FORUM REVIEW ARTICLE

Abnormal Ca^{2+} Cycling in Failing Ventricular Myocytes: Role of NOS1-Mediated Nitroso-Redox Balance

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Abstract

Significance: Heart failure (HF) results from poor heart function and is the leading cause of death in Western society. Abnormalities of Ca^{2+} handling at the level of the ventricular myocyte are largely responsible for much of the poor heart function. **Recent Advances:** Although studies have unraveled numerous mechanisms for the abnormal Ca^{2+} handling, investigations over the past decade have indicated that much of the contractile dysfunction and adverse remodeling that occurs in HF involves oxidative stress. **Critical Issues:** Regrettably, antioxidant therapy has been an immense disappointment in clinical trials. Thus, redox signaling is being reassessed to elucidate why antioxidants failed to treat HF. **Future Directions:** A recently identified aspect of redox signaling (specifically the superoxide anion radical) is its interaction with nitric oxide, known as the nitroso-redox balance. There is a large nitroso-redox imbalance with HF, and we suggest that correcting this imbalance may be able to restore myocyte contraction and improve heart function. *Antioxid. Redox Signal.* 21, 2044–2059.

Introduction

HEART FAILURE (HF) is the leading cause of death in Western society. It is a syndrome characterized by the inability of the diseased heart to pump blood normally. It has many different causes (ischemic heart disease and chronic hypertension are most common), but all culminate in a heart with poor pump performance with a progressive decline in function. In these HF conditions, the myocytes must develop higher than normal forces to pump blood. These persistent demands for enhanced myocyte contraction are associated with myocyte hypertrophy, an increased propensity for cell death, and functional remodeling that ultimately culminates in reducing the contractile capacity of the myocyte (46, 161). Thus, the failing heart has increased contractile demands but fewer myocytes (122) with reduced contractility reserve. This is an inherently unstable condition that increases the risk of death from arrhythmias and hemodynamic collapse. Numerous studies have explored the fundamental cellular and molecular bases of myocyte contractility alterations in HF (79). Increases in cytosolic $[\text{Ca}^{2+}]_i$ are essential for activating cardiac contraction. There is a robust literature documenting that alterations in myocyte Ca^{2+} handling are present in HF and that these changes are largely responsible

for altered contractility of the failing heart (80). The cellular and molecular bases for abnormal Ca^{2+} regulation in HF have been studied with the idea that correcting these alterations might restore myocyte contraction and improve heart function. However, many therapeutic approaches that increase myocyte contraction have been tested over the past few decades, and most have been shown to increase death, primarily by inducing lethal cardiac arrhythmias (72). There is a need to develop strategies that can increase myocyte contractile capacities while reducing arrhythmia risk. In this review, we discuss the idea that post-translational modifications of Ca^{2+} regulatory proteins *via* aberrant neuronal nitric oxide synthase (NOS1)-mediated nitroso-redox balance contribute to contractility defects in HF and suggest that restoring the normal nitroso-redox balance may be the promising strategy.

Excitation–Contraction Coupling

In diastole, cytosolic Ca^{2+} ($[\text{Ca}^{2+}]_i$) is low and there is little or no Ca^{2+} bound to the myofilaments. During systole, $[\text{Ca}^{2+}]_i$ increases and binds to troponin C (TnC) to induce a strong interaction between TnC and troponin I (TnI). This will destabilize the interaction between TnI and actin causing a shift in the troponin–tropomyosin complex allowing

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myosin to bind actin resulting in myocyte force production (and/or shortening) (41). The rate and magnitude of myocyte force development are determined by systolic $[Ca^{2+}]$. Thus, altering the force of cardiac contraction largely occurs by regulating systolic Ca^{2+} levels (contractility regulation) (summarized in Fig. 1).

The increases in myocyte Ca^{2+} that initiate contraction occur *via* a process termed excitation–contraction coupling (ECC) (19). ECC is initiated with an action potential, in which the opening of Na^+ channels causes membrane depolarization *via* the entry of positive charges (Na^+ ions). This depolarization will activate voltage-gated Ca^{2+} channels causing the influx of Ca^{2+} . In a process termed Ca^{2+} -induced Ca^{2+} release, this Ca^{2+} influx will activate the Ca^{2+} release channel (ryanodine receptor [RyR]) in the junctional sarcoplasmic reticulum (SR). The resultant increase in $[Ca^{2+}]_i$ is the sum of Ca^{2+} influx through L-type Ca^{2+} channels (LTCC) and SR Ca^{2+} release. The amount of Ca^{2+} influx and the amount of Ca^{2+} available for release from the SR (the SR Ca^{2+} load) are the major determinants of the amplitude of the Ca^{2+} transient amplitude (145) and thus myocyte contraction. Relaxation is initiated by a decrease in $[Ca^{2+}]_i$ (*i.e.*, the decline of the Ca^{2+} transient). This is primarily due to the SR Ca^{2+} -ATPase (SERCA), which re-sequesters released Ca^{2+} back into the SR, and the sarcolemmal Na/Ca exchanger (NCX), which eliminates the Ca^{2+} that has entered with each heartbeat. In rodents, the SR Ca^{2+} uptake is responsible for >90% of the Ca^{2+} transient while Ca^{2+} efflux plays a significantly larger role in larger species, including humans.

The normal heart has the ability to increase its pump performance when needed, as during aerobic exercise (172). Increases in the amount of Ca^{2+} delivered to the contractile apparatus are essential for increases in cardiac contraction, termed contractility. The Ca^{2+} regulatory proteins responsible for physiological regulation of cardiac contractility are

those that determine Ca^{2+} influx (LTCC), SR Ca^{2+} uptake (SERCA), and SR Ca^{2+} release (RyR). In the normal heart, changes in the activity of these proteins are largely caused by phosphorylation of these proteins *via* the activation of beta-adrenergic (β -AR) receptors (*i.e.*, sympathetic regulation of cardiac function) (20).

A critical modulator of SERCA activity, and thus SR Ca^{2+} uptake, is the phosphoprotein phospholamban (PLB) (110). Under basal conditions, PLB phosphorylation is low and PLB in an unphosphorylated state slows SERCA-mediated SR Ca^{2+} uptake, and this results in a modest SR Ca^{2+} load and relatively slow SR Ca^{2+} uptake (*i.e.*, prolonged Ca^{2+} transient decline). This is a low contractility state as might be seen in a person with low metabolic demands, such as occurring with sleep or during rest. With the activation of the sympathetic nervous system, there is activation of β -AR signaling pathway and a resultant activation of protein kinase A (PKA), with phosphorylation of PKA target proteins (20). PKA-mediated PLB phosphorylation at Serine16 disinhibits SERCA and results in an increase in the SERCA activity (131). Increased PKA activity also increases Ca^{2+} entry *via* LTCC. These phosphorylation changes cause greater Ca^{2+} influx, SR Ca^{2+} uptake, and SR Ca^{2+} load. The net result is an increase in the rate and amplitude of the systolic Ca^{2+} transient and a decrease in the duration of the transient. These PKA-mediated increases in systolic Ca^{2+} also lead to the activation of Ca^{2+} -calmodulin-dependent protein kinase (CaMKII), which further enhances both Ca^{2+} entry and SERCA activity (PLB Threonine 17 phosphorylation) (111). PKA- and CaMKII-mediated phosphorylation of RyR can also have a minor effect on myocyte contractility by altering the Ca^{2+} dependence of RyR openings (58, 144, 173); however, this effect is not universally observed (185).

The *in vivo* contractile demands on myocytes within the failing heart are greater than in healthy hearts due to increased systolic wall stress, and these demands increase with HF progression. This persistent demand for enhanced myocyte function is associated with alterations in myocyte Ca^{2+} regulation (summarized in Fig. 2). LTCCs are

FIG. 1. Regulation of Ca^{2+} and Na^+ handling in a healthy myocyte by LTCC, RyR, SERCA/PLB, NCX, NKA, and Nav1.5. Thickness of arrows is indicative of activity (compare to Fig. 2). Font size of Ca^{2+} in SR is indicative of SR Ca^{2+} load. LTCC, L-type Ca^{2+} channel; NCX, Na^+/Ca^{2+} exchanger; NKA, Na^+/K^+ ATPase; PLB, phospholamban; RyR, ryanodine receptor; SERCA, SR Ca^{2+} -ATPase; SR, sarcoplasmic reticulum. To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/ars

FIG. 2. Regulation of Ca^{2+} and Na^+ handling in a failing myocyte by LTCC, RyR, SERCA/PLB, NCX, NKA, and Nav1.5. Thickness of arrows is indicative of activity (compare to Fig. 1). Font size of Ca^{2+} in SR is indicative of SR Ca^{2+} load. To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/ars

hyperphosphorylated, but channel density appears to be reduced (32); SERCA abundance and PLB phosphorylation are reduced (70, 136, 141); NCX activity is increased (125); and RyRs are hyperphosphorylated (3, 112). These modifications limit the maximal Ca^{2+} entry, SR uptake and storage, and induce a more pronounced efflux of Ca^{2+} from the SR via RyR during diastole (*i.e.*, diastolic Ca^{2+} leak). The sum of these changes limits the ability of failing myocytes to enhance systolic Ca^{2+} and thus contractility. Therefore, the failing heart must generate much higher than normal force (*via* activation of neurohormonal regulatory pathways) while the inherent ability of the resident myocytes to increase their contractility (contractility reserve) is reduced. The failing heart also loses its ability to increase force at higher heart rates (blunted force-frequency response [FFR]) (133) and has a depressed functional response to β -AR stimulation (193). Myocytes isolated from failing hearts and studied *versus* nonfailing myocytes under identical conditions have a decrease in SR Ca^{2+} load resulting in a decreased amplitude of the Ca^{2+} transient and a slowing of its decline (124). HF-associated changes in Ca^{2+} regulation also contribute to arrhythmias in HF. The increase in diastolic SR Ca^{2+} leak will activate electrogenic Ca^{2+} efflux *via* the NCX, and this causes afterdepolarizations that can trigger arrhythmias (36, 146). Restoring a normal Ca^{2+} regulatory system should improve cardiac function and is a therapeutic goal. However, those approaches that increase PKA signaling (which is already activated in HF) have been linked to arrhythmias and sudden death (119). Standard of care therapy for HF includes β -blockers, which are thought to prevent excessive PKA signaling and related arrhythmias (6). Therefore, strategies that can restore a more normal Ca^{2+} transient without activating PKA might increase contractility reserve without increasing arrhythmia risk. In the remainder of this review, we discuss the idea that abnormal post-translational modifications of Ca^{2+} regulatory proteins by redox reactions contribute to aberrant contractility in HF. If true, then restoring a normal redox state could improve myocyte contractility and cardiac pump function in HF.

Redox Signaling

There is a precise regulation of reactive oxygen species (ROS) levels in which the production of ROS is counterbalanced by its degradation. Oxidative stress arises when there is an accumulation of ROS due to an increase in the production of ROS and/or a decrease in the degradation of ROS. The superoxide anion radical ($\text{O}_2^{\bullet-}$) is the main radical since it can be converted into other ROS (hydrogen peroxide [H_2O_2], $\bullet\text{OH}$, peroxynitrite, *etc.*) *via* various enzymes and chemical reactions (Fig. 3). Within healthy cardiac myocytes, the major producers of $\text{O}_2^{\bullet-}$ are the mitochondria (171), nicotinamide adenine dinucleotide phosphate (NADPH) oxidase (104), and xanthine oxidase (51) (Fig. 4). Production of $\text{O}_2^{\bullet-}$ in the mitochondria occurs as a reactionary byproduct of respiration in the electron transport chain of complex I and III (77, 159). Unlike the mitochondria, the sole purpose of NADPH oxidases (NOX) is to produce $\text{O}_2^{\bullet-}$ (163). Within ventricular myocytes, the NOX isoforms that are expressed are NOX2 and NOX4 (2, 15, 179). Xanthine oxidase is involved in purine degradation and produces $\text{O}_2^{\bullet-}$ from the reduction of molecular O_2 and is also expressed in ventricular

FIG. 3. Superoxide ($\text{O}_2^{\bullet-}$) signaling within ventricular myocytes. ($\text{O}_2^{\bullet-}$) is the main radical since it can be converted into additional reactive species. For example, upon reaction with nitric oxide ($\text{NO}^\bullet$), peroxynitrite (ONOO^-) is formed. In addition, $\text{O}_2^{\bullet-}$ can form H_2O_2 by SOD, which can then form the hydroxyl radical ($\bullet\text{OH}$) *via* the Fenton reaction. $\bullet\text{OH}$ can also be directly formed from $\text{O}_2^{\bullet-}$ *via* the Haber-Weiss reaction. $\text{O}_2^{\bullet-}$ can also directly modulate protein function by targeting cysteine residues. Reversible cysteine modifications (oxidation to sulfenic acid or the formation of disulfide bridges) will occur with low $\text{O}_2^{\bullet-}$ levels. Irreversible cysteine modifications (oxidation to sulfenic acid or sulfonic acid) will occur with high $\text{O}_2^{\bullet-}$ levels. H_2O_2 , hydrogen peroxide; SOD, superoxide dismutase.

