Targeting Mitochondria During CPR

13

Raúl J. Gazmuri

13.1 Mitochondria and Cardiac Resuscitation

Sudden cardiac arrest is a major public health problem with ~360,000 cases assessed every year by Emergency Medical Services in the United States yielding a survival rate to hospital discharge that averages only 9.5 % [1]; a percentage that has improved very little over the past decade. Restoration of cardiac activity requires reperfusion by external means (i.e., CPR) of a myocardium that has been ischemic for a variable period of time. Reperfusion is obligatory to deliver the oxygen required for mitochondria to restore capability to regenerate ATP (i.e., bioenergetic function) and thus create the conditions required for resumption of an electrically organized and mechanically competent cardiac activity. Yet, reperfusion also triggers injury that largely involves generation of reactive oxygen species [2] and mitochondrial calcium overload [3, 4]. This injury further compromises mitochondrial bioenergetic function and thus the conditions required for successful cardiac resuscitation [5].

Current resuscitation methods focus almost exclusively on means to generate blood flow and terminate ventricular fibrillation (VF) but lack therapies directed at protecting mitochondria. In this chapter, basic concepts of mitochondrial function are discussed along with experimental evidence pointing to mitochondrial involvement and interventions to protect their function in helping to restore cardiac activity and lessen post-resuscitation myocardial dysfunction.

R. J. Gazmuri (✉)
Resuscitation Institute at Rosalind Franklin University of Medicine and Science,
3333 Green Bay Road, North Chicago, IL 60064, USA
e-mail: raul.gazmuri@rosalindfranklin.edu

A. Gullo and G. Ristagno (eds.), *Resuscitation*,
DOI: 10.1007/978-88-470-5507-0_13,

13.2 Mitochondrial Function and Dysfunction

13.2.1 Bioenergetic Function

Mitochondria are highly abundant in myocardial tissue encompassing ~35 % of the cardiomyocyte volume, and are "strategically" located to power contractile activity adopting a "crystal-like" structure with one mitochondrion per sarcomere [6]. Transfer of energy contained in nutrients to molecules of ATP starts with the reduction of nicotinamide adenine dinucleotide (NAD^+) to NADH and flavin adenine dinucleotide (FAD) to $FADH_2$ in the mitochondrial matrix. NADH and $FADH_2$ transfer their electrons down a redox potential through complexes I, II, III, and IV of the electron transport chain to oxygen; the final electron acceptor. Complexes I, III, and IV are also proton pumps and translocate H^+ against their electrochemical gradient from the mitochondrial matrix to the inter-mitochondrial membrane space creating a proton motive force that powers the enzyme F_oF_1 ATPsynthase to regenerate ATP from ADP and inorganic phosphate (Fig. 13.1). The newly synthesized ATP is then exchanged for ADP across the inner-mitochondrial membrane by the adenine nucleotide translocator (ANT). The newly synthesized and translocated ATP is used to phosphorylate creatine which is then

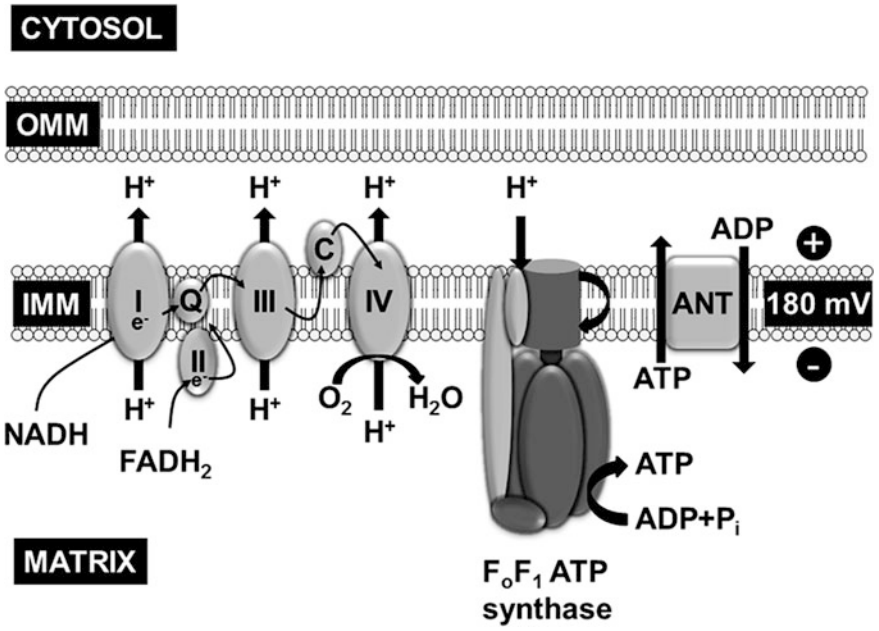

Fig. 13.1 Schematic rendition of key mitochondrial components involved in ATP synthesis via oxidative phosphorylation. *OMM*, outer mitochondrial membrane; *IMM*, inner-mitochondrial membrane; *I*, *II*, *III*, and *IV*, electron transport complexes of the respiratory chain; e^-, electrons; Q, coenzyme Q; C, cytochrome *c*; *ANT*, adenine nucleotide translocator; *NADH*, reduced nicotinamide adenine dinucleotide; $FADH_2$, reduced flavin adenine dinucleotide

