SIGNA VITAE 2008; 3(1): 7 - 12

REVIEW

# Myocardial effects of cardiac arrest and resuscitation with especial reference to mitochondrial injury

RAÚL J. GAZMURI ( ✉ ),
IYAD M. AYOUB,
JEEJABAI RADHAKRISHNAN
Rosalind Franklin University
of Medicine and Science
North Chicago VA Medical Center
Chicago, USA
Phone: 224-610-3681
Fax: 224-610-3741
E-mail: raul.gazmuri@rosalindfranklin.edu

**RAÚL J. GAZMURI • IYAD M. AYOUB • JEEJABAI RADHAKRISHNAN**

## ABSTRACT

*The underlying mechanism of cell injury during ischemia and reperfusion is complex and timesesnsitive. Some processess develop coincidentally with the onset of ischemia and during reperfusion leading to abnormalities in energy metabolism, acid base status, and intracellular ion homeostasis. Other processes develop later and encompass activation of various signalling pathways that have deleterious or beneficial effects on specific effectors, but associated with sustained disruption of energy production contractile dysfunction and activation of apoptotic pathways. Discussion on the various cell mechanisms resposible for cell injury is beyond the scope of this review. However, pertinent to our discussion is the mounting evidence pointing to mitochondria as key target organelles of reperfusion injury.*

**Key words: cardiac arrest, mitochondrial injury, cardiopulmonary resuscitation, apoptosis**

The working heart is a highly metabolically active organ that consumes close to 10% of the total body oxygen consumption. It extracts nearly 70% of the oxygen supplied by the coronary circuit but has little capability for extracting additional oxygen. Accordingly, increased metabolic demands are met through coronary vasodilation resulting in increased blood flow and oxygen delivery. (1,2) As a result, a severe energy imbalance develops during cardiac arrest shortly after coronary blood flow ceases. The severity of the energy deficit is contingent on the metabolic requirements and is particularly high when ventricular fibrillation (VF) is present; because the energy needs of the fibrillating heart are the same or more than the normally beating heart. (3) A lesser energy requirement occurs when cardiac arrest occurs in the quiescent or minimally active heart (i.e., asystole or pulseless electrical activity as a result of asphyxia or exsanguinations). (4) However, most experimental studies have examined the myocardial manifestations of cardiac arrest and resuscitation in animal models of VF.

After cessation of coronary blood flow and oxygen delivery, the mitochondrial capability for ATP regenerating through oxidative phosphorylation stops prompting anaerobic regeneration of limited amount of ATP at the substrate level from breakdown of creatine phosphate and oxidation of pyruvate to lactate. (5-7) This leads to rapid depletion of creatine phosphate, marked elevation in lactate, and a relatively slow depletion of ATP. (6) We observed in a rat model of VF, that 10 minutes of untreated VF were accompanied by decreases in myocardial creatine phosphate and ATP to levels corresponding to 7% and 19% of baseline, respectively, whereas the lactate content increased by more than 50-fold. (8) Along with the energy deficit, ischemia is accompanied by accumulation of $CO_2$ and $H^+$ yielding profound myocardial acidosis. (9) Acidosis, however, is believed to be protective. Several studies have shown that hypoxic cells and tissues are preserved better in an acidic environment. (10-12)

Conventional closed-chest resuscitation is hemodynamically limited failing to generate a coronary blood flow greater than 20% of normal. (13) Such low flow fails to reverse myocardial ischemia but is sufficient to activate multiple pathogenic mechanisms triggering what is known as reperfusion injury. Accordingly, resuscitation typically proceeds during and in spite of severe myocardial ischemia and in the midst of reperfusion injury compounded by specific resuscitation interventions which can also injure the myocardium, such as electrical shocks and adrenergic vasopressor

agents. The end result is that several functional myocardial abnormalities develop during cardiac resuscitation which may compromise resuscitability and survival. These myocardial abnormalities can be grouped into those that manifest during the resuscitation effort and those that manifest after return of spontaneous circulation. The former include decreased left ventricular compliance and increased resistance to electrical defibrillation; the latter include reperfusion arrhythmias and myocardial dysfunction.

