Responses of Cardiac Protein Kinase C Isoforms to Distinct Pathological Stimuli Are Differentially Regulated

Yasuchika Takeishi, Thunder Jalili, Nancy A. Ball, Richard A. Walsh

Abstract—Currently at least 11 protein kinase C (PKC) isoforms have been identified and may play different roles in cell signaling pathways leading to changes in cardiac contractility, the hypertrophic response, and tolerance to myocardial ischemia. The purpose of the present study was to test the hypothesis that responses of individual PKC isoforms to distinct pathological stimuli were differentially regulated in the adult guinea pig heart. Isolated hearts were perfused by the Langendorff method and were exposed to ischemia, hypoxia, H2O2, or angiotensin II. Hypoxia and ischemia induced translocation of PKC isoforms α, β2, γ, and ζ, and H2O2 translocated PKC isoforms α, β2, and ζ. Angiotensin II produced translocation of α, β2, γ, ζ, and H2O2 blocked hypoxia-induced (α, β2, and ζ) and angiotensin II–induced (α, β2, γ, and ζ) translocation of PKC isoforms. Inhibition of translocation of PKC isoforms with genistein blocked translocation of PKC isoforms by hypoxia (β2, and ζ) and by angiotensin II (β2). By contrast, neither D609 nor genistein blocked H2O2-induced translocation of any PKC isoform. We conclude that hypoxia-induced activation of PKC isoforms is mediated through pathways involving phospholipase C and tyrosine kinase; however, oxidative stress may activate PKC isoforms independently of Gq-phospholipase C coupling and tyrosine kinase signaling. Because oxidative stress may directly activate PKC, and PKC activation appears to be involved in human heart failure, selective inhibition of the PKC isoforms may provide a novel therapeutic strategy for the prevention and treatment of this pathological process. (Circ Res. 1999;85:264-271.)

Key Words: hypoxia—oxidative stress—ischemia—myocardium—signal transduction

Protein kinase C (PKC) has been implicated as the intracellular mediator of several neurotransmitters, growth factors, and tumor promoters through multiple signal transduction pathways.1 Currently at least 11 isoforms have been identified in vivo. Recently we reported that PKC plays a critical role in the development of cardiac hypertrophy and failure.2,3 Acute mechanical stretch of the left ventricle induces translocation of the PKCα isoform,4 and chronic pressure overload by aortic banding activates the PKCα and -ε isoforms in guinea pig heart.5 Furthermore, the expression and activity of PKCα and -β isoforms are elevated in failing human heart.6 Finally, transgenic mice with cardiac-specific postnatal overexpression of the PKCβ2 isoform display cardiac hypertrophy, cardiomyocyte necrosis, multifocal fibrosis, and depressed in vivo left ventricular performance.7 The PKCβ2 isoform regulates cardiomyocyte contractility, at least in part, through phosphorylation of cardiac regulatory protein, troponin I, and a resultant decrease in myofilament calcium sensitivity.8 Taken together, PKC isoforms may play different functional roles in cell signaling, although the exact significance of individual isoforms is not yet known.

Probably the most pathologically relevant forms of cardiac stress in vivo are ischemia/hypoxia9 and oxidative stress.10 Goldberg et al11 have reported hypoxia-induced translocation of PKCsα and -ε isoforms in the neonatal rat cardiomyocyte. However, insights from results of in vitro studies using the neonatal cardiomyocyte may not be completely relevant to the adult heart in vivo, because it is known that PKC isoform expression is developmentally altered.12,13 Although valuable information regarding mechanotransduction and hypertrophy has been learned from the study of neonatal cardiomyocytes, there is little information regarding the mechanisms wherein this process occurs in the adult left ventricle. Recently, Ping et al14 have reported in the adult rabbit heart that ischemic preconditioning induces selective translocation of PKCε and -η isoforms, which suggests an important role of these 2 isoforms in the genesis of this pathological process.

An increase in oxidative stress due to an increase in free radicals and/or a relative deficit in the endogenous antioxidant reserve can cause contractile depression.15 Dhalla et al16 have reported that oxidative stress is one of the contributing factors in the transition from compensated hypertrophy to decompensated heart failure. It has been reported that reduction-oxidation (redox) reactions generate reactive oxygen species, including H2O2, O2−, and OH−. Although reactive oxygen species have been identified as important chemical processes that can regulate cellular signaling,10,17 the effect of oxidative stress on PKC signaling has not been rigorously examined.