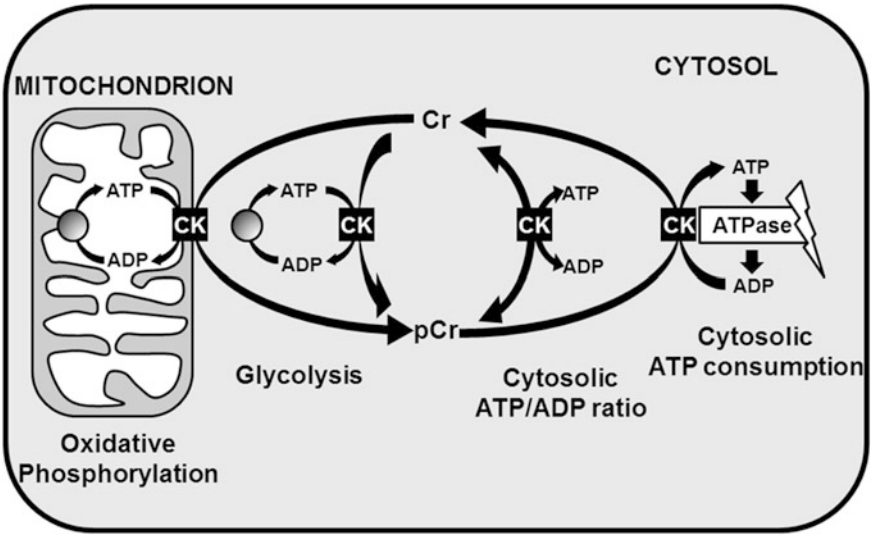

Fig. 13.2 Schematic rendition of mitochondrial *ATP* synthesis and translocation to the cytosol through the creatine phosphate shuttle. *CK*, creatine kinase; *pCr*, phosphocreatine; *Cr*, creatine

exported outside mitochondria to regenerate ATP being used in various energy requiring processes (Fig. 13.2). Measuring the amount of creatine phosphate relative to total creatine is indeed a useful indirect measurement of mitochondrial function.

13.2.2 Cell Death Signaling and Cytochrome *c* Release as Marker of Mitochondrial Injury

In addition to its bioenergetic function, mitochondria also participate in processes leading to cell death via necrosis or apoptosis. Various distinctive mechanisms have been identified including opening of the so-called mitochondrial permeability transition pore (leading to collapse of the proton motive force and uncoupling of respiration) [7] and release of various pro-apoptotic proteins, including cytochrome *c*, apoptosis-inducing factor, Smac/DIABLO, endonuclease G, and a serine protease Omi/HtrA2 [8, 9]. Of these proteins, cytochrome *c* has been the most widely studied, including work in our laboratory [10, 11].

Cytochrome *c* is a 14-kDa hemoprotein that normally resides in the outer surface of the inner-mitochondrial membrane bound to cardiolipin [12]. Cytochrome *c* plays a crucial role in oxidative phosphorylation enabling transfer of electrons from complex III to complex IV (Fig. 13.1). However, cytochrome *c* can also translocate to the cytosol under various pathological conditions including (among others) oxidative stress [13], calcium overload [14], and injury by hypoxia and reoxygenation [15, 16]. In the cytosol, cytochrome *c* forms an oligomeric

complex with 2-deoxy-ATP and the apoptotic protease activating factor-1 [17]. This complex recruits procaspase-9 forming what is known as the apoptosome leading to cleavage and release of active caspase-9, which in turn cleaves and activates caspases-3, -6, and -7 [18–20]; the effectors of apoptosis.

Cytochrome *c* can also leave the cell and reach the bloodstream through mechanisms apparently unrelated to cell necrosis [21, 22]. In patients, elevated levels of circulating cytochrome *c* have been reported associated with conditions able to injure mitochondria such as cancer [23, 24], chemotherapy [21, 25], acute myocardial infarction [26], reperfusion after coronary intervention [27], possibly cardiomyopathies [28], fulminant hepatitis [29], the systemic inflammatory response syndrome [30], and influenza-associated encephalopathy [31, 32].

In a rat model of VF and CPR, we reported the release of cytochrome *c* to the cytosol in left ventricular tissue with activation of the mitochondrial apoptotic pathway through formation of the apoptosome as described earlier [10, 11]. However, in this model, activation of the mitochondrial apoptotic pathway did not cause cell death or was responsible for the severe myocardial dysfunction that characteristically occurs post-resuscitation [11]. In the same rat model, cytochrome *c* reached the bloodstream and progressively increased during CPR and the post-resuscitation period attaining levels that were inversely related to survival [10]. Thus, in rats that survived, plasma cytochrome *c* increased modestly to levels <2 μg/ml returning to baseline within 48–96 h. In rats that did not survive, plasma cytochrome *c* increased at a much faster rate and attained levels substantially higher than 2 μg/ml before demise, which was characteristically the consequence of hemodynamic deterioration.

Based on these findings, we have postulated that plasma cytochrome *c* could serve as biomarker of mitochondrial injury severity and be useful not only to prognosticate outcome but also to assess therapies designed to attenuate or reverse mitochondrial injury.

13.3 Mitochondrial Protection by Inhibition of the Sodium-Hydrogen Exchanger Isoform-1

Our laboratory had investigated for more than a decade the potential beneficial effects of inhibiting the sodium-hydrogen exchanger isoform-1 (NHE-1) during cardiac resuscitation, showing protective mitochondrial effects leading to functional myocardial effects that would be clinically relevant [5, 33–43].

