Heart Failure, Oxidative Stress, and Ion Channel Modulation

The balance of reactive oxygen species (ROS) and nitric oxide, the cell redox state, appears to be important in the mechanisms of heart failure. This balance has significant impact on calcium-handling proteins, affecting excitation-contraction coupling. Both ROS and nitric oxide appear to be elevated in heart failure and are accompanied by significant impairments in the number and function of calcium-handling proteins. These proteins contain sulphydryl groups or disulfide linkages involving cysteine residues, making them susceptible to the action of oxidizing-reducing agents and nitrosylation, thereby altering their properties. Initial increases in nitric oxide may be an adaptive response to myocardial dysfunction, elevated cytokines, and increases in ROS, while a further increase in nitric oxide and overwhelming ROS can be damaging. Abundant nitric oxide and ROS can cause formation of peroxynitrite, a strong oxidant, or nitric oxide can activate alternate pathways aiding the ROS, causing impaired calcium handling contributing to contractile dysfunction. (CHF. 2002;8:148–155) ©2002 CHF, Inc.

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Congestive heart failure is a leading cause of morbidity in the adult population. It accounts for a significant proportion of hospital admissions and health care dollars spent. In the last few decades, significant advances have been made in understanding the pathophysiology and molecular basis leading to the failing heart. The balance of reactive oxygen species (ROS) and nitric oxide (NO), the cell redox state, appears to be important in the pathogenesis of heart failure. This balance has a significant impact on ion channel function. In this review, we will summarize the evidence pointing toward the role of ROS and NO in modulation of myocardial ion channels in heart failure.

Calcium plays an important role in excitation-contraction (EC) coupling. Calcium levels are set by ion-handling proteins (Figure 1). Changes in cellular calcium levels have been implicated in the mechanical dysfunction and arrhythmogenesis observed in congestive heart failure.1,2 During the action potential plateau, sarcoplasmic reticulum-gated L-type calcium channels are activated. These channels are the targets of all clinically used calcium channel blockers. A rise in calcium, mediated predominantly by these channels initiates the release of Ca2+ from the sarcoplasmic reticulum (SR), mainly via the calcium-gated ryanodine receptors (RyR2). Also, in the SR are the inositol 1,4,5-triphosphate receptors that can allow Ca2+ release, but their role in EC coupling is less clear. The free Ca2+ in the cytosol increases from nanomolar to micromolar levels, binding to various intracellular proteins, including the thin filament protein troponin C, and activating the contractile machinery. In diastole, calcium declines so as to reverse this process. The SR Ca2+ adenosine triphosphatase (Ca2+-ATPase), sarcolemmal Na+-Ca2+ exchanger (NCX), sarcolemmal Ca2+-ATPase, and mitochondrial Ca2+ uniporter are involved, the first two contributing most.

All these calcium-handling proteins contain sulphydryl groups or disulfide linkages involving cysteine residues that are susceptible to the action of oxidizing-reducing agents and available for nitrosylation, thereby altering channel properties. The balance of NO and ROS determines the extent of these processes. NO has emerged as a ubiquitous messenger involved in various pathophysiologic processes. In cardiomyocytes, NO synthases (NOS) convert L-
arginine to L-citrulline to produce NO. Constitutive NOS is regulated by a calcium-calmodulin interaction that positively responds to increasing cytosolic calcium, as occurs with muscarinic cholinergic receptor activation. The production of NO by NOS is a coupled redox reaction that, when uncoupled, can involve generation of superoxide radical ($O_2^-$). $O_2^-$ from this or other sources can combine with NO to form peroxynitrite, can oxidize other species directly, or can be scavenged by superoxide dismutase. NO can combine with glutathione to form S-nitrosogluthathione, in the presence of $O_2^-$ or as free NO. S-nitrosogluthathione can yield NO in the presence of certain copper-containing catalysts. NO acts on various cellular processes either directly or by a cyclic guanosine monophosphate (cGMP)-mediated mechanism. In the failing myocardium, the levels of NO are higher, most likely because of the increased expression and activity of inducible NOS$^{4,5}$ mediated via inflammatory cytokines$^6$ or increased β-adrenergic stimulation.$^7$ The effect of NO on the EC coupling has been studied and shows a biphasic response. Physiologic levels of NO augment cardiac contractility and higher NO levels cause depressed myocardial function. NO might be a physiologically important modulator of EC coupling in the absence of a disease state.

