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J Mol Cell Cardiol. Author manuscript; available in PMC 2012 February 20.

Published in final edited form as:
J Mol Cell Cardiol. 2008 November ; 45(5): 625–632. doi:10.1016/j.yjmcc.2008.07.015.

Nitric Oxide Signaling and the Regulation of Myocardial Function

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Abstract

Nitric oxide, which is produced endogenously within cardiac myocytes by three distinct isoforms of nitric oxide synthase, is a key regulator of myocardial function. This review will focus on the regulation of myocardial function by each nitric oxide synthase isoform during health and disease, with a specific emphasis on the proposed end-targets and signaling pathways.

Keywords

NOS; Peroxynitrite; cGMP; Excitation-contraction coupling; L-type Ca^{2+} channel; Phospholamban; Ryanodine receptor

1.1 Nitric oxide and the myocardium

The role of nitric oxide (NO) signaling has been well defined in such processes as neural transmission and the dilation of blood vessels. Although the function of NO remains less well defined in the heart, NO has been shown to be a key regulator of excitation-contraction coupling (ECC) [1]. The process of ECC underlies myocardial contraction [2]. The β-adrenergic receptor (β-AR) signaling pathway is also a critical modulator of ECC and produces positive inotropic and lusitropic effects upon activation [3]. Balligand et al. first demonstrated that endogenous NO plays a role in the mediation of β-AR signaling as well [4].

NO is synthesized upon the cleavage of L-arginine into L-citrulline by three distinct isoforms of NO synthase (NOS) within the myocardium [5, 6]. Neuronal NOS (nNOS, NOS1) and endothelial NOS (eNOS, NOS3) are constitutively expressed in cardiac myocytes. These two isoforms are considered to be low output enzymes and produce NO in phase with myocyte contraction due to Ca-calmodulin regulation. In early studies, the use of NO donors or nonspecific NOS inhibitors made it difficult to distinguish between NOS1 and NOS3 signaling. However, recent studies have found that although NO is a highly diffusible signaling molecule, signaling via NOS1 and NOS3 is compartmentalized, and NOS1 and NOS3 differentially modulate cardiac function [5, 7, 8]. Inducible NOS (iNOS, NOS2), on the other hand, is only expressed during inflammatory responses and has been shown to be

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present during many pathophysiological conditions of the myocardium (e.g. ischemia-reperfusion injury, septicemia, aging, heart failure, etc.). When expressed, NOS2 produces much higher levels of NO independent of $[Ca^{2+}]_i$, compared to the constitutive NOS isoforms [6, 9].

1.2 Nitric Oxide Signaling

NO has been shown to signal through at least two distinct pathways: cGMP-dependent and cGMP-independent [10]. The cGMP-dependent effects of NO result from the NO-induced activation of guanylate cyclase, leading to increased cGMP levels, which modulate the activity of protein kinase G (PKG), as well as cGMP-regulated phosphodiesterases (PDE; cGMP-stimulated: PDE2; cGMP-inhibited: PDE3). cGMP-independent effects occur mainly via *S*-nitrosylation, an important protein modification related to cell signaling [11]. NO can also directly activate adenylate cyclase, thus increasing cAMP levels and myocardial contractility [12]. Additionally, NO may couple with other reactive oxygen and nitrogen species, leading to the formation of related congeners, such as peroxynitrite ($ONOO^-$). These related species may also influence cardiac contractility, and in some cases produce markedly differing effects from those observed with NO alone. Therefore, it is not surprising that paradoxical results have been reported in the literature, as both positive and negative effects of NO and related congeners have been observed. However, recent studies are resolving these apparent contradictions by determining that the contractile effects of NO are greatly influenced by NOS isoform localization [5, 6], and the activation of distinct cGMP-dependent and cGMP-independent signaling pathways which target individual ECC proteins in the cardiac myocyte. Additional studies have determined that these contractile effects are further confounded by such factors as gender [13], site of production [14, 15], species produced [16-18], concentration [19, 20], and cardiac myocyte contractile state [17, 20]. These factors are relevant to the contractile effects of NO and related congeners during both health and disease and are sensitive to cellular redox state.

