Am J Transl Res 2013;5(3):316-326
www.ajtr.org /ISSN:1943-8141/AJTR1303008

Original Article

Erythropoietin facilitates resuscitation from ventricular fibrillation by signaling protection of mitochondrial bioenergetic function in rats

Jeejabai Radhakrishnan[1*], Madhav P Upadhyaya[2*], Matthew Ng[2], Ari Edelheit[2], Hawnyeu M Moy[2], Iyad M Ayoub[3], Raúl J Gazmuri[4,5]

[1]Department of Medicine and Resuscitation Institute at Rosalind Franklin University of Medicine and Science; [2]Rosalind Franklin University of Medicine and Science; [3]Resuscitation Institute at Rosalind Franklin University of Medicine and Science; [4]Department of Medicine, Physiology and Biophysics, Resuscitation Institute at Rosalind Franklin University of Medicine and Science, 3333 Green Bay Road, North Chicago, Illinois 60064, USA; [5]Critical Care Medicine and ICU, Captain James A. Lovell Federal Health Care Center, 3001 Green Bay Road, North Chicago, Illinois 60064, USA. [*]Authors contributed equally to this work.

Received March 25, 2013; Accepted April 9, 2013; Epub April 19, 2013; Published April 30, 2013

Abstract: Objective: We previously reported beneficial myocardial effects during chest compression after administration of high-dose erythropoietin. We hypothesized that erythropoietin also elicits post-resuscitation myocardial benefits partly linked to protection of mitochondrial bioenergetic function. Methods: Two series of 10 rats each underwent ventricular fibrillation for 10 minutes (*series-1*) and 8 minutes (*series-2*) and were randomized to erythropoietin (5,000 U/kg) or 0.9% NaCl before chest compression. Dobutamine was infused post-resuscitation in *series-2* harvesting their hearts at 120 minutes. Results: During chest compression, a statistically insignificant trend showing progressively higher coronary perfusion pressure in the erythropoietin group was observed consistent with previously reported preservation of left ventricular distensibility. Post-resuscitation, in the absence of dobutamine (*series-1*) erythropoietin failed to improve post-resuscitation myocardial function or survival; in the presence of dobutamine (*series-2*) all rats survived and those treated with erythropoietin reversed post-resuscitation myocardial dysfunction yielding higher cardiac work index (CWI; 39±3 *vs* 25±10 mmHg·ml/kg, $p<0.01$) and higher mean aortic pressure (MAP; 99±4 *vs* 83±16, $p<0.01$) at 120 minutes post-resuscitation. Better myocardial function was associated with lesser increases in plasma cytochrome *c*, attaining levels which inversely correlated with CWI ($p=0.026$) and MAP ($p=0.025$). Hearts from erythropoietin-treated rats had higher phosphorylation levels of cytosolic Akt and higher phosphorylation levels of cytosolic and mitochondrial PKCε and maintained cytochrome *c* oxidase activity. Conclusion: Erythropoietin activated mitochondrial protective mechanisms that helped maintain bioenergetic function enabling reversal of post-resuscitation myocardial dysfunction in the presence of dobutamine.

Keywords: Cardiopulmonary resuscitation, erythropoietin, mitochondrial function, myocardial function, rats, ventricular fibrillation

Introduction

Erythropoietin is a glycoprotein hormone normally produced in the adult kidney and known primarily for regulating red blood cell production. However, studies within the past 14 years have shown that erythropoietin also exerts non-hematopoietic effects which activate cell protective mechanisms ameliorating injury caused by ischemia and reperfusion in a broad array of tissues [1-4]. In previous studies using a rat model of VF [5], we reported generation of higher coronary perfusion pressures (CPP) for a given compression depth after administration of erythropoietin consistent with preservation of left ventricular distensibility [6]; an effect which we have previously reported associated with preservation of mitochondrial bioenergetic function [7]. In a subsequent clinical study assessing the effects of erythropoietin during out-of-hospital resuscitation, we reported higher end-tidal PCO_2 ($P_{ET}CO_2$) indicative of higher

forward blood flow generation [8], also consistent with preservation of left ventricular distensibility. In addition, a trend was observed in which resuscitated victims who had received erythropoietin had higher survival rates than victims who had been resuscitated without receiving erythropoietin [8]. We conducted the present study in a rat model of VF and closed-chest resuscitation to examine whether erythropoietin could promote favorable post-resuscitation myocardial effects. We hypothesized that erythropoietin activates mechanisms protective of mitochondrial bioenergetic function, specifically investigating whether these effects could be mediated by protein kinase B (Akt) [9], protein kinase C epsilon (PKCε) [10, 11], and effects on the electron transport chain activity.

Methods

The studies were approved by our Institutional Animal Care and Use Committee and conducted according to the *Guide for the Care and Use of Laboratory Animals* published by the National Research Council.

Rat model of VF and resuscitation

Animal preparation

Anesthesia was induced in adult male Sprague-Dawley rats (443 to 538 grams) with sodium pentobarbital (45 mg/kg ip) and maintained administering 10 mg/kg iv every 30 minutes if required. Catheters were advanced for pressure measurements, blood sampling, VF induction, and cardiac output measurements as described in detail before [12].

Experimental protocol

VF was induced by delivering a 60-Hz alternating current to the right ventricular endocardium for 3 minutes. VF was allowed to continue spontaneously for 7 additional minutes in *series-1* and for 5 additional minutes in *series-2*. Chest compression was then initiated using a piston device delivering 200 compressions per minute with a 50% duty cycle [12]. The depth of compression was gradually increased to attain by minute 2 an aortic diastolic pressure between 26 and 28 mmHg. The depth was further increased by increments of 2 mm every minute to a maximum depth of 17 mm, measured with a displacement transducer and making small adjustments to the site of compression (sidewise and rostrocaudal) seeking to obtain the highest aortic diastolic pressure for a given compression depth. Positive pressure ventilation was provided with a volume controlled ventilator delivering 25 unsynchronized breaths per minute (tidal volume = 6 ml/kg) and 100% oxygen. After 8 minutes of chest compression up to two 5-J biphasic waveform electrical shocks (Heartstream XL, Philips Medical Systems, MA) were delivered five seconds apart. If VF persisted or an organized rhythm with a mean aortic pressure ≤ 25 mm Hg ensued, chest compression was resumed for 30 seconds. The defibrillation-compression cycle was repeated for up to three additional times, increasing the energy of individual shocks to 7-J for the subsequent two cycles if VF persisted. Successful defibrillation was defined as the return of an organized rhythm with a mean aortic pressure > 60 mmHg for > 5 minutes. Resuscitated rats were ventilated initially with 100% oxygen for the initial 15 minutes and 50% oxygen for the remaining post-resuscitation interval. In *series-2*, dobutamine HCl (2,000 μg/ml; Hospira Inc.) was infused for inotropic support into the right atrium at a rate of 15 $\mu g/kg{\cdot}min^{-1}$. Concomitantly, 0.9% NaCl was infused for preload support – also into the right atrium – at a rate of 24 $ml/kg{\cdot}h^{-1}$. Both infusions were started upon return of spontaneous circulation or after delivery of the initial electrical shocks and were maintained throughout the post-resuscitation interval.

