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S-nitrosylation of cardiac ion channels

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Abstract

Nitric oxide exerts ubiquitous signaling via post-translational modification of cysteine residues, a reaction termed *S*-nitrosylation. Important substrates of *S*-nitrosylation that influence cardiac function include receptors, enzymes, ion channels, transcription factors, and structural proteins. Cardiac ion channels subserving excitation-contraction coupling are potentially regulated by *S*-nitrosylation. Specificity is achieved in part by spatial co-localization of ion channels with nitric oxide synthases (NOS), enzymatic sources of NO in biologic systems, and by coupling of NOS activity to localized calcium/second messenger concentrations. Ion channels regulate cardiac excitability and contractility in millisecond timescales raising the possibility that NO-related species modulate heart function on a beat-to beat basis. This review focuses on recent advances in understanding of NO regulation of the cardiac action potential, and of the calcium release channel ryanodine receptor, which is crucial for the generation of force. *S*-nitroso (SNO) signaling is disrupted in pathological states in which the redox state of the cell is dysregulated, including ischemia, heart failure, and atrial fibrillation.

Keywords

Heart; nitrosylation; nitric oxide; ryanodine receptor; oxidation

Introduction

S-nitrosylation has emerged as an important and ubiquitous post-translational modification system, participating in cellular signaling throughout the phylogeny. *S*-nitrosylation is involved in cell signaling in bacteria[1], plants[2] and animals. In mammalian cells, *S*-nitrosylation affects crucial and diverse processes including neurotransmission and memory, gene expression, cellular excitability, mitochondrial energetics, control of blood flow, and respiration[3, 4].

S-nitrosylation consists of the addition of a NO group to the thiol moiety of a cysteine residue. This covalent reaction can occur upon formal oxidation of NO or by trans-nitrosylation in which the NO group is exchanged between donor and acceptor thiol. The growing list of proteins that are targets for *S*-nitrosylated is vast [5, 6]. Cysteine residues susceptible to S-nitrosylation are often located in an acid-base motif in which acidic and basic aminoacids surround the Cys-site of modification in primary and/or quaternary

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structure; an acid (D, E) in the +1 position appears to particularly important[3, 7]. In addition, hydrophobic motifs[8] containing signature aromatic residues within a 6-7 angstrom sphere of influence have been shown to influence NO targeting [3, 9].

S-nitrosylation and denitrosyaltion reactions

Molecular mechanisms of *S*-nitrosylation and denitrosylation in biological systems are considered below.

Acidification of nitrite—At low pH, nitrite undergoes protonation to HNO_2 (nitrous acid), which disproportionates to N_2O_3 (dinitrogen trioxide), a nitrosating agent. The reaction may be of relevance under ischemic conditions.

Trans-nitros(yl)ation—The transnitrosation reaction comprises the transfer of a NO^+ group between two thiol groups (or between transition metal and thiol) The transnitrosation reaction involves nucleophilic attack of a thiolate on the nitrogen of SNO group. Acid-base catalysis is favored by the presence of basic amino acids surrounding the cysteine, which may lower thiol pKa ($R\text{-}S^-$).

Metal-catalyzed—Accumulating evidence indicates that transition metals catalyze *S*-nitrosylation reactions *in vivo* [10, 11]. These studies point to the possibility that enzymatic formation of SNO is an important and relevant signaling mechanism. Examples of metal catalyzed *S*-nitrosylation include the NO and nitrite dependent auto-*S*-nitrosylation of hemoglobin [12, 1314] and the *S*-nitrosylation of glutathione by ceruloplasmin.

