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UNCOUPLING THE COUPLED CALCIUM AND ZINC DYSHOMEOSTASIS IN CARDIAC MYOCYTES AND MITOCHONDRIA SEEN IN ALDOSTERONISM

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Abstract

Intracellular $[Ca^{2+}]_i$ overloading in cardiomyocytes is a fundamental pathogenic event associated with chronic aldosterone/salt treatment (ALDOST) and accounts for an induction of oxidative stress that leads to necrotic cell death and consequent myocardial scarring. This prooxidant response to Ca^{2+} overloading in cardiac myocytes and mitochondria is intrinsically coupled to simultaneous increased Zn^{2+} entry serving as an antioxidant. Herein, we investigated whether Ca^{2+} and Zn^{2+} dyshomeostasis and prooxidant:antioxidant dysequilibrium seen at 4 wks, the pathologic stage of ALDOST, could be uncoupled in favor of antioxidants, using cotreatment with a $ZnSO_4$ supplement, pyrrolidine dithiocarbamate (PDTC), a Zn^{2+} ionophore, or $ZnSO_4$ in combination with amlodipine (Amlod), a Ca^{2+} channel blocker. We monitored and compared responses in cardiomyocyte free $[Ca^{2+}]_i$ and $[Zn^{2+}]_i$ together with biomarkers of oxidative stress in cardiac myocytes and mitochondria. At wk 4 ALDOST and compared to controls, we found: i) an elevation in $[Ca^{2+}]_i$ coupled with $[Zn^{2+}]_i$; and ii) increased mitochondrial H_2O_2 production, and increased mitochondrial and cardiac 8-isoprostane levels. Cotreatment with the $ZnSO_4$ supplement alone, PDTC, or $ZnSO_4$ +Amlod augmented the rise in cardiomyocyte $[Zn^{2+}]_i$ beyond that seen with ALDOST alone, while attenuating the rise in $[Ca^{2+}]_i$ which together served to reduce oxidative stress. Thus, a coupled dyshomeostasis of intracellular Ca^{2+} and Zn^{2+} was demonstrated in cardiac myocytes and mitochondria during 4 wks ALDOST, where prooxidants overwhelm antioxidant defenses. This intrinsically coupled Ca^{2+} and Zn^{2+} dyshomeostasis could be uncoupled in favor of antioxidant defenses by selectively increasing free $[Zn^{2+}]_i$ and/or reducing $[Ca^{2+}]_i$ using cotreatment with $ZnSO_4$ or PDTC alone or $ZnSO_4$ +Amlod in combination.

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Keywords

aldosteronism; calcium; zinc; cardiomyocytes; mitochondria; oxidative stress; antioxidant defenses

Introduction

Microscopic scarring is present throughout the right and left ventricles of the explanted failing human heart (1,2). This extensive and adverse myocardial remodeling by replacement fibrosis implicates the pathogenic significance of cardiomyocyte necrosis (vis-à-vis apoptosis which begets neither inflammatory cell/fibroblast responses nor consequent fibrosis), and suggests that a cumulative loss of cardiomyocytes contributes to the progressive nature of heart failure. Indeed, elevations in serum troponins, biomarkers of myocardial necrosis, have been reported in patients hospitalized with congestive heart failure (CHF), despite the absence of an acute coronary event or significant renal dysfunction, where they are associated with increased in-hospital and overall cardiac mortality (3-6). Insights into relevant pathophysiologic mechanisms of cardiomyocyte necrosis would offer potential clues for developing novel cardioprotective strategies. The biventricular scarring calls into question the role of a circulating substance(s).

In this context, the deleterious consequences of inappropriate neurohormonal activation, which accounts for the ongoing salt and water retention eventuating in the symptoms and signs of CHF, includes the adrenergic nervous and the renin-angiotensin-aldosterone systems. Their effector hormones are not only linked to this salt-avid state, but also causal to cardiomyocyte necrosis (7-9). In rodents, the administration of a single-dose catecholamine leads to acute intracellular Ca^{2+} overloading of cardiac myocytes and mitochondria, as well as the excessive generation of reactive oxygen (ROS) and nitrogen (RNS) species that overwhelm endogenous antioxidant defenses (10). Cardiomyocyte necrosis occurs within hours of isoproterenol treatment and can be prevented by cotreatment with either a Ca^{2+} channel blocker or β_1 adrenergic receptor antagonist (11-13). The induction of oxidative stress that accompanies such excessive intracellular Ca^{2+} accumulation (EICA) begins in mitochondria with the necrotic cell death pathway initiated by the opening of their permeability transition pore (mPTP) regulated by cyclophilin D (10,13-15). Inhibitors of mPTP opening or cyclophilin D have proven cardioprotective in the isoproterenol rodent model, as well as in ischemia/reperfusion (I/R) injury, another example of acute EICA (16).