13.3.1 Underlying Pathophysiology

The benefit associated with NHE-1 inhibition is linked to the pathophysiological process of cell injury triggered by the intense and sustained intracellular myocardial acidosis that develops during cardiac arrest after cessation of coronary blood flow

[44–46]. Intracellular acidosis activates the sarcolemmal NHE-1 bringing Na^+ into the cell in exchange for H^+ [47]. During the ensuing resuscitation effort, reperfusion of the ischemic myocardium washes-out H^+ that have accumulated in the extracellular space during no-flow ischemia intensifying the sarcolemmal Na^+–H^+ exchange [33, 47, 48]. Na^+ may also enter the cell through Na^+ channels and the Na^+–HCO_3^- co-transporter. The Na^+ entering the cell is not extruded as it normally would because of concomitant reduction of the Na^+–K^+ ATPase activity [49], such that progressive and prominent increases in cytosolic Na^+ occur.

The cytosolic Na^+ excess drives sarcolemmal Ca^{2+} influx through reverse mode operation of the sarcolemmal Na^+–Ca^{2+} exchanger leading to cytosolic Ca^{2+} overload [50] and subsequent mitochondrial Ca^{2+} entry; a process which is regulated by the Ca^{2+} uniporter for influx and the Na^+–Ca^{2+} exchanger for efflux [51]. Mitochondria can buffer large amounts of Ca^{2+} in its matrix up to a limit when free mitochondrial Ca^{2+} rises, the mitochondrial Na^+–Ca^{2+} exchanger becomes saturated, and mitochondrial Ca^{2+} overload ensues [51] worsening cell injury in part by compromising its capability to sustain oxidative phosphorylation [52] and by promoting the release of pro-apoptotic factors [53].

13.3.2 Relevance to Cardiac Resuscitation

The relevance of this mechanism of injury and potential therapeutic target is highlighted by preclinical work at the Resuscitation Institute using various animal models and other capabilities at the cellular and subcellular levels over more than a decade, strongly supporting a role of NHE-1 inhibition for resuscitation from cardiac arrest [5, 33–43].

Effects during VF: Initial observations were made in an isolated rat model of VF and simulated resuscitation using the NHE-1 inhibitor cariporide [33, 34]. In these studies, infusion of the NHE-1 inhibitor cariporide during simulated resuscitation markedly attenuated left ventricular pressure increases suggesting that NHE-1 inhibition could help preserve left ventricular distensibility during cardiac resuscitation. Post-resuscitation, hearts treated with cariporide had their end-diastolic pressure–volume curves preserved suggesting a beneficial effect preventing post-resuscitation diastolic dysfunction. These observations were followed by work in a clinically more relevant swine model of VF and CPR, showing that cariporide given as bolus dose immediately before starting chest compression could also preserve left ventricular distensibility during CPR in the intact animal, evidenced by preservation of wall thickness and cavity size. Preservation of left ventricular distensibility enabled chest compression to sustain the generation of coronary perfusion pressures at stable levels in contrast to controls animals in which the coronary perfusion pressure progressively declined. As a result, higher resuscitability was observed in animals treated with cariporide (2/8 vs. 8/8; $p < 0.05$) [36].

We hypothesized that the observed hemodynamic benefits in the swine model could reflect the ability of chest compression to generate a greater cardiac output

for a given compression depth as a result of preservation of left ventricular distensibility. In other words, a more distensible left ventricle would allow a larger volume of blood to fill the cavity before compression resulting in more blood ejected by the ensuing compression. To test this hypothesis, we conducted studies on an intact rat model of VF and CPR and measured cardiac output and regional organ blood flow using fluorescent microspheres while varying the depth of compression [38].

Two series of 14 experiments each were conducted in which rats were subjected to 10 min of untreated VF followed by 8 min of chest compression before attempting defibrillation. Compression depth was adjusted to maintain an aortic diastolic pressure between 26 and 28 mmHg in the first series and between 36 and 38 mmHg in the second series. Within each series, rats were randomized to receive cariporide (3 mg/kg) or NaCl 0.9 % (control) before starting chest compression. In rats that received cariporide, higher cardiac output and higher regional organ blood flow (including heart and brain) were generated for a given compression depth. In other words, cariporide causes a very favorable leftward shift of the flow-depth relationship as a result of maintaining left ventricular distensibility.

Because pressure is a function of flow and resistance, we further reasoned that administration of a vasopressor agent could potentiate the hemodynamic effect of shifting the flow-depth relationship to the left resulting in an even higher systemic and coronary perfusion pressure. This was indeed the case as we demonstrated in the same rat model of VF and closed-chest resuscitation [37]. These studies involved two series of 16 experiments each using epinephrine in one series and vasopressin in the other. Within each series, rats were randomized to receive cariporide or NaCl control immediately before starting chest compression with the vasopressor agents given during chest compression. A significantly higher coronary perfusion pressure was generated when either vasopressor agent was given in rats that had received cariporide. The effect was not mediated through a vascular effect as the vasoconstrictive effects of epinephrine or vasopressin were not enhanced by cariporide [37]. A similar effect was subsequently demonstrated associated with the administration of epinephrine in our pig model of VF and closed-chest resuscitation [39]. These effects on coronary perfusion pressure are important; if translated clinically they could be highly relevant because only a small increase in coronary perfusion pressure is required to have a dramatic effect on resuscitability [54].

