PKC Isozymes in Chronic Cardiac Disease: Possible Therapeutic Targets?

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Key Words

atherosclerosis, fibrosis, hypertrophy, heart failure, protein kinase C, signal transduction, rational drug design, novel therapeutics

Abstract

Cardiovascular disease is the leading cause of death in the United States. Therefore, identifying therapeutic targets is a major focus of current research. Protein kinase C (PKC), a family of serine/threonine kinases, has been identified as playing a role in many of the pathologies of heart disease. However, the lack of specific PKC regulators and the ubiquitous expression and normal physiological functions of the 11 PKC isozymes has made drug development a challenge. Here we discuss the validity of therapeutically targeting PKC, an intracellular signaling enzyme. We describe PKC structure, function, and distribution in the healthy and diseased heart, as well as the development of rationally designed isozyme-selective regulators of PKC functions. The review focuses on the roles of specific PKC isozymes in atherosclerosis, fibrosis, and cardiac hypertrophy, and examines principles of pharmacology as they pertain to regulators of signaling cascades associated with these diseases.

INTRODUCTION

PKC: protein kinase C

According to the American Heart Association, 37% of Americans suffered from some form of cardiovascular disease in 2004 (1). Mortality owing to cardiovascular disease accounted for 36% of all deaths, with a staggering 2400 people dying daily. Cardiovascular disease is composed of several different pathologies, including coronary ischemic heart disease, rheumatic heart disease, congenital cardiovascular defects, diseases of the arteries, high blood pressure, heart failure, and stroke. Because of the role of protein kinase C (PKC) in the incidence of chronic cardiac disease and the growing interest in PKC as a therapeutic target, this review focuses on PKC and the heart. However, the role of PKC in ischemia/reperfusion injury, heart failure, and ischemic preconditioning are not evaluated as they have been recently reviewed by us and others (2–7).

PKC STRUCTURE, FUNCTION AND REGULATION

PKC was initially identified by Nishizuka and coworkers as a nucleotide-independent, Ca^{2+}-dependent serine kinase (8). Molecular cloning identified at least 11 isozymes of PKC that were further divided into subfamilies based on sequence homology and mode of stimulation. The classical PKCs (α, β_I, β_{II}, and γ) are diacylglycerol (DAG) and calcium-dependent enzymes, whereas the novel PKCs (δ, ε, θ, and η) require DAG, but not calcium, for activation. The atypical PKCs (ζ, ι/λ) are not responsive to activation by DAG or calcium, but are activated by other lipid-derived second messengers. It is perhaps a little misleading that the different isozymes are grouped into subfamilies, as members of each family can have different even opposing functions. PKCs contain N-terminal regulatory and C-terminal catalytic domains separated by a flexible hinge region. In the absence of activating cofactors, the catalytic domain is subject to autoinhibition by the regulatory domain mediated, in part, by a pseudosubstrate (PS) sequence motif that resembles the consensus sequence for phosphorylation by PKC (9). Other intramolecular interactions between the catalytic and regulatory domains stabilize the inactive form (10, 11).

The activating factors DAG, Ca^{2+}, and phosphatidylserine promote membrane association resulting in activation of the catalytic activity. Generation of DAG often accompanied by a rise in calcium occurs due to activation of transmembrane receptors that are coupled to phospholipase C and leads to the hydrolysis of phosphatidylinositol bisphosphate (PIP_2). The resulting DAG activates PKC and phosphatidylinositol trisphosphate (IP3) releases calcium from internal stores. For the classical PKC isozymes, binding of Ca^{2+} and phosphatidylserine to the C2 domain leads to increased membrane association. Binding of DAG to the zinc finger–rich region of the C1 domain causes a conformational change enabling the release of the autoinhibition and activation of the enzyme. The lack of several aspartate residues in the C2 calcium binding site confers calcium independence to the novel isozymes of PKC, whereas absence of part of the C1 DAG-binding domain and the C2 binding domain renders the atypical isotypes insensitive to both DAG and Ca^{2+}. Binding of PKC to the membrane allows for phosphorylation of critical residues in the regulatory and catalytic

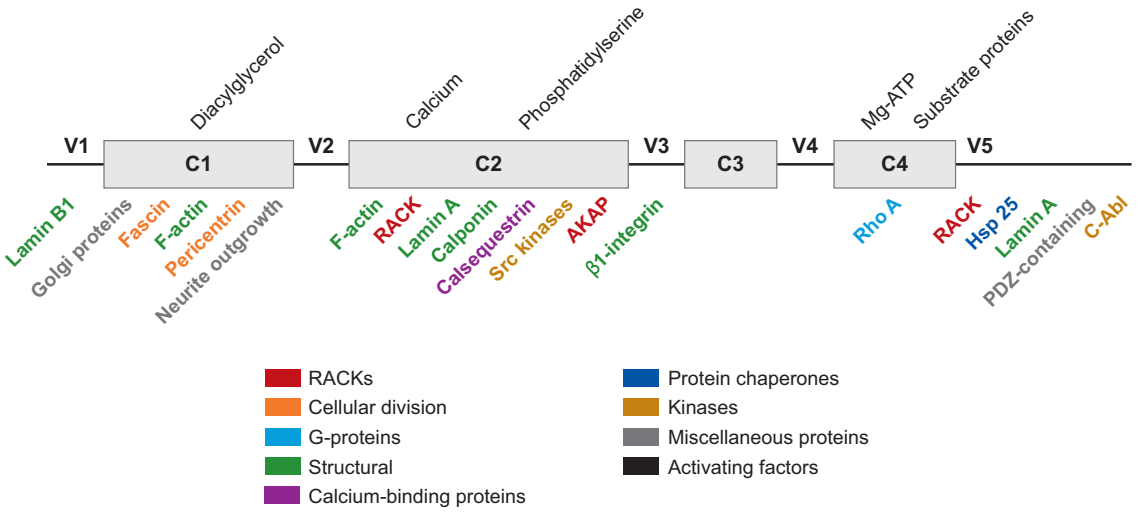


Figure 1

PKC domains and interacting proteins. PKC is composed of both variable (V) and conserved (C) domains. Proteins involved in many aspects of cellular function have been shown to interact with PKC at these domains. These are listed in the figure near their sites of interaction.

domains conferring full activation (12). Many protein-protein interactions between PKC and other proteins have been described. **Figure 1** provides the organization of the PKC domains and identifies some of the intermolecular binding sites for select PKC-binding proteins within these domains based on studies of many laboratories.

PKC ISOZYMES IN THE HEART

PKC Isozyme Expression in Healthy Myocardium

PKC is ubiquitously expressed in all tissues. Early studies utilizing RT-PCR identified the presence of α, δ, ε, η, and ζPKCs in rat cultured cardiomyocytes (13, 14). However, other researchers have shown abundant expression of both βI and βIIPKC in human and rat cardiomyocytes (15–17). Further species-specific differences in expression of η, θ, and εPKC were also reported (18). This suggests that caution must be taken when translating potential therapeutic PKC targets from animal models because expression patterns of individual isozymes varies between species.

PKC Isozyme Expression in Cardiac Disease States

In addition to changes in phosphorylation and translocation of PKC, alterations in PKC levels are associated with normal cardiac development, as well as with pathology. For example, α, β, ε, and ζPKC expression is high in fetal and neonatal hearts, and it decreases in adult hearts (19). However, during transition to heart failure, in

humans, the levels of α and βPKC isozymes increase, suggesting a reversion back to a neonatal phenotype (15). In a model of aortic banding, increased δPKC expression or a decreased expression in α, β, ε, and ζ (18) without any changes in εPKC and ζPKC was associated with pressure-overload cardiac hypertrophy (20). Therefore, when targeting individual PKC isozymes to combat pathophysiological conditions, changes in expression patterns must be taken into account.