Chronic aldosterone/salt treatment (ALDOST) in rats leads to a structural remodeling of myocardium, but its propagation is more gradual in onset. Herein, plasma aldosterone levels are raised to those found in human CHF. This is accompanied by subacute EICA of cardiomyocytes and mitochondria with an imbalance in the prooxidant:antioxidant ratio and mPTP opening that lead to cell death (17-19). Myocardial scarring of the right and left atria and ventricles is first evident at wk 4 ALDOST (17,18,20-22). Furthermore, we identified secondary hyperparathyroidism (SHPT) with marked bone resorption to accompany ALDOST (23,24). In this context, it is parathyroid hormone (PTH), a potent calcitropic hormone and not aldosterone, which is mechanistically responsible for EICA in cardiac tissue, including its myocytes and mitochondria, and inevitably coupled to the induction of oxidative stress. We further had shown cotreatment with the following agents to prevent intracellular Ca^{2+} overloading either with or without SHPT: spironolactone, an aldosterone receptor antagonist, attenuates the heightened urinary and fecal excretion of Ca^{2+} and consequent appearance of ionized hypocalcemia that begets SHPT (23,24); a dietary Ca^{2+} supplement, together with vitamin D, to prevent hypocalcemia (25); parathyroidectomy, performed prior to introducing ALDOST; cinacalcet, a calcimimetic that resets the threshold of the Ca^{2+} -sensing receptor of the parathyroid glands to prevent SHPT (26); and amlodipine, an L-type Ca^{2+} channel blocker

(27). Antioxidants have also proven cardioprotective in the ALDOST model. These included: N-acetylcysteine, a ROS scavenger; and pyrrolidine dithiocarbamate (PDTC), a Zn^{2+} ionophore (28).

Unlike the acute EICA seen with isoproterenol where cardiac tissue Zn^{2+} levels decline consequent to reduced Zn^{2+} transporters concomitant with cardiomyocyte necrosis, we found the subacute intracellular Ca^{2+} overloading seen with ALDOST to be accompanied by a contemporaneous antioxidant response invoked by the intrinsically coupled Ca^{2+} and Zn^{2+} dyshomeostasis (18). Therefore, the increased intracellular Zn^{2+} translocation observed in our ALDOST rats is predominantly mediated by upregulation of Zn^{2+} transporters, and partially augmented by Zn^{2+} coentry via L-type Ca^{2+} channels (17,18). We had shown increased cytosolic Zn^{2+} serves to activate its sensor, the metal-responsive transcription factor (MTF)-1 which regulates the expression of endogenous antioxidant defenses that include metallothionein (MT)-1, Cu/Zn-superoxide dismutase (SOD), and glutathione synthase (18, 29). We also had found cotreatment of ALDOST with a $ZnSO_4$ supplement to prevent cardiomyocyte necrosis and scarring (17). Thus, it seems intriguing that the pathophysiologic balance between prooxidants and antioxidants would dictate the fate of cardiomyocytes during chronic aldosteronism. The intrinsically coupled dyshomeostasis of Ca^{2+} and Zn^{2+} , which respectively serve as prooxidant and antioxidant, calls into question whether they could be uncoupled in favor of Zn^{2+} and antioxidant defenses, thereby laying the foundation for novel and yet simple cardioprotective strategies that could salvage endangered cardiomyocytes and prevent their progressive attrition to mitochondria-dependent EICA and necrosis. To fill this gap in our knowledge, the present study was undertaken. Cotreatment of rats receiving 4 wks ALDOST included either a $ZnSO_4$ supplement alone, PDTC, a Zn^{2+} ionophore alone, or the combination of $ZnSO_4$ plus amlodipine. We had previously shown these agents to attenuate myocardial scarring, a footprint of cardiomyocyte necrosis (17,27,28). In this study we carefully evaluated the effects of these treatments on the balance of Ca^{2+} and Zn^{2+} in cardiomyocytes and on markers of oxidative stress in purified cardiomyocytes and mitochondria.

