation and normothermic reperfusion

	Baseline	Control (saline)	Cardioplegia
+dP/dt _{max} (mm Hg/s)	3626 $\pm$ 274	1116 $\pm$ 174	2937 $\pm$ 424*
Recovery (%)		31	81*
–dP/dt _{min} (mm Hg/s)	3611 $\pm$ 209	1108 $\pm$ 74	2781 $\pm$ 447*
Recovery (%)		32	77*
LVEDP (mm Hg)	9.2 $\pm$ 3.3	59.5 $\pm$ 13.1	18.6 $\pm$ 4.2*
HR (beats/min)	400 $\pm$ 1	400 $\pm$ 2	400 $\pm$ 2

n = 9 in each group. All hearts were paced. LVEDP, Left ventricular end-diastolic pressure; HR, heart rate. **P* < .05 versus control.

Results

Myocardial Functional Recovery

These studies were performed in nontransgenic mice to investigate the effects of cardioplegic arrest and hypothermic preservation on myocardial function and to establish the protective effect of UW solution in this murine model. Hearts were treated with antegrade perfusion of 3 mL of saline (control, SA group) or 3 mL of UW preservation solution (CP group) after 30 minutes of baseline normothermic perfusion. Baseline systolic and diastolic cardiac function were identical between experimental groups (Table 1). The control (SA) group hearts, which received 3 mL of cold saline followed by hypothermic storage for 4 hours at 4°C and normothermic reperfusion for 90 minutes, recovered only 31% of basal dP/dt_{max} and 32% of basal dP/dt_{min}. In addition, left ventricular end-diastolic pressure was markedly increased to 59.5 $\pm$ 13.1 mm Hg from a baseline of 9.2 $\pm$ 3.3 mm Hg (Table 1). The CP group, in contrast, in which hearts were arrested with 3 mL of UW solution followed by 4 hours of hypothermic storage and 90 minutes of normothermic reperfusion, showed recovery of dP/dt_{max} to 81% of baseline and dP/dt_{min} to 77% of baseline. Left ventricular end-diastolic pressure after ischemia and reperfusion in the CP group was also much lower compared with that seen in the control group at 18.6 $\pm$ 4.2 mm Hg (Table 1). Heart rate during basal conditions and after the experimental protocol was not different between groups because external pacing was used to maintain a heart rate of 400 beats/min. CK activity was measured in the effluent from the perfusion system at the end of the reperfusion period to assess myocyte damage after cold preservation and normothermic reperfusion. The total CK activity was nearly 2.5-fold greater in the control hearts compared with that seen in the CP group (Figure 1). The hemodynamic data and the degree of myocyte injury demonstrated that this was a valid murine model of cardioplegic arrest and cold preservation because there was a highly significant difference in myocardial preservation with UW solution versus hypothermia alone.

Figure 1. Creatine kinase activity in 1 mL of myocardial effluent after 90 minutes of normothermic reperfusion. * $P < .01$ versus control (SA group); $n = 9$ in each group.

UW solution is very commonly used for preservation of donor hearts in the clinical setting.

Assessment of PKC α Activity

Myocardial PKC α isoform activity was studied in both groups after 4 hours of cold preservation and 90 minutes of normothermic reperfusion. The ratio of PKC isoform expression in the membrane fraction to the cytosolic fraction is an indirect measure of kinase activity because it translocates from the cytosolic fraction to the membrane fraction, where it phosphorylates other signaling molecules and membrane proteins. In the saline control group there was a 2-fold increase in myocardial PKC α activity relative to that seen in the CP group (Figure 2). The PKC ϵ isoform has been shown to play an important role in myocardial recovery after normothermic ischemia-reperfusion injury.^{E1} There was no significant difference in PKC ϵ activity between groups at the end of the cold preservation and reperfusion period in our model (Figure 3). These results demonstrate that inhibition of PKC α might be an important mechanism of cardioplegia-mediated myocardial protection from ischemia-reperfusion injury.