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The purpose of the present study was to test the hypothesis that responses of PKC isoforms to distinct pathological stimuli were differentially regulated in the adult heart. Isolated hearts were subjected to hypoxia, ischemia, and oxidative stress using H₂O₂. Angiotensin II, a known ligand of the Gq–phospholipase C signaling cascade, was also used to activate PKC through a receptor-mediated pathway. Translocation of PKC isoforms from cytosolic to membranous fractions was examined using isoform-specific antibodies. To examine the signal transduction pathways leading to PKC isoform translocation, specific phospholipase C and PKC activators were used to activate PKC through a Gq-coupled receptor-mediated pathway. The dose of angiotensin II and perfusion time were chosen on the basis of a previous study from our laboratory reporting that angiotensin II produced inositol phosphate accumulation and translocation of PKC under these conditions. In some hearts exposed to angiotensin II, hypoxia, and H₂O₂, tricyclodecan-9-yl-xanthogenate (D609, Sigma), a phospholipase C inhibitor, or genistein (Sigma), a tyrosine kinase inhibitor, was added to the perfusate, starting 10 minutes before the stimulation with angiotensin II (1, 10, and 100 μmol/L of D609 [n = 3 at each dose]) and genistein [n = 3 at each dose]), hypoxia (D609 100 μmol/L [n = 3], genistein 100 μmol/L [n = 3]), or H₂O₂ (D609 100 μmol/L [n = 3], genistein 100 μmol/L [n = 3]). After completion of perfusion, the left ventricle was flash frozen with liquid nitrogen– precooled Wollenberger clamp, powdered in liquid nitrogen, and stored at −80°C.  

Separation of Membranous and Cytosolic Fractions for PKC Localization

Membrane and cytosolic fractions of detergent-extracted PKC were prepared as previously described. 4,5 Briefly, left ventricular tissue was homogenized in lysis buffer containing (in mmol/L) Tris-HCl 113.8, NaCl 4.7, and HEPES 25, MgSO⁴ 1.1, KH₂PO⁴ 0.12, NaHCO₃ 23.6, CaCl₂ 2.5, manni tol 6.0, and glucose 11.0, as previously reported. 5,18 The solution was saturated with 95% O₂/5% CO₂ (pH 7.4) at 37°C. A saline-filled latex balloon attached to a 3F micromanometer catheter (Millar Instruments) was inserted into the left ventricle through the mitral valve for pressure measurements. 5,18 The balloon was inflated to achieve 10 mm Hg initial minimum diastolic pressure and was kept isovolumic during the perfusion. Heart rate and aortic and left ventricular pressure were continuously monitored on a Gould MK200 multichannel recorder interfaced to an IBM computer. Analog signals were digitized online at a sampling frequency of 1000 Hz, and hemodynamic parameters were derived by custom-designed software. Ten to fifteen beats were averaged for each condition, and premature contractions were excluded from the analysis. The maximal rate of isovolumic pressure development (+dP/dt) was calculated and used as an index of left ventricular contractility. The minimum rate of pressure development (−dP/dt) was measured to assess changes in the rate of isovolumic relaxation. The coronary flow rate was adjusted to 10 mL·min⁻¹·g⁻¹ net heart weight and was kept constant throughout the experiment. After basal hemodynamic recording for 10 to 15 minutes, guinea pig hearts were subjected to 8 minutes of global ischemia (n = 6), 8 minutes of hypoxia (n = 6), 100 μmol/L (n = 3), or 180 μmol/L (n = 6) of H₂O₂ for 20 minutes. Six guinea pigs perfused with Krebs-Henseleit buffer alone were used as a control. Hypoxia was produced by saturating the perfusion buffer with 5% CO₂/95% N₂. 19 Ischemia was induced by suspending the circulation of the perfusion pump. It has been reported that 8 minutes of global ischemia and/or hypoxia is sufficient to alter cell signaling or phosphorylation of proteins in the myocardium. 20 The dose and perfusion time with H₂O₂ was chosen on the basis of previous reports. 21,22 Angiotensin II, 10 μmol/L for 20 minutes (n = 3), was also used as a potent ligand to activate PKC through a Gq-coupled receptor-mediated pathway. The dose of angiotensin II and perfusion time were chosen on the basis of a previous study from our laboratory reporting that angiotensin II produced inositol phosphate accumulation and translocation of PKC under these conditions. 4 In some hearts exposed to angiotensin II, hypoxia, and H₂O₂, tricyclodecan-9-yl-xanthogenate (D609, Sigma), a phospholipase C inhibitor, or genistein (Sigma), a tyrosine kinase inhibitor, was added to the perfusate, starting 10 minutes before the stimulation with angiotensin II (1, 10, and 100 μmol/L of D609 [n = 3 at each dose] and 1, 10, and 100 μmol/L of genistein [n = 3 at each dose]), hypoxia (D609 100 μmol/L [n = 3], genistein 100 μmol/L [n = 3]), or H₂O₂ (D609 100 μmol/L [n = 3], genistein 100 μmol/L [n = 3]). After completion of perfusion, the left ventricle was flash frozen with liquid nitrogen– precooled Wollenberger clamp, powdered in liquid nitrogen, and stored at −80°C. 5,18

