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Original article

# δPKC-mediated activation of εPKC in ethanol-induced cardiac protection from ischemia

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**Abstract**

Previous studies have demonstrated that acute ethanol exposure induces activation of δ protein kinase C (δPKC) and εPKC, and mimics ischemic preconditioning *via* εPKC activation. However, the role of δPKC isozyme in ischemia and reperfusion is still controversial. Here, we investigated the role of δPKC in ethanol-induced cardioprotection using a selective δPKC activator (ψδRACK), or inhibitor (δV1-1), and a selective εPKC inhibitor (εV1-2) in isolated mouse hearts. Mice were injected intraperitoneally or by gavage with ethanol, regulators of δ and εPKC or an adenosine $A_1$ receptor blocker (DPCPX). Isolated perfused mouse hearts were subjected to a 30-min global ischemia and a 120-min reperfusion, ex vivo. Injection of 0.5 g/kg ethanol 1 h, but not 10 min, before ischemia reduced infarct size and CPK release. Pretreatment with εV1-2 abolished this ethanol-induced cardioprotection. Pretreatment with δV1-1 induced cardioprotection when injected with ethanol (0.5 g/kg) 10 min before ischemia, but δV1-1 partly inhibited ethanol-induced cardioprotection when injected with ethanol 1-h before the onset of ischemia. ψδRACK injection 1 h, but not 10 min, before ischemia induced cardioprotection and translocation of εPKC from the cytosol to the particulate fraction. Pretreatment with DPCPX or εV1-2 inhibited ψδRACK-induced cardioprotection and translocation of εPKC. Therefore, activation of δPKC-induced by ethanol or by the δPKC activator is cardioprotective, provided that sufficient time passes to allow δPKC-induced activation of εPKC, an $A_1$ adenosine receptor-dependent process.


*Keywords:* Alcohol; Ischemia; Protein kinase C; Cardioprotection; Adenosine

## 1. Introduction

The benefit of moderate amounts of ethanol consumption for patients at risk of coronary artery disease is well documented [1–3]. Moreover, animal studies indicate that acute exposure to ethanol also exerts direct protection to the heart, if administered *prior* to the ischemic event [4–6]. This effect of ethanol mimics ischemic preconditioning, as demonstrated in rat cardiomyocytes, isolated perfused mouse and rabbit hearts, and in vivo rabbit hearts [4–6]. However, The limitation in the timing of ethanol administration is detrimental for optimal protection [6]. This narrow 'therapeutic' window of ethanol strongly suggests that it activates opposing mechanisms. Supporting this hypothesis are our findings that ethanol activates two protein kinase C (PKC) isozymes [4]: εPKC, an isozyme that mediates cardioprotection from ischemia [4,5,7–12], and δPKC, an isozyme that mediates reperfusion injury [9,13].

Furthermore, there is still some controversy as to which PKC isozyme mediates cardioprotection from ischemia/reperfusion (I/R). Translocation of δPKC and εPKC is induced by ischemic preconditioning in neonatal rat cultured cardiomyocytes [11] and in rat whole hearts [14]. An εPKC-selective activator conferred cardioprotective effects against I/R in rats and mice [5,7–9], and an εPKC-selective inhibitor inhibited cardioprotective effects induced by ischemic preconditioning in mice, rats, and rabbits [7,11,12]. We have previously found that δPKC mediates reperfusion injury [9,13], however, some earlier studies suggested that δPKC plays a cardioprotective role in ischemic preconditioning [15–18].

Here, we found that δPKC activation by intraperitoneal injection (IP) of ethanol an hour before the ischemic event mediated cardioprotection from I/R-induced injury by acti-

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vating the cardioprotective enzyme, εPKC. Furthermore, injection of the selective δPKC activator, ψδRACK, also induced cardioprotection by activating εPKC. Finally, we found that this activation of εPKC is blocked by the adenosine $A_1$ receptors antagonist, DPCPX. These data are discussed in view of the narrow time window of cardiprotection from I/R injury afforded by ethanol treatment.

## 2. Methods

### 2.1. Peptide synthesis

We synthesized ψδRACK (δPKC activator, amino acids 74–81 [MRAAEDPM]) [7], δV1–1 (δPKC inhibitor, amino acids 8–17 [SFNSYELGSL]) [9], ψεRACK (εPKC activator, amino acids 85–92 [HDAPIGYD]) [7], and εV1–2 (εPKC inhibitor, amino acids 14–21 [EAVSLKPT]) [11] at Stanford's Protein and Nucleic Acid facility and conjugated them to TAT (carrier peptide, amino acids 47–57 [YGRKKRRQRRR]) [19] *via* a cysteine–cysteine S–S bond at their N termini, as previously described [20].