2.1 NOS1 expression in the myocardium

The neuronal isoform of NOS (NOS1) was originally characterized in the forebrain [21], but has also been found to be constitutively expressed in cardiac myocytes. NOS1 has been shown to be localized to the sarcoplasmic reticulum (SR), and co-immunoprecipitates with the SR Ca^{2+} release channel or ryanodine receptor (RyR) under physiological conditions [5, 7]. Although sex hormones such as estradiol have been demonstrated to increase NOS1 mRNA levels [22], the effect of gender on the expression of NOS1 remains less well defined. One study demonstrated higher levels of NOS1 expression in female hearts compared to male [23], while another study found no difference between male and female hearts [24]. However, this discrepancy may result from species differences (rat vs. mouse) and/or female estrus cycle variance.

2.2 Contractile effects of NOS1-derived NO

The force frequency response (FFR) is an important mediator of contractility [25], and is partly modulated by NOS1 signaling. For instance, *in vivo* measurements have demonstrated that NOS1 knockout ($NOS1^{-/-}$) mice exhibit a blunted FFR (contraction and relaxation), which was also apparent in isolated $NOS1^{-/-}$ trabeculae and myocytes [5, 26, 27].

Several studies have shown that NOS1 is also capable of regulating the β-AR pathway. Specifically, *in vivo* and whole heart experiments demonstrated that the knockout of NOS1 leads to a reduced contractile response to β-AR stimulation [5, 28, 29]. We have recently demonstrated that myocytes isolated from $NOS1^{-/-}$ hearts also had a blunted response to β-

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AR stimulation, observed as a decrease in $[Ca^{2+}]_i$ transient and cell shortening amplitudes compared to WT [27].

NOS1 expression and activity may also be upregulated in certain disease states. For example, one study noted gender-dependent changes in NOS1 activity following pressure overload [30]. NOS1 has also been shown to translocate to the sarcolemma and localize with caveolin-3 during disease states [31, 32], or with conditional overexpression [33]. Conditional cardiac-specific overexpression of NOS1 resulted in decreased contractile function, while $NOS1^{-/-}$ mice exhibited increased mortality, hypertrophy, and left-ventricular dilation after myocardial infarction [28, 34]. Although NOS1 appears to be cardioprotective, the mechanism(s) for these effects are unknown.

2.3 End-targets and signaling pathways of NOS1

Phospholamban (PLB) is a key ECC protein which modulates SR Ca^{2+}-ATPase activity (SERCA). As such, PLB is a participant in the FFR and is also the major phosphoprotein in the β-AR pathway [35]. We and others have demonstrated that PLB is a key target of NOS1 signaling [27, 36]. In WT myocytes, acute NOS1 inhibition resulted in decreased basal and β-AR-stimulated contraction, and slowed $[Ca^{2+}]_i$ decline (Fig. 1B; similar to $NOS1^{-/-}$). However, with acute NOS1 inhibition in $PLB^{-/-}$ myocytes, we noted no effect on contraction or $[Ca^{2+}]_i$ decline. We further examined the effects of NOS1 signaling on PLB, and observed that NOS1 inhibition decreased PLB phosphorylation [27], which was shown to be due to enhanced protein phosphatase activity [36]. We also observed a decreased SR Ca^{2+} load, an important determinant of myocyte contraction [37], with NOS1 knockout or inhibition. Interestingly, $NOS1^{-/-}$ hearts have decreased expression of PLB and increased expression of RyR and calsequestrin [26, 38]. These changes appear to be compensatory in an attempt to increase SR Ca^{2+} uptake, SR Ca^{2+} load, and SR Ca^{2+} release. During β-AR stimulation, PLB phosphorylation levels are similar between $NOS1^{-/-}$ and WT myocytes [36]. This normalized PLB phosphorylation leads to similar $[Ca^{2+}]_i$ decline and myocyte re-lengthening rates between $NOS1^{-/-}$ and WT myocytes [27, 36]. However, we have shown that there is still a reduced contractile response to β-AR stimulation in $NOS1^{-/-}$ myocytes [27], suggesting additional protein targets.