Effects on β AR Signaling

Because the β AR signaling system is critical in the regulation of cardiac contractility and can be modulated by PKC, this pathway was investigated before and after cardioplegic arrest, cold preservation, and normothermic reperfusion with this model. Total myocardial sarcolemmal membrane β AR density was not different before or after the preservation period in both the SA and CP groups (Table 2). Both basal and isoproterenol-stimulated adenylyl cyclase activity

Figure 2. Myocardial PKC α activity after cardioplegic arrest, cold preservation, and normothermic reperfusion. *M*, Membrane fraction; *C*, cytosolic fraction; *CP*, cardioplegia (University of Wisconsin) solution. * $P < .05$ versus control; $n = 9$ in each group.

was significantly lower in the SA group after reperfusion compared with that seen in the CP group (Table 2). Cyclase activity stimulated by NaF was not different between groups, indicating preservation of G protein–adenylyl cyclase coupling. These data also indicate that the defect in β AR signaling involves uncoupling of these receptors from their downstream effector, adenylyl cyclase. Activity of

Figure 3. Myocardial PKC ϵ activity after cardioplegic arrest, cold preservation, and normothermic reperfusion. *M*, Membrane fraction; *C*, cytosolic fraction; *CP*, cardioplegia (University of Wisconsin) solution. * $P < .05$ versus control; $n = 7$ in each group.

TABLE 2. Myocardial β -adrenergic receptor signaling

	Saline-treated group	Cardioplegia-treated group
β AR density, fmol/mg membrane protein (baseline)	62.5 $\pm$ 5.8	67.2 $\pm$ 7.4
β AR density, fmol/mg membrane protein (after reperfusion)	65.9 $\pm$ 7.1	64.0 $\pm$ 4.9
Basal AC activity, pmol cAMP $\cdot$ mg ⁻¹ $\cdot$ min ⁻¹ (baseline)	45.8 $\pm$ 6.4	52.1 $\pm$ 8.3
ISO AC activity, pmol cAMP $\cdot$ mg ⁻¹ $\cdot$ min ⁻¹ (baseline)	103.6 $\pm$ 18.3	110.7 $\pm$ 14.9
Basal AC activity, pmol cAMP $\cdot$ mg ⁻¹ $\cdot$ min ⁻¹ (after reperfusion)	21.2 $\pm$ 5.7*	45.5 $\pm$ 9.2
ISO AC activity, pmol cAMP $\cdot$ mg ⁻¹ $\cdot$ min ⁻¹ (after reperfusion)	32.8 $\pm$ 6.6*	106.1 $\pm$ 18.5

N = 8 in each group. β AR, β -Adrenergic receptor density; AC, adenylyl cyclase activity; cAMP, cyclic adenosine monophosphate; ISO, stimulation with 10⁻⁴ mol/L isoproterenol. *P < .05 versus CP group.

GRK2, a member of the family of serine–threonine kinases known as G protein–coupled receptor kinases, which phosphorylates and desensitizes agonist-occupied β ARs, was significantly increased after reperfusion in the SA group compared with that in the CP group (Figure 4). There was no difference in GRK2 activity between groups at baseline. These results suggest that β AR desensitization plays an important role in myocardial dysfunction in the SA group and might be associated with activation of PKC α .

Functional Consequences of Myocardial PKC α Activation and Inhibition

Because PKC α is the predominant Ca²⁺-dependent PKC isoform in the murine heart and activity of this isoform was significantly inhibited by cardioplegic arrest with UW solution, we studied cardiac function after cardioplegic arrest,