myocytes (18, 90). The myocyte has an array of defenses to ascertain that ROS levels do not increase. These defenses include various enzymes such as superoxide dismutase (SOD), catalase, glutathione peroxidase, and thioredoxin. The myocyte also has various compounds (*e.g.*, vitamin E, ascorbic acid) and peptides (glutathione) that are also antioxidants. While there is a decline in the antioxidant defense with HF, it appears that oxidative stress is primarily due to an increase in ROS generation (Fig. 5). That is, mitochondrial $\text{O}_2^{\bullet-}$ production is increased in HF patients due to state 3 respiration and dysregulation of cytosolic Ca^{2+} and Na^+ (discussed further below in the $[\text{Na}^+]_i$ regulation section) (83, 93). Furthermore, under these oxidative conditions, the mitochondria will also produce large amounts of H_2O_2 (9, 150). NOX activity is also increased with HF (75, 104, 107, 128). While there do not appear to be changes in NOX2 expression

FIG. 4. Regulation of nitroso-redox balance in a healthy myocyte by NOS1. Thickness of arrows is indicative of activity. Font size is indicative of concentration. Size of NOS1 octagon is indicative of expression. $\text{NO}^\bullet$, nitric oxide; NOX, NADPH oxidase; $\text{O}_2^{\bullet-}$, superoxide anion; XO, xanthine oxidase. To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/ars

FIG. 5. Aberrant NOS1 activity and nitroso-redox imbalance in a failing myocyte. Thickness of arrows is indicative of activity. Font size is indicative of concentration. Size of NOS1 octagon is indicative of expression. MAO, monoamine oxidase. To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/ars

levels, post-translational modification and membrane translocation of subunits (e.g., p47^{phox}, p67^{phox}) are responsible for the increased activity. Unfortunately, it also appears that there is a connection between NOX activity and mitochondrial $O_2^{\bullet-}$ production (47, 78). Thus, in the setting of HF, the increased mitochondrial $O_2^{\bullet-}$ production may be further augmented *via* NOX. In addition, the dismutation of $O_2^{\bullet-}$ to H_2O_2 can further augment production of $O_2^{\bullet-}$ from neighboring mitochondria in cardiac myocytes termed ROS-induced-ROS release (8, 165, 194). Xanthine oxidase activity is also upregulated due to increased expression and altered modulation by NO (discussed further below in the Neuronal NOS section) (134). Unfortunately, the HF syndrome also results in additional ROS contributors within ventricular myocytes not observed under physiological states. Monoamine oxidases (MAO) are responsible for the oxidative deamination of neurotransmitters, such as epinephrine (epi) and norepinephrine (norepi). With the enhanced β -AR drive and increased epi/norepi in HF, MAO activity and expression (both MAO A and B) are increased resulting in enhanced H_2O_2 levels (86, 87). In addition, under certain conditions (as occurs in HF), NO production *via* NOS is interrupted resulting in the production of $O_2^{\bullet-}$, termed NOS uncoupling (154).

Oxidative stress occurs in HF, and many studies have focused on the effects of ROS on myocyte contraction. Acute exposure of myocytes to ROS ($O_2^{\bullet-}$, H_2O_2 , $\bullet OH$) results in decreased Ca^{2+} transient amplitude, slowed Ca^{2+} decline, and reduced SR Ca^{2+} load (60, 61, 64, 67, 100, 109, 121). These effects of ROS on myocyte Ca^{2+} handling are similar to what is observed in failing myocytes. These data suggest that oxidative stress contributes to the observed phenotype in failing myocytes (especially in which there are no changes in protein expression). Further studies have shown that scavenging of ROS, inhibiting the sources of ROS, or increasing antioxidants (e.g., glutathione peroxidase) in disease are able to reverse the contractile dysfunction (2, 54, 63, 65, 105, 128, 135, 148, 183). Hence, these studies unequivocally prove that oxidative stress does indeed contribute to the contractile dysfunction in failing hearts.

Studies have further investigated the molecular mechanisms for the altered Ca^{2+} handling induced by oxidative stress and found that all the major Ca^{2+} handling proteins (RyR, SERCA/PLB, and NCX) are modified. ROS is able to acutely modulate protein function *via* reversible post-translational modifications of the amino acids methionine and cysteine (i.e., redox signaling) (177). ROS are able to oxidize the sulfur atom in methionine to yield methionine sulfoxide, which is reversed *via* methionine sulfoxide reductase (24). Low levels of ROS will oxidize cysteine residues to yield sulfenic acid and then disulfide formation (which can occur between adjacent cysteines, between two proteins, or a protein thiol and glutathione, S-glutathionylation). These post-translational modifications can be reversed back by thioredoxin and glutaredoxin (11, 17, 74, 155). With higher levels of ROS, there is further oxidation of cysteines to generate sulfinic acid and then sulfonic acid. These modifications are irreversible (although sulfiredoxin can reverse some of the sulfinic acid) (178). Thus, depending on the redox state of the cell, this range of cysteine modifications (Fig. 3) allows for the acute modulation of protein function during physiological stresses or can result in protein dysfunction during pathological stresses.

Ryanodine receptor

This large protein exists as a tetramer, with each monomer containing 89 cysteine residues. Thus, there can be an assortment of modifications (number and location of residues) resulting in varied effects. Indeed, this seems to be the case as ROS has a bidirectional effect of RyR activity (180, 184). Marginal levels of RyR oxidation seem to have little effect on activity, whereas low levels of oxidation result in an increase in activity (23, 89, 182, 189). Importantly, high levels of ROS have been reported to reduce or irreversibly increase RyR activity (50). Once again this may be due to distinctive cysteine modifications as well as time dependence of ROS exposure. However, with HF, it has been reported that RyR activity is increased due to ROS (113, 157). This ROS-mediated increase in activity has been shown to contribute to the generation of arrhythmogenic Ca^{2+} waves *via* increased diastolic Ca^{2+} leak from the SR. In addition to cause arrhythmias, this leak will empty the SR Ca^{2+} load (a hallmark of HF) and decrease contraction.

SR Ca^{2+} -ATPase

Another cause of the decreased SR Ca^{2+} load in HF is decreased SR Ca^{2+} uptake *via* reduced SERCA activity. Many studies have shown that ROS inhibits SERCA function. This effect occurs *via* a direct attack on the ATP binding site, uncoupling Ca^{2+} uptake from ATP hydrolysis, and *via* sulfination at cysteine674 (101, 114, 139, 181).

Na/Ca exchanger

Since Ca^{2+} transport into the SR is reduced in HF, to maintain proper diastolic function, an increase in NCX activity could be beneficial by reducing $[Ca^{2+}]_i$ (71). It has also been postulated that increased NCX activity (reverse mode) can increase SR Ca^{2+} load, which will occur when there is a concomitant increase in $[Na^+]_i$ (see below in the $[Na^+]_i$ regulation section) (124, 174). Studies have shown an increase in NCX activity (usually without changes in expression) in HF

(125). Consistent with this increase in activity with HF, ROS has also been shown to increase NCX activity *via* oxidation (59, 100, 130). However, there is a negative consequence of this increased NCX activity, which is to increase the propensity to generate arrhythmias (7).

[Na⁺]_i regulation

With NCX (and other pathways), there is a strong interdependence between [Ca²⁺]_i and [Na⁺]_i levels. Similar to Ca²⁺ handling, there are many proteins involved in the regulation of [Na⁺]_i levels (Figs. 1 and 2). These proteins are also regulated by ROS. The voltage-gated Na⁺ channel (Nav1.5) inactivation is greatly impeded by oxidation of methionine residues (increasing Na⁺ entry) (56, 88). While NCX and Nav1.5 can increase the amount of Na⁺ influx into the myocyte, [Na⁺]_i levels are largely determined by the Na⁺/K⁺-ATPase (NKA) (45). Studies have shown that NKA activity is reduced *via* ROS, in part *via* glutathionylation of Cysteine46 (55). Thus, with oxidative stress and direct protein modifications to NCX, Nav1.5, and NKA (along with the Ca²⁺ handling proteins), there will be a dysregulation of myocyte [Na⁺]_i and [Ca²⁺]_i homeostasis contributing to the contractile dysfunction and arrhythmias observed in HF. Furthermore, the dysregulation of [Na⁺]_i and [Ca²⁺]_i will alter mitochondrial dynamics resulting in the generation of more ROS setting up a vicious cycle (57, 93).

Kinase/phosphatase activity

While ROS can directly modulate ECC protein function *via* oxidation (*i.e.*, redox signaling), it is also known that various kinases and phosphatases are redox sensitive. Thus, there may be an additional layer of complexity of ROS in which it may also regulate ECC protein function *via* changing phosphorylation status. As previously mentioned, PLB phosphorylation is decreased in HF. This decreased phosphorylation is partly due to the desensitization of the β -AR pathway *via* downregulation of β -AR receptors, increased Gxi, and increased GRK2 (53, 193). Another beneficial effect of β -blockers is to resensitize this pathway (27). However, protein phosphorylation levels will remain low due to an increase in phosphatase activity (22, 66, 118). Studies have shown that PP2a can be activated *via* various ROS to decrease PLB phosphorylation and blunt contraction (98, 99). It has also been shown that CaMKII can be activated *via* ROS (52). CaMKII can phosphorylate RyR at serine2814 to increase diastolic Ca²⁺ leak to empty the SR of Ca²⁺ and increase the propensity for arrhythmias (3, 36, 37, 162, 173). Furthermore, CaMKII phosphorylation of Nav1.5 will increase the late current resulting in [Na]_i dysregulation and arrhythmias (166). Thus, oxidative stress manifests a “double whammy” on the ventricular myocyte by directly oxidizing and indirectly changing phosphorylation status of Na⁺ and Ca²⁺ handling proteins.

Antioxidant therapy

With the many studies considered above, one would expect that antioxidants could be used to treat HF. While antioxidant therapy (either scavenging ROS or inhibiting a source of ROS) has beneficial effects in small animal models of HF (4, 76, 117, 128, 147), clinical trials have been disappointing (21,

69, 108, 116). Thus, the field is reexamining redox signaling and why the failure in the treatment of HF. One likely reason is that, similar to other signaling pathways, redox signaling is compartmentalized. That is, redox signaling occurs with the production of specific species confined to spatially and temporally defined microdomains *via* specific antioxidant defenses (proteins, *etc.*). Thus, our current ways of antioxidant treatment may not be reaching or affecting these microdomains. Furthermore, by completely reducing ROS levels, our treatments may be inhibiting the beneficial effects of redox signaling. For example, studies have shown that angiotensin II and endothelin-1 stimulates ROS production *via* NOX to increase contraction (34, 43, 44). ROS production also plays an important role in the positive inotropic effects of β -AR stimulation (5). However, prolonged β -AR stimulation (>6 min) results in greater ROS production resulting in arrhythmogenic waves (25). It also appears that ROS (specifically H₂O₂ *via* NOX4) is important for vasodilation and lowering of blood pressure (129). This mechanism is also important during pressure overload to preserve capillary density to prevent cardiovascular dysfunction. Vasodilation also occurs *via* the cyclic GMP (cGMP)-dependent protein kinase (PKG), which can be activated *via* ROS independent of cGMP (29). In knock-in mice with a redox “dead” PKG, these mice were not able to vasodilate in response to acetylcholine and developed hypertension (127). We speculate that NOX4 signaling leads to vasodilation *via* activation of PKG; however, work needs to be done to confirm this. Another physiological function of NOX (specifically NOX2) is the stretch-dependent increase in contraction, termed X-ROS (126). Thus, with nonspecific antioxidants, we may be limiting the physiological effects of ROS, which can further deteriorate cardiac function during disease. In fact, this has been reported in some of the clinical trials (21, 108). Another important factor in redox signaling (specifically O₂^{•-}) is its interaction with NO, known as the nitroso-redox balance (68).

Nitroso-Redox Balance

There is an interdependence between O₂^{•-} and NO known as the nitroso-redox balance, in which the myocyte strives to keep NO levels greater than O₂^{•-} (Fig. 4). The O₂^{•-} and NO pathways communicate with each other *via* multiple means to make sure this nitroso-redox balance is maintained. One way is that NO acts as a buffer by reacting with O₂^{•-} [one of the fastest reactions in nature (81)] to form peroxynitrite, which itself is a potent cardiac signaling molecule in health and disease (95). In addition, each signaling molecule can modulate the activity of enzymes on the other side of the balance. For example, NO is able to inhibit xanthine oxidase activity to limit O₂^{•-} production (73, 82, 103). NO is produced *via* enzymes termed NOS (143). Within ventricular myocytes, the neuronal (NOS1) and endothelial (NOS3) NOS isoforms are constitutively expressed (191).