NOS1 has also been shown to target RyR [39], as one study demonstrated that $NOS1^{-/-}$ myocytes had an enhanced diastolic leak via RyR. This enhanced leak could also contribute to the reduction in SR Ca^{2+} load. NOS1 signaling has also been found to target the L-type Ca^{2+} current (I_{Ca}). Interestingly, this NOS1-mediated decrease in I_{Ca} led to decreased basal and β-AR-stimulated contraction [38]. NOS1 also interacts with non-ECC proteins, as NOS1 has been shown to bind with sarcolemmal Ca^{2+} pump 4b (PMCA) [40], which regulates NOS1 activity by modulating $[Ca^{2+}]_i$ levels. Overexpression of PMCA was shown reduce NOS1 activity, and decrease the response to β-AR stimulation, with a trend toward decreased basal contraction (similar to $NOS1^{-/-}$). Notably, several of these studies showed that these effects were through cGMP-independent signaling pathways (Fig. 1A, solid lines) [27, 36, 39].

Since NOS1 co-immunoprecipitates with xanthine oxidoreductase [41], a superoxide ($O_2{\cdot}^-$) producing enzyme, low levels of peroxynitrite may be formed. Additionally, it is possible for NOS1 to produce both NO and superoxide [42], although this is more likely to occur with uncoupling in disease states [43]. Thus peroxynitrite, produced by the reaction of NO and superoxide, may be a potential signaling molecule for NOS1. We investigated the role of peroxynitrite in NOS1 signaling and upon perfusion with FeTPPS, a peroxynitrite decomposition catalyst, we were able to mimic the effects of NOS1 knockout or acute inhibition (decreased contraction, slowed $[Ca^{2+}]_i$ decline, decreased PLB phosphorylation)

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[27]. In another study, we demonstrated that a low concentration of peroxynitrite increased basal contraction and further increased the response to β-AR stimulation in WT myocytes through a PLB-dependent mechanism [20]. These data are consistent with NOS1 signaling occurring via peroxynitrite.

RyR activity is also regulated by *S*-nitrosylation, which increases RyR open probability [44]. Xu et al. confirmed reversible *S*-nitrosylation of up to 12 cysteines of the RyR tetramer, which led to progressive channel activation [45]. More recently, Gonzalez et al. [39] reported that NOS1 knockout decreased RyR *S*-nitrosylation levels. They also observed increased RyR oxidation, which can lead to enhanced RyR activity. However, prolonged oxidation of RyR leads to irreversible inactivation [46]. As a result, $NOS1^{-/-}$ myocytes may also have decreased RyR activity. Indeed, our preliminary data suggests that RyR activity is decreased in $NOS1^{-/-}$ myocytes [47]. In summary, NOS1 modulates cardiac myocyte contraction mainly through cGMP-independent signaling pathways by targeting multiple ECC proteins, including PLB, RyR, and the L-type Ca^{2+} channel (Fig. 1A).

3.1 NOS3 expression in the myocardium

As with NOS1, the endothelial isoform of NOS (NOS3) is also constitutively expressed in cardiac myocytes. NOS3 has been shown to be localized to the caveolaeand co-immunoprecipitates with caveolin-3 (Fig. 1A) [5, 48]. NOS3 expression also appears to be graded in the myocardium, such that NOS3 expression in left ventricular epicardial myocytes is significantly increased compared to left ventricular endocardial myocytes [49]. Additionally, the expression of NOS3 in the myocardium is not limited to the cardiac myocyte, as NOS3 is also expressed in endothelial cells [50], where NOS3-dervied NO serves to regulate vascular tone and contraction [15, 51]. Sex hormones have also been shown to increase NOS3 mRNA levels [22, 52], but the effects of gender on NOS3 expression remain inconsistent [23, 24]. This is likely due the preparation used (myocyte vs. whole heart) and/or variances in the female estrus cycle. Phosphorylation of NOS3 by protein kinase AKT can also increase the enzyme activity of NOS3 [53]. Interestingly, studies have also shown that estrogen can bind to membrane receptors, leading to the phosphorylation and activation of NOS3 [54, 55]. However, gender-based differences in NOS3 phosphorylation in the myocardium are currently unknown.

3.2 Contractile effects of NOS3-derived NO

In contrast to NOS1, NOS3 signaling does not regulate the FFR or basal function, as studies have found no difference in isolated cardiac myocytes with NOS3 knockout ($NOS3^{-/-}$) or overexpression compared to WT [5, 26, 56, 57]. Conversely, some *in vivo* and whole heart studies have found NOS3 to modulate basal function [5, 58, 59]. However, endothelial cell-derived NO may cause indirect effects on cardiac myocytes through diffusion [15]. NOS3 signaling has also been shown to play a pivotal role in the stretch-dependent enhancement of $[Ca^{2+}]_i$ transients and cardiac myocytecontraction [8]. This phenomenon allows the heart to increase contraction in response to an increase in preload beyond that achieved by ordinary length-dependent activation mechanisms.

