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Protective role of protein kinase C epsilon activation in ischemia–reperfusion arrhythmia

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Abstract

Purpose: Ischemic heart disease carries an increased risk of malignant ventricular tachycardia (VT), fibrillation (VF), and sudden cardiac death. Protein kinase C (PKC) epsilon activation has been shown to improve the hemodynamics in hearts subjected to ischemia/reperfusion. However, very little is known about the role of epsilon PKC in reperfusion arrhythmias. Here we show that epsilon PKC activation is anti-arrhythmic and its inhibition is pro-arrhythmic.

Method: Langendorff-perfused isolated hearts from εPKC agonist (εPKC activation), antagonist (εPKC inhibition) transgenic (TG), and wild-type control mice were subjected to 30 min stabilization period, 10 min global ischemia, and 30 min reperfusion. Action potentials (APs) and calcium transients (Ca_iT) were recorded simultaneously at 37 °C using optical mapping techniques. The incidence of VT and VF was assessed during reperfusion.

Results: No VT/VF was seen in any group during the stabilization period in which hearts were perfused with Tyrode's solution. Upon reperfusion, 3 out of the 16 (19%) wild-type mice developed VT but no VF. In εPKC antagonist group, in which εPKC activity was downregulated, 10 out of 13 (76.9%) TG mice developed VT, of which six (46.2%) degenerated into sustained VF upon reperfusion. Interestingly, in εPKC agonist mice, in which the activity of εPKC was upregulated, no VF was observed and only 1 out of 12 mice showed only transient VT during reperfusion. During ischemia and reperfusion, Ca_iT decay was exceedingly slower in the antagonist mice compared to the other two groups.

Conclusion: Moderate in vivo activation of εPKC exerts beneficial antiarrhythmic effect vis-a-vis the lethal reperfusion arrhythmias. Abnormal Ca_iT decay may, in part, contribute to the high incidence of reperfusion arrhythmias in the antagonist mice. These findings have important implications for the development of PKC isozyme targeted therapeutics and subsequently for the treatment of ischemic heart diseases.

Keywords: Optical mapping; εPKC; Arrhythmia; Ischemia–reperfusion

Ischemic heart disease is the leading cause of congestive heart failure and death in the Western world [1]. Early reperfusion after coronary occlusion improves cardiac function and reduces infarct size, but it is invariably accompanied by an overload of intracellular Ca that mediates cellular damage, contractile dysfunction [2,3] and fatal ventricular arrhythmias, such as ventricular fibrillation (VF). Effort has thus been made to minimize the adverse arrhythmic events related to myocardial ischemia–reperfusion.

PKC is a key enzyme in signal transduction involved in a variety of cellular functions [4] and was the first kinase examined in detail in ischemia–reperfusion models. PKC is a serine/threonine kinase, consisting of an N-terminal regulatory and C-terminal catalytic region, and is activated by diacylglycerol (DAG) produced in response to hormones such as α-adrenergic agonist, angiotensin II, or endothelin (ET-1) [5,6]. Three PKC subgroups have been identified: conventional PKC (α, βI, βII, and γ), novel

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PKC (ε, δ, and θ), and atypical PKC (ζ and ι/λ) [7,8]. PKC plays a major role in cardioprotection from ischemia–reperfusion injury in various cell culture studies and in animal models [9–11]. Of the 11 PKC isozymes, α, β_I, β_{II}, ε, λ, δ, ζ, and γ PKC have been identified in the heart in different animal species [12–15]. The role of individual isozymes in cardioprotection was difficult to assess because the pharmacological tools were not PKC isozyme selective. Mochly–Rosen's group has developed several PKC translocation inhibitor and activator peptides that, when introduced into cells, cause selective regulation of translocation and function of the corresponding PKC isozymes [16]. Using these peptides, they have shown that εPKC activator peptide, ψεPACK, reduced the damage of isolated rat cardiomyocytes and of isolated perfused mouse and rat hearts from simulated ischemia–reperfusion [17–19]. Consistent with the results obtained when peptides were acutely introduced into isolated cardiomyocytes or to perfused hearts [17–21], or transgenic mice heart overexpressing εPKC [22] demonstrated cardioprotection from ischemia–reperfusion injuries. In addition, loss of ischemic preconditioning was observed in εPKC knockout mice [23].