Experimental series

Two sequential series of experiments were conducted with each series including 10 rats each randomized 1:1 to receive either 5,000 U/kg of erythropoietin (Procrit, epoietin alfa, 20,000 units/ml, 0.25 ml/kg) or an equivalent volume of 0.9% NaCl into the right atrium immediately before starting chest compression. In the initial series, cardiac activity was reestablished in each instance but failed to restore sustained circulation in most animals, precluding assessing post-resuscitation myocardial function (*series-1*). Thus, a second series of experiments was conducted in which the duration of untreated VF was shortened from 10 minutes to 8 minutes and post-resuscitation myocardial function supported by preload and inotropic intervention – as described above – enabling

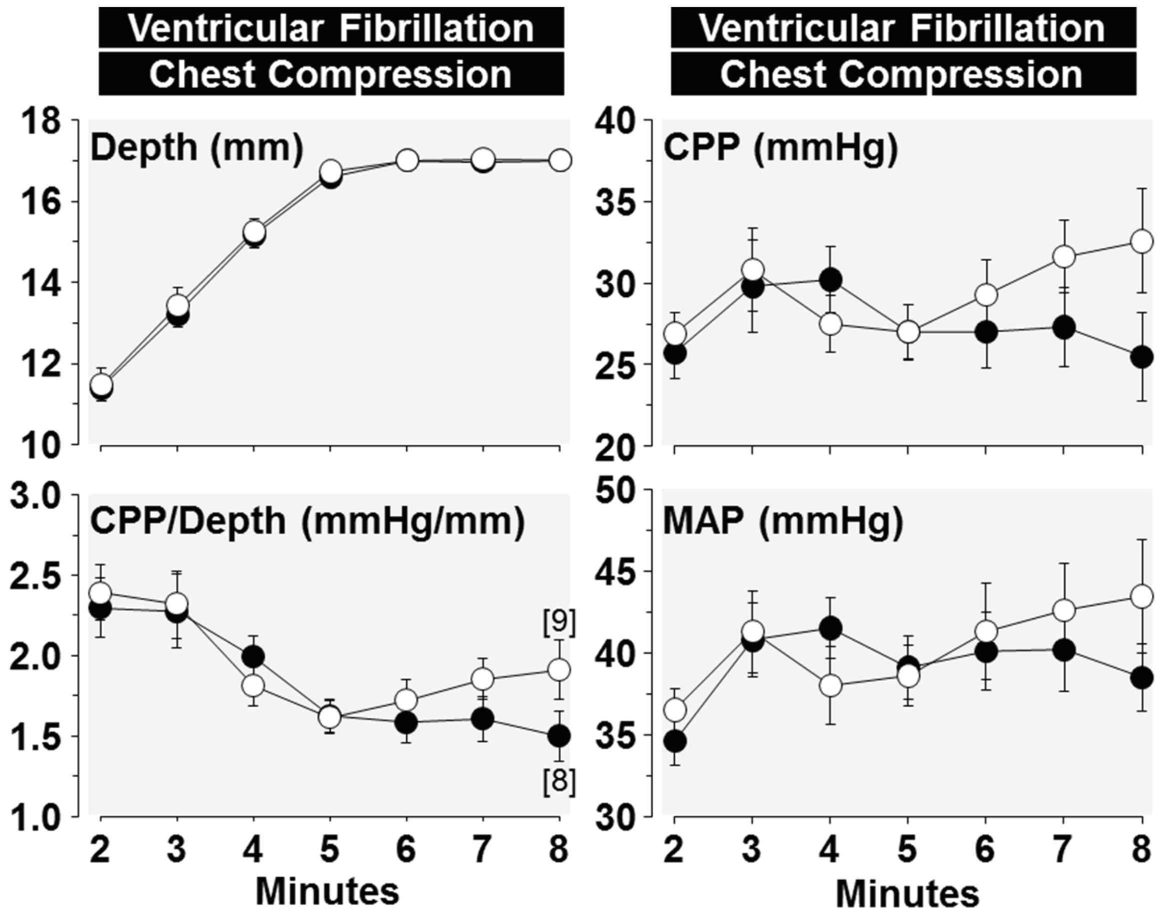

Figure 1. Effects of erythropoietin (open circles) compared to vehicle control (closed circles) during chest compression. Each group is represented by 10 rats until minute 7, after which one rat in the erythropoietin group and two rats in the control group had spontaneous defibrillation with return of spontaneous circulation not requiring additional chest compression. Depth, depth of compression; CPP, coronary perfusion pressure; and MAP, mean aortic pressure. Mean ± SEM. Differences between groups were analyzed by two-way repeated measures ANOVA without achieving more than borderline statistical significances.

return of sustained spontaneous circulation throughout the post-resuscitation interval in each rat (*series-2*). The investigators performing the experiments were blind to the treatment assignment.

Measurements

Hemodynamic measurements

Transduced signals were conditioned, sampled and digitized at 250 scans per second using a 16-bit data acquisition board (AT-MIO-16XE-50; National Instruments), and analyzed using custom developed programs in LabVIEW (National Instruments). The coronary perfusion pressure (CPP), cardiac index (CI), and cardiac work index (CWI) were calculated as described previously [12].