Western Blot Analysis

The subcellular localization of PKC isoforms was examined by quantitative immunoblotting. 4,5 Equal amounts of cytosolic and membranous protein extracts for each group were separated by 10% SDS-PAGE and transferred to nitrocellulose membranes. To ensure equivalent quantitative transfer efficiency of proteins, the nitrocellulose membrane was stained with Ponceau S. Membranes were blocked with 5% nonfat dry milk overnight at 4°C and incubated overnight with PKC isofrom–specific primary antibodies (Santa Cruz Biotechnology) at 4°C. To ensure the specificity of immunoreactive proteins, transferred membranes were incubated with primary antibodies in the presence and absence of the corresponding blocking peptide (Santa Cruz Biotechnology). To additionally ensure equivalent loading of proteins, the nitrocellulose membrane of the fraction was cut into 2 pieces, ...
and the lower half was probed with a primary antibody for calsequestrin (a gift from Dr L.R. Jones, Krannert Institute of Cardiology, Indiana University School of Medicine, Indianapolis, Ind) at room temperature for 2 hours. Calsequestrin was chosen as a marker protein for equal loading, given that the expression of this protein was not modulated in hearts with pressure-overload hypertrophy and failure.

Then, blots were incubated for 1 to 2 hours with a secondary antibody (horseradish peroxidase–conjugated, KPL Laboratories) and visualized by enhanced chemiluminescence (Amersham Life Science). The degree of labeling was quantified by a computer program (NIH) and expressed in relative scan units.

**Statistical Analysis**

Data are presented as mean±SEM. Reported data were analyzed by analysis of variance followed by Student Newman-Keuls test. If data were not normally distributed or failed equal variance tests after log10 transformations, they were analyzed by nonparametric statistics. Values with P<0.05 were considered to be statistically significant.

**Results**

**Hemodynamic Changes by Angiotensin II, Ischemia, Hypoxia, and Oxidative Stress**

The Table summarizes isovolumic left ventricular mechanics in response to angiotensin II, ischemia, hypoxia, and H$_2$O$_2$ measured with a high-fidelity micromanometer. Angiotensin II produced no significant alterations in isovolumic left ventricular mechanics. Hypoxia, ischemia, and 100 and 180 μmol/L of H$_2$O$_2$ induced significant reduction of left ventricular peak systolic pressure, developed pressure, maximal dP/dt, and minimum dP/dt. The end-diastolic pressure was significantly increased by hypoxia and H$_2$O$_2$.

**Alterations in Subcellular Distribution of PKC Isoforms by Distinct Stimuli**

We had examined the subcellular distribution of 7 PKC isoforms (α, β, β', δ, ε, γ, and ζ) by immunoblotting with the use of isoform-specific antibodies. We found that the left ventricle of adult guinea pig expressed the 5 PKC isoforms α, β', δ, ε, and ζ, whereas no significant immunoreactivity was detected for β, δ. Therefore, alterations in the subcellular localization of these 5 PKC isoforms were examined in the present study.

