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Mitochondria Play a Central Role in Nonischemic Cardiomyocyte Necrosis: Common to Acute and Chronic Stressor States

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Abstract

The survival of cardiomyocytes must be ensured as the myocardium adjusts to a myriad of competing physiologic and pathophysiologic demands. A significant loss of these contractile cells, together with their replacement by stiff fibrillar collagen in the form of fibrous tissue accounts for a transition from a usually efficient muscular pump into one that is failing. Cellular and subcellular mechanisms involved in the pathogenic origins of cardiomyocyte cell death have long been of interest. This includes programmed molecular pathways to either necrosis or apoptosis which are initiated from ischemic or nonischemic origins. Herein we focus on the central role played by a mitochondriocentric signal-transducer-effector pathway to nonischemic cardiomyocyte necrosis which is common to acute and chronic stressor states. We begin by building upon the hypothesis advanced by Albrecht Fleckenstein and coworkers some 40 years ago based on the importance of calcitropic hormone-mediated intracellular Ca^{2+} overloading which predominantly involves subsarcolemmal mitochondria and is the signal to pathway activation. Other pathway components, which came to be recognized in subsequent years, include the induction of oxidative stress and opening of the mitochondrial inner membrane permeability transition pore. The ensuing loss of cardiomyocytes and consequent replacement fibrosis, or scarring, represents a disease of adaptation and a classic example of when homeostasis begets dyshomeostasis.

Keywords

Mitochondria; Cardiomyocytes; Necrosis; Calcium overloading; Oxidative stress; mPTP opening

Introduction

The heart has classically been viewed as a postmitotic organ: its parenchyma, composed of terminally differentiated highly specialized cardiomyocytes, is unable to replicate. In essence, the myocardium has a fixed number of cardiomyocytes. This perspective, however, has more recently been challenged. For example, Anversa and coworkers [30] and Houser et

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al. [3] have given evidence of cell cycle activation and documented nuclear and cellular division of a subpopulation of cardiomyocytes. They would argue such endogenous progenitor cells exist and could be mobilized to regenerate myocardium following injury [30,3]. Dawn et al. [66] have focused on the mobilization of progenitor cells derived from bone marrow and their homing to the site of myocardial injury, where they would transdifferentiate and replace lost cardiomyocytes. One remains hopeful that in the future such pluripotent cells, irrespective of their origin, could be strategically mobilized to rescue and rebuild injured myocardium.

These provocative possibilities notwithstanding, the survival of cardiomyocytes must be ensured as this muscular pump adapts to diverse physiologic and pathophysiologic stimuli. These include: the hemodynamic burden imposed by short-term pressure or volume loading, which respectively accompanies the physiologic stress of isometric and isotonic exercise; the provocation incurred during elevations in circulating catecholamines associated with acute bodily injury; and the cumulative and persistent hemodynamic overloading and neurohormonal activation which are integral to chronic stressor states, such as congestive heart failure. Its pumping efficiency is compromised when the cumulative loss of cardiomyocytes crosses the biologic threshold and their replacement by stiff fibrillar collagen in the form of fibrous tissue poses an impediment to both the stretching and contraction of the myocardium while the hypertrophy of remaining muscle cells reaches its physiologic limits. Such pathologic remodeling accounts for the clinical transition to heart failure and holds true irrespective of the etiologic origins of cell loss or whether necrotic or apoptotic cell death pathways, or both, are involved. A critical loss of cardiomyocytes and the appearance of fibrosis are fundamental structural abnormalities accounting for the appearance of a failing muscular pump while an ongoing loss of cardiomyocytes contributes to the progressive nature of heart failure (HF) [9,18].

The cellular and subcellular mechanisms involved in the pathogenic origins of cardiomyocyte cell death have long been of interest, be they related to reduced coronary blood flow with myocardial infarction, ischemia/reperfusion injury, or nonischemic necrosis. In a series of studies, many appearing in *Pflügers Archives* 40 years ago [83,20,44], Fleckenstein and coworkers drew attention to the importance of excessive intracellular Ca^{2+} accumulation (EICA) and the role of Ca^{2+} overloaded mitochondria in particular, together with ATP depletion, in leading to nonischemic cardiomyocyte necrosis. Herein, we build upon their hypothesis and the central role played by a mitochondriocentric signal-transducer-effector pathway in accounting for necrotic cell loss and which is common to both acute and chronic stressor states. In so doing, we draw attention to *mitochondria-targeted interventions* and their potential as cardioprotective strategies in salvaging myocardium. For the reader interested in cardiomyocyte apoptosis several reviews are recommended [47,28,77].

The Death of Cardiomyocytes: Necrosis and Apoptosis

The death of cardiomyocytes involves complex programmed molecular pathways: one is termed necrosis [48]; the other apoptosis [47,28,77]. The potential contribution of another yet-to-be-fully-elucidated pathway, termed autophagy with lysosomal-mediated autocannibalism, is in evolution and its biologic significance in the failing heart remains to be fully established [46,58,12,4].

Necrosis

Necrotic cell death is viewed as “dirty” death because the hyperpermeable cell on its terminal trajectory spills intracellular contents (e.g., troponins) which serve as “danger” signals to the innate immune system [54]. The ensuing invasion of inflammatory cells to the

site of necrosis, together with recruited myofibroblasts, results in tissue repair and a subsequent reparative fibrosis. This myocardial scarring is a morphologic footprint of previous necrosis while elevated serum troponin levels represent molecular markers of recent cardiomyocyte necrosis.

Foci of fibrosis are found scattered throughout the myocardium of the right and left heart in the explanted failing human heart, where fibrosis is considered the major component to the adverse structural remodeling of myocardium [6]. It follows *pari passu* that necrosis is a major form of cardiomyocyte loss in the failing heart. The diffuse patchy distribution to myocardial scarring, often most prevalent in the endomyocardium, suggests that cardiomyocyte necrosis is widespread and likely to be an ongoing process. Each episode of necrosis and tissue repair is coupled to myofibroblast-derived collagen fibrillogenesis and the accumulation of stiff type I collagen [89]. This fibrillar collagen, which predominates in scar tissue, has the tensile strength equivalent to steel. In time, the progressive fibrosis of myocardium contributes to diastolic and systolic ventricular dysfunction [90,9,18].

Apoptosis

Apoptosis, on the other hand, is considered a “sterile” form of cell death with cardiomyocytes rapidly engulfed by macrophages never to spill their contents, nor to provoke the immune system or to invoke the invasion of inflammatory cells to the site of injury. Hence, tissue repair eventuating in fibrous tissue does not take place after apoptotic cell death. Accordingly, the apoptotic loss of cardiomyocytes leaves neither a morphologic trail nor a biochemical footprint. Some would question the pathophysiologic role of apoptosis in the loss of cardiomyocytes and the progression to HF [69,81,31,80].

