Protein Kinase C and Myocardial Biology and Function

Keiko Naruse, George L. King

Activation of protein kinase C (PKC) and its various isoforms has been postulated to have multiple cardiovascular functions, including vascular permeability, cell migration and growth, extracellular matrix production, and expression of various cytokines. The ability of PKC to regulate many cardiovascular functions is not surprising, because PKC, a family of serine-threonine kinases, is an intracellular signal for many cardiovasotropic growth factors, such as angiotensin, endothelin, and vascular endothelial growth and permeability factor. In addition, PKC activation can indirectly modulate other signal pathways, such as the Raf-MEK1–MAP kinase and PI3 kinase–Akt cascades.

The physiological importance of PKC can also be surmised by the existence of multiple isoforms, of which 12 members have been documented to date. These are usually arranged according to their structure and substrate requirements into the following groups: conventional PKCs (cPKCs) (α, β1/2, and γ), which are Ca²⁺ dependent and activated by binding to diacylglycerol (DAG) and phosphatidylserine (PS); novel PKCs (nPKCs) (δ, ε, η, and θ), which are Ca²⁺ independent but are activated by DAG and PS; and atypical PKCs (aPKCs) (ζ and ηα), which are Ca²⁺ and DAG dependent but are PS sensitive. The distribution of the various PKC isoforms is tissue and species dependent. In the heart, PKC isoforms α, β1/2, δ, ε, and ζ have been identified in rat neonatal cardiomyocytes. In adult rat cardiomyocytes and myocardium, PKC isoforms δ and ε seem to be maintained with age, whereas other PKC isoforms may decline. In human myocardium, PKC isoforms α, β1/2, δ, and ε have also been reported. Similarly, all PKC isoforms with the exception of PKCγ have been identified in the microvessels and macrovessels.

That the various PKC isoforms have specific cellular or cardiovascular functions is suggested by their specific intracellular locations and apparent preferential activation in response to hormonal or biological stimuli. For example, PKCβ2 is associated with fibrillar structures in unstimulated rat cardiomyocytes and translocates to the perinuclear and plasma membranes on activation. PKCβ1 translocates from the cytosol and perinuclear regions into the nucleus when activated. In contrast, PKCδ and PKCe are reported to localize to the perinucleus and nucleus at basal state and translocate to the fibrillar cytoskeletal and cross-striated structures when activated.

Changes in specific PKC isoforms located in the myocardium have also been reported, particularly in ischemic preconditioning, ischemia-reperfusion, heart failure due to cardiomyopathy, and diabetes. The exact PKC isoforms that are preferentially activated in these conditions have been difficult to determine. To date, PKCe and PKCβ are believed to be important for ischemic preconditioning, and PKCe and PKCβ1/2 are activated in heart failure associated with diabetes or nonviral cardiomyopathy. Difficulties in determining the involvement of specific PKC isoforms exist, because PKC activation, as measured by immunoblot analysis to assess translocation, provides only indirect evidence of activation and often does not reflect the extent of activation quantitatively.

To determine the specific biological effects of each PKC isoform, several laboratories have used transgenic animals that overexpress or have one PKC isoform deleted in a general or tissue-specific manner (Table). PKCβ-null mice exhibited mild immunological dysfunctions, whereas PKCγ-null mice showed neurological deficit with regard to neuropathic pain.

Another approach to specifically inhibit or activate a particular PKC isoform has been achieved by manipulating the interaction of the PKC isoforms with their specific anchoring proteins, termed receptors for the activated C kinases (RACKs). Much of the information on the RACK protein has been reported by Mochly-Rosen and coworkers. RACKs are 30- to 36 000-Da proteins that are postulated to bind and translocate each PKC isoform. Specific peptide fragments of PKCβ or PKCe introduced into cardiomyocytes have been reported to either activate or inhibit each respective PKC isoform specifically. Furthermore, PKC peptides derived from PKC RACK-binding or pseudo-RACK sites in cardiomyocytes have been reported to either enhance or abolish ischemic preconditioning, depending on their design.