## Decreased left ventricular compliance

A progressive reduction in left ventricular compliance characterized by reductions in cavity size and wall thickening but without changes in cavity pressure has been documented by our group and others during closed-chest resuscitation. (14-17) A comparable phenomenon has been reported in humans during open-chest direct cardiac compression. (18) Decreases in left ventricular compliance can compromise preload-dependent blood flow generation and partly explain time-dependent reductions in the hemodynamic efficacy of closed-chest resuscitation. (16,17) We have reported that this phenomenon can be prevented by administration of inhibitors of the sodium-hydrogen exchanger isoform-1 (NHE-1), enabling in closed-chest models to prolong the duration of hemodynamically effective chest compression. (16,17)

We have previously interpreted decreases in left ventricular compliance as a manifestation of ischemic contracture. (3,16) However, ischemic contracture is characteristically preceded by reductions in myocardial ATP to less than 10% of normal, (19) and typically occurring after long intervals of no-flow ischemia. In recent studies we have reported decreases in ventricular compliance with myocardial ATP levels of $\approx$ 55% of baseline and prevention by the NHE-1 inhibitor zoniporide. (20) This would suggest that reperfusion leading to $Na^+$-induced cytosolic $Ca^{2+}$ overload may play a critical role. However, delayed reversal of intracellular acidosis by NHE-1 inhibition at the time of reperfusion may also contribute to preserve compliance. (21)

## Resistance to defibrillation

Electrical shocks delivered immediately after onset of VF are consistently effective in terminating VF and reestablishing mechanical cardiac activity. Even short delays (i.e. up to 3 minutes) may not be substantially detrimental resulting in more than 50% likelihood of successful resuscitation. (22) However, longer intervals of untreated VF, as characteristically occurs in out-of-hospital settings, are associated with decreased effectiveness of defibrillation attempts. Under these conditions electrical shocks typically fail to terminate VF, or terminate VF precipitating asystole or a pulseless electrical activity. (23) Additional resuscitation interventions are required to restore myocardial conditions favorable to successful defibrillation. New approaches are being developed to optimize the effectiveness of electrical defibrillation by identifying the proper timing for shock delivery and by using safer and more effective defibrillation waveforms. (24,25)

## Reperfusion arrhythmias

Electrical instability manifested by premature ventricular complexes and episodes of ventricular tachycardia and VF commonly occurs during the early minutes after return of cardiac activity. Episodes of VF have been reported to occur in up to 79% of patients, with the number of episodes inversely correlated with ultimate survival. (26) The mechanisms responsible for postresuscitation arrhythmias are complex but probably involve prominently cytosolic $Ca^{2+}$ overload with afterdepolarizations triggering ventricular ectopic activity. (27) In addition, there are repolarization abnormalities that include shortening of the action potential (AP) duration, decreased AP amplitude, and development of AP duration alternant creating conditions for reentry. (28) Experimentally, these repolarization abnormalities are short-lived (5 to 10 minutes) and coincide with the interval of increased propensity for ventricular arrhythmias and recurrent VF. (16) We have reported marked attenuation of reperfusion arrhythmias and prevention of episodes of refibrillation by NHE-1 inhibitors given during cardiac resuscitation in various animal models. (14,16,29)

## Postresuscitation myocardial dysfunction

Variable degrees of left ventricular systolic and diastolic dysfunction develop after resuscitation from cardiac arrest despite full restoration of coronary blood flow. Left ventricular dysfunction is largely reversible, consistent with the definition of myocardial stunning. (30-33)

Systolic dysfunction has been documented using load-independent indices of contractility showing decreases in the slope of the end-systolic pressure-volume relationship, known as elastance, and increases in the volume intercept at a left ventricular pressure of 100 mm Hg ($V_{100}$). (31) Impaired contractility leads to reductions on global indices of ventricular performance such as cardiac index, ejection fraction, and left ventricular stroke work (32,34,35) and renders the heart susceptible to afterload increases during the postresuscitation phase. In a pig model of VF and closed chest resuscitation, the administration of vasopressin during cardiac resuscitation was associated with decreased left ventricular performance with reversal by administration of a specific antagonist of the $V_1$ receptor. (36)

Diastolic dysfunction is characterized by left ventricular wall thickening with reductions in end-diastolic volume and impaired relaxation, (16) and appears to be maximal immediately after restoration of spontaneous circulation. The magnitude of diastolic dysfunction correlates closely with the magnitude of ischemic contracture, (37) suggesting a common pathogenic thread with diastolic dysfunction being a manifestation of resolving ischemic contracture. From a functional perspective, diastolic dysfunction may limit the compensatory

ventricular dilatation required to overcome decreased contractility according to the Frank-Starling mechanism.