Mitochondriocentric Pathway to Nonischemic Cardiomyocyte Necrosis

Historical Perspective

Research conducted by Albrecht Fleckenstein and coworkers at the Physiologisches Institute of the University of Freiburg some 40 years ago, led to the novel hypothesis that catecholamine-mediated EICA in cardiomyocytes and mitochondrial Ca^{2+} overloading in particular, combined with ATP depletion, lead to necrosis of these cells. Because the calcitropic hormones, parathyroid hormone (PTH) and vitamin D, potentiate Ca^{2+} overloading and ATP loss, they sensitize cardiomyocytes to catecholamine-induced necrosis [22]. This contrasts to Ca^{2+} channel blockers, cations (e.g., Zn^{2+}) which compete with Ca^{2+} entry, or β_1 adrenergic receptor antagonists, each of which were cardioprotective in response to catecholamine treatment of rats using isoproterenol (Isop) [45,36,75,16,76]. The multidisciplinary team of talented investigators from the Institute contributed importantly to this “new principle in cardiac pathophysiology” [21]. They included H. Antonio, H. J. Doering; J. Janke, H. Kammermeier, R. Kaufmann, H. Krause, and H. Tritthart. The Ca^{2+} overloading-to-cardiomyocyte-necrosis hypothesis was extended by these investigators and by Bhattacharya et al. to the hereditary cardiomyopathy of the Syrian Hamster, where the cardioprotective properties of Ca^{2+} channel blockade were likewise demonstrated [51,19,8].

The Fleckenstein hypothesis of nonischemic cardiomyocyte necrosis has recently been broadened into a central mitochondriocentric signal-transducer-effector (MSTE) pathway and found to be common to both acute and chronic stressor states [74,41]. As Fleckenstein envisaged, the *signal* for this pathway includes catecholamine- or PTH-mediated intracellular Ca^{2+} overloading (see Figure 1). This initial event involves the subsarcolemmal mitochondria spatially closest to Ca^{2+} channels (vis-à-vis the interfibrillar population closer to contractile proteins) [23,41,63,17,11]. The pathway *transducer* is embodied by induction of oxidative stress within Ca^{2+} overloaded organelles and where their rate of reactive oxygen species generation overwhelms their rate of detoxification by endogenous

metalloenzyme-based antioxidant defenses whose activity depends on intracellular Cu^{2+} , Zn^{2+} and Se^{2+} . A more terminal event and pathway *effector* is a cyclophilin D-dependent opening of the mitochondrial inner membrane permeability transition pore (mPTP) [34]. The consequent osmotic-based entry of solutes and ongoing swelling accounts for the degeneration of these organelles, loss of ATP synthesis, and ensuing cardiomyocyte necrosis.

How cardiomyocytes determine the activation of apoptotic or necrotic cell death pathways is not fully understood and is under investigation [34,56,60]. The functional roles of these specific pathway components was selectively validated (vide infra) in acute and chronic stressor states using pharmacologic agents that interrupted the cascade at specific downstream targets in rats receiving either Isop or 4 wks aldosterone/salt treatment (ALDOST), respectively [74,73,38,39]. We hypothesize that a lesser level of pathway activation, without ATP depletion, may lead to apoptosis whereas a more severe activation and ATP depletion would result in irreparable damage with cardiomyocyte necrosis and reparative fibrosis in the heart as observed in our animal studies.

Acute Stressor States

Following a single subcutaneous dose of Isop, ionized hypocalcemia is evident within 2 h and attributable to the translocation of Ca^{2+} to diverse tissues that includes the heart, skeletal muscle and peripheral blood mononuclear cells [74]. In cardiomyocytes this EICA includes increased mitochondrial $[\text{Ca}^{2+}]_m$ coupled with increased H_2O_2 production and increased plasma and cardiac tissue 8-isoprostane, a biomarker of lipid peroxidation, and enhanced propensity to mPTP opening, as evidenced by the response to experimental CaCl_2 stimulation. Intracellular Ca^{2+} overloading and consequent necrosis with myocardial scarring that accompanies Isop-mediated hyperadrenergic state favors the endomyocardium of the left ventricular apex [59,88,64,7,74]. We believe this propensity to catecholamine-induced injury at the apex is in keeping with its greater β_1 adrenergic receptor density [74,7,14] and which is driven by the apical to basal activation of the myocardium needed for the sequential peristaltic-like emptying of the ventricle into the aorta [65,70,10]. These pathophysiologic events were abrogated by pretreatment with either carvedilol, a β -adrenergic receptor antagonist, or quercetin, a flavonoid, each of which have mitochondria-targeted antioxidant properties [74,67,42,72,84]. In transgenic mice with enhanced sarcolemmal L-type Ca^{2+} channel activity, a progressive necrosis of cardiomyocytes is seen together with consequent appearance of reparative fibrosis and pump dysfunction which are accentuated by provocation with Isop [5]. In other rodent models, the loss of cyclophilin D blocked Ca^{2+} overloading-based cardiomyocyte necrosis while overexpression of Bcl-2, an antiapoptotic factor, proved ineffective [57]. Collectively, these findings support the central role of the MSTE pathway in nonischemic cardiomyocyte necrosis during acute hyperadrenergic states.

Chronic Stressor States

Chronic inappropriate (relative to dietary Na^+) aldosteronism appears with congestive heart failure (CHF), low-renin hypertension, or the adrenal glands' autonomous production of aldosterone. ALDOST has been used in rats to induce a chronic stressor state to interrogate the specific MSTE pathway components. However, we have been unable to reproduce the expected cardiac remodeling in mice receiving ALDOST. Hence, we have not pursued genetic manipulations to further elucidate the pathway components in mice.

Chronic ALDOST in rats is associated with marked increments in fecal and urinary Ca^{2+} excretion, and which are already evident at wk 1. For the fixed Ca^{2+} intake attendant with laboratory chow provided to these rats, these excretory losses quickly lead to the appearance

of plasma ionized hypocalcemia at wk 1 generating a major stimulus to the parathyroid glands' increased secretion of PTH. The accompanying secondary hyperparathyroidism (SHPT) is further evidenced by bone resorption and a marked fall in bone mineral density and strength seen at wks 4–6 ALDOST [14,15]. PTH-mediated intracellular Ca^{2+} overloading includes both cardiomyocyte cytosolic free $[\text{Ca}^{2+}]_i$ and subsarcolemmal mitochondrial $[\text{Ca}^{2+}]_m$, and is evident at wk 1 and persistent over 4 wks, together with increased production of H_2O_2 by these mitochondria.