Endothelial NOS

While NOS3 activity can be modulated by ROS (*e.g.*, O₂^{•-} and H₂O₂) (31, 138), we do not believe that NOS3 contributes to the nitroso-redox balance that is involved in the regulation of myocyte Ca²⁺ handling. Our and others' previous data have shown that NOS3 signaling has no effect on basal myocyte Ca²⁺ handling and only modestly reduces

β -AR-stimulated Ca^{2+} transient amplitude (10, 169). NO signals *via* the cGMP-independent (which is affiliated with the nitroso-redox balance) or the cGMP-dependent pathway (190). Our data demonstrated that NOS3 signals *via* the cGMP pathway in a spatially constricted microdomain to regulate caveolar LTCC (168). We believe that this is the basis for the minor role NOS3 plays in modulating contractile Ca^{2+} . Furthermore, NOS3 colocalizes with SOD (26), an enzyme that reduces $\text{O}_2^{\bullet-}$ levels, making NO more likely to signal *via* the cGMP pathway. This is further confirmed in studies that showed no change in myocyte ROS levels with NOS3 knockout (90). Thus, NOS3 signaling plays a minor role in the nitroso-redox balance that modulates myocyte Ca^{2+} handling. Our and others' data reveal that it is NOS1 that has this responsibility (90, 158).

Neuronal NOS

NOS1 is localized to the SR *via* binding to RyR (10) (Fig. 4). Unlike NOS3, NOS1 modulates ventricular myocyte contractile Ca^{2+} (192). That is, NOS1 signaling increases basal Ca^{2+} transient amplitude and accelerates Ca^{2+} decline (91, 167). In addition, physiological mediators of contraction such as the FFR and β -AR stimulation increase contraction and enhance lusitropy, in part, *via* altering Ca^{2+} handling (20, 85). Our and others' work has shown that NOS1 is a key protein involved in these pathways (10, 91, 167, 170). That is, in NOS1 knockout ($\text{NOS1}^{-/-}$) mice, there is a blunted FFR and functional response to β -AR stimulation, which is similar to failing myocytes (*i.e.*, decreased contractile reserve).

Similar to redox signaling, NO can also modify cysteine residues. This occurs by the addition of the NO moiety to a cysteine thiol to form an S-nitrosothiol (SNO) and is termed S-nitrosylation (74). There are also enzymes that reverse this reaction termed denitrosylation. One enzyme is the S-nitrosoglutathione reductase (106). However, this is not a direct denitrosylation as SNO proteins are not substrates. Instead, it reduces protein SNO levels by influencing the cellular redox environment. Another enzyme family, thioredoxin, has been found to be a direct denitrosylase (17). Thus, this cysteine modification (SNO) allows for the acute modulation of protein function, and we and others have found that NOS1 signals *via* the activation of this pathway (cGMP-independent) (167, 187). As with ROS, NO is also able to modulate various Ca^{2+} and Na^+ handling proteins.

Ryanodine receptor. Since NOS1 localizes with RyR, studies have investigated the effects of NOS1 signaling on RyR function. We have found that NOS1 signaling increases RyR activity *via* S-nitrosylation (170). Specifically, by using various techniques to measure RyR activity that ranged from direct single RyR protein activity to indirect measurements in intact myocytes, we observed that $\text{NOS1}^{-/-}$ myocytes had reduced RyR activity associated with decreased S-nitrosylation. Additionally, when we added NO (SNAP), we could increase $\text{NOS1}^{-/-}$ RyR S-nitrosylation and activity. This is consistent with data obtained in mice with increased NOS1 activity (30). It should be noted that the Hare group has found the opposite results in which they report that $\text{NOS1}^{-/-}$ myocytes have increased RyR activity (62). We believe that our disparate results on RyR activity can be explained by nitroso-redox imbalance.

Nitroso-redox imbalance occurs in $\text{NOS1}^{-/-}$ mice *via* decreased NO bioavailability due to genetic deletion of NOS1 and increased $\text{O}_2^{\bullet-}$ production *via* xanthine oxidase (90, 92, 158). Along with RyR, NOS1 colocalizes with xanthine oxidase. NO is able to inhibit xanthine oxidase and limit $\text{O}_2^{\bullet-}$ production (73, 82, 103). Thus, with the loss of NOS1 and NO, xanthine oxidase is no longer inhibited and will produce an oversupply of $\text{O}_2^{\bullet-}$. It has also been shown that $\text{O}_2^{\bullet-}$ production *via* NOX (most likely NOX2) is also increased in $\text{NOS1}^{-/-}$ myocytes (186). This loss of NO and S-nitrosylation will have two effects. First, the loss of SNO will have direct effects on protein function; second, S-nitrosylation of proteins protects them from oxidation (115, 152). Thus, RyR is now more susceptible to oxidation. Under low stress conditions, we hypothesize that loss of NO results in nitroso-redox imbalance leading to a decrease in RyR activity due to less RyR SNO. However, RyR in $\text{NOS1}^{-/-}$ myocytes is more susceptible to oxidative injury. We believe that under various stress conditions that will worsen the nitroso-redox imbalance by producing more $\text{O}_2^{\bullet-}$ (*e.g.*, FFR, β -AR stimulation, HF); there will be augmented oxidative injury to RyR. Data support this concept as restoring the nitroso-redox balance under low stress conditions increases RyR activity (158), whereas it decreases the activity under high stress conditions (48, 63). Thus, under low stress conditions, $\text{NOS1}^{-/-}$ myocytes have decreased Ca^{2+} transient amplitude due to decreased RyR activity, whereas high-stress (FFR, β -AR) $\text{NOS1}^{-/-}$ myocyte have decreased Ca^{2+} transient amplitude due to decreased SR Ca^{2+} load caused by excessive Ca^{2+} leak due to enhanced RyR activity. Furthermore, a similar situation was observed when investigating the effects of nitroso-redox imbalance, RyR, and arrhythmias (38). There were no observed arrhythmias with acute inhibition of NOS1. However, with the additional stress of elevated $[\text{Ca}^{2+}]_i$, the nitroso-redox imbalance now resulted in RyR-dependent arrhythmias.

SR Ca^{2+} -ATPase. Unlike RyR, studies have not investigated if NOS1 is able to directly modulate SERCA activity. However, a study demonstrated that SERCA is S-nitrosylated (14), but the NO source responsible for this post-translational modification was not investigated. It has also been reported that nitroxyl (the 1 electron reduction product of NO) is able to modify SERCA *via* S-glutathiolation of cysteine674 (102). In addition to directly activating SERCA, we have shown that nitroxyl is able to target cysteines on PLB resulting in the formation of less monomeric PLB (the form that inhibits SERCA) (149). This may be important since it has been suggested that a product of NOS1 is nitroxyl (84, 140), and we have shown that the functional effects of nitroxyl are similar to NOS1 signaling (94). Thus, one would presume with NOS1 localized at the SR that it can directly activate SERCA activity *via* S-nitrosylation/glutathiolation, or indirectly by thiol modification of PLB, which would be consistent with the functional effects of NOS1 signaling. However, this remains to be established.

NCX and Na^+ handling. Using an indirect method to measure NCX (decline of the caffeine-induced Ca^{2+} transient), we and others have found that there was no difference between WT and $\text{NOS1}^{-/-}$ myocytes (142, 167). These data suggest that NOS1 signaling does not modulate NCX. It does

appear that NOS1 is able to modulate other Na^+ handling proteins. For example, it has been shown that NOS1 increases Nav1.5 current amplitude and late current *via* S-nitrosylation (160). Furthermore, it has been shown that NO (possibly *via* NOS1) is able to stimulate NKA activity (123, 175), whereas NOS1^{-/-} mice have decreased NKA activity (188). However, it appears that these effects are due to a differentially located NOS1. There is a subset of NOS1 that is localized to the plasma membrane by binding to the plasma membrane Ca^{2+} -ATPase (PMCA4) (176). Studies have found that this subgroup of NOS1 does regulate myocyte contraction *via* cyclic nucleotide signaling (cyclic AMP [cAMP] and cGMP *via* phosphodiesterase type 2) (120). Another subset of NOS1 is bound to caveolin-3, which is an important interaction for the regulation of Nav1.5 (33). Hence, the NOS1 located at the SR is the specific class of NOS1 that modulates the nitroso-redox balance to regulate contraction (Fig. 6).

Kinase/phosphatase activity. NOS1 can directly modulate ECC protein function *via* S-nitrosylation. But, similar to ROS, may also modulate the activity of various kinases and phosphatases to change the protein phosphorylation status. We have previously shown that a peroxynitrite donor (SIN-1) in a low dose was able to increase PLB Serine16 phosphorylation *via* activation of PKA (96, 97). Surprisingly, the peroxynitrite-mediated increase in PKA activity occurred in the absence of cAMP, and most likely occurs *via* S-nitrosylation (28). NOS1 signals, in part, *via* peroxynitrite and NOS1^{-/-} myocytes have decreased PLB Serine16 phosphorylation (167). Therefore, a segment of NOS1 signaling (and the nitroso-redox balance) is to activate PKA to maintain basal contraction. The decrease in PLB phosphorylation in NOS1^{-/-} myocytes and increased PLB phosphorylation in NOS1 overexpressing myocytes may also result from altered phosphatase activity. NOS1^{-/-} myocytes do indeed have increased phosphatase activity (187). Although not examined, one can speculate that the increased phosphatase activity could be due to oxidative stress (as discussed above in the kinase/phosphatase activity section). However,

we believe that loss of S-nitrosylation levels is also important. In most cases, oxidation and S-nitrosylation elicit opposing functional affects. As has been shown in other cell types, S-nitrosylation decreases PP2a activity (153). We believe that the same phenomenon will exist in cardiac myocytes in which S-nitrosylation will decrease PP activity, whereas oxidation increases PP activity. Thus, we envisage that the NOS1-mediated nitroso-redox balance increases protein phosphorylation *via* activation of PKA and inhibition of PP. This is what we precisely observed in a model of increased NOS1 expression and activity due to chronic exercise (132). Further work needs to be done to specifically examine how (and if) the nitroso-redox balance cross talks with kinases and phosphatases to modulate cardiac function. Thus, the nitroso-redox regulation of contraction may involve an intricate signaling network for proper modulation of the heart at rest and during various physiological stresses such as FFR and β -AR signaling. The nitroso-redox imbalance mediated by altered NOS1 activity has also been shown to be important in HF (Fig. 5).

Heart Failure

Oxidative stress contributes to the contractile dysfunction of HF, but antioxidant therapy has been ineffective. Since there is a strong connection between ROS and NO (nitroso-redox balance), one must also consider what occurs to NO with HF.

NOS in HF

Examining total NOS activity and NO bioavailability in HF, one finds that there is no difference or slightly decreased when compared with control. However, upon further evaluation, there is in fact a vast difference between HF and control when specifically examining NOS1 and NOS3. In animal models and humans with HF, it has been shown that NOS3 expression and activity are decreased (1, 39); however, NOS1 expression and activity are greatly increased (39, 40). Interestingly, NOS1 translocates from the SR to the plasma membrane and specifically to caveolin-3 after myocardial

FIG. 6. Regulation of heart function by the NOS1-mediated nitroso-redox balance in a healthy heart (left) or imbalance in a failing heart (right). To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/ars

infarction (MI) and with HF. It has been hypothesized that this occurs to surmount the decrease in NOS3. NOS1 and NOS3 signaling are spatially compartmentalized due, in part, to their localization (191). Thus, with NOS3 localized to the caveolae, NO produced *via* NOS3 inhibits β -AR stimulated L-type Ca^{2+} current in a cGMP-dependent manner (as discussed above in the Endothelial NOS section). NOS1 localized to the SR augments SR Ca^{2+} handling to lead to positive inotropy, enhance the FFR, and enhance the functional response to β -AR stimulation *via* the nitroso-redox balance (as discussed above in the Neuronal NOS section). As expected, NOS1 translocation to the plasma membrane in HF now behaves like NOS3 to inhibit the β -AR stimulated Ca^{2+} current (16), which is protective. Nevertheless, there is a detrimental effect of this translocation and there is less NOS1 at the SR (Fig. 5).