## Effects of cardiac arrest and resuscitation on cardiac mitochondria

The underlying mechanism of cell injury during ischemia and reperfusion is complex and time-sensitive. Some processes develop coincident with the onset of ischemia and during reperfusion leading to abnormalities in energy metabolism, acid base status, and intracellular ion homeostasis. Other processes develop later and encompass activation of various signaling pathways that have deleterious or beneficial effects on specific effectors, but associated with sustained disruption of energy production, contractile dysfunction, and activation of apoptotic pathways. Discussion on the various cell mechanisms responsible for cell injury is beyond the scope of this review. However, pertinent to our discussion is the mounting evidence pointing to mitochondria as key target organelles of reperfusion injury. (38-47)

## Energy production

The mitochondria are organelles present in all eukaryotic cells and responsible for production of more than 90% of the ATP requirements. Mitochondria have an inner membrane that is highly impermeable and folds inwardly into the mitochondrial matrix forming multiple cristae where proteins responsible for oxidative phosphorylation reside. The outer mitochondrial membrane is more permeable given the abundance of channel-forming proteins, such as the voltage dependent anion channel (VDAC). Generation of ATP results from oxidation of NADH in the electron transport chain. This chain is composed of protein complexes assembled along the inner mitochondrial membrane where electrons are transferred down their redox potential while $H^+$ are pumped into the intermembrane space. The accumulation of $H^+$ in the intermembrane space establishes along with its voltage gradient (negative in the matrix side) a protonmotive force which is used by $F_oF_1$ ATP synthase to form ATP from ADP and inorganic phosphate.

Mitochondrial ATP is used to phosphorylates creatine, forming creatine phosphate which is then shuttled outside mitochondria to key sites where ATP is utilized to support contractile activity (acto-myosin ATPase), $Ca^{2+}$ reuptake (sarcoplasmic reticulum $Ca^{2+}$ ATPase), and resting membrane potential (sarcolemmal $Na^+$-$K^+$ ATPase). (48,49) Creatine phosphate rephosphorylates ADP, ensuring that adequate free energy for ATP hydrolysis is available at these reaction sites. (48) Dephosphorylated creatine is shuttled back to mitochondria for rephosphorylation closing the phosphotransfer circuit. (50,51)

Disruption of the inner membrane permeability leads to collapse of the $H^+$ gradient compromising the protonmotive force required for ATP synthesis. One important mechanism of disruption of the inner membrane permeability at the time of reperfusion is opening of the mitochondrial permeability transition pore (mPTP). The mPTP is a high-conductance mega channel formed by apposition of transmembrane proteins from the inner and the outer mitochondrial membrane. (40) Opening of the pore – in addition to causing collapse of the inner membrane voltage and uncoupling oxidative phosphorylation – allows molecules up to 1.5 kDa to enter the mitochondrial matrix along with water and solutes. This leads to mitochondrial swelling with stretching and disruption of the outer mitochondrial membrane. (40) Factors present at the time of reperfusion and responsible for mPTP opening include $Ca^{2+}$ overload, production of reactive oxygen species, depletion of ATP and ADP, and increased inorganic phosphate. (40) Intracellular acidosis at the time of reperfusion appears to prevent mPTP opening.

## Apoptotic signaling

In addition to the key role on energy production, mitochondria can also signal cell death by activation of the intrinsic apoptotic pathway through release of cytochrome c. Cytochrome c is a 14 kDa hemoprotein normally present in the intermembrane mitochondrial space that plays a key role transferring electrons from complex III to complex IV. However, cytochrome c can be released to the cytosol prompting the formation of an oligomeric complex with dATP and the apoptotic protease activating factor-1 (Apaf-1). (38) This complex recruits procaspase-9 forming the so-called apoptosome. In the apoptosome, procaspase-9 is activated and then released as caspase-9, which in turn, activates the executioner caspases 3, 6, and 7. (52,53) Active executioner caspases cleave several cytoplasmic proteins including α-spectrin and actin and nuclear proteins including poly (ADP-ribose) polymerase (PARP), lamin A, and the inhibitor of caspase activated DNase (ICAD). Cleavage of ICAD leads to activation of caspase activated DNase (CAD) which in turn cleaves chromatin into 180 to 200 bp fragments. Other substrates activated during apoptosis include components of DNA repair machinery and a number of protein kinases, (52) ultimately culminating in cell death. However, activation of apoptotic cascades does not necessarily end in cell death. We have recently reported activation of caspase-3 without evidence of DNA fragmentation. Other investigators have coined the term “apoptosis interruptus” (54) to indicate the same concept. In myocytes, apoptosis interruptus is associated with disruption of contractile proteins, (55) suggesting that it could represent and adaptive response to lower metabolic needs under conditions of metabolic stress.