This prooxidant state is counterbalanced by an increment in antioxidant defenses which also appears at wk 1. This includes the increased activities of Cu/Zn-superoxide dismutase and glutathione peroxidase and total antioxidant capacity, which consists of nonenzymatic low molecular weight antioxidants [38]. However, the persistent Ca^{2+} overloading of cardiomyocytes present over 4 wks leads to a dysequilibrium between pro- and antioxidants and altered redox state. In the absence of additional increments in endogenous defenses, a marked rise in reactive oxidant species generation by these organelles follows coupled with the subsequent opening of their inner membrane mPTP leading to cardiomyocyte necrosis and consequent tissue repair. Cardiac pathology with microscopic scarring is first evident at 4 wks ALDOST [79,41].

The crucial importance of PTH-mediated Ca^{2+} overloading-induced oxidative stress in leading to the proinflammatory cardiac failure phenotype of this model is evidenced by the upregulation of the redox-sensitive nuclear transcription factor (NF)- κB and the proinflammatory gene cascade it regulates, including ICAM-1, MCP₁ and TNF- α , and which are abrogated by cotreatment with an antioxidant N-acetylcysteine [79]. The importance of NF κB signaling in the heart and its role as a signaling integrator has led some to propose targeting this mediator of cytokine responses in preventing pathologic remodeling with HF [37,26].

The crucial importance of SHPT in mediating this pathophysiologic scenario was demonstrated by a number of interventions. They included: cotreatment with spironolactone, an aldosterone receptor antagonist, which attenuated the marked excretory losses of Ca^{2+} in feces and urine, and thereby prevented ionized hypocalcemia with SHPT [15]; cotreatment with a Ca^{2+} and vitamin D₃-supplemented diet to prevent ionized hypocalcemia and SHPT [25]; surgical parathyroidectomy performed prior to initiating ALDOST [87]; medical parathyroidectomy using cotreatment with a calcimimetic to raise the threshold of the Ca^{2+} -sensing receptor of the parathyroids and prevent SHPT [71]; and cotreatment with a Ca^{2+} channel blocker [1]. In subsequent studies the MSTE pathway components were addressed using mitochondria-targeted interventions (see Figure 2). They included quercetin and cyclosporine A, an mPTP opening inhibitor. We found the marked increments in $[\text{Ca}^{2+}]_m$ and mitochondrial H_2O_2 production, together with increased 8-isoprostane and propensity to their mPTP opening and myocardial scarring seen at wk 4 ALDOST, were each attenuated by cotreatment with either of these agents. Mitochondrial electron transport as a source of reactive oxygen species has also been targeted for cardioprotection [85] while in the vasculature, NADPH oxidase as a source of oxygen radical generation has been targeted [61,49].

Intrinsically Coupled Ca^{2+} and Zn^{2+} Dyshomeostasis: Prooxidant and Antioxidant

An excessive accumulation of cytosolic $[\text{Ca}^{2+}]_i$ and mitochondrial $[\text{Ca}^{2+}]_m$ do occur in cardiomyocytes under acute and chronic stressor states, in which each operates as endogenous prooxidant. Mg^{2+} and Mn^{2+} are natural antagonists to Ca^{2+} entry. Subsequently, we identified Zn^{2+} to be intrinsically coupled to cardiomyocyte Ca^{2+} entry

whereas Zn^{2+} is distributed within the cytosol and mitochondria functioning as antioxidant [41,13] (see Table 1).

Acute Stressor States

Soon after a single subcutaneous dose of Isop, cardiac tissue Ca^{2+} levels rise. Because of cell necrosis, Zn^{2+} transporters are no longer operative and therefore tissue Zn^{2+} levels decline at the site of injury [74,16,76]. In surrounding tissues, however, Zn^{2+} levels rise with the upregulation in its binding protein, metallothionein (MT)-1 [82]. Pretreatment with supplemental $ZnSO_4$ enhances $[Zn^{2+}]_i$ levels and antioxidant potential, and has proven cardioprotective against Isop-induced nonischemic cardiomyocyte necrosis [16,76].

Chronic Stressor States

At 4 wks ALDOST, the expression of Zn^{2+} transporters and MT-1 is upregulated and correspondingly cytosolic and mitochondrial Zn^{2+} levels are increased (see Table 1). In an effort to raise antioxidant defenses, cotreatment with a $ZnSO_4$ supplement was used. It raised cardiomyocyte $[Zn^{2+}]_i$ to 2.0 nM, which was significantly greater than that seen with ALDOST alone (see Table 1) [24,39]. The attendant increased antioxidant capacity of these cells abrogated the rise in mitochondrial H_2O_2 production and levels of 8-isoprostane in cardiac tissue and mitochondria. Moreover, the rise in $[Zn^{2+}]_i$ attenuated intracellular Ca^{2+} overloading. Given the attenuation in scarring, expressed as reduced myocardial collagen volume fraction, myocardium was salvaged from necrotic cell death by the $ZnSO_4$ supplement [38].

The response in $[Zn^{2+}]_i$ was examined in rats receiving ALDOST and simultaneously cotreated with either carvedilol or nebivolol [13]. These β_1 receptor antagonists are unique in that they have an additional property as a β_3 receptor agonist, where the latter provokes nitric oxide (NO) formation from endothelial NO synthase which, in turn, releases Zn^{2+} which had been inactive being bound to MT-1 (see Figure 3). In response to carvedilol, $[Zn^{2+}]_i$ rose to 2.5 nM and with nebivolol to 2.8 nM, which were greater than that seen with $ZnSO_4$. The rise in $[Zn^{2+}]_i$ activates its sensor, metal-responsive transcription factor (MTF)-1, which upon its translocation to the nucleus upregulates the expression of antioxidant defense genes, including Cu/Zn-SOD, glutathione synthase and MT-1, that collectively raise endogenous antioxidant defenses [38]. Furthermore, the increase in $[Zn^{2+}]_i$ gained with either carvedilol or nebivolol served to attenuate Ca^{2+} entry, and consequently $[Ca^{2+}]_i$ and $[Ca^{2+}]_m$ levels were each reduced. By enhancing endogenous antioxidant capacity and reducing EICA, the multifactorial antioxidant potential of these newer beta antagonists served to markedly attenuate mitochondrial and cardiac tissue 8-isoprostane levels, and the appearance of myocardial scarring [13].