Nitroso-redox imbalance in HF

We believe that this NOS1 translocation is akin to knockout NOS1. Ventricular myocytes from NOS1^{-/-} mice exhibit slowed relaxation, a blunted FFR, and decreased response to β -AR stimulation. This is exactly what has been reported in failing myocytes. These data indicate that the translocation of NOS1 with HF results in nitroso-redox imbalance and depressed Ca^{2+} handling and thus contraction (Fig. 6). The significant role of the nitroso-redox imbalance in HF can be observed in NOS1^{-/-} mice with MI. These mice had a larger nitroso-redox imbalance compared with WT mice with MI. Studies have shown that this massive nitroso-redox imbalance resulted in a faster and more severe ventricular remodeling, greater contractile dysfunction, and increased mortality (42, 137). Nitroso-redox imbalance was further investigated in an animal model of HF, the spontaneous hypertensive-heart failure (SHHF) rat (63). Similar to other HF models, this rat exhibited increased xanthine oxidase expression and activity with increased NOS1 expression and translocation. Similar to NOS1^{-/-}, the SHHF rat displayed oxidative stress, which resulted in enhanced RyR oxidation and decreased S-nitrosylation. These RyR post-translational modifications increased RyR diastolic SR Ca^{2+} leak to reduce the SR Ca^{2+} load and blunt the FFR. Inhibition of xanthine oxidase with allopurinol decreased ventricular myocyte superoxide levels. The decrease in oxidative stress with allopurinol resulted in enhanced RyR S-nitrosylation. Since SNO and oxidative modifications can occur on the same cysteines, by decreasing ROS and thiol oxidation, these residues can now be nitrosylated given that NOS1 is still present (which is, although reduced at the SR, in the SHHF rat). Thus, the RyR SNO levels are highly determined by the level of ROS production by xanthine oxidase. With increased RyR S-nitrosylation and decreased oxidation, diastolic SR Ca^{2+} leak was reduced to increase SR Ca^{2+} load to improve the FFR. Interestingly, it has been shown in various skeletal muscle diseases (impaired exercise capacity, dystrophic muscle, environmental heat stroke, and sudden death) that the major contributor is hypernitrosylation of the skeletal muscle RyR isoform (RyR1) (12, 13, 49). Thus, maintaining proper RyR post-translational modifications appears to be vital to prevent pathological conditions. So, in HF, the decrease in RyR SNO due to increased ROS production *via* xanthine oxidase (nitroso-redox imbalance) will result in damaging oxidation to activate RyR and negatively impact contraction.

Correcting nitroso-redox imbalance: current status and perspectives

A previous study has shown that treatment with the xanthine oxidase inhibitor, allopurinol, was ineffective to increase contractility in a canine HF model in the presence of NOS inhibition (134). Thus, the effectiveness of antioxidants is highly dependent on NO bioavailability, suggesting that one needs to correct both NO (increase) and $\text{O}_2^{\bullet-}$ (decrease) levels (*i.e.*, restore nitroso-redox balance). Spin traps are used in the detection and identification of free radicals, including $\text{O}_2^{\bullet-}$, using electron paramagnetic resonance spectroscopy. Nitrones are a unique class of spin traps that produce NO as a byproduct after binding of $\text{O}_2^{\bullet-}$ (164). We recently generated a novel spin trap that is able to permeate cell membranes (2-(2-ethoxy-2-oxoethyl)-2-(ethoxycarbonyl)-3,4-dihydro-2H-pyrrole 1-oxide [EMEPO]) (158). We hypothesized that EMEPO would be more beneficial for contraction and myocyte Ca^{2+} handling than antioxidants since it is both an $\text{O}_2^{\bullet-}$ scavenger and NO donor. We tested the effects of EMEPO on NOS1^{-/-} myocyte Ca^{2+} handling since these myocytes mimic the nitroso-redox imbalance observed in HF. As expected, EMEPO increased NO bioavailability and decreased $\text{O}_2^{\bullet-}$ levels. Correcting the nitroso-redox balance resulted in an increased basal Ca^{2+} transient amplitude and enhanced the rate of relaxation and increased the FFR and functional response to β -AR stimulation. In fact, EMEPO significantly increased contraction greater than antioxidant treatment, which we believe is due to an additive effect of elevated NO and reduced $\text{O}_2^{\bullet-}$ levels. We further showed that the improved Ca^{2+} handling was due to normalizing SR Ca^{2+} release and uptake. Specifically, EMEPO increased RyR activity and increased PLB Serine16 phosphorylation. The change in phosphorylation status suggests that we were able to reverse the harmful shift in the kinase/phosphatase balance in NOS1^{-/-} myocytes. We also observed similar contractile effects of EMEPO on a disease model, in which acute treatment with EMEPO of ventricular myocytes from a canine MI model increased contraction by improving Ca^{2+} handling. Thus, designing a single therapeutic agent that can increase NO and simultaneously decrease $\text{O}_2^{\bullet-}$ (*i.e.*, correct the nitroso-redox imbalance) may be a promising approach for the treatment of the contractile dysfunction and arrhythmias associated with HF.

There have been clinical trials (HeFT and A-HeFT) that have examined the effects of isosorbide dinitrate (NO donor) and hydralazine (vasodilator) in HF patients (35, 156). These studies found that on top of standard care (β -blockers, digoxin, diuretics), the group treated with isosorbide dinitrate and hydralazine had decreased mortality, increased ejection fraction, and improvement in the quality of life compared with the placebo group. Although the molecular mechanisms of these beneficial effects of isosorbide dinitrate and hydralazine are unknown, it was suggested that this treatment increased NO bioavailability. However, we believe that a component of these beneficial effects of isosorbide dinitrate and hydralazine is by correcting the nitroso-redox imbalance and not just by increasing NO levels. Indeed, this was proven to be accurate (48). Once again, using the NOS1^{-/-} mice as a model system of nitroso-redox imbalance, isosorbide dinitrate and hydralazine decreased $\text{O}_2^{\bullet-}$ levels. This resulted in an increase in FFR due to improved Ca^{2+} handling and SR

Ca^{2+} load *via* normalizing RyR activity and PLB phosphorylation. Interestingly, this treatment also increased myofilament Ca^{2+} sensitivity. It is known that sarcomeric proteins are also targets of redox signaling. However, this review is focused on Ca^{2+} handling, and the effects of ROS and reactive nitrogen species (RNS) on sarcomeric proteins has been reviewed elsewhere (151). Acute treatment with isosorbide dinitrate and hydralazine also increased the FFR in SHHF myocytes. Thus, it appears that the beneficial effects of isosorbide dinitrate and hydralazine are, in part, to restore the nitroso-redox balance by improving ventricular myocyte Ca^{2+} handling. While this combination of drugs works, in part, by correcting the nitroso-redox imbalance, cells may not equally uptake these drugs and the nitroso-redox imbalance will remain. We believe that a better approach will be the design of single compounds to correct the nitroso-redox imbalance.

Summary

A final common pathway of HF resulting in contractile dysfunction and arrhythmias is altered myocyte Ca^{2+} handling. It has been found that a major contributor to the altered Ca^{2+} handling in failing myocytes is oxidative stress targeting RyR, kinases, and phosphatases. Unfortunately, antioxidants have been clinically ineffective. We believe that the reason for the lack of effect of antioxidants is that they will only fix one side of the balance. That is, ROS (specifically $\text{O}_2^{\bullet-}$) is tightly connected with NO (nitroso-redox balance), which is reduced at the SR. NO also modulates RyR, kinases, and phosphatases. Recent data now demonstrate that it is important to simultaneously restore both NO and $\text{O}_2^{\bullet-}$ levels to normalize Ca^{2+} handling in the setting of HF. Thus, re-establishing the nitroso-redox balance may be an important therapeutic target for the treatment of HF patients.

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References

1. Agnoletti L, Curello S, Bachetti T, Malacarne F, Gaia G, Comini L, Volterrani M, Bonetti P, Parrinello G, Cadei M, Grigolato PG, and Ferrari R. Serum from patients with severe heart failure downregulates eNOS and is proapoptotic: role of tumor necrosis factor- α . *Circulation* 100: 1983–1991, 1999.
2. Ago T, Kuroda J, Pain J, Fu C, Li H, and Sadoshima J. Upregulation of Nox4 by hypertrophic stimuli promotes apoptosis and mitochondrial dysfunction in cardiac myocytes. *Circ Res* 106: 1253–1264, 2010.
3. Ai X, Curran JW, Shannon TR, Bers DM, and Pogwizd SM. Ca^{2+} /calmodulin-dependent protein kinase modulates cardiac ryanodine receptor phosphorylation and sarcoplasmic reticulum Ca^{2+} leak in heart failure. *Circ Res* 97: 1314–1322, 2005.
4. Amado LC, Saliaris AP, Raju SV, Lehrke S, St John M, Xie J, Stewart G, Fitton T, Minhas KM, Brawn J, and Hare JM. Xanthine oxidase inhibition ameliorates cardiovascular dysfunction in dogs with pacing-induced heart failure. *J Mol Cell Cardiol* 39: 531–536, 2005.
5. Andersson DC, Fauconnier J, Yamada T, Lacampagne A, Zhang SJ, Katz A, and Westerblad H. Mitochondrial production of reactive oxygen species contributes to the beta-adrenergic stimulation of mouse cardiomyocytes. *J Physiol* 589: 1791–1801, 2011.
6. Anh D and Marine JE. Beta blockers as anti-arrhythmic agents. *Heart Fail Rev* 9: 139–147, 2004.
7. Antoons G, Willems R, and Sipido KR. Alternative strategies in arrhythmia therapy: evaluation of Na/Ca exchange as an anti-arrhythmic target. *Pharmacol Ther* 134: 26–42, 2012.
8. Aon MA, Cortassa S, Marban E, and O'Rourke B. Synchronized whole cell oscillations in mitochondrial metabolism triggered by a local release of reactive oxygen species in cardiac myocytes. *J Biol Chem* 278: 44735–44744, 2003.
9. Aon MA, Cortassa S, and O'Rourke B. Redox-optimized ROS balance: a unifying hypothesis. *Biochim Biophys Acta* 1797: 865–877, 2010.
10. Barouch LA, Harrison RW, Skaf MW, Rosas GO, Cappola TP, Kobeissi ZA, Hobai IA, Lemmon CA, Burnett AL, O'Rourke B, Rodriguez ER, Huang PL, Lima JA, Berkowitz DE, and Hare JM. Nitric oxide regulates the heart by spatial confinement of nitric oxide synthase isoforms. *Nature* 416: 337–339, 2002.
11. Beer SM, Taylor ER, Brown SE, Dahm CC, Costa NJ, Runswick MJ, and Murphy MP. Glutaredoxin 2 catalyzes the reversible oxidation and glutathionylation of mitochondrial membrane thiol proteins: implications for mitochondrial redox regulation and antioxidant DEFENSE. *J Biol Chem* 279: 47939–47951, 2004.
12. Bellinger AM, Reiken S, Carlson C, Mongillo M, Liu X, Rothman L, Matecki S, Lacampagne A, and Marks AR. Hypernitrosylated ryanodine receptor calcium release channels are leaky in dystrophic muscle. *Nat Med* 15: 325–330, 2009.
13. Bellinger AM, Reiken S, Dura M, Murphy PW, Deng SX, Landry DW, Nieman D, Lehnart SE, Samaru M, Lacampagne A, and Marks AR. Remodeling of ryanodine receptor complex causes “leaky” channels: a molecular mechanism for decreased exercise capacity. *Proc Natl Acad Sci U S A* 105: 2198–2202, 2008.
14. Bencsik P, Kupai K, Giricz Z, Gorbe A, Huliak I, Furst S, Dux L, Csont T, Jancso G, and Ferdinandy P. Cardiac capsaicin-sensitive sensory nerves regulate myocardial relaxation via S-nitrosylation of SERCA: role of peroxynitrite. *Br J Pharmacol* 153: 488–496, 2008.
15. Bendall JK, Cave AC, Heymes C, Gall N, and Shah AM. Pivotal role of a gp91(phox)-containing NADPH oxidase in angiotensin II-induced cardiac hypertrophy in mice. *Circulation* 105: 293–296, 2002.
16. Bendall JK, Damy T, Ratajczak P, Loyer X, Monceau V, Marty I, Milliez P, Robidel E, Marotte F, Samuel JL, and Heymes C. Role of myocardial neuronal nitric oxide synthase-derived nitric oxide in beta-adrenergic hyporesponsiveness after myocardial infarction-induced heart failure in rat. *Circulation* 110: 2368–2375, 2004.
17. Benhar M, Forrester MT, Hess DT, and Stamler JS. Regulated protein denitrosylation by cytosolic and mitochondrial thioredoxins. *Science* 320: 1050–1054, 2008.
18. Berry CE and Hare JM. Xanthine oxidoreductase and cardiovascular disease: molecular mechanisms and pathophysiological implications. *J Physiol* 555: 589–606, 2004.

