Protein kinase C in heart failure: a therapeutic target?

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Heart failure (HF) afflicts about 5 million people and causes 300 000 deaths a year in the United States alone. An integral part of the pathogenesis of HF is cardiac remodelling, and the signalling events that regulate it are subject to intense research. Cardiac remodelling is the sum of responses of the heart to causes of HF, such as ischaemia, myocardial infarction, volume and pressure overload, infection, inflammation, and mechanical injury. These responses, including cardiomyocyte hypertrophy, myocardial fibrosis, and inflammation, involve numerous cellular and structural changes and ultimately result in a progressive decline in cardiac performance. Pharmacological and genetic manipulation of cultured heart cells and animal models of HF and the analysis of cardiac samples from patients with HF are all used to identify the molecular and cellular mechanisms leading to the disease. Protein kinase C (PKC) isozymes, a family of serine-threonine protein kinase enzymes, were found to regulate a number of cardiac responses, including those associated with HF. In this review, we describe the PKC isozymes that play critical roles in specific aspects of cardiac remodelling and dysfunction in HF.

1. Protein kinase C: an introduction

Protein kinase C (PKC) is a group of closely related serine-threonine protein kinases, further classified as (1) the classical PKCs (α, βI, βII, and γ), the diacylglycerol (DAG)-, and calcium-dependent enzymes, (2) the novel PKCs (δ, ε, η, and θ), which require DAG, but not calcium, for activity, and (3) the atypical PKCs (ζ, λ), which are not stimulated by DAG or calcium, but are stimulated by other lipid-derived second messengers.

1.1 Protein kinase C in the normal and diseased myocardium

PKC isozymes are expressed in all tissues. mRNA expression of α, δ, ε, η, and ζPKCs is found in rat cultured cardiomyocytes.1,2 Abundant expression of both βI and βII PKC in human and rat cardiomyocytes has also been reported,3–6 whereas the mouse myocardium expresses low levels of these βPKCs.7 Further species-specific differences in the expression of η, θ, and εPKC were also reported.8 Therefore, the interpretation of animal studies must be done with caution as species to species variation in PKC isozyme expression is substantial. Western blot analyses of human cardiac tissue using polyclonal antibodies (PKC-α, -βI, -βII, -ε, -δ, -γ, and -η) or monoclonal antibodies (PKC-λ, -μ, and -θ) demonstrated the presence of these isozymes in human heart tissue.4 This study also demonstrated differences in the distribution of PKC isozymes between the atria and ventricles. The calcium-dependent isozymes, α, βI, and βII PKC, reside predominantly in the ventricle, whereas δ and ζPKC are mainly expressed in the atria and ε and λPKC are evenly distributed in both atria and ventricles.

PKC isozymes are involved in a variety of chronic cardiac diseases9 as well as in acute cardiac injuries and preconditioning.10 We and others have demonstrated that select PKC isozymes contribute to heart failure (HF).9,11–16 Isozyme-selective tools that were generated in the past few years, including pharmacological peptide regulators (Figure 1) and use of genetic manipulation and RNAi, demonstrated that the same isozyme may mediate different functions in acute vs. chronic heart diseases. For example, εPKC activation prior to MI is protective,17 whereas in hypertension-induced HF εPKC activation is detrimental.15 Here we review the role of PKC isozymes in cardiac remodelling and HF.

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2. Heart failure: an introduction

HF is a clinical syndrome characterized by impaired ability of the left ventricle to fill or eject blood. Currently, the life-time risk to develop HF after the age of 40 is ~20% for men or women. The aetiology of HF is diverse and includes ischaemia, hypertension, idiopathic cardiomyopathy, bacterial endocarditis, congenital cardiovascular defects, and valvular diseases. However, the most common aetiologies of HF are coronary artery disease and myocardial infarction (MI); each year, about one million people suffer from acute MI and within 6 years, HF will disable about 300 000 of these patients.

