Calcium and zinc dyshomeostasis during isoproterenol-induced acute stressor state

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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 300: H636–H644, 2011. First published November 12, 2010; doi:10.1152/ajpheart.00900.2010.—Acute hyperadrenergic stressor states are accompanied by cation dyshomeostasis, together with the release of cardiac troponins predictive of necrosis. The signal-transducer-effector pathway accounting for this pathophysiological scenario remains unclear. We hypothesized that a dyshomeostasis of extra- and intracellular Ca2+ and Zn2+ occurs in rats in response to isoproterenol (Isop) including excessive intracellular Ca2+ accumulation (EICA) and mitochondrial [Ca2+]m-induced oxidative stress. Contemporaneously, the selective translocation of Ca2+ and Zn2+ to tissues contributes to their fallen plasma levels. Rats received a single subcutaneous injection of Isop (1 mg/kg body wt). Other groups of rats received pretreatment for 10 days with either carvedilol (C), a β-adrenergic receptor antagonist with mitochondrial Ca2+ uniporter-inhibiting properties, or quercetin (Q), a flavonoid with mitochondrial-targeted antioxidant properties, before Isop. We monitored temporal responses in the following: [Ca2+] and [Zn2+] in plasma, left ventricular (LV) apex, equator, and base, skeletal muscle, liver, spleen, and peripheral blood mononuclear cells (PBMC), indices of oxidative stress and antioxidant defenses, mitochondrial permeability transition pore (mPTP) opening, and myocardial fibrosis. We found ionized hypocalcemia and hypozincemia attributable to their tissue translocation and also a heterogeneous distribution of these cations among tissues with a preferential Ca2+ accumulation in the LV apex, muscle, and PBMC, whereas Zn2+ declined except in liver, where it increased corresponding with upregulation of metallothionein, a Zn2+-binding protein. EICA was associated with a simultaneous increase in tissue 8-isoprostane and increased [Ca2+]m accompanied by a rise in H2O2 generation, mPTP opening, and scarring, each of which were prevented by either C or Q. Thus excessive [Ca2+]m, coupled with the induction of oxidative stress and increased mPTP opening, suggests that this signal-transducer-effector pathway is responsible for Isop-induced cardiomyocyte necrosis at the LV apex.

catecholamines; mitochondria; oxidative stress; fibrosis

Patients hospitalized with acute bodily injury often present with ionized hypocalcemia and hypozincemia, together with elevated plasma catecholamines and parathyroid hormone (15, 17–20, 22, 24, 26, 29, 38). The degree of these disturbances is related to the extent of injury and therefore serves as a prognostic marker. The accompanying hyperadrenergic state can be associated with elevation in plasma cardiac troponins, indicative of cardiomyocyte necrosis (10, 28, 54). Pathophysiological responses invoked by an acute stressor state involving the signal-transducer-effector pathway mechanistically account for the dyshomeostasis of Ca2+ and Zn2+, serving as prooxidant and antioxidant, respectively, and appearance of cardiomyocyte necrosis, all of which are of the focus of our ongoing investigative effort.

In rats with an acute hyperadrenergic state induced by isoproterenol (Isop), a synthetic catecholamine, Rona et al. (55) reported on cardiomyocyte necrosis and the unusual propensity of the left ventricular (LV) apex to develop more extensive necrosis relative to its equator or base. Later, Fleckenstein and coworkers (30, 31) characterized the catecholamine-induced intracellular Ca2+ overloading as the signal-transduction pathway initiating cardiomyocyte necrosis and predicted that associated mitochondrial dysfunction with reduced ATP synthetic activity is responsible for consequent cardiotoxicity. Subsequent studies have demonstrated that intracellular Ca2+ overloading is coupled to induction of oxidative stress and serves as a transducer in the involved myocardium, where the rate of reactive oxygen species generation overwhelms the rate of their elimination by endogenous antioxidant defenses that include a rise in intracellular free Zn2+ concentration ([Zn2+]i) (21, 46, 49).