Denitrosylation—A number of enzymes have been shown to break down *S*-nitrosothiols; several meet stringent genetic criteria of being physiologically relevant. *S*-nitrosoglutathione reductase (GSNOR) and the thioredoxins (Trx1 and Trx2) have emerged as key denitrosylases. GSNOR metabolizes GSNO to GSNHOH, albeit a mixture of products will form depending on conditions, including GSSG, N-hydroxysulfenimide hydroxylamine, and NH_3[15]. The activity of GSNOR is critically dependent on glutathione and NADH. Mice deficient in GSNOR are protected from myocardial infarction[16] but more susceptible to septic shock[17]. On the other hand, thioredoxin/thioredoxin reductase directly denitrosylates proteins (and potentially also low molecular weight *S*-nitrosothiols)[18-21]. Reduced thioredoxin operates with thioredoxin reductase and NADPH. Deficiency of thioredoxin/TrxR promotes Fas induced apoptosis. Xanthine oxidoreductase[22], an enzyme involved in purine catabolism, is also able to degrade *S*-nitroglutathione and *S*-nitrosocysteine[23, 24], but the physiological relevance of this activity has not been demonstrated.

Detection of S-nitrosylated proteins

The detection and quantification of S-nitrosylated proteins is technically challenging, in part due to the labile nature of the S-NO bond. In this section, we discuss the methods that have been most commonly used to evaluate the S-nitrosylation of ion channels.

Biotin switch protocol—The most widely used method for detecting and quantifying S-nitrosylated proteins is the “biotin switch” technique. This protocol developed by Jaffrey and colleagues[25, 26], involves the formal reduction of S-nitrosylated proteins with ascorbate followed by tagging of the nascent thiols with a biotinylated compound N-[6-(biotinamido)hexyl]-3’-(2’-pyridyldithio)propionamide (biotin –HPDP), a thiol-specific biotinylating agent. If performed appropriately, the reaction is highly specific for S-nitrosylated *vs.* alternatively oxidized proteins[27].

Photolysis-Chemiluminiscence—This assay involves homolysis of the SNO bond with UV light, followed by detection of NO by chemiluminescense[28]. Assays are performed in the presence and absence of $HgCl_2$, which eliminates SNOs but not other NO complexes. ,This assay provided the first measurements of endogenous ion channel S-nitrosylation (RyR1 and RyR2)[29-31], and remains a gold standard for SNO quantification, with limit of detection being in the range of pmol (the limit of detection for NO in the nitric oxide analyzer[32]). However, the assay is not available in many laboratories and is not amenable to preoteomic analysis.

Anti-S-nitrosocysteine antibodies—Antibodies have been raised against the SNO moiety in proteins (monoclonal and polyclonal), and are commercially available. These antibodies have demonstrated their usefulness in immunohistochemical studies of SNO-proteins[33]. In addition, anti-SNO antibodies have used been in Western blots of S-nitrosylated RyR1 [34-37] and RyR2[38].

Impact of S-nitrosylation on the excitation-contraction coupling process

In the cardiac myocyte, contraction and relaxation is governed by excitation-contraction coupling[39]. During membrane depolarization triggered by voltage-gated Na^+ channels, L-type Ca^{2+} channels (which are also voltage-sensitive) become activated. This inward Ca^{2+} current triggers massive release of stored Ca^{2+} from the sarcoplasmic reticulum (SR), through the Ca^{2+} release channel ryanodine receptor 2, a process termed calcium-induced calcium release. The SR Ca^{2+} release leads to Ca^{2+} binding to troponin C in the myofilaments and activation of ATPase activity of myosin, generating contraction. Relaxation of the muscle starts as Ca^{2+} dissociates from troponin C and is transported back into the SR by the pump SERCA2a and is extruded from the cell via a Na^+/Ca^{2+} exchanger. NO appears to play a significant role in Ca^{2+} handling, principally by *S*-nitrosylation, as discussed below.

Voltage-gated sodium channels—In atrial and ventricular myocytes, the voltage-gated sodium (Nav) channels are responsible for fast depolarization. The main Nav expressed in the mammalian myocardium is encoded by the gene *SCN5A*. It consists of a single pore-forming α subunit of approximately 260 kDa. Nav channels typically inactivate very quickly at the more positive potentials. However, in cardiac myocytes, a late current is observed. Persistence of this current has been shown to be dependent on *S*-nitrosylation of Nav1.5. Nitrosylation is coupled to NOS1 activity[40]. Conversely, *S*-nitrosylation of sodium channels in sensory neurons blocks the current[41-43].