Plasma cytochrome c measurements (series-2)

Arterial blood samples (250 µl) were collected at baseline and at 60 and 120 minutes post-resuscitation and their plasma aliquoted (100 µl) and stored at -80°C for subsequent analysis of cytochrome *c*. Cytochrome *c* was measured by reverse phase HPLC as previously described [13].

Left ventricular tissue measurements (series-2)

Hearts were harvested from healthy rats not subjected to cardiac arrest (baseline hearts) and from *series-2* at 120 minutes post-resuscitation. Their left ventricles were quickly isolated, frozen in liquid N_2, and stored at -80°C for

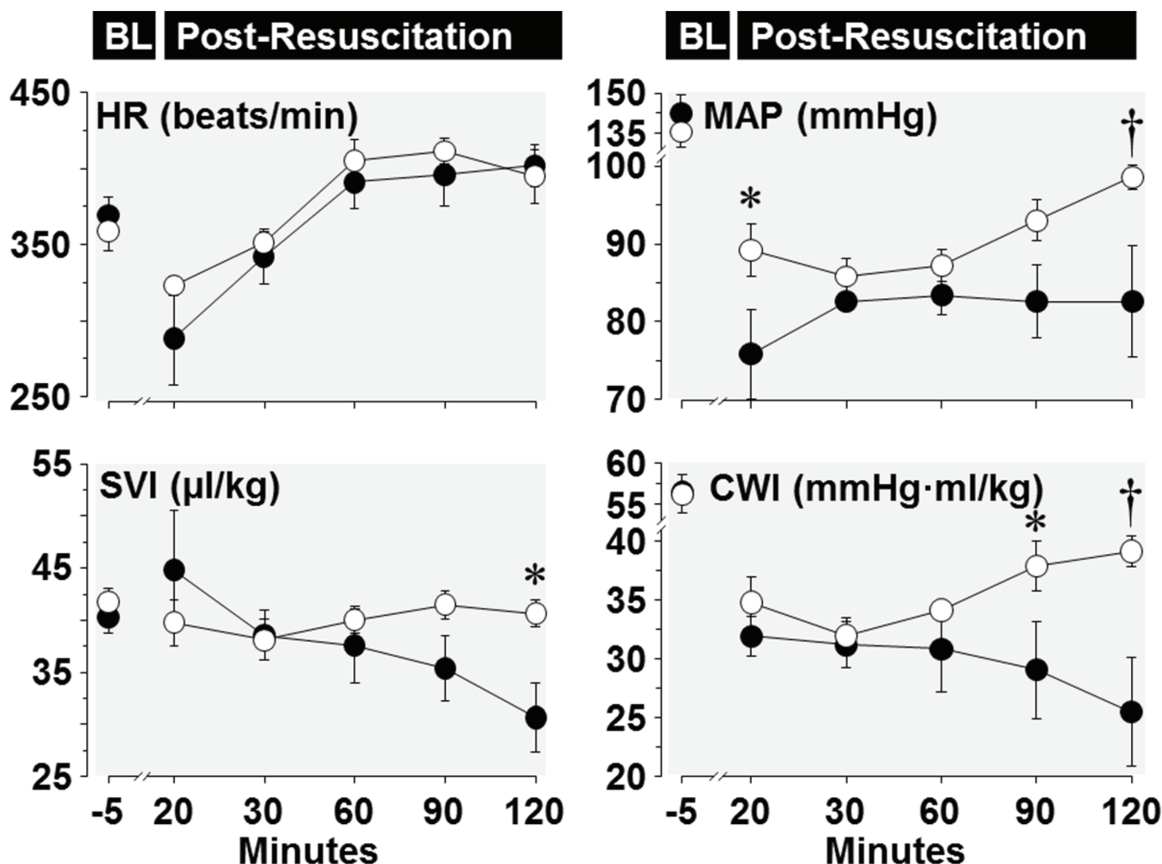

Figure 2. Effects of erythropoietin (open circles) compared to vehicle control (closed circles) on post-resuscitation hemodynamic and left ventricular function. Each group is represented by 5 rats from *series-2*. HR, heart rate; MAP, mean aortic pressure; SVI, stroke volume index; and CWI, cardiac work index. Mean ± SEM. Differences between groups were analyzed by two-way repeated measures ANOVA, showing a statistically significant interaction between treatment and time for CWI (p=0.012) and SVI (p=0.035). *p<0.05; †p<0.01 between groups at the specified time point.

subsequent analysis. Mitochondrial and cytosolic fractions were separated from the frozen ventricles as previously described using buffers containing protease and phosphatase inhibitors [13]. Aliquots of the mitochondrial fractions were used immediately for electron transport complex activity assays and the rest stored at -80°C until further analysis by western blotting. Protein concentration was determined by Bradford (Bio-Rad) method.

Western blotting

Mitochondrial (40 µg) and cytosolic fractions (20 µg) were separated using 4 % to 12 % Novex NuPAGE gels and western blotting was performed as previously described [13]. Phosphatase inhibitors were included in the buffers to inhibit phosphatase activity [14]. Chemiluminescent detection was performed using west femto maximum sensitivity kit. After detection, membranes were stripped using Restore PLUS stripping buffer and probed again with GAPDH and prohibitin as loading controls for cytosolic and mitochondrial fractions, respectively. Densitometry was measured using Gel Logic 200 Imaging System and 1D image analysis software (Kodak).