2.1 Cardiac remodelling

Cardiac remodelling is an early and progressive response of the heart to insults, such as ischaemia, volume and pressure overload, infection, inflammation, mechanical injury, and stimulation by cytokines and enzymes. Depending on the extent of cardiomyocyte loss by these insults, fibroblast proliferation and inflammatory processes, namely fibrosis, is triggered to maintain the shape and structure of the myocardium. Injuries to coronary vasculature, stimulation by stress factors, and fibrosis also result in changes in structure and function of blood vessels. Infiltration of inflammatory cells into the jeopardized myocardium leads to continuous release of cytokines, chemokines, enzymes, and growth factors, which further contribute to the remodelling process. Therefore, a better understanding of the cellular and molecular basis of cardiac remodelling events, including adaptive and maladaptive hypertrophy, perivascular and interstitial fibrosis, and inflammation, will further clarify the pathogenesis of HF.

3. Cardiac hypertrophy: adaptive and maladaptive responses

Adaptive cardiac hypertrophy is characterized by an increase in heart mass and wall thickening due to an increase in cardiac myocyte size and protein synthesis, and is associated with improved cardiac function. If cardiac overload continues, a transition from adaptive to maladaptive hypertrophy takes place. This is associated with left ventricle dilation, a decrease in contractile elements, and reduced cardiac output. The use of culture studies, animal models, and human samples with HF provided insight into the role of PKC in this pathology.

3.1 Protein kinase C isozymes in cardiomyocyte hypertrophy in cell culture models

Neonatal cardiomyocytes are commonly used to study hypertrophic signalling. Whether this model represents developmental hypertrophy only or whether it also provides an appropriate model of pathological hypertrophy is debated. However, because cardiac remodelling activates many of the cardiac embryonic developmental programs, this culture model may provide an insight into HF. A variety of stimulants, such as phorbol myristate acetate (PMA), angiotensin-II (AngII), phenylephrine (PE), and...
| Model                          | Cardiac phenotype | PKC isoyme   | Stimulus/treatment | Main response                                                                 | Reference |
|-------------------------------|-------------------|--------------|--------------------|-------------------------------------------------------------------------------|-----------|
| Streptozotocin-induced diabetic rats | Hypertrophy      | cPKCs        | Cardiac-specific expression of εPKC inhibitor, εV1 activator, εRACK          | Increased cardiac activity and heart failure                                | 86        |
| Transgenic mice               | Hypertrophy      | εPKC         | Over-expression constitutively active εPKC                                  | Concentric cardiac hypertrophy                                             | 36        |
| Transgenic mice               | Hypertrophy      | εPKC         | Active calcineurin over-expression                                           | Increased cardiac activity and heart failure                                | 36        |
| Pressure-overload aortic banding rats | Hypertrophy      | cPKCs and nPKCs | Increased cardiac activity and heart failure                                | Concentric cardiac hypertrophy                                             | 87        |
| Transgenic mice               | Hypertrophy      | βIIPKC and εPKC | Over-expression constitutively active βIPKC                                | Concentric cardiac hypertrophy                                             | 32        |
| Adult rat ventricle myocyte   | Hypertrophy      | εPKC         | Pharmacological: εV1-2 (specific εPKC isoyme inhibitor)                      | Attenuated isoproterenol-induced apoptosis                                | 91        |
| Dahl salt-sensitive hypertensive rats | Hypertrophy      | cPKCs and nPKCs | Perfusion with angiotensin II                                                | Increased cardiac activity and heart failure                                | 14        |
| Pressure-overload heart failure rats | Hypertrophy      | εPKC         | Sustained treatment with ACE inhibitor                                       | Pathological cardiac hypertrophy                                             | 95        |
| βIIPKC transgenic mice        | Hypertrophy/heart failure | βIIPKC       | Cardiac-specific βIIPKC over-expression                                      | Increased contractility and cardioprotection                               | 32, 96    |
| αPKC transgenic mice          | Hypertrophy/heart failure | αPKC         | Wild-type and dominant negative αPKC expression                             | Increased cardiac activity and heart failure                                | 3, 94     |
| Human end-stage dilated or ischaemic cardiomyopathy | Heart failure | cPKCs        | ACE inhibitor attenuates increased αPKC and εPKC translocation               | Pathological cardiac hypertrophy                                             | 32        |
| Human end-stage Dilated cardiomyopathy | Heart failure | cPKCs and nPKCs | Increased cardiac activity and heart failure                                | Pathological cardiac hypertrophy                                             | 32        |
| Dahl Salt hypertensive rats    | Heart failure     | cPKCs and nPKCs | Increased cardiac activity and heart failure                                | Pathological cardiac hypertrophy                                             | 32        |
| MLP transgenic mice           | Heart failure     | εPKC         | Increased cardiac activity and heart failure                                | Pathological cardiac hypertrophy                                             | 32        |
| Dahl Salt hypertensive rats    | Fibrosis/heart failure | εPKC         | Sustained treatment with εV1-2 (specific εPKC inhibitor)                    | Decreased cardiac fibrosis                                                 | 15        |
| βIIPKC transgenic mice        | Hypertrophy/fibrosis | βIIPKC       | Cardiac-specific over-expression of βIIPKC                                | Pathological cardiac hypertrophy and fibrosis                              | 11        |
| Pressure-overload aortic banding mice | Fibrosis | βPKC and εPKC | εPKC knock-out mouse                                                        | Increased fibrosis                                                         | 54        |
| Neonatal rat cardiac fibroblast | Fibroblast proliferation | βPKC and εPKC | TGFβ1 and isozyme-specific inhibitors                                        | Stimulated cardiac fibroblast proliferation                                  | 39        |