Dorn et al previously reported that overexpression of ψeRACK, an analogue of the anchoring and activation protein for PKCe, induced translocation of PKCe in the myocardium. In ψeRACK-overexpressing mice, they showed that PKCe was activated by 20% and the heart was resistant to ischemic injury. In this issue of Circulation Research, Mochly-Rosen et al, using an opposite approach, studied the role of PKCe in the heart by inhibiting endogenous PKCe translocation and function by overexpressing an inhibitor of PKCe RACK-binding site (eV1), specifically in the myocardium. They reported that the amount of PKCe in the cardiac particulate fraction decreased by 15% in eV1-overexpressing mice. Their results showed that inhibition of cardiomyocyte PKCe by eV1 induced expression of α-skeletal actin mRNA,
increased cardiomyocyte cell size, modestly impaired left ventricular fractional shortening, decreased posterior wall thickness, and, at high levels, caused lethal dilated cardiomyopathy. In contrast, activation of PKC\(\varepsilon\) by deRACK was associated with increased \(\beta\)-myosin heavy chain expression, decreased myocyte cell size, increased posterior wall thickness, and normal left ventricular function. These results provide strong evidence that PKC\(\varepsilon\) signaling is important for normal postnatal maturation of myocardial development and ischemic preconditioning. In addition, the results suggest the potential for activation of PKC\(\varepsilon\) as a therapeutic agent for improving cardiac growth and survival after ischemic insult.

The role of PKC\(\beta\) activation has also been defined by overexpressing the PKC\(\beta_2\) isoform, specifically in the myocardium of mice. Wakasaki et al\(^{23}\) reported myocyte hypertrophy, myocardial necrosis, ventricular thickening, calcification, impaired ventricular systolic performance, and increased expression of atrial natriuretic factor, transforming growth factor-\(\beta\), collagen types IV and VI, c-fos, and myosin heavy chain-\(\beta\) in PKC\(\beta_2\)-overexpressing mice. Bowman et al\(^{24}\) have also shown that in an inducible model of PKC\(\beta\) overexpression in the myocardium, adult mice developed ventricular hypertrophy and impaired diastolic relaxation, whereas changes in Ca\(^{2+}\) flux and sudden death were noted in neonatal mice. A specific inhibitor of PKC\(\beta\), LY333531, prevented cardiac pathologies in the PKC\(\beta\)-overexpressing transgenic mice and many other vascular changes in diabetic animals.\(^{23,26}\)

The results of the transgenic animal studies have provided clear and definitive evidence that PKC\(\varepsilon\) has important effects on cardiomyocyte growth and can facilitate the protective responses of ischemic preconditioning.\(^{13}\) In contrast, PKC\(\beta_2\) activation may not be as beneficial, with decreases in cardiac contractility and increases in fibrosis reported. However, many questions remain about the role of other PKC isoforms, such as PKC\(\delta\), which is also activated in vivo by diabetes and ischemia.\(^{27,28}\) In addition, it is critical to decipher the mechanism by which different PKC isoforms mediate their specific actions in the myocardium and other vascular tissues. For the activation of PKC\(\beta_{1/2}\) and PKC\(\varepsilon\), specificity can be conferred partially by the stimuli. The stimuli that can increase Ca\(^{2+}\) flux will preferentially activate PKC\(\beta_{1/2}\). Interestingly, stress-related factors such as ischemia, oxidants, and UV irradiation seem to activate PKC\(\varepsilon\) and stress-related c-Jun NH\(_2\)-terminal protein kinase and p38 mitogen-activated protein kinase in parallel.\(^{29-31}\) However, evidence also indicates that both PKC\(\beta\) and PKC\(\varepsilon\) can be activated by growth factors, such as epidermal growth factor, and can activate ERK\(_{1/2}\) mitogen-activated protein kinases.\(^{32}\) Thus, it is likely that PKC isoforms have overlapping effects but mediate some of their specific effects via different signaling pathways, which will need to be elucidated to understand important processes such as ischemic preconditioning, myocardial contractility, and growth.