Studies have consistently reported that NOS3 signaling decreases the cardiac functional response to β-AR stimulation [5, 58-61]. For example, $NOS3^{-/-}$ myocytes exhibited a greater β-AR-stimulated $[Ca^{2+}]_i$ transient and cell shortening amplitude compared to WT [5, 61]. Additionally, cardiac specific NOS3 overexpression resulted in a reduced response to β-AR-stimulation [62-64]. In contrast, one study observed no difference in the response to β-AR stimulation in $NOS3^{-/-}$ myocytes compared to myocytes from WT littermate hearts [56]. However, we observed that acute NOS3 inhibition in WT myocytes increased the contractile response to β-AR stimulation [61].

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NOS3 signaling may also play a cardioprotective role. We observed that the largest effector of NOS3 signaling was action potential (AP) waveform, as β-AR-stimulated myocytes with NOS3 knockout or inhibition exhibited a substantially larger increase in AP duration (measured as time to 90% repolarization – APD_{90}) compared to WT myocytes (Fig. 1C) [61]. This greatly prolonged APD in $NOS3^{-/-}$ 90 myocytes may contribute to arrhythmias. Previous studies have found that $NOS3^{-/-}$ mice had a greater incidence of digoxin-induced premature ventricular beats and ventricular tachycardia, and increased oubain-induced aftercontractions in isolated myocytes [65, 66]. We also observed greater spontaneous activity in $NOS3^{-/-}$ myocytes during β-AR stimulation, manifested as a higher incidence of early and delayed afterdepolarizations in $NOS3^{-/-}$ myocytes [61]. Early and delayed afterdepolarizations occur from increased Ca^{2+} influx, which subsequently produces SR Ca^{2+} overload and spontaneous release [67, 68]. However, NOS3 appears to protect the myocardium from this arrhythmogenic activity during β-AR stimulation.

In addition to protecting the heart from arrhythmias, it appears NOS3 signaling is also beneficial by limiting remodeling. $NOS3^{-/-}$ mice showed increased hypertrophy, fibrosis, and contractile dysfunction after chronic pressure overload compared to WT [69]. Conversely, cardiac-specific NOS3 overexpression limited hypertrophy and contractile dysfunction following chronic pressure overload or myocardial infarction [62, 70]. Therefore, the anti-adrenergic effects of NOS3 signaling are beneficial, acting as an endogenous β-blocker. Unfortunately, it appears that NOS3 expression and activity is decreased in pathophysiological states (e.g., heart failure) [30, 31]. Further, NOS3 can become uncoupled during such disease states as pressure overload, thus leading to the formation of supraphysiological levels of superoxide and additional pathophysiological remodeling [71]. Therefore, NOS3 may represent a novel therapeutic target [72].

3.3 End-Targets and Signaling Pathways of NOS3

The L-type Ca^{2+} channel is a key ECC protein. Ca^{2+} influx via the L-type Ca^{2+} current (I) provides the trigger Ca^{2+} for the release of additional Ca^{2+} Ca from the SR (Ca^{2+}-induced Ca^{2+}-release). I -derived Ca^{2+} can also increase SR Ca^{2+} Ca load, and may directly activate the myofilaments to contract. NOS3 localizes to the caveolae, along with the L-type Ca^{2+} channel [48, 73]. Importantly, the L-type Ca^{2+} channel is a target for the β-AR pathway as we observed that NOS3 signaling has only anti-adrenergic effects. I_{Ca} also contributes to the development of early and delayed afterdepolarizations, and the activation of apoptotic and hypertrophic signaling pathways [67, 74, 75]. Our recent data demonstrated that NOS3 signaling is able to limit β-AR-stimulated I_{Ca} [61]. Consistent with these data, as well as that of others, a NOS3-induced reduction in β-AR-stimulated I_{Ca} will decrease contraction, and protect against arrhythmias and hypertrophic signaling. In addition, the L-type Ca^{2+} channels located in the caveolae may play a minor role in ECC, and this may be the reason only modest effects were observed on contraction. NOS3 signaling has also been demonstrated to affect the slow component of the delayed rectifier K^+ current (I_{Ks}). Bai et al. showed that NOS3 activation resulted in the depression of I_{Ca}, the enhancement of I_{Ks}, and the shortening of AP duration [76]. Altered I_{Ca} and I_{Ks} are both known to play critical roles in the genesis of arrhythmias.