Electron transport complex activity assays

Complex I: Complex I (NADH dehydrogenase) activity was measured as described by Janssen et al [15]. The technique is based on coupling the oxidation of NADH by complex I to the reduction of decylubiquinone and the subsequent reduction of 2,6-dichloroindophenol (DCIP); measuring reduced DCIP absorption at 600 nm using a spectrophotometer (Biomate 3, Thermo Scientific). The reaction mixture included 25 mM potassium phosphate buffer, 3.5 mg/ml BSA, 60 µM DCIP, 70 µM decylubiquinone, 1

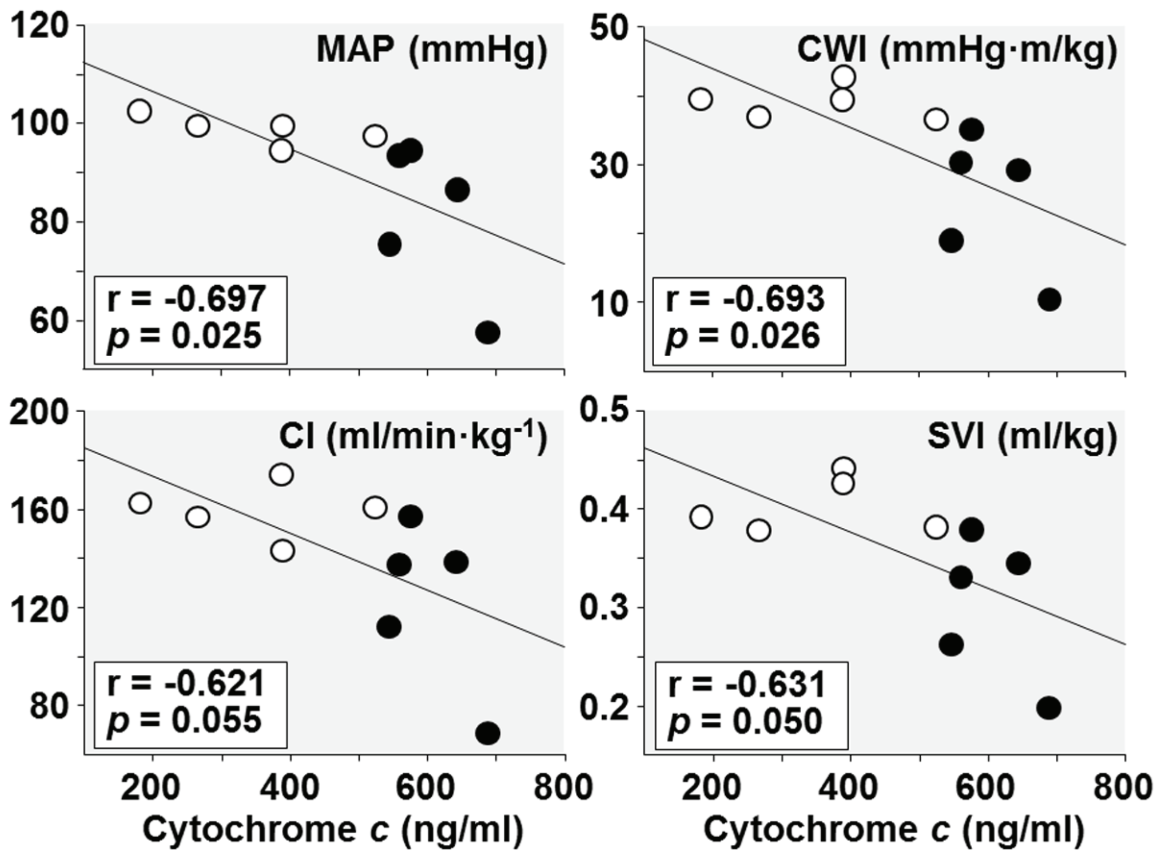

Figure 3. Scatterplots depicting linear regressions and correlations between plasma cytochrome *c* and mean aortic pressure (MAP), cardiac work index (CWI), cardiac index (CI), and stroke volume index (SVI) at post-resuscitation 120 minutes in rats treated with erythropoietin (o) and controls (●).

µM antimycin A, and 5 µg of mitochondrial protein in 750 µl of reaction volume. Non-specific absorbance was determined by parallel assay with 4 µM rotenone (complex I inhibitor).

Complex I and III: Complex I (NADH dehydrogenase) and complex III (cytochrome *c* reductase) combined activity was measured as described by Jarreta et al [16]. The technique is based on coupling the oxidation of NADH by complex I to the reduction of cytochrome *c* by complex III; measuring ferrocytochrome *c* (reduced cytochrome *c*) absorption at 550 nm. The reaction mixture included 50 mM potassium phosphate, 80 µM ferricytochrome *c* (oxidized cytochrome *c*), 100 µM NADH, 2 mM KCN, and 30 µg of mitochondrial protein in 750 µl of reaction volume. Non-specific absorbance was determined by parallel assay with 0.5 µM rotenone.

Complex IV: Complex IV [Cytochrome *c* oxidase (COX)] activity was determined using a commercially available kit (CYTOCOX1, Sigma). The technique is based on measuring the amount of ferricytochrome *c* produced from exogenously supplied ferrocytochrome *c* in the presence of COX; measuring ferricytochrome *c* at 550 nm. A purified COX standard provided in the kit was used to generate a standard curve and it was used to calculate the COX activity and data expressed as enzyme units/mg protein.

Complex V: Complex V (FoF1 ATPase) activity was determined as described by Tzagoloff [17] and Mueller [18]. The technique is based on measuring the amount of phosphate liberated after ATP hydrolysis by FoF1 ATPase. Briefly, 25 µg of mitochondrial suspension was added to 90 µl of reaction buffer (0.05 M Tris-SO_4, pH 8.5, 0.004 M $MgSO_4$). The reaction was started by adding 10 µl of 0.1 M ATP. Oligomycin was used at a concentration of 5 µg/ml to control for contamination by other ATPases. The reaction was stopped by adding 20 µl of 50% (w/v) ice-cold trichloroacetic acid (TCA) and the samples were centrifuged at 20,000g for 30 min-