GENERAL CONSIDERATIONS; IN SEARCH OF THE PERFECT DRUG TARGET

Pharmacological Targets within Signal Transduction Pathways

When examining the approximately 480 approved drugs on the market (21), it is obvious that most affect the uppermost events in the signaling cascade, thus blocking the cascade at its origin (**Figure 2**, step 1). Drugs in this category, such as β-adrenergic receptor blockers, include competitive and noncompetitive inhibitors of the natural hormones that trigger the signaling cascade. There are also inhibitors that affect the hormone directly, including decoy peptides that bind receptors and inhibit the activation of the hormone (22). Inhibitors of the processing enzymes for the hormones, such as the angiotensin-converting enzyme inhibitors or acetycholine esterase inhibitors, are drugs that affect the processing of the hormones. Finally, inhibitors of ion channels, such as digoxin, and of transporters, such as amiloride, also represent drugs that regulate very early events in the signaling cascades.

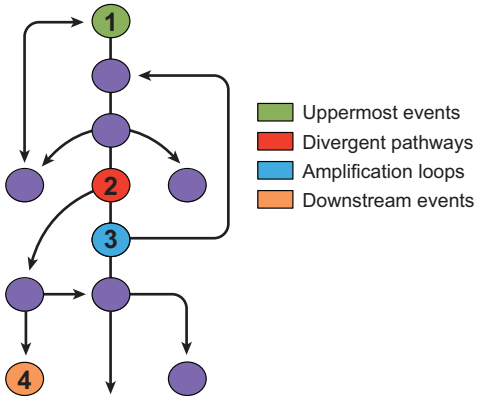


Figure 2

Molecular dissection of a signal transduction pathway to determine pharmacological targets. Signal transduction cascades often include side branches, which must be considered when designing effective therapeutics. Currently, most approved drugs on the market target the upper-most events in a signaling pathway thus blocking the cascade at its origin (*step 1*). However, this strategy neglects downstream divergent pathways such as an intracascade kinase (*step 2*), or amplification loops (*step 3*), which will be coincidentally targeted, thereby potentially limiting the safety of the treatment. This understanding led to a drug discovery effort that focused on inhibition at the most downstream event, such as inhibition of the transcription factors (*step 4*).

However, signaling cascades often include side branches (**Figure 2**, step 2), and thus divergent pathways that are not involved in the disease states are inhibited as well—potentially limiting the safety of the treatment. Furthermore, cascades, by nature, include many amplification steps but often end with an on-off switch-like event, such as turning on expression of a new set of genes. This understanding led to a drug discovery effort that focused on inhibition at the most downstream event, such as inhibition of the transcription factors (**Figure 2**, step 3). When tested genetically (by expressing a dominant negative form or creating a null allele), inhibiting the most downstream event proves very effective. However, even though this approach has been a focus for the pharmaceutical industry and, more often, for the biotech industry in the past decade, there are no drugs on the market that target gene transcription (21), with the exception of drugs that affect steroid receptors (receptors that are themselves transcription factors). Concomitantly, the use of therapeutic antibodies is gaining momentum, with a total of 14 therapeutics on the market as of 2006 (21). However, because without further modification, antibodies cannot cross cell membranes, many more extracellular targets and early signaling events have been added to the already crowded receptor targeting approach.

Does the current drug repertoire represent all the signaling events that can be targeted or does the current status reflect difficulties in targeting intracellular events in the signaling cascade? Eighty-eight recent inhibitors of intracellular enzymes have been approved. These include phosphodiesterase inhibitors (such as caffeine and sildenafil), histone deacetylases (such as valproic acid and carbamezepine), and phosphatase inhibitors (such as cyclosporine).

Intracellular signaling enzymes may be a challenge because it is not clear which step is best to target (**Figure 2**). Furthermore, intracellular enzymes maybe hard to target because they often come as a family of homologous enzymes with highly conserved catalytic domains, which are not only conserved within the family but also among the families. Nevertheless, selective inhibitors targeting the active site of protein kinases have been approved recently for the treatment of cancer, including Gleevec, a tyrosine kinase inhibitor, approved for treatment of chronic myelogenous leukemia (CML) (23). Relevant to this review, several inhibitors targeting the catalytic site and the activator site of PKC have been tested in clinical trials and were found to have unacceptable toxicity, thus restricting their use as experimental tools. Importantly, the selectivity of those inhibitors for individual isozymes is limited. For example, even the βIIPKC-selective inhibitor that is currently in clinical trials has been reported to affect other PKC isozymes (24).

Isozyme-Specific Pharmacological Tools

We have taken another approach to generate isozyme-selective pharmacological tools. Based on our early observation that individual isozymes are each localized to distinct subcellular sites following activation (25), we suggested and subsequently demonstrated that each isozyme binds to a selective anchoring protein, a RACK, in addition to binding to the lipid-derived second messengers (26). We next used a rational approach to identify the interaction site between each isozyme and its RACK and

identified peptides corresponding to these sites that selectively interfere with the interaction. The approaches that we used to identify these PKC peptide regulators were reviewed elsewhere (11). The peptide regulators of PKC can be delivered into cells in culture and in vivo by crosslinking them to cell-permeating peptides, such as TAT_{47-57} (27). These PKC-regulating peptides are highly selective and efficacious. In a porcine model of cardiac ischemia ~500 ng Kg^{-1} of the δPKC inhibitor δV1-1, for example, caused a selective reduction of δPKC translocation, and a decrease in infarct size and an improvement in cardiac function of ~70% when delivered at reperfusion (28). Importantly, the peptide caused no adverse effects in a variety of species, even when delivered for a prolonged period (using an Alzet pump) at very high doses. Finally, a recent safety Phase IIa clinical trial in humans showed that doses up to 5 mg per patient delivered into the coronary arteries during intervention of myocardial infarction, showed no adverse effects. Although the study was not powered for efficacy, a variety of endpoints, including infarct size, CKMB AUC, and restoration of electrical activity, were improved (29). Therefore, although protein-protein interactions are thought to be difficult to target for therapeutic, short peptides derived from the interaction sites of the interacting proteins may provide a new set of drugs or drug leads.

In addition, we suggest that although RACKs can anchor the activated PKCs at all these subcellular sites, additional unique protein-protein interactions between PKC and each substrate provide further anchoring at each subcellular site (**Figure 3*a***). This takes into account the findings that RACKs may bind the substrates directly (11) and that upon PKC phosphorylation, phosphorylated substrates detach from the PKC isozyme (30). This was demonstrated, for example, for Src and β_{II}PKC binding to RACK1 (31) and calsequestrin and εPKC (32). Finally, if our hypothesis is correct, each PKC isozyme is likely to have unique interactions with different substrate proteins at different subcellular sites (**Figure 3*a***). Thus, we should be able to identify

