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Review

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 a subject of 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, θ, and η), 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 βIIPKC 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 βIIPKC, 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 diseases[9] 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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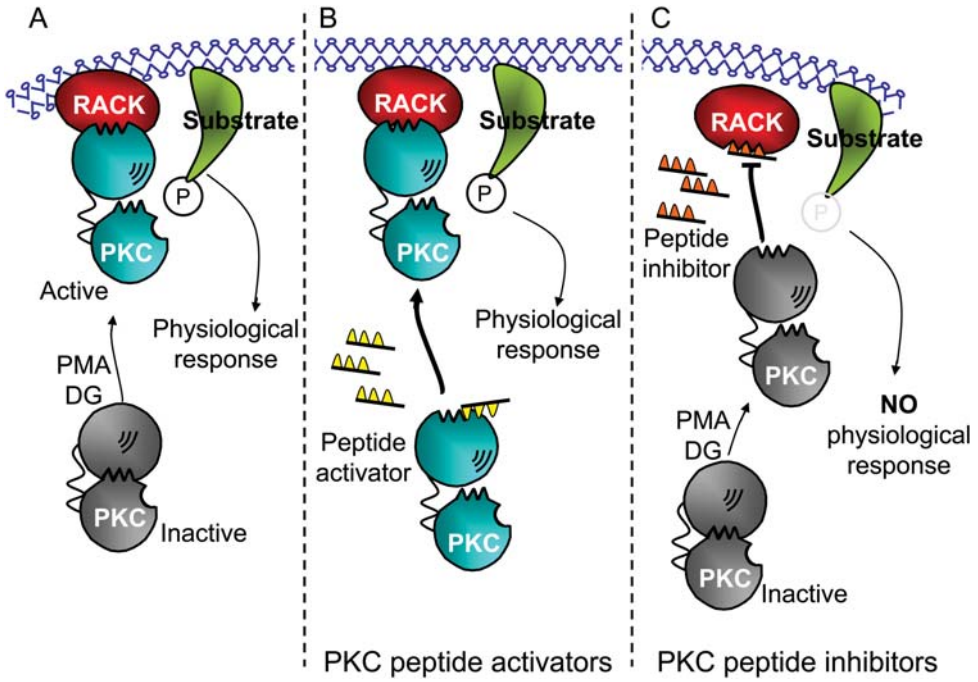

Figure 1 Protein kinase C peptide modulators. (*A*) Inactive protein kinase C (gray) undergoes a conformational change exposing both the RACK-binding site and the active site when diacylglycerol (DG) or PMA are elevated. Active protein kinase C (blue) binds to its RACK (red), anchoring the activated isozyme near its substrate (green). Phosphorylation (P) of that substrate leads to the physiological responses of that isozyme. (*B*) Alternatively, a peptide that mimics the RACK-binding site, pseudo-RACK (ΨRACK, yellow) can also cause these conformational changes. ΨRACK binds to protein kinase C with a lower affinity than the intact RACK and thus does not always occupy the RACK-binding site on the enzyme. During the time that the peptide is not bound, the activated enzyme may bind to its RACK (red), resulting in anchoring of the activated isozyme near its substrate (green) followed by substrate phosphorylation (P) and physiological responses. This process is isozyme-specific. (*C*) A peptide corresponding to the RACK-biding site on protein kinase C (orange) inhibits translocation and function of its corresponding isozyme. The translocation inhibitor peptide binds to the RACK and blocks binding of the activated isozyme to that RACK. Therefore, the physiological responses mediated by that isozyme are blocked.

2. Heart failure: an introduction

HF is a clinical syndrome characterized by impaired ability of the left ventricle to fill or eject blood.[18] Currently, the life-time risk to develop HF after the age of 40 is ~20% for men or women.[19] 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);[20] each year, about one million people suffer from acute MI and within 6 years, HF will disable about 300 000 of these patients.[19]

2.1 Cardiac remodelling

Cardiac remodeling 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 collagen secretion, 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.[21] 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.[21] 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

Table 1 The role of individual protein kinase C isozymes in cardiac remodelling and heart failure