Effects on post-resuscitation arrhythmias and refibrillation: Another prominent effect elicited by cariporide was the suppression of ventricular ectopic activity and refibrillation that typically occurs early after return of cardiac activity [34, 36, 39, 55]. This effect was associated with preservation of the action potential duration [36]; an effect that would facilitate preservation of the impulse wavelength and thus reducing the risk of reentry [55]. This is also an important effect, which if translated clinically could help stabilize initially resuscitated victim of out-of-hospital cardiac arrest and avert re-arrest episodes during initial post-resuscitation period while enroute to a hospital.

Effects on post-resuscitation myocardial function: Variable degrees of systolic dysfunction occur after resuscitation from cardiac arrest despite full restoration of coronary blood flow. This phenomenon, known as myocardial stunning, is reversible but reversibility may take hours or days and contingent on severity compromise hemodynamic function and survival. Myocardial stunning is amenable to inotropic stimulation [56, 57] and use of dobutamine has been shown to facilitate hemodynamic stabilization post-resuscitation [58]. Diastolic dysfunction also occurs in the post-resuscitation period and is linked to the same pathophysiological abnormalities responsible for decreases in distensibility; namely increases in diastolic Ca^{2+} overload and energy deficit precluding full relaxation of cardiomyocytes. Administration of NHE-1 inhibitors during CPR in our animal models also attenuated post-resuscitation left ventricular systolic and diastolic dysfunction [41, 55].

13.3.3 Mechanism of the Resuscitation Effects

We also investigated the underlying mechanism of the benefit associated with use of NHE-1 inhibitors. In a rat model of VF and closed-chest resuscitation, we examined the effects of NHE-1 inhibition and of Na^+ channel blockade (interventions collectively referred to as "Na^+-limiting interventions") on intracellular Na^+, mitochondrial Ca^{2+}, cardiac function, and plasma levels of cardiac troponin I (cTnI) [40]. For these studies, hearts were removed at specific time events; namely (i) at baseline, (ii) at 15 min of untreated VF, (iii) at 15 min of VF with chest compression provided during the last 5 min of VF, and (iv) at 60-min post-resuscitation. Rats from the last two time events were randomized to receive an Na^+-limiting intervention immediately before starting chest compression or vehicle control. The Na^+-limiting interventions included a newly developed NHE-1 inhibitor AVE4454 (1 mg/kg), lidocaine (5 mg/kg), and the combination of AVE4454 and lidocaine.

Limiting sarcolemmal Na^+ entry attenuated increases in cytosolic Na^+ and mitochondrial Ca^{2+} overload during chest compression and the post-resuscitation phase. Attenuation of cytosolic Na^+ and mitochondrial Ca^{2+} increases was accompanied by preservation of left ventricular distensibility during chest compression, less post-resuscitation myocardial dysfunction, and lower levels of cTnI. In similar studies, attenuation of post-resuscitation myocardial dysfunction by NHE-1 inhibitors was associated with lesser increases in plasma cytochrome *c* in inverse relationship with left ventricular function [43].

We also used an open-chest pig model of electrically induced VF and extracorporeal circulation to study the myocardial energy effects of inhibiting NHE-1 under conditions of controlled coronary perfusion pressure [41]. For this study, VF was induced by epicardial delivery of an alternating current and left untreated for 8 min. After this interval, extracorporeal circulation was started and the systemic (extracorporeal) blood flow adjusted to maintain a coronary perfusion pressure at

10 mmHg for 10 min before attempting defibrillation. The target coronary perfusion pressure was chosen to mimic the low coronary perfusion pressure generated by closed-chest resuscitation. Two groups of eight pigs each were randomized to receive the NHE-1 inhibitor zoniporide (3 mg/kg) or vehicle control as a right atrial bolus immediately before starting extracorporeal circulation. Like in previous studies using the NHE-1 inhibitor cariporide [36], zoniporide also prevented reductions in left ventricular distensibility during the interval of VF and extracorporeal circulation, which in control pigs was characterized by progressive reductions in cavity size and progressive thickening of the left ventricular wall. Importantly, these effects occurred without changes in coronary blood flow or coronary vascular resistance indicating that the favorable myocardial effects of NHE-1 inhibition during resuscitation are not likely to be mediated through increases in blood flow and oxygen availability.

Myocardial tissue measurements indicated that administration of zoniporide prevented progressive loss of oxidative phosphorylation during the interval of simulated resuscitation. This effect was supported by a higher creatine phosphate-to-creatine (pCr/Cr) ratio, higher ATP/ADP ratio, and lesser increases in adenosine in animals treated with zoniporide. These measurements are consistent with regeneration of ADP into ATP by mitochondria instead of downstream degradation to adenosine, with the newly formed ATP being used to regenerate creatinine phosphate; all indicative of preserved mitochondrial bioenergetic function.

These changes were accompanied with prominent amelioration of myocardial lactate increases, attaining levels which were inversely proportional to the pCr/Cr ratio at 8 min of VF and extracorporeal circulation, suggesting a shift away from anaerobic metabolism consequent to preservation of mitochondrial bioenergetic function in pigs treated with zoniporide.

These energy effects are consistent with NHE-1 inhibition protecting mitochondrial bioenergetic function—probably as a result of limiting mitochondrial Ca^{2+} overload—and supportive of the concept that left ventricular distensibility during resuscitation is likely to be preserved by activating mitochondrial mechanisms capable of maintaining bioenergetic function.