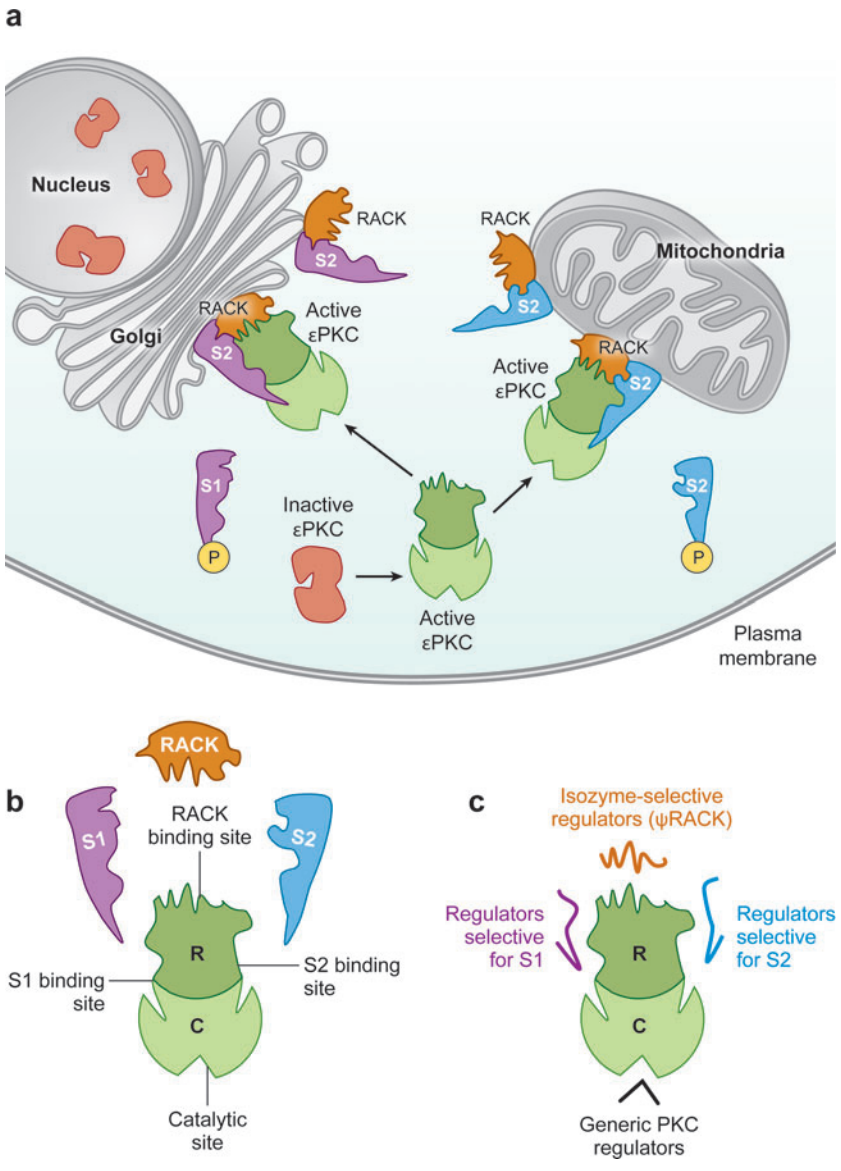
Figure 3

Scheme depicting the interaction of activated εPKC with its RACK and with two substrates in different cellular compartments. (*a*) A scheme depicting inactive (*red*) εPKC isozyme undergoing a series of conformational changes (involving also interruption of autoinhibitory intramolecular interactions) leading to activation of εPKC (*green*) and its anchoring to two subcellular sites: mitochondria and Golgi. At each site, the activated isozyme is anchored to its εRACK (*orange*) and is selectively binding also a unique substrate (S1 in the Golgi and S2 in the mitochondria). εRACK may serve as a scaffold to bind both the substrate and the active εPKC. Once phosphorylated (P), the substrate detaches, as shown by Jaken and collaborators (30), and may translocate to the cytosol. (*b*) An important aspect of our hypothesis is that each PKC isozyme has several distinct sites of protein-protein interactions (see also **Figure 2**). (*c*) We propose that in addition to interfering with PKC binding to its RACK (an isozyme-selective interaction) or interfering with the catalytic site (which often affects many of the PKC isozymes similarly because this site is conserved), it should be possible to design select activators and inhibitors of each isozyme that will affect interaction with one substrate and not another. Depicted, as an example, are regulator peptides of each interaction (*colored regions*). Peptide regulators that mimic the PKC-binding site for the cognate molecules will compete with the interaction and thus will be selective peptide inhibitors of interaction with S1, but not S2, for example.

inhibitors and activators of each protein-protein interaction, thereby conferring even greater specificity of PKC isozyme regulation (**Figure 3*b*,*c***).

THE ROLE OF PKC IN ATHEROSCLEROSIS AND ASSOCIATED INFLAMMATORY RESPONSES

Coronary heart disease is characterized by the development of atherosclerotic lesions in the coronary arteries leading to sustained ischemic events and/or acute myocardial



infarction (AMI). The disease involves both cardiac and noncardiac-resident cells, including coronary arterial wall constituents [endothelial cells and vascular smooth muscle cells (VSMCs)], fibroblasts, inflammatory cells, and circulating blood components (33, 34), and it can be defined by distinct stages (33, 34). The following section examines the role of PKC in atherosclerosis and identifies the isozyme(s) that provide a therapeutic target for the prevention of the disease.

PKC and LDL Metabolism and Oxidation

The controlled removal of low-density lipoprotein (LDL) through LDL receptor activation in the liver is critical in preventing the pathologic accumulation of serum LDL (35). Early work demonstrated that stimulation of a human hepatic cell line (HepG2) with PMA, or with DAG and calcium, upregulates LDL receptor mRNA (36) resulting in increased LDL binding (37) and increased translocation of αPKC to the particulate fraction (38). Furthermore, overexpression of εPKC and βPKC significantly induces LDL receptor promoter activity (39, 40), possibly through a mechanism involving βPKC-mediated hyperphosphorylation of histone H3 (41). In vivo evidence shows that treatment of rats with the LDL regulator 17-β-estradiol activates αPKC, resulting in LDL receptor upregulation in the liver (42). Therefore, activation of αPKC seems to be required for LDL receptor upregulation and the resulting decrease in serum LDL (**Figure 4*a***).

Oxidized LDL (oxLDL) in the subendothelial space of coronary arteries serves as a chemotactant for monocytes, allowing for extravasation into the subendothelial space and increased endocytosis of oxLDL. Activation of human monocytes increases superoxide anion ($O_2^{-\bullet}$) generation and subsequent LDL oxidation (43–45); inhibition of PKC blocks both of these effects (46). αPKC and βI, βII PKCs are upregulated upon monocyte stimulation, and whereas αPKC and βIPKC translocate to the membrane particulate fraction, only αPKC appears to be involved in $O_2^{-\bullet}$-mediated oxidation of LDL (47) (**Figure 4*b***).

Endothelial Cell Reactive Oxygen Species Generation

Endothelial dysfunction is a major contributing factor in the progression of atherosclerosis, and the decline in nitric oxide (NO) production by endothelial nitric oxide synthase (eNOS) contributes substantially to disease progression (48). Dephosphorylation of human eNOS Thr495 (Thr497 in cows) is associated with increased eNOS activity (49), increased calmodulin binding (49), and increased superoxide production (50). Initially, PKC was shown to regulate this by phosphorylating eNOS on Thr495 (51, 52). Further studies suggested that a feedback loop in which oxLDL inactivates αPKC may result in dephosphorylation of Thr495, resulting in inactivation of eNOS and increased $O_2^{-\bullet}$ generation (53). In addition to eNOS-mediated $O_2^{-\bullet}$ generation, ζPKC has also been shown to associate with and phosphorylate p47phox in TNF-α-stimulated endothelial cells, thus activating NADPH oxidase (54), which may further uncouple eNOS through enhanced reactive oxygen species generation (55). Taken together, these findings suggest that PKC may increase