*Continued*
endothelin-1 (ET1), are used to induce hypertrophy in culture (Table 1). PMA, which activates both conventional and novel PKC isozymes, or transfection of either wild-type (WT) or dominant-negative (DN) αPKC mutant demonstrated that αPKC is both necessary and sufficient to induce certain features of cardiomyocyte hypertrophy including increases in protein synthesis, the protein-to-DNA ratio, and cell surface area. Further, αPKC antisense treatment reduced PE-induced increases in α-actin mRNA and atrial natriuretic peptide (ANP) secretion, but not PE-induced β-myosin heavy chain, ANP, or B-type natriuretic peptide (BNP) gene expression, therefore causing a loss of only some to the pathological hypertrophic markers. In other studies, overexpression of αPKC increased cell surface area, [3H]-leucine incorporation, and mRNA levels of ANP, together indicating that αPKC activation induced cardiomyocyte hypertrophy in cultured cardiomyocytes. We found that βI and βIIIPKC are required for PMA-induced cardiomyocyte hypertrophy. Later, we substantiated that RBCK-1 (RBCC protein that interacts with βIPIPKC) is essential for PE-induced cardiomyocyte hypertrophy. Overexpression of RBCK1 increased the cardiac myocyte cell surface by 50% in the absence of PE treatment and the βI- and βIIIPKC-specific peptide inhibitors prevented that effect. A role for εPKC has also been suggested; treatment with εPKC antisense reduced myotrophin-induced stimulation of protein synthesis in neonatal myocytes. This study also found that δ and εPKC are not involved in this process. Therefore, at least four PKC isoforms, α, βI, βII, and ε induce hypertrophy in neonatal cardiac myocytes.