Clinical Correlates: Nonischemic Cardiomyocyte Necrosis

Acute Stressor States

A hyperadrenergic response accompanies acute bodily injury, such as acute myocardial infarction (MI), major surgery including coronary artery bypass surgery, thermal burns, head injury or subarachnoid hemorrhage, and musculoskeletal trauma. Sepsis and diabetic ketoacidosis are systemic inflammatory illnesses accompanied by a hyperadrenergic state.

Nonischemic myocardial necrosis secondary to catecholamine-induced cell death can lead to elevations in serum troponins. These elevations, however, are modest in comparison to the segmental loss of myocardium with an acute reduction in coronary blood flow and MI. Elevated troponins have been reported in acute stressor states that include sepsis, gastrointestinal bleeding, pulmonary embolus and subarachnoid hemorrhage [27,35,53,86].

Nonischemic necrosis is a risk factor for multiorgan failure and mortality associated with ventricular dysfunction [2,91].

Chronic Stressor States

Cardiomyocyte necrosis with elevations in serum troponins indicative of cardiomyocyte necrosis but not due to MI or related to renal failure, are found in patients hospitalized with CHF and are associated with increased in-hospital and overall cardiac mortality [33,50,62,92,52,29,78,32,68,55]. A consistent association between troponin elevation and poor outcomes is found in hospitalized and ambulatory patients with HF. A concentration-dependent relationship exists between the magnitude of troponin elevation and adverse outcomes. Consequently, there is the need for serial troponin measurements to assess changing levels of risk. Increased frequency and magnitude of troponin “leak” results in worsened outcomes and transition from compensated to decompensated HF [43].

Summary and Conclusions

Cardiomyocyte survival must be sustained if the efficiency of this muscular pump is to be preserved. Mitochondria play a central role in nonischemic cardiomyocyte necrosis in a pathophysiologic scenario which is common to both acute and chronic stressor states. A mitochondriocentric signal-transducer-effector pathway leads to necrosis. Its major components include: catecholamine or PTH-mediated intracytosolic and intramitochondrial Ca^{2+} overloading; the induction of oxidative stress by these organelles; and the subsequent opening of their inner mPTP with ensuing osmotic swelling, loss of ATP synthesis, organellar degeneration and a hyperpermeable sarcoplasmic membrane with spillage of cell contents that includes troponins. The necrotic loss of cells leads to a subsequent wound healing response with invading inflammatory cells and myofibroblasts that eventuates in a replacement fibrosis. Necrosis contrasts to apoptosis, which leaves neither a morphologic footprint of fibrosis nor a biochemical trail with elevated troponins. The scattered foci of myocardial scarring found in the explanted failing human heart and the presence of increased serum troponins at the time of hospitalization for episodes of decompensated CHF underscores the importance of cardiomyocyte necrosis and which is ongoing.

Strategic interruption of this pathway by pharmaceuticals and/or nutraceuticals offers a mitochondria-targeted approach toward salvaging cardiomyocytes from necrotic cell death. Experimental evidence gathered to date holds promise for the potential efficacy of such cardioprotective strategies.

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References

1. Ahokas RA, Sun Y, Bhattacharya SK, Gerling IC, Weber KT. Aldosteronism and a proinflammatory vascular phenotype. Role of Mg^{2+} , Ca^{2+} and H_2O_2 in peripheral blood mononuclear cells. *Circulation*. 2005; 111:51–57. [PubMed: 15611366]
2. Ammann P, Maggiorini M, Bertel O, Haenseler E, Joller-Jemelka HI, Oechslin E, Minder EI, Rickli H, Fehr T. Troponin as a risk factor for mortality in critically ill patients without acute coronary syndromes. *J Am Coll Cardiol*. 2003; 41:2004–2009. [PubMed: 12798573]

3. Angert D, Berretta RM, Kubo H, Zhang H, Chen X, Wang W, Ogorek B, Barbe M, Houser SR. Repair of the injured adult heart involves new myocytes potentially derived from resident cardiac stem cells. *Circ Res.* 2011; 108:1226–1237. [PubMed: 21454756]
4. Arbustini E, Brega A, Narula J. Ultrastructural definition of apoptosis in heart failure. *Heart Fail Rev.* 2008; 13:121–135. [PubMed: 18172761]
5. Baines CP, Kaiser RA, Purcell NH, Blair NS, Osinska H, Hambleton MA, Brunskill EW, Sayen MR, Gottlieb RA, Dorn GW, Robbins J, Molkentin JD. Loss of cyclophilin D reveals a critical role for mitochondrial permeability transition in cell death. *Nature.* 2005; 434:658–662. [PubMed: 15800627]
6. Beltrami CA, Finato N, Rocco M, Feruglio GA, Puricelli C, Cigola E, Quaini F, Sonnenblick EH, Olivetti G, Anversa P. Structural basis of end-stage failure in ischemic cardiomyopathy in humans. *Circulation.* 1994; 89:151–163. [PubMed: 8281642]
7. Benjamin IJ, Jalil JE, Tan LB, Cho K, Weber KT, Clark WA. Isoproterenol-induced myocardial fibrosis in relation to myocyte necrosis. *Circ Res.* 1989; 65:657–670. [PubMed: 2527639]
8. Bhattacharya SK, Palmieri GM, Bertorini TE, Nutting DF. The effects of diltiazem in dystrophic hamsters. *Muscle Nerve.* 1982; 5:73–78. [PubMed: 7057811]
9. Boluyt MO, Bing OH, Lakatta EG. The ageing spontaneously hypertensive rat as a model of the transition from stable compensated hypertrophy to heart failure. *Eur Heart J.* 1995; 16(Suppl N):19–30. [PubMed: 8682057]
10. Buchalter MB, Rademakers FE, Weiss JL, Rogers WJ, Weisfeldt ML, Shapiro EP. Rotational deformation of the canine left ventricle measured by magnetic resonance tagging: effects of catecholamines, ischaemia, and pacing. *Cardiovasc Res.* 1994; 28:629–635. [PubMed: 8025907]
11. Camello-Almaraz C, Gomez-Pinilla PJ, Pozo MJ, Camello PJ. Mitochondrial reactive oxygen species and Ca^{2+} signaling. *Am J Physiol Cell Physiol.* 2006; 291:C1082–1088. [PubMed: 16760264]
12. Cao DJ, Gillette TG, Hill JA. Cardiomyocyte autophagy: remodeling, repairing, and reconstructing the heart. *Curr Hypertens Rep.* 2009; 11:406–411. [PubMed: 19895751]
13. Cheema Y, Sherrod JN, Shahbaz AU, Zhao W, Zhao T, Ahokas RA, Sun Y, Bhattacharya SK, Gerling IC, Weber KT. Mitochondriocentric pathway to cardiomyocyte necrosis in aldosteronism: cardioprotective responses to carvedilol and nebivolol. *J Cardiovasc Pharmacol.* 2011; 58:80–86. [PubMed: 21558884]
14. Chhokar VS, Sun Y, Bhattacharya SK, Ahokas RA, Myers LK, Xing Z, Smith RA, Gerling IC, Weber KT. Loss of bone minerals and strength in rats with aldosteronism. *Am J Physiol Heart Circ Physiol.* 2004; 287:H2023–H2026. [PubMed: 15475529]
15. Chhokar VS, Sun Y, Bhattacharya SK, Ahokas RA, Myers LK, Xing Z, Smith RA, Gerling IC, Weber KT. Hyperparathyroidism and the calcium paradox of aldosteronism. *Circulation.* 2005; 111:871–878. [PubMed: 15710759]
16. Chvapil M, Owen JA. Effect of zinc on acute and chronic isoproterenol induced heart injury. *J Mol Cell Cardiol.* 1977; 9:151–159. [PubMed: 839549]
17. Coatesworth W, Bolsover S. Spatially organised mitochondrial calcium uptake through a novel pathway in chick neurones. *Cell Calcium.* 2006; 39:217–225. [PubMed: 16338004]
18. Conrad CH, Brooks WW, Hayes JA, Sen S, Robinson KG, Bing OHL. Myocardial fibrosis and stiffness with hypertrophy and heart failure in the spontaneously hypertensive rat. *Circulation.* 1995; 91:161–170. [PubMed: 7805198]
19. Crawford AJ, Bhattacharya SK. Excessive intracellular zinc accumulation in cardiac and skeletal muscles of dystrophic hamsters. *Exp Neurol.* 1987; 95:265–276. [PubMed: 3803514]
20. Döring HJ, Leder O, Jaedicke W, Reindell A, Fleckenstein A. [Limitation of the disappearance of energy-rich phosphate compounds in the hyperactive atrial and ventricular myocardium by divalent Ca-antagonistic inhibitors of electromechanical coupling (iprovdratil, D 600, prenylamine)]. *Pflügers Arch.* 1969; 312:R7–R8.
21. Fleckenstein A, Janke J, Döring HJ, Leder O. Myocardial fiber necrosis due to intracellular Ca overload—a new principle in cardiac pathophysiology. *Recent Adv Stud Cardiac Struct Metab.* 1974; 4:563–580. [PubMed: 4468468]