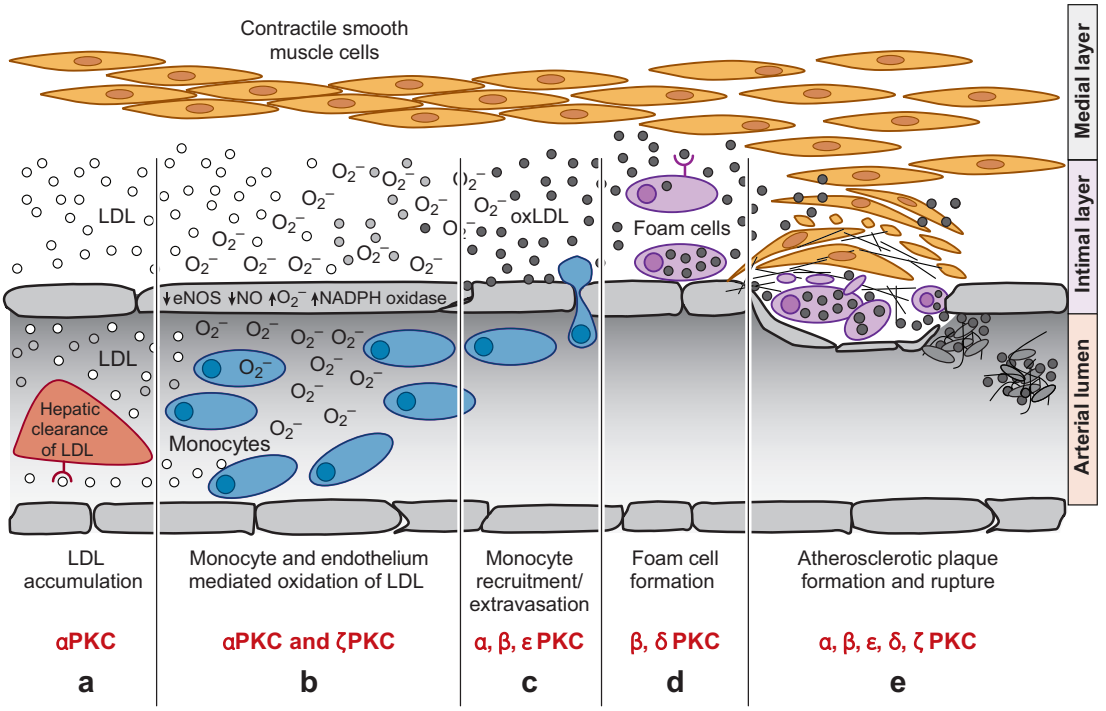


Figure 4

PKC is intimately involved with many stages of atherosclerotic disease progression. The progression of atherosclerosis is marked by distinct stages with PKC playing a role in each stage. (*a*) Hepatic clearance of LDL is regulated, in part, by αPKC. (*b*) α and ζPKC-mediated increases in reactive oxygen species generated from monocytes, endothelium, and smooth muscle oxidizes LDL. (*c*) Oxidized LDL in the subendothelial space acts as a chemotractant for monocytes. Recruitment, adherence, and extravasation of monocytes are regulated in part by α, β, and εPKC. (*d*) β and δPKC-mediated engulfment of oxLDL by macrophages leads to foam cell formation. (*e*) Recruitment and apoptosis of smooth muscle cells and apoptosis of foam cells, as well as accumulation of fibrous tissue, leads to further atherosclerotic plaque progression and subsequent rupture, resulting in thrombi formation into the arterial lumen.

the pro-oxidant environment of the growing atherosclerotic lesion by increasing $O_2^{-\bullet}$ production, thus leading to apoptosis and plaque instability (56) (**Figure 4*b***).

Monocyte Recruitment and Adhesion

LDL induces monocyte adhesion to the vascular endothelium and subsequent extravasation into the intimal space by activating both monocytes and endothelial cells; the process in both cell types is dependent on PKC (57, 58). Inhibition of PKC with the broad spectrum PKC blocker, H7, prevents thrombin-, PMA-, and interleukin-1-mediated monocyte adhesion to the endothelium (59). oxLDL treatment of human monocytes induces CD-11b-dependent endothelial cell adhesion, which is blocked

by another PKC inhibitor, staurosporin (60). Phospholipase A_2 activation and increased monocyte PECAM-1 expression resulting in endothelial cell adhesion has been associated with PKCε/α (61). PKC may also play a role in transendothelial cell migration because inhibition of PKC prevented F-actin polymerization (62), PECAM-1 phosphorylation, and subsequent monocyte transmigration (63). Furthermore, PKC activation also results in the upregulation of P-selectin expression on the surface of coronary endothelium (57), and the LDL constituent, apolipoprotein CIII, induces activation and translocation of βPKC and increased expression of VCAM-1 and ICAM-1 (64). Therefore, PKC plays a role in monocyte adherence (by increasing adhesion molecule expression) and monocyte transendothelial cell migration (by activating proteins involved in extravasation). Inhibition of α, ε, and/or βPKC may provide a mechanism to inhibit the inflammation associated with LDL-induced atherosclerotic progression (**Figure 4*c***).

Macrophage Proliferation and LDL Accumulation

Once in the subendothelial space, monocytes are activated to become macrophages, leading to simple fatty streak formation. Matsumura et al. found that oxLDL treatment of murine macrophages resulted in the release of granulocyte macrophage–colony stimulating growth factor (GM-CSF), which is blocked by the PKC inhibitor calphostin C (65). However, the study does not identify which PKC isozyme is responsible for this effect. Accumulation of oxLDL by macrophages results in the formation of cholesterol-laden foam cells and characteristic atherosclerotic lesions. Although some controversy exists, LDL uptake has been hypothesized to occur through either micropinocytosis (<0.1-μm vesicles), macropinocytosis (0.5–5.0-μm vacuoles), or receptor-mediated endocytosis. Incubation of human monocyte-derived macrophages with the PKC activator, PMA, increased LDL uptake, which was blocked with PKC inhibitors (66, 67). The use of various PKC inhibitors and PMA-induced isozyme downregulation indicates that βPKC and δPKC are most likely responsible for macrophage oxLDL accumulation through macropinocytosis (68) (**Figure 4*d***).

PKC may also play a role in receptor-mediated LDL endocytosis by an LDL receptor and/or scavenger receptor. Upregulation of CD36, a scavenger receptor for oxLDL, was shown to be controlled by PKC (69). However, another study showed that PMA-stimulation of macrophages actually decreases the uptake of oxLDL through phosphorylation of LDL receptors (70). Because the expression levels of LDL receptors are low in human atherosclerotic plaques (71), this decreased LDL uptake may be a result of PMA-mediated downregulation of PKC. Furthermore, PKC activation inhibits cholesterol esterification, leading to foam cell formation within the macrophage, and ERK activation counters this pathway (72). Regardless of the controversy surrounding which pathway mediates the uptake of LDL into macrophages, it is evident that PKC plays a central role in LDL accumulation. This raises the possibility that inhibition of LDL uptake through PKC inhibition may prevent foam cell formation and generation of atherosclerotic lesions (**Figure 4*d***). Whereas decreasing monocyte recruitment and oxLDL macrophage accumulation may

diminish subendothelial inflammation, it would also result in the accumulation of extracellular oxLDL, which may have pathophysiological implications as well. Therefore, a better therapy may be to target PKC in the early stages of LDL accumulation in the vascular system.

Smooth Muscle Cell Migration, Proliferation, and Apoptosis

The transition to more complex atherosclerotic lesions occurs when VSMCs migrate from the medial layer to the subendothelial space (intimal layer) (**Figure 4*e***). Whereas phospholipase-D (PLD) is activated in VSMCs upon oxLDL stimulation, PKC is not involved (73). However, this does not rule out the possibility that PKC activation is downstream of PLD activity. In support of this, treatment of VSMC with a calcium antagonist blocks PDGF- and phorbol ester-stimulated VSMC proliferation with a concomitant inhibition of δPKC translocation to the membrane particulate fraction (74). Because δPKC is a calcium-insensitive isozyme, it is likely that activation of VSMC proliferation is downstream of another calcium-sensitive enzyme. Indeed, PKC activation was necessary for oxLDL and native LDL-mediated activation of ERK in rat VSMC (75, 76). Although the necessity of calcium in oxLDL-mediated VSMC proliferation is debated (75, 76), a role for PKC is indicated (77). Further, treatment of VSMC with ATP or PDGF-β induces proliferation through translocation and activation of δPKC (78, 79). However, atherosclerotic lesions in δPKC null mice ($\delta PKC^{-/-}$) increases the number of VSMC and decreases apoptosis as compared with controls, suggesting an antiproliferative role of δPKC (80). Additionally, in a porcine model of atherosclerosis, εPKC and αPKC have been implicated in the process (81). Luft et al. found that VSMC proliferation can be induced by activation of different membrane-bound receptors, resulting in activation of different PKC isozymes (82).