The sodium channel is rich in cysteine: 33-40 Cys are present in various isoforms from brain, skeletal muscle, and heart. A full *S*-nitrosylation motif of the acid base type is found in the Nav of sensory neurons, but only partial in the heart [42]. It is possible that beside *S*-nitrosylation, cardiac and non cardiac Nav channels also could be regulated by cysteine oxidation[44, 45], perhaps under oxidative stress conditions.

Interestingly, a mutation in syntrophin associated with a form of the long QT syndrome (LQT3)[46], results in aberrant S-nitrosylation of the sodium channel. Syntrophin, a member of the dystrophin-associated proteins normally serves as scaffold protein for NOS1 and the plasmalemmal calcium pump PMCA, an interaction that results in inhibition of NO production. Syntrophin mutation results in a disruption of the PMCA-NOS1 complex and favors interaction of NOS1 with the Na channel (subunit SCN5A). Release of PMCA increases NOS1 activity promoting *S*-nitrosylation of SCN5a and thereby increasing late Na^+ currents. The impact of this process on the action potential duration was not

investigated by the authors, but mutations of the sodium channel that lead to similar increased late currents prolong the duration of the action potential[47].

L-type calcium channel—The L-type calcium channels consist of a major α1 subunit, which is pore-forming, and β, α_2/δ and γ auxiliary subunits. In the heart the gene $Ca_v1.2$ encodes for the α_{1C}, which subserves sensitivity to dihydropyridines[48].

It has been reported that the L-type calcium channel can be activated by the NO donors SIN-1, nitrosocysteine (CysNO), and GSNO, in a manner that is independent of cGMP[49]. On the other hand, nitrosothiols apparently inhibit L-type channels that are expressed in heterologous systems[50, 51]. The mechanism of inhibition involves both reduction of open probability of single channels and a reduction of conductance. Although *S*-nitrosylation was not confirmed in these studies, the effects of *S*-nitrosothiols were independent of cGMP.

Recently, Sun and colleagues reported that the α1-subunit of the channel is constitutively *S*-nitrosylated in the mouse heart [52]. They found that nitrosylation increases in ischemia and is associated with inhibition of the channel. Preconditioning of hearts with GSNO likewise increased the degree of nitrosylation[53]. In addition, GSNO reduced the damage after reperfusion and was associated with decreased Ca^{2+} influx through L-type calcium channels. Collectively, these data support the view that *S*-nitrosylation serves as a mechanism of cardioprotection.

In patients with atrial fibrillation (AF), it has been reported that the α_{1C} subunit of the channel is hyper-nitrosylated, and an inverse relationship between nitrosylation and total glutathione cell content was observed [54]. Hypernitrosylation of the channel may therefore contribute to the disease. The number and specific cysteine residues of L-type calcium channel that are *S*-nitrosylated remain unknown.

The L-type channel is also regulated by the cGMP-PKG pathway, probably through phosphorylation. This leads to decreased Ca^{2+} influx. L-type channel inhibition that is seen following stimulation of adrenergic receptors is mediated by NOS3[55], but when NOS1 is over expressed in the mouse heart, it may be responsible for the cGMP-mediated inhibition[56]. Reduced influx through the L-type would lead to a decreased load in the sarcoplasmic reticulum, reducing the amplitude of the Ca^{2+} transients. NO derived from both NOS3 and NOS1 may therefore serve as a protective mechanism to avoid Ca^{2+} overload after ischemia-reperfusion.

Voltage-gated potassium channels—Cardiac voltage-gated potassium channels determine the resting membrane potential and the duration of the cardiac action potential. Several channels participate, including the rapidly activating and inactivating transient outward current (I_{to}), the ultrarapid (I_{Kur}), rapid (I_{Kr}) and slow (I_{Ks}) components of the delayed rectifier and the inward rectifier (I_{K1})[48].