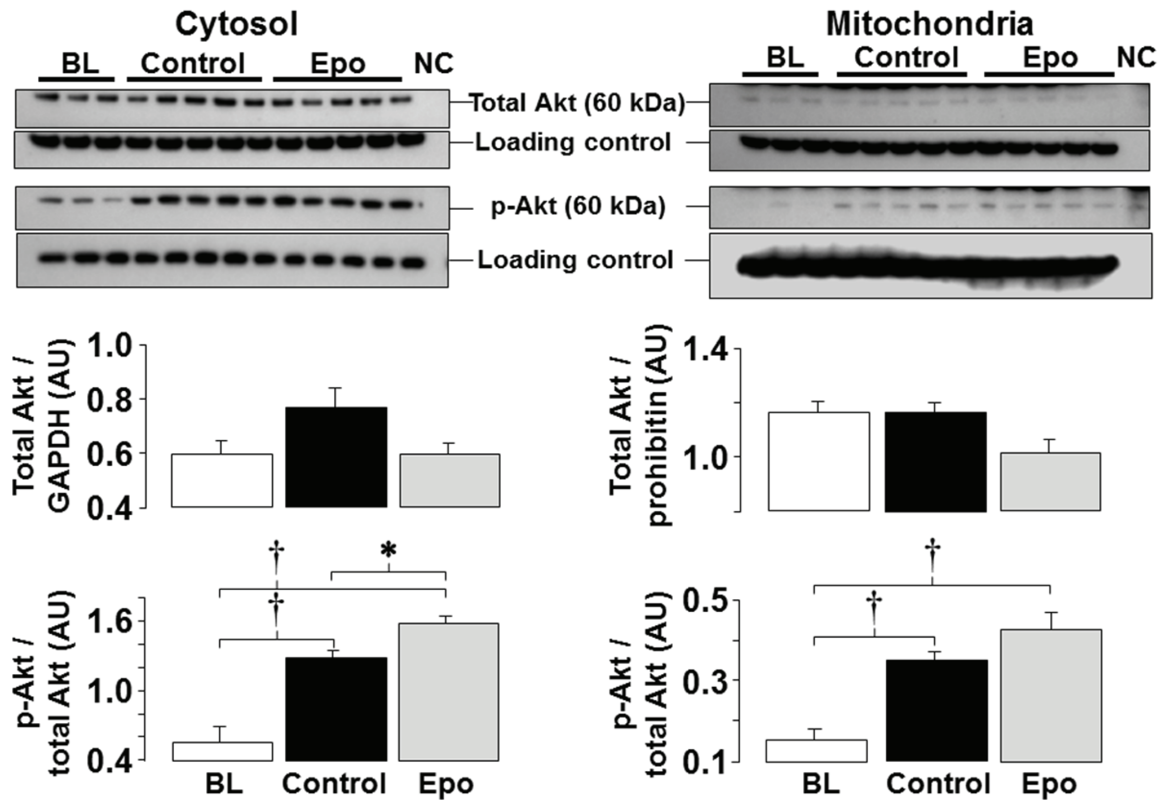

Figure 4. Western blots and their corresponding densitometries showing total Akt and phospho ser-473 Akt (p-Akt) levels in left ventricular cytosolic and mitochondrial fractions in baseline hearts (BL, n = 3) and in hearts harvested at 120 minutes post-resuscitation in *series-2* from rats randomized to receive 0.9% NaCl (control, n = 5) or erythropoietin (Epo, n = 5). Bar graphs show the densitometry of total Akt normalized to GAPDH for cytosolic fraction and to prohibitin for mitochondrial fraction and the p-Akt to total Akt ratio. AU = arbitrary units. NC = negative control (lane probed only with secondary antibody). Loading control = Glyceraldehyde 3-phospho dehydrogenase (GAPDH) for cytosol and prohibitin for mitochondria. *$p<0.02$; †$p<0.005$ by one-way ANOVA and Holm-Sidak test for multiple comparisons.

utes and the supernatants (50 μl) were collected for estimating inorganic phosphate according to King [19]. The FoF1 ATPase activity was expressed as μmoles of inorganic phosphate generated/mg protein·hour^{-1}.

Statistical analysis

For continuous and repetitive measurements, two-way repeated measures ANOVA was used. For continuous but non-repetitive variables, Student's *t*-test was used when comparing two groups and one-way ANOVA with the Holm-Sidak test for multiple comparisons. Kaplan-Meier survival analysis was performed using the Gehan-Breslow method. The strength of association between variables was analyzed using Pearson's product moment correlation test. The data were presented as means ± SD unless otherwise stated. A two-tail value of $p<0.05$ was considered significant.

Results

Hemodynamic and survival effects

Baseline hemodynamic parameters were comparable between groups in both series.

Series-1 and *series-2* were combined to analyze the effects during chest compression (**Figure 1**). Erythropoietin was associated with progressive increase in CPP for the same depth of compression attaining borderline statistical significance at the last minute of chest compression (27 ± 8 *vs* 33 ± 10 mmHg, $p = 0.106$). The effect on CPP was explained mostly by an effect on aortic diastolic (29 ± 7 *vs* 34 ± 9