In addition to cardiac-related pathophysiology of a hyperadrenergic state that accompanies bodily injury, there is a systemic response involving diverse tissues, such as skeletal muscle, liver, and peripheral blood mononuclear cells (PBMC; lymphocytes and monocytes), which includes intrinsically coupled Ca2+ and Zn2+ dyshomeostasis (1, 18–20, 65).

On the basis of these cumulative findings, we hypothesized that, in rats with Isop-induced acute stressor state, a dyshomeostasis of extra- and intracellular Ca2+ and Zn2+ ensues in the myocardium and systemic tissues. In the heart, excessive intracellular Ca2+ accumulation (EICA) and consequent increased mitochondrial [Ca2+]m preferentially target the endocardium of the LV apex, leading to the induction of oxidative stress and opening of mitochondrial permeability transition pores (mPTP). A simultaneous fall in Zn2+ may exacerbate this oxidative stress because of its critical role in the activity of Cu/Zn-superoxide dismutase. The ensuing necrosis of cardiomyocytes results in a reparative fibrosis at this site. In our experiments, two groups of age- and sex-matched rats also received pretreatment with either carvedilol, a β-adrenergic receptor antagonist with mitochondrial Ca2+ uniporter inhibitor properties, or quercetin, a naturally occurring flavonoid found in fruit with mitochondrial antioxidant properties. In a series of strategically designed targeted interventions described in MATERIALS AND METHODS, we monitored and contrasted plasma and tissue levels of Ca2+ and Zn2+, regions of LV that included its apex, equator, and base, skeletal muscle, PBMC,
liver, and spleen, together with indices of oxidative stress, antioxidant defenses, and mPTP opening of subsarcolemmal cardiac mitochondria to elucidate the mechanism(s) of action at play for these observed iterations.

MATERIALS AND METHODS

Animal Model

Eight-week-old male Sprague-Dawley rats were used throughout this series of experiments approved by the Animal Care and Use Committee of the University of Tennessee Health Science Center. Untreated age- and sex-matched rats served as controls. Treatment consisted of rats given Isop (1 mg/kg) as a single subcutaneous injection. According to the specific aims of our studies, rats were killed at various time points (vide infra) following Isop treatment. Two separate groups of rats received pretreatment with either carvedilol (5 mg/kg per day) or quercetin (25 mg/kg per day), given by gavage once daily for 10 days before Isop injection.

Experimental Protocol

To test our hypothesis, a series of sequential interventions were carried out, and major findings are summarized herein. First, we examined the accumulation and distribution of Ca\(^{2+}\) that appeared within the heart at 8 h after Isop treatment, including the base, equator, and apex of the LV. This study demonstrated the preferential Ca\(^{2+}\) overloading in the LV apex compared with other regions. Thereafter, our follow-up studies focused on the LV apex only.

We next addressed the temporal responses in tissue Ca\(^{2+}\) and Zn\(^{2+}\) in plasma, LV apex, skeletal muscle (rectus femoris), PBMC, liver, and spleen at 2, 4, 8, and 24 h and at 7 days after Isop treatment. We also addressed the perturbations in prooxidant:antioxidant equilibrium present in LV apex and muscle at these time points on the basis of respective rise in 8-isoprostane and levels of total antioxidant capacity.

Third, and having identified LV apex as the site of predominant intracellular Ca\(^{2+}\) overloading and inferring that 2 h after Isop treatment coincided with the onset of cardiomyocyte necrosis (9), we focused our attention only to this time point and the apical region of the LV for the isolation of subsarcolemmal cardiac mitochondria to determine their responses in [Ca\(^{2+}\)]\(_{m}\) and mPTP opening.

Finally, two subgroups of rats received either carvedilol or quercetin as pretreatment before Isop administration to address the specific impact of these mechanistically targeted interventions on mitochondrial free [Ca\(^{2+}\)]\(_{m}\) and mPTP opening at 2 h after Isop.