3.2 Protein kinase C in cardiac hypertrophy of animal models

αPKC expression and activity were unaltered in early HF but were up-regulated in two distinct rat models of end-stage HF. Depletion of myocardial αPKC by gene knock-out increased myocardial contractility, whereas transgenic overexpression of αPKC led to marked ventricular dysfunction and alterations in Ca$^{2+}$ homeostasis. Phosphorylation studies suggest that αPKC depresses myofilament contractility through phosphorylation of cTnI and/or cTnT. Skinned left-ventricular myocytes isolated from rats subjected to chronic (8–9 months) pressure overload or MI-induced HF supported these conclusions; myofilament function is severely depressed in these experimental HF models. In an earlier study, βPKC levels were elevated in hypertension-induced HF rats. Treatment with angiotensin-receptor blocker improved cardiac function and decreased βPKC activation and levels. In a recent study, we found that a selective inhibition of βIIIPKC improved cardiac function and calcium handling in rats with post-MI HF, and improved function and prolonged the life span of rats with hypertension-induced HF. In addition to reducing mortality, selective and sustained inhibition of βIIIPKC by βIIV5-3 (a selective inhibitor of βIIIPKC) in rats with post-MI HF improved cardiac function compared with that prior to treatment initiation. The beneficial effect was associated with enhanced calcium handling and normalization of the levels and phosphorylation of SERCA2, NCX, and troponin I. Further, the reduction in cardiomyocyte width, HW/BW, and increased fractional shortening following βIIV5-3 treatment in rats with end-stage pathological conditions.
hypertrophy were observed, thereby indicating that βII PKC activation is a critical mediator of cardiac hypertrophy in rats (Figure 2).

Although the basal level of β PKC in the hearts of adult mice is low,13 a number of reports suggest that β PKC is an important isozyme in cardiac diseases in mice, as well.11,32 Targeted over-expression of βII PKC in mice resulted in cardiac hypertrophy with myocardial dysfunction similar to that of HF.11 Over-expression of activated βII PKC in neonatal mice is fatal and, in the case of adult mice, it induces hyper-trophy and myocardial dysfunction.32 Pharmacological inhibition of α and β PKC by Ro-32-0432 improved myocardial contractility and left-ventricular developed pressure in mouse hearts.34 In contrast to these observations, another study using β PKC knockout mice demonstrated no role for β PKC in HF development.35 Hence the role of β PKC in cardiac hypertrophy in mice using genetic manipulation is controversial.

Transgenic mice that express ε or δ PKC activator or inhibitor peptides only postnatally and only in cardiac myocytes (using the α MHC promoter)36 revealed potential redundant roles for these enzymes in cardiac hypertrophy. Mice expressing the ε PKC-selective inhibitor, εV1, developed dilated eccentric cardiomyopathy and HF, an effect associated with a 10% increase in myocyte size when compared with the non-transgenic mice. Transgenic mice expressing the ε PKC-selective activator, εRACK, exhibited normal cardiac function, increased cardiac muscle mass (concentric hypertrophy) (Figure 2), and had no increase in fibrosis. However, upon examination of cardiac myocyte cell size, it was found that myocytes were 10% smaller (P < 0.01). Since the number and size of other cardiac cells remained unchanged, this indicated that the number of cardiomyocytes has increased. As a result of ε PKC activation, hyperplasia, a phenomenon restricted mainly to perinatal cardiac development, may have occurred.36 These data suggest that ε PKC signalling may be part of a compensatory signalling pathway that is pro-proliferative at least during early post-natal development. This conclusion was further supported by work examining a cross between Gαq over-expressing mice, which develop a dilated cardiomyopathy phenotype, and transgenic mice with mild activation of ε PKC. The double-transgenic progeny exhibited a reduction in cardiac hypertrophy and an improvement in cardiac function compared with the Gαq over-expressing mice.37 Therefore, ε PKC appears to be a positive modulator of compensatory cardiac hypertrophy in this mouse model. Because in this model activation of ε PKC and Gαq were induced from day 1 after birth, the phenotype may reflect restoration of ε PKC activity in the Gαq-phenotype.37

![Figure 2](https://academic.oup.com/cardiovascres/article-abstract/82/2/229/277656)