The delayed rectifier K^+ current is one of the major components that determine the timing of repolarization of cardiac myocytes. The delayed rectifier K^+ current consists of two components: the rapidly activating (I_{Kr}) and the slowly activating component (I_{Ks}).

It has been reported that *S*-nitrosylation increases I_{Ks} in a manner dependent on NOS3 in guinea pig cardiomyocytes. This activation resulted in a shortening of the action potential duration[57, 58]. Cysteine 445 has been recently identified as the site *S*-nitrosylated in the pore-forming subunit KCNQ1 [59]. Interestingly, this cysteine is located within a consensus motif for nitrosylation (Lys,Arg,His)-Cys-(Asp,Glu), and mutation of the acid in the +1 position prevented *S*-nitrosylation. *S*-nitrosylation was also dependent on calmodulin, a

situation analogous to that described for the RyR[3]. Also, in guinea pig cardiomyocytes, the overexpression of CAPON (also known as *NOS1AP*), a binding protein for NOS1, resulted in increased I_{Ks}[60]. This effect, along with a reduction in $I_{Ca,L,}$ shortened action potential duration by 30%. NOS1 presumably translocated to the sarcolemma, although it was not confirmed if these effects were due to *S*-nitrosylation of the channels or cGMP-dependent events. The NOS1 effect on I_{Ks} was modest, probably due to co-existing regulation by NOS3.

In the atria, KV1.5 generates the ultrarapid component of the delayed rectifier (I_{Kur}), determining the duration and membrane potential of the plateau phase. *S*-nitrosylation inhibits this current[61]. A mechanism has been proposed based on three-dimensional modeling: nitrosylated Cys331 and Cys346 (which are located in the voltage-sensor region of the channel) are suggested to be stabilized by Ile262 and Arg342, effecting a conformational change.

Further, in the atria, KV4.3 which generates the transient outward K^+ current (I_{to}) is also *S*-nitrosylated, although this has not been shown to change the gating properties[62].

HERG channels (I_{Kr}) are reportedly inhibited by NO in a cGMP independent manner when expressed in *X. laevis* oocytes, although *S*-nitrosylation was not directly evaluated[63].

Calcium release channel ryanodine receptor

Overview—The ryanodine receptor (RyR1 in skeletal muscle, RyR2 in the heart and RyR3 in brain) is a massive channel located in the membrane of the sarcoplasmic reticulum. In cardiac myocytes, RyR2 mediates SR Ca^{2+} release in response to the trans-sarcolemmal Ca^{2+} influx through L-type calcium channels, following membrane depolarization (calcium-induced calcium release). The RyR functions as a tetramer of 560 kDa subunits, and has a conductivity of around 150 pS for Ca^{2+}. The channel is endogenously inhibited by Mg^{2+} and activated by Ca^{2+}. The cardiac isoform RyR2 displays a sigmoid response to activating Ca^{2+} (Figure 3), whereas RyR1 displays a bell-shaped response, being inhibited by higher concentrations of cytosolic Ca^{2+}. Additionally, the Ca^{2+} -dependent activity of the channel is modulated by Mg^{2+} and ATP[64]. Mg^{2+} inhibits the channel activity by competing with Ca^{2+} for high affinity binding sites. Redox modifications of RyR alter the effects of both Ca^{2+} and Mg^{2+}.

The activity of the channel can be modified by NO donors and molecules that affect the redox state of cysteine residues. Canine heart RyR and rabbit skeletal muscle RyR possess 85-100 Cys residues, about half as many of which are reactive thiols, that is, they can be *S*-nitrosylated, *S*-glutathionylated or oxidized to disulfide bonds.

In the case of RyR1, Cys3635 (which resides within a hydrophobic motif for S-nitrosylation), mediates regulation by NO[65]. NO nitrosylates Cys3635 and thereby displaces calcium-calmodulin[34], activating the channel. A further dependence of S-nitrosylation on calmodulin has been observed previously[29] and rationalized by a hydrophobic requirement for the NO effect. The corresponding cysteine in the RyR2 (rabbit heart) Cys3602 is also part of a putative hydrophobic site for S-nitrosylation, but is apparently not nitrosylated by NO. Instead, RyR2 is nitrosylated and activated by GSNO[30].