Plasma Ionized Calcium and Plasma Zinc

Plasma ionized [Ca\(^{2+}\)]\(_{i}\) was measured by direct ion-selective electrode technique using a Nova 8 Analyzer (Nova Biomedical, Waltham, MA) and expressed in mmol/l as we previously reported (23, 64). Plasma total Zn\(^{2+}\) was measured by atomic absorption spectroscopy and expressed in \(\mu\)g/dl as we previously reported (5, 11).

Tissue Calcium and Zinc

Total Ca\(^{2+}\) and Zn\(^{2+}\) contents in heart, skeletal muscle, liver, and spleen were measured using atomic absorption spectroscopy as we previously reported (43, 62) and expressed as nEq/mg fat-free dry tissue (FFDT) and ng/mg FFDT, respectively. PBMC were isolated, and their cytosolic free [Ca\(^{2+}\)]\(_{c}\), concentration in nM was measured using a ratiometric method and fluorescent molecular probe Fura-2 (Molecular Probes, Eugene, OR) as we previously reported (3, 4, 23). PBMC cytosolic-free [Zn\(^{2+}\)]\(_{c}\), concentration in nM was measured by flow cytometry (BD FACS Calibur; Becton Dickson, Franklin Lakes, NJ) using a Zn\(^{2+}\)-specific probe (FluoZin3; Invitrogen, Eugene, OR) according to Hasse et al. (37) and as we previously reported (3, 4, 23).

Differential and regional heterogeneity in cardiac tissue Ca\(^{2+}\) and Zn\(^{2+}\) distribution were addressed by sectioning the LV transversely into three coronal sections representing the base, equator, and apex with each LV section further subdivided to distinguish between epicardium and endocardium.

Metallothionein-1 Expression

Metallothionein-1 (MT-1) protein levels in heart and liver were measured by Western blot as previously reported (62). The relative abundance of protein present was measured by a computer image analysis system (NIH Image, version 1.6).

Oxidative Stress: Prooxidants

Total 8-isoprostane. This biomarker of lipid peroxidation (free and esterified) was measured in plasma, heart, skeletal muscle, and PBMC from control rats and those after Isop treatment using a competitive enzyme immunoassay kit (Cayman Chemical, Ann Arbor, MI) as we previously reported (32).

\(\mathrm{H}_2\mathrm{O}_2\) production. The \(\mathrm{H}_2\mathrm{O}_2\) generation within mitochondria, stimulated by succinate, was measured using the Amplex Red (Invitrogen) protocol of Mohanty et al. (50) as we previously reported (43).

Oxidative Stress: Antioxidants

Total antioxidant capacity. The total antioxidant capacity found in plasma, heart, and skeletal muscle was measured using a commercially available reagent kit (Cayman Chemical) as we previously reported (41).

Isolation of Cardiac Mitochondria

Subsarcolemmal cardiac mitochondria were isolated from the LV apex by differential centrifugation of whole myocardial homogenates. The purity of mitochondrial preparation was assessed by flow cytometry and mitochondria-specific dye, Mito Tracker Red (Invitrogen), as we previously reported (32). Given the paucity of mitochondrial population density in endothelial and smooth muscle cells and fibroblasts, we presume that these cells may contribute as a very miniscule source of contamination, if any.

Mitochondrial Free [Ca\(^{2+}\)]\(_{m}\) and Total [Zn\(^{2+}\)]\(_{m}\)

Free Ca\(^{2+}\) concentration ([Ca\(^{2+}\)]\(_{m}\)) in cardiac mitochondria was determined by ratiometric method using Fura-2 and expressed in nM as we previously reported (3, 4). Mitochondrial total Zn\(^{2+}\) concentration ([Zn\(^{2+}\)]\(_{m}\)) was determined by atomic absorption spectroscopy as we previously reported (64).

mPTP Opening

Opening potential of the mPTP was stimulated by Ca\(^{2+}\)-induced swelling of isolated cardiac mitochondria and measured according to Baines et al. (6) with the addition of 40 \(\mu\)M of 200 \(\mu\)M CaCl\(_2\), thus providing a reproducible and stable decline in mitochondrial optical density as we previously reported (41). This decrease in optical density measured by change in absorption at 560 nm was completely prevented by preincubation of the organelles with 30 nM cyclosporine A, thus confirming the specificity of our mPTP opening assay, as previously reported.