cold preservation, and normothermic reperfusion in transgenic hearts with specific activation (PKC ACT) or inhibition (PKC INH) of PKC α and also compared these values with those seen in nontransgenic control hearts. These studies with transgenic mice might more specifically delineate the effects of PKC α on cardiac function after preservation and reperfusion. The transgenic mice used in this study had a modest 1.5-fold cardiac-specific increase in PKC α activity (PKC ACT) or a 25% decrease in myocardial PKC α activity (PKC INH) relative to that seen in control mice.^{E7} Baseline cardiac function was similar between the transgenic and nontransgenic hearts (Table 3). After CP arrest, cold preservation, and the warm reperfusion period, there was a highly significant difference in functional recovery because PKC α activation led to severely impaired myocardial recovery of both systolic function (1822 $\pm$ 111 vs 2918 $\pm$ 107 mm Hg/s, P < .01) and diastolic function (1518 $\pm$ 125 vs 2601 $\pm$ 118 mm Hg/s, P < .05) compared with baseline values, which were not different between groups (Table 3). In contrast, inhibition of PKC α activity led to significantly better recovery of myocardial systolic function (3499 $\pm$ 188 vs 2918 $\pm$ 107 mm Hg/s, P < .01) and diastolic function (3214 $\pm$ 145 vs 2601 $\pm$ 118 mm Hg/s, P < .01) after preservation and reperfusion compared with that seen in the nontransgenic hearts (Table 3). The transgenic hearts were arrested with cold saline followed by hypothermic storage and normothermic reperfusion (n = 10 in each group) to further evaluate the significance of PKC α activity on myocardial protection. The PKC ACT hearts treated with saline recovered only 14% of baseline +dP/dt_{max} and 12% of baseline -dP/dt_{min}. In contrast, the PKC INH group treated with saline recovered 62% of baseline +dP/dt_{max} and 58% of baseline -dP/dt_{min} (P < .01 vs PKC ACT). The recovery of left ventricular systolic and diastolic function in the saline-treated PKC ACT hearts was also significantly worse than that in the saline-treated nontransgenic hearts (P < .05), and the PKC INH hearts treated with saline had significantly improved recovery (approximately 2-fold) relative to that of the nontransgenic group arrested with saline (P < .01).

Figure 4. G protein–coupled receptor kinase (GRK2) activity before and after cold preservation and normothermic reperfusion. SA, Saline; CP, cardioplegia (University of Wisconsin) solution. *P < .001 versus control (SA group); n = 8 in each group.

TABLE 3. Hemodynamic measurements of left ventricular function before and after cold preservation and normothermic reperfusion

	Control Ntg	PKC α ACT	PKC α INH
Baseline +dP/dt _{max} (mm Hg/s)	3741 $\pm$ 291	3795 $\pm$ 142	3804 $\pm$ 202
Postreperfusion +dP/dt _{max} (mm Hg/s)	2918 $\pm$ 107	1822 $\pm$ 111*	3499 $\pm$ 188†
Recovery (%)	78	48*	92†
Baseline -dP/dt _{min} (mm Hg/s)	3467 $\pm$ 147	3613 $\pm$ 163	3694 $\pm$ 184
Postreperfusion -dP/dt _{min} (mm Hg/s)	2601 $\pm$ 118	1518 $\pm$ 125*	3214 $\pm$ 145†
Recovery (%)	75	42*	87†
Heart rate (beats/min)	400 $\pm$ 3	400 $\pm$ 1	400 $\pm$ 3

N = 11 in each group. All hearts were paced. * P < .05 versus control and PKC INH. † P < .05 versus PKC ACT and control. Ntg, Nontransgenic; PKC, protein kinase C; ACT, specific activation; INH, specific inhibition.

Myocardial GRK2 Activity and β -adrenergic Signaling in Transgenic Mice

Total myocardial β AR density was not different between PKC ACT and PKC INH hearts under basal conditions or after cold preservation and reperfusion (Table 4). In contrast, basal and isoproterenol-stimulated adenylyl cyclase activity in myocardial sarcolemmal membranes was significantly lower in the PKC ACT mice at baseline and after cold preservation and reperfusion (Table 4). These data indicate that activation of PKC α is associated with β AR desensitization as cyclase activity is inhibited. To determine a potential mechanism, we measured myocardial GRK2 activity using rhodopsin phosphorylation. GRK2 activity was greater in the PKC ACT hearts compared with that seen in the PKC INH and nontransgenic control hearts both before and after the preservation and reperfusion period (Figure 5, A). In contrast, there was no difference between groups in myocardial GRK2 protein expression either before or after hypothermic preservation and reperfusion (Figure 5, B). It appears that activation of PKC α can lead to activation of GRK2 in vivo and that the resulting β AR desensitization or receptor-effector uncoupling might represent an important mechanism of myocardial dysfunction after cardiac preservation.