19. Bers DM. Cardiac excitation-contraction coupling. *Nature* 415: 198–205, 2002.
20. Bers DM and Ziolo MT. When is cAMP not cAMP? Effects of compartmentalization. *Circ Res* 89: 373–375, 2001.
21. Bjelakovic G, Nikolova D, Gluud LL, Simonetti RG, and Gluud C. Mortality in randomized trials of antioxidant supplements for primary and secondary prevention: systematic review and meta-analysis. *JAMA* 297: 842–857, 2007.
22. Boknik P, Fockenbrock M, Herzig S, Knapp J, Linck B, Luss H, Muller FU, Muller T, Schmitz W, Schroder F, and Neumann J. Protein phosphatase activity is increased in a rat model of long-term beta-adrenergic stimulation. *Naunyn Schmiedebergs Arch Pharmacol* 362: 222–231, 2000.
23. Boraso A and Williams AJ. Modification of the gating of the cardiac sarcoplasmic reticulum Ca^{2+} -release channel by H_2O_2 and dithiothreitol. *Am J Physiol* 267: H1010–H1016, 1994.
24. Boschi-Muller S, Gand A, and Branlant G. The methionine sulfoxide reductases: catalysis and substrate specificities. *Arch Biochem Biophys* 474: 266–273, 2008.
25. Bovo E, Lipsius SL, and Zima AV. Reactive oxygen species contribute to the development of arrhythmogenic Ca^{2+} waves during beta-adrenergic receptor stimulation in rabbit cardiomyocytes. *J Physiol* 590: 3291–3304, 2012.
26. Brahmajothi MV and Campbell DL. Heterogeneous basal expression of nitric oxide synthase and superoxide dismutase isoforms in mammalian heart: implications for mechanisms governing indirect and direct nitric oxide-related effects. *Circ Res* 85: 575–587, 1999.
27. Brodde OE. Beta-adrenoceptor blocker treatment and the cardiac beta-adrenoceptor-G-protein(s)-adenylyl cyclase system in chronic heart failure. *Naunyn Schmiedebergs Arch Pharmacol* 374: 361–372, 2007.
28. Burgoyne JR and Eaton P. Transnitrosylating nitric oxide species directly activate type I protein kinase A, providing a novel adenylyl cyclase-independent cross-talk to beta-adrenergic-like signaling. *J Biol Chem* 284: 29260–29268, 2009.
29. Burgoyne JR, Madhani M, Cuello F, Charles RL, Brennan JP, Schroder E, Browning DD, and Eaton P. Cysteine redox sensor in PKG α enables oxidant-induced activation. *Science* 317: 1393–1397, 2007.
30. Carnicer R, Hale AB, Suffredini S, Liu X, Reilly S, Zhang MH, Surdo NC, Bendall JK, Crabtree MJ, Lim GB, Alp NJ, Channon KM, and Casadei B. Cardiomyocyte GTP cyclohydrolase 1 and tetrahydrobiopterin increase NOS1 activity and accelerate myocardial relaxation. *Circ Res* 111: 718–727, 2012.
31. Chen CA, Wang TY, Varadharaj S, Reyes LA, Hemann C, Talukder MA, Chen YR, Druhan LJ, and Zweier JL. S-glutathionylation uncouples eNOS and regulates its cellular and vascular function. *Nature* 468: 1115–1118, 2010.
32. Chen X, Piacentino V, 3rd, Furukawa S, Goldman B, Margulies KB, and Houser SR. L-type Ca^{2+} channel density and regulation are altered in failing human ventricular myocytes and recover after support with mechanical assist devices. *Circ Res* 91: 517–524, 2002.
33. Cheng J, Valdivia CR, Vaidyanathan R, Balijepalli RC, Ackerman MJ, and Makielski JC. Caveolin-3 suppresses late sodium current by inhibiting nNOS-dependent S-nitrosylation of SCN5A. *J Mol Cell Cardiol* 61: 102–110, 2013.
34. Cingolani HE, Villa-Abrille MC, Cornelli M, Nolly A, Ennis IL, Garcarena C, Suburo AM, Torbidoni V, Correa MV, Camilionde Hurtado MC, and Aiello EA. The positive inotropic effect of angiotensin II: role of endothelin-1 and reactive oxygen species. *Hypertension* 47: 727–734, 2006.
35. Cohn JN, Archibald DG, Ziesche S, Franciosa JA, Harston WE, Tristani FE, Dunkman WB, Jacobs W, Francis GS, Flohr KH, et al. Effect of vasodilator therapy on mortality in chronic congestive heart failure. Results of a Veterans Administration Cooperative Study. *N Engl J Med* 314: 1547–1552, 1986.
36. Curran J, Brown KH, Santiago DJ, Pogwizd S, Bers DM, and Shannon TR. Spontaneous Ca waves in ventricular myocytes from failing hearts depend on Ca^{2+} -calmodulin-dependent protein kinase II. *J Mol Cell Cardiol* 49: 25–32, 2010.
37. Curran J, Tang L, Roof SR, Velmurugan S, Millard A, Shonts S, Wang H, Santiago D, Ahmad U, Perryman M, Bers DM, Mohler PJ, Ziolo MT, and Shannon TR. Nitric oxide-dependent activation of CaMKII increases diastolic sarcoplasmic reticulum calcium release in cardiac myocytes in response to adrenergic stimulation. *PLoS One* 9: e87495, 2014.
38. Cutler MJ, Plummer BN, Wan X, Sun QA, Hess D, Liu H, Deschenes I, Rosenbaum DS, Stamler JS, and Laurita KR. Aberrant S-nitrosylation mediates calcium-triggered ventricular arrhythmia in the intact heart. *Proc Natl Acad Sci U S A* 109: 18186–18191, 2012.
39. Damy T, Ratajczak P, Robidel E, Bendall JK, Oliviero P, Boczkowski J, Ebrahimian T, Marotte F, Samuel JL, and Heymes C. Up-regulation of cardiac nitric oxide synthase 1-derived nitric oxide after myocardial infarction in senescent rats. *FASEB J* 17: 1934–1936, 2003.
40. Damy T, Ratajczak P, Shah AM, Camors E, Marty I, Hasenfuss G, Marotte F, Samuel JL, and Heymes C. Increased neuronal nitric oxide synthase-derived NO production in the failing human heart. *Lancet* 363: 1365–1367, 2004.
41. Davis JP and Tikunova SB. Ca^{2+} exchange with troponin C and cardiac muscle dynamics. *Cardiovasc Res* 77: 619–626, 2008.
42. Dawson D, Lygate CA, Zhang MH, Hulbert K, Neubauer S, and Casadei B. nNOS gene deletion exacerbates pathological left ventricular remodeling and functional deterioration after myocardial infarction. *Circulation* 112: 3729–3737, 2005.
43. De Giusti VC, Correa MV, Villa-Abrille MC, Beltrano C, Yeves AM, de Cingolani GE, Cingolani HE, and Aiello EA. The positive inotropic effect of endothelin-1 is mediated by mitochondrial reactive oxygen species. *Life Sci* 83: 264–271, 2008.
44. De Giusti VC, Garcarena CD, and Aiello EA. Role of reactive oxygen species (ROS) in angiotensin II-induced stimulation of the cardiac $\text{Na}^{+}/\text{HCO}_3^{-}$ cotransport. *J Mol Cell Cardiol* 47: 716–722, 2009.
45. Despa S and Bers DM. Na^{+} transport in the normal and failing heart—remember the balance. *J Mol Cell Cardiol* 61: 2–10, 2013.
46. Dorn GW, 2nd. Apoptotic and non-apoptotic programmed cardiomyocyte death in ventricular remodelling. *Cardiovasc Res* 81: 465–473, 2009.
47. Doughan AK, Harrison DG, and Dikalov SI. Molecular mechanisms of angiotensin II-mediated mitochondrial

- dysfunction: linking mitochondrial oxidative damage and vascular endothelial dysfunction. *Circ Res* 102: 488–496, 2008.
48. Dulce RA, Yiginer O, Gonzalez DR, Goss G, Feng N, Zheng M, and Hare JM. Hydralazine and organic nitrates restore impaired excitation-contraction coupling by reducing calcium leak associated with nitroso-redox imbalance. *J Biol Chem* 288: 6522–6533, 2013.
 49. Durham WJ, Aracena-Parks P, Long C, Rossi AE, Goonasekera SA, Boncompagni S, Galvan DL, Gilman CP, Baker MR, Shirokova N, Protasi F, Dirksen R, and Hamilton SL. RyR1 S-nitrosylation underlies environmental heat stroke and sudden death in Y522S RyR1 knockin mice. *Cell* 133: 53–65, 2008.
 50. Eager KR, Roden LD, and Dulhunty AF. Actions of sulfhydryl reagents on single ryanodine receptor Ca^{2+} -release channels from sheep myocardium. *Am J Physiol* 272: C1908–C1918, 1997.
 51. Ekelund UE, Harrison RW, Shokek O, Thakkar RN, Tunin RS, Senzaki H, Kass DA, Marban E, and Hare JM. Intravenous allopurinol decreases myocardial oxygen consumption and increases mechanical efficiency in dogs with pacing-induced heart failure. *Circ Res* 85: 437–445, 1999.
 52. Erickson JR, Joiner ML, Guan X, Kutschke W, Yang J, Oddis CV, Bartlett RK, Lowe JS, O'Donnell SE, Aykin-Burns N, Zimmerman MC, Zimmerman K, Ham AJ, Weiss RM, Spitz DR, Shea MA, Colbran RJ, Mohler PJ, and Anderson ME. A dynamic pathway for calcium-independent activation of CaMKII by methionine oxidation. *Cell* 133: 462–474, 2008.
 53. Feldman DS, Elton TS, Sun B, Martin MM, and Ziolo MT. Mechanisms of disease: detrimental adrenergic signaling in acute decompensated heart failure. *Nat Clin Pract Cardiovasc Med* 5: 208–218, 2008.
 54. Ferdinandy P, Dhanil H, Ambrus I, Rothery RA, and Schulz R. Peroxynitrite is a major contributor to cytokine-induced myocardial contractile failure. *Circ Res* 87: 241–247, 2000.
 55. Figtree GA, Liu CC, Bibert S, Hamilton EJ, Garcia A, White CN, Chia KK, Cornelius F, Geering K, and Rasmussen HH. Reversible oxidative modification: a key mechanism of Na^{+} - K^{+} pump regulation. *Circ Res* 105: 185–193, 2009.
 56. Fukuda K, Davies SS, Nakajima T, Ong BH, Kupersmidt S, Fessel J, Amarnath V, Anderson ME, Boyden PA, Viswanathan PC, Roberts LJ, 2nd, and Balser JR. Oxidative mediated lipid peroxidation recapitulates proarrhythmic effects on cardiac sodium channels. *Circ Res* 97: 1262–1269, 2005.
 57. Garciarena CD, Caldiz CI, Correa MV, Schinella GR, Mosca SM, Chiappe de Cingolani GE, Cingolani HE, and Ennis IL. $\text{Na}^{+}/\text{H}^{+}$ exchanger-1 inhibitors decrease myocardial superoxide production via direct mitochondrial action. *J Appl Physiol* (1985) 105: 1706–1713, 2008.
 58. Ginsburg KS and Bers DM. Modulation of excitation-contraction coupling by isoproterenol in cardiomyocytes with controlled SR Ca^{2+} load and Ca^{2+} current trigger. *J Physiol* 556: 463–480, 2004.
 59. Goldhaber JI. Free radicals enhance $\text{Na}^{+}/\text{Ca}^{2+}$ exchange in ventricular myocytes. *Am J Physiol* 271: H823–H833, 1996.
 60. Goldhaber JI, Ji S, Lamp ST, and Weiss JN. Effects of exogenous free radicals on electromechanical function and metabolism in isolated rabbit and guinea pig ventricle. Implications for ischemia and reperfusion injury. *J Clin Invest* 83: 1800–1809, 1989.
 61. Goldhaber JI and Liu E. Excitation-contraction coupling in single guinea-pig ventricular myocytes exposed to hydrogen peroxide. *J Physiol* 477: 135–147, 1994.
 62. Gonzalez DR, Beigi F, Treuer AV, and Hare JM. Deficient ryanodine receptor S-nitrosylation increases sarcoplasmic reticulum calcium leak and arrhythmogenesis in cardiomyocytes. *Proc Natl Acad Sci U S A* 104: 20612–20617, 2007.
 63. Gonzalez DR, Treuer AV, Castellanos J, Dulce RA, and Hare JM. Impaired S-nitrosylation of the ryanodine receptor caused by xanthine oxidase activity contributes to calcium leak in heart failure. *J Biol Chem* 285: 28938–28945, 2010.
 64. Greensmith DJ, Eisner DA, and Nirmalan M. The effects of hydrogen peroxide on intracellular calcium handling and contractility in the rat ventricular myocyte. *Cell Calcium* 48: 341–351, 2010.
 65. Guo P, Nishiyama A, Rahman M, Nagai Y, Noma T, Namba T, Ishizawa M, Murakami K, Miyatake A, Kimura S, Mizushige K, Abe Y, Ohmori K, and Kohno M. Contribution of reactive oxygen species to the pathogenesis of left ventricular failure in Dahl salt-sensitive hypertensive rats: effects of angiotensin II blockade. *J Hypertens* 24: 1097–1104, 2006.
 66. Gupta RC, Mishra S, Rastogi S, Imai M, Habib O, and Sabbah HN. Cardiac SR-coupled PP1 activity and expression are increased and inhibitor 1 protein expression is decreased in failing hearts. *Am J Physiol Heart Circ Physiol* 285: H2373–H2381, 2003.
 67. Haizlip KM, Hiranandani N, Biesiadecki BJ, and Janssen PM. Impact of hydroxyl radical-induced injury on calcium handling and myofilament sensitivity in isolated myocardium. *J Appl Physiol* 113: 766–774, 2012.
 68. Hare JM. Nitroso-redox balance in the cardiovascular system. *N Engl J Med* 351: 2112–2114, 2004.
 69. Hare JM, Mangal B, Brown J, Fisher C, Jr., Freudenberger R, Colucci WS, Mann DL, Liu P, Givertz MM, and Schwarz RP. Impact of oxypurinol in patients with symptomatic heart failure. Results of the OPT-CHF study. *J Am Coll Cardiol* 51: 2301–2309, 2008.
 70. Hasenfuss G, Reinecke H, Studer R, Meyer M, Pieske B, Holtz J, Holubarsch C, Posival H, Just H, and Drexler H. Relation between myocardial function and expression of sarcoplasmic reticulum Ca^{2+} -ATPase in failing and nonfailing human myocardium. *Circ Res* 75: 434–442, 1994.
 71. Hasenfuss G, Schillinger W, Lehnart SE, Preuss M, Pieske B, Maier LS, Prestle J, Minami K, and Just H. Relationship between Na^{+} - Ca^{2+} -exchanger protein levels and diastolic function of failing human myocardium. *Circulation* 99: 641–648, 1999.
 72. Hasenfuss G and Teerlink JR. Cardiac inotropes: current agents and future directions. *Eur Heart J* 32: 1838–1845, 2011.
 73. Hassoun PM, Yu FS, Zulueta JJ, White AC, and Lanzillo JJ. Effect of nitric oxide and cell redox status on the regulation of endothelial cell xanthine dehydrogenase. *Am J Physiol* 268: L809–L817, 1995.
 74. Hess DT, Matsumoto A, Kim SO, Marshall HE, and Stamler JS. Protein S-nitrosylation: purview and parameters. *Nat Rev Mol Cell Biol* 6: 150–166, 2005.