22. Fleckenstein A, Kanke J, Döring HJ, Leder O. Key role of Ca in the production of noncoronagenic myocardial necroses. *Recent Adv Stud Cardiac Struct Metab.* 1975; 6:21–32. [PubMed: 1197883]
23. Gallitelli MF, Schultz M, Isenberg G, Rudolf F. Twitch-potential increases calcium in peripheral more than in central mitochondria of guinea-pig ventricular myocytes. *J Physiol.* 1999; 518(Pt 2):433–447. [PubMed: 10381590]
24. Gandhi MS, Deshmukh PA, Kamalov G, Zhao T, Zhao W, Whaley JT, Tichy JR, Bhattacharya SK, Ahokas RA, Sun Y, Gerling IC, Weber KT. Causes and consequences of zinc dyshomeostasis in rats with chronic aldosteronism. *J Cardiovasc Pharmacol.* 2008; 52:245–252. [PubMed: 18806605]
25. Goodwin KD, Ahokas RA, Bhattacharya SK, Sun Y, Gerling IC, Weber KT. Preventing oxidative stress in rats with aldosteronism by calcitriol and dietary calcium and magnesium supplements. *Am J Med Sci.* 2006; 332:73–78. [PubMed: 16909053]
26. Gordon JW, Shaw JA, Kirshenbaum LA. Multiple facets of NF- κ B in the heart: to be or not to NF- κ B. *Circ Res.* 2011; 108:1122–1132. [PubMed: 21527742]
27. Gunnewiek JM, Van Der Hoeven JG. Cardiac troponin elevations among critically ill patients. *Curr Opin Crit Care.* 2004; 10:342–346. [PubMed: 15385749]
28. Gustafsson AB, Gottlieb RA. Mechanisms of apoptosis in the heart. *J Clin Immunol.* 2003; 23:447–459. [PubMed: 15031632]
29. Horwich TB, Patel J, MacLellan WR, Fonarow GC. Cardiac troponin I is associated with impaired hemodynamics, progressive left ventricular dysfunction, and increased mortality rates in advanced heart failure. *Circulation.* 2003; 108:833–838. [PubMed: 12912820]
30. Hosoda T, Rota M, Kajstura J, Leri A, Anversa P. Role of stem cells in cardiovascular biology. *J Thromb Haemost.* 2011; 9(Suppl 1):151–161. [PubMed: 21781250]
31. Hughes SE. Detection of apoptosis using in situ markers for DNA strand breaks in the failing human heart. Fact or epiphenomenon? *J Pathol.* 2003; 201:181–186. [PubMed: 14517834]
32. Ilva T, Lassus J, Siirilä-Waris K, Melin J, Peuhkurinen K, Pulkki K, Nieminen MS, Mustonen H, Porela P, Harjola VP. Clinical significance of cardiac troponins I and T in acute heart failure. *Eur J Heart Fail.* 2008; 10:772–779. [PubMed: 18599345]
33. Ishii J, Nomura M, Nakamura Y, Naruse H, Mori Y, Ishikawa T, Ando T, Kurokawa H, Kondo T, Nagamura Y, Ezaki K, Hishida H. Risk stratification using a combination of cardiac troponin T and brain natriuretic peptide in patients hospitalized for worsening chronic heart failure. *Am J Cardiol.* 2002; 89:691–695. [PubMed: 11897211]
34. Javadov S, Karmazyn M. Mitochondrial permeability transition pore opening as an endpoint to initiate cell death and as a putative target for cardioprotection. *Cell Physiol Biochem.* 2007; 20:1–22. [PubMed: 17595511]
35. Jeremias A, Gibson CM. Narrative review: alternative causes for elevated cardiac troponin levels when acute coronary syndromes are excluded. *Ann Intern Med.* 2005; 142:786–791. [PubMed: 15867411]
36. Jin YT, Hasebe N, Matsusaka T, Natori S, Ohta T, Tsuji S, Kikuchi K. Magnesium attenuates isoproterenol-induced acute cardiac dysfunction and beta-adrenergic desensitization. *Am J Physiol Heart Circ Physiol.* 2007; 292:H1593–1599. [PubMed: 17114241]
37. Jones WK, Brown M, Ren X, He S, McGuinness M. NF- κ B as an integrator of diverse signaling pathways: the heart of myocardial signaling? *Cardiovasc Toxicol.* 2003; 3:229–254. [PubMed: 14555789]
38. Kamalov G, Ahokas RA, Zhao W, Johnson PL, Shahbaz AU, Bhattacharya SK, Sun Y, Gerling IC, Weber KT. Temporal responses to intrinsically coupled calcium and zinc dyshomeostasis in cardiac myocytes and mitochondria during aldosteronism. *Am J Physiol Heart Circ Physiol.* 2010; 298:H385–H394. [PubMed: 19915175]
39. Kamalov G, Ahokas RA, Zhao W, Zhao T, Shahbaz AU, Johnson PL, Bhattacharya SK, Sun Y, Gerling IC, Weber KT. Uncoupling the coupled calcium and zinc dyshomeostasis in cardiac myocytes and mitochondria seen in aldosteronism. *J Cardiovasc Pharmacol.* 2010; 55:248–254. [PubMed: 20051880]