Superoxide, hydroxyl radicals 'OH, hydrogen peroxide H$_2$O$_2$, and singlet oxygen comprise some of the ROS mediating the oxidative stress in cardiac cells (Figure 2). Several metabolic pathways are known to produce ROS, including uncoupled NOS, the xanthine/xanthine oxidase system, the cyclooxygenase pathway of arachidonic acid metabolism, and the mitochondrial electron transport system.$^8$ Enzymes such as superoxide dismutase, glutathione peroxidase, and catalase keep the toxic effects of these ROS in check in physiologic conditions. In chronic heart failure, the levels of ROS increase$^{9-11}$ and myocardial antioxidant reserve decreases.$^{12-14}$ This leads to a cellular redox imbalance in favor of ROS, which exerts toxic effects on myocardial contractility.$^{15}$ Effects of ROS on cellular processes and structure are largely deleterious, with reports of cell death by apoptosis, impaired cellular respiration, structural damage to proteins, and impaired contractility.

The interplay of ROS and NO in the setting of heart failure is largely unexplored. There are a few possibilities that can be hypothesized, based on what is known about other disease processes. Atherosclerosis appears
to be accompanied by an imbalance of ROS in excess of NO. It has been suggested that ROS can induce NOS activity and increase NOS protein levels.\textsuperscript{16,17} Also, NO may limit ROS-mediated damage in the cells. Nevertheless, beyond a certain limit, NO itself can suppress myocardial contractility. Elevated ROS and NO lead to peroxynitrite and peroxynitrous acid formation. Peroxynitrite, in turn, can nitrosylate thiol groups of proteins. There are controversial data regarding the effect of peroxynitrite on myocardial contractility, with both augmentation\textsuperscript{18,19} and depression\textsuperscript{20,21} reported. Peroxynitrous acid, the protonated form, is very toxic, causing protein nitration and oxidation. The temporal, spatial, and concentration factors influencing the point at which NO assumes a pathologic role remain to be investigated.

EC coupling and myocardial contractility are controlled by various ion transport mechanisms and calcium-handling proteins in the myocytes. Redox balance is affected in congestive heart failure, and this balance appears to play an important role in impairment of myocardial contractility, as in chronic heart failure. This review explores the known ion channel alterations affecting calcium handling that are mediated by NO and ROS.

**L-Type Calcium Channels**

L-type or dihydropyridine-sensitive calcium channels initiate the calcium flux and contractile cascade in myocytes in response to the cardiac action potential. They are multimeric proteins consisting of up to five subunits: \( \alpha_1 \), \( \alpha_2 \), \( \beta \), \( \gamma \), and \( \delta \). The \( \alpha_1 \) subunit contains the Ca\(^{2+}\)-conducting pore and is the binding site for calcium channel blockers. The rest of the subunits play regulatory roles. The \( \gamma \) subunit is expressed only in skeletal muscle cells. The amount of L-type Ca\(^{2+}\) current after depolarization is modulated via cyclic adenosine monophosphate (cAMP)-dependent protein kinase A phosphorylation of subunits such as \( \alpha_{1C} \),\textsuperscript{22} the cardiac-specific \( \alpha_1 \) subunit, and the \( \beta \) subunit.\textsuperscript{23} c AMP increases calcium influx by increasing the open probability and the number of functional channels.\textsuperscript{24-26} Increased cAMP in heart failure plays an important role in regulation of the number and function of L-type Ca\(^{2+}\) channels.