NOS3 signaling can also directly alter cardiac myocyte contraction by targeting myofilament proteins. For example, NOS3 has been shown to target TnI, thus decreasing myofilament Ca^{2+} sensitivity [77]. This decrease in myofilament sensitivity will also decrease myocyte shortening amplitude.

Studies have yet to completely elucidate the signaling pathway(s) for NOS3. However, since NOS3 has been shown to co-localize with superoxide dismutase [49], an enzyme which

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catalyzes the decomposition of superoxide, the reaction of NO with superoxide is prevented. Thus, NOS3-derived NO leads to the enhanced activation of guanylate cyclase and increased cGMP production (Fig. 1A, dashed lines). The cGMP-dependent pathway in cardiac myocytes is primarily through the activation of PKG [78]. α_{1C} subunit of the L-type Ca^{2+} channel at Ser533 within cardiac myocytes [79, 80]. PKG is also able to phosphorylate TnI [81]. Interestingly, a study reported that inhibition of the cGMP-specific phosphodiesterase (PDE5A) depressed β-AR-stimulated cardiac contraction through increased PKG activity in WT mice, with no effect in $NOS3^{-/-}$ [82]. We also observed that PDE5 inhibition decreased β-AR-stimulated I_{Ca} through increased PKG activity [83]. These data suggest that NOS3 and PDE5 co-localize, further implicating cGMP as the signaling molecule of NOS3.

NOS3 may also signal independent of cGMP (Fig. 1A, solid lines). Sun et al. demonstrated that NOS3 signaling in female myocytes had decreased I_{Ca} via increased *S*-nitrosylation of the α_{1C} subunit of the L-type Ca^{2+} channel compared to male myocytes [84]. Thus, NOS3 modulates cardiac myocyte contraction through cGMP-dependent and cGMP-independent signaling pathways, primarily by targeting I_{Ca}, I_{Ks}, and TnI.

4.1 NOS2 expression in the myocardium

The inducible isoform of NOS (NOS2) is only expressed during immune responses [85], and therefore during pathophysiological conditions of the myocardium that are associated with an upregulated inflammatory response. These include such conditions as ischemia-reperfusion injury [86], septicemia [87-89], aging [90], and heart failure [91]. NOS2 is widely considered to be a cytosolic protein [6].

4.2 Contractile effects of NOS2 expression

Numerous studies have shown that in many pathophysiological conditions of the myocardium, the observed cardiac dysfunction is partly due to NOS2. For example, NOS2 expression following ischemia-reperfusion injury was shown to contribute to basal cardiac dysfunction and an increase in infarct size [86, 92]. Additionally, genetic deletion or inhibition of NOS2 afforded protection against myocardial dysfunction in sepsis [87, 88]. In the aged myocardium, whole-heart function was shown to be decreased compared to young hearts, but was normalized with NOS2 inhibition [90]. Furthermore, we have demonstrated that β-AR-stimulated $[Ca^{2+}]_i$ transients and cell shortening were reduced in failing human cardiac myocytes expressing NOS2 [91]. Similarly, we observed dysfunctional contraction in myocytes isolated from rejecting transplanted hearts that was reversible with NOS2 inhibition [9, 93].

4.3 End-targets and signaling pathways of NOS2

NOS2 has been shown to target several Ca^{2+}-handling proteins within the cardiac myocyte, including troponin I [94], RyR [89], and I_{Ca} [9]. Many cGMP-dependent effects of NOS2 signaling have been observed through the activation of PKG. Yasuda et al. demonstrated that NOS2 induced a reduction in myofilament Ca^{2+} sensitivity [94], likely mediated by troponin I phosphorylation via PKG [81], thus decreasing myocardial contraction (Fig. 2A). The dysfunction that we observed with NOS2 expression in myocytes isolated from rejecting transplanted hearts resulted from a reduction in basal I_{Ca}, that was reversible upon NOS2 inhibition [9]. This effect was shown to be dependent upon the phosphorylation of the L-type Ca^{2+} channel by PKG. Other cGMP-dependent effects of NOS2 have been shown to be mediated through alterations in phosphodiesterase activity (Fig. 2A). Joe et al. demonstrated that NOS2 attenuated the response to β-AR stimulation by decreasing cAMP levels [95], possibly via PDE2 activation.

