Review

Epsilon protein kinase C as a potential therapeutic target for the ischemic heart

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Abstract

Ischemic heart disease is the leading cause of morbidity and mortality in the western world. Ischemic damage can occur by acute myocardial infarction, stable angina, cardiac stunning, and myocardial hibernation. In addition, ‘scheduled’ ischemic events, occurring during cardiac surgery, heart transplantation, and elective angioplasty, can also result in cardiac damage. Ischemic or pharmacological preconditioning can decrease the extent of damage to the myocardium. Although the mechanism of preconditioning-mediated cardioprotection is not fully understood, εPKC has been implicated as a critical mediator of this process in animal studies. The use of isozyme-specific pharmacological tools has permitted a better elucidation of the upstream stimuli and the downstream transducers of εPKC in the pathways leading to cardioprotection. While little is known about the role of εPKC in these pathways in humans, animal studies suggest a potential therapeutic role of εPKC. This review will focus on the role of εPKC in cardiac protection and on the signal transduction cascades that have been implicated in this protection.

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1. Introduction

Ischemic heart disease is the leading cause of death in the western world and the ability to reduce this damage by subjecting the heart to short bouts of ischemia prior to the prolonged ischemic episode, a process termed ischemic preconditioning, has been a focus of research since its first description [1]. Preconditioning reduces damage to the myocardium when the time of cardiac ischemia is predictable, such as the damage occurring during bypass surgery, cardiac transplantation, and elective angioplasty. The elucidation of signaling molecules that mediate ischemic/hypoxic preconditioning allowed for the identification of pharmacological agents that can act as preconditioning mimetics. Here, we will review the role of epsilon protein kinase C (εPKC) in the molecular events leading to preconditioning. We will also describe pharmacological preconditioning mimetics and the potential of PKC regulating drugs as therapeutic agents in a clinically relevant setting.

2. PKC isozymes in ischemic/hypoxic preconditioning

Over 10 years ago, Ytrehus et al., showed that inhibition of PKC blocks the protection afforded by ischemic/hypoxic preconditioning in a rabbit model [2]. However, which isozyme mediates this effect is still controversial (Table 1). Whereas εPKC and ηPKC translocate from the cytosolic fraction to the particulate fraction in ischemic/hypoxic preconditioned rabbit myocardium [3], δPKC and εPKC

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1 DMR is the founder of KAI Pharmaceuticals, Inc, a company that plans to bring PKC regulators to the clinic. However, none of the work described in the review is based on or supported by the company.

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translocate in ischemic/hypoxic preconditioned neonatal rat cultured cardiomyocytes and in isolated rat hearts [4,5].

Cardiac-specific overexpression of εPKC or expression of a εPKC-activating peptide confers cardioprotection against ischemia/reperfusion-mediated damage [6,7], and ischemic/hypoxic preconditioning fails to diminish infarct size in εPKC knockout mice [8]. Therefore, εPKC is required and sufficient to induce cardioprotection. Cardiac specific expression of a δPKC-activating peptide increases ischemia/reperfusion mediated damage [9] and expression of a δPKC-inhibitory peptide decreases it [10,11]. In contrast, some studies suggest that δPKC has a cardioprotective role in ischemic/hypoxic preconditioning [12–14] and that δPKC knockout mice exhibit increased damage following ischemic preconditioning (Table 1) [12].

The use of transgenic or knockout mice was instrumental in identifying the role of specific PKC isozymes during ischemia/reperfusion. However, the challenge in relying on genetically modified mice is that sustained gain or loss of a PKC function may alter cardiac development and functions not related to the response to the acute ischemic event. A better approach is the use of pharmacological tools to study the roles of PKC isozymes in ischemic/hypoxic preconditioning. These could then be applied clinically as therapeutic agents for ischemic heart disease.