METHODS

Animal Model

Eight-week-old male Sprague-Dawley rats were used throughout this series of experiments approved by our institution's Animal Care and Use Committee. As reported previously and following uninephrectomy, an osmotic minipump containing ALDO was implanted subcutaneously. It releases ALDO (0.75 μ g/h) to raise circulating ALDO levels to those commonly found in human CHF which suppresses plasma renin activity and circulating levels of angiotensin II. Drinking water was fortified with 1% NaCl and with 0.4% KCl to prevent hypokalemia. A detailed accounting of this model, including various controls (e.g., uninephrectomy, ALDO, or 1% NaCl treatment alone) can be found elsewhere (18,30).

Separate groups of rats received ALDOST together with various cotreatments: a) a $ZnSO_4$ supplement (40 mg/day by gavage) for 4 wks, which prevents the hypozincemia that appears in this model due to heightened excretory Zn^{2+} losses and translocation to injured tissues, including the heart; or b) PDTC (200 mg/kg subcutaneous/day), serving as a Zn^{2+} ionophore to putatively raise intracellular Zn^{2+} levels; and c) $ZnSO_4$ combined with amlodipine (10 mg/kg/day by gavage) to retard Ca^{2+} entry as we previously reported (17,18,31,32). Since the evidence of cardiac pathology is first seen at wk 4 ALDOST, we restricted the present study to this time point. Rats were sacrificed at 4 weeks of ALDOST alone or at wk 4 with each of the various cotreatments. Unoperated, untreated age-/gender-matched rats served as controls.

Isolation of Cardiomyocytes and Mitochondria

Cardiomyocytes were harvested by retrograde collagenase perfusion of the crystalloid perfused heart, and mitochondria were isolated by differential centrifugation of whole heart homogenates. The purity of our mitochondrial preparation was assessed by flow cytometry and mitochondrial-specific dye MitoTracker Red (Invitrogen, Eugene, OR, USA) as we have previously reported (17). Given the paucity of mitochondria found in endothelial and smooth muscle cells and fibroblasts, we would suggest that, in aggregate, these cells represent a very minor source of contamination.

Cardiomyocyte Cytosolic Free $[Ca^{2+}]_i$ and $[Zn^{2+}]_i$

Cytosolic free Ca^{2+} concentration ($[Ca^{2+}]_i$, nM) was measured ratiometrically using the Ca^{2+} -specific fluorophore Fura-2 (Invitrogen) as we have previously reported (27,33). Cytosolic free Zn^{2+} concentration ($[Zn^{2+}]_i$; nM) of viable cardiomyocytes was measured by 2-color flow cytometry (BD FACSCalibur™, Becton, Dickinson & Co., Franklin Lakes, NJ, USA) using zinc-specific dye FluoZin-3 (Invitrogen) and propidium iodide (Sigma, St. Louis, MO, USA) for detection of non-viable cells as we reported previously (17).

Mitochondrial and Cardiac Tissue 8-Isoprostane

Mitochondrial and cardiac tissue total 8-isoprostane (free and esterified) was measured using a competitive enzyme immunoassay kit (Cayman Chemical, Ann Arbor, MI, USA) according to the manufacturer's instructions and as reported previously (17).

Mitochondrial H_2O_2 Production

Mitochondrial production of ROS is potentiated by a rise in intramitochondrial $[Ca^{2+}]$, partially through stimulation of Krebs's cycle enzymes with an increased flux of electrons to the respiratory chain. To measure the release of H_2O_2 from isolated cardiac mitochondria stimulated by succinate, the Amplex Red (Invitrogen) protocol of Mohanty et al. (34) was used with modifications as previously reported (18).

Statistical Analysis

Group data are presented as mean±SEM. Data were analyzed by Mann-Whitney rank sum test using SigmaStat statistical software (version 2.0; Systat Software, Inc., Point Richmond, CA, USA). Significant differences between individual group means were assigned when p values were <0.05.