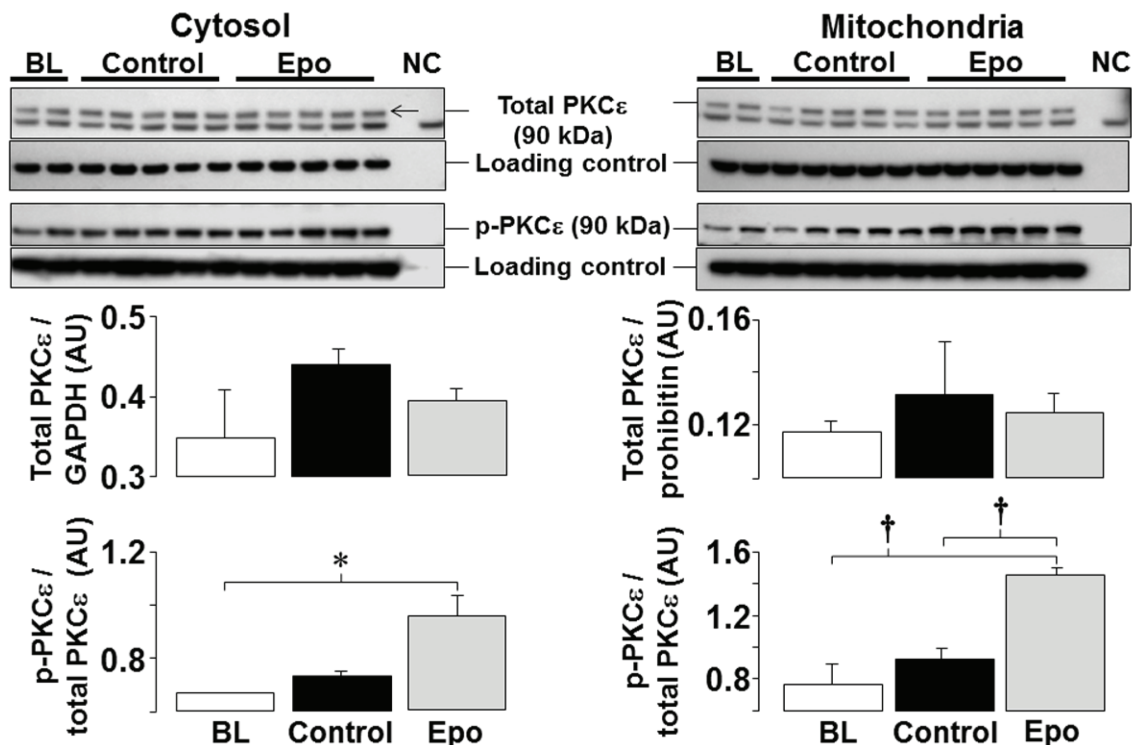

Figure 5. Western blots and their corresponding densitometries showing total PKCe and phospho ser-729 PKCe (p-PKCe) levels in left ventricular cytosolic and mitochondrial fractions in baseline hearts (BL, n = 2) and in hearts harvested at 120 minutes post-resuscitation in in *series-2* from rats randomized to receive 0.9% NaCl (control, n = 5) or erythropoietin (Epo, n = 5). Bar graphs show the densitometry of total PKCe normalized to GAPDH for cytosolic fraction and to prohibitin for mitochondrial fraction and the p-PKCe to total PKCe ratio. AU = arbitrary units. NC = negative control. Loading control = Glyceraldehyde 3-phospho dehydrogenase (GAPDH) for cytosol and prohibitin for mitochondria. *$p<0.05$ by one-way ANOVA and Dunn's test for multiple comparisons. †$p<0.001$ by one-way ANOVA and Holm-Sidak test for multiple comparisons.

mmHg, p = 0.170) rather than right atrial diastolic (2 ± 1 *vs* 1 ± 1 mmHg, p = 0.043) pressure. A similar trend was observed in mean aortic pressure, consistent with preservation of left ventricular distensibility as previously reported [5]. The possibility of a vasoconstrictive effect was unlikely given that systemic vascular resistance measured during spontaneous circulation at baseline in a previous study [5] and post-resuscitation in the present (see below) was not increased by erythropoietin.

An organized cardiac activity was restored in all rats. However, survival differed between series. In *series-1* survival at 120 minutes averaged only 50% without differences between erythropoietin (2/5) or control (3/5) rats. In *series-2*, all rats survived the 120 minutes; an effect that was statistically significant when compared with *series-1* and attributed to the inotropic support of dobutamine (p = 0.012 by the Gehan-Breslow method).

In *series-2*, progressive myocardial dysfunction after return of spontaneous circulation was evident despite inotropic support as previously reported [20, 21]. However, rats treated with erythropoietin reversed myocardial dysfunction evidenced by progressive increases in mean aortic pressure, stroke volume index, and cardiac work index with the maximal effect at the end of the post-resuscitation interval (**Figure 2**). These effects of erythropoietin occurred without increases in systemic vascular resistance index [22], which was essentially the same − if not lower − in the erythropoietin group at 120 minutes post-resuscitation (0.61 ± 0.05 *vs* 0.66 ± 0.05 $mmHg/ml \cdot min^{-1} \cdot kg^{-1}$).

Plasma cytochrome c levels (series-2)

Plasma cytochrome *c* levels (ng/ml) were comparable between control and erythropoietin treated rats at baseline (286 ± 123 *vs* 187 ± 65). However, erythropoietin treated rats had

Table 1. Mitochondrial electron transport complex activities

	Complex I	Complex I & III	Complex IV	Complex V
	Enzyme units/mg protein			μmoles of phosphate/μg protein·hr^{-1}
Baseline (n=3)	0.94 ± 0.12	0.79 ± 0.25	26.12 ± 4.88	12.22 ± 2.56
Control (n=5)	0.90 ± 0.14	0.95 ± 0.18	21.00 ± 4.12	11.26 ± 0.32
EPO (n=5)	1.01 ± 0.14	0.91 ± 0.33	29.37 ± 1.68*	11.66 ± 1.21

Activities were measured in mitochondria isolated from the left ventricles of hearts harvested at baseline (n=3, healthy rats) and at 120 minutes post-resuscitation in rats from *series-2* that received vehicle control (n=5) or EPO (n=5). Mean ± SD. *p = 0.004 *vs* control by one-way ANOVA and Holm-Sidak test for multiple comparisons.

an attenuated increase in plasma cytochrome *c* at post-resuscitation 60 minutes (656 ± 124 *vs* 463 ± 68, p = 0.016) and 120 minutes (588 ± 70 *vs* 356 ± 149, p = 0.014). The levels of cytochrome *c* were inversely correlated with mean aortic pressure and cardiac work index at 120 minutes post-resuscitation (**Figure 3**).

Effects on signaling pathways and activity of mitochondrial respiratory complexes (series-2)

Analysis of left ventricular cytosolic and mitochondrial fractions of hearts from *series-2* demonstrated higher post-resuscitation levels of phosphorylated Akt in cytosolic and mitochondrial fraction in both erythropoietin and control hearts relative to baseline hearts, with erythropoietin prompting levels higher than controls in the cytosolic fraction (**Figure 4**). The levels of PKCe phosphorylation, however, were only elevated in rats treated with erythropoietin in both the cytosolic and the mitochondrial fraction (**Figure 5**). The activity of mitochondrial respiratory complexes I, II & III combined, and of V (i.e., FoF1 ATP synthase) remained unchanged (**Table 1**). The activity of complex IV, however, was higher in erythropoietin treated rats compared to controls but comparable to baseline activity. Linear regression demonstrated correlations between the levels of phosphorylated mitochondrial PKCε and levels of phosphorylated cytosolic Akt (r = 0.869, p = 0.001), between complex IV activity and levels of phosphorylated mitochondrial PKCε (r = 0.786, p = 0.007), and between complex IV activity and plasma cytochrome *c* (r = -0.607, p = 0.063) at 120 minutes post-resuscitation.