3. Pharmacological tools to study the role of PKC in cardiac ischemia

PKC activators, such as phorbol 12-myristate 13-acetate (PMA) and diacylglycerol (DAG), or various PKC inhibitors have been used to study the roles of PKC during ischemia/reperfusion. However, these agents show poor selectivity for individual PKC isozymes [15–18]. Because of the opposing roles of many of the individual isozymes in the cardiac response to ischemia/reperfusion injury [9,19,20], these non-selective pharmacological tools may have contributed to the conflicting data [21].

To generate isozyme-selective pharmacological tools, we have focused on the observation that when activated, PKC isozymes translocate from one cellular compartment to another, placing the activated PKCs near their corresponding substrates. We showed that the location of the activated PKC isozymes is determined, in part, by their anchoring proteins, termed RACKs [22]. We have capitalized on this observation and have rationally designed PKC isozyme-selective regulating peptides [23] that modulate the subcellular location of PKCs [22,23]. Peptide inhibitors compete with the binding of an isozyme to its RACK, whereas peptide activators promote this interaction. The peptides (6–10 amino acids in length) are highly selective and effective (IC50 at 1–10nM) [23], and membrane permeable due to conjugation with carrier peptides such as TAT4–57 [24]. Use of these isozyme-selective PKC regulators in several laboratories has helped in determining the role of PKC isozymes both in vitro and in vivo.

The use of the εPKC-selective peptide activator, ψεRACK, prior to or early during the ischemic event, conferred cardioprotection against ischemia/reperfusion injury, whereas the εPKC-selective peptide inhibitor, εV1-2, prevented ischemic/hypoxic or pharmacologic preconditioning in mice, rats, rabbits and pigs [4,7,9,19,25,26]. Infusion of the δPKC-selective peptide inhibitor, δV1-1, during reperfusion confers cardioprotection against reperfusion injury but exerts no effect when delivered just prior to ischemia [11,27].

Although treatment with a δPKC-selective peptide activator, ψδRACK immediately before ischemia exagger-
ated cardiac damage, δPKC activation an hour prior to the ischemic event resulted in cardioprotection in the mouse heart [9,28]. The protection afforded by δPKC activation was inhibited by both an A1 receptor antagonist, DPCPX, and by the δPKC inhibitor V1-2, suggesting that δPKC-mediated cardioprotection is dependent upon both δPKC and adenosine-mediated pathways [28]. The role of δPKC-induced adenosine-dependent cardioprotection was also suggested in early studies in myocytes where δPKC was overexpressed [14]. PKC activation increases 5′nucleotidase activity, which then dephosphorylates 5′AMP to form adenosine [29,30]. Adenosine transporter function is also regulated by δ and/or εPKC [31,32]. Non-selective PKC regulators suggest that δPKC may also be protective in opiate-induced cardioprotection [33]. Therefore, εPKC activation is required for preconditioning and activation of δPKC during reperfusion is detrimental whereas transient activation of δPKC early prior to ischemia may induce cardioprotection by εPKC activation (Fig. 1).

4. The signal transduction pathways of εPKC in cardiac preconditioning

Both rational search and the systematic proteomic studies identifying proteins that are complexed with εPKC assisted in elucidating the pathway of εPKC in cardiac preconditioning [34–41]. In the following sections, some of the upstream stimuli and downstream transducers of εPKC, as well as end-effectors of preconditioning will be discussed.

4.1. Upstream stimuli of εPKC

Most of the preconditioning mimetic agents activate εPKC through G protein-coupled heptahelical transmembrane receptors (GPCR). Ligands of the GPCR are released during a brief ischemic event (including adenosine, catecholamines, angiotensin II, bradykinin, and endothelin) and induce preconditioning through activation of the corresponding G proteins [5,42–46]. The active G proteins then stimulate phospholipases to generate diacylglycerol and in some cases inositol-triphosphate, which releases calcium from internal stores. Diacylglycerol (with or without calcium) then activates PKC (Fig. 1) [47]. As discussed later, it is possible that reactive oxygen species and/or nitric oxide, which are both generated during the brief ischemic event, can cause direct modification of εPKC leading to its activation [96,100].