As VSMCs continue to proliferate and engulf oxLDL, the increased cell-to-cell interactions and oxidized sterols in the LDL can lead to apoptosis of the VSMC (83). The accumulation of apoptosed VSMCs and macrophages contributes to the necrotic core that makes up the atherosclerotic plaque and contributes to its vulnerability to rupture (**Figure 4*e***). Pro-oxidants, such as hydrogen peroxide, play a key role in the induction of apoptosis of VSMCs, and hydrogen peroxide treatment of rat aortic VSMCs activates εPKC and αPKC followed by apoptosis (84). Inhibition of PKC blocked apoptosis and increased cellular necrosis. Conversely, other researchers have shown that δPKC is responsible for VSMC apoptosis in a kinase-independent manner (80, 85).

Atherosclerotic Plaque Stability

The generation of stable atherosclerotic plaques causes narrowing of the vessel lumen, which can lead to subsequent downstream ischemia. However, rupturing of the plaque (**Figure 4*e***) leads to activation of humoral responses and coagulation, resulting in acute myocardial infarction and greater damage. Matrix metalloproteinases (MMPs) are secreted factors that degrade extracellular matrix proteins, thus

contributing to plaque instability (86). The first indication that PKC is involved in MMP secretion was shown in human umbilical vein endothelial cells; PMA stimulation increased expression of MMP-9 (type IV collagenase) mRNA through a Ras/MAPK pathway (87). Stimulation of endothelial cells with homocysteine (commonly found in atherosclerotic plaques) activates calcium-sensitive PKCs through a G protein–coupled receptor–dependent mechanism, resulting in ERK phosphorylation and increased MMP-9 mRNA transcription (88). This process is controlled by the transcription factor Ets-1, which also regulates MMP-1 (type-1 collagenase) expression in endothelial cells and is blocked by PKC inhibition (89). Stimulation of human arterial endothelial cells with oxLDL increases the expression of MMP-1 and MMP-3 (stromelysin-1), a process that is blocked by the βPKC inhibitor, hispidin (90). Additionally, increased MMP-2 expression by Il-1β-treatment in endothelial cells is partly dependent on PKCα/β activation (91). Therefore, in endothelial cells, at least two different PKC isozymes are responsible for the upregulation of four different MMPs. The activation of different isozymes seems to be dependent upon the type of stimulus administered (**Figure 4*e***).

The current understanding of the role of PKC in MMP secretion from VSMCs and macrophages is quite limited. However, PKCζ may play a critical role in VSMC secretion of MMP as its activity is increased 13-fold and overexpression of a dominant negative PKCζ significantly blocks MMP-1, -3, and -9 expression (92). MMPs may also be important for the migration of VSMCs to growing atherosclerotic lesions.

Therapeutic Potential for PKC in Atherosclerosis

Each of the steps in the process of atherosclerosis in which PKC has been found to play a role are indicated in **Figure 4** and detailed in **Table 1**. This literature review highlights potential isozymes that should be targeted to inhibit the progression of many steps leading to the catastrophic plaque rupture. PKC-mediated LDL uptake in the liver may prove to be one of the most promising therapeutic targets to combat this disease. In that case, selective activators of αPKC, the isozyme that appears to be responsible for hepatic uptake of LDL (42) thereby diminishing the amount of LDL in the arteries (42), may provide a means to block the most potent initiating atherosclerotic signal, LDL. However, although activation of αPKC in the endothelium would diminish superoxide production (53) and therefore LDL oxidation, the opposite is true in monocytes (47). Because αPKC targets in the liver, endothelial cells, and monocytes are likely to be different, selective regulators of αPKC interaction with each substrate, as depicted in **Figure 2*c***, may provide a superior, more selective drug to modulate αPKC in these tissues. δPKC may be another target of therapeutic importance because of its role in macrophage oxLDL accumulation (68) as well as in VSMC apoptosis (80, 85). This suggests that selective δPKC inhibition might decrease the extent of damage that occurs as oxLDL-laden macrophages build up in the subendothelial space. Based on published studies, we suggest that selective regulators of α and δPKC isozymes are the most promising candidates in the prevention and regression of atherosclerosis, respectively. Direct studies examining this suggestion should make use of highly selective pharmacological tools.

Table 1 Isozyme-specific effects of PKC in etiologies leading to heart failure

PKC isozyme	Cardiac etiology	Activator/inhibitor	Species/ cell type	Effect	Reference
αPKC	Atherosclerosis (LDL accumulation)	100nM TPA	HepG2	Upregulation of LDL receptor mRNA	(38)
αPKC	Atherosclerosis (LDL accumulation)	17-β-estradiol	Rat	LDL receptor upregulation and αPKC translocation	(42)
εPKC	Atherosclerosis (LDL accumulation)	Overexpression/antisense oligonucleotide	HepG2	Increased/decreased LDL receptor promoter activity	(40)
βPKC	Atherosclerosis (LDL accumulation)	Overexpression	HepG2	Increased LDL receptor promoter activity	(39)
αPKC	Atherosclerosis (LDL oxidation)	Antisense oligonucleotide	Activated monocyte	Decreased superoxide production and LDL oxidation	(47)
αPKC	Atherosclerosis (endothelial dysfunction)	oxLDL	Human endothelium	Inactivation of αPKC and superoxide generation	(53)
ζPKC	Atherosclerosis (endothelial dysfunction)	TNF-α	Human endothelium	Generation of active NADPH oxidase	(54)
α,εPKC	Atherosclerosis (monocyte adhesion)	Lactosyl-ceramide	U937 cells	PLA2 activation, PECAM1 expression and adhesion	(61)
βPKC	Atherosclerosis (monocyte adhesion)	Apolipoprotein CIII	Human endothelium	βPKC translocation, VCAM1, ICAM1 expression, adhesion	(64)
βPKC	Atherosclerosis (macrophage LDL)	Pseudosubstrate peptide inhibitor	Activated macrophage	Inhibition of βPKC blocks LDL accumulation	(68)
δPKC	Atherosclerosis (VSMC proliferation)	Phorbol ester, PDGF-β, ATP	Rat aortic VSMC	Translocation, VSMC proliferation ERK activation	(78, 79)
α,εPKC	Atherosclerosis (VSMC proliferation)	oxLDL	Rat aortic VSMC	Translocation and VSMC vasoconstriction	(81)
α,εPKC	Atherosclerosis (VSMC apoptosis)	Hydrogen peroxide	Rat aortic VSMC	Translocation and VSMC apoptosis	(84)
δPKC	Atherosclerosis (VSMC apoptosis)	Hydrogen peroxide	$\delta PKC^{-/-}$ VSMC	Diminished apoptosis and VSMC accumulation	(80, 85)
βPKC	Atherosclerosis (plaque stability)	oxLDL	Human endothlieum	Increased expression of MMP1, MMP3	(90)

(*Continued*)

Table 1 (*Continued*)