Model	Cardiac phenotype	PKC isozyme	Stimulus/treatment	Main response	Reference
Streptozotocin-induced diabetic rats	Hypertrophy	cPKCs		Increased cardiac ßIIPKC activity	86
Transgenic mice	Hypertrophy	εPKC	Cardiac-specific expression of εPKC inhibitor, εV1	Lethal dilated cardiomyopathy	36
Transgenic mice	Hypertrophy	εPKC	Cardiac-specific expression of εPKC activator, ψεRACK	Concentric cardiac hypertrophy	36
Transgenic mice	Hypertrophy	εPKC	Over-expression constitutively active εPKC	Concentric cardiac hypertrophy	87
Transgenic mice	Hypertrophy	cPKCs and nPKCs	Active calcineurin over-expression	Increased cardiac α and βPKC translocation	88
Pressure-overload aortic banding rats	Hypertrophy	cPKCs and nPKCs		Increased cardiac βIPKC, βIIPKC, εPKC, and θPKC translocation	89
Transgenic mice	Hypertrophy	βIIPKC and εPKC	Over-expression constitutively active εPKC	Pathological cardiac hypertrophy	90
Adult rat ventricle myocyte	Hypertrophy	εPKC	Pharmacological: εV1-2 (specific εPKC isozyme inhibitor)	Attenuated isoproterenol-induced apoptosis	91
Dahl salt-sensitive hypertensive rats	Hypertrophy	cPKCs and nPKCs		Increased cardiac εPKC levels in compensatory stage and βIIPKC levels during cardiac dysfunction	14
Pressure-overload aortic banding rats	Hypertrophy	αPKC and δPKC		Increased cardiac levels of αPKC and δPKC	92
Adult guinea pig heart (*ex vivo*)	Hypertrophy	cPKCs and nPKCs	Perfusion with angiotensin II	Increased cardiac αPKC, βIIPKC, and γPKC	93
Dahl Salt hypertensive rats	Hypertrophy/heart failure	cPKCs, nPKCs, and aPKCs		Increased cardiac levels of cPKC, nPKC, and aPKC	94
Pressure-overload heart failure rats	Hypertrophy/heart failure	αPKC and εPKC	Sustained treatment with ACE inhibitor	ACE inhibitor attenuates increased αPKC and εPKC translocation	95
βIIPKC transgenic mice	Hypertrophy/heart Failure	βIIPKC	Cardiac-specific βIIPKC over-expression	Pathological cardiac hypertrophy	32
αPKC transgenic mice	Hypertrophy/heart failure	αPKC	Wild-type and dominant negative αPKC expression	Increased contractility and cardioprotection	29,96
Human end-stage dilated or ischaemic cardiomyopathy	Heart failure	cPKCs		Increased cardiac βPKC activity	3
Human end-stage Dilated cardiomyopathy	Heart failure	cPKCs and nPKCs		Increased cardiac βIIPKC levels	4
Dahl Salt hypertensive rats	Heart failure	cPKCs and nPKCs		Increased cardiac βIPKC, βIIPKC levels, and translocation	14
MLP transgenic mice	Heart failure		Ro-31-8220 (cPKC and εPKC inhibitors)	PKC inhibition reverted cardiac hypertrophy	34
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	δPKC and εPKC	εPKC knock-out mouse	Increased fibrosis	54
Neonatal rat cardiac fibroblast	Fibroblast proliferation	δPKC and ζPKC	TGFβ1 and isozyme-specific inhibitors	Stimulated cardiac fibroblast proliferation	39

Continued

Table 1 *Continued*

Model	Cardiac phenotype	PKC isozyme	Stimulus/treatment	Main response	Reference
Neonatal rat cardiac fibroblast	Fibroblast proliferation	cPKC, nPKC, and aPKC	Endothelin-1	Increased cardiac fibroblast proliferation	49
Adult rat cardiac fibroblast	Fibroblast proliferation	δPKC	Angiotensin II	Increased δPKC and fibroblast proliferation	97
Adult rat cardiac fibroblast	Collagen synthesis	cPKCs and nPKC	Mechanical load and non-specific PKC inhibitor (RO-31-8220)	Non-specific PKC inhibition and decreased collagen synthesis	98
Myocardial infarction-induced heart failure rats	Fibroblast proliferation	αPKC, βIPKC, and βIIPKC	Non-specific PKC inhibitor (LY333531)	Decreased fibrosis and TGFβ1 expression	12

PKC, protein kinase C.