13.3.4 Barriers to Clinical Translation

Unfortunately, efforts by pharmaceutical companies to develop NHE-1 inhibitors for clinical use have been modest at best and targeted only myocardial infarction [59–61] and myocardial protection during coronary artery bypass surgery (CABG) [60, 62]. Although the studies in acute myocardial infarction were inconclusive—with only one of three studies showing myocardial benefits [59]—studies in patients undergoing CABG—best represented by the EXPEDITION trial [62]—demonstrated a prominent myocardial protective effect providing proof-of-concept and lending support for NHE-1 inhibition in this clinical setting. The EXPEDITION trial compared cariporide with placebo in 5,761 high risk patients

undergoing CABG. Cariporide—given intravenously before surgery and after surgery for 48 h—reduced the incidence of postoperative myocardial infarction from 18.9 % in the placebo group to 14.4 % in the treatment group ($p < 0.001$). Unfortunately and unexpectedly, patients who received cariporide had higher incidence of occlusive strokes. In subsequent analysis, the risk of stroke was linked to an enhanced platelet aggregation effect related to a very high dose of cariporide used in the study. However, the effect was unrelated to the mode of action and was not observed with other NHE-1 inhibitors.

Experts in the field have attributed the inconclusive findings of NHE-1 inhibition for acute myocardial infarction to the diminishing efficacy of NHE-1 inhibition when given only at the time of reperfusion after an extended period of coronary occlusion [63, 64]; a concept that is also supported by studies in a porcine model of coronary occlusion and reperfusion [65]. Likewise, the benefit observed in the CABG population can be explained by the administration of NHE-1 inhibitors before the anticipated episodes of myocardial ischemia [62]. In contrast to acute myocardial infarction and CABG, cardiac arrest is characterized by rapid development of intense myocardial ischemia (and other organs including the brain) but without infarction thus enabling to intervene on tissues suffering potentially reversible injury.

13.3.5 Alternative Strategies

Pending clinical development of NHE-1 inhibitors, we examined alternative mitochondrial protective strategies using compounds that are clinically available for other uses hypothesizing that mitochondrial protection through non-genomic activation of protective pathways such as Akt or the use of antioxidants could be beneficial. Applying this paradigm with first examined whether erythropoietin administered at the start of CPR could be as effective as an NHE-1 inhibitor. Studies in rat models of VF and CPR demonstrated a similar effect on left ventricular distensibility and an effect favoring reversal of post-resuscitation myocardial dysfunction in the presence of dobutamine [58, 66]. In these studies, use of erythropoietin was associated with activation of Akt and PKCε in myocardial tissue and preservation of activity of complex IV of the electron transport chain. These effects, consistent with activation of mitochondrial protective mechanisms, were also associated with an inverse relationship between plasma cytochrome *c* and left ventricular function. However, in a more recent study using a swine model of VF and resuscitation by ECC, we could not reproduce the beneficial effects on myocardial distensibility observed in rats. Moreover, no effects on myocardial energy metabolism or mitochondrial protective pathways could be demonstrated despite a modest favorable effect on post-resuscitation left ventricular systolic function [67].

Examination of other potential interventions in our rat model, including vitamin C [68] and estrogens (Unpublished) was not only ineffective but also associated with decreased resuscitability and survival.

13.4 Conclusions

Our experience using various animal models of VF and resuscitation over the last 15 years indicates that mitochondria play a key role in resuscitation from cardiac arrest and that therapies aimed at protecting mitochondrial bioenergetic function have the potential for facilitating initial resuscitation and subsequent survival. Based on our work we continue to look forward to the clinical development of NHE-1 inhibitors for reducing mitochondrial Ca^{2+} overload as the most promising experimental pharmacological intervention for cardiac resuscitation.

References

1. Go AS, Mozaffarian D, Roger VL, Benjamin EJ, Berry JD, Borden WB, Bravata DM, Dai S, Ford ES, Fox CS, Franco S, Fullerton HJ, Gillespie C, Hailpern SM, Heit JA, Howard VJ, Huffman MD, Kissela BM, Kittner SJ, Lackland DT, Lichtman JH, Lisabeth LD, Magid D, Marcus GM, Marelli A et al (2013) Heart disease and stroke statistics–2013 update: a report from the American Heart Association. Circulation 127(1):e6–e245
2. Weisfeldt ML, Zweier J, Ambrosio G, Becker LC, Flaherty JT (1988) Evidence that free radicals result in reperfusion injury in heart muscle. Basic Life Sci 49:911–919
3. Dong Z, Saikumar P, Weinberg JM, Venkatachalam MA (2006) Calcium in cell injury and death. Annu Rev Pathol 1:405–434
4. Halestrap AP (2006) Calcium, mitochondria and reperfusion injury: a pore way to die. Biochem Soc Trans 34(Pt 2):232–237
5. Gazmuri RJ, Radhakrishnan J (2012) Protecting mitochondrial bioenergetic function during resuscitation from cardiac arrest. Crit Care Clin 28(2):245–270
6. Vendelin M, Beraud N, Guerrero K, Andrienko T, Kuznetsov AV, Olivares J, Kay L, Saks VA (2005) Mitochondrial regular arrangement in muscle cells: a "crystal-like" pattern. Am J Physiol Cell Physiol 288(3):C757–C767
7. Halestrap AP, Clarke SJ, Javadov SA (2004) Mitochondrial permeability transition pore opening during myocardial reperfusion–a target for cardioprotection. Cardiovasc Res 61(3):372–385
8. Cai J, Yang J, Jones DP (1998) Mitochondrial control of apoptosis: the role of cytochrome c. Biochim Biophys Acta 1366(1–2):139–149
9. Green DR, Reed JC (1998) Mitochondria and apoptosis. Science 281(5381):1309–1312
10. Radhakrishnan J, Wang S, Ayoub IM, Kolarova JD, Levine RF, Gazmuri RJ (2007) 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 292:H767–H775
11. Radhakrishnan J, Ayoub IM, Gazmuri RJ (2009) Activation of caspase-3 may not contribute to postresuscitation myocardial dysfunction. Am J Physiol Heart Circ Physiol 296(4):H1164–H1174
12. Ott M, Robertson JD, Gogvadze V, Zhivotovsky B, Orrenius S (2002) Cytochrome c release from mitochondria proceeds by a two-step process. Proc Natl Acad Sci USA 99(3):1259–1263