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cGMP-independent effects of NOS2 expression have also been demonstrated. Since NOS2 is a high output isoform, NOS2 can easily become uncoupled, leading to the production of both NO and superoxide , which couple to form high levels of peroxynitrite [96, 97]. At high concentrations, peroxynitrite is a potent oxidant capable of causing cellular damage, often by nitrating tyrosine residues [98], and is most likely the major signaling molecule of NOS2 [99]. Additionally, NADPH oxidase and xanthine oxidoreductase can increase superoxide production during pathophysiological conditions of the myocardium [100, 101]. Studies examining peroxynitrite have demonstrated dysfunctional effects via direct exposure or using peroxynitrite donors [99, 102, 103]. Previous studies have also shown that peroxynitrite can directly inactivate SERCA at high concentrations [104, 105], thus reducing SR Ca^{2+} load and myocardial contraction. Peroxynitrite has also been demonstrated to affect other ECC proteins (Fig. 2A), including RyR [89], and PLB [16]. In a previous study, we demonstrated a reduction in the β-AR response, observed as a decrease in myocyte $[Ca^{2+}]_i$ transients and shortening, upon perfusion with a high concentration of peroxynitrite with no effects during basal stimulation (Fig. 2B) [16]. We further demonstrated that high peroxynitrite exerted anti-adrenergic effects by reducing cAMP-dependent PLBSerine16 phosphorylation via activation of protein phosphatases, resulting in reduced SERCA uptake of Ca^{2+} and decreased myocardial contraction. These results are strikingly similar to the effects that we observed in failing human myocytes expressing NOS2, where β-AR-stimulated myocyte $[Ca^{2+}]_i$ transients and shortening were increased following NOS2 inhibition with no effect during basal stimulation (Fig. 2C) [91]. The expression of NOS2 in heart failure may be a key component of the observed β-AR dysfunction, as studies have demonstrated increased peroxynitrite production [99, 106, 107], increased protein phosphatase activity [108, 109], and decreased PLBSerine16 phosphorylation in heart failure [110, 111]. Thus, NOS2 modulates cardiac myocyte contraction through cGMP-dependent (Fig. 2A, dashed lines) and cGMP-independent (Fig. 2A, solid lines) signaling pathways by targeting multiple ECC proteins, as well as many components of the β-AR pathway. NOS2 is likely capable of producing these vast effects through the production of such high levels of NO compared to the constitutive NOS isoforms, thus resulting in signaling that is not compartmentalized. In addition, it has been hypothesized that the high NO production of NOS2 may result in the loss of local NOS1 and NOS3 signaling during many of these pathophysiological states [112].

5. Conclusions

NO signaling plays a critical role in the modulation of myocardial function. The resulting functional effects of NO signaling in the myocardium, as many studies have demonstrated, are multifaceted and highly dependent on such factors as NOS isoform localization, the activated signaling pathway, species produced, concentration, gender, site of production, and myocyte contractile state. These effects can also be influenced by additional factors, including the redox state of the myocyte and disease states. Therefore, it comes as no surprise that results contrary to those detailed herein have been previously reported in the literature [36, 38, 56, 72, 113-118]. However, recent studies are beginning to resolve these apparent controversies, and although conflicting reports regarding NO signaling exist, these results indicate that NO signaling does indeed play a key role, albeit complex, in the regulation of myocardial function.

Acknowledgments

Supported by the American Heart Association (Pre-doctoral Fellowship 0715159B, MJK; Post-doctoral Fellowship, 0725560B HW) and the National Institutes of Health (R01HL079283, MTZ).