Cardiac Pathology

The presence of myocardial scarring, a footprint of cardiomyocyte necrosis, was elucidated in hearts harvested at 7 days after Isop treatment using a collagen-specific picrosirius red stain applied to coronal sections (6 \(\mu\)m) of the ventricles and examined by light microscopy as previously reported (61).
Statistical Analysis

Group data are presented as means ± SE. Data were analyzed by one-way ANOVA in SPSS software (version 18.0; SPSS, Chicago, IL). Multiple-group comparisons were made by Scheffé’s F-test. Significant differences between individual group means were assigned for P values <0.05.

RESULTS

Regional Responses in Cardiac Tissue Ca2+ and Zn2+ After Isoproterenol

Tissue Ca2+. We examined myocardial Ca2+ and Zn2+ concentrations in different regions of the rat myocardium in control tissue and those occurring at 8 h of Isop treatment. This included the LV, further subdivided into its basal, equatorial, and apical segments, and in their corresponding epi- and endocardial regions. As seen in the control tissue in Fig. 1 (top), there was no significant difference in baseline Ca2+ concentration between the LV apex and its endo- or epicardium. Furthermore, there were no differences between the endo- and epicardium of the LV apex and its equator or base.

At 8 h after Isop treatment, a marked rise (P < 0.01) in tissue Ca2+ was seen in the LV apex contrasting to the LV equator and base. In the endocardium of LV apex tissue, Ca2+ rose significantly, as was the case for the apical epicardium, whereas tissue Ca2+ concentrations at the endo- and epicardium of the LV equator and base remained unaltered from control levels after Isop treatment.

![Tissue Ca2+ LV Apex](image)

Tissue Zn2+. In contrast to Ca2+ overloading of the LV apex that appeared at 8 h after Isop, a decline (P < 0.01) in tissue Zn2+ occurred at this site (see Fig. 1, bottom). In control tissue, Zn2+ concentrations did not differ between the LV apex and its endo- or epicardium or the LV equator and LV base. At 8 h following Isop, however, tissue Zn2+ fell (P < 0.01) in the apical LV endocardium (P < 0.01) and epicardium. In contrast, tissue Zn2+ levels found at the endo- and epicardium of the LV equator and base remained unchanged from control levels after Isop treatment.

![Tissue Zn2+ LV Apex](image)

Temporal Responses in Ca2+ and Zn2+ Dyshomeostasis After Isoproterenol

As seen in Fig. 2 (top) and compared with controls, plasma ionized [Ca2+]i, was unchanged at 2 h, but fell (P < 0.05) at 4 h after Isop and continued to fall at 8 h before returning to control levels at 24 h and 7 days. This early and significant ionized hypocalcemia was coupled to the precipitous hypozincemia (see Fig. 3) that appeared at 4 h compared with control levels and remained subnormal at 8 h before returning to control levels at 24 h and 7 days.

In LV apical tissue, a marked rise (P < 0.0001) in Ca2+ above controls was found at 2 and 4 h, reaching a peak at 8 h, thereafter declining at 24 h and returning to control levels at 7 days.

Contemporaneous to Ca2+ overloading of the LV apex, a progressive rise (P < 0.05) in tissue Ca2+ appeared in skeletal muscle and PBMC at 2 h and continued thereafter over 24 h before returning to control levels at 7 days (see Fig. 2). In rectus femoris tissue, total tissue Ca2+ rose (P < 0.0001) above control levels at 2 h and reached a peak at 24 h, thereafter returning to control levels at 7 days. In PBMC, cytosolic free [Ca2+]i was elevated (P < 0.01) above control levels at 2 h, continuing to rise at 8 h and reaching a peak at 24 h. No significant change in tissue Ca2+ content was found either in the liver or spleen over the various time points (data not shown).