13. von Harsdorf R, Li PF, Dietz R (1999) Signaling pathways in reactive oxygen species-induced cardiomyocyte apoptosis. Circulation 99(22):2934–2941
14. Petrosillo G, Ruggiero FM, Pistolese M, Paradies G (2004) Ca2+-induced reactive oxygen species production promotes cytochrome c release from rat liver mitochondria via mitochondrial permeability transition (MPT)-dependent and MPT-independent mechanisms: role of cardiolipin. J Biol Chem 279(51):53103–53108
15. de Moissac D, Gurevich RM, Zheng H, Singal PK, Kirshenbaum LA (2000) Caspase activation and mitochondrial cytochrome C release during hypoxia-mediated apoptosis of adult ventricular myocytes. J Mol Cell Cardiol 32(1):53–63
16. Loor G, Kondapalli J, Iwase H, Chandel NS, Waypa GB, Guzy RD Vanden Hoek TL, Schumacker PT (2011) Mitochondrial oxidant stress triggers cell death in simulated ischemia-reperfusion. Biochim Biophys Acta 1813(7):1382–1394
17. Li P, Nijhawan D, Budihardjo I, Srinivasula SM, Ahmad M, Alnemri ES, Wang X (1997) Cytochrome c and dATP-dependent formation of Apaf-1/caspase-9 complex initiates an apoptotic protease cascade. Cell 91(4):479–489
18. Budihardjo I, Oliver H, Lutter M, Luo X, Wang X (1999) Biochemical pathways of caspase activation during apoptosis. Annu Rev Cell Dev Biol 15:269–290
19. Saleh A, Srinivasula SM, Acharya S, Fishel R, Alnemri ES (1999) Cytochrome c and dATP-mediated oligomerization of Apaf-1 is a prerequisite for procaspase-9 activation. J Biol Chem 274(25):17941–17945
20. Zou H, Li Y, Liu X, Wang X (1999) An APAF-1.cytochrome c multimeric complex is a functional apoptosome that activates procaspase-9. J Biol Chem 274(17):11549–11556
21. Renz A, Burek C, Mier W, Mozoluk M, Schulze-Osthoff K, Los M (2001) Cytochrome c is rapidly extruded from apoptotic cells and detectable in serum of anticancer-drug treated tumor patients. Adv Exp Med Biol 495:331–334
22. Zager RA, Johnson AC, Hanson SY (2004) Proximal tubular cytochrome c efflux: determinant, and potential marker, of mitochondrial injury. Kidney Int 65(6):2123–2134
23. Osaka A, Hasegawa H, Tsuruda K, Inokuchi N, Yanagihara K, Yamada Y, Aoyama M, Sawada T, Kamihira S (2009) Serum cytochrome c to indicate the extent of ongoing tumor cell death. Int J Lab Hematol 31(3):307–314
24. Liu X, Xie W, Liu P, Duan M, Jia Z, Li W, Xu J (2006) Mechanism of the cardioprotection of rhEPO pretreatment on suppressing the inflammatory response in ischemia-reperfusion. Life Sci 78(19):2255–2264
25. Barczyk K, Kreuter M, Pryjma J, Booy EP, Maddika S, Ghavami S, Berdel WE, Roth J, Los M (2005) Serum cytochrome c indicates in vivo apoptosis and can serve as a prognostic marker during cancer therapy. Int J Cancer 116(2):167–173
26. Alleyne T, Joseph J, Sampson V (2001) Cytochrome-c detection: a diagnostic marker for myocardial infarction. Appl Biochem Biotechnol 90(2):97–105
27. Marenzi G, Giorgio M, Trinei M, Moltrasio M, Ravagnani P, Cardinale D, Ciceri F, Cavallero A, Veglia F, Fiorentini C, Cipolla CM, Bartorelli AL, Pelicci P (2010) Circulating cytochrome c as potential biomarker of impaired reperfusion in ST-segment elevation acute myocardial infarction. Am J Cardiol 106(10):1443–1449
28. Narula J, Pandey P, Arbustini E, Haider N, Narula N, Kolodgie FD, Dal Bello B, Semigran MJ, Bielsa-Masdeu A, Dec GW, Israels S, Ballester M, Virmani R, Saxena S, Kharbanda S (1999) Apoptosis in heart failure: release of cytochrome c from mitochondria and activation of caspase-3 in human cardiomyopathy. Proc Natl Acad Sci USA 96(14):8144–8149
29. Sakaida I, Kimura T, Yamasaki T, Fukumoto Y, Watanabe K, Aoyama M, Okita K (2005) Cytochrome c is a possible new marker for fulminant hepatitis in humans. J Gastroenterol 40(2):179–185
30. Adachi N, Hirota M, Hamaguchi M, Okamoto K, Watanabe K, Endo F (2004) Serum cytochrome c level as a prognostic indicator in patients with systemic inflammatory response syndrome. Clin Chim Acta 342(1–2):127–136