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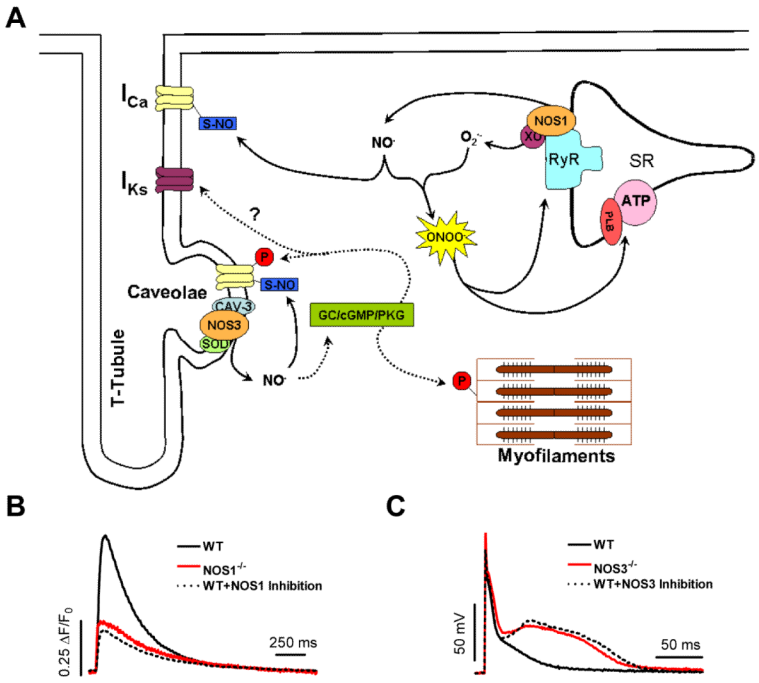

Figure 1. Physiological (non-pathophysiological) signaling of constitutive NOS isoforms in the myocardium

A.) Proposed cGMP-dependent (dashed lines) and cGMP-independent (solid lines) signaling pathways and end targets of NOS1 and NOS3 in the normal functioning myocardium. B.) Basal cardiac myocyte $[Ca^{2+}]_i$ transients in WT, NOS1$^{-/-}$, and WT+NOS1 Inhibition (S-methyl-L-thiocitrulline). C.) Cardiac myocyte action potential waveform during perfusion with 1 μmol/L isoproterenol (ISO, non-specific β-AR agonist) in WT, NOS3$^{-/-}$, and WT +NOS3 Inhibition (N5-(1-Iminoethyl)-L-ornithine dihydrochloride). NOTE: Data modified from references [27, 61].

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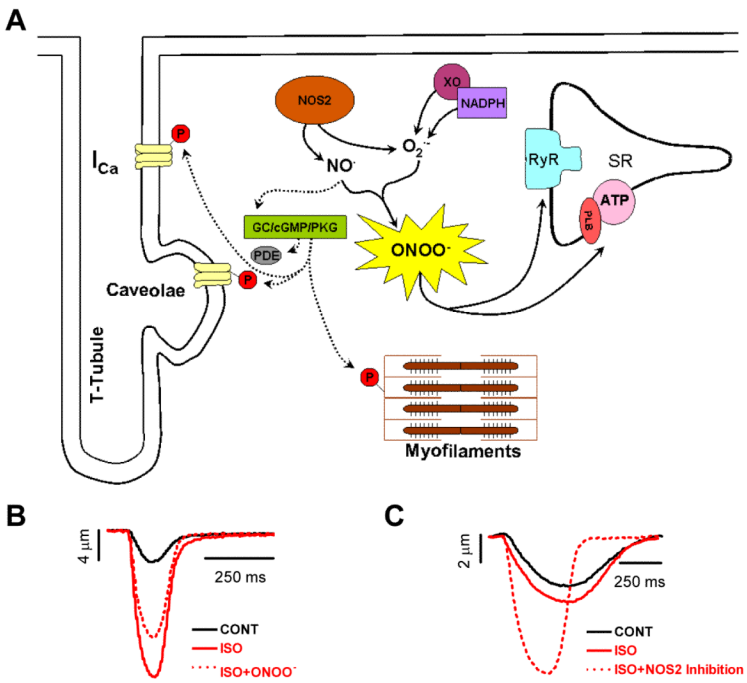

Figure 2. NOS2 signaling in the myocardium

A.) Proposed cGMP-dependent (dashed lines) and cGMP-independent (solid lines) signaling pathways and end targets of NOS2. B.) Cell shortening in a mouse cardiac myocyte during perfusion with control, 1 μmol/L isoproterenol (ISO, non-specific β-AR agonist), and ISO +SIN-1 (peroxynitrite donor). C.) Cell shortening in a failing human cardiac myocyte during perfusion with control, 1 μmol/L isoproterenol, and ISO+NOS2 inhibition (L-N6-[1-Iminoethyl]-lysine dihydrochloride). NOTE: Data modified from references [16, 91].