From these studies, it is clear that it is no longer adequate to correlate total PKC activity changes with biological or functional changes in the myocardium. It is exciting that molecular approaches have identified functional significance of several PKC isoforms that could be either beneficial or detrimental to cardiac functions. Thus, it should be possible to design specific activators or inhibitors of various PKC isoforms as therapeutic agents to improve cardiac function and potentially decrease myocardial damage from ischemia.

References

2. Koya D, Jirosek MR, Lin Y-W, Ishi H, Kuboki K, King GL. Characteristics of protein kinase C \(\beta\) isoform activation on the gene expression of transforming growth factor \(\beta\), extracellular matrix components and pro-

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**Summary of Transgenic Mice Either Overexpressing or Removing a PKC Isoform**

<table>
<thead>
<tr>
<th>Knockout</th>
<th>Tissue</th>
<th>Authors</th>
<th>Features</th>
</tr>
</thead>
<tbody>
<tr>
<td>PKC(\beta_1 + \beta_2)</td>
<td>Whole body</td>
<td>Leitges et al(^{15})</td>
<td>Impaired humoral immune responses, reduced cellular responses of B cells</td>
</tr>
<tr>
<td>PKC(\varepsilon)</td>
<td>Whole body</td>
<td>Abeliovich et al(^{23})</td>
<td>Mild deficits in spatial and contextual learning, impaired synapse elimination during cerebellar development, altered function of GABA receptors</td>
</tr>
<tr>
<td>PKC(\delta)</td>
<td>Whole body</td>
<td>Harris et al(^{15})</td>
<td>Supersensitivity of GABA receptors to ethanol and allosteric modulators</td>
</tr>
<tr>
<td>Transgenic</td>
<td>Heart</td>
<td>Wakasaki et al(^{23})</td>
<td>Left ventricular hypertrophy, multifocal fibrosis, impaired diastolic relaxation, abnormalities of Ca(^{2+}) flux</td>
</tr>
<tr>
<td>PKC(\beta_2)</td>
<td>Intestine</td>
<td>Murray et al(^{17})</td>
<td>Hyperproliferation, increased sensitivity to colon carcinogenesis</td>
</tr>
<tr>
<td>PKC(\delta)</td>
<td>Epidermis</td>
<td>Reddig et al(^{18})</td>
<td>Resistant to skin tumor promotion by TPA</td>
</tr>
<tr>
<td>PKC(\varepsilon)</td>
<td>Epidermis</td>
<td>Reddig et al(^{19})</td>
<td>Enhanced carcinoma formation</td>
</tr>
<tr>
<td>(\Psi)eRACK (PKC(\varepsilon) agonist octapeptide)</td>
<td>Heart</td>
<td>Dorn et al(^{13})</td>
<td>Decreased myocyte cell size, increased posterior wall thickness, cardioprotection from ischemia</td>
</tr>
<tr>
<td>(\varepsilon)1 (PKC(\varepsilon) pseudosubstrate)</td>
<td>Heart</td>
<td>Mochly-Rosen et al(^{22})</td>
<td>Increased cardiomyocyte cell size, modestly impaired left ventricular fractional shortening, decreased posterior wall thickness, lethal dilated cardiomyopathy at high levels of (\varepsilon)1</td>
</tr>
</tbody>
</table>

GABA indicates \(\gamma\)-aminobutyric acid; TPA, 12-O-tetradecanoylphorbol-13-acetate.


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**Key Words**: protein kinase C • cardiac • growth • contractility • ischemic preconditioning
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*Circ Res.* 2000;86:1104-1106
doi: 10.1161/01.RES.86.11.1104

*Circulation Research* is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circres.ahajournals.org/content/86/11/1104

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