Discussion

Myocardial protection in cardiac surgery has continued to improve and has led to better outcomes after elective oper-

ations, as well as cardiac transplantation. Previous studies have described the improvements in myocardial metabolic function with cardioplegic arrest and hypothermia, and these have been the foundation of current cardioplegia solutions, as well as organ-preservation solutions, such as UW solution.^{E9} Pharmacologic strategies to minimize tissue acidosis and cellular hypercalcemia and to preserve high-energy phosphates have been quite effective.^{E10-E12} Unfortunately, there has been very little progress in cardiac donor organ-preservation strategies to further increase the safe duration of ischemia, which might provide a greater number of available donor organs. In addition, much of the early myocardial dysfunction that is occasionally present after allograft reperfusion might be minimized with more effective means of myocardial protection.

PKC has been implicated as a critical signaling molecule in mediating cardiac functional recovery after ischemia-reperfusion injury and particularly in mediating ischemic preconditioning.^{E13-E15} PKC was first described as a phospholipid-dependent serine-threonine kinase that is activated as a result of receptor-dependent activation of phospholipase C and the hydrolysis of membrane phosphoinositides.^{E16} Activation of PKC isoforms involves translocation from the cytosolic fraction of cells to the membrane or particulate fraction. PKC has been shown to phosphorylate β ARs in vitro and can also phosphorylate particular contractile proteins, such as troponin I.^{E17} The

TABLE 4. Myocardial β -adrenergic receptor signaling in transgenic mice

	PKC α ACT	PKC α INH
β AR density, fmol/mg membrane protein (baseline)	56.9 $\pm$ 3.8	60.2 $\pm$ 6.9
β AR density (after reperfusion)	55.2 $\pm$ 6.1	56.8 $\pm$ 5.0
Basal AC activity, pmol cAMP $\cdot$ mg ⁻¹ $\cdot$ min ⁻¹ (baseline)	40.8 $\pm$ 5.3	45.9 $\pm$ 6.3
ISO AC activity, pmol cAMP $\cdot$ mg ⁻¹ $\cdot$ min ⁻¹ (baseline)	87.4 $\pm$ 15.5*	125.2 $\pm$ 17.1
Basal AC activity, pmol cAMP $\cdot$ mg ⁻¹ $\cdot$ min ⁻¹ (after reperfusion)	23.9 $\pm$ 4.2*	44.8 $\pm$ 7.4
ISO AC activity, pmol cAMP $\cdot$ mg ⁻¹ $\cdot$ min ⁻¹ (after reperfusion)	42.0 $\pm$ 6.9*	118.4 $\pm$ 15.9

N = 8 in each group. PKC, Protein kinase C; ACT, specific activation; INH, specific inhibition; β AR, β -adrenergic receptor density; AC, adenylyl cyclase activity; cAMP, cyclic adenosine monophosphate; ISO, stimulation with 10⁻⁴ mol/L isoproterenol. * P < 0.05 versus PKC α INH.

Figure 5. A, G protein–coupled receptor kinase (GRK2) activity in transgenic protein kinase C (PKC) α hearts with specific inhibition (INH) and PKC α hearts with specific activation (ACT) before and after cold preservation and normothermic reperfusion. B, Baseline; P, after reperfusion; NTg, nontransgenic. * $P < .001$ versus NTg control and PKC α INH hearts; $n = 8$ in each group. B, Total myocardial GRK2 protein expression. There was no difference between groups. B, Baseline; P, after reperfusion; NTg, nontransgenic. $N = 8$ in each group.