4.2. Downstream transducers of εPKC: ERKs and Lck in ischemic/hypoxic preconditioning

Ischemic/hypoxic preconditioning result in the phosphorylation and activation of p42 and p44 extracellular signal-
regulated kinases (ERKs) and the ERK inhibitor, PD98059, attenuates the cardioprotection afforded by ischemic/hypoxic preconditioning [48,49]. Administration of the εPKC activator, ψεRACK, which reduces infarct size and creatine phosphokinase release in isolated perfused mouse hearts, also resulted in ERKs phosphorylation [26,28]. Finally, εPKC co-immunoprecipitates with and phosphorylated ERKs at the mitochondria, where the εPKC-ERKs complex can phosphorylate the pro-apoptotic protein BAD [37]. Therefore, the phosphorylation of ERKs by εPKC during ischemic/hypoxic preconditioning may confer cardioprotection by inhibition of pro-apoptotic proteins (Fig. 1) [28,37,50].

Another downstream transducer of εPKC is a member of the Src family of tyrosine kinases, Lck. Preconditioning induces a signaling complex with εPKC resulting in phosphorylation and activation of Lck in cardiac cells [6]. Disruption of the complex by ablation of the Lck gene, abrogated the infarct-sparing effects of preconditioning or εPKC overexpression, indicating that the formation of εPKC-Lck signaling modules is required for the manifestation of a cardioprotective phenotype [6].

4.3. Effectors of preconditioning: K_{ATP} channels, mitochondrial permeability transition pore and εPKC in ischemic/hypoxic preconditioning

εPKC translocates from the cytosol to the sarcolemmal membrane and to mitochondria upon activation [33,51]. The ATP-sensitive K⁺ channel (K_{ATP} channel) exists both in the sarcolemmal membrane and in the mitochondria, and is likely an end-effector of ischemic/hypoxic preconditioning [52]. The K_{ATP} channel is inhibited by high levels of adenosine triphosphate (ATP) and opens as ATP levels fall [53]. Other studies suggest that the surface or sarcolemmal K_{ATP} channel might be one of the critical effectors of the cardioprotective effects of ischemic/hypoxic preconditioning [54]. Opening of the sarcolemmal K_{ATP} channel by hypoxia or pharmacological K_{ATP} channel openers may increase cell viability by shortening cardiac action potential duration, thereby reducing calcium overload during ischemia and early reperfusion [55,56]. PKC can directly activate single sarcolemmal K_{ATP} channels at physiological levels of ATP in rabbit ventricular myocytes [57]. Stimulation of PKC by PMA activates the sarcolemmal K_{ATP} channel and mimics the cardioprotective effect of ischemic preconditioning. These studies also showed that glibenclamide or 5-hydroxydecanoate (K_{ATP} channel blockers) inhibits the PMA-induced preconditioning in chick embryo ventricular myocytes [58].

Garlid et al. demonstrated that a potent opener of mitochondrial K_{ATP}, diazoxide, has little effect on cardiac action potential duration, but it decreases cardiac damage from ischemia in isolated perfused rat hearts [59], an effect blocked with the mitochondrial K_{ATP} channel blocker 5-hydroxydecanoate (5-HD). Therefore, the mitochondrial K_{ATP} channel is a likely end-effector in preconditioning-mediated cardioprotection. Opening of the mitochondrial K_{ATP} channel stabilized the mitochondrial membrane potential, reduces mitochondrial Ca^{2+} overload, prevents ATP depletion and the generation of reactive oxygen species [60,61]. The PKC activator phorbol ester was found to increase the mitochondrial K_{ATP} channel activity [62], and infarct size limitation by a selective mitochondrial K_{ATP} channel opener (diazoxide) was abolished by a selective inhibitor of the mitochondrial K_{ATP} channel 5-HD but not by a PKC inhibitor (calphostin C) [63]. These data indicate that the mitochondrial K_{ATP} channel is an end-effector of preconditioning and that possibly εPKC is upstream of this.