40. Kamalov G, Bhattacharya SK, Weber KT. Congestive heart failure: where homeostasis begets dyshomeostasis. *J Cardiovasc Pharmacol.* 2010; 56:320–328. [PubMed: 20588190]
41. Kamalov G, Deshmukh PA, Baburyan NY, Gandhi MS, Johnson PL, Ahokas RA, Bhattacharya SK, Sun Y, Gerling IC, Weber KT. Coupled calcium and zinc dyshomeostasis and oxidative stress in cardiac myocytes and mitochondria of rats with chronic aldosteronism. *J Cardiovasc Pharmacol.* 2009; 53:414–423. [PubMed: 19333130]
42. Kametani R, Miura T, Harada N, Shibuya M, Wang R, Tan H, Fukagawa Y, Kawamura S, Matsuzaki M. Carvedilol inhibits mitochondrial oxygen consumption and superoxide production during calcium overload in isolated heart mitochondria. *Circ J.* 2006; 70:321–326. [PubMed: 16501300]
43. Kociol RD, Pang PS, Gheorghiade M, Fonarow GC, O'Connor CM, Felker GM. Troponin elevation in heart failure prevalence, mechanisms, and clinical implications. *J Am Coll Cardiol.* 2010; 56:1071–1078. [PubMed: 20863950]
44. Kohlhardt M, Bauer B, Krause H, Fleckenstein A. Differentiation of the transmembrane Na and Ca channels in mammalian cardiac fibres by the use of specific inhibitors. *Pflügers Arch.* 1972; 335:309–322.
45. Kohlhardt M, Bauer B, Krause H, Fleckenstein A. Selective inhibition of the transmembrane Ca conductivity of mammalian myocardial fibres by Ni, Co and Mn ions. *Pflügers Arch.* 1973; 338:115–123.
46. Kostin S, Pool L, Elsässer A, Hein S, Drexler HC, Arnon E, Hayakawa Y, Zimmermann R, Bauer E, Klövekorn WP, Schaper J. Myocytes die by multiple mechanisms in failing human hearts. *Circ Res.* 2003; 92:715–724. [PubMed: 12649263]
47. Kumar D, Lou H, Singal PK. Oxidative stress and apoptosis in heart dysfunction. *Herz.* 2002; 27:662–668. [PubMed: 12439637]
48. Kung G, Konstantinidis K, Kitsis RN. Programmed necrosis, not apoptosis, in the heart. *Circ Res.* 2011; 108:1017–1036. [PubMed: 21493924]
49. Kuroda J, Sadoshima J. NADPH oxidase and cardiac failure. *J Cardiovasc Transl Res.* 2010; 3:314–320. [PubMed: 20559780]
50. Kuwabara Y, Sato Y, Miyamoto T, Taniguchi R, Matsuoka T, Isoda K, Yamane K, Nishi K, Fujiwara H, Takatsu Y. Persistently increased serum concentrations of cardiac troponin in patients with acutely decompensated heart failure are predictive of adverse outcomes. *Circ J.* 2007; 71:1047–1051. [PubMed: 17587709]
51. Lossnitzer K, Janke J, Hein B, Stauch M, Fleckenstein A. Disturbed myocardial calcium metabolism: a possible pathogenetic factor in the hereditary cardiomyopathy of the Syrian hamster. *Recent Adv Stud Cardiac Struct Metab.* 1975; 6:207–217. [PubMed: 1197882]
52. Löwbeer C, Gustafsson SA, Seeberger A, Bouvier F, Hulting J. Serum cardiac troponin T in patients hospitalized with heart failure is associated with left ventricular hypertrophy and systolic dysfunction. *Scand J Clin Lab Invest.* 2004; 64:667–676. [PubMed: 15513324]
53. Maeder M, Fehr T, Rickli H, Ammann P. Sepsis-associated myocardial dysfunction: diagnostic and prognostic impact of cardiac troponins and natriuretic peptides. *Chest.* 2006; 129:1349–1366. [PubMed: 16685029]
54. Matzinger P. The danger model: a renewed sense of self. *Science.* 2002; 296:301–305. [PubMed: 11951032]
55. Miller WL, Hartman KA, Burritt MF, Grill DE, Jaffe AS. Profiles of serial changes in cardiac troponin T concentrations and outcome in ambulatory patients with chronic heart failure. *J Am Coll Cardiol.* 2009; 54:1715–1721. [PubMed: 19850213]
56. Mughal W, Kirshenbaum LA. Cell death signalling mechanisms in heart failure. *Exp Clin Cardiol.* 2011; 16:102–108. [PubMed: 22131851]
57. Nakayama H, Chen X, Baines CP, Kleivitsky R, Zhang X, Zhang H, Jaleel N, Chua BH, Hewett TE, Robbins J, Houser SR, Molkentin JD. Ca^{2+} - and mitochondrial-dependent cardiomyocyte necrosis as a primary mediator of heart failure. *J Clin Invest.* 2007; 117:2431–2444. [PubMed: 17694179]
58. Nishida K, Yamaguchi O, Otsu K. Crosstalk between autophagy and apoptosis in heart disease. *Circ Res.* 2008; 103:343–351. [PubMed: 18703786]