PKC isozyme	Cardiac etiology	Activator/inhibitor	Species/ cell type	Effect	Reference
α,βPKC	Atherosclerosis (plaque stability)	IL-1β	Human endothelium	Increased MMP-2 gene expression	(91)
ζPKC	Atherosclerosis (plaque stability)	Inflammatory cytokines	Rabbit VSMC	Increased MMP1,3,9 release	(92)
δPKC	Fibroblast proliferation	δV1-1 peptide inhibitor	Rat cardiac fibroblasts	Increased basal fibroblast proliferation	(116)
ζPKC	Fibroblast proliferation	ζPKC pseudosubstrate inhibitor	TGF-βI treated fibroblasts	Reduced basal and TGF-βI induced fibroblast proliferation	(116)
εPKC	Fibrosis	Pressure overload by aortic constriction	$\varepsilon PKC^{-/-}$ mice hearts	Elevated fibrosis and increased collagen deposition	(103)
εPKC	Fibrosis	Ang II	Rat cardiac fibroblasts	Increased βI-Integrin interaction	(104)
εPKC	Fibrosis	ET-1	Rat cardiac fibroblast	Translocation and fibroblast proliferation	(119)
β_{II}PKC	Fibrosis	β_{II}PKC overexpression	Mouse myocardium	TGF-β1, CTGF upregulation and increased collagen deposition	(123)
δPKC	Fibrosis	Adenoviral overexpression of dominant negative δPKC	Rat cardiomyocytes	Increased CTGF expression	(120)
εPKC	Hypertrophy	Hypertrophic stimuli, ψεRACK overexpression	Myocytes, rat heart, transgenic mice	Translocation and increased hypertrophic cellular growth	(124–129, 132)
δPKC	Hypertrophy	ψδRACK overexpression	Transgenic mice	Increased hypertrophic cellular growth	(129)
β_{II}PKC	Hypertrophy	Overexpression of constituitively active	β_{II}PKC	Cultured cardiomyocytes	(123, 134)
β_{I}PKC	Hypertrophy	$\beta_{I}V_{5-3}$ treatment	Phorbol treated neonatal cardiomyocytes	Developmental hypertrophy	(135)

PKC AND FIBROSIS

Cardiac fibrosis, which results from the expansion of the cardiac extracellular matrix, is essential for scar formation after AMI. However, fibrosis also contributes to the pathology of chronic heart failure. Fibrosis reduces the flexibility of myocardial tissue resulting in diastolic dysfunction; cardiac contraction becomes progressively impeded by an increasingly thickened extracellular matrix. Furthermore, increased collagen content disrupts electrical connectivity between cardiomyocytes, resulting

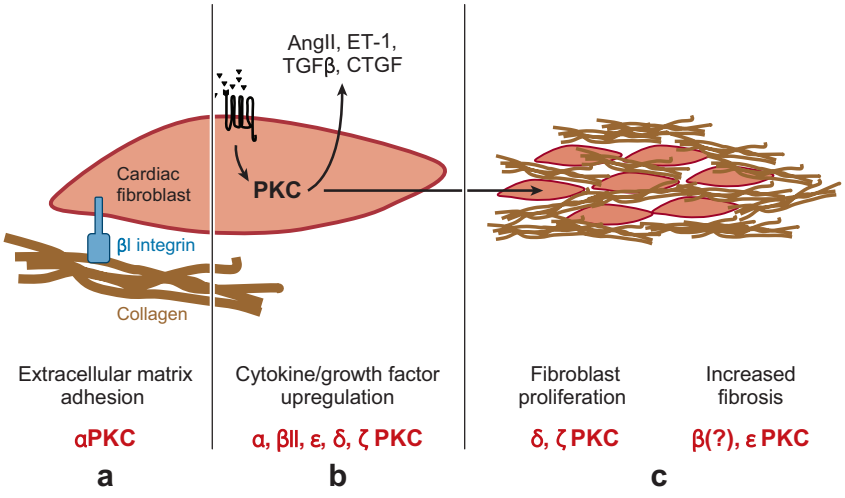


Figure 5

Role of PKC isozymes in cardiac fibroblast proliferation. Ischemic injury results in increased levels of circulating cytokines, growth factors, and hormones that stimulate cell surface receptors on cardiac fibroblasts. The resulting increase in fibroblast proliferation and collagen synthesis causes expansion of the extracellular matrix, leading to cardiac fibrosis. Distinct PKC isozymes contribute to different stages of cardiac fibrosis. (*a*) Fibroblast adhesion to the extracellular matrix is regulated through εPKC (via βI-integrin). (*b*) Upregulation of cytokine and growth factors is mediated by α, βII, δ, ε, and ζPKC. (*c*) δ, ε, and ζPKC have been demonstrated to regulate fibroblast proliferation.

in arrhythmogenesis (93). Cardiac fibroblasts are the cell type primarily responsible for homeostatic maintenance of the extracellular matrix and account for 90%–95% of noncardiomyocyte cell mass in the myocardium (93, 94). The rennin-angiotensin system (RAS) plays a key role in the neurohumoral response that results in fibroblast proliferation and fibrogenesis. The myocardium contains an endogenous RAS, which is distinct and regulated independently from the renovascular system (95–97). Myocardial ischemia leads to increased cardiac levels of aldosterone, which results in increased local levels of angiotensin II (AngII). Stimulation with AngII results in cardiac fibroblast proliferation and a net accumulation of fibrillar collagen in vitro and cardiac fibrosis in vivo (93). These responses are transduced by angiotensin-type-1 (AT_1) receptors (98) and through upregulation of a variety of cytokines and growth factors, including transforming growth factor-βI (TGFβI), endothelin-1 (ET-1), and connective tissue growth factor (CTGF) (93, 99). Whereas the postischemic events that culminate in fibrosis are diverse, many of the pathways involved share common intracellular signaling pathways, including activating the PKC family (**Figure 5**).

AngII Signaling: Role of PKC Isozymes

The AT_1 receptor is coupled to $G\alpha_{q/11}$ and stimulates PLCβ, generating inositol 1,4,5triphosphate (IP_3) and DAG, increasing $[Ca^{2+}]_i$, and activating PKC. In neonatal

rat cardiac fibroblasts, AngII activates PKC within 5 min (100). Early studies found that activation of PKC with phorbol ester does not elicit fibroblast proliferation (101) and AngII-induced proliferation in isolated human fibroblasts was shown to be independent of PKC (102). However, more recent studies have found that the εPKC isozyme plays a crucial role in mediating fibrogenesis in vivo. Hearts from εPKC null mice ($\varepsilon PKC^{-/-}$) demonstrate normal baseline hemeodynamics and normal levels of interstitial fibrosis. However, when exposed to pressure overload by thoracic aortic constriction, $\varepsilon PKC^{-/-}$ mice demonstrate elevated cardiac fibrosis and increased collagen deposition when compared with wild-type animals (103). Recent evidence suggests that the εPKC-sensitive mechanism of fibroblast proliferation induced by AngII may be due to regulation of cell matrix adhesion mediated via β_1-integrins (104). Integrins are transmembrane receptors, regulating the interaction of fibroblasts with their surrounding matrix (105). Integrin function can be modified by extracellular stimuli (outside-in signaling) and by intracellular signal transduction pathways (inside-out signaling) providing a dynamic interaction between environmental cues and intracellular signaling events (106). AngII treatment of adult rat cardiac fibroblasts induces εPKC phosphorylation (but not αPKC or δPKC phosphorylation) and εPKC:β_1-integrin complex formation. Furthermore, AngII treatment induces increased adhesion of rat cardiac fibroblasts to collagen, an effect that is blocked by PKC inhibition (104). AngII also fails to induce collagen adhesion in myofibroblasts isolated from $\varepsilon PKC^{-/-}$ mice (104). Other studies have shown that εPKC is critical for β_1-integrin-mediated adhesion (107, 108). In human glioma cells, εPKC:β_1-integrin complex formation is induced by PMA (107), and εPKC has also been shown to be essential for intracellular trafficking of β_1-integrin in mouse embryo fibroblast (MEF) cells (108). In addition, combined treatment with εV1-2 (a specific inhibitor of εPKC) and an AngII receptor blocker was superior in the treatment of hypertension-induced heart failure more than treatment with the AngII receptor blocker alone (109). Thus, εPKC appears to be critical in mediating fibroblast adhesion to the extracellular matrix (**Figure 5*a***). However, it remains to be determined whether εPKC mediates an increase or decrease in fibrosis in the context of postinfarct remodeling.