Representative immunoblots of each PKC isoform are shown in Figure 1. The membrane-associated immunoreactivity of PKCα was markedly increased in response to angiotensin II, hypoxia, and H$_2$O$_2$ compared with a control heart perfused with Krebs buffer only. The immunoreactivity was specific to PKCα, given that it was blocked by a competing peptide. The nitrocellulose membrane was cut into 2 pieces, and the lower half was probed separately with an antibody specific to calsequestrin. Protein level of calsequestrin in the membrane fraction did not differ among 5 animals. Positions of the molecular mass markers (in kDa) are indicated on the right. Con indicates control.
The group data of PKC immunoblots are summarized in Figure 2. Membrane/cytosol ratios of immunoreactivity were used as indices of the extent of PKC isoform translocation. Significant increases of membrane/cytosol ratios in PKC\(\alpha\), -\(\beta_2\), and -\(\zeta\) were observed in response to angiotensin II, hypoxia, ischemia, and 100 and 180 \(\mu\)mol/L of H\(_2\)O\(_2\). PKC\(\gamma\) showed significant increases of membrane/cytosol ratios by angiotensin II, hypoxia, and ischemia, but not by H\(_2\)O\(_2\). Only angiotensin II, but not hypoxia, ischemia, and H\(_2\)O\(_2\), produced a significant increase in the membrane/cytosol ratio of PKC\(\epsilon\). These data suggested that responses of PKC isoforms to distinct pathological stimuli were differentially regulated.

Role of Phospholipase C and Tyrosine Kinase in the Translocation of PKC Isoforms

To examine the role of phospholipase C and tyrosine kinase in the translocation of PKC isoforms, hearts were perfused with tricyclodecan-9-yl-xanthogenate (D609), a phospholipase C inhibitor,\(^{11}\) or genistein, a tyrosine kinase inhibitor.\(^{24}\) D609 or genistein was continuously infused by coronary artery perfusion in the buffer during experiments, starting 10 minutes before exposing hearts to angiotensin II, hypoxia, or H\(_2\)O\(_2\).

First, we tested the effects of 3 different doses of D609 (1, 10, and 100 \(\mu\)mol/L [n=3 at each dose]) on angiotensin II–mediated translocation of PKC isoforms,\(^{11}\) because the angiotensin II receptor is coupled with phospholipase C. D609 inhibited translocation of PKC\(\alpha\), -\(\beta_2\), -\(\gamma\), and -\(\zeta\) in a dose-dependent manner (data not shown), and D609 of 100 \(\mu\)mol/L significantly blocked angiotensin II–mediated translocation of PKC\(\alpha\), -\(\beta_2\), -\(\gamma\), and -\(\zeta\) as shown in Figure 3 (data not shown for PKC\(\gamma\)). Thus, a dose of 100 \(\mu\)mol/L of D609 was applied to hearts exposed to hypoxia and H\(_2\)O\(_2\). PKC\(\epsilon\) was not examined, because this isoform did not show translocation with hypoxia, ischemia, or H\(_2\)O\(_2\). As shown in Figure 4, hypoxia-induced translocation of PKC\(\alpha\), -\(\beta_2\), and -\(\zeta\), but not -\(\gamma\), was blocked by 100 \(\mu\)mol/L of D609. These findings suggested that hypoxia activates PKC isoforms with a pathway involving phospholipase C. By contrast, D609 failed to block H\(_2\)O\(_2\)-induced translocation of any PKC isoform. PKC\(\gamma\) was not examined, because this isoform did not show translocation with hypoxia, ischemia, or H\(_2\)O\(_2\). Group data of effects of D609 on PKC signaling are summarized in Figure 3. D609 blocked hypoxia-induced translocation of PKC\(\alpha\), -\(\beta_2\), and -\(\zeta\) but failed to block H\(_2\)O\(_2\)-induced translocation of any PKC.