59. Nykamp D, Titak JA. Takotsubo cardiomyopathy, or broken-heart syndrome. *Ann Pharmacother*. 2010; 44:590–593. [PubMed: 20124462]
60. Ong SB, Gustafsson AB. New roles for mitochondria in cell death in the reperfused myocardium. *Cardiovasc Res*. 2011 In press.
61. Paravicini TM, Touyz RM. NADPH oxidases, reactive oxygen species, and hypertension: clinical implications and therapeutic possibilities. *Diabetes Care*. 2008; 31(Suppl 2):S170–S180. [PubMed: 18227481]
62. Peacock WF 4th, De Marco T, Fonarow GC, Diercks D, Wynne J, Apple FS, Wu AH. Cardiac troponin and outcome in acute heart failure. *N Engl J Med*. 2008; 358:2117–2126. [PubMed: 18480204]
63. Rizzuto R, Pinton P, Brini M, Chiesa A, Filippin L, Pozzan T. Mitochondria as biosensors of calcium microdomains. *Cell Calcium*. 1999; 26:193–199. [PubMed: 10643557]
64. Rona G. Catecholamine cardiotoxicity. *J Mol Cell Cardiol*. 1985; 17:291–306. [PubMed: 3894676]
65. Rushmer RF, Thal N. The mechanics of ventricular contraction; a cinefluorographic study. *Circulation*. 1951; 4:219–228. [PubMed: 14859398]
66. Sanganalmath SK, Abdel-Latif A, Bolli R, Xuan YT, Dawn B. Hematopoietic cytokines for cardiac repair: mobilization of bone marrow cells and beyond. *Basic Res Cardiol*. 2011; 106:709–733. [PubMed: 21541807]
67. Santos DL, Moreno AJ, Leino RL, Froberg MK, Wallace KB. Carvedilol protects against doxorubicin-induced mitochondrial cardiomyopathy. *Toxicol Appl Pharmacol*. 2002; 185:218–227. [PubMed: 12498738]
68. Sato Y, Nishi K, Taniguchi R, Miyamoto T, Fukuhara R, Yamane K, Saijyo S, Tanada Y, Yamamoto E, Goto T, Takahashi N, Fujiwara H, Takatsu Y. In patients with heart failure and non-ischemic heart disease, cardiac troponin T is a reliable predictor of long-term echocardiographic changes and adverse cardiac events. *J Cardiol*. 2009; 54:221–230. [PubMed: 19782259]
69. Schaper J, Lorenz-Meyer S, Suzuki K. The role of apoptosis in dilated cardiomyopathy. *Herz*. 1999; 24:219–224. [PubMed: 10412645]
70. Sedmera D, Reckova M, Bigelow MR, Dealmeida A, Stanley CP, Mikawa T, Gourdie RG, Thompson RP. Developmental transitions in electrical activation patterns in chick embryonic heart. *Anat Rec A Discov Mol Cell Evol Biol*. 2004; 280:1001–1009. [PubMed: 15368341]
71. Selektor Y, Ahokas RA, Bhattacharya SK, Sun Y, Gerling IC, Weber KT. Cinacalcet and the prevention of secondary hyperparathyroidism in rats with aldosteronism. *Am J Med Sci*. 2008; 335:105–110. [PubMed: 18277117]
72. Sgobbo P, Pacelli C, Grattagliano I, Villani G, Cocco T. Carvedilol inhibits mitochondrial complex I and induces resistance to H₂O₂-mediated oxidative insult in H9C2 myocardial cells. *Biochim Biophys Acta*. 2007; 1767:222–232. [PubMed: 17346667]
73. Shahbaz AU, Kamalov G, Zhao W, Zhao T, Johnson PL, Sun Y, Bhattacharya SK, Ahokas RA, Gerling IC, Weber KT. Mitochondria-targeted cardioprotection in aldosteronism. *J Cardiovasc Pharmacol*. 2011; 57:37–43. [PubMed: 20966765]
74. Shahbaz AU, Zhao T, Zhao W, Johnson PL, Ahokas RA, Bhattacharya SK, Sun Y, Gerling IC, Weber KT. Calcium and zinc dyshomeostasis during isoproterenol-induced acute stressor state. *Am J Physiol Heart Circ Physiol*. 2011; 300:H636–H644. [PubMed: 21076021]
75. Shechter M. Magnesium and cardiovascular system. *Magnes Res*. 2010; 23:60–72. [PubMed: 20353903]
76. Singal PK, Dhillon KS, Beamish RE, Dhallan NS. Protective effect of zinc against catecholamine-induced myocardial changes electrocardiographic and ultrastructural studies. *Lab Invest*. 1981; 44:426–433. [PubMed: 7230729]
77. Singh SS, Kang PM. Mechanisms and inhibitors of apoptosis in cardiovascular diseases. *Curr Pharm Des*. 2011 In press.
78. Sukova J, Ostadal P, Widimsky P. Profile of patients with acute heart failure and elevated troponin I levels. *Exp Clin Cardiol*. 2007; 12:153–156. [PubMed: 18650997]
79. Sun Y, Zhang J, Lu L, Chen SS, Quinn MT, Weber KT. Aldosterone-induced inflammation in the rat heart. Role of oxidative stress. *Am J Pathol*. 2002; 161:1773–1781. [PubMed: 12414524]