Several mechanisms may explain cytochrome c release. One involves mPTP opening causing matrix swelling and disruption of the outer mitochondrial membrane. However, cytochrome c can also be released without mPTP opening through formation of pores in the outer mitochondrial membrane. This mechanism is best explained by permeabilization of the outer membrane by pro-apoptotic proteins such as Bcl-

2–associated X protein (Bax), Bcl-2 homologous antagonist killer (Bak), or truncated BH3 interacting domain death agonist (Bid). (56) Anti-apoptotic proteins such as Bcl-2, Bcl-x, and Bcl-w, however, may play important roles by counterbalancing the aforementioned pro-apoptotic effects. (57)

We investigated in a rat model of ventricular fibrillation (VF) and closed-chest resuscitation the effects of ischemia and reperfusion on cytosolic and systemic release of cytochrome c. (58) We found that cytochrome c is released to the cytosol after resuscitation from VF in association with activation of caspase-3 and marked reduction in left ventricular function. We then measured changes in plasma cytochrome c in 12 rats successfully resuscitated after 8 minutes of untreated VF and 8 minutes of closed-chest resuscitation. In 3 rats that survived, plasma cytochrome c increased and decreased gradually within a 96 hour interval to levels not exceeding 2 $\mu$g/ml. In 9 rats, which died between 1.3 and 32.5 hours post-resuscitation, plasma cytochrome c increased faster ($0.7 \pm 0.5$ vs $0.1 \pm 0.05$ $\mu$g/ml/hr; $p = 0.046$) and reached higher levels ($4.6 \pm 2.0$ vs $1.6 \pm 0.3$ $\mu$g/ml; $p = 0.029$) than in survivor rats. Plasma cytochrome c increased despite reversal of whole-body ischemia, which was documented by declining blood lactate levels in both survivor and non-survivor rats.

Increased plasma levels of cytochrome c, inversely related to outcomes, have also been reported in patients presenting with the systemic inflammatory response syndrome, (59) influenza-associated encephalopathy, (60) and fulminant hepatitis. (61) Accordingly, release of cytochrome c to the bloodstream may serve to identify mitochondrial injury, quantitate its severity, and predict survival. Future work is planned to assess the effects of interventions targeting mechanisms of mitochondrial injury during reperfusion, anticipating preservation of organ function and improved survival in relation to interventions that (i) ameliorate mitochondrial $Ca^{2+}$ overload and (ii) reduce the sensitivity of the mPTP. Amelioration of mitochondrial $Ca^{2+}$ overload can be accomplished by inhibition of the sodium-hydrogen exchanger isoform-1 using cariporide (16,29) and by inhibition of the mitochondrial $Ca^{2+}$ uniporter using Ru360. (62) The sensitivity of mPTP can be reduced by use of cyclosporine A (63,64) and the nonimmunosuppressive cyclosporine derivative NIM811. (65,66)