TGFβI Signaling: Role of PKC Isozymes

Transforming growth factor beta-I (TGFβI) is released in an autocrine or paracrine fashion after MI (98, 99, 110, 111). TGFβI binds to a specific cell surface receptor (112), thus activating intracellular signaling pathways, promoting collagen synthesis and fibroblast proliferation (99). The intracellular signaling pathways downstream of TGFβI include TGF-β-activated kinase 1 (TAK1) (113) and the Smad family of proteins (99, 114). Stimulation with TGFβI also results in translocation of specific PKC isozymes, each of which translocates to distinct subcellular regions of the cell, suggesting PKC-izozyme-selective events in response to TGFβI stimulation (115). To determine which PKC isozymes are involved in mediating TGFβI-stimulated fibroblast proliferation, we used PKC-isozyme-selective inhibitors (116). Selective inhibition of δPKC increased basal proliferation of rat neonatal cardiac

fibroblasts, whereas inhibition of ζPKC reduced basal and TGFβI-induced proliferation. Other isozyme-specific inhibitors used in this study had no effect on proliferation similar to results obtained in adult cardiac fibroblasts (M. Braun and D. Mochly-Rosen, unpublished). These findings suggest that the δPKC and ζPKC isozymes mediate opposing effects; δPKC suppresses fibroblast proliferation, whereas ζPKC plays a permissive role in basal and TGFβI-mediated cardiac fibroblast proliferation (**Figure 5*c***).

ET-1 Signaling: Role of PKC Isozymes

Endothelin-1 (ET-1) is another growth-promoting peptide that is upregulated by AngII and has a mitogenic effect on cardiac fibroblasts (117). PKC has been shown to be central to ET-1 signaling in cardiac myocytes (118); however, less is known about the role of PKC in mediating ET-1-dependent cardiac fibroblast proliferation. Inhibition of PKC using either staurospaurine or chelerythrine abolished the ET-1-induced increases in rat cardiac fibroblast DNA synthesis and cell proliferation (119) and downregulation of PKC following 24 h incubation with phorbol 12-myristate 13-acetate (PMA) prevents it. Immunoblotting and immunofluorescence studies revealed that the only isozyme that translocates following ET-1 stimulation is εPKC (119), implicating εPKC in mediating ET-1-induced proliferation in cardiac fibroblasts. However, further work is required to clarify the signaling events downstream of εPKC that result in cardiac fibroblast proliferation.

CTGF Signaling: Role of PKC Isozymes

Connective tissue growth factor (CTGF) is activated by TGFβI and has also been implicated in the regulation of fibroblast proliferation (120). Although the CTGF receptor has yet to be identified, tyrosine phosphorylation of LDL receptor-related protein after binding with CTGF has been reported (121) and has been suggested to be associated with fibroblast proliferation induced by TGFβI (122). Targeted overexpression of PKCβ2 in murine cardiac myocytes results in upregulation of TGF-β1 and CTGF and is accompanied by increased deposition of collagen type IV and VI, resulting in severe fibrosis (123). Whether the observed fibrosis is a direct consequence of PKCβ2 overexpression, or whether it is a compensatory response is unclear as these mice develop multifocal myocardial necrosis after only 3 weeks of age. To assess the involvement of PKC isoforms in the regulation of CTGF expression by AngII, neonatal rat cardiac myocytes were infected with adenoviral vectors containing dominant-negative PKC isoforms (120). AngII induced a twofold increase in CTGF expression which was inhibited by blockade of α, ζ, or εPKC. Interestingly, dominant-negative δPKC increased AngII-induced CTGF expression by ∼10-fold (120). Furthermore, in mice with targeted overexpression of δPKC in cardiomyocytes, CTGF expression induced by AngII was significantly reduced when compared with wild-type mice. Thus, whereas α, ζ, or εPKC may play a permissive role in AngII-induced upregulation of CTGF, δPKC may play a role as a negative regulator of CTGF expression.

Therapeutic Potential for PKC in Fibrosis

There is now growing evidence to support the hypothesis that PKC isozymes play a critical role in regulating cardiac fibrosis. However, owing to the complex progression of fibrosis, coupled with the number of PKC isozymes that appear to be involved, the exact role of each isozyme remains to be fully characterized. PKC isozymes can elicit distinct and in many cases opposing roles in the progression of fibrosis. In the case of εPKC, the same isozyme was reported to mediate both profibrosis and antifibrosis actions depending on the cell type or model used. For example, in rat cardiac fibroblasts, endothelin-1 stimulates εPKC phosphorylation and this is associated with proliferation (119). On the other hand, fibrosis induced by pressure overload is considerably worsened when cardiac εPKC is knocked out in mice (103). We have recently found that sustained and selective activation of εPKC (by continuous infusion of ψεRACK) in hypertensive rats increased cardiac fibrosis and that selective inhibition of this isozyme, by sustained treatment with the εPKC inhibitor, εV1-2, inhibited cardiac fibrosis in this model (K. Inagaki, T. Koyanagi, N. Berry, L. Sun, and D. Mochly-Rosen, submitted). These data suggest a benefit for εPKC inhibition in two animal models of excessive fibrosis. Activation of δPKC decreases fibroblast proliferation (116) and prevents upregulation of CTGF (120), and when δPKC is overexpressed in murine hearts, there is no overt pathophysiology [in contrast to mice overexpressing PKCβII, which develop severe fibrosis (123)], suggesting that selective activation of δPKC may be a possible therapeutic target. However, in our recent study using hypertensive rats that develop heart failure, inhibition of δPKC by sustained treatment with the selective δPKC inhibitor, δV1-1, did not affect cardiac fibrosis (K. Inagaki, T. Koyanagi, N. Berry, L. Sun, and D. Mochly-Rosen, submitted). Therefore, at this stage, the role of specific PKC isozymes in cardiac fibrosis remains to be fully resolved and warrants further investigation using isozyme-selective pharmacological compounds or gene-targeting strategies, such as knockout mice or RNAi, in a variety of animal models of cardiac disease.

PKC SIGNALING IN CARDIAC HYPERTROPHY AND HEART FAILURE

During the postnatal period, the myocardium undergoes hypertrophic growth associated with development of the heart. This hypertrophic growth is characterized by an increase in cardiomyocyte size but not in an increase in overall cell number. In the adult heart, several mechanical and neurohormonal stimuli can induce the reappearance of the neonatal cardiac hypertrophic phenotype. Cardiac myocyte hypertrophy is an adaptive mechanism triggered by decreased cardiac output. However, if cardiac hypertrophy is sustained, a further decrease in cardiac function and output develops, leading to heart failure and death. Many studies have investigated the intracellular molecular mechanisms that regulate developmental and pathological cardiac hypertrophy. Among the data obtained, PKC isozymes emerged as potential mediators of hypertrophic stimuli. Indeed, PKC activation with PMA causes cellular hypertrophy, and drugs that inhibit PKC also inhibit the hypertrophic response (135).