Discussion

Variable degrees of left ventricular systolic and diastolic dysfunction [20, 21] typically develop after resuscitation from cardiac arrest. If dysfunction is severe and persistent, it may preclude reestablishment of sustained circulation and contribute to the approximately 40% fatality rate reported in victims of out-hospital cardiac arrest before admission to a hospital [23]. Previous work in our laboratory showed that protection of mitochondrial bioenergetic function during cardiac resuscitation resulted in improved post-resuscitation myocardial function [7]. In the present study, erythropoietin elicited similar myocardial effects while concomitantly activating signaling pathways linked to mitochondrial protection. However, improved myocardial function was observed only in the presence of dobutamine; in the absence of dobutamine (*series-1*) there was a 50% fatality rate within the initial 120 minutes post-resuscitation associated with severe myocardial dysfunction. In *series-2*, administration of dobutamine improved myocardial function resulting in 100% survival at 120 minutes post-resuscitation, allowing assessment of additional effects of erythropoietin on myocardial function.

The effect of dobutamine improving post-resuscitation myocardial dysfunction has been reported [24] and is consistent with actions on a myocardium that is stunned (i.e., reversible post-ischemic dysfunction). However, such dobutamine effect in the present studies only partially reversed myocardial dysfunction resulting in a cardiac work index of 45% of baseline levels at 120 minutes post-resuscitation in control rats. In contrast, the cardiac work index in rats that received erythropoietin reached 70% of baseline levels at 120 minutes post-resuscitation, thus markedly improving post-resuscitation myocardial dysfunction and demonstrating a positive interaction between dobutamine and erythropoietin. This is a novel finding of potential clinical relevance considering that post-resuscitation myocardial dysfunction accounts for a substantial percentage of deaths early after resuscitation from cardiac arrest.

The positive effect of erythropoietin on myocardial function was associated with activation of pathways known to signal mitochondrial protection. Specifically, there was enhanced phosphorylation of Akt in the cytosol and prominent phosphorylation of PKCe in both the cytosol and mitochondria, along with lesser increases in plasma cytochrome *c* indicating attenuation of mitochondrial injury [13]. Erythropoietin also affected electron transport complex IV, maintaining its activity at levels comparable to baseline whereas control rats had complex IV activity reduced to 80% of baseline. The activity of the remaining electron transport complexes and ATP synthase remained unaltered in both the control and erythropoietin treated rats.

Reductions in complex IV activity after ischemia and reperfusion have been reported in rabbit hearts subjected to global ischemia [25] and in human hearts of patients undergoing elective aortic or mitral valve surgery after aortic cross clamping [26], and credited with shifting the electron transport chain complexes to a more reduced state prompting generation of reactive oxygen species from complexes I and III and limiting ATP synthesis [27]. Reductions in complex IV activity can be prevented by preconditioning through mechanisms involving phosphorylation of mitochondrial PKCe and interaction with subunit IV of the electron transport complex IV [28]. In the present study, complex IV activity was statistically higher in rats resuscitated with erythropoietin than in control rats resuscitated without erythropoietin but neither group was statistically different than baseline. Yet, judging by the values (**Table 1**), it is more likely that complex IV activity declined in the control group rather than increased in the erythropoietin group. The strong correlation between phosphorylation of cytosolic Akt and phosphorylation of mitochondrial PKCe and between phosphorylation of mitochondrial PKCe and complex IV activity, which in turn, was inversely correlated with plasma cytochrome *c*, suggest that these effects could be mechanistically linked and elicited by erythropoietin. However, further work using pathway-specific inhibitor would be required to establish causality.

Dobutamine exerts its inotropic action via ß-adrenoceptor stimulation leading to cyclic AMP signaling resulting in enhanced calcium uptake by the sarcoplasmic reticulum and consequently larger calcium-induced calcium-release after action potential activation, thus enhancing sarcomeric contraction among other effects. These are energy requiring process and the ability of mitochondria to appropriately respond to increases in energy demands could be at the core of the positive interaction between dobutamine and erythropoietin.

Conclusions

Erythropoietin activated mitochondrial protective mechanisms that helped maintain bioenergetic function enabling reversal of post-resuscitation myocardial dysfunction in the presence of dobutamine. The effects of erythropoietin herein reported in conjunction with earlier observations on left ventricular distensibility – enabling hemodynamically more effective chest compression – could collectively help improve survival from cardiac arrest.

Acknowledgments

This work was partially supported by a Veterans Affairs Merit Review Grant (to RJG) entitled "Myocardial Effects of Erythropoietin during Resuscitation from Cardiac Arrest." We wish to thank the Pathology Laboratory and the Pharmacy Services at Captain James A. Lovell Federal Health Care Center for their continuous support of our research work.

Address correspondence to: Dr. Raúl J Gazmuri, Resuscitation Institute, Rosalind Franklin University of Medicine and Science, 3333 Green Bay Road; North Chicago, Illinois, 60064, USA. Phone: 224-610-3681; Fax: 847-578-3863; E-mail: raul.gazmuri@rosalindfranklin.edu

References

[1] Sakanaka M, Wen TC, Matsuda S, Masuda S, Morishita E, Nagao M, Sasaki R. In vivo evidence that erythropoietin protects neurons from ischemic damage. Proc Natl Acad Sci U S A 1998; 95: 4635-4640.

[2] Ehrenreich H, Hasselblatt M, Dembowski C, Cepek L, Lewczuk P, Stiefel M, Rustenbeck HH, Breiter N, Jacob S, Knerlich F, Bohn M, Poser W, Ruther E, Kochen M, Gefeller O, Gleiter C, Wessel TC, De Ryck M, Itri L, Prange H, Cerami A, Brines M, Siren AL. Erythropoietin therapy for acute stroke is both safe and beneficial. Mol Med 2002; 8: 495-505.

[3] Calvillo L, Latini R, Kajstura J, Leri A, Anversa P, Ghezzi P, Salio M, Cerami A, Brines M. Recom-

binant human erythropoietin protects the myocardium from ischemia-reperfusion injury and promotes beneficial remodeling. Proc Natl Acad Sci U S A 2003; 100: 4802-4806.

[4] Hu L, Yang C, Zhao T, Xu M, Tang Q, Yang B, Rong R, Zhu T. Erythropoietin Ameliorates Renal Ischemia and Reperfusion Injury via Inhibiting Tubulointerstitial Inflammation. J Surg Res 2012; 176: 260-266.