## REFERENCES


1. Klocke FJ, Ellis AK. Control of coronary blood flow. Annu Rev Med 1980;31:489-508.
2. Hoffman JIE. Maximal coronary flow and the concept of coronary vascular reserve. Circulation 1984;70:153-9.
3. Gazmuri RJ, Berkowitz M, Cajigas H. Myocardial effects of ventricular fibrillation in the isolated rat heart. Crit Care Med 1999;27:1542-50.
4. Kamohara T, Weil MH, Tang W, Sun S, Yamaguchi H, Klouche K, et al, editors. A comparison of myocardial function after primary cardiac and primary asphyxial cardiac arrest. Am J Respir Crit Care Med 2001;164:1221-4.
5. Neumar RW, Brown CG, Van Ligten P, Hoekstra J, Altschuld RA, Baker P. Estimation of myocardial ischemic injury during ventricular fibrillation with total circulatory arrest using high-energy phosphates and lactate as metabolic markers. Ann Emerg Med 1991;20:222-9.
6. Kern KB, Garewal HS, Sanders AB, Janas W, Nelson J, Sloan D, et al, editors. Depletion of myocardial adenosine triphosphate during prolonged untreated ventricular fibrillation: effect on defibrillation success. Resuscitation 1990;20:221-9.
7. Noc M, Weil MH, Gazmuri RJ, Sun S, Bisera J, Tang W. Ventricular fibrillation voltage as a monitor of the effectiveness of cardiopulmonary resuscitation. J Lab Clin Med 1994;124:421-6.
8. Kolarova JD, Radhakrishnan J, Sufen W, Gopalakrishnan P, Ayoub IM, Gazmuri RJ. Effects of cariporide on myocardial energy metabolism during resuscitation from VF. Circulation 2004;110:III-532.
9. Kette F, Weil MH, Gazmuri RJ, Bisera J, Rackow EC. Intramyocardial hypercarbic acidosis during cardiac arrest and resuscitation. Crit Care Med 1993;21:901-6.
10. Bing OH, Brooks WW, Messer JV. Heart muscle viability following hypoxia: Protective effect of acidosis. Science 1973;180:1297-8.
11. Bernard M, Menasche P, Canioni P, Fontanarava E, Grousset C, Piwnica A, et al, editors. Influence of the pH of cardioplegic solutions on intracellular pH, high-energy phosphates, and postarrest performance. Protective effects of acidotic, glutamate-containing cardioplegic perfusates. J Thorac Cardiovasc Surg 1985;90:235-42.
12. Gores GJ, Nieminen AL, Fleishman KE, Dawson TL, Herman B, Lemasters JJ. Extracellular acidosis delays onset of cell death in ATP-depleted hepatocytes. Am J Physiol 1988;255:C315-C322.
13. Duggal C, Weil MH, Gazmuri RJ, Tang W, Sun S, OžConnell F, et al, editors. Regional blood flow during closed-chest cardiac resuscitation in rats. J Appl Physiol 1993;74:147-52.
14. Gazmuri RJ, Ayoub IM, Hoffner E, Kolarova JD. Successful ventricular defibrillation by the selective sodium-hydrogen exchanger isoform-1 inhibitor cariporide. Circulation 2001;104:234-9.
15. Klouche K, Weil MH, Sun S, Tang W, Povoas HP, Kamohara T, et al, editors. Evolution of the stone heart after prolonged cardiac arrest. Chest 2002;122:1006-11.