31. Hosoya M, Nunoi H, Aoyama M, Kawasaki Y, Suzuki H (2005) Cytochrome c and tumor necrosis factor-alpha values in serum and cerebrospinal fluid of patients with influenza-associated encephalopathy. Pediatr Infect Dis J 24(5):467–470
32. Hosoya M, Kawasaki Y, Katayose M, Sakuma H, Watanabe M, Igarashi E, Aoyama M, Nunoi H, Suzuki H (2006) Prognostic predictive values of serum cytochrome c, cytokines, and other laboratory measurements in acute encephalopathy with multiple organ failure. Arch Dis Child 91:469–472
33. Gazmuri RJ, Hoffner E, Kalcheim J, Ho H, Patel M, Ayoub IM, Epstein M, Kingston S, Han Y (2001) Myocardial protection during ventricular fibrillation by reduction of proton-driven sarcolemmal sodium influx. J Lab Clin Med 137(1):43–55
34. Gazmuri RJ, Ayoub IM, Hoffner E, Kolarova JD (2001) Successful ventricular defibrillation by the selective sodium-hydrogen exchanger isoform-1 inhibitor cariporide. Circulation 104:234–239
35. Gazmuri RJ, Ayoub IM, Kolarova JD, Karmazyn M (2002) Myocardial protection during ventricular fibrillation by inhibition of the sodium-hydrogen exchanger isoform-1. Crit Care Med 30(4 Suppl):S166–S171
36. Ayoub IM, Kolarova JD, Yi Z, Trevedi A, Deshmukh H, Lubell DL, Franz MR, Maldonado FA, Gazmuri RJ (2003) Sodium-hydrogen exchange inhibition during ventricular fibrillation: Beneficial effects on ischemic contracture, action potential duration, reperfusion arrhythmias, myocardial function, and resuscitability. Circulation 107:1804–1809
37. Kolarova J, Yi Z, Ayoub IM, Gazmuri RJ (2005) Cariporide potentiates the effects of epinephrine and vasopressin by nonvascular mechanisms during closed-chest resuscitation. Chest 127(4):1327–1334
38. Kolarova JD, Ayoub IM, Gazmuri RJ (2005) Cariporide enables hemodynamically more effective chest compression by leftward shift of its flow-depth relationship. Am J Physiol Heart Circulatory Physiol 288:H2904–H2911
39. Ayoub IM, Kolarova J, Kantola RL, Sanders R, Gazmuri RJ (2005) Cariporide minimizes adverse myocardial effects of epinephrine during resuscitation from ventricular fibrillation. Crit Care Med 33(11):2599–2605
40. Wang S, Radhakrishnan J, Ayoub IM, Kolarova JD, Taglieri DM, Gazmuri RJ (2007) Limiting sarcolemmal Na+ entry during resuscitation from VF prevents excess mitochondrial Ca2+ accumulation and attenuates myocardial injury. J Appl Physiol 103:55–65
41. Ayoub IM, Kolarova J, Kantola R, Radhakrishnan J, Gazmuri RJ (2007) Zoniporide preserves left ventricular compliance during ventricular fibrillation and minimizes post-resuscitation myocardial dysfunction through benefits on energy metabolism. Crit Care Med 35:2329–2336
42. Ayoub IM, Kolarova J, Gazmuri RJ (2010) Cariporide given during resuscitation promotes return of electrically stable and mechanically competent cardiac activity. Resuscitation 81(1):106–110
43. Radhakrishnan J, Kolarova JD, Ayoub IM, Gazmuri RJ (2011) AVE4454B—a novel sodium-hydrogen exchanger isoform-1 inhibitor—compared less effective than cariporide for resuscitation from cardiac arrest. Transl Res 157(2):71–80
44. von Planta M, Weil MH, Gazmuri RJ, Bisera J, Rackow EC (1989) Myocardial acidosis associated with CO2 production during cardiac arrest and resuscitation. Circulation 80:684–692
45. Kette F, Weil MH, Gazmuri RJ, Bisera J, Rackow EC (1993) Intramyocardial hypercarbic acidosis during cardiac arrest and resuscitation. Crit Care Med 21(6):901–906
46. Noc M, Weil MH, Gazmuri RJ, Sun S, Bisera J, Tang W (1994) Ventricular fibrillation voltage as a monitor of the effectiveness of cardiopulmonary resuscitation. J Lab Clin Med 124:421–426
47. Karmazyn M, Sawyer M, Fliegel L (2005) The na(+)/h(+) exchanger: a target for cardiac therapeutic intervention. Curr Drug Targets Cardiovasc Haematol Disord 5(4):323–335