Figure 2 Protein kinase C isozymes are closely involved with different remodelling events in myocardial infarction-induced heart failure. Heart failure progression is noticeably characterized by cardiac remodelling, whereas specific protein kinase C isozymes play a crucial role in this time-related event. Cardiomyocyte death, inflammation, cardiac hypertrophy, and fibrosis are directly regulated by specific protein kinase C isozymes such as α, βII, δ, and ε protein kinase C as depicted in the figure. The TUNEL staining image is from Murriel et al. (2004)33 and the hypertrophy image from www.ipmc.cnrs.fr.
In hypertensive rats, εPKC levels increase during the compensatory stage of cardiac hypertrophy, but εPKC was detrimental in this response. Further activation of εPKC (by a sustained treatment with the PKC-selective activator, ψ2RACK) increased cardiac fibrosis and HF, whereas εPKC inhibition (by sustained treatment with the εPKC-selective inhibitor, εV1-2) prolonged survival, reduced hypertrophy, excessive fibrosis, vascular remodelling, and inflammation and corrected cardiac dysfunction.15,16

Thus studies in mice and rats are not in agreement, suggesting either species differences and/or differences due to the tools that were used (pharmacological vs. genetic manipulation of the animals) as well as the timing of regulation of PKC (i.e. before and/or during the disease course.). A summary of a number of studies using pharmacological and genetic approaches to determine the role of PKC isoforms in hypertrophy and in HF is provided in Table 1.

### 3.3 Protein kinase C in human hypertrophy and heart failure

Although animal studies were inconclusive using genetic manipulations and pharmacological PKC modulators (Table 1), studies characterizing the level and activity of PKC isoforms in human HF3,4 provide insight into which isoforms should be focused on as therapeutic targets. For instance, αPKC was found to be critical in cardiomyocyte hypertrophy by knock-out and over-expression studies in mice.22,28,29 Though activation of PKC is elevated in cardiomyocytes of human HF,4,33 provide insight into which isoforms should be focused on as therapeutic targets.

For example, αPKC was found to be critical in cardiomyocyte hypertrophy by knock-out and over-expression studies in mice. Though activation of PKC is critical in mouse model of HF,6,34 activated αPKC levels were found to be low in samples of patients with end-stage HF when compared with normal subjects.4 Further this study demonstrated that αPKC is activated in the myocardium of patients with aortic stenosis, a condition in which heart functions are not jeopardized. In contrast, both Bowling et al.3 and Simonis et al. also found a significant 70 and 150% increase in activation of βPKC, respectively and immuno-histochemical staining and mRNA labelling indicated that βPKC is elevated in cardiomyocytes of human HF samples.3,4 LY333531, an inhibitor reported initially to be specific for βPKC,38 reduced total PKC activity in membrane fractions of failing hearts by 209 pmol min⁻¹mg⁻¹ suggesting that βPKC constitutes for the majority PKC activity in the failing hearts. Together, these studies indicate that changes in βPKC correlate better with the human HF, suggesting that focusing on this PKC isozyme in considering therapeutic intervention is advisable.

### 4. Cardiac fibrosis

Fibrosis refers to accumulation of fibroblasts due to increased proliferation, migration, and adhesion of fibroblasts to the site of injury and/or leading to the accumulation of extracellular matrix proteins, such as collagen, by augmented release from fibroblasts or reduced degradation of collagen. Replacement fibrosis, interstitial fibrosis, and perivascular fibrosis are different types of myocardial fibrotic processes, which may occur sequentially or simultaneously. However, an excess of any of these processes interferes with myocardial metabolism, particularly the supply of oxygen and removal of cellular metabolic waste, leading to myocardial malfunctioning, and thus posing detrimental effects to failing hearts. Excess fibrosis can also decrease cardiac elasticity and thus affect cardiac contraction. A variety of pathological stressors, such as ischaemia and hypertension, can trigger cardiac fibrosis. The occurrence of cardiac fibrosis requires a series of coordinated molecular and cellular events that alter the properties of the extracellular matrix (ECM) and cardiac fibroblasts. PKC has been shown to regulate the specific events leading to the deposition of collagen.