G protein–coupled receptor agonists such as phenylephrine, isoproterenol, and angiotensin II, as well as mechanical stretch, have been shown to induce cardiac hypertrophy through the activation of PKC (124, 127, 128). Moreover, the activity and expression of the PKC isozymes present in the myocardium are increased in various in vivo models of cardiac hypertrophy (125, 126, 128). Although those studies demonstrate that PKC activation is a major component of cardiac hypertrophy, they do not allow determination of whether it is causal or an epiphenomenon. Therefore, the involvement of individual PKC isozymes in the development of cardiac hypertrophy was addressed in several studies based on experimental approaches using cultured myocytes, transgenic mice overexpressing individual PKC isozymes, PKC knockout mice, and isoform selective agonist or antagonist peptides.

εPKC is activated in response to hypertrophic stimuli in cultured myocytes and in vivo (129), and overexpression and activation of εPKC results in myocardial hypertrophy (128). Transgenic mice overexpressing constitutively active εPKC develop a concentric hypertrophy with normal in vivo cardiac function (128). On the other hand, εPKC-knockout mice subjected to a transverse aortic constriction develop cardiac hypertrophy similar to wild-type mice, suggesting that εPKC is not required for the development of a pressure overload-induced cardiac hypertrophy (103). Cardiac myocyte-restricted εPKC activation in transgenic mice expressing the εPKC-specific activator (ψεRACK) induces a physiologic form of hypertrophy (132), and inhibition of εPKC by a selective inhibitor, εV1 fragment, expressed in low levels in transgenic mice results in a thin ventricular wall and an increased myocyte cell size (132). High levels of expression of εV1 causes a lethal form of heart failure from dilated cardiomyopathy (132). Transgenic mice expressing the selective δPKC activator ψδRACK in their cardiomyocytes exhibit hypertrophy similar to ψεRACK mice, indicating a common role for δPKC and εPKC in regulating postnatal cardiac hypertrophy (129).

Other PKC isozymes may also play a role in cardiac hypertrophy. The expression of βPKC is restricted to embryonic and neonatal cardiac myocytes and its gene expression is increased during pathological cardiac hypertrophy in adult animals and in failing human hearts (15, 123, 125, 126, 130). The direct role of βPKC in the development of cardiac hypertrophy emerged from studies using specific overexpression of βPKC in cardiac myocytes. Transient transfection of cultured cardiac myocytes with constitutively active β_{II}PKC increases the level of atrial natriuretic factor (ANF) and beta myosin heavy chain, all indicators of cardiac hypertrophy (123). Targeted overexpression of β_{II}PKC in mouse cardiomyocytes results in left ventricular hypertrophy and fibrosis (123). More importantly, oral treatment of the transgenic animal with LY333531, a βPKC inhibitor, prevented hypertrophy (123), supporting a direct relationship between β_{II}PKC and the pathological response. The conditional overexpression of constitutively active β_{II}PKC in adult cardiac myocytes also induces hypertrophy (134). In addition, the peptides βIV5-3 and βIIV5-3, specific translocation inhibitors of β_{I}PKC and β_{II}PKC, respectively, inhibited phorbol ester-induced hypertrophy of cultured rat neonatal cardiac myocytes, indicating a role for βPKC in the molecular events leading to developmental cardiac hypertrophy (135). However, mice with targeted disruption of the βPKC gene developed hypertrophy in response to phenylephrine or aortic banding similar to wild-type animals, suggesting

that βPKC is not necessary for cardiac hypertrophy (136). Further, treatment of hypertensive Dahl salt-sensitive rats with the β_{II}PKC-specific inhibitor, but not with the β_{I}PKC-specific inhibitor, greatly delayed the development of heart failure and death, suggesting a negative role of β_{II}PKC in heart failure (K. Inagaki, T. Koyanagi, N. Berry, L. Sun, and D. Mochly-Rosen, in preparation). These paradoxical results may be due to related PKC isozymes with an overlapping function compensating for the βPKC knockout. Taken together, studies using cultured cells and transgenic animals suggest that the signaling pathways leading to cardiac hypertrophy involves βPKC as well as εPKC.

The conflicting data concerning the involvement of individual PKC isoforms in the development of cardiac hypertrophy using genetically manipulated mice can sometimes complicate the identification of their role. We therefore suggest that the use of pharmacological tools specifically stimulating or inhibiting PKC translocation may be informative and more suited to determine the roles of individual PKC isozymes. In contrast to genetically modified animals, such tools would allow for better temporal control of each isozyme during the progression of the disease as well as better control of the dose and extent of PKC isozyme inhibition.

CONCLUSIONS

It is apparent that PKC isozymes play critical roles in atherosclerosis, cardiac fibrosis and heart failure (reviewed here), as well as in ischemic heart disease (3–5, 7). Sifting through hundreds of publications implicating PKC in a variety of pathologies related to cardiac disease is daunting, in part, because of what may appear to be conflicting data. If the goal is to identify the appropriate candidate for therapy, sole reliance on genetically manipulated mice or in-culture studies can be misleading. This is not only because the models do not completely recapitulate the human disease state but also because early pre- and postnatal events, which can be triggered by culturing or by genetic manipulations in vivo, contribute to the outcome. The pharmacological approach is advantageous as treatment and withdrawal of treatment during various stages of the disease provide better assessment of causality. Nevertheless, the value of these data solely depends on the selectivity of the pharmacological tools.

We suggest that the current focus on receptors and early events in the signaling pathways leading to cardiac pathology needs to be changed. Intracellular signaling enzymes, such as PKC isozymes that are in the middle of the signaling cascade (**Figure 1**), provide excellent targets for drug development. We also suggest that a great degree of selectivity can be obtained by targeting protein-protein interactions rather than the catalytic site per se. The approach employed in our lab, using rationally designed peptide inhibitors and activators for each PKC isozyme is a cheap and easy way of doing this. The peptides are effective as pharmacological tools in culture and in vivo and can be delivered in acute and chronic disease models. As mentioned earlier, one such inhibitor of δPKC recently completed Phase II clinical studies for the treatment of acute myocardial infarction with encouraging results (29). Whether peptides can be delivered readily into humans for chronic indications remains to be determined. However, many new approaches, including injection of slow release

nanoparticles, inhaled particles, and transdermal delivery may enable treatment of chronic disease with intracellularly acting peptides. Finally, we hope that promising data using small-molecule regulators of PKC in clinical trials will encourage the pharmaceutical industry to explore PKC for chronic diseases again, including the number one cause of death in the United States and the world, heart disease.

SUMMARY POINTS

1. PKC isozymes could serve as targets for inhibition of atherosclerotic disease progression. Selective activators of αPKC, the isozyme that appears to be responsible for hepatic uptake of LDL, may provide a means to block the most potent initiating atherosclerotic signal, LDL. δPKC may be another target of therapeutic importance because of its role in macrophage oxLDL accumulation as well as in VSMC apoptosis.
2. The role of specific PKC isozymes in cardiac fibrosis remains to be fully resolved and warrants further investigation using isozyme-selective pharmacological compounds or gene-targeting strategies, such as knockout mice or RNAi in a variety of animal models of cardiac disease. However, the novel isozymes, ε and δPKC, are potential targets.
3. Studies using cultured cells and transgenic animals suggest that the signaling pathways leading to cardiac hypertrophy involve βPKC as well as εPKC.
4. When targeting the early events in signal transduction pathways, caution must be taken because cascades often include side branches, amplification steps, and feedback loops. Intracellular signaling enzymes, such as PKC isozymes, that are in the middle of the signaling cascade provide excellent targets for drug development.
5. We also suggest that a great degree of selectivity can be obtained by targeting protein-protein interactions rather than the catalytic site per se. Future work can determine whether inhibitors of protein-protein inhibitors are effective and well tolerated for the treatment of chronic diseases such as those discussed above.

DISCLOSURE STATEMENT

D.M.-R. is the founder of KAI Pharmaceuticals, a company that plans to bring PKC regulators to the clinic. This work was supported by NIH grant HL 76675 to D.M.-R.

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