The mitochondrial permeability transition pore (MPTP) is downstream of εPKC [64,65], and is another candidate effector of preconditioning [65–67]. Opening of the MPTP allows water and solutes to enter the mitochondria, increasing matrix volume, and rupturing the outer mitochondrial membrane. This can lead to the release of intermembrane cytochrome c, which in turn can trigger apoptosis. In addition, pore opening uncouples mitochondria, leading to ATP hydrolysis and collapse of the mitochondrial membrane potential. The MPTP is closed during ischemia and only opens in the first few minutes of reperfusion [68]. Baines et al. demonstrated that εPKC interacts with MPTP, leading to phosphorylation of MPTP associated voltage-dependent anion channel [64]. Additionally, incubation of isolated cardiac mitochondria with recombinant εPKC inhibits Ca^{2+}-induced mitochondrial swelling, a correlate of MPTP opening [64]. Overexpressed εPKC interacts with the MPTP components and inhibits Ca^{2+}-induced MPTP opening [64], and the MPTP opener atracylloside significantly attenuated this infarct-sparing effect of εPKC overexpression [64]. Therefore, the cardioprotective effects of εPKC may partly or solely be due to the enzyme’s ability to interact with and maintain the MPTP in a closed conformation.

4.4. εPKC pathway in late preconditioning

Activation of εPKC also mediates the protective effects of late preconditioning. This is a delayed protective adaptation, whereby preconditioning stimuli enhance the resistance of the heart to ischemia/reperfusion injury 12–72 h later [69]. Late preconditioning results from upregulation of several cardioprotective genes, including inducible nitric oxide synthase, cyclooxygenase-2, heme oxygenase-1, aldose reductase and Mn superoxide dismutase, by activation of transcription factors, such as nuclear factor-kB (NFkB) and activator protein (AP)-1 [69,71–73].

PKC activates the I/B kinase that phosphorylates IκB, an inhibitor of the transcription factor NFκB. Phosphorylated IκB dissociates from NFκB, allowing its entry into the nucleus to exert transcriptional activity [71,72]. Finally, εPKC has also been shown to translocate to the nucleus suggesting a possible role in the mediation of transcription...
there [5,51]. Li et al. showed that activation of εPKC increased the DNA-binding activity of NFκB and AP-1 in adult rabbit cardiomyocytes, and this effect is completely abolished by inhibition of ERK with PD98059 [73]. Additionally, Xuan et al. showed that late preconditioning causes STAT1 and STAT3 phosphorylation by the εPKC-ERK pathway, resulting in the upregulation of COX-2 [74]. These data indicate that the εPKC-ERKs complex or εPKC alone activates transcription factors, leading to the upregulation of cardioprotective genes in late preconditioning.

5. ROS, PKC and preconditioning-mediated cardioprotection

There is a correlation between the generation of reactive oxygen species (ROS) and cardiac protection [75–78]. Exogenous ROS mimic preconditioning whereas inclusion of anti-oxidants during preconditioning blocks cardioprotection [76,79,80]. Furthermore, pharmacological preconditioning with agents such as angiotensin II [77,81] and KATP channel opener [79,82–85] increase the cardioprotective ROS production. What is the source of ROS? Mitochondrial respiratory chain inhibitors suggest that ROS such as superoxide generated by the mitochondria are important in cardioprotection (Fig. 1, [79,85]). In contrast, the protective effects of ischemic/hypoxic preconditioning is lost in NADPH oxidase subunit, NOX-2, knock-out hearts suggesting that this enzyme is also a source of ROS [86]. Per Kimura et al., this can be reconciled if protection is first mediated by a mitochondrial source of ROS, which is subsequently enhanced through NADPH oxidase activation by JNK and p38 [81].