The effect of NO on L-type Ca\(^{2+}\) channels has been studied in different experimental models with variable results, depending on the animal species and the concentration of NO. The effect is probably biphasic, with low levels increasing and high levels decreasing L-type Ca\(^{2+}\) current. Both cGMP-dependent and -independent mechanisms of NO action have been implicated (Figure 3). NO causes activation of soluble guanylyl cyclase,\textsuperscript{27} increasing cellular cGMP levels. The cGMP-dependent mechanisms include: 1) cGMP-activated protein kinase G phosphorylation of the Ca\(^{2+}\) channel, decreasing the Ca\(^{2+}\) current, especially after initial stimulation by cAMP;\textsuperscript{28-30} 2) cGMP-activated type 2 phosphodiesterase-mediated decrease in cAMP concentration, decreasing L-type Ca\(^{2+}\) current. This effect is mainly to antagonize the actions of elevated cAMP and there is little NO effect, if any, on basal contractile state;\textsuperscript{7,26,31} and 3) GMP-inhibited type 3 phosphodiesterase-mediated increased cAMP concentration and potentiation of adrenergic actions. Some variability in experimental results may be that these cGMP pathways vary among species.\textsuperscript{28,29,32} NO can have a direct, cGMP-independent action to decrease L-type Ca\(^{2+}\) current by S-nitrosylation of intrinsic thiols sensitive to redox modulation.\textsuperscript{10,20}

ROS have been shown to decrease L-type Ca\(^{2+}\) current in cardiomyocytes.\textsuperscript{33,34} The mechanism is unclear but may involve modulation of redox-sensitive thiols on the Ca\(^{2+}\) channel,\textsuperscript{18} an action opposite that of NO.

Multiple studies have looked at the number and function of the L-type channel in heart failure by using Northern blot analysis, measuring dihydropyridine binding, or recording whole-cell calcium current density in various models of heart failure. The findings are inconsistent, with increases,\textsuperscript{35} no change,\textsuperscript{36-38} and decreases\textsuperscript{39,40} in the number and function reported. In human material, a decrease in the expression of L-type calcium channels was seen,\textsuperscript{39} but the total Ca\(^{2+}\) current remained unchanged.\textsuperscript{37,38} Single-channel recordings of L-type channels from failing human hearts have revealed an increased activity, probably because of an increased phosphorylation state.\textsuperscript{41} This may compensate in part for the decrease in the number of channels and may explain the constant Ca\(^{2+}\) current.

The net result of NO and ROS modulation of L-type Ca\(^{2+}\) current during the pathophysiologic development of heart failure remains unresolved. It is conceivable that they play a role in reversible myocardial depression and \( \beta \)-adrenergic desensitization mediated by cGMP antagonism of elevated cAMP levels. In pacing-induced heart failure, initially there is a decrease in \( \beta \)-adrenergic receptor-augmented L-type Ca\(^{2+}\) current, followed by absolute loss of steady-state L-type Ca\(^{2+}\) current and channel protein. This might be explained by an adaptive cGMP-mediated response of NO to the hyperadrenergic state in compensated heart failure that becomes maladaptive with increasing levels of NO and ROS, leading to decompensation.

**SR Ca\(^{2+}\) Release Channels**

RyR2 and the inositol 1,4,5-triphosphate receptors constitute the cardiac SR Ca\(^{2+}\) release channels. RyR2 are 50 times more abundant than inositol...
1,4,5-triphosphate receptors and play the major role in Ca\(^{2+}\) regulation and EC coupling in cardiac tissue. Therefore, we limit our discussion to these SR release channels. They are tetrameric structures, and their activity is modulated by luminal Ca\(^{2+}\) concentration in the SR; a decrease in SR Ca\(^{2+}\) leads to a decrease in open probability. A defect in Ca\(^{2+}\) release can lead to impaired ventricular contractility or cause after-depolarization-mediated triggering arrhythmias.

NO has mixed effects on RyR2. NO decreases Ca\(^{2+}\) release from cardiac SR and reduces the steady-state activity or open probability of the RyR2. On the other hand, poly-S-nitrosylation of RyR2, as might occur with higher NO concentrations, reversibly activates these channels.

A cellular net oxidative state has been postulated to affect RyR2, and the RyR2 response to changes in cytosolic Ca\(^{2+}\) depends on the redox state. Initially, oxidation of sulfhydryl residues leads to an increase in channel activity as well as the sensitivity to activation by calcium. Continued oxidation leads to irreversible loss of channel function, however.

In sheep heart, RyR2 Ca\(^{2+}\) release channels, H\(_2\)O\(_2\) increases the probability of being in open state, with no effects on channel conductance. This is thought to be a direct effect on the gating mechanism mediated via alteration of the redox state of cysteines on the channel.