![Figure 2. Group data for translocation of PKC isoforms \(\alpha\), \(\beta_2\), \(\epsilon\), \(\gamma\), and \(\zeta\) in response to distinct stimuli. Membrane/cytosol ratios of immunoreactivity were calculated for each isoform as indices of PKC translocation. Data were obtained from 6 control guinea pigs and 3 exposed to angiotensin II, 6 to hypoxia, 6 to ischemia, 3 to H\(_2\)O\(_2\) (100 \(\mu\)mol/L), and 6 to H\(_2\)O\(_2\) (180 \(\mu\)mol/L). Abbreviations as in Figure 1. *P<0.01, #P<0.05 vs control (Cont).](#)

![Figure 3. Effects of D609 or genistein on translocation of PKC\(\alpha\), -\(\beta_2\), and -\(\zeta\) isoforms. Hearts were perfused with either D609 or genistein and subjected to angiotensin II (AII), hypoxia, or H\(_2\)O\(_2\). Data were obtained from 3 animals in each group. *P<0.01, +P<0.05 vs angiotensin II; #P<0.01 vs hypoxia.](#)
isoform. These findings suggested that phospholipase C was not involved in H₂O₂-induced translocation of PKC isoforms. Then we tested the effect of 3 different doses of genistein (1, 10, and 100 μmol/L [n=3 at each dose]) on angiotensin II–mediated PKC signaling. A dose of 100 μmol/L of genistein blocked translocation of PKCβ₂ by angiotensin II, but not other isoforms (Figures 3 and 4). Hypoxia-induced translocation of PKCβ₂ and -ζ, but not -α and -γ, was also blocked by 100 μmol/L of genistein. These findings indicated that tyrosine kinase was involved in the angiotensin II–mediated translocation of PKCβ₂ and hypoxia-induced translocation of PKCβ₂ and -ζ. However, genistein failed to block translocation of any PKC isoform by H₂O₂. These findings suggested that H₂O₂-mediated PKC signaling is independent of tyrosine kinase signaling.

Discussion

In the present study, we demonstrated differential activation of PKC isoforms in response to distinct pathological stimuli such as angiotensin II, hypoxia, ischemia, and oxidative stress using H₂O₂. Hypoxia and angiotensin II induced translocation of PKC isoforms through pathways involving phospholipase C and tyrosine kinase, but H₂O₂ activated PKC isoforms independently of phospholipase C and tyrosine kinase signaling.

PKC Activation in Neonatal and Adult Cardiomyocytes

It has been reported that PKC redistribution does not correlate in extent or duration with phosphorylation of PKC substrates, which suggests that translocation may not always equate to activity. Measurements of PKC activity are not sufficiently sensitive to detect the involvement of PKC in cardiac hypertrophy (Y.T., R.A.W., unpublished data, 1998) or ischemic preconditioning. Actual decreases in absolute protein abundance of cytosolic PKC associated with translocation are not typically observed in the intact heart. One can see the absolute decreases in cytosolic PKC in cultured neonatal cardiac myocytes when acutely stimulated by phorbol esters. However, when whole hearts are subjected to pathophysiological stimuli such as mechanical stretch, chronic pressure overload, ischemia and reperfusion, and streptozotocin-induced diabetes, translocation of PKC isoforms is not accompanied by a reciprocal absolute decrease in protein abundance of cytosolic PKC. Those reports showed the decreases in relative abundance of cytosolic PKC, but not in absolute abundance, as we showed in the present study.

It has also been reported that hypoxia induces PKC translocation in neonatal rat ventricular myocytes. However, insights from results of in vitro studies using neonatal cardiomyocytes may or may not be applied to the intact adult heart, because PKC isoform expression is differentially regulated during development. Rybin and Steinberg have reported that the PKCα, -δ, -ε, and -ζ isoforms are detected in fetal and neonatal cardiomyocytes. Among them, PKCα and -ζ isoforms show developmental decline. Furthermore, Puceat et al have shown that responses of PKC isoforms to neurohormones are also different between neonatal and adult cardiomyocytes. Endothelin-1 and carbachol activate PKC isoforms δ and ε in the neonatal cardiomyocyte, but not in the adult cardiomyocyte. Although valuable information has been learned from the study of neonatal cardiomyocytes, there is little information regarding the mechanisms wherein this process occurs in the adult left ventricle. In the present study, we reported for the first time a direct comparison of distinct pathological stimuli such as hypoxia, ischemia, H₂O₂, and angiotensin II to activate individual PKC isoforms in the adult heart under identical experimental conditions and found differential selective translocation of PKC isoforms in response to those stimuli.

