Protein Kinase C and Myocardial Biology and Function

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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.1,2 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 cardioatrophic growth factors, such as angiotensin, endothelin, and vascular endothelial growth and permeability factor.3-5 In addition, PKC activation can indirectly modulate other signal pathways, such as the Raf-MEK1–MAP kinase and PI3 kinase–Akt cascades.6,7

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, β2, 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 ηA), which are Ca2+ and DAG independent but are PS sensitive. The distribution of the various PKC isoforms is tissue and species dependent. In the heart, PKC isoforms α, β1, δ, ε, and ζ have been identified in rat neonatal cardiomyocytes.8 In adult rat cardiomyocytes and myocardium, PKC isoforms δ and ε seem to be maintained with age, whereas other PKC isoforms may decline.9,10 In human myocardium, PKC isoforms α, β1, δ, and ε have also been reported.11 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, PKCβ1 is associated with fibrillar structures in unstimulated rat cardiomyocytes and translocates to the perinuclear and plasma membranes on activation.8 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.11-14 The exact PKC isoforms that are preferentially activated in these conditions have been difficult to determine. To date, PKCe and PKCδ 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 specific manner (Table). PKCδ-null mice exhibited mild immunological dysfunctions, whereas PKCγ-null mice showed neurological deficit with regard to neuropathic pain.15,16

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).17 Much of the information on the RACK protein has been reported by Moehly-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 PKCβ1 or PKCe introduced into cardiomyocytes have been reported to either activate or inhibit each respective PKC isoform specifically.18-21 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 al13 previously reported that overexpression of ψεRACK, an analogue of the anchoring and activation protein for PKCe, induced translocation of PKCe in the myocardium. In ψεRACK-overexpressing mice, they showed that PKCe was activated by 20% and the heart was resistant to ischemic injury. In this issue of Circulation Research, Moehly-Rosen et al,22 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 (εV1), specifically in the myocardium. They reported that the amount of PKCe in the cardiac particulate fraction decreased by 15% in εV1-overexpressing mice. Their results showed that inhibition of cardiomyocyte PKCe by εV1 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 PKCε by deRACK was associated with increased β-myosin heavy chain expression, decreased myocyte cell size, increased posterior wall thickness, and normal left ventricular function. These results provide strong evidence that PKCε signaling is important for normal postnatal maturation of myocardial development and ischemic preconditioning. In addition, the results suggest the potential for activation of PKCε as a therapeutic agent for improving cardiac growth and survival after ischemic insult.

The role of PKCβ activation has also been defined by overexpressing the PKCβ2 isoform, specifically in the myocardium of mice. Wakasaki et al23 reported myocyte hypertrophy, myocardial necrosis, ventricular thickening, calcification, impaired ventricular systolic performance, and increased expression of atrial natriuretic factor, transforming growth factor-β, collagen types IV and VI, c-fos, and myosin heavy chain-β in PKCβ2-overexpressing mice. Bowman et al24 have also shown that in an inducible model of PKCβ overexpression in the myocardium, adult mice developed ventricular hypertrophy and impaired diastolic relaxation, whereas changes in Ca2+ flux and sudden death were noted in neonatal mice. A specific inhibitor of PKCβ, LY333531, prevented cardiac pathologies in the PKCβ2-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 PKCε has important effects on cardiomyocyte growth and can facilitate the protective responses of ischemic preconditioning.13 In contrast, PKCβ2 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 PKCδ, 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 PKCβ2 and PKCε, specificity can be conferred partially by the stimuli. The stimuli that can increase Ca2+ flux will preferentially activate PKCβ2. Interestingly, stress-related factors such as ischemia, oxidants, and UV irradiation seem to activate PKCε and stress-related c-Jun NH2-terminal protein kinase and p38 mitogen-activated protein kinase in parallel.29–31 However, evidence also indicates that both PKCβ and PKCε can be activated by growth factors, such as epidermal growth factor, and can activate ERK1/2 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


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