### 4.1 Protein kinase C isozymes in cultured cardiac fibroblasts

α, β1, βII, δ, ε, and ϵPKC have been found in both neonatal and adult cardiac fibroblasts and the non-selective PKC activator, PMA, inhibits basal and TGFβ-induced thymidine incorporation in these rat fibroblasts.39 Using isozyme-selective inhibitors, we found that δPKC and ϵPKC have opposing roles in TGFβ-induced fibroblast proliferation, whereas other PKC isoforms have no role in this process.39 We showed that selective inhibition of δPKC blocked TGFβ1-induced cardiac fibroblast proliferation. In contrast, the ϵPKC-selective peptide inhibitor, ϵVI-1, had an opposite effect to that of the δPKC inhibitor; it increased TGFβ1-induced proliferation. Therefore, δ and ϵPKC act downstream of TGFβ1, yielding opposing roles in fibroblast proliferation.

PKC regulates the levels and activity of matrix metalloproteinases (MMP), a family of zinc-containing proteases, that degrade ECM and facilitate the motility of cardiac fibroblasts.40–44 For example, α and βPKC increase the activity of MMP-9, but not MMP-2, primarily through the JNK-dependent pathway.44 Other PKCs, such as 3 and εPKC, increase both MMP-2 and MMP-9 via ERK and NFκB pathways in adult rat cardiac fibroblasts.44 Ang II binds to angiotensin-type 1 receptor and activates PKC which ultimately leads to fibroblast proliferation.46–48 The pro-fibrotic effects of other profibrotic stimuli, such as ET-1, were also attenuated by inhibition of PKC with either chelerythrine or staurosporine in neonatal cardiac fibroblasts.49

A critical role for εPKC in regulating fibroblast adhesion and migration has also been reported; the effect of Ang II treatment, which induces adhesion and migration in cardiac fibroblasts, was blocked by PKC inhibition, and was abolished in cardiac myofibroblasts obtained from εPKC knockout mice.48 Additional mechanistic studies demonstrated that εPKC forms a tight complex with β1-integrin to regulate the interaction between the cell and ECM.50–52 These findings corroborate a role for εPKC in mediating cardiac fibroblast adhesion and migration.

### 4.2 Protein kinase C in cardiac fibrosis, in vivo

A role for PKC in cardiac fibrosis has also been suggested by in vivo studies using animal models of HF. Inhibition of classical PKCs, namely, α and βPKC, by ruboxistaurin attenuated pathological fibrosis and improved cardiac function following MI in rats, suggesting a role for classical PKCs in fibrogenesis in the heart.12 In a recent study, selective βIIIPKC inhibition with B1IVS-3 attenuated collagen deposition in the remote region of the myocardium of post-MI HF rats13 (Figure 2). Hearts from εPKC knock-out mice demonstrated elevated interstitial fibrosis when subjected to pressure overload by transverse aortic constriction.54 In contrast,
sustained inhibition of εPKC by its isozyme-selective peptide inhibitor, εV1-2, suppressed cardiac fibrosis and ameliorated cardiac function, in part by inhibiting MMP-2 activity, in a rat model of hypertension-induced HF. Moreover, εPKC, an activator of εPKC, augmented the fibrotic process and accelerated mortality in these hypertensive animals. Further, in a rat or mouse cardiac transplantation model, we found that inhibition of εPKC by εV1-2 also blocked parenchymal fibrosis and the increase in TGF-β1 in the grafted heart, corroborating a role for εPKC in cardiac fibrosis in vivo. (Figure 2). The contradicting findings using εPKC knock-out mice and pharmacological regulation of εPKC in rats suggest that regulation by PKC isozymes may differ according to the aetiology of fibrosis, the species, and/or the extent of activation of compensatory mechanisms (i.e. εPKC null mice have 60% increase in δPKC activity, which may have compensated for εPKC, whereas there is no change in the levels or activity of any PKC isozyme, other than εPKC, when using the εPKC-selective inhibitor). (These and other studies are also listed in Table 1.). Understanding the exquisite control of cardiac fibrosis by PKC could potentially translate to novel effective treatments for cardiac dysfunction and HF.