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.[22] 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,[23] therefore causing a loss of only some to the pathological hypertrophic markers. In other studies, overexpression of αPKC increased cell surface area, [^{3}H]-leucine incorporation, and mRNA levels of ANP,[24] together indicating that αPKC activation induced cardiomyocyte hypertrophy in cultured cardiomyocytes. We found that βI and βIIPKC are required for PMA-induced cardiomyocyte hypertrophy.[25] Later, we substantiated that RBCK-1 (RBCC protein that interacts with βIPKC) is essential for PE-induced cardiomyocyte hypertrophy.[26] Overexpression of RBCK1 increased the cardiac myocyte cell surface by 50% in the absence of PE treatment and the βI- and βIIPKC-specific peptide inhibitors prevented that effect.[26] A role for εPKC has also been suggested; treatment with εPKC antisense reduced myotrophin-induced stimulation of protein synthesis in neonatal myocytes.[27] This study also found that δ and ζPKC are not involved in this process. Therefore, at least four PKC isozymes, 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.[28] 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.[29] Phosphorylation studies suggest that αPKC depresses myofilament contractility through phosphorylation of cTnI and/or cTnT.[30] Skinned left-ventricular myocytes isolated from rats subjected to chronic (8–9 months) pressure overload or MI-induced HF in rats supported these conclusions; myofilament function is severely depressed in these experimental HF models.[28]

In an earlier study, βPKC levels were elevated in hypertension-induced HF rats.[14] Treatment with angiotensin-receptor blocker improved cardiac function and decreased βPKC activation and levels.[14] In a recent study, we found that a selective inhibition of βIIPKC 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.[31] In addition to reducing mortality, selective and sustained inhibition of βIIPKC by βIIV5-3 (a selective inhibitor of βIIPKC[25]) in rats with post-MI HF improved cardiac function compared with that prior to treatment initiation.[31] The beneficial effect was associated with enhanced calcium handling and normalization of the levels and phosphorylation of SERCA2, NCX, and troponin I.[31] Further, the reduction in cardiomyocyte width, HW/BW, and increased fractional shortening following βIIV5-3 treatment in rats with end-stage pathological