While the protective role of εPKC activation in myocardial ischemia–reperfusion injury was extensively characterized regarding the alteration in hemodynamics, cellular damage, and infarction size [17–23], the potential protective effect of εPKC activation on the fatal ventricular arrhythmia associated with ischemia–reperfusion remains to be explored.

In the present study, we used optical mapping technique to focus on the potential protective role of εPKC modulation on ischemia–reperfusion arrhythmias in two lines of transgenic mice which moderately overexpress εPKC activator peptide (εPKC agonist mice) [17,20,21] resulting in 20% increase in εPKC activity, and εPKC inhibitor peptide (εPKC antagonist mice) [24] resulting in 15% downregulation of εPKC in the heart.

Methods

Breeding of εPKC transgenic mice. All studies were approved by the Institutional Animal Care and Use Committee at the VA New York Harbor Healthcare System. εPKC agonist and antagonist founder mice were kindly provided by Dr. Daria Mochly–Rosen from Stanford University, CA, and previously described in detail [17,20,21,24]. Heterozygous εPKC agonist and εPKC antagonist transgenic mice were bred with FVB wild-type controls to generate εPKC agonist and antagonist mice. Mice aged 4–6 months were used for the current studies. For all studies, εPKC transgenic and control mice were age, sex, and strain matched.

Perfusion of mouse hearts. The heart was rapidly excised under anesthesia, cannulated, and placed in a chamber specifically designed to immobilize the heart which was perfused with an oxygenated (95% O_2–5% CO_2) modified Tyrode's solution containing (in mM): 130 NaCl, 25 $NaHCO_3$, 1.20 $MgSO_4$, 4.75 KCl, 10 dextrose, and 1.8 $CaCl_2$, pH 7.4, at 37 °C. The coronary flow rate was adjusted to maintain constant perfusion pressures of 50–60 mm Hg. The temperature of the perfusate was maintained at 37 °C by feedback control as previously described [25]. All hearts were subjected to a 30 min stabilization period, 10 min global ischemia followed by 30 min reperfusion. Hearts which demonstrated contractile or other abnormalities during stabilization period were discarded.

Optical mapping of action potentials. The optical mapping apparatus and computer interface have been previously described [26–28]. The heart was placed in a chamber specifically designed to immobilize, and focus an image of the left ventricular free wall on a 12×12 element photodiode array as previously described [25]. Hearts were stained with the voltage-sensitive dye 1-(3-sulfonatopropyl)-8-[-[2-(di-*n*-butylamino)-6-naphthyl]vinyl]pyridium betaine (di-4-ANEPPS, 10–15 μl of a 3 mM stock solution in DMSO) delivered through the port of a bubble trap, which results in homogeneous dye loading throughout the heart. Light from a tungsten halogen lamp was collimated, passed through an interference filter (520 ± 20 nm), and focused on the surface of the stained heart. The fluorescence from the dye was passed through a cutoff filter (>630 nm) and focused on the 124-element photodiode array. The array viewed an area of ~4×4 mm on the free wall of the left ventricle, meaning that each element recorded electrical activity from a region of ~310×310 μm with a depth of field of ~40 μm. The photocurrent of each photodiode was passed through a current to a voltage converter and a second stage amplifier, digitized with temporal resolution >1 kHz, and stored in computer.

Optical mapping of intracellular calcium. For intracellular Ca transients (Ca_iT) recordings, hearts were stained with the Ca-sensitive dye Rhod 2-AM (50 μg dissolved in 50 μl of DMSO added to the coronary perfusate). Rhod 2-AM is membrane permeable and becomes Ca sensitive and trapped in the cytosol when esterified to Rhod 2 intracellularly. With excitation light (ex = 520 ± 20 nm), Rhod 2 exhibits a more than 100-fold increase in fluorescence at its emission wavelength (em = 585 nm) on binding Ca and is typically used as a single excitation and single emission wavelength dye [29]. APs and Ca_iT are recorded simultaneously.

Data analysis. Data are presented as means ± SD. The number of experiments (n) indicates the number of hearts (or mice) used. The hearts were not stimulated but were beating spontaneously to mimic physiological conditions. Therefore no measurements of action potential duration was performed. Arrhythmia and optical mapping data for TG-mice and controls are compared by paired or unpaired Student's t-test as appropriate. A value of $P < 0.05$ was considered significant.