5. Cardiac inflammation

Irrespective of aetiology, myocardial inflammation is an integral part of HF (i.e. inflammation is found in the myocardium following ischaemia, cardiac infection, autoimmune response, pressure and volume overload, etc.). Though inflammation is often secondary to the trigger for specific cardiac disease, inflammatory cells are a constant source for cytokines, enzymes, and growth factors, which regulate remodelling events such as hypertrophy, fibrosis, and vascularization.

5.1 Protein kinase C in cardiac inflammation

Numerous in vitro studies point out the role of PKC isozymes in pro-inflammatory mediator production (transcription and translation) and release. One aspect of cardiac inflammation that is better described is the induction of cell damage by pro-inflammatory cytokines in cardiac diseases or cardiac cells. For example, TNF-α induced apoptosis in coronary vascular endothelial cells seems to be a PKC-mediated event. Sustained inhibition of εPKC by εV1-2 decreased the infiltration of inflammatory cells into the myocardium in the hearts of hypertensive rats with HF. In an Ml-induced HF rat model, βIIIP5-3, the specific βIIIPK inhibiting, also attenuated infiltration of inflammatory cells (Figure 2). Both of these PKC inhibitors attenuated degranulation of mast cells (MCs), the important inflammatory cells that are involved in HF progression (Figure 2). Likewise, TNF-α production in macrophages is blocked by PKC inhibition with bisindolylmaleimide, a pan PKC inhibitor, or by Go-6976, a classical PKC inhibitor. Further studies on the role of PKC isozymes in the function of important inflammatory cells such as macrophages, T-cells, MCs, and neutrophils in HF progression are needed, because these cells are an integral part of cardiac remodelling and HF.

6. Downstream targets of protein kinase C in cardiac remodelling

As discussed earlier, PKC isozymes regulate fibrosis, inflammation, and cardiac muscle dysfunction and a number of downstream mediators of PKC effects have been identified. Stimulation of primary cultures of adult feline cardiomyocytes with ET-1, PE, PMA, or insulin resulted in phosphorylation and activation of several pro-survival kinases, including mTOR (aka mammalian Target of Rapamycin; at S2448) and S6K (at T389/4S21/T424) via PKC activation. Expression of DN-εPKC abolished ET-1-stimulated mTOR and S6K phosphorylation, but not insulin-stimulated S6K phosphorylation. ET-1- and insulin-stimulated mTOR and S6K phosphorylation in cardiomyocytes was inhibited by expression of DN-δPKC or pre-treatment with rottlerin, a δPKC inhibitor. However, treatment with Go6976, a specific classical PKC (cPKC) inhibitor did not affect mTOR/S6K1 activation. This study demonstrates that ε and δPKC activate mTOR and S6 kinase and subsequently lead to cardiomyocyte hypertrophy in cultured feline cardiomyocytes. Another important kinase that has been implicated in hypertrophic signalling is glycogen synthase kinase (GSK). Up-regulation of GSK-3α suppresses cardiac growth and pressure-overload-induced cardiac hypertrophy in mice. Knock-down of GSK-3α increased the phosphorylation of ERK (extracellular signal-regulated kinase), an effect that was inhibited by pharmacological inhibitors of ε and δPKC and MEK (mitogen-activated protein kinase kinase), suggesting that GSK-3α inhibits ERK through PKC-MEK-dependent mechanisms and further regulates cardiac hypertrophy. In another study, involvement of PKC in TGF-β1-induced cardiac hypertrophic responses by activating TAK1 (another member of the mitogen-activated kinase kinase kinase family) and ultimately activating transcription factor (ATF). The PKC inhibitors, Go6976 and GF109203X, blocked TGF-β1-induced TAK1 kinase activity and subsequent downstream signalling pathways including ATF-2 phosphorylation, leading to suppression of ATF-2 transcriptional activity. The transcription factor GATA-4 plays a key role in ANF promoter activation in response to pro-hypertrophic Ang II through PKC activation and ultimately resulting in enhanced DNA binding activity. Inhibition of PKC prevents nuclear export of histone deacetylase 5 (HDAC5, a protein regulating myogenesis) in response to hypertrophic agonists. Moreover, a mutation in HDAC5 is refractory to PKC activation. Protein kinase D (PKD), another downstream effector of PKC, directly phosphorylates HDAC5 and stimulates its nuclear export. The stretch-induced increase in cardiac hypertrophy is blocked by inhibition of the small G protein, Rho, or by overexpression of dominant negative α and δPKC, suggesting that α and δPKC are both required for stretch-induced hypertrophy, through Rho GTPase-mediated signalling pathways. Also, phosphorylation of MEK1/ERK1/2 and the MEK kinase, MKK4, and jun kinase, JNK, was inhibited by over-expression of dominant negative α and δPKC.