For RyR, S-nitrosylation is principally activating, although very high supraphysiological concentrations of NO or other NO donors inhibit RyR1 and RyR2 *in vitro*[66]. More generally, the effects of NO may depend on the nature and concentration of the NO donor[67], the redox state of the channel and the PO_2. Studies performed in lipid bilayers have shown increase in open probability (*P*o) as well as a decrease. Generally, GSNO activates the

channel independently of PO_2 and redox state of the channel. Suko and colleagues showed that NOC-7 (1-hydroxy-2-oxo-3-(N-methyl-3-aminopropyl)-3 methyl-1-triazine) 0.1-0.3 mM and nitrosocysteine increased the P_O while nitrosocysteine (4 mM) decrease it[68]. Hart *et. al.* found that S-nitroso-N-acetylpenicillamine (SNAP) at 10 μM increased the P_O of the channel, while SNAP at higher concentration (1 mM) produced inactivation of the channel[69]. Nitroxyl anion, a reduced congener of NO (NO^-), has been shown to activate the channel by cysteine oxidation[70-72].

RyR may exist in different conformations depending on its redox state[73] (Figure 3). The redox state of the channel is critical to RyR1 activity and its subsequent modulation by NO. Oxidants increase the activity and reducing agents inhibit it (GSH). High pO_2 increases the open probability and a physiologic pO_2 decreases it. At low (physiological) PO_2, NO activates RyR1 through S-nitrosylation, whereas this effect is abrogated at high PO_2. Given the intracellular concentrations of glutathione (~mM), it is possible that S-nitrosoglutathione acts as an intermediate or the direct species that nitrosylates the channel. In heart failure, the RyR is oxidized excessively[74, 75] .

Both the cardiac and skeletal RyR are endogenously S-nitrosylated *in vivo*[29, 31]. In both cardiac and skeletal muscle, RyR nitrosylation is coupled to NOS1 activity[76]. In cardiac muscle, RyR2 co-localizes with NOS1 (Figure 1). NOS1 deficiency but not NOS3 deficiency impairs RyR2 nitrosylation indicating that NOS1 is the primary source of NO [76]. However, S-nitrosylation of RyR2 in response to physiological stretch may be mediated by NOS3[77]. Hypernitrosylation of RyR1 has been causally linked to pathologies of skeletal muscle, like malignant hyperthermia[37] , muscular dystrophy[36] and muscular fatigue[35].

A number of ROS containing enzymes are found in or at the SR. Xanthine oxidoreducase co-precipitates with NOS1[78]. Additionally, an NADH oxidase[79]and possibly an NADPH oxidase are found in the SR[80] (Figure 1), altering RyR activity. Redox homeostasis, which becomes disturbed in pathological conditions like heart failure results in oxidation of RyR2 thiols, which may in turn impair *S*-nitrosylation.

Allosteric effectors—FK506-binding protein (FKBP12) is a small (12 kDa) protein that coordinates RyR gating. Removal of FKBP12 with the immunosuppressants FK506 or rapamycin increase the channel *P*o and promotes the emergence of subconductance states. In skeletal muscle triads, RyR1 *S*-nitrosylation with GSNO or NOR-3 leads to a four-fold increase in the K_d for FKBP12[34]. By contrast, oxidation with GSSG has no effect.

In cardiac SR vesicles, the oxidizing agents H_2O_2 and dioxide reduced the binding between RyR2 and FKBP12.6, the cardiac isoform of FKBP12. Use of a cysteine-null mutant FKBP12.6 did not prevent the redox-sensitive changes in binding. Interestingly, in a canine model of heart failure, RyR2 exists in an oxidized state (assessed by the content of free-cisterns) when FKBP12.6 binding is reduced[75]. Antioxidant treatment restores free cysteine content and FKP12.6 binding. The phosphorylation and nitrosylation states of the channel, which also affect FKP12.6 binding, were not assessed.