### 2.2. Isolated perfused mouse heart model

Isorated perfused mouse heart model was prepared as previously reported [21]. FVB/N mice (20 g) were heparinized (4000 U/kg IP) and anesthetized with sodium pentobarbital (200 mg/kg IP). The hearts were rapidly excised, and then perfused with an oxygenated Krebs–Henseleit solution containing (in mmol/l); NaCl 120, KCl 5.8, $NaHCO_3$ 25, $NaH_2PO_4$ 1.2, $MgSO_4$ 1.2, $CaCl_2$ 2.0, and dextrose 10, pH 7.4, at 37 °C in a Langendorff coronary perfusion system. Coronary flow rate was kept constant during the experiment at 2.5 ml/min. Hearts were submerged into a heat-jacketed organ bath set at 37 °C. Coronary effluent was collected to determine creatine phosphokinase (CPK) release. Hearts were subjected to a 30-min global ischemia and a 120-min full reperfusion after 10 min of equilibration. At the end of the reperfusion period, hearts were sliced into 1-mm-thick transverse sections and incubated in triphenyltetrazolium chloride solution (TTC) (1% in phosphate buffer, pH 7.4) at 37 °C for 15 min as previously reported [9]. Infarct size was expressed as a percentage of the risk zone (equivalent to total left ventricular muscle mass).

### 2.3. Experimental protocol in isolated perfused mouse hearts

A PKC isozyme regulating peptide at 20 nmol in 200 μl of saline was injected intraperitoneally before ischemia, and ethanol was injected or administrated by gavage. In the first set of experiments, mice were treated with ethanol (0.1, 0.5 or 4.0 g/kg) 10, 60 or 90 min before sacrifice (Fig. 1). In the second set of experiments, mice were pretreated with ethanol (0.1 g/kg, 100 μl) by gavage 60 min before sacrifice (Fig. 2). In the third set of experiments, mice were pretreated with saline, δV1-1 or εV1-2 (20 nmol) 10 min before injecting

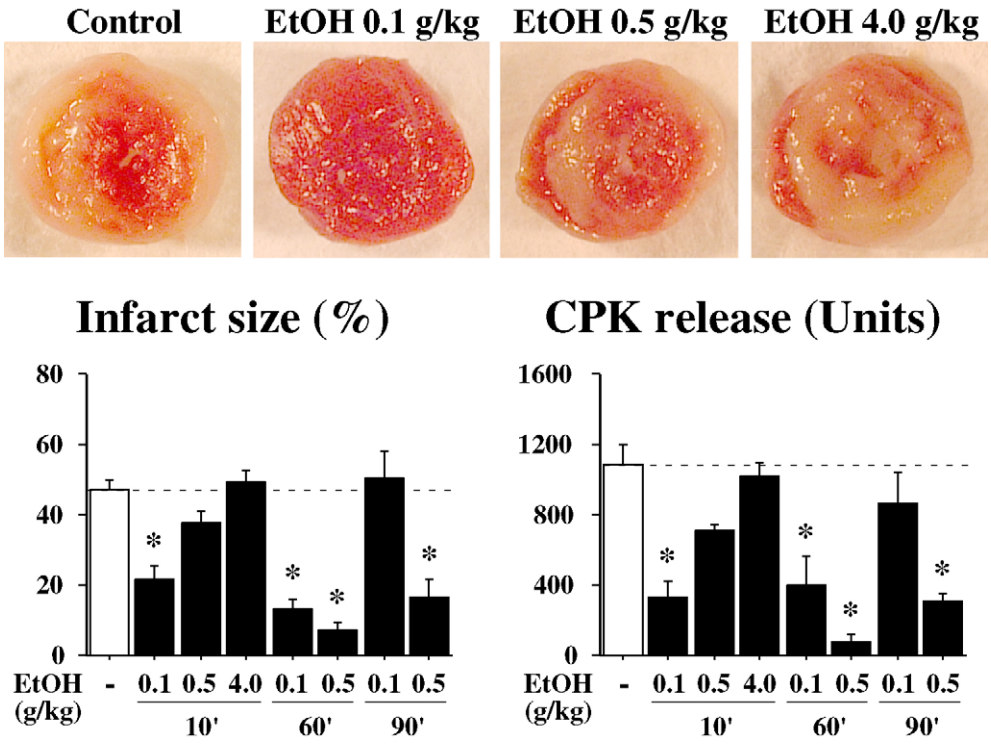


Fig. 1. Time–course and dose-dependence of ethanol (EtOH)-induced cardioprotection. Representative pictures shows infarcted area (white) and non-infarcted area (red) stained by TTC in mouse hearts subjected to I/R ex vivo, 10 min after injection of ethanol (0.1–4 g/kg), in vivo. Cardiac damage was evaluated by infarct size and CPK release. *$P < 0.002$ vs. control. $n = 4$–6 for each group.