RESULTS

Coupled Ca^{2+} and Zn^{2+} Dyshomeostasis in Cardiomyocytes

As seen in Figure 1, top panel, cardiomyocyte cytosolic free $[Ca^{2+}]_i$ was significantly ($p<0.05$) increased to 80 ± 5 nM at wk 4 ALDOST compared to controls (29 ± 4 nM). In keeping with the intrinsically coupled dyshomeostasis of Ca^{2+} and Zn^{2+} , the intracellular Ca^{2+} overloading of cardiomyocytes seen at 4 wks ALDOST was accompanied by (see Figure 1, lower panel) a significant ($p<0.05$) increase in intracellular $[Zn^{2+}]_i$ (1.64 ± 0.08 nM) compared to controls (0.76 ± 0.12 nM).

The various cotreatments used were intended to uncouple the coupled dyshomeostasis of Ca^{2+} and Zn^{2+} found at 4 wks ALDOST. In the case of $ZnSO_4$ supplement and PDTC, the Zn^{2+} ionophore, each was able to significantly ($p<0.05$) increase cardiomyocyte $[Zn^{2+}]_i$ (1.94 ± 0.12 and 2.30 ± 0.12 nM, respectively) above that seen during ALDOST (see Figure 1, lower panel). The Zn^{2+} ionophore-mediated elevation in $[Zn^{2+}]_i$, however, was greater ($p<0.05$) than that seen with $ZnSO_4$ alone. The combination of $ZnSO_4$ and amlodipine prevented intracellular

Ca^{2+} overloading of cardiomyocytes and their $[\text{Ca}^{2+}]_i$ did not differ (36 ± 6 nM) from control values.

In keeping with the concept of Zn^{2+} as a competitor to Ca^{2+} entry through L-type Ca^{2+} channels (LTCC), each of these interventions attenuated intracellular Ca^{2+} overloading during ALDOST. The reduction in $[\text{Ca}^{2+}]_i$ seen with PDTC was greater ($p < 0.05$) than the reduction that accompanied ZnSO_4 (45 ± 3 and 60 ± 7 nM, respectively) and appeared to be related to the extent of enhanced $[\text{Zn}^{2+}]_i$ seen with each intervention. The rise in $[\text{Zn}^{2+}]_i$ during cotreatment with ZnSO_4 plus amlodipine (1.15 ± 0.17 nM) was significant, but attenuated compared to 4 wks ALDOST or to cotreatments with ZnSO_4 or PDTC alone, and likely reflects the attenuation of Zn^{2+} entry through LTCC due to amlodipine.

Oxidative Stress in Cardiac Tissue

Oxidative damage of the myocardium due to lipid peroxidation was gauged by increased tissue levels of 8-isoprostane. Coincident with the pathologic stage of myocardial remodeling, where cardiomyocyte necrosis with scarring first appears at wk 4 ALDOST, is the marked increase ($p < 0.01$) in tissue levels of 8-isoprostane (341.8 ± 50.1 pg/mg protein) compared to controls (32.8 ± 5.8 pg/mg protein). The uncoupling of Ca^{2+} and Zn^{2+} was demonstrated by the selective elevation in $[\text{Zn}^{2+}]_i$ and an attenuated rise in $[\text{Ca}^{2+}]_i$ associated with the three different cotreatments, together with a simultaneous reduction in 8-isoprostane levels in cardiac tissue at 4 wks ALDOST (see Figure 2). In the case of the ZnSO_4 supplement or ZnSO_4 plus amlodipine, the ALDOST-related rise in cardiac tissue 8-isoprostane was attenuated (57.6 ± 6.1 and 87.7 ± 8.5 pg/mg protein, respectively); with the Zn^{2+} ionophore it was prevented (35.1 ± 2.3 pg/mg protein) compared to controls.

Oxidative Stress in Mitochondria

Levels of 8-isoprostane found in mitochondria harvested from control rats was 134 ± 18 pg/mg protein. As seen in Figure 3, it was significantly ($p < 0.05$) elevated at 4 wks ALDOST (324 ± 87 pg/mg protein). This biomarker of oxidative stress was attenuated by cotreatment with ZnSO_4 (147 ± 8), PDTC (111 ± 25) and ZnSO_4 plus amlodipine (146 ± 11).

H_2O_2 production by mitochondria has also been used as a biomarker of oxidative stress. As seen in Figure 4, it was significantly ($p < 0.05$) increased at 4 wks ALDOST (158 ± 13 pmol/mg/min) compared to controls (91 ± 13), and was attenuated by cotreatments with ZnSO_4 (111 ± 15), the Zn^{2+} ionophore (101 ± 12), and the addition of amlodipine to ZnSO_4 produced a further significant reduction (62 ± 12).