[5] Singh D, Kolarova JD, Wang S, Ayoub IM, Gazmuri RJ. Myocardial protection by erythropoietin during resuscitation from ventricular fibrillation. Am J Ther 2007; 14: 361-368.

[6] Kolarova JD, Ayoub IM, Gazmuri RJ. Cariporide enables hemodynamically more effective chest compression by leftward shift of its flow-depth relationship. Am J Physiol Heart Circ Physiol 2005; 288: H2904-H2911.

[7] Ayoub IM, Kolarova J, Kantola R, Radhakrishnan J, Gazmuri RJ. Zoniporide preserves left ventricular compliance during ventricular fibrillation and minimizes post-resuscitation myocardial dysfunction through benefits on energy metabolism. Crit Care Med 2007; 35: 2329-2336.

[8] Grmec S, Strnad M, Kupnik D, Sinkovic A, Gazmuri RJ. Erythropoietin facilitates the return of spontaneous circulation and survival in victims of out-of-hospital cardiac arrest. Resuscitation 2009; 80: 631-637.

[9] Hanlon PR, Fu P, Wright GL, Steenbergen C, Arcasoy MO, Murphy E. Mechanisms of erythropoietin-mediated cardioprotection during ischemia-reperfusion injury: role of protein kinase C and phosphatidylinositol 3-kinase signaling. FASEB J 2005; 19: 1323-1325.

[10] Cai Z and Semenza GL. Phosphatidylinositol-3-kinase signaling is required for erythropoietin-mediated acute protection against myocardial ischemia/reperfusion injury. Circulation 2004; 109: 2050-2053.

[11] Shi Y, Rafiee P, Su J, Pritchard KA Jr, Tweddell JS, Baker JE. Acute cardioprotective effects of erythropoietin in infant rabbits are mediated by activation of protein kinases and potassium channels. Basic Res Cardiol 2004; 99: 173-182.

[12] Radhakrishnan J, Kolarova JD, Ayoub IM, Gazmuri RJ. AVE4454B--a novel sodium-hydrogen exchanger isoform-1 inhibitor--compared less effective than cariporide for resuscitation from cardiac arrest. Transl Res 2011; 157: 71-80.

[13] Radhakrishnan J, Wang S, Ayoub IM, Kolarova JD, Levine RF, Gazmuri RJ. Circulating levels of cytochrome *c* after resuscitation from cardiac arrest: a marker of mitochondrial injury and predictor of survival. Am J Physiol Heart Circ Physiol 2007; 292: H767-H775.

[14] Sharma SK and Carew TJ. Inclusion of phosphatase inhibitors during Western blotting enhances signal detection with phospho-specific antibodies. Anal Biochem 2002; 307: 187-189.

[15] Janssen AJ, Trijbels FJ, Sengers RC, Smeitink JA, van den Heuvel LP, Wintjes LT, Stoltenborg-Hogenkamp BJ, Rodenburg RJ. Spectrophotometric assay for complex I of the respiratory chain in tissue samples and cultured fibroblasts. Clin Chem 2007; 53: 729-734.

[16] Jarreta D, Orus J, Barrientos A, Miro O, Roig E, Heras M, Moraes CT, Cardellach F, Casademont J. Mitochondrial function in heart muscle from patients with idiopathic dilated cardiomyopathy. Cardiovasc Res 2000; 45: 860-865.

[17] Tzagoloff A. Oligomycin-sensitive ATPase of Saccharomyces cerevisiae. Methods Enzymol 1979; 55: 351-358.

[18] Mueller DM. Arginine 328 of the beta-subunit of the mitochondrial ATPase in yeast is essential for protein stability. J Biol Chem 1988; 263: 5634-5639.

[19] King EJ. The colorimetric determination of phosphorus. Biochem J 1932; 26: 292-297.

[20] Gazmuri RJ, Weil MH, Bisera J, Tang W, Fukui M, McKee D. Myocardial dysfunction after successful resuscitation from cardiac arrest. Crit Care Med 1996; 24: 992-1000.

[21] Kern KB, Hilwig RW, Rhee KH, Berg RA. Myocardial dysfunction after resuscitation from cardiac arrest: An example of global myocardial stunning. J Am Coll Cardiol 1996; 28: 232-240.

[22] Tojo A, Doumoto M, Oka K, Numabe A, Kimura K, Yagi S. Endothelin-mediated effect of erythropoietin on blood pressure and renal hemodynamics in hypertensive rats. Am J Physiol 1996; 270: R744-R748.

[23] Laurent I, Monchi M, Chiche JD, Joly LM, Spaulding C, Bourgeois B, Cariou A, Rozenberg A, Carli P, Weber S, Dhainaut JF. Reversible myocardial dysfunction in survivors of out-of-hospital cardiac arrest. J Am Coll Cardiol 2002; 40: 2110-2116.

[24] Kern KB, Hilwig RW, Berg RA, Rhee KH, Sanders AB, Otto CW, Ewy GA. Postresuscitation left ventricular systolic and diastolic dysfunction. Treatment with dobutamine. Circulation 1997; 95: 2610-2613.

[25] Prabu SK, Anandatheerthavarada HK, Raza H, Srinivasan S, Spear JF, Avadhani NG. Protein kinase A-mediated phosphorylation modulates cytochrome c oxidase function and augments hypoxia and myocardial ischemia-related injury. J Biol Chem 2006; 281: 2061-2070.

[26] Corbucci GG, Perrino C, Donato G, Ricchi A, Lettieri B, Troncone G, Indolfi C, Chiariello M, Avvedimento EV. Transient and reversible de-

oxyribonucleic acid damage in human left ventricle under controlled ischemia and reperfusion. J Am Coll Cardiol 2004; 43: 1992-1999.
[27] Dawson TL, Gores GJ, Nieminen AL, Herman B, Lemasters JJ. Mitochondria as a source of reactive oxygen species during reductive stress in rat hepatocytes. Am J Physiol 1993; 264: C961-C967.
[28] Guo D, Nguyen T, Ogbi M, Tawfik H, Ma G, Yu Q, Caldwell RW, Johnson JA. Protein kinase C-epsilon coimmunoprecipitates with cytochrome oxidase subunit IV and is associated with improved cytochrome-c oxidase activity and cardioprotection. Am J Physiol Heart Circ Physiol 2007; 293: H2219-H2230