75. Heymes C, Bendall JK, Ratajczak P, Cave AC, Samuel JL, Hasenfuss G, and Shah AM. Increased myocardial NADPH oxidase activity in human heart failure. *J Am Coll Cardiol* 41: 2164–2171, 2003.
76. Hill MF, Palace VP, Kaur K, Kumar D, Khaper N, and Singal PK. Reduction in oxidative stress and modulation of heart failure subsequent to myocardial infarction in rats. *Exp Clin Cardiol* 10: 146–153, 2005.
77. Hirst J, King MS, and Pryde KR. The production of reactive oxygen species by complex I. *Biochem Soc Trans* 36: 976–980, 2008.
78. Ho HT, Liu B, Snyder JS, Lou Q, Brundage EA, Velez-Cortes F, Wang H, Ziolo MT, Anderson ME, Sen CK, Wehrens XH, Fedorov VV, Biesiadecki BJ, Hund TJ, and Gyorke S. Ryanodine receptor phosphorylation by oxidized CaMKII contributes to the cardiotoxic effects of cardiac glycosides. *Cardiovasc Res* 101: 165–174, 2014.
79. Houser SR and Margulies KB. Is depressed myocyte contractility centrally involved in heart failure? *Circ Res* 92: 350–358, 2003.
80. Houser SR, Piacentino V, 3rd, and Weisser J. Abnormalities of calcium cycling in the hypertrophied and failing heart. *J Mol Cell Cardiol* 32: 1595–1607, 2000.
81. Huie RE and Padmaja S. The reaction of NO with superoxide. *Free Radic Res Commun* 18: 195–199, 1993.
82. Ichimori K, Fukahori M, Nakazawa H, Okamoto K, and Nishino T. Inhibition of xanthine oxidase and xanthine dehydrogenase by nitric oxide. Nitric oxide converts reduced xanthine-oxidizing enzymes into the desulfo-type inactive form. *J Biol Chem* 274: 7763–7768, 1999.
83. Ide T, Tsutsui H, Kinugawa S, Utsumi H, Kang D, Hattori N, Uchida K, Arimura K, Egashira K, and Takeshita A. Mitochondrial electron transport complex I is a potential source of oxygen free radicals in the failing myocardium. *Circ Res* 85: 357–363, 1999.
84. Ishimura Y, Gao YT, Panda SP, Roman LJ, Masters BS, and Weintraub ST. Detection of nitrous oxide in the neuronal nitric oxide synthase reaction by gas chromatography-mass spectrometry. *Biochem Biophys Res Commun* 338: 543–549, 2005.
85. Janssen PM and Periasamy M. Determinants of frequency-dependent contraction and relaxation of mammalian myocardium. *J Mol Cell Cardiol* 43: 523–531, 2007.
86. Kaludercic N, Carpi A, Nagayama T, Sivakumaran V, Zhu G, Lai EW, Bedja D, De Mario A, Chen K, Gabrielson KL, Lindsey ML, Pacak K, Takimoto E, Shih JC, Kass DA, Di Lisa F, and Paolocci N. Monoamine oxidase B prompts mitochondrial and cardiac dysfunction in pressure overloaded hearts. *Antioxid Redox Signal* 20: 267–280, 2014.
87. Kaludercic N, Takimoto E, Nagayama T, Feng N, Lai EW, Bedja D, Chen K, Gabrielson KL, Blakely RD, Shih JC, Pacak K, Kass DA, Di Lisa F, and Paolocci N. Monoamine oxidase A-mediated enhanced catabolism of norepinephrine contributes to adverse remodeling and pump failure in hearts with pressure overload. *Circ Res* 106: 193–202, 2010.
88. Kassmann M, Hansel A, Leipold E, Birkenbeil J, Lu SQ, Hoshi T, and Heinemann SH. Oxidation of multiple methionine residues impairs rapid sodium channel inactivation. *Pflugers Arch* 456: 1085–1095, 2008.
89. Kawakami M and Okabe E. Superoxide anion radical-triggered Ca^{2+} release from cardiac sarcoplasmic reticulum through ryanodine receptor Ca^{2+} channel. *Mol Pharmacol* 53: 497–503, 1998.
90. Khan SA, Lee K, Minhas KM, Gonzalez DR, Raju SV, Tejani AD, Li D, Berkowitz DE, and Hare JM. Neuronal nitric oxide synthase negatively regulates xanthine oxidoreductase inhibition of cardiac excitation-contraction coupling. *Proc Natl Acad Sci U S A* 101: 15944–15948, 2004.
91. Khan SA, Skaf MW, Harrison RW, Lee K, Minhas KM, Kumar A, Fradley M, Shoukas AA, Berkowitz DE, and Hare JM. Nitric oxide regulation of myocardial contractility and calcium cycling: independent impact of neuronal and endothelial nitric oxide synthases. *Circ Res* 92: 1322–1329, 2003.
92. Kinugawa S, Huang H, Wang Z, Kaminski PM, Wolin MS, and Hintze TH. A defect of neuronal nitric oxide synthase increases xanthine oxidase-derived superoxide anion and attenuates the control of myocardial oxygen consumption by nitric oxide derived from endothelial nitric oxide synthase. *Circ Res* 96: 355–362, 2005.
93. Kohlhaas M, Liu T, Knopp A, Zeller T, Ong MF, Bohm M, O'Rourke B, and Maack C. Elevated cytosolic Na^{+} increases mitochondrial formation of reactive oxygen species in failing cardiac myocytes. *Circulation* 121: 1606–1613, 2010.
94. Kohr MJ, Kaludercic N, Tocchetti CG, Dong Gao W, Kass DA, Janssen PM, Paolocci N, and Ziolo MT. Nitroxyl enhances myocyte Ca^{2+} transients by exclusively targeting SR Ca^{2+} -cycling. *Front Biosci* 2: 614–626, 2010.
95. Kohr MJ, Roof SR, Zweier JL, and Ziolo MT. Modulation of myocardial contraction by peroxynitrite. *Front Physiol* 3: 468, 2012.
96. Kohr MJ, Traynham CJ, Roof SR, Davis JP, and Ziolo MT. cAMP-independent activation of protein kinase A by the peroxynitrite generator SIN-1 elicits positive inotropic effects in cardiomyocytes. *J Mol Cell Cardiol* 48: 645–648, 2010.
97. Kohr MJ, Wang H, Wheeler DG, Velayutham M, Zweier JL, and Ziolo MT. Biphasic effect of SIN-1 is reliant upon cardiomyocyte contractile state. *Free Radic Biol Med* 45: 73–80, 2008.
98. Kohr MJ, Wang H, Wheeler DG, Velayutham M, Zweier JL, and Ziolo MT. Targeting of phospholamban by peroxynitrite decreases $\{\beta\}$ -adrenergic stimulation in cardiomyocytes. *Cardiovasc Res* 77: 353–361, 2008.
99. Kubin AM, Skoumal R, Tavi P, Konyi A, Perjes A, Leskinen H, Ruskoaho H, and Szokodi I. Role of reactive oxygen species in the regulation of cardiac contractility. *J Mol Cell Cardiol* 50: 884–893, 2011.
100. Kuster GM, Lancel S, Zhang J, Communal C, Trucillo MP, Lim CC, Pfister O, Weinberg EO, Cohen RA, Liao R, Siwik DA, and Colucci WS. Redox-mediated reciprocal regulation of SERCA and Na^{+} - Ca^{2+} exchanger contributes to sarcoplasmic reticulum Ca^{2+} depletion in cardiac myocytes. *Free Radic Biol Med* 48: 1182–1187, 2010.
101. Lancel S, Qin F, Lennon SL, Zhang J, Tong X, Mazzini MJ, Kang YJ, Siwik DA, Cohen RA, and Colucci WS. Oxidative posttranslational modifications mediate decreased SERCA activity and myocyte dysfunction in Galphaq-overexpressing mice. *Circ Res* 107: 228–232, 2010.
102. Lancel S, Zhang J, Evangelista A, Trucillo MP, Tong X, Siwik DA, Cohen RA, and Colucci WS. Nitroxyl activates SERCA in cardiac myocytes via glutathiolation of cysteine 674. *Circ Res* 104: 720–723, 2009.