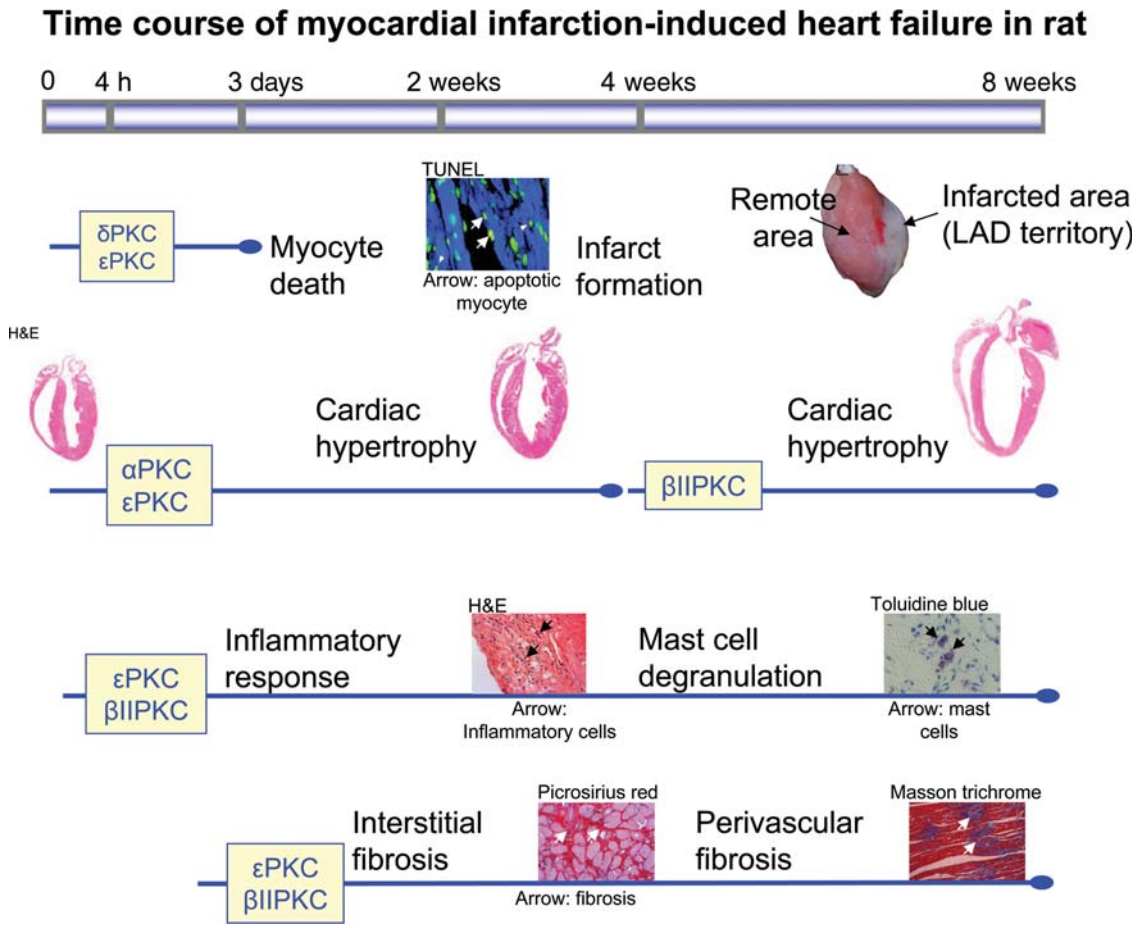

Figure 2 Protein kinase C isozymes are closely involved with different remodelling events in myocardial infarction induced-heart failure. Heart failure progression is noticeably characterized by cardiac remodelling, whereas specific protein kinase C isozyme plays a crucial role in this time-related event. 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.

hypertrophy were observed, thereby indicating that βIIPKC activation is a critical mediator of cardiac hypertrophy in rats (*Figure 2*).

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

Transgenic mice that express ε or δPKC activator or inhibitor peptides only postnatally and only in cardiac myocytes (using the αMHC promoter)[36] revealed potential redundant roles for these enzymes in cardiac hypertrophy. Mice expressing the εPKC-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 εPKC-selective activator, ψεRACK, 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 εPKC activation, hyperplasia, a phenomenon restricted mainly to perinatal cardiac development, may have occurred.[36] These data suggest that εPKC 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 εPKC. 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, εPKC appears to be a positive modulator of compensatory cardiac hypertrophy in this mouse model. Because in this model activation of εPKC and Gαq were induced from day 1 after birth, the phenotype may reflect restoration of εPKC activity in the Gαq-phenotype.[37]

In hypertensive rats, εPKC levels increase during the compensatory stage of cardiac hypertrophy,[14] but εPKC was detrimental in this response. Further activation of εPKC (by a sustained treatment with the PKC-selective activator, ψεRACK) 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 isozymes 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 pharmacologcial PKC modulators (*Table 1*), studies characterizing the level and activity of PKC isozymes in human HF[3,4] provide insight into which isozymes 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 αPKC is critical in mouse model of HFs,[29,34] 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[4] found a significant 70 and 150% increase in activation of βPKC, respectively and imunohistochemical 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 $min^{-1}mg^{-1}$ 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, βI, βII, δ, ε, and ζPKC 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 ζPKC have opposing roles in TGFβ-induced fibroblast proliferation, whereas other PKC isozymes have no role in this process.[39] We showed that selective inhibition of ζPKC blocked TGF-β1-induced cardiac fibroblast proliferation. In contrast, the δPKC-selective peptide inhibitor, δV1-1, had an opposite effect to that of the ζPKC inhibitor; it increased TGF-β1-induced proliferation. Therefore, δ and ζPKC act downstream of TGF-β1, 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 βIPKC increase the activity of MMP-9, but not MMP-2, primarily through the JNK-dependent pathway.[44] Other PKCs, such as θ and ζPKC, 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[45] which ultimately leads to fibroblast proliferation.[46–48] The pro-proliferative 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 βIIPKC inhibition with βIIV5-3 attenuated collagen deposition in the remote region of the myocardium of post-MI HF rats[53] (*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.[15] Moreover, ψεRACK, 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*[55,56] (*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,[45] whereas there is no change in the levels or activity of any PKC isozyme, other than εPKC, when using the εPKC-selective inhibitor[15]). (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.[57–62] 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.[63] Sustained inhibition of εPKC by εV1-2 decreased the infiltration of inflammatory cells into the myocardium in the hearts of hypertensive rats with HF.[16] In an MI-induced HF rat model, βIIV5-3, the specific βIIPKC inhibitor, also attenuated infiltration of inflammatory cells[53] (*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.[64] 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.[65,66]