80. Takemura G, Fujiwara H. Morphological aspects of apoptosis in heart diseases. *J Cell Mol Med*. 2006; 10:56–75. [PubMed: 16563222]
81. Tamura T, Said S, Lu W, Harris J, Neufeld D, Burbach JA, Gerdes AM. Is apoptosis present in progression to chronic hypertensive heart failure? *J Card Fail*. 2000; 6:37–42. [PubMed: 10746817]
82. Thomas M, Vidal A, Bhattacharya SK, Ahokas RA, Sun Y, Gerling IC, Weber KT. Zinc dyshomeostasis in rats with aldosteronism. Response to spironolactone. *Am J Physiol Heart Circ Physiol*. 2007; 293:H2361–H2366. [PubMed: 17616752]
83. Tritthart H, Fleckenstein A, Kaufmann R. [Specific increase in relaxation velocity of the isolated ventricular myocardium of rhesus monkeys, guinea pigs and frogs produced by sympathetic transmitters, and the neutralization of this effect by beta-receptor blockade]. *Pflügers Arch*. 1968; 303:350–365.
84. Trumbeckaite S, Bernatoniene J, Majiene D, Jakstas V, Savickas A, Toleikis A. The effect of flavonoids on rat heart mitochondrial function. *Biomed Pharmacother*. 2006; 60:245–248. [PubMed: 16777369]
85. Tsutsui H, Kinugawa S, Matsushima S. Mitochondrial oxidative stress and dysfunction in myocardial remodelling. *Cardiovasc Res*. 2009; 81:449–456. [PubMed: 18854381]
86. Vasile VC, Babuin L, Rio Perez JA, Alegria JR, Song LM, Chai HS, Afessa B, Jaffe AS. Long-term prognostic significance of elevated cardiac troponin levels in critically ill patients with acute gastrointestinal bleeding. *Crit Care Med*. 2009; 37:140–147. [PubMed: 19050617]
87. Vidal A, Sun Y, Bhattacharya SK, Ahokas RA, Gerling IC, Weber KT. Calcium paradox of aldosteronism and the role of the parathyroid glands. *Am J Physiol Heart Circ Physiol*. 2006; 290:H286–H294. [PubMed: 16373592]
88. Weber HW, Van Der Walt JJ. Cardiomyopathy in crowded rabbits. *Recent Adv Stud Cardiac Struct Metab*. 1975; 6:471–477. [PubMed: 128082]
89. Weber KT. Cardiac interstitium in health and disease: the fibrillar collagen network. *J Am Coll Cardiol*. 1989; 13:1637–1652. [PubMed: 2656824]
90. Weber KT, Brilla CG, Janicki JS. Myocardial fibrosis: functional significance and regulatory factors. *Cardiovasc Res*. 1993; 27:341–348. [PubMed: 8490934]
91. Wu TT, Yuan A, Chen CY, Chen WJ, Luh KT, Kuo SH, Lin FY, Yang PC. Cardiac troponin I levels are a risk factor for mortality and multiple organ failure in noncardiac critically ill patients and have an additive effect to the APACHE II score in outcome prediction. *Shock*. 2004; 22:95–101. [PubMed: 15257080]
92. Zairis MN, Tsiaousis GZ, Georgilas AT, Makrygiannis SS, Adamopoulou EN, Handanis SM, Batika PC, Prekates AA, Velissaris D, Kouris NT, Mytas DZ, Babalis DK, Karidis KS, Foussas SG. Multimarker strategy for the prediction of 31 days cardiac death in patients with acutely decompensated chronic heart failure. *Int J Cardiol*. 2009; 141:284–290. [PubMed: 19157603]

Fig. 1.

A mitochondriocentric signal-transducer-effector pathway to nonischemic cardiomyocyte necrosis. Catecholamines and the calcitropic hormone, parathyroid hormone (PTH), each raises Ca^{2+} entry leading to intracellular Ca^{2+} overloading with the ensuing increment in cytosolic $[Ca^{2+}]_i$ and mitochondrial $[Ca^{2+}]_m$, specifically the subsarcolemmal (SSM) population of these organelles. In response to mitochondrial Ca^{2+} overloading and ATP depletion, there follows the induction of oxidative stress by these organelles and subsequent opening of their inner membrane permeability transition pore (mPTP) with solute entry, osmotic swelling and structural degeneration of these organelles. This programmed sequence of events leads to cardiomyocyte necrosis with a leakage of intracellular contents, including troponins. Tissue repair follows the loss of necrotic cardiomyocytes. This healing eventuates in a reparative fibrosis that preserves the structural integrity of the myocardium. Microscopic scarring represents a morphologic trail, with elevated serum troponins, the biochemical footprints of cardiomyocyte necrosis

Fig. 2.

Acute and chronic stressor states are accompanied by neurogenic and hormonal responses which lead to intracellular Ca^{2+} and Zn^{2+} entry via L-type Ca^{2+} channels (LTCC) with Ca^{2+} acting as prooxidant and Zn^{2+} as antioxidant. Cytosolic $[\text{Ca}^{2+}]_i$ and $[\text{Zn}^{2+}]_i$, as well as mitochondrial $[\text{Ca}^{2+}]_m$ and $[\text{Zn}^{2+}]_m$ are each increased. Intracellular Ca^{2+} overloading and the ensuing induction of oxidative stress upregulates the expression of Zn^{2+} transporters that further raises Zn^{2+} entry while releasing inactive intracellular Zn^{2+} which had been bound to its binding protein metallothionein (MT)-1. Collectively, these responses further raise $[\text{Zn}^{2+}]_i$ and $[\text{Zn}^{2+}]_m$ and antioxidant defenses. Maintenance of this critical equilibrium between pro- and antioxidants determine the fate of cardiomyocytes with oxidative stress leading to necrosis and myocardial scarring.

Mitochondria-targeted interventions can be used as cardioprotective strategies. For example, the prooxidant pathway to necrosis can be interrupted by pharma- and nutraceuticals: a) reducing mitochondrial Ca^{2+} uptake with either carvedilol or nebivolol; b) a flavonoid, quercetin, as antioxidant; and c) with cyclosporine A, a mPTP opening inhibitor.

Antioxidant defenses, on the other hand, can be upregulated by ZnSO_4 supplementation that raises $[\text{Zn}^{2+}]_i$ and $[\text{Zn}^{2+}]_m$. See text. Adapted with permission from [40]

Fig. 3.

Acute and chronic stressor states are accompanied by fallen levels of ionized $[Ca^{2+}]_o$ with elevated plasma PTH, or secondary hyperparathyroidism. Cardiomyocyte prooxidant pathway represented by increments in $[Ca^{2+}]_i$ and $[Ca^{2+}]_m$ while augmentations in $[Zn^{2+}]_i$ and $[Zn^{2+}]_m$ serve as an antioxidant pathway. Increments in cytosolic free $[Zn^{2+}]_i$ activate its sensor, metal-responsive transcription factor (MTF)-1, which upon its translocation to the nucleus induces the transcription of antioxidant defense genes, including Cu/Zn-superoxide dismutase, glutathione synthase and MT-1. Nebivolol and carvedilol are unique β_1 adrenergic receptor blockers in that they are also β_3 agonists which upregulates nitric oxide (NO) production from endothelial nitric oxide synthase (eNOS). In turn, NO releases Zn^{2+} bound to MT-1 which promotes antioxidant defenses while it reduces LTCC-based Ca^{2+} entry via upregulated cGMP. ROS, reactive oxygen species; GC, guanylyl cyclase. Adapted with permission from [13]

Table 1Coupled dyshomeostasis of Ca^{2+} and Zn^{2+} in cardiomyocytes: response to chronic aldosteronism [38,13]

	Cytosolic Free		Mitochondrial	
	$[\text{Ca}^{2+}]_i$ (nM)	$[\text{Zn}^{2+}]_i$ (nM)	$[\text{Ca}^{2+}]_m$ (nM)	$[\text{Zn}^{2+}]_m$ (ng/mg mitochondrial protein)
Control	29±4	0.76±0.12	82±11	26±3
ALDOST (4 wks)	80±5 *	1.64±0.08 *	139±9 *	42±3 *

*
p<0.05 vs. control