Results

General observations

Three groups of mice, wild-type control (WT), εPKC agonist, and εPKC antagonist mice, were subjected to the ischemia–reperfusion protocol described in the method. During the 30 min stabilization period, none of the mice from the three groups developed VT or VF, except that 6 of the 13 mice in the εPKC antagonist group exhibited premature ventricular beat (PVB). The spontaneous heart rate during basal conditions was not statistically different between the three groups (wild-type, 313 ± 15 beat/min ($n = 16$); agonist, 315 ± 10 beat/min ($n = 13$) and antagonist, 323 ± 12 beat/min ($n = 13$)).

Arrhythmias in εPKC agonist, εPKC antagonist and wild-type mice

To test the hypothesis that εPKC activation protects the ischemic heart from arrhythmias, reperfusion arrhythmias were assessed in all three groups. The summary of the arrhythmic events is shown in Table 1. In the WT control group, 3 of the 16 hearts (19%) developed VT which lasted less than 3 min and 8 of the 16 mice (50%) showed PVB

Table 1
Frequency of reperfusion arrhythmias in the mice

Groups	*n*	VT	VF	PVC	Duration VF/VT (min)
Wild-type	16	3	0	8	3
εPKC antagonist	13	10	6	13	>30
εPKC agonist	13	1	0	5	⩽1

upon reperfusion. Of the 13 εPKC agonist mice studied, no VF was observed in any of the hearts. Only one heart developed VT which lasted less than 1 min and five hearts had PVB upon reperfusion. We next hypothesized that if εPKC activation protects the heart from reperfusion arrhythmia, then downregulation of εPKC should be expected to develop exacerbated ischemia–reperfusion arrhythmia in εPKC antagonist mice. Indeed, of the 13 εPKC antagonist mice studied, 6 developed VF (46.2%), 10 developed VT (76.9%), and all showed PVB upon reperfusion. The total duration of VF/VT lasted more than 30 min and most VT/VF occurred at 3–7 min reperfusion. Altogether, the data above support the hypothesis that εPKC activation in the heart confers protection against reperfusion arrhythmia.

Alteration of action potentials during basal, ischemia, and reperfusion periods

In the wild-type mice, during basal conditions, no arrhythmia was observed. During the first 3 min of ischemia, bradychardia was observed and at 10 min of ischemia, most APs were suppressed. Upon 5 min of reperfusion, APs were irregular, however APs recovery was obtained at 30 min of reperfusion. Fig. 1 shows representative AP recordings during basal, ischemia, and reperfusion periods from a wild-type mouse heart. In the agonist group, bradycardia was also observed during 3 min of ischemia and APs were suppressed at 10 min of ischemia. Interestingly, at the end of reperfusion, there was better AP recovery when compared to the antagonist group. Fig. 2 shows representative AP recordings from εPKC agonist mouse heart. Unlike the WT and εPKC agonist mice, AP shortening was exacerbated during ischemia and AP recovery was the worst in the εPKC antagonist mice. Fig. 3 shows representative AP recordings in εPKC antagonist mouse heart. A selected example of a run of VT which degenerated into VF at 7 min of reperfusion is shown in Fig. 3.

Relationship of optical APs and Ca_iT

The unique advantage of the optical mapping system used in this study is the simultaneous recordings of Ca_iT and APs. As expected at basal condition, the Ca_iT upstroke followed the AP upstroke in all three groups (Fig. 4). Ischemia resulted in a slow decay of Ca_iT in relation to the AP repolarization for wild-type mice and more significantly for εPKC antagonist mice. This slow decay in Ca_iT did remain upon reperfusion. However, these Ca_iT abnormalities were not observed in the εPKC agonist mice during ischemia and reperfusion.