Unlike the rise in total Ca2+ concentration that appeared in multiple tissues and likely accounted for ionized hypocalcemia following Isop treatment, the precipitous hypozincemia that appeared was associated with a variable response in total tissue Zn2+ concentration (see Fig. 3). A fall in tissue Zn2+ was seen in the heart over 24 h and in muscle over 4 h. In LV apex, tissue Zn2+ decreased from controls at 2 and 4 h and remained equally low at 8 and 24 h before returning to control levels at 7 days. This fall in skeletal muscle Zn2+ was even more marked, declining from control levels and then slowly returning to normal range by 7 days.

In contrast, there was a progressive increase in liver Zn2+ over 24 h rising from control levels at 2 and 4 h and reaching a peak at 8 h before declining at 24 h and falling to near control levels at 7 days. This hepatospecific sequestration of Zn2+ to the liver was accompanied by an increased hepatic upregulation of MT-1, a Zn2+–binding protein, before returning to basal levels at 7 days. Corresponding change in the MT-1 expression was not found in the heart at any time point. Splenic tissue Zn2+ was unchanged following Isop over the course of the study (data not shown), whereas cytosolic free Zn2+ concentration in PBMC increased at 2 h before returning to control levels, and it remained there thereafter.

Fig. 1. Tissue Ca2+ and Zn2+ from the left ventricular (LV) apex for endocardium (endo) and epicardium (epi) found in control hearts and those harvested 8 h following isoproterenol (Isop) injection. *P < 0.05 vs. controls. FFDT, fat-free dry tissue.
Prooxidant: Antioxidant Responses After Isop

Figure 4 depicts the results on total 8-isoprostanate for plasma and heart. A marked rise ($P < 0.0001$) in cardiac tissue.

Fig. 2. The temporal responses in plasma ionized $[\text{Ca}^{2+}]_o$ and tissue $\text{Ca}^{2+}$ for the LV apex, skeletal muscle, and peripheral blood mononuclear cells (PBMC) at 2, 4, 8, and 24 h and 7 days after a single subcutaneous dose of isoproterenol. *$P < 0.05$ vs. controls.

Fig. 3. The temporal responses in plasma $\text{Zn}^{2+}$ and tissue $\text{Zn}^{2+}$ for the LV apex, skeletal muscle and liver after isoproterenol administration. *$P < 0.05$ vs. controls.

Prooxidant: Antioxidant Responses After Isop

Figure 4 depicts the results on total 8-isoprostanate for plasma and heart. A marked rise ($P < 0.0001$) in cardiac tissue.
8-isoprostane occurred at 2 h and remained elevated at 4 h before returning to controls levels at 8 and 24 h and 7 days. This sudden burst in oxidative stress in the heart virtually mirrored the rise in cardiac tissue Ca\(^{2+}\) seen at 2 and 4 h (see Fig. 2) and the continuing fall in cardiac Zn\(^{2+}\) that was present over 24 h (see Fig. 3). A contemporaneous rise (P < 0.05) in muscle 8-isoprostane levels was also seen at 2 and 4 h and in PBMC at 2 h before each returned to control values. The rapid induction of oxidative stress in these multiple tissues led to a temporal and progressive rise (P < 0.001) in plasma 8-isoprostane at 2, 4, and 8 h after Isop compared with controls (see Fig. 4) and returned to basal values at 24 h and 7 days.

Total antioxidant capacity embodies cumulative antioxidant defenses derived from nonenzymatic low molecular weight antioxidants and vitamins A, C, and E. The response in this biomarker in plasma and heart is shown in Fig. 5, in which a sustained rise (P < 0.05) in these antioxidant defenses was found in both tissue over the course of 24 h.

Cardiac Pathology

In hearts harvested 7 days after Isop treatment, the appearance of myocardial fibrosis was evident within the LV, in which it appeared primarily within the endocardium of the LV apex (not shown). These findings are consistent with those reported by others (9, 13, 36, 40).