In vitro experiments using sarcoplasmic reticulum vesicles have shown that S-nitrosylation of RyR2 is enhanced at activating pCa values (around pCa 5) [31, 81, 82]. In contrast, the reaction is inhibited by high Mg^{2+}, which promotes channel closure (at low free Ca^{2+})[31]. This may have important physiological consequences: *S*-nitrosylation may enhance CICR under in conditions of high activity, increasing Ca^{2+} release in systole (high free Ca^{2+}), but not in diastole (low free Ca^{2+} but high Mg^{2+}). This is in contrast to the effect of oxidizing agents, which promote activity at low Ca^{2+} or high Mg^{2+} [83] .

Nitrosylation vs. glutathionylation—S-nitrosylation and glutathionylation have been shown to occur in RyR1 and RyR2 [34, 80, 84]. However, it is nitrosylation not glutathionylation that has been causally linked to physiological RyR activity. Skeletal muscle disorders exhibit increased nitrosylation that displaces FKBP12 from the complex. Both RyR1 and RyR2 show increased oxidation in disease but nature of the coincident oxidative modifications is not known.

Clinical Relevance

Since S-nitrosylation signaling is involved in multiple physiological processes, it is expected that aberrant nitrosylation could result in undesired behavior of the proteins involved. Consistent with this idea, arrhythmias and heart failure may be frequently associated with altered cellular redox state, which will tend to disrupt cellular S-nitrosylation and altered S-nitrosylation of specific ion channels or affiliate proteins may be relevant in the examples reviewed below.

Atrial Fibrillation—Atrial fibrillation (AF) is the most common sustained arrhythmia observed in adults. In addition, it is a common complication of cardiac surgery. Among the electrophysiological alterations associated with AF, there is a decrease in I_{Ca}, which shortens the action potential and descreases atrial contractility[85, 86].

A role for oxidative/nitrosative stress has recently been appreciated to contribute to this condition[86]. NO production has been shown to be reduced in a porcine model of AF[87]. NO blocks I_{Kur} both by S-nitrosylation and the cGMP pathway, accelerating repolarization. It has been hypothesized that a reduction in NO bioavailability (as is observed in experimental models of AF) would reduce the tonic inhibition of I_{Kur}, shortening the plateu phase of the AP. This remains to be tested in both experimental settings of AF and in human samples. Interestingly, and consistent with these ideas, the use of an nitrosylaing agent, sodium nitroprusside, has been tested for the prevention of AF after cardiac surgey[88] with encouraging results. Whether this treatment resulted in S-nitrosylation or other redox modification of atrial ion channels is unknown.

Long QT syndrome—Long QT syndrome is a defect in cardiac repolarization identified by prolongation of the QT interval in the EKG. It is characterized by malignant ventricular arrhythmias and syncopal episodes. The syndrome has two main forms, acquired and congenital. Acquired long QT syndrome is often associated with heart failure and with the drugs such as antiarrhythmics, antidepressants, and phenothiazines. Heritable long QT is associated with mutations in genes that encode for ion channels (channelopathies)[89].

A mutation in syntrophin associated with a form of the long QT syndrome (LQT3)[46], results in aberrant S-nitrosylation of the sodium channel. Syntrophin mutation results in disruption of the PMCA-nNOS1 complex, which keeps NOS quiescent, and favors interaction of NOS1 with the Na channel (subunit SCN5A), promoting *S*-nitrosylation of SCN5a and thereby increasing late Na currents.

NOS1AP is a gene that encodes for nitric oxide synthase 1 adaptor protein, also known as CAPON (*ca*rboxy-terminal *P*DZ ligand of *n*NOS)[90], a protein that regulates NOS1 subcellular location. A genome-wide study originally identified *NOS1AP* variants (single nucleotides polymorphisms, SNPs) with variations in the QT interval in a population of European origin[91]. Latter studies confirmed this observation and showed the association of *NOS1AP* SNPs with long QT[92]. Furthermore, some SNPs were associated with increased risk of cardiac sudden death[93], and one particular SNP was associated with an increase in QT induced by the calcium channel blocker verapamil [94]. The use of calcium channel blockers (verapamil and diltiazem) in carriers of a *NOS1AP* polymorphism was associated

with increased cardiovascular mortality (not only cardiac sudden death) [95]. Interestingly, non dihydropyridines blockers such as amilodipine and nifedipine did not increase the incidence of cardiovascular death in carriers of a *NOS1AP* polymorphism.