Myocyte dysfunction in αPKC transgenic mice was caused by alterations in Ca2+ homeostasis. As discussed, in mice, αPKC depresses myofilament contractility through site-specific phosphorylation of cTnT at the threonine-206 residue in cardiomyocytes. On the other hand, a decreased myofilament responsiveness to Ca2+ was seen in the...
myocardium of βIIIPKC overexpressing transgenic mice as well as a significant increase in the degree of phosphorylation of troponin I. The depressed cardiomyocyte function improved after the sequential superfusion of LY333531, a βPKC inhibitor. This study shows that βIIIPKC-mediated phosphorylation of troponin I in vivo may decrease myofilament Ca\(^{2+}\) responsiveness, and thus causes cardiomyocyte dysfunction.\(^{73}\)

Other protein targets of PKC are those involved in ECM regulation. αPKC regulates β1-integrin complex formation with ECM and further participates in the fibrotic events.\(^{50–52}\) α, β1, ε, θ, and δPKC isozymes activate different MMPs to degrade ECM, thereby facilitating the motility of cardiac fibroblasts\(^{44}\) and inflammatory cells. In inflammatory cells, PKC activation enhances the transcription of cytokine production through phosphorylation of the inhibitor of transcription factor NFκB, IκB.\(^{74}\) A summary of these and other downstream targets of PKC activation leading to HF is given in Figure 3.

7. Summary and conclusion: should protein kinase C be a target for heart failure treatment?

Preventing maladaptive cardiac remodelling is a goal of therapy for HF.\(^{75}\) In this review, we provide evidence that select PKC isozymes play different roles in many aspects of cardiac remodelling in HF (see Table 1 and Figures 2 and 3). Because modulators of PKC isozymes are already in clinical trials for a variety of indications,\(^{76–84}\) it may now be possible to consider using such PKC isozyme regulators to treat human HF. Although PKC isozymes are present in many tissues, recent clinical trials suggest that systemic delivery of inhibitors and activators of PKC isozymes is well tolerated (see studies with the βPKC small molecule inhibitor,\(^{77,78}\) with the peptide inhibitor of δPKC.\(^{76}\) Further, new developments in drug delivery suggest that organ-selective delivery may also be possible in the future, for example by delivering slow drug releasing particles to the organ of interest.\(^{85}\) Therefore, clinical trials with PKC inhibitors to address HF should be considered using either systemic or cardiac-specific drug delivery. Further in vivo studies using animal models, including larger animals like dogs, sheep, and pigs, will help guide the identification of the right PKC isozymes that should serve as therapeutic targets for the treatment of pathological cardiac remodelling and HF in humans.

Conflict of interest: D.M.-R. is a founder, stock option holder, paid consultant, chair of the scientific advisory board, and member of the board of directors of KAI Pharmaceuticals, a company whose goal is to bring peptide regulators of PKC to the clinic. However, none of the research proposed here and none of the detail of the work done in my laboratory at Stanford is disclosed to the company before it is disclosed publicly elsewhere. D.M.-R. has provided reports on her relationship with KAI to Stanford University (last report was provided in April 2008). S.S.P., L.S., and J.C.B.F. have no conflict of interest to disclose.

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PKC in heart failure

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