16. Ayoub IM, Kolarova JD, Yi Z, Trevedi A, Deshmukh H, Lubell DL, et al, editors. Sodium-hydrogen exchange inhibition during ventricular fibrillation: Beneficial effects on ischemic contracture, action potential duration, reperfusion arrhythmias, myocardial function, and resuscitability. Circulation 2003;107:1804-9.
17. 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.
18. Takino M, Okada Y. Firm myocardium in cardiopulmonary resuscitation. Resuscitation 1996;33:101-6.
19. Lowe JE, Jennings RB, Reimer KA. Cardiac rigor mortis in dogs. J Mol Cell Cardiol 1979;11:1017-31.
20. Ayoub IM, Kolarova JD, Radhakrishnan J, Wang S, Gazmuri RJ. Zoniporide ameliorates post-resuscitation myocardial dysfunction by flow independent mechanisms. Crit Care Med 2004:32, A57.
21. Portman MA, Panos AL, Xiao Y, Anderson DL, Ning X. HOE-642 (cariporide) alters pH (i) and diastolic function after ischemia during reperfusion in pig hearts in situ. Am J Physiol Heart Circ Physiol 2001;280:H830-H834.
22. Valenzuela TD, Roe DJ, Nichol G, Clark LL, Spaite DW, Hardman RG. Outcomes of rapid defibrillation by security officers after cardiac arrest in casinos. N Engl J Med 2000;343:1206-9.
23. Niemann JT, Burian D, Garner D, Lewis RJ. Monophasic versus biphasic transthoracic countershock after prolonged ventricular fibrillation in a swine model. J Am Coll Cardiol 2000;36:932-8.
24. Kolarova J, Ayoub IM, Yi Z, Gazmuri RJ. Optimal timing for electrical defibrillation after prolonged untreated ventricular fibrillation. Crit Care Med 2003;31:2022-8.
25. Tang W, Weil MH, Sun S, Jorgenson D, Morgan C, Klouche K, et al, editors. The effects of biphasic waveform design on post-resuscitation myocardial function. J Am Coll Cardiol 2004;43:1228-35.
26. van Alem AP, Post J, Koster RW. VF recurrence: characteristics and patient outcome in out-of-hospital cardiac arrest. Resuscitation 2003;59:181-8.
27. Koretsune Y, Marban E. Cell calcium in the pathophysiology of ventricular fibrillation and in the pathogenesis of postarrhythmic contractile dysfunction. Circulation 1989;80:369-79.
28. Franz MR. Monophasic action potentials recorded by contact electrode method. Genesis, measurement, and interpretations. In: Franz MR, editor. Monophasic Action Potentials. Bridging Cell and Bedside.Armonk, New York: Futura Publishing Company, Inc.; 2000. p. 19-45.
29. Ayoub IM, Kolarova J, Kantola RL, Sanders R, Gazmuri RJ. Cariporide minimizes adverse myocardial effects of epinephrine during resuscitation from ventricular fibrillation. Crit Care Med 2005;33:2599-605.
30. Deantonio HJ, Kaul S, Lerman BB. Reversible myocardial depression in survivors of cardiac arrest. PACE 1990;13:982-5.
31. 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.
32. 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;28:232-40.
33. Kern KB. Postresuscitation myocardial dysfunction. Cardiol Clin 2002;20:89-101.
34. Mullner M, Domanovits H, Sterz F, Herkner H, Gamper G, Kurkciyan I, et al, editors. Measurement of myocardial contractility following successful resuscitation: quantitated left ventricular systolic function utilizing non-invasive wall stress analysis. Resuscitation 1998;39:51-9.
35. Laurent I, Monchi M, Chiche JD, Joly LM, Spaulding C, Bourgeois B, et al, editors. Reversible myocardial dysfunction in survivors of out-of-hospital cardiac arrest. J Am Coll Cardiol 2002;40:2110-6.
36. Kern KB, Heidenreich JH, Higdon TA, Berg RA, Hilwig RW, Sanders AB, et al, editors. Effect of vasopressin on postresuscitation ventricular function: unknown consequences of the recent Guidelines 2000 for Cardiopulmonary Resuscitation and Emergency Cardiovascular Care. Crit Care Med 2004;32:S393-S397.
37. Gazmuri RJ. Effects of repetitive electrical shocks on postresuscitation myocardial function. Crit Care Med 2000;28:N228-N232.
38. Li P, Nijhawan D, Budihardjo I, Srinivasula SM, Ahmad M, Alnemri ES, et al, editors. Cytochrome c and dATP-dependent formation of Apaf-1/caspase-9 complex initiates an apoptotic protease cascade. Cell 1997;91:479-89.
39. Green DR, Reed JC. Mitochondria and apoptosis. Science 1998;281:1309-12.
40. Crompton M. The mitochondrial permeability transition pore and its role in cell death. Biochem J 1999;341:233-49.
41. Weiss JN, Korge P, Honda HM, Ping P. Role of the mitochondrial permeability transition in myocardial disease. Circ Res 2003;93:292-301.
42. Halestrap AP, Clarke SJ, Javadov SA. Mitochondrial permeability transition pore opening during myocardial reperfusion--a target for cardioprotection. Cardiovasc Res 2004;61:372-85.
43. Juhaszova M, Zorov DB, Kim SH, Pepe S, Fu Q, Fishbein KW, et al, editors. Glycogen synthase kinase-3beta mediates convergence of protection signaling to inhibit the mitochondrial permeability transition pore. J Clin Invest 2004;113:1535-49.
44. Murphy E. Primary and secondary signaling pathways in early preconditioning that converge on the mitochondria to produce cardioprotection. Circ Res 2004;94:7-16.
45. Pan G, Humke EW, Dixit VM. Activation of caspases triggered by cytochrome c in vitro. FEBS Lett 1998;426:151-4.
46. Iwai T, Tanonaka K, Inoue R, Kasahara S, Kamo N, Takeo S. Mitochondrial damage during ischemia determines post-ischemic contractile dysfunction in perfused rat heart. J Mol Cell Cardiol 2002;34:725-38.