The mechanisms by which polymorphism of *NOS1AP* (CAPON) influence the QT period remain unknown, but it has been recently shown in an animal model that CAPON, through effects on NOS1, can reduce I_{Ca} and increase I_{Ks}, increasing the duration of the action potential[60]. In theory, if mutations of *NOS1AP* interfere with the proper location/activity of NOS1, this in turn will modify S-nitrosylation of the cardiac ion channels.

Heart failure—In canine models of heart failure, oxidation of RyR2 as measured as loss of free cysteines[74, 75, 96] , has been observed. The oxidation is associated with diastolic Ca^{2+} leak, which is partially corrected by in vitro treatment with reductants agents like DTT, or by a variety of systemic treatments, including antioxidants (edaravone[97]), beta blockers[96], and ACE inhibitors[98]. Heart failure is characterized by increased production of ROS that may derive from different sources. NADPH oxidase[99], xanthine oxidase[100] are up-regulated; uncoupled NOS3[101, 102] and mitochondria[4, 103] are additional sources of ROS, which may disrupt nitrosylation either by oxidation of cysteines that are target of nitrosylation or by elimination of NO. Adding to this complexity, nitric oxide synthase expression varies in heart failure. NOS1 has been reported to be up-regulated and translocated from the SR to the sarcolemma[104, 105]. Collectively, these changes will favor diminished S-nitrosylation of RyR by NO derived from nNOS in heart failure.

Conclusions

Cell signaling mediated by *S*-nitrosylation is ubiquitous in nature. Many cardiac ion channels are evidently regulated by *S*-nitrosylation. Specifically, *S*-nitrosylation modulates the major currents involved in the generation of the action potential and in the development of the calcium transient that is transduced into force generation. *S*-nitrosylation is regulated by the activity of the nitric oxide synthases and GSNO reductase, compartmentation of signals, and the redox state of the subcellular domain. Oxidative stress profoundly modifies NO effects especially in the case of the ryanodine receptor, which may function as a redox sensor.

The enzymatic systems that govern nitrosylation/denitrosylation reactions in the heart remain to be fully understood. Aberrant S-nitrosylation has been implicated in a variety of cardiac pathologies, including arrhythmias, reperfusion injury, preconditioning and heart failure. It is probable that many current pharmacological treatments including organic nitrates, beta blockers[96], ACE inhibitors[98], and statins[106], mediate their actions, at least in a significant part, by restoring *S*-nitrosylation homeostasis.

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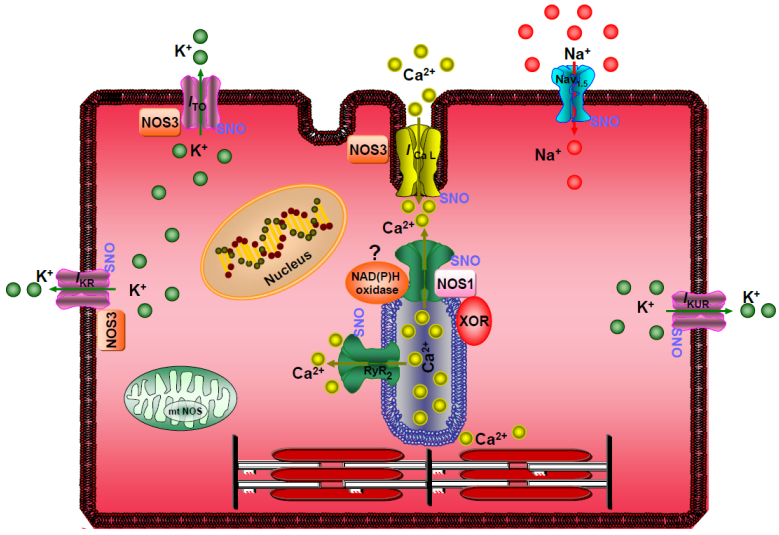