G Protein–Phospholipase C Coupling and PKC

It has been demonstrated that in neonatal cardiomyocytes, mechanical deformation activates the phospholipase C signaling
pathway. In the present study, hypoxia-induced (α, βz, and ζ) and angiotensin II–induced (α, βz, γ, and ζ) translocation of PKC isoforms in adult guinea pig hearts was blocked by D609, a phospholipase C inhibitor. These findings suggest that hypoxia and angiotensin II activate PKC isoforms through a pathway involving phospholipase C. Whereas D609 is reported to be more selective for the phosphatidylcholine-specific phospholipase C compared with the phosphatidylinositol-specific phospholipase C in an in vitro assay system, it has been found that D609 inhibits agonist- or stretch-dependent activation of phosphatidylinositol-specific phospholipase C in vivo in neonatal rat ventricular myocytes. Genistein, a tyrosine kinase inhibitor, blocked angiotensin II–induced translocation of PKCβ1 and hypoxia-induced translocation of PKCβ1 and γ. However, both D609 and genestein failed to block H2O2-induced translocation of any PKC isoform. These results indicate that H2O2 may activate PKC isoforms directly or through a pathway that does not involve Gα-phospholipase C coupling and tyrosine kinase. Taken together, the signaling pathways leading to activation of various PKC isoforms in response to distinct stimuli are differentially regulated in the adult heart.

Ischemic Preconditioning and PKC
Although still controversial, evidence has implicated PKC in ischemic preconditioning. In cultured cardiomyocytes, hypoxic preconditioning activates PKCε and -δ isoforms. In the isolated rat heart, Mitchell et al have reported that ischemic preconditioning induces translocation of PKCε and -δ isoforms using immunohistochemistry. Ping et al have shown in the conscious rabbit that ischemic preconditioning causes selective translocation of PKC isoforms ε and η. Although PKCε was not activated by either ischemia or hypoxia in the present study, Qiu et al have recently demonstrated that the PKCε translocation may be responsible for the protective mechanism of late preconditioning against myocardial stunning in the rabbit heart. A possible reason for the differences in PKC isoform translocation among available studies compared with our data are the fact that ischemia was followed by reperfusion, whereas the current study examined ischemia alone. The oxidative stress and/or calcium overload associated with reperfusion could cause PKC isoform translocation during the reflow phase. Differences in species may also account for the discrepant findings in those studies. Taken together, the results caution against broad generalizations regarding PKC isoform translocation that do not take into account species and experimental conditions.

Oxidative Stress and PKC
Recently, Konishi et al have reported that H2O2 activates PKC in vitro by tyrosine phosphorylation. COS-7 cells were transfected with the expression plasmids of PKC isoforms and treated with H2O2. However, H2O2-induced alterations in subcellular PKC distribution has not been examined in either neonatal cardiomyocytes or the intact adult heart, and signal transduction pathways leading to PKC activation by H2O2 are still unclear. We showed in the present study that H2O2 produced direct and selective translocation of PKC isoforms α, βz, and ζ associated with significant decreases in left ventricular systolic and developed pressure in the adult guinea pig heart. This translocation appears to be independent of both phospholipase C and tyrosine kinase signal transduction. PKC isoform activation may contribute in part to oxidative-stress-induced contractile depression by phosphorylation of myofilament proteins and resultant decreased calcium sensitivity.

It has been reported that there is a dose-response relation in extracellular signal-regulated kinase (ERK1 and ERK2) activation by H2O2 in the range of 10 μmol/L to 1 mmol/L. In the study by Konishi et al, the cells were exposed to a 5 mmol/L concentration of H2O2. Clerk et al have recently reported the activation of p38-mitogen-activated protein kinase (MAPK) and c-jun N-terminal kinase (JNK) in the adult rat heart perfused by 200 μmol/L to 1 mmol/L of H2O2. The hemodynamic data from our laboratory and another have shown that the left ventricular dysfunction caused by 180 μmol/L of H2O2 is transient. These lines of evidence indicate that doses of H2O2 (100 and 180 μmol/L) that we used in the present study do not produce nonspecific toxicity.