Mitochondrial Free [Ca\(^{2+}\)]\(_{m}\) and Total [Zn\(^{2+}\)]\(_{m}\)

At 2 h after Isop treatment, [Ca\(^{2+}\)]\(_{m}\) rose (P < 0.01) from control levels (see Fig. 6, top), whereas mitochondrial [Zn\(^{2+}\)]\(_{m}\) remained unchanged (not shown).

Mitochondrial 8-Isoprostane and H\(_{2}\)O\(_{2}\) Production

The rise in [Ca\(^{2+}\)]\(_{m}\) at 2 h after Isop treatment was accompanied by increased (P < 0.005) mitochondrial 8-isoprostane levels compared with controls.

As seen in Fig. 6, (bottom), succinate-stimulated mitochondrial H\(_{2}\)O\(_{2}\) generation was increased (P < 0.05) from control levels following Isop.

Ten days of pretreatment with either carvedilol or quercetin before Isop administration attenuated (P < 0.01) both the rise in [Ca\(^{2+}\)]\(_{m}\) and H\(_{2}\)O\(_{2}\) production that followed in these organelles harvested from the LV apex (see Fig. 6).

mPTP Opening

In cardiac mitochondria harvested from the LV apex in control tissue and 2 h after Isop treatment, opening of a nonspecific pore in the inner mPTP is followed by an abnormal and abrupt rise in permeability to solutes (molecular weight <1,500 Da) and osmotic swelling. This was assessed by the reduction in absorbance, measured spectrophotometrically at 560 nm, and with pore opening induced by 40 μl of 200 μM CaCl\(_{2}\). The response in mPTP opening is shown in Fig. 7 for mitochondria harvested from the LV apex in control tissue and after 2 h Isop treatment alone. As presented, there is an increase in mPTP opening in response to this hyperadrenergic state (ΔA560 118.0 ± 3.6) compared with 70.1 ± 2.3 in controls, whereas this propensity to mPTP opening was attenuated by pretreatment with either carvedilol or quercetin.
Our study led to several major findings. First, the acute stressor state induced in young adult male Sprague-Dawley rats by a single subcutaneous dose of the synthetic catecholamine, Isop, was accompanied by a dyshomeostasis of extracellular and intracellular Ca$^{2+}$/Zn$^{2+}$ involving diverse tissues. In plasma, the appearance of ionized hypocalcemia and hypozincemia was concordant with each appearing within 4 h and remaining subnormal for 8 h before returning to control levels at 24 h and 7 days. The presence of ionized hypocalcemia would inevitably provoke the parathyroid glands to secrete parathyroid hormone, which we documented to be the case throughout weeks 1–4 of aldosterone/salt treatment (23). As we and others have shown, this calcitropic hormone promotes intracellular Ca$^{2+}$ overloading (see Fig. 8) (23, 59, 64). We envisaged that the single subcutaneous dose of Isop would quickly raise its plasma concentration, peaking within 20 min and lasting over 60 min or less, thereafter rapidly metabolizing within the liver by catechol-O-methyltransferase and monoamine oxidase (39). However, metabolites of Isop may remain active for longer duration. As we have demonstrated with plasma concentrations of Ca$^{2+}$/Zn$^{2+}$ and with their temporal responses within the heart and systemic tissues (vide infra), the carryover effects to this hyperadrenergic burst appeared to have lasted for 24 h, at the very least.

Second, the perturbations in Ca$^{2+}$ and Zn$^{2+}$ that followed Isop treatment differed considerably among the tissues examined. In myocardium, there was a discordance in which a preferential accumulation of Ca$^{2+}$ occurred within the LV apex (vis-à-vis the LV equator and base), whereas the tissue Zn$^{2+}$ content declined and remained subnormal for 24 h. This regional heterogeneity to intracellular Ca$^{2+}$ accumulation that favors apical LV tissue is likely predestined by the high abundance of $\beta_1$-adrenergic receptors present at this site and not attributable to a differential expression of L-type Ca$^{2+}$ channels (34, 45, 51). An alternate explanation would suggest...
the binding affinity for $\beta_1$ receptors is greater at the LV apex (63). In either scenario, we would speculate that this difference in $\beta_1$ receptor density or affinity innately relates to the sequence of depolarization that occurs within the LV, involving apical to basal activation, with the concurrent contraction and torsion (twisting) of myocardium along the vertical axis contributing to the efficient emptying and propulsion of blood from its chamber into the aorta (14, 56, 57).