Reports linking heart failure and RyR are unclear. In failing hearts, RyR2 levels may be downregulated. On the other hand, some authors have not found significant change. Altered function of RyR has been suggested by some authors including impaired gating ability. Impaired EC coupling and the amount of SR Ca\(^{2+}\) released for a given trigger, the gain, have been noted in some animal models of cardiomyopathy. Perhaps as a result of inhibited coupling among the Ca\(^{2+}\) release channels. In failing hearts, there is hyperphosphorylation of the channel by protein kinase A, causing increased sensitivity to Ca\(^{2+}\)-dependent activation. Excessive stimulation of this pathway may lead to depletion of SR Ca\(^{2+}\) stores and/or aberrant release of SR Ca\(^{2+}\) during diastole, triggering arrhythmias.

The temporal sequence of and net response to increasing ROS and NO are unclear. Early, increased NO levels and poly-S-nitrosylation of RyR may reversibly activate these channels, an adaptive response to the impaired contractility. On the other hand, high NO inactivates the RyR channels, thus contributing to the impaired EC coupling and EC gain. ROS, when present in increasing amounts, leads to early Ca\(^{2+}\) responsiveness and finally, irreversible inactivation, contributing to the contractile dysfunction. The transition may be related to the switch from compensated to decompensated heart failure.

**SR Ca\(^{2+}\)-ATPase**

SR Ca\(^{2+}\)-ATPase and sarcosomal NCX pump constitute the major mechanisms of cytosolic Ca\(^{2+}\) removal during the relaxation phase. The SR Ca\(^{2+}\)-ATPase transports from the cytosol to the SR two calcium ions per molecule of phosphate hydrolyzed. It is crucial for cytosolic Ca\(^{2+}\) removal and for availability of sufficient Ca\(^{2+}\) for systolic release. The pump is regulated by phospholamban. Phosphorylation of phospholamban by Ca\(^{2+}\)/calmodulin-dependent protein kinase and protein kinase A results in stimulation of SR Ca\(^{2+}\)-ATPase.

Phosphorylation by these enzymes results in an increased affinity for Ca\(^{2+}\) and a shift of K\(_{1/2}\) for the calcium dependence of calcium transport.

Recently, a possible new isomform of the neuronal type NOS was localized on cardiac SR, and evidence for a molecular interaction between NO and cardiac SR Ca\(^{2+}\)-ATPase was provided. NO can down-regulate the SR Ca\(^{2+}\)-ATPase directly. The mechanism underlying the regulatory effect of NO on SR Ca\(^{2+}\) active transport is not clear.

Also, inhibition of SR Ca\(^{2+}\)-ATPase is observed with ROS. Hydroxyl radicals denature the SR Ca\(^{2+}\)-ATPase by directly attacking the ATP binding site, since ATP at the active site protects against OH\(^{-}\)-induced loss of SR Ca\(^{2+}\). Oxidative modulation of a cysteine residue near the ATP binding site was postulated as the probable target.
NO may regulate SR Ca\textsuperscript{2+}-ATPase by reacting with regulatory thiols of these proteins. It is possible that SR Ca\textsuperscript{2+}-ATPase regulation by phospholamban is altered with the redox state, but there have been no studies of this. ROS can damage the channel by denaturation. In failing hearts, there is a defect in communication between ATP-producing and -consuming sites, leading to impaired energetics.\textsuperscript{67} This may further aggravate the effect of ROS on SR Ca\textsuperscript{2+}-ATPase.

**Sarcolemmal NCX**

The NCX constitutes the dominant mechanism for Ca\textsuperscript{2+} efflux from myocytes. It extrudes one calcium ion for the entry of three Na\textsuperscript{+} ions, using the energy of the Na\textsuperscript{+} gradient produced by the Na\textsuperscript{+} pump. The protein is a tetramer linked with disulfide bonds, consistent with the possibility of redox modulation.\textsuperscript{68,69}

There are no studies looking at the role of NO in modulation of NCX activity in mammalian heart tissue. With the presence of sulphydryl groups and the known ATP-dependent stimulation of activity, it is possible that NO plays some role in the regulation of the transporter, directly or indirectly. The evidence is lacking, however. In one study, oxygen-derived free radicals arising during cardiac reperfusion stimulated NCX activity.\textsuperscript{70} Others have reported contradictory results. This could be because of the different ROS involved, the different conformations of the exchanger at the time of application, or the alteration of regulators, such as Ca\textsuperscript{2+} and Na\textsuperscript{+}, phosphorylation, and phos-