Angiotensin II and PKC
Translocation of the PKCζ isoform was observed after angiotensin II in the present study. PKCζ represents an atypical PKC isoform, which lacks the C2 domain and consequently does not bind to calcium and cannot be activated by diacylglycerol. However, Liao et al have clearly shown that angiotensin II activates PKCζ in vascular smooth muscle cells. The mechanism of PKCζ regulation remains unclear. Phosphatidylinositol 3-kinase may regulate PKCζ by generation of an activating molecule such as phosphatidylinositol triphosphate and/or by acting as a linker protein to bring PKCζ in contact with other activating molecules. It has been reported that phosphatidylinositol-3,4,5-triphosphate and phosphatidylserine selectively activate the PKCζ isoform. These lines of evidence suggest the presence of multiple mechanisms for activation of PKCζ.

Angiotensin II has a positive inotropic effect in some, but not all, isolated heart preparations. In isolated guinea pig heart, it has been reported that this species lacked an inotropic response to angiotensin II, although there is a dose-dependent increase in inositol phosphate production in response to angiotensin II, and this response was blocked by a selective angiotensin II antagonist. In the intact organism, a positive inotropic effect has been difficult to demonstrate. We have reported a lack of a measurable positive inotropic effect of angiotensin II in the conscious baboon heart. Thus, it is possible that experimental preparation and species explain the lack of a positive inotropic effect in the present study.

Implications of Selective PKC Isoform Activation
One of the consequences of PKC activation is the activation of transcription. Responses of selective PKC isoforms to distinct stimuli in the present study thereby initiate a phosphorylation cascade and may lead to changes in gene expression characteristics of the cardiac hypertrophic response. Postnatal cardiac-specific overexpression of the PKCβ1 isoform in transgenic mice causes left ventricular hypertrophy, cardiomyocyte necrosis, multifocal fibrosis, and decreased in vivo left ventricular performance. It has been also shown that
transgenic cardiac-specific Goq overexpression results in PKCε activation, cardiac hypertrophy, and decreased cardiac function. Taken together, these findings strongly implicated overactivity of the phospholipase C-PKC signaling pathway in the pathogenesis of cardiac hypertrophy and failure, and PKC isoforms play different functional roles in this process.

PKC has been implicated in the modulation of cardiac contractile performance through phosphorylation of its substrate as well as in the control of cardiomyocyte hypertrophy. PKC isoforms expressed in rat cardiomyocytes have displayed distinct substrate specificities in phosphorylating troponin I and troponin T subunits. In in vivo mouse heart, we have reported that the PKCβ1 isoform phosphorylates troponin I with a resultant decrease in myofilament sensitivity to calcium that may cause depressed cardiomyocyte function. In the present study, although the PKCβ1 isoform was activated, maximal dP/dt was not significantly decreased after angiotensin II. Stimulation of the angiotensin II receptor results in not only PKC activation but also an increase in inositol triphosphate levels. The inositol triphosphate may cause an increase in intracellular calcium by binding to the inositol triphosphate receptor on the sarcoplasmic reticulum membrane. This may mask the negative inotropic effect mediated by phosphorylation of troponin I.

We have reported that depressed cardiomyocyte function of the PKCβ1-overexpressing mouse improves and approaches normal by a superfusion of a highly selective inhibitor of the PKCβ isoform. The morphological and functional changes observed in PKCβ1 transgenic mouse are also prevented or reversed by chronic administration of a PKCβ inhibitor. Therefore, it is important to clarify specific responses of PKC isoforms to distinct pathological stimuli, because they may be potential targets for therapeutic interventions. The fact that both a phospholipase C inhibitor and a tyrosine kinase inhibitor failed to block H2O2-induced PKC activation in the present study also supports the important therapeutic implication of direct PKC isoform–specific inhibition.

Conclusion
PKC isoform activation, which may selectively contribute to cardiac hypertropy, contractile function, and tolerance to ischemia, was differentially regulated by pathological stimuli such as angiotensin II, hypoxia, ischemia, and oxidative stress using H2O2. Hypoxia-induced activation of PKC isoforms was mediated through pathways involving phospholipase C and tyrosine kinase, but H2O2 may activate PKC isoforms independent of phospholipase C and tyrosine kinase signal transduction. Because oxidative stress activates PKC isoforms directly or through an unknown pathway, and PKC isoform activation appears to be involved in the structural and functional changes observed in human heart failure, selective inhibition of PKC isoforms may provide a novel therapeutic strategy for the prevention and treatment of this pathological process.

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References


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