In the case of skeletal muscle and liver, $Ca^{2+}$ and $Zn^{2+}$ responses were discordant with respect to one another. Total tissue $Ca^{2+}$ rose in rectus femoris, a fast-twitch muscle, soon after Isop and remained so for 24 h, whereas the $Zn^{2+}$ levels fell at 2 and 4 h before returning to control levels. In this context, slow-twitch muscles (e.g., soleus) are considered less susceptible to catecholamine-induced injury than fast-twitch muscles (16, 52). The temporal translocation of $Ca^{2+}$ from blood to these tissues accounts for the early appearance of ionized hypocalcemia. Oxidative stress in skeletal muscle is manifest with mitochondrial $Ca^{2+}$ overloading and myocyte necrosis associated with EICA, whether attributable to catecholamines, muscular dystrophy, prolonged ischemia, or electrical trauma (12, 16, 31, 35, 52). In liver, a conservation of $Zn^{2+}$ and progressive rise in hepatic $Zn^{2+}$ concentrations occurred, accounting for hypozincemia and in accordance with the upregulation of the $Zn^{2+}$-binding protein MT-1 seen in other acute stressor states (8, 44, 66). Liver serves as a storage site for $Zn^{2+}$, which becomes a repository when needed for wound healing (58). A persistent rise in $[Ca^{2+}]_i$ was noted in PBMC over 24 h after Isop treatment. Intracellular $Ca^{2+}$ overloading of PBMC mirrors that seen in the LV apex, raising the prospect that these cells could be monitored as a novel noninvasive surrogate biomarker for $Ca^{2+}$ levels in heart tissue.

Third, the EICA associated with Isop-induced injury was accompanied by the induction of oxidative stress and early (2 hrs) rise in 8-isoprostane found in such diverse tissues as the LV apex, skeletal muscle, and PBMC, which contributed to the rise in plasma levels of this endogenous biomarker of lipid peroxidation. Also contributory to the appearance of oxidative stress, when the rate of reactive oxygen and nitrogen species overwhelm their rate of elimination by endogenous antioxidant defenses, including the increment in total antioxidant capacity of tissues, was the contemporaneous decline in tissue $Zn^{2+}$ in heart, muscle, and plasma. The fall in cardiac tissue $Zn^{2+}$ we observed in response to Isop has also been reported by others (2, 48). Intracellular $Zn^{2+}$, acting as an antioxidant, is essential to the activity of Cu/Zn-superoxide dismutase, a metalloenzyme integral to these defenses and degradation of cytotoxic oxygen-rich intermediaries that appear in these tissues in response to intracellular $Ca^{2+}$ overloading. The fall in cardiac tissue $Zn^{2+}$ following Isop treatment contrasted to its rise in $Zn^{2+}$ in cardiomyocytes that occurs in response to ischemia/reperfusion and the appearance of chronic stressor state associated with aldosterone/salt treatment (21, 32, 43, 46, 49). This begs the question whether supplemental $Zn^{2+}$ would be clinically useful if given soon after the acute hyperadrenergic state is set in motion following bodily injury. Relative to the heart, we do not believe this to be the case given that the activity of $Zn^{2+}$ transporters needed for $Zn^{2+}$ entry and intracellular $Zn^{2+}$ accumulation would be lost to cardiomyocyte necrosis. This contrasts to $ZnSO_4$ supplement given as pretreatment before Isop (25) or with the salutary responses to this supplement when given to prevent the chronic cardiotoxicity associated with diabetes or aldosterone/salt treatment (32, 42, 60). Finally, the rise in total antioxidant capacity found in heart and plasma, including those derived from vitamins A, C, and E, was not adequate to prevent the appearance of oxidative stress over the 24 h that followed Isop treatment.

