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. 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 angiotsenin, endothelin, and angiotensin II. PKC isoforms 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 Monchy-Rosen and coworkers. RACKs are 30- to 36-kDa 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 ζRACK, an analogue of the anchoring and activation protein for PKCe, induced translocation of PKCe in the myocardium. In ζRACK-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 (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 PKCe 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 PKCe signaling is important for normal postnatal maturation of myocardial development and ischemic preconditioning. In addition, the results suggest the potential for activation of PKCe 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 al. 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 al. 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, LY335351, prevented cardiac pathologies in the PKCβ-overexpressing transgenic mice and many other vascular changes in diabetic animals.

The results of the transgenic animal studies have provided clear and definitive evidence that PKCe has important effects on cardiomyocyte growth and can facilitate the protective responses of ischemic preconditioning. 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. 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 PKCβ1 and PKCε, specificity can be conferred partially by the stimuli. The stimuli that can increase Ca²⁺ flux will preferentially activate PKCβ1/2. 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. However, evidence also indicates that both PKCβ and PKCe can be activated by growth factors, such as epidermal growth factor, and can activate ERK1/2 mitogen-activated protein kinases. 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


| Summary of Transgenic Mice Either Overexpressing or Removing a PKC Isoform |
|-----------------------------|-----------------|---------------------------------------------------------------|
|                            | Tissue          | Authors                     | Features                                                                 |
| Knockout                    | Whole body      | Leitges et al.15            | Impaired humoral immune responses, reduced cellular responses of B cells   |
| PKCβ2 + β2                 | Whole body      | Abeliovic et al.23          | Mild deficits in spatial and contextual learning, impaired synapse         |
| PKCγ                        | Whole body      | Harris et al.26             | Elimination during cerebellar development, altered function of GABA        |
| PKCe                       | Whole body      | Hodge et al.26              | Supersensitivity of GABA receptors to ethanol and allosteric                |
| Transgenic                  | Heart           | Dorn et al.13               | Decreased myocyte cell size, increased posterior wall thickness,           |
| PKCβ2                      | Intestine       | Murray et al.24             | Enhanced carcinoma formation                                              |
| PKCβ2                      | Epidermis       | Reddig et al.18             | Resistant to skin tumor promotion by TPA                                    |
| PKCe                       | Epidermis       | Reddig et al.29             | Enhanced carcinoma formation                                              |
| εV1 (PKCe pseudosubstrate)  | Heart           | Mochly-Rosen et al.22       | Increased cardiomyocyte cell size, modestly impaired left                  |

GABA indicates γ-aminobutyric acid; TPA, 12-O-tetradecanoylphorbol-13-acetate.


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