103. Lee CI, Liu X, and Zweier JL. Regulation of xanthine oxidase by nitric oxide and peroxynitrite. *J Biol Chem* 275: 9369–9376, 2000.
104. Li JM, Gall NP, Grieve DJ, Chen M, and Shah AM. Activation of NADPH oxidase during progression of cardiac hypertrophy to failure. *Hypertension* 40: 477–484, 2002.
105. Li SY, Yang X, Ceylan-Isik AF, Du M, Sreejayan N, and Ren J. Cardiac contractile dysfunction in Lep/Lep obesity is accompanied by NADPH oxidase activation, oxidative modification of sarco(endo)plasmic reticulum Ca^{2+} -ATPase and myosin heavy chain isozyme switch. *Diabetologia* 49: 1434–1446, 2006.
106. Liu L, Hausladen A, Zeng M, Que L, Heitman J, and Stamler JS. A metabolic enzyme for S-nitrosothiol conserved from bacteria to humans. *Nature* 410: 490–494, 2001.
107. Liu Y, Huang H, Xia W, Tang Y, Li H, and Huang C. NADPH oxidase inhibition ameliorates cardiac dysfunction in rabbits with heart failure. *Mol Cell Biochem* 343: 143–153, 2010.
108. Lonn E, Bosch J, Yusuf S, Sheridan P, Pogue J, Arnold JM, Ross C, Arnold A, Sleight P, Probstfeld J, and Dagenais GR. Effects of long-term vitamin E supplementation on cardiovascular events and cancer: a randomized controlled trial. *JAMA* 293: 1338–1347, 2005.
109. Luo J, Xuan YT, Gu Y, and Prabhu SD. Prolonged oxidative stress inverts the cardiac force-frequency relation: role of altered calcium handling and myofilament calcium responsiveness. *J Mol Cell Cardiol* 40: 64–75, 2006.
110. MacLennan DH and Kranias EG. Phospholamban: a crucial regulator of cardiac contractility. *Nat Rev Mol Cell Biol* 4: 566–577, 2003.
111. Maier LS and Bers DM. Role of Ca^{2+} /calmodulin-dependent protein kinase (CaMK) in excitation-contraction coupling in the heart. *Cardiovasc Res* 73: 631–640, 2007.
112. Marx SO, Reiken S, Hisamatsu Y, Jayaraman T, Burkhoff D, Rosemblyt N, and Marks AR. PKA phosphorylation dissociates FKBP12.6 from the calcium release channel (ryanodine receptor): defective regulation in failing hearts. *Cell* 101: 365–376, 2000.
113. Mochizuki M, Yano M, Oda T, Tateishi H, Kobayashi S, Yamamoto T, Ikeda Y, Ohkusa T, Ikemoto N, and Matsuzaki M. Scavenging free radicals by low-dose carvedilol prevents redox-dependent Ca^{2+} leak via stabilization of ryanodine receptor in heart failure. *J Am Coll Cardiol* 49: 1722–1732, 2007.
114. Morris TE and Sulakhe PV. Sarcoplasmic reticulum Ca^{2+} -pump dysfunction in rat cardiomyocytes briefly exposed to hydroxyl radicals. *Free Radic Biol Med* 22: 37–47, 1997.
115. Murphy E, Kohr M, Sun J, Nguyen T, and Steenbergen C. S-nitrosylation: a radical way to protect the heart. *J Mol Cell Cardiol* 52: 568–577, 2012.
116. Nasr G and Maurice C. Allopurinol and global left myocardial function in heart failure patients. *J Cardiovasc Dis Res* 1: 191–195, 2010.
117. Naumova AV, Chacko VP, Ouwerkerk R, Stull L, Marban E, and Weiss RG. Xanthine oxidase inhibitors improve energetics and function after infarction in failing mouse hearts. *Am J Physiol Heart Circ Physiol* 290: H837–H843, 2006.
118. Neumann J, Eschenhagen T, Jones LR, Linck B, Schmitz W, Scholz H, and Zimmermann N. Increased expression of cardiac phosphatases in patients with end-stage heart failure. *J Mol Cell Cardiol* 29: 265–272, 1997.
119. O'Connor CM, Gattis WA, Uretsky BF, Adams KF, Jr., McNulty SE, Grossman SH, McKenna WJ, Zannad F, Swedberg K, Gheorghiade M, and Califf RM. Continuous intravenous dobutamine is associated with an increased risk of death in patients with advanced heart failure: insights from the Flolan International Randomized Survival Trial (FIRST). *Am Heart J* 138: 78–86, 1999.
120. Oceandy D, Cartwright EJ, Emerson M, Prehar S, Baudoin FM, Zi M, Alatwi N, Schuh K, Williams JC, Armesilla AL, and Neyses L. Neuronal nitric oxide synthase signaling in the heart is regulated by the sarcolemmal calcium pump 4b. *Circulation* 115: 483–492, 2007.
121. Okabe E, Kuse K, Sekishita T, Suyama N, Tanaka K, and Ito H. The effect of ryanodine on oxygen free radical-induced dysfunction of cardiac sarcoplasmic reticulum. *J Pharmacol Exp Ther* 256: 868–875, 1991.
122. Orogo AM and Gustafsson AB. Cell death in the myocardium: my heart won't go on. *IUBMB Life* 65: 651–656, 2013.
123. Pavlovic D, Hall AR, Kennington EJ, Aughton K, Boguslavskyi A, Fuller W, Despa S, Bers DM, and Shattock MJ. Nitric oxide regulates cardiac intracellular Na^{+} and Ca^{2+} by modulating Na/K ATPase via PKCepsilon and phospholamban-dependent mechanism. *J Mol Cell Cardiol* 61: 164–171, 2013.
124. Piacentino V, 3rd, Weber CR, Chen X, Weisser-Thomas J, Margulies KB, Bers DM, and Houser SR. Cellular basis of abnormal calcium transients of failing human ventricular myocytes. *Circ Res* 92: 651–658, 2003.
125. Pogwizd SM, Qi M, Yuan W, Samarel AM, and Bers DM. Upregulation of $\text{Na}^{+}/\text{Ca}^{2+}$ exchanger expression and function in an arrhythmogenic rabbit model of heart failure. *Circ Res* 85: 1009–1019, 1999.
126. Prosser BL, Ward CW, and Lederer WJ. X-ROS signaling: rapid mechano-chemo transduction in heart. *Science* 333: 1440–1445, 2011.
127. Prysazhna O, Rudyk O, and Eaton P. Single atom substitution in mouse protein kinase G eliminates oxidant sensing to cause hypertension. *Nat Med* 18: 286–290, 2012.
128. Qin F, Simeone M, and Patel R. Inhibition of NADPH oxidase reduces myocardial oxidative stress and apoptosis and improves cardiac function in heart failure after myocardial infarction. *Free Radic Biol Med* 43: 271–281, 2007.
129. Ray R, Murdoch CE, Wang M, Santos CX, Zhang M, Alom-Ruiz S, Anilkumar N, Ouattara A, Cave AC, Walker SJ, Grieve DJ, Charles RL, Eaton P, Brewer AC, and Shah AM. Endothelial Nox4 NADPH oxidase enhances vasodilatation and reduces blood pressure *in vivo*. *Arterioscler Thromb Vasc Biol* 31: 1368–1376, 2011.
130. Reeves JP, Bailey CA, and Hale CC. Redox modification of sodium-calcium exchange activity in cardiac sarcolemmal vesicles. *J Biol Chem* 261: 4948–4955, 1986.
131. Roof SR, Shannon TR, Janssen PM, and Ziolo MT. Effects of increased systolic Ca^{2+} and phospholamban phosphorylation during beta-adrenergic stimulation on Ca^{2+} transient kinetics in cardiac myocytes. *Am J Physiol Heart Circ Physiol* 301: H1570–H1578, 2011.
132. Roof SR, Tang L, Ostler JE, Periasamy M, Gyorke S, Billman GE, and Ziolo MT. Neuronal nitric oxide synthase is indispensable for the cardiac adaptive effects of exercise. *Basic Res Cardiol* 108: 332, 2013.
133. Rossman EI, Petre RE, Chaudhary KW, Piacentino V, 3rd, Janssen PM, Gaughan JP, Houser SR, and Margulies KB.

- Abnormal frequency-dependent responses represent the pathophysiologic signature of contractile failure in human myocardium. *J Mol Cell Cardiol* 36: 33–42, 2004.
134. Saavedra WF, Paolocci N, St John ME, Skaf MW, Stewart GC, Xie JS, Harrison RW, Zeichner J, Mudrick D, Marban E, Kass DA, and Hare JM. Imbalance between xanthine oxidase and nitric oxide synthase signaling pathways underlies mechanoenergetic uncoupling in the failing heart. *Circ Res* 90: 297–304, 2002.
 135. Saliaris AP, Amado LC, Minhas KM, Schuleri KH, Lehrke S, St John M, Fitton T, Barreiro C, Berry C, Zheng M, Kozielski K, Eneboe V, Brawn J, and Hare JM. Chronic allopurinol administration ameliorates maladaptive alterations in Ca^{2+} cycling proteins and beta-adrenergic hyporesponsiveness in heart failure. *Am J Physiol Heart Circ Physiol* 292: H1328–H1335, 2007.
 136. Sande JB, Sjaastad I, Hoen IB, Bokenes J, Tonnessen T, Holt E, Lunde PK, and Christensen G. Reduced level of serine(16) phosphorylated phospholamban in the failing rat myocardium: a major contributor to reduced SERCA2 activity. *Cardiovasc Res* 53: 382–391, 2002.
 137. Saraiva RM, Minhas KM, Raju SV, Barouch LA, Pitz E, Schuleri KH, Vandegaer K, Li D, and Hare JM. Deficiency of neuronal nitric oxide synthase increases mortality and cardiac remodeling after myocardial infarction: role of nitroso-redox equilibrium. *Circulation* 112: 3415–3422, 2005.
 138. Sartoretto JL, Kalwa H, Pluth MD, Lippard SJ, and Michel T. Hydrogen peroxide differentially modulates cardiac myocyte nitric oxide synthesis. *Proc Natl Acad Sci U S A* 108: 15792–15797, 2011.
 139. Scherer NM and Deamer DW. Oxidative stress impairs the function of sarcoplasmic reticulum by oxidation of sulfhydryl groups in the Ca^{2+} -ATPase. *Arch Biochem Biophys* 246: 589–601, 1986.
 140. Schmidt HH, Hofmann H, Schindler U, Shutenko ZS, Cunningham DD, and Feelisch M. No .NO from NO synthase. *Proc Natl Acad Sci U S A* 93: 14492–14497, 1996.
 141. Schwinger RH, Munch G, Bolck B, Karczewski P, Krause EG, and Erdmann E. Reduced Ca^{2+} -sensitivity of SERCA 2a in failing human myocardium due to reduced serin-16 phospholamban phosphorylation. *J Mol Cell Cardiol* 31: 479–491, 1999.
 142. Sears CE, Bryant SM, Ashley EA, Lygate CA, Rakovic S, Wallis HL, Neubauer S, Terrar DA, and Casadei B. Cardiac neuronal nitric oxide synthase isoform regulates myocardial contraction and calcium handling. *Circ Res* 92: e52–e59, 2003.
 143. Sessa WC. The nitric oxide synthase family of proteins. *J Vasc Res* 31: 131–143, 1994.
 144. Shan J, Kushnir A, Betzenhauser MJ, Reiken S, Li J, Lehnart SE, Lindegger N, Mongillo M, Mohler PJ, and Marks AR. Phosphorylation of the ryanodine receptor mediates the cardiac fight or flight response in mice. *J Clin Invest* 120: 4388–4398, 2010.
 145. Shannon TR, Ginsburg KS, and Bers DM. Potentiation of fractional sarcoplasmic reticulum calcium release by total and free intra-sarcoplasmic reticulum calcium concentration. *Biophys J* 78: 334–343, 2000.
 146. Shannon TR, Pogwizd SM, and Bers DM. Elevated sarcoplasmic reticulum Ca^{2+} leak in intact ventricular myocytes from rabbits in heart failure. *Circ Res* 93: 592–594, 2003.
 147. Shi Z, Chen AD, Xu Y, Chen Q, Gao XY, Wang W, and Zhu GQ. Long-term administration of tempol attenuates postinfarct ventricular dysfunction and sympathetic activity in rats. *Pflugers Arch* 458: 247–257, 2009.
 148. Shiomi T, Tsutsui H, Matsusaka H, Murakami K, Hayashidani S, Ikeuchi M, Wen J, Kubota T, Utsumi H, and Takeshita A. Overexpression of glutathione peroxidase prevents left ventricular remodeling and failure after myocardial infarction in mice. *Circulation* 109: 544–549, 2004.
 149. Sivakumaran V, Stanley BA, Tocchetti CG, Ballin JD, Caceres V, Zhou L, Keceli G, Rainer PP, Lee DI, Huke S, Ziolo MT, Kranias EG, Toscano JP, Wilson GM, O'Rourke B, Kass DA, Mahaney JE, and Paolocci N. HNO enhances SERCA2a activity and cardiomyocyte function by promoting redox-dependent phospholamban oligomerization. *Antioxid Redox Signal* 19: 1185–1197, 2013.
 150. Stanley BA, Sivakumaran V, Shi S, McDonald I, Lloyd D, Watson WH, Aon MA, and Paolocci N. Thioredoxin reductase-2 is essential for keeping low levels of H_2O_2 emission from isolated heart mitochondria. *J Biol Chem* 286: 33669–33677, 2011.
 151. Steinberg SF. Oxidative stress and sarcomeric proteins. *Circ Res* 112: 393–405, 2013.
 152. Sun J, Steenbergen C, and Murphy E. S-nitrosylation: NO-related redox signaling to protect against oxidative stress. *Antioxid Redox Signal* 8: 1693–1705, 2006.
 153. Switzer CH, Glynn SA, Cheng RY, Ridnour LA, Green JE, Ambs S, and Wink DA. S-nitrosylation of EGFR and Src activates an oncogenic signaling network in human basal-like breast cancer. *Mol Cancer Res* 10: 1203–1215, 2012.
 154. Takimoto E, Champion HC, Li M, Ren S, Rodriguez ER, Tavazzi B, Lazzarino G, Paolocci N, Gabrielson KL, Wang Y, and Kass DA. Oxidant stress from nitric oxide synthase-3 uncoupling stimulates cardiac pathologic remodeling from chronic pressure load. *J Clin Invest* 115: 1221–1231, 2005.
 155. Tang H, Viola HM, Filipovska A, and Hool LC. $\text{Ca}_v1.2$ calcium channel is glutathionylated during oxidative stress in guinea pig and ischemic human heart. *Free Radic Biol Med* 51: 1501–1511, 2011.
 156. Taylor AL, Ziesche S, Yancy C, Carson P, D'Agostino R, Jr., Ferdinand K, Taylor M, Adams K, Sabolinski M, Worcel M, and Cohn JN. Combination of isosorbide dinitrate and hydralazine in blacks with heart failure. *N Engl J Med* 351: 2049–2057, 2004.
 157. Terentyev D, Gyorke I, Belevych AE, Terentyeva R, Sridhar A, Nishijima Y, de Blanco EC, Khanna S, Sen CK, Cardounel AJ, Carnes CA, and Gyorke S. Redox modification of ryanodine receptors contributes to sarcoplasmic reticulum Ca^{2+} leak in chronic heart failure. *Circ Res* 103: 1466–1472, 2008.
 158. Traynham CJ, Roof SR, Wang H, Prosak RA, Tang L, Viatchenko-Karpinski S, Ho HT, Racoma IO, Catalano DJ, Huang X, Han Y, Kim SU, Gyorke S, Billman GE, Villamena FA, and Ziolo MT. Diesterified nitron rescues nitroso-redox levels and increases myocyte contraction via increased SR Ca^{2+} handling. *PLoS One* 7: e52005, 2012.
 159. Turrens JF. Mitochondrial formation of reactive oxygen species. *J Physiol* 552: 335–344, 2003.
 160. Ueda K, Valdivia C, Medeiros-Domingo A, Tester DJ, Vatta M, Farrugia G, Ackerman MJ, and Makielski JC.