The predilection for cardiac injury and myocardial scarring following Isop that involves the LV apex is related to a preferential $Ca^{2+}$ overloading at this site. This importantly includes increased mitochondrial free $[Ca^{2+}]_m$ and the associated induction of oxidative stress by these organelles, demonstrated by their increased $H_2O_2$ generation and 8-isoprostane concentration, leading to consequent mPTP opening and ensuing cardiomyocyte necrosis. In attenuating mitochondrial $Ca^{2+}$ accumulation with carvedilol or mitochondrial oxidative stress with quercetin, the greater propensity for mPTP opening in response to Isop could be largely attenuated. Furthermore, when quercetin binds to $Zn^{2+}$, forming a flavonoid-metal complex, it is an even more potent antioxidant than free quercetin (27). We would suggest that these responses represent a crucial pathophysiological overview confirming that this signal-transducer-effector pathway (see Fig. 8) preferentially begets cytotoxicity at the LV apex in response to hyperadrenergic stressor state.

It is intriguing to speculate on the relevance of this pathway in leading to the predilection for hypokinesia of the apical segment of LV myocardium, which has been clinically termed apical ballooning, "broken heart," ampulla, or takotsubo cardiomyopathy that accompanies the hyperadrenergic state seen with profound emotional stress, pheochromocytoma, or dobutamine-stress echocardiography (33, 47, 67). In this context, carvedilol has been used to effectively treat apical ballooning cardiomyopathy (53). Our study of the acute hyperadrenergic stressor state induced by Isop and assessment of $Ca^{2+}$ and $Zn^{2+}$ dyshomeostasis and oxidative stress that appeared in the heart and systemic organs could be explored to further address other pertinent issues. For example, we did not specifically monitor stressor signaling pathways, such as the mitogen-activated kinases (p38MAPK, JNK and ERK), in each of these tissues. This family of enzymes will relay and propagate external stimuli using phosphorylation cascades to generate coordinated and adaptive cellular responses that target downstream substrates, such as redox-sensitive nuclear transcription factor (NF)-kB and the proinflammatory cytokines it regulates. In response to acute burn injury, p38MAPK activation importantly contributes to cytokine-mediated organ injury (7). In future studies, it will be important to critically examine and address many of these relevant issues, including their responses to targeted pharmacological interventions.

In summary, we demonstrated that a dyshomeostasis of $Ca^{2+}$ and $Zn^{2+}$ occurs in the heart and systemic tissues of rats in response to an acute hyperadrenergic stressor state induced by a single dose of Isop in which pathophysiological derangements persisted for many hours. In plasma, $Ca^{2+}$ and $Zn^{2+}$ responses were discordant, whereas in tissues these were both discordant and persistent. Within the heart the EICA was heterogeneous, favoring the LV apex (vis-à-vis the LV equator and base), whereas the total tissue $Zn^{2+}$ levels fell early and remained subnormal over 24 h. Liver, on the other hand, with
its upregulation of MT-1 served as a strategic repository for Zn$^{2+}$. The Ca$^{2+}$-overloading of heart, muscle, and PBMC was accompanied by the contemporaneous appearance of oxidative stress and the concordant rise in endogenous total antioxidant capacity although it was insufficient to completely abrogate cardiotoxicity. In mitochondria harvested from the LV apex, Ca$^{2+}$-overloading was accompanied by increased H$_2$O$_2$ generation and opening potential of mPTP, resulting in myocardial necrosis and subsequent scarring. These iterations in mitochondrial Ca$^{2+}$ and their corresponding pathological redox state could each be prevented by cotreatment with either carvedilol or quercetin.

We therefore would suggest that signal-transducer-effector pathway, leading to LV apical injury and scarring of the endocardium in response to catecholamine excess, involves Ca$^{2+}$-overloading of cardiac mitochondria, induction of oxidative stress, and increased opening potential of their inner membrane mPTP.

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CALCIUM AND ZINC DYSHOMEOSTASIS