Discussion

The present data demonstrate that moderate in vivo activation of εPKC (preconditioning mimicking agent)

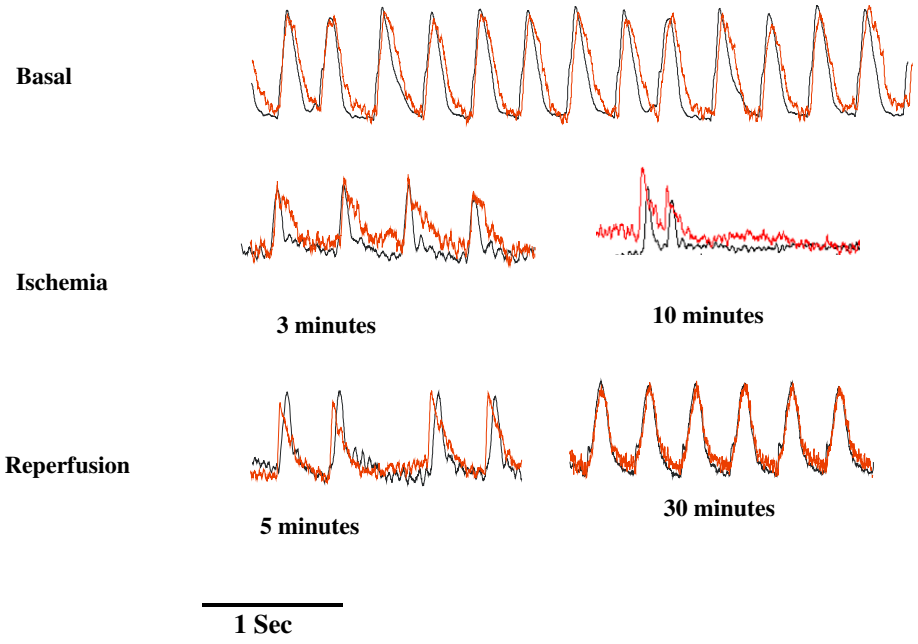

Fig. 1. Simultaneous recordings of action potentials (APs) and intracellular calcium transient (Ca_iT) from a representative pixel at the left ventricle free wall of a Langendorff perfused wild-type mouse heart. APs (black lines) recording and Ca_iT (red lines) are shown during basal, ischemia, and reperfusion periods.

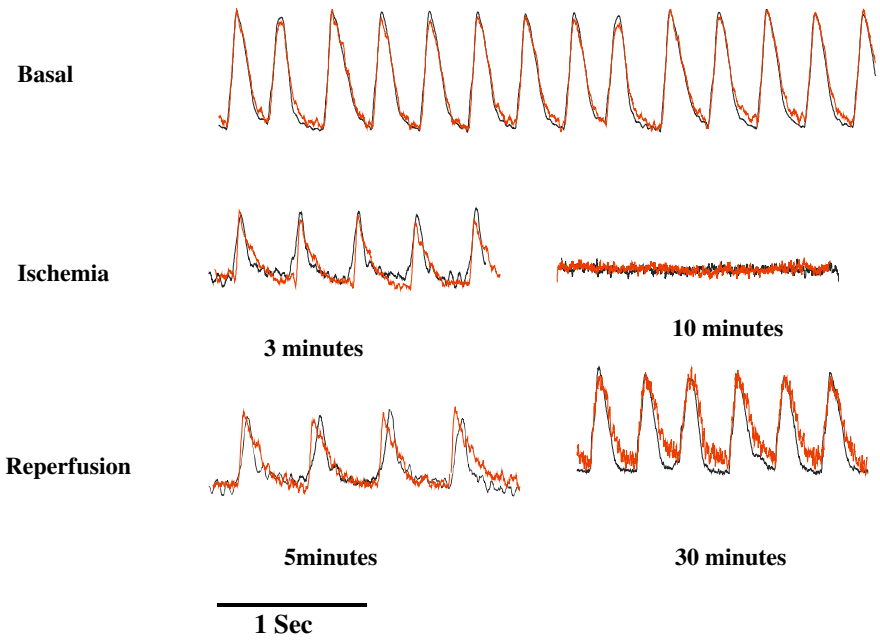

Fig. 2. Simultaneous recordings of action potentials (APs) and intracellular calcium transient (Ca_iT) from a representative pixel at the left ventricle free wall of a Langendorff perfused εPKC agonist mouse heart. APs (black lines) recording and Ca_iT (red lines) are shown during basal, ischemia, and reperfusion periods.