The protective effect of ROS during preconditioning does not contradict the role of ROS during prolonged reperfusion in mediating cellular damage and death [87]. Real-time measurement of superoxide production in guinea pig hearts showed that ischemic/hypoxic preconditioning decreases superoxide production during both ischemia and reperfusion [88]. In addition, small amounts of superoxide are produced during the ischemic/hypoxic preconditioning stimulus [76,78,80]. Baines et al. showed that whereas the initial preconditioning stimulus affords protection through the generation of ROS, subsequent preconditioning stimuli may protect through other mechanisms involving adenosine [76]. Therefore, a small burst of ROS during the ischemic/hypoxic preconditioning stimulus may mediate a decrease in ROS production during reperfusion and/or may sensitize the cell to the burst of ROS by increasing antioxidant defenses; data exist to support these scenarios [78,88,89].

Work by Bolli et al. demonstrated that nitric oxide (NO) generation during ischemic preconditioning may act as a trigger of preconditioning-mediated cardioprotection [90,91]. Additionally, NO donors afforded the same protection seen during ischemic/hypoxic preconditioning. Inclusion of peroxynitrate and hydroxyl radical scavengers completely abrogated the effects of the NO donors, suggesting that the NO-mediated mechanism of protection involves the generation of pro-oxidant species [92]. Together these data suggest that whereas elevated levels of ROS can be deleterious to the cell, small amounts of ROS may be protective by triggering cardiac preconditioning.

What is the relationship between δPKC and ROS generation? Ischemic/hypoxic preconditioning in a δPKC knockout heart resulted in diminished ROS production and decreased cardiac protection, placing δPKC upstream in the cascade leading to ROS generation [12]. Furthermore, δPKC translocation to the mitochondria increases ROS production and selective inhibition of this translocation reduces ROS levels and improves mitochondrial function [93]. δPKC is also activated by ROS [94]. Specifically, exposure of cells to hydrogen peroxide and ultraviolet radiation (a ROS generator) results in tyrosine phosphorylation and activation of this isozyme [95,96] as well as translocation of δPKC to the mitochondria [97,98]. Therefore, δPKC may be both upstream and downstream of ROS generation (Fig. 1).

In addition to the ROS-mediated activation of δPKC, recent studies delineate the involvement of ROS in the activation of εPKC. Removal of hydrogen peroxide during ischemic/hypoxic preconditioning of chick cardiomyocytes resulted in the abrogation of εPKC activation [99]. Additionally, treatment of these cells with hydrogen peroxide activates εPKC and induces cardioprotection from hypoxia/reoxygenation injury, which is blocked by inhibition of K_{ATP} channel opening [99].

In addition to the activation of εPKC by hydrogen peroxide, treatment with NO selectively activates εPKC in the rabbit heart, leading to late preconditioning-like effects [100]. Ischemia-induced generation of NO is also sufficient to activate εPKC [100] and association with Src, which when blocked, can abolish NO-induced late preconditioning [39]. These data suggest that oxidant-mediated activation of εPKC may play a role in cardioprotection. Conversely, εPKC may also inhibit ROS production by preserving cellular ATP levels: preconditioning places this isozyme at the mitochondrial pore, leading to protection of mitochondrial function, which should decrease ROS generation as well as maintain ATP levels (Fig. 1) [64,101].