DISCUSSION

The cumulative loss of cardiomyocytes to necrotic cell death promotes the progressive adverse structural remodeling of the failing heart. Replaced by fibrous tissue, extensive necrotic cell loss is readily recognized by the microscopic scarring found throughout the myocardium of the explanted failing human heart, where multiple foci of replacement fibrosis are considered the major cause of myocardial remodeling (1,2). Fibrosis contributes to ventricular dysfunction and the heightened arrhythmogenic potential of the failing heart. Necrosis takes place when oxidative stress in cardiomyocytes overwhelms endogenous antioxidant defenses, leading to the opening of the mitochondrial transition pore (mPTP) (13). The subsequent increase in solute permeability and resultant osmotic dysequilibrium accounts for the destabilization of mitochondrial structure and function. Mitochondria are integral to maintaining intracellular Ca^{2+} homeostasis and cellular energetics; their integrity must be preserved to sustain cell survival.

Microscopic scarring of the right and left heart, together with biomarker evidence of oxidative stress in cardiomyocytes and mitochondria, are found at 4 wks ALDOST. Their pathogenic covariant is the intrinsically coupled dyshomeostasis of Ca^{2+} and Zn^{2+} that respectively serve as prooxidant and antioxidant. The overall objectives of our study were to explore whether a) this intrinsically coupled interplay between these divalent cations could be uncoupled, and if so whether b) this can be selectively uncoupled in favor of intracellular Zn^{2+} excess and thereby enhanced antioxidant defenses. Toward this end, we targeted several cotreatments which we previously have shown to attenuate myocardial scarring during chronic aldosteronism (17, 27,28). Our goal was to evaluate the effect of these treatments, and their combination, on cation balances and oxidative stress markers.

We previously described that 4 wks ALDOST is associated with intracellular Ca^{2+} overloading of cardiac myocytes and mitochondria. This EICA originates from increased Ca^{2+} entry via L-type Ca^{2+} channels and can be prevented by amlodipine (18). Excessive intracellular Ca^{2+} influx is facilitated by parathyroid hormone seen with SHPT consequent to ionized hypocalcemia, whose appearance is precipitated by the increased urinary and fecal excretion of Ca^{2+} promoted by ALDOST and which can be prevented by: spironolactone; enhanced dietary Ca^{2+} combined with vitamin D; parathyroidectomy; or a calcimimetic (24-26,35). Contemporaneous with intracellular Ca^{2+} overloading is the induction of oxidative stress in mitochondria as reflected by their increased production of H_2O_2 and elevated 8-isoprostane level; cardiac myocytes also encounter oxidative stress with increased tissue levels of 8-isoprostane. As we previously reported, this EICA is accompanied by increased $[\text{Zn}^{2+}]_i$ and mitochondrial $[\text{Zn}^{2+}]_m$ and is causally linked with a concomitant rise in antioxidant defenses (18,22). Increased intracellular Zn^{2+} , arising largely from intravascular sources (32), activates its sensor, metal-responsive transcription factor (MTF)-1 and, in turn, MTF-1 regulates several genes pertinent to antioxidant defenses, including Cu/Zn-SOD, glutathione synthase, and MT-1 (18). We previously reported the increase in cytosolic free $[\text{Zn}^{2+}]_i$ seen at 4 wks ALDOST to mirror the upregulation of MTF-1 and MT-1 in the injured and remodeling heart (18,31,32). The expression of metallothionein MT-1, a Zn^{2+} -binding cysteine-rich protein, serves to regulate $[\text{Zn}^{2+}]_i$, thereby minimizing cytotoxicity (17,31,32). Using ^{65}Zn to monitor its whole-body distribution pattern in rats with ALDOST, we found that the preferential translocation of circulating Zn^{2+} to injured tissues, including the heart, contributed to hypozincemia where compensatory Zn^{2+} resorption from bone and skin was invoked in an attempt to counterbalance this intracompartamental shift (32). Also contributory to the hypozincemia was a marked increase in the urinary and fecal Zn^{2+} excretion (31). The rise in myocardial Zn^{2+} seen during ALDOST contributes to enhanced endogenous antioxidant defenses as manifested by increased Cu/Zn-superoxide dismutase activity (31).