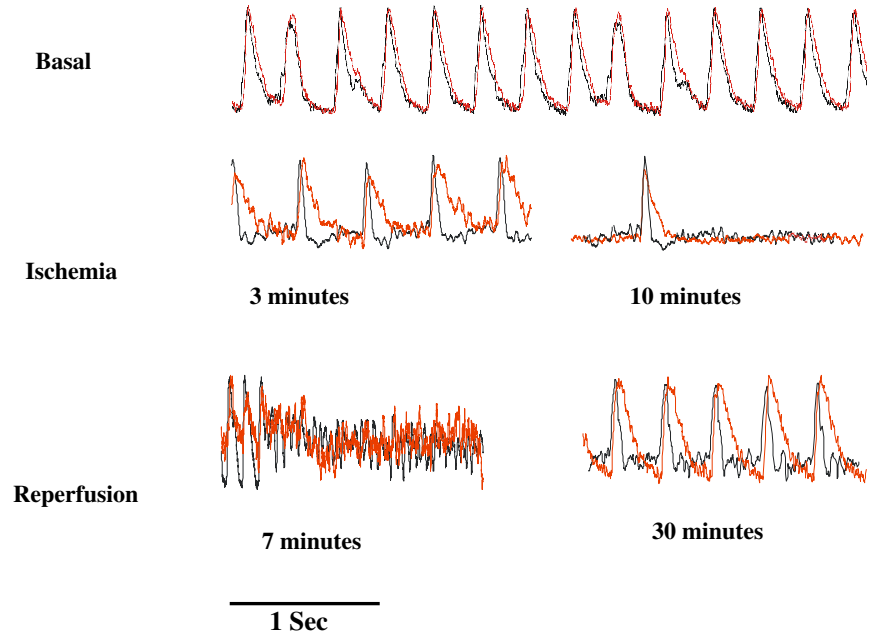

Fig. 3. Simultaneous recordings of action potentials (APs) and intracellular calcium transient (Ca_iT) from a representative pixel at the left ventricle free wall of a Langendorff perfused εPKC antagonist mouse heart. APs (black lines) recording and Ca_iT (red lines) are shown during basal, ischemia, and reperfusion periods.

protects the ischemic heart from reperfusion arrhythmias. Conversely, moderate inhibition (downregulation) of εPKC exacerbates the incidence of reperfusion arrhythmia. These findings have important clinical therapeutic significance since treatment of patients with ischemic heart diseases includes preconditioning mimicking agents.

Pathophysiology of εPKC

Activation of εPKC confers cardioprotection from ischemia–reperfusion injury in various cell culture and isolated perfused heart models [17–21]. However, the role of εPKC in the ischemia–reperfusion related ventricular arrhythmias is poorly understood. Here, our data show

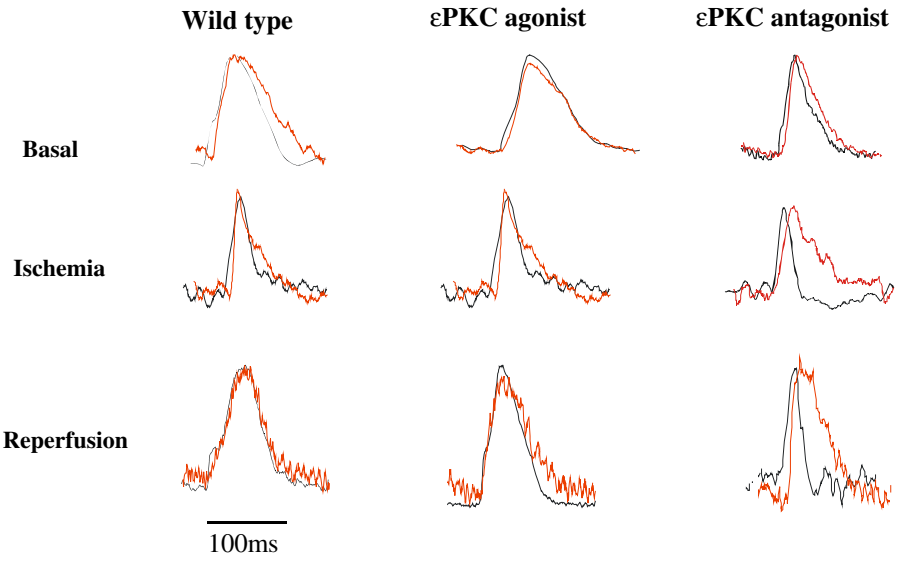

Fig. 4. Expended individual APs (black lines) and Ca_iT (red lines) recordings from the three groups of mice (wild-type, εPKC agonist, and εPKC antagonist mice) during basal, ischemia, and reperfusion period. Note the clear differences in Ca_iT decay during ischemia, and reperfusion.

that activation εPKC alone in the heart confers protection from ischemia–reperfusion arrhythmia.