Figure 1. Ion channels regulated by S-nitrosylation in the cardiac myocyte

So far, characterized: the sodium channel, which generates a rapid depolarization; the L-type calcium channel, which generates the inward Ca^{2+} current; the ryanodine receptor (RyR2) calcium release channel of the sarcoplasmic reticulum; the transient outward potassium channel I_{To}, which activates rapidly upon depolarization; I_{Kur}, I_{Kr} and I_{Ks} which generates the delayed rectifier. The figure also includes the source of NO, the NO synthases NOS1 and NOS3 and the oxidases that are present in the cardiac cell. XOR, xanthine oxidoreductase, NOS, nitric oxide synthase.

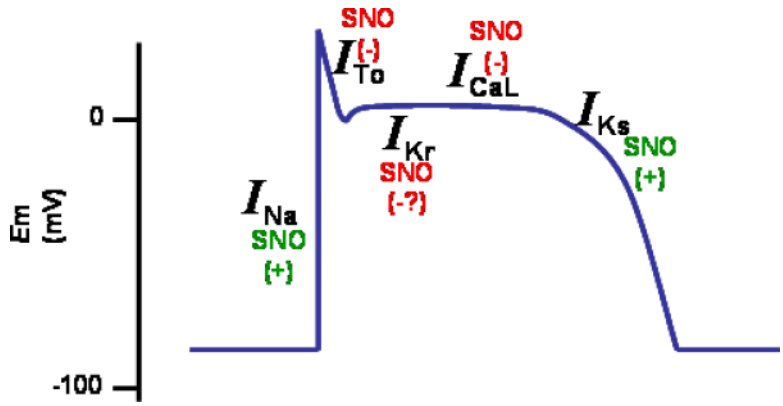

Figure 2. Impact of S-nitrosylation on the cardiac action potential

Between parenthesis, the effect of nitrosylation on the ionic currents.

The sodium current is increased by S-nitrosylation; the L-type calcium channel is inhibited, the transient-outward and the rapidly activating (I_{Kr}) potassium currents are inhibited; the slow component of the delayed rectifier is increased.

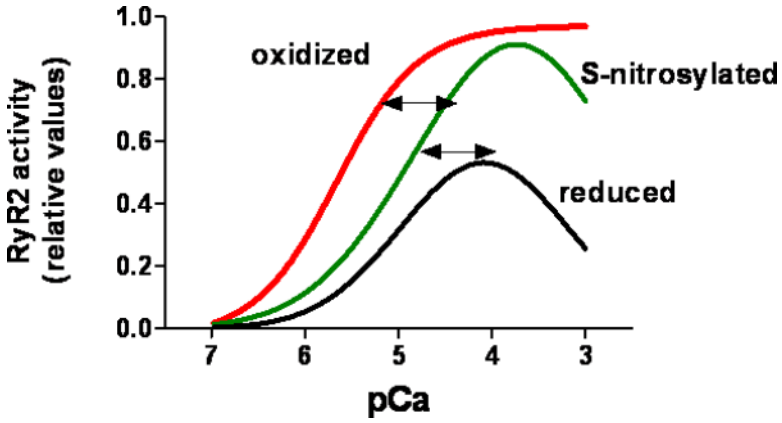

Figure 3. Effect of oxidative and nitrosative modifications on the calcium dependence of the cardiac ryanodine receptor (RyR2)

RyR is constitutively and reversibly S-nitrosylated, which increases RyR2 activity under normal conditions. Hyper- and hypo-nitrosylation may contribute to disease. Cysteine oxidation, likely a pathological modification, progressively increases RyR2 responsiveness to activating cytosolic Ca^{2+}. Oxidative activation may be irreversible. The values for RyR2 activity are relative and were obtained from the literature as open probability and ^{3}[H]ryanodine binding.