48. Imahashi K, Kusuoka H, Hashimoto K, Yoshioka J, Yamaguchi H, Nishimura T (1999) Intracellular sodium accumulation during ischemia as the substrate for reperfusion injury. Circ Res 84(12):1401–1406
49. Avkiran M, Ibuki C, Shimada Y, Haddock PS (1996) Effects of acidic reperfusion on arrhythmias and Na(+)-K(+)-ATPase activity in regionally ischemic rat hearts. Am J Physiol 270(3 Pt 2):H957–H964
50. An J, Varadarajan SG, Camara A, Chen Q, Novalija E, Gross GJ, Stowe DF (2001) Blocking Na(+)/H(+) exchange reduces [Na(+)](i) and [Ca(2+)](i) load after ischemia and improves function in intact hearts. Am J Physiol 281(6):H2398–H2409
51. Gunter TE, Buntinas L, Sparagna G, Eliseev R, Gunter K (2000) Mitochondrial calcium transport: mechanisms and functions. Cell Calcium 28(5–6):285–296
52. Yamamoto S, Matsui K, Ohashi N (2002) Protective effect of Na+/H+ exchange inhibitor, SM-20550, on impaired mitochondrial respiratory function and mitochondrial Ca2+ overload in ischemic/reperfused rat hearts. J Cardiovasc Pharmacol 39(4):569–575
53. Borutaite V, Brown GC (2003) Mitochondria in apoptosis of ischemic heart. FEBS Lett 541(1–3):1–5
54. Paradis NA, Martin GB, Rivers EP, Goetting MG, Appleton TJ, Feingold M, Nowak RM (1990) Coronary perfusion pressure and the return of spontaneous circulation in human cardiopulmonary resuscitation. JAMA 263:1106–1113
55. Ayoub IM, Kolarova J, Gazmuri RJ (2009) Cariporide given during resuscitation promotes return of electrically stable and mechanically competent cardiac activity. Resuscitation 81:106–110
56. Ellis SG, Wynne J, Braunwald E, Henschke CI, Sandor T, Kloner RA (1984) Response of reperfusion-salvaged, stunned myocardium to inotropic stimulation. Am Heart J 107(1):13–19
57. Meyer RJ, Kern KB, Berg RA, Hilwig RW, Ewy GA (2002) Post-resuscitation right ventricular dysfunction: delineation and treatment with dobutamine. Resuscitation 55(2):187–191
58. Radhakrishnan J, Upadhyaya MP, Ng M, Edelheit A, Moy HM, Ayoub IM, Gazmuri RJ (2013) Erythropoietin facilitates resuscitation from ventricular fibrillation by signaling protection of mitochondrial bioenergetic function in rats. Am J Transl Res 5(3):316–326
59. Rupprecht HJ, vom DJ, Terres W, Seyfarth KM, Richardt G, Schultheibeta HP, Buerke M, Sheehan FH, Drexler H (2000) Cardioprotective effects of the Na(+)/H(+) exchange inhibitor cariporide in patients with acute anterior myocardial infarction undergoing direct PTCA. Circulation 101(25):2902–2908
60. Boyce SW, Bartels C, Bolli R, Chaitman B, Chen JC, Chi E, Jessel A, Kereiakes D, Knight J, Thulin L, Theroux P (2003) Impact of sodium-hydrogen exchange inhibition by cariporide on death or myocardial infarction in high-risk CABG surgery patients: results of the CABG surgery cohort of the GUARDIAN study. J Thorac Cardiovasc Surg 126(2):420–427
61. Zeymer U, Suryapranata H, Monassier JP, Opolski G, Davies J, Rasmanis G, Linssen G, Tebbe U, Schroder R, Tiemann R, Machnig T, Neuhaus KL (2001) The Na(+)/H(+) exchange inhibitor eniporide as an adjunct to early reperfusion therapy for acute myocardial infarction. Results of the evaluation of the safety and cardioprotective effects of eniporide in acute myocardial infarction (ESCAMI) trial. J Am Coll Cardiol 38(6):1644–1650
62. Mentzer RM Jr, Bartels C, Bolli R, Boyce S, Buckberg GD, Chaitman B, Haverich A, Knight J, Menasche P, Myers ML, Nicolau J, Simoons M, Thulin L, Weisel RD (2008) Sodium-hydrogen exchange inhibition by cariporide to reduce the risk of ischemic cardiac events in patients undergoing coronary artery bypass grafting: results of the EXPEDITION study. Ann Thorac Surg 85(4):1261–1270
63. Murphy E, Allen DG (2009) Why did the NHE inhibitor clinical trials fail? J Mol Cell Cardiol 46(2):137–141
64. Karmazyn M (2013) NHE-1: still a viable therapeutic target. J Mol Cell Cardiol. 61:77–82

65. Klein HH, Pich S, Bohle RM, Lindert-Heimberg S, Nebendahl K (2000) Na(+)/H(+) exchange inhibitor cariporide attenuates cell injury predominantly during ischemia and not at onset of reperfusion in porcine hearts with low residual blood flow. Circulation 102(16):1977–1982
66. Singh D, Kolarova JD, Wang S, Ayoub IM, Gazmuri RJ (2007) Myocardial protection by erythropoietin during resuscitation from ventricular fibrillation. Am J Ther 14:361–368
67. Borovnik-Lesja V, Whitehouse K, Baetiong A, Artin B, Radhakrishnan J, Gazmuri RJ (2013) High-dose erythropoietin during cardiac resuscitation lessens post-resuscitation myocardial stunning in swine. Transl Res 162(2):110–121
68. Motl J, Radhakrishnan J, Ayoub IM, Grmec S, Gazmuri RJ (2012) Vitamin C compromises cardiac resuscitability in a rat model of ventricular fibrillation. Am J Ther. Jun 16 [Epub]