PKC family of ubiquitous serine–threonine kinases now numbers 12 distinct isoforms.^{E8} Cardiac myocytes coexpress multiple PKC isoforms, and there is general consensus that ventricular myocytes coexpress calcium-sensitive PKC α , novel PKC δ and PKC ϵ , and atypical PKC λ .^{E18} Prior studies have shown that the PKC α iso-

form represents nearly 80% of total PKC expression in the rat and murine heart^{E7,E19} and is an important isoform in the human heart.^{E3,E20}

Our data show that cardioplegic arrest inhibits activation of PKC α . This was associated with significantly better ventricular function after normothermic reperfusion and much less myocyte damage, as measured by CK release. In addition, activation of PKC α was also associated with impaired β AR signaling, as assessed on the basis of myocardial sarcolemmal membrane adenylyl cyclase activity. GRK2 activity was increased with resultant β AR desensitization. Previous studies have shown that PKC can phosphorylate and activate GRK2 in vitro.^{E21,E22} Isoform-specific studies have not been performed, however, with regard to activation of GRK2. PKC α has been shown to have important effects on cardiac function in vivo. Cardiac-specific PKC α overexpression in transgenic mice resulted in a blunting of basal and β -agonist–stimulated contractility.^{E6} Mechanistically, modulation of PKC α activity affects dephosphorylation of the sarcoplasmic reticulum Ca^{2+} ATPase 2 pump inhibitory protein phospholamban and alters sarcoplasmic reticulum Ca^{2+} loading and the Ca^{2+} transient. PKC α was found to directly phosphorylate protein phosphatase inhibitor 1, altering the activity of protein phosphatase 1, which might account for the effects of PKC α on phospholamban phosphorylation and blunted contractility.^{E6} In contrast, homozygous deletion of PKC α in gene-targeted mice led to enhanced basal and β -agonist–stimulated contractility.^{E6}

To determine the effects of PKC α activation on myocardial preservation and recovery after cardioplegic arrest in a more specific fashion, we used transgenic mice with cardiac-specific activation (PKC ACT) or inhibition (PKC INH) of PKC α activity. PKC α activation resulted from overexpression of a specific receptor for activated C kinase, which is critical in the targeted translocation of PKC α from the cytosolic to the membrane fraction of cardiac myocytes.^{E7} As previously described, this leads to a modest 1.5-fold increase in myocardial PKC α activity.^{E7} The transgenic mice with cardiac-specific inhibition of PKC α activity showed a 25% decrease in PKC α activity compared with that seen in nontransgenic control animals through expression of an inhibitory peptide.^{E7} The PKC ACT hearts had significantly worse recovery of both systolic and diastolic left ventricular function compared with the nontransgenic control hearts. In contrast, PKC INH hearts had even greater recovery of left ventricular function after cardioplegic arrest and reperfusion versus the nontransgenic hearts. It appears that inhibition of PKC α activity is an important mechanism in myocardial protection and preservation with cardioplegic arrest.

Our data also demonstrate, for the first time, that PKC α activation can lead to enhanced GRK2 activity and β AR

desensitization in vivo and, more specifically, in the heart. This might represent another important mechanism by which PKC α can alter myocardial function. Impaired cardiac β AR signaling and increased GRK2 activity are classical characteristics of chronic heart failure in human subjects and experimental animal models.^{E23,E24} Activation of PKC isoforms in cardiac hypertrophy might lead to enhanced GRK2 activity and contribute to β AR desensitization in the transition from compensatory hypertrophy to heart failure.

Recently, it has been reported that gene therapy–based inhibition of PKC α/β enhances cardiac contractility and attenuates heart failure in a rat model of postinfarction cardiomyopathy.^{E25} This report clearly demonstrates the important effect of PKC α as a negative regulator of cardiac contractility

and shows the potentially beneficial effect of inhibiting this PKC isoform on cardiac function after myocardial injury. Our data also show that even modest inhibition of PKC α activity has dramatic effects on left ventricular recovery in a model of cardioplegic arrest and hypothermic preservation followed by normothermic reperfusion. This appears to be an important mechanism by which UW solution provides myocardial protection. It is possible that the development of specific pharmacologic inhibitors of PKC α might further improve current techniques of cardiac preservation in the setting of cardiac surgery and particularly in cardiac transplantation, where organ preservation remains very limited. In addition, novel approaches to inhibit GRK2-mediated β AR desensitization might represent another potential opportunity to improve myocardial protection.

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