6. Anesthetic-mediated cardioprotection

Because preconditioning is particularly applicable to cardiac protection from scheduled ischemia, such as that occurring during surgeries, the possible preconditioning-mimetic effect of some general anesthetics is important to review. Halogenated volatile anesthetics mimic preconditioning in a mechanism that may depend on εPKC activation [102,103]. For example, inhibition of εPKC with εV1-2 inhibits isoflurane-induced protection of human myocardi um [104]. In addition to halogenated volatile anesthetics, the
noble gas xenon induces translocation of εPKC and pharmacological preconditioning in rat hearts, in vivo. This effect is blocked by a non-selective PKC inhibitor, calphostin C [105]. In another model of isoflurane-induced preconditioning, δPKC is activated by ROS in a K_{ATP} channel-independent mechanism, and preconditioning by isoflurane sensitizes the sarcolemmal K_{ATP} channel that is blocked in the presence of the δPKC-specific inhibitor, δV1-1 [106]. Finally, opioids (analgesics that are often used in conjunction with general anesthetics) also mimic preconditioning, and require activation and translocation of δPKC to the mitochondria [13,48]. Together these data suggest that in addition to εPKC-mediated protection by some general anesthetics, there is a possible role of δPKC in anesthetic preconditioning that is downstream of ROS generation.

A few clinical studies of anesthetic preconditioning on a small number of patients have been conducted, with somewhat limited success. Five-minute exposure to isoflurane before aortic cross-clamping and cardioplegic arrest reduced cardiac damage assessed by the release of troponin I and creatine kinase-MB [107]. Sevoflurane and desflurane preserved left ventricular function after coronary bypass surgery in patients with three-vessel disease and an ejection fraction below 50% [108]. However, no clinical investigation to date has shown decreased long-term morbidity and mortality by anesthetic preconditioning nor the role of PKC isoymes in this process.

7. PKC and cardiac transplant

Although 1-year allograft survival and patient survival after cardiac transplantation continues to improve, the incidence of heart failure caused by graft coronary artery disease has not changed [109]. Ischemia/reperfusion injury is the strongest alloantigen-independent factor for the subsequent development of this graft coronary disease [110]. Furthermore, transplant ischemia induces a pro-inflammatory environment, which includes an influx of injurious cytokines and chemokines and upregulation of adhesion molecules on the vascular endothelium [111].

The potential benefit of PKC regulation and preconditioning mimetics around the time of transplantation was recently examined in two rodent models of cardiac transplantation. Donor hearts were harvested into solution containing the εPKC activator, ψεRACK, as a preconditioning agent, and the recipient animals were intraperitoneally treated once with the δPKC inhibitor, δV1-1, before reperfusion, to prevent reperfusion injury in genetically mismatched transplantation of hearts in mice [112] and in rats [113]. Although the animals were treated with the immunosuppressive drug, cyclosporine, acute treatment with these PKC regulators reduced immediate pro-inflammatory responses. Importantly, graft coronary artery disease was inhibited by more than 50% and improved cardiac allograft function was also noted by 30 and 90 days post-transplantation [112,113]. Therefore, pharmacological preconditioning including an εPKC activator with or without treatment with δPKC inhibitor may be beneficial in improving patient survival after cardiac transplantation surgery.

8. Potential clinical implications

The seminal discovery of preconditioning over 20 years ago stirred extensive studies to elucidate the molecular basis of this phenomenon, mainly because of its potential to ameliorate human health. The therapeutic intervention by pharmacological preconditioning may be possible in situations when the timing of ischemia is predetermined, such as during cardiac surgery with extracorporeal circulation or, as discussed above, just prior to cardiac transplant surgery. Further, patients at high risk for eminent infarction may also benefit from preconditioned mimetics. Based on data generated in several independent laboratories using five animal models (mice, rats, rabbits and pigs) and human tissue [4,7,9,11,25,26,112,113], it appears that selective activation of εPKC provides such protection against ischemic damage. These studies also indicate that εPKC activation is an early step in this pathway. Furthermore, the finding that treatment with ψεRACK during ischemia also reduced the incidence of malignant ventricular arrhythmia in an in vivo model of acute myocardial infarction [26] suggest an additional benefit for this treatment. Although the sustained treatment with ψεRACK is well tolerated and did not result in desensitization to the cardioprotective effect in animals [26,114], it remains to be determined whether activation of εPKC is well tolerated in humans having other co-morbidity factors including age, diabetes and obesity.

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