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 S2248) and S6K1 (at and T389 S421/T424) via PKC activation. Expression of DN-εPKC abolished ET-1-stimulated mTOR and S6K1 phosphorylation, but not insulin-stimulated S6K1 phosphorylation. ET-1- and insulin-stimulated mTOR and S6K1 phosphorylation in cardiomyocytes was inhibited by expression of DN-δPKC or pre-treatment with rottlerin, a δPKC inhibitor. However, treatment with Gö6976, 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.[67] 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.[68] 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.[69] 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.[70] 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.[71] 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.[72]

Myocyte dysfunction in αPKC transgenic mice was caused by alterations in Ca^{2+} homeostasis.[29] As discussed, in mice, αPKC depresses myofilament contractility through site-specific phosphorylation of cTnT at the threonine-206 residue in cardiomyocytes.[30] On the other hand, a decreased myofilament responsiveness to Ca2+ was seen in the

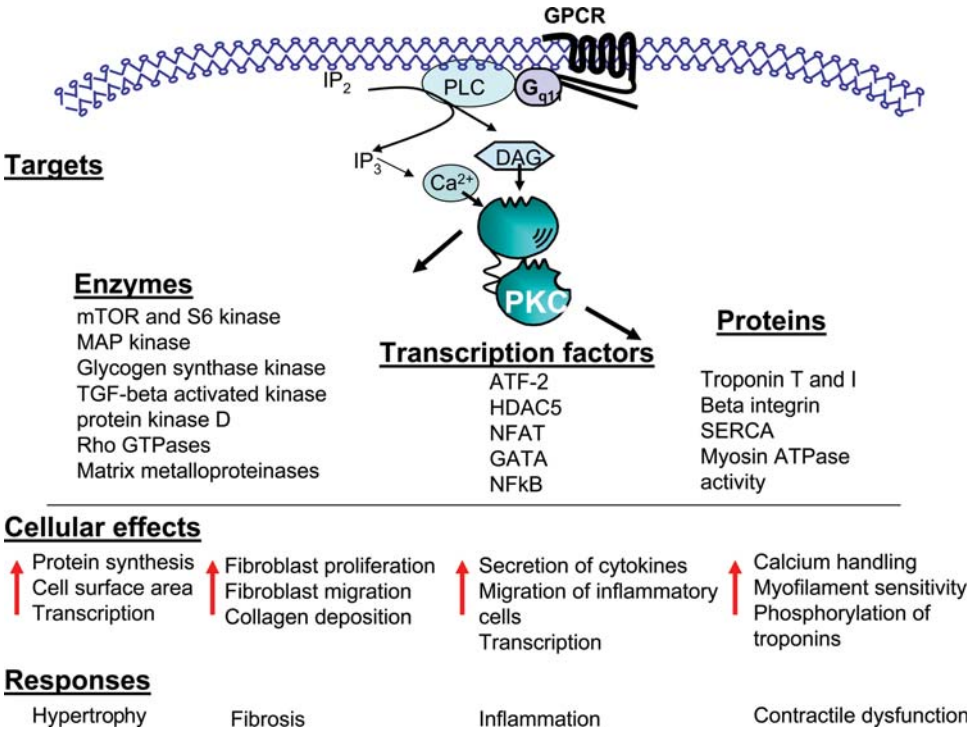

Figure 3 Schematic protein kinase C isozyme signalling pathways and downstream targets in the heart. The activation of different protein kinase C isozymes contributes to the establishment of heart failure through phosphorylation of isozyme-selective substrates in the failing heart.

myocardium of βIIPKC 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 βIIPKC-mediated phosphorylation of troponin I *in vivo* may decrease myofilament Ca2+ responsiveness, and thus causes cardiomyocyte dysfunction.[73]

Other protein targets of PKC are those involved in ECM regulation. εPKC regulates β1-integrin complex formation with ECM and further participates in the fibrotic events.[50–52] α, βI, ε, θ, and ζPKC 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 NFkB, IkB.[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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