Ischemia is known to cause elevated Ca_iT which in turn has been shown to initiate spontaneous Ca oscillations and arrhythmia [30,31]. This is likely the underlying mechanism for arrhythmia in the εPKC antagonist hearts, which showed the highest incidence of VT/VF and slow decay of Ca_iT. Similarly the protection against arrhythmia in the εPKC agonist mice hearts may be due to the "normal" Ca_iT decay during ischemia. Further support is provided by the patch-clamp experiments showing that L-type Ca current is significantly reduced in myocytes from hearts of εPKC agonist mice [24,32]. Using optical maps, we have recently showed that spontaneous Ca oscillations during ischemia resulted in PVBs that initiated runs of polymorphic VT/VF in a guinea-pig heart [31]. This tachyarrhythmia results in further increase in the level of Ca_iT. Tachyarrhythmia-induced increase in Ca_iT and the degeneration of the arrhythmia to VF may be related to the development of fast spontaneous Ca oscillations and/or Ca induced cell-to-cell uncoupling [31].

Altogether, the significant slow Ca_iT decay during ischemia and reperfusion periods in εPKC antagonist mice could account for the high incidence of VT/VF during reperfusion. Absence of these Ca_iT changes in the εPKC agonist mice hearts is suggested as one important mechanism of cardioprotection against reperfusion arrhythmia.

While alterations in Ca_iT decay during ischemia/reperfusion in wild-type mice and in εPKC antagonist mice were expected, and are consistent with previous reports [33,34], it is surprising to observe that slow Ca_iT decay was not present in εPKC agonist mice. The activity of both the SR Ca-ATPase and the ryanodine receptor (RyR) are regulated by ATP. During ischemia, therefore, when ATP falls, uptake of Ca into the SR as well as the release would be compromised [33]. It is well established that the open probability of the RyR can be affected by metabolic inhibition, which was shown to delay the upstroke and prolong the duration of the Ca_iT [35]. Of potential relevance to ischemia, the open probability is decreased by acidification or a decrease of cytoplasmic ATP concentration [33]. Whether and how εPKC activation prevents these from happening in the εPKC agonist mice remains unknown.

The unique alteration of Ca_iT during reperfusion in εPKC antagonist mice compared to the wild-type and εPKC agonist mice also reflected the dramatic intracellular Ca overload upon reperfusion when εPKC activity was inhibited in εPKC antagonist mice. It has been established that an overload of intracellular Ca will mediate cellular damage and contractile dysfunction [2,3]. In addition, a rise in cytosolic Ca is known to produce inward current through the Na/Ca exchanger [36] as well as outward current carried by Ca-activated chloride channel [37] and faster inactivation of the L-type Ca current through Ca-medicated inactivation [38]. This in turn will affect the AP duration and subsequently create proarrhythmic conditions. Multiple ion channels have been shown to be the substrate of εPKC, including our previously reported work on human cardiac Na channels, human delayed rectifying K channels, and L-type Ca channel [32,39,40]. The exact molecular mechanism(s) of how εPKC activation manipulate these ion channels and/or Ca handling proteins to attenuate the intracellular Ca overload during reperfusion in the εPKC agonist mice heart warrant further investigations.

Previous in vivo and in vitro studies using εPKC modulating peptides and εPKC transgenic mice have shown that εPKC activation significantly improved the hemodynamic of the heart, attenuated cellular damage, and reduced infarction size [17–23]. Our study demonstrates that in addition to these cardioprotective effects, the εPKC

activation also dramatically suppressed the ischemia–reperfusion related ventricular tachyarrhythmia, indicating that εPKC as potential pharmacological target in managing patients with ischemic heart diseases.

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