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**Table. The Effects of NO, ROS, and the Net Effect of the Redox State in Heart Failure on Major Ca\textsuperscript{2+} Handling Proteins**

<table>
<thead>
<tr>
<th><strong>NITRIC OXIDE</strong></th>
<th><strong>REACTIVE OXYGEN SPECIES</strong></th>
<th><strong>HEART FAILURE</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart failure</td>
<td>Increased cellular as well as extracellular NO\textsuperscript{5-7}</td>
<td>Increased ROS\textsuperscript{6-11}</td>
</tr>
<tr>
<td>L-type Ca\textsuperscript{2+} channels</td>
<td>- Biphasic response\textsuperscript{7}</td>
<td>Decreased L-type current\textsuperscript{52,55}</td>
</tr>
<tr>
<td></td>
<td>- Increased\textsuperscript{17} decreased L-type current\textsuperscript{26-30}</td>
<td>Increased\textsuperscript{54}/decreased\textsuperscript{27-30}/no change\textsuperscript{32,36} in number and function</td>
</tr>
<tr>
<td>SR Ca\textsuperscript{2+}-release channels</td>
<td>Increased\textsuperscript{49}/decreased\textsuperscript{44} activity</td>
<td>Increase in P\textsubscript{a} followed by irreversible loss of channel function\textsuperscript{45,46}</td>
</tr>
<tr>
<td></td>
<td>Decreased number\textsuperscript{60}</td>
<td>Decreased activity\textsuperscript{61,62}</td>
</tr>
<tr>
<td>SR Ca\textsuperscript{2+}-ATPase</td>
<td>Decreased activity\textsuperscript{64}</td>
<td>Downregulation\textsuperscript{84}</td>
</tr>
<tr>
<td>Sarcolemmal Na\textsuperscript{+}-Ca\textsuperscript{2+} exchange pump</td>
<td>Increased activity\textsuperscript{71}</td>
<td>Upregulation\textsuperscript{70-72}</td>
</tr>
</tbody>
</table>

NO=nitric oxide; ROS=reactive oxygen species; SR=sarcoplasmic reticulum; RyR2=ryanodine receptors; Ca\textsuperscript{2+}-ATPase=Ca\textsuperscript{2+} adenosine triphosphatase
Summary

Studies concerning the redox modulation of calcium handling and EC coupling in heart failure are incomplete. Little consideration has been given to redox modulation of gene transcription. Contributing to experimental variability are species differences, model differences, the duration of failure, and the state of compensation. Still, it is possible to discern some trends (Table).

In heart failure, NO appears to act in a biphasic manner. Early on, NO activates a second messenger cascade to regulate EC coupling, improving the contractile response. NO does this by increasing L-type calcium current and by stimulating the release of SR Ca²⁺. A prolonged, higher NO level results in pathologic effects that include a decrease in L-type Ca²⁺ current, down-regulation of SR Ca²⁺-ATPases, and inactivation of RyR2 calcium channels, leading to impaired EC coupling.

Increased ROS in heart failure appear to decrease L-type calcium current, irreversibly inactivate RyR2 channels, denature SR Ca²⁺-ATPases, and lead to enhanced Ca²⁺ efflux from the cells via the sarcoplasmic NCX pump. The net result is low SR Ca²⁺, lower contractility, and less efficient energy utilization.

Both ROS and NO are elevated in heart failure. Initial increases in NO may be an adaptive response to myocardial dysfunction, elevated cytokines, and increased ROS, while further increases in NO and overwhelming ROS can be damaging. Elevations in NO and ROS contribute to formation of peroxynitrite, a strong oxidant, and high NO levels can activate alternate signaling pathways, increasing the ROS-induced impairment of calcium handling. A complete understanding of the relationship of the redox state and heart failure is lacking, although it is clear that NO and ROS have major effects on calcium-handling proteins and that heart failure alters the redox state. This should present a fruitful area for future research. Many drugs shown to affect mortality in heart failure alter the redox state, and a better understanding of the importance of redox balance in heart failure should open the possibility of drug therapy to inhibit the development and progression of heart failure.

REFERENCES

channel by sarcoplasmic reticulum luminal Ca\(^{2+}\). \textit{Biophys J.} 1998;75:2302-2312.