Furthermore, relative to uncoupling Ca^{2+} and Zn^{2+} dyshomeostasis is the oxidative stress-mediated upregulation of cardiomyocyte Zn^{2+} transporters. This includes Zip1, which accounts for transmembrane Zn^{2+} entry, and ZnT-1, whose primary role is to regulate Zn^{2+} efflux and intracellular Zn^{2+} redistribution to prevent acute cytotoxicity (18). We would also speculate the rise in cytosolic Zn^{2+} that accompanies ALDOST could be related to Zn^{2+} released from its pool bound to MT-1. Increased cytosolic Zn^{2+} can be prevented by inhibiting the appearance of oxidative stress and, in turn, the expressions of Zip1 and MT-1 (18).

Cotreatment of ALDOST with a nutraceutical, ZnSO_4 , was shown to provide several salutary effects. It prevented the associated hypozincemia and fall in plasma Cu/Zn-superoxide dismutase activity that accompany increased excretory Zn^{2+} losses and its translocation to injured tissues and the liver (17,31,32). As shown herein, this ZnSO_4 supplement served to raise $[\text{Zn}^{2+}]_i$. The pharmaceutical, PDTC, also raised cardiac myocyte cytosolic $[\text{Zn}^{2+}]_i$ beyond that seen with ALDOST alone. The increased expression of Zn^{2+} transporters, invoked by oxidative stress (18), facilitated a further rise in $[\text{Zn}^{2+}]_i$ during ZnSO_4 supplementation and

with the Zn^{2+} ionophore. In raising intracellular levels of this antioxidant, mitochondrial H_2O_2 production and 8-isoprostane levels were attenuated, and the rise in cardiac tissue 8-isoprostane level was abrogated.

Another major finding of this study was that by raising $[\text{Zn}^{2+}]_i$ we could attenuate intracellular Ca^{2+} overloading with Zn^{2+} serving as an antagonist to Ca^{2+} entry via LTCC. Thus, ZnSO_4 and PDTC each revealed distinct dual properties: a) to increase intracellular Zn^{2+} on the one hand; and b) to reduce intracellular $[\text{Ca}^{2+}]_i$ by competitive blocking Ca^{2+} entry via LTCC on the other. As such, these two treatments have similar outcomes to what we reported for treatment with a calcium channel blocker, albeit by a different mechanism. Corollary to these observations, amlodipine, the LTCC blocker not only blocked Ca^{2+} overloading, but also attenuated Zn^{2+} entry and corresponding $[\text{Zn}^{2+}]_i$.

Notwithstanding, we also recognize several limitations to our study. We did not monitor the interaction between Zn^{2+} and selenium, where Se^{2+} affects the binding and release of Zn^{2+} bound to cysteine-rich MT-1 and its delivery to Zn^{2+} -dependent metalloenzymes (36,37). We also did not monitor antioxidant defenses, including Se-GSHPx activity, but have done so and reported previously (18,19). The response in mPTP opening, a prerequisite to cardiomyocyte necrosis, to each intervention remains uncertain and will be addressed in future studies.

The translational and therapeutic relevance of our findings regarding the intrinsically coupled dyshomeostasis of Ca^{2+} and Zn^{2+} found in rat cardiac myocytes and mitochondria during chronic aldosteronism, and which can be uncoupled in favor of antioxidants defenses, are several-fold. Increased intracellular Ca^{2+} and Zn^{2+} are also found in the myopathic heart of the Syrian hamster with muscular dystrophy, and where a Ca^{2+} channel blocker or parathyroidectomy have each proven cardioprotective (38). Abnormalities in plasma and cellular Ca^{2+} and Zn^{2+} have been reported in other cardiomyopathies, including those associated with pregnancy, adriamycin, coxsackievirus B3, rheumatic valvular heart disease, and those whose origins are uncertain (39-44). They also have been observed in patients with arterial hypertension and in atherosclerotic tissue (45,46). In experimental animals and in patients hospitalized following trauma, burn injury or sepsis, hypocalcemia and hypozincemia, together with elevated plasma parathyroid hormone (PTH), have been related to the extent of injury and are determinants of survival (47-54). Ionized hypocalcemia with elevated serum PTH levels are commonly found among patients presenting to the emergency department with diverse disorders, and where these are related to disease severity and mortality (51). In outpatients with heart failure (reduced ejection fraction), elevated serum PTH levels are predictive of the impending need for hospitalization (55). From the perspective of treatment, micronutrient supplementation that includes Zn^{2+} has proven cardioprotective in various stressor states (56-63). A Zn^{2+} ionophore has been shown to be a cardioprotective strategy against I/R injury (16). Thus it would appear that the coupled dyshomeostasis of Ca^{2+} and Zn^{2+} that occurs with either acute or chronic stressor states, and which relates to the critical balance between prooxidant and antioxidant is, as Selye suggested (64), adaptation gone awry—when homeostasis begets dyshomeostasis.