47. Vanden Hoek TL, Qin Y, Wojcik K, Li CQ, Shao ZH, Anderson T, et al, editors. Reperfusion, not simulated ischemia, initiates intrinsic apoptosis injury in chick cardiomyocytes. Am J Physiol 2003;284:H141-H150.
48. Kaasik A, Veksler V, Boehm E, Novotova M, Minajeva A, Ventura-Clapier R. Energetic crosstalk between organelles: architectural integration of energy production and utilization. Circ Res 2001;89:153-9.
49. Ingwall JS. Transgenesis and cardiac energetics: new insights into cardiac metabolism. J Mol Cell Cardiol 2004;37:613-23.
50. Bessman SP, Carpenter CL. The creatine-creatine phosphate energy shuttle. Annu Rev Biochem 1985;54:831-62.
51. Saks V, Dzeja P, Schlattner U, Vendelin M, Terzic A, Wallimann T. Cardiac system bioenergetics: metabolic basis of the Frank-Starling law. J Physiol 2006;571:253-73.
52. Earnshaw WC, Martins LM, Kaufmann SH. Mammalian caspases: structure, activation, substrates, and functions during apoptosis. Annu Rev Biochem 1999;68:383-424.
53. Zou H, Li Y, Liu X, Wang X. An APAF-1.cytochrome c multimeric complex is a functional apoptosome that activates procaspase-9. J Biol Chem 1999;274:11549-56.
54. Narula J, Arbustini E, Chandrashekhar Y, Schwaiger M. Apoptosis and the systolic dysfunction in congestive heart failure. Story of apoptosis interruptus and zombie myocytes. Cardiol Clin 2001;19:113-26.
55. Communal C, Sumandea M, de Tombe P, Narula J, Solaro RJ, Hajjar RJ. Functional consequences of caspase activation in cardiac myocytes. Proc Natl Acad Sci U S A 2002;99:6252-6.
56. Schlesinger PH, Gross A, Yin XM, Yamamoto K, Saito M, Waksman G, Korsmeyer SJ. Comparison of the ion channel characteristics of proapoptotic BAX and antiapoptotic BCL-2. Proc Natl Acad Sci U S A 1997;94:11357-62.
57. Niquet J, Wasterlain CG. Bim, Bad, and Bax: a deadly combination in epileptic seizures. J Clin Invest 2004;113:960-2.
58. 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.
59. Adachi N, Hirota M, Hamaguchi M, Okamoto K, Watanabe K, Endo F. Serum cytochrome c level as a prognostic indicator in patients with systemic inflammatory response syndrome. Clin Chim Acta 2004;342:127-36.
60. Hosoya M, Nunoi H, Aoyama M, Kawasaki Y, Suzuki H. Cytochrome c and tumor necrosis factor-alpha values in serum and cerebrospinal fluid of patients with influenza-associated encephalopathy. Pediatr Infect Dis J 2005;24:467-70.
61. Sakaida I, Kimura T, Yamasaki T, Fukumoto Y, Watanabe K, Aoyama M, et al, editors. Cytochrome c is a possible new marker for fulminant hepatitis in humans. J Gastroenterol 2005;40:179-85.
62. Jesus Garcia-Rivas G, Guerrero-Hernandez A, Guerrero-Serna G, Rodriguez-Zavala JS, Zazueta C. Inhibition of the mitochondrial calcium uniporter by the oxo-bridged dinuclear ruthenium amine complex (Ru360) prevents from irreversible injury in postischemic rat heart. FEBS J 2005;272:3477-88.
63. Soriano ME, Nicolosi L, Bernardi P. Desensitization of the permeability transition pore by cyclosporin a prevents activation of the mitochondrial apoptotic pathway and liver damage by tumor necrosis factor-alpha. J Biol Chem 2004;279:36803-8.
64. Shanmuganathan S, Hausenloy DJ, Duchen MR, Yellon DM. Mitochondrial permeability transition pore as a target for cardioprotection in the human heart. Am J Physiol Heart Circ Physiol 2005;289:H237-H242.
65. Waldmeier PC, Feldtrauer JJ, Qian T, Lemasters JJ. Inhibition of the mitochondrial permeability transition by the nonimmunosuppressive cyclosporin derivative NIM811. Mol Pharmacol 2002;62:22-9.
66. Argaud L, Gateau-Roesch O, Muntean D, Chalabreysse L, Loufouat J, Robert D, et al, editors. Specific inhibition of the mitochondrial permeability transition prevents lethal reperfusion injury. J Mol Cell Cardiol 2005;38:367-74.