- Syntrophin mutation associated with long QT syndrome through activation of the nNOS-SCN5A macromolecular complex. *Proc Natl Acad Sci U S A* 105: 9355–9360, 2008.
161. van Berlo JH, Maillet M, and Molkenin JD. Signaling effectors underlying pathologic growth and remodeling of the heart. *J Clin Invest* 123: 37–45, 2013.
 162. van Oort RJ, McCauley MD, Dixit SS, Pereira L, Yang Y, Respress JL, Wang Q, De Almeida AC, Skapura DG, Anderson ME, Bers DM, and Wehrens XH. Ryanodine receptor phosphorylation by calcium/calmodulin-dependent protein kinase II promotes life-threatening ventricular arrhythmias in mice with heart failure. *Circulation* 122: 2669–2679, 2010.
 163. Vignais PV. The superoxide-generating NADPH oxidase: structural aspects and activation mechanism. *Cell Mol Life Sci* 59: 1428–1459, 2002.
 164. Villamena FA. Superoxide radical anion adduct of 5,5-dimethyl-1-pyrroline N-oxide. 5. Thermodynamics and kinetics of unimolecular decomposition. *J Phys Chem A* 113: 6398–6403, 2009.
 165. Viola HM and Hool LC. Qo site of mitochondrial complex III is the source of increased superoxide after transient exposure to hydrogen peroxide. *J Mol Cell Cardiol* 49: 875–885, 2010.
 166. Wagner S, Ruff HM, Weber SL, Bellmann S, Sowa T, Schulte T, Anderson ME, Grandi E, Bers DM, Backs J, Belardinelli L, and Maier LS. Reactive oxygen species-activated Ca/calmodulin kinase II is required for late I(Na) augmentation leading to cellular Na and Ca overload. *Circ Res* 108: 555–565, 2011.
 167. Wang H, Kohr MJ, Traynham CJ, Wheeler DG, Janssen PM, and Ziolo MT. Neuronal nitric oxide synthase signaling within cardiac myocytes targets phospholamban. *Am J Physiol Cell Physiol* 294: C1566–C1575, 2008.
 168. Wang H, Kohr MJ, Traynham CJ, and Ziolo MT. Phosphodiesterase 5 restricts NOS3/Soluble guanylate cyclase signaling to L-type Ca²⁺ current in cardiac myocytes. *J Mol Cell Cardiol* 47: 304–314, 2009.
 169. Wang H, Kohr MJ, Wheeler DG, and Ziolo MT. Endothelial nitric oxide synthase decreases beta-adrenergic responsiveness via inhibition of the L-type Ca²⁺ current. *Am J Physiol Heart Circ Physiol* 294: H1473–H1480, 2008.
 170. Wang H, Viatchenko-Karpinski S, Sun J, Gyorke I, Benkusky NA, Kohr MJ, Valdivia HH, Murphy E, Gyorke S, and Ziolo MT. Regulation of myocyte contraction via neuronal nitric oxide synthase: role of ryanodine receptor S-nitrosylation. *J Physiol* 588: 2905–2917, 2010.
 171. Wang W, Fang H, Groom L, Cheng A, Zhang W, Liu J, Wang X, Li K, Han P, Zheng M, Yin J, Wang W, Mattson MP, Kao JP, Lakatta EG, Sheu SS, Ouyang K, Chen J, Dirksen RT, and Cheng H. Superoxide flashes in single mitochondria. *Cell* 134: 279–290, 2008.
 172. Warburton DE, Haykowsky MJ, Quinney HA, Blackmore D, Teo KK, and Humen DP. Myocardial response to incremental exercise in endurance-trained athletes: influence of heart rate, contractility and the Frank-Starling effect. *Exp Physiol* 87: 613–622, 2002.
 173. Wehrens XH, Lehnart SE, Reiken SR, and Marks AR. Ca²⁺/calmodulin-dependent protein kinase II phosphorylation regulates the cardiac ryanodine receptor. *Circ Res* 94: e61–e70, 2004.
 174. Weisser-Thomas J, Piacentino V, 3rd, Gaughan JP, Margulies K, and Houser SR. Calcium entry via Na/Ca exchange during the action potential directly contributes to contraction of failing human ventricular myocytes. *Cardiovasc Res* 57: 974–985, 2003.
 175. White CN, Hamilton EJ, Garcia A, Wang D, Chia KK, Figtree GA, and Rasmussen HH. Opposing effects of coupled and uncoupled NOS activity on the Na⁺-K⁺ pump in cardiac myocytes. *Am J Physiol Cell Physiol* 294: C572–C578, 2008.
 176. Williams JC, Armesilla AL, Mohamed TM, Hagarty CL, McIntyre FH, Schomburg S, Zaki AO, Oceandy D, Cartwright EJ, Buch MH, Emerson M, and Neyses L. The sarcolemmal calcium pump, alpha-1 syntrophin, and neuronal nitric-oxide synthase are parts of a macromolecular protein complex. *J Biol Chem* 281: 23341–23348, 2006.
 177. Winterbourn CC and Hampton MB. Thiol chemistry and specificity in redox signaling. *Free Radic Biol Med* 45: 549–561, 2008.
 178. Woo HA, Jeong W, Chang TS, Park KJ, Park SJ, Yang JS, and Rhee SG. Reduction of cysteine sulfinic acid by sulfiredoxin is specific to 2-cys peroxiredoxins. *J Biol Chem* 280: 3125–3128, 2005.
 179. Xiao L, Pimentel DR, Wang J, Singh K, Colucci WS, and Sawyer DB. Role of reactive oxygen species and NAD(P)H oxidase in alpha(1)-adrenoceptor signaling in adult rat cardiac myocytes. *Am J Physiol Cell Physiol* 282: C926–C934, 2002.
 180. Xie H and Zhu PH. Biphasic modulation of ryanodine receptors by sulfhydryl oxidation in rat ventricular myocytes. *Biophys J* 91: 2882–2891, 2006.
 181. Xu KY, Zweier JL, and Becker LC. Hydroxyl radical inhibits sarcoplasmic reticulum Ca(2+)-ATPase function by direct attack on the ATP binding site. *Circ Res* 80: 76–81, 1997.
 182. Xu L, Eu JP, Meissner G, and Stamler JS. Activation of the cardiac calcium release channel (ryanodine receptor) by poly-S-nitrosylation. *Science* 279: 234–237, 1998.
 183. Xu Q, Dalic A, Fang L, Kiriazis H, Ritchie RH, Sim K, Gao XM, Drummond G, Sarwar M, Zhang YY, Dart AM, and Du XJ. Myocardial oxidative stress contributes to transgenic beta(2)-adrenoceptor activation-induced cardiomyopathy and heart failure. *Br J Pharmacol* 162: 1012–1028, 2011.
 184. Yan Y, Liu J, Wei C, Li K, Xie W, Wang Y, and Cheng H. Bidirectional regulation of Ca²⁺ sparks by mitochondria-derived reactive oxygen species in cardiac myocytes. *Cardiovasc Res* 77: 432–441, 2008.
 185. Zhang H, Makarewich CA, Kubo H, Wang W, Duran JM, Li Y, Berretta RM, Koch WJ, Chen X, Gao E, Valdivia HH, and Houser SR. Hyperphosphorylation of the cardiac ryanodine receptor at serine 2808 is not involved in cardiac dysfunction after myocardial infarction. *Circ Res* 110: 831–840, 2012.
 186. Zhang YH, Dingle L, Hall R, and Casadei B. The role of nitric oxide and reactive oxygen species in the positive inotropic response to mechanical stretch in the mammalian myocardium. *Biochim Biophys Acta* 1787: 811–817, 2009.
 187. Zhang YH, Zhang MH, Sears CE, Emanuel K, Redwood C, El-Armouche A, Kranias EG, and Casadei B. Reduced phospholamban phosphorylation is associated with impaired relaxation in left ventricular myocytes from neu-

- ronal NO synthase-deficient mice. *Circ Res* 102: 242–249, 2008.
188. Zhou L, Burnett AL, Huang PL, Becker LC, Kuppusamy P, Kass DA, Kevin Donahue J, Proud D, Sham JS, Dawson TM, and Xu KY. Lack of nitric oxide synthase depresses ion transporting enzyme function in cardiac muscle. *Biochem Biophys Res Commun* 294: 1030–1035, 2002.
 189. Zima AV, Copello JA, and Blatter LA. Effects of cytosolic NADH/NAD(+) levels on sarcoplasmic reticulum Ca(2+) release in permeabilized rat ventricular myocytes. *J Physiol* 555: 727–741, 2004.
 190. Ziolo MT. The fork in the nitric oxide road: cyclic GMP or nitrosylation? *Nitric Oxide* 18: 153–156, 2008.
 191. Ziolo MT and Bers DM. The real estate of NOS signaling: location, location, location. *Circ Res* 92: 1279–1281, 2003.
 192. Ziolo MT, Kohr MJ, and Wang H. Nitric oxide signaling and the regulation of myocardial function. *J Mol Cell Cardiol* 45: 625–632, 2008.
 193. Ziolo MT, Maier LS, Piacentino V, 3rd, Bossuyt J, Houser SR, and Bers DM. Myocyte nitric oxide synthase 2 contributes to blunted beta-adrenergic response in failing human hearts by decreasing Ca²⁺ transients. *Circulation* 109: 1886–1891, 2004.
 194. Zorov DB, Juhaszova M, and Sollott SJ. Mitochondrial ROS-induced ROS release: an update and review. *Biochim Biophys Acta* 1757: 509–517, 2006.

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Abbreviations Used

β-AR = beta-adrenergic
 [Ca]_i = cytosolic Ca²⁺
 CaMKII = Ca²⁺-calmodulin-dependent protein kinase
 cAMP = cyclic AMP
 cGMP = cyclic GMP
 ECC = excitation-contraction coupling
 EMEPO = 2-(2-ethoxy-2-oxoethyl)-2-(ethoxycarbonyl)-3,4-dihydro-2H-pyrrole 1-oxide
 epi = epinephrine
 FFR = force-frequency response
 H₂O₂ = hydrogen peroxide
 HF = heart failure
 LTCC = L-type Ca²⁺ channel
 MAO = monoamine oxidase
 MI = myocardial infarction
 NADPH = nicotinamide adenine dinucleotide phosphate
 Nav1.5 = Na⁺ channel
 NCX = Na⁺/Ca²⁺ exchanger
 NKA = Na⁺/K⁺ ATPase
 norepi = norepinephrine
 NO = nitric oxide
 NOS = NO synthase
 NOS1 = neuronal NOS
 NOS3 = endothelial NOS
 NOX = NADPH oxidase
 O₂^{•−} = superoxide anion radical
 •OH = hydroxyl radical
 ONOO = peroxynitrite
 PLB = phospholamban
 PMCA4 = plasma membrane Ca²⁺-ATPase
 PKA = protein kinase A
 ROS = reactive oxygen species
 RNS = reactive nitrogen species
 RyR = ryanodine receptor
 SERCA = SR Ca²⁺-ATPase
 SHHF = spontaneous hypertensive-heart failure
 SOD = superoxide dismutase
 SNAP = S-nitroso-N-acetyl-DL-penicillamine
 SNO = S-nitrosothiol
 SR = sarcoplasmic reticulum
 TnC = troponin C
 TnI = troponin I
 XO = xanthine oxidase