In summary, chronic aldosteronism, such as that associated with the salt-avid state seen with congestive heart failure, is accompanied by an intrinsically coupled dyshomeostasis of Ca^{2+} and Zn^{2+} in cardiac myocytes and mitochondria, and where increased $[\text{Ca}^{2+}]_i$ serves as a prooxidant while $[\text{Zn}^{2+}]_i$ is an antioxidant. Herein, we were able to uncouple this coupled dyshomeostasis in favor of raising $[\text{Zn}^{2+}]_i$ while reducing $[\text{Ca}^{2+}]_i$ and in so doing the biomarkers of oxidative stress in these cells and their organelles were attenuated. These interventions included cotreatment with a nutraceutical, ZnSO_4 , a pharmaceutical PDTC, a Zn^{2+} ionophore, or the combination of ZnSO_4 with a Ca^{2+} channel blocker. In attenuating oxidative stress, these relatively simple interventions have the potential to prevent

cardiomyocyte necrosis, and the multiple foci of scarring that constitute a major determinant of the adverse structural remodeling of the failing heart.

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Figure 1.

Cardiomyocyte cytosolic $[Ca^{2+}]_i$ (upper panel) and $[Zn^{2+}]_i$ (lower panel) in untreated controls, 4 wks aldosterone/salt treatment (ALDOST) alone, and combined with either a $ZnSO_4$ supplement (ALDOST+ $ZnSO_4$), a Zn^{2+} ionophore, pyrrolidine dithiocarbamate (ALDOST +PDTC), or the combination of $ZnSO_4$ plus amlodipine (ALDOST+ $ZnSO_4$ +Amlod). The increment in $[Zn^{2+}]_i$ that occurs at wk 4 ALDOST and which is coupled to intracellular Ca^{2+} overloading, can be uncoupled by further raising $[Zn^{2+}]_i$ with either $ZnSO_4$ or PDTC, each of which also serves to reduce $[Ca^{2+}]_i$, whereas the rise in $[Ca^{2+}]_i$ and $[Zn^{2+}]_i$ seen with ALDOST can both be attenuated by $ZnSO_4$ +Amlod cotreatment. * $p < 0.05$ vs. control; † $p < 0.05$ vs. ALDOST; ‡ $p < 0.05$ PDTC vs. $ZnSO_4$ +Amlod.

Figure 2.

Eight-isoprostane levels for cardiac tissue found in control rats, 4 wks ALDOST, or the three cotreatment regimens are presented in Figure 1. The ten-fold rise in this biomarker of oxidative stress found at 4 wks ALDOST could be prevented by cotreatment with either ZnSO₄, PDTC, or ZnSO₄+Amlod. *p<0.05 vs. controls; †p<0.05 vs. ALDOST.

Figure 3.

Eight-isoprostane levels found in cardiac mitochondria for controls, ALDOST alone or together with the cotreatments are presented in Figure 1. The marked rise in 8-isoprostane found in mitochondria at 4 wks ALDOST could be abrogated by cotreatment with either the ZnSO₄ supplement, the Zn²⁺ ionophore PDTC, or the combination of ZnSO₄+amlodipine.

*p<0.05 vs. controls; †p<0.05 vs. ALDOST.

Figure 4.

Further evidence of oxidative stress in cardiac mitochondria is demonstrated by monitoring their production of H₂O₂. Mitochondrial H₂O₂ production is almost doubled at 4 wks ALDOST and can be attenuated by cotreatment with ZnSO₄, PDTC, or ZnSO₄+Amlod. *p<0.05 vs. control; †p<0.05 vs. ALDOST; ‡p<0.05 PDTC vs. ZnSO₄+Amlod.