Editorials

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Myocyte Nitroso-Redox Imbalance in Sepsis

NO Simple Answer

Dan E. Berkowitz

Our initial understanding that the inhibition of inducible nitric oxide synthase (iNOS or NOS2) dependent NO production in cardiac myocytes would restore contractile function and improve outcome in septic shock failed.1 This was, in part, because of our then lack of understanding of the complexity of NO signaling in the cardiovascular system in health and disease. The initial paradigm that NO acts as a diffusible molecule to depress cardiovascular function uniformly and diffusely has been replaced by a more sophisticated yet incomplete model of NO signaling. It calls for NO to act in very defined signaling domains, through distinct signal transduction mechanism, sub-serving focused physiologic functions.2 It is now well established that NO and NO-related species are significant modulators of cardiac muscle excitation-contraction coupling and that these species affect a number of molecules and channels that coordinate this process.2 It has been further established that mammalian cardiac myocytes express all 3 of the isoforms of nitric oxide synthase: endothelial (eNOS or NOS3), neuronal (nNOS or NOS1), and iNOS or NOS2. Furthermore what determines the modulatory effects (sometimes directionally opposite) with regard to physiologic function is not only the signal transduction mechanism, but the spatial confinement of the enzyme within the cardiac myocyte. For example, NOS3 is targeted to the sarcolemmal caveolae associated with the caveolar membrane protein caveolin 3. There, it is coupled through the β-3 adrenergic3 and muscarinic receptor, guanylyl cyclase and the second messenger cGMP to negatively regulate l-type Ca2+ channel-dependent Ca2+ influx. It thus acts as a negative modulator of adrenergic signaling to balance and constrain positive inotropy. On the other hand nNOS is confined to the sarcoplasmic reticulum (SR)4 where it is associated with the ryanodine receptor (RYR 2), and positively modulates channel function, enhancing contractile response to β adrenergic stimuli5 and the contractile response to increasing pacing frequency.5 This signal transduction mechanism involves S-nitrosylation of cysteine thiols (an emerging critical redox-sensitive post translational protein regulatory mechanism7) of the RYR which in turn regulates its gating function.8 Functional studies in NOS3−/− and NOS1−/− mice have confirmed these directionally opposite roles of NOS isoforms on contractile function which are dependent on their location within the cell.9 With regard to the iNOS however, it is present at very low concentrations in the cell and its expression is primarily induced in disease states.9

Dysregulated NO signaling and NOS isoform function is implicated in a number of adaptive and disease processes such as heart failure, ischemia/reperfusion injury, aging and the contractile dysfunction associated with the septic shock.10 In the current issue,1 Ichinohe and colleagues provide a tour de force of in vivo and in vitro physiologic, as well as biochemical data to support the protective role of cardiac myocyte-specific NOS3 overexpression (NOS3TG) in the myocardial depression mediated by sepsis. Using a multitude of techniques in vivo (pressure-volume loops), and in vitro (isolated myocytes studies), and using 2 models of sepsis (lipopolysacharide [LPS] administration and the cecal ligation and puncture technique), the investigators demonstrate that cardiac myocyte-specific overexpression of NOS3 markedly attenuates the myocardial contractile depression seen with sepsis. The investigators demonstrate that this preservation is in part a function of a significant but less depressed [Ca2+]i (compared with wild type [WT] LPS treated mice), as well an increase in myofilament Ca2+ sensitivity, which compensates in part for a depressed myocardial [Ca2+]i. This increased Ca2+ sensitivity in the NOS3TG is likely mediated by nitroso-redox modulation of the proteins involved in excitation-contraction coupling because the relatively depressed Ca2+ sensitivity in WT (compared with TG) is restored by the reducing agent dithiothreitol (DTT [DTT reduces thiols to the SH form]). Furthermore, the investigators demonstrate that NOS3TG animals have attenuated reactive oxygen species (ROS) production and that increased ROS production in LPS treated WT animals can be suppressed by the xanthine oxidase inhibitor allopurinol. This supports the enzyme as the primary source of ROS in this pathophysiologic process. Thus, overexpression of NOS clearly alters the nitroso-redox state of the myocyte promoting protection and maintenance of contractile function. Finally, and most impressively is the demonstration that the protective effect of the NOS3TG on myocardial oxidant stress and function translates into a markedly enhanced survival.

Because the global “amount” of NO measured in both the TG (before) and WT mice following LPS administration are the same, we are left to speculate that it is the both the spatial and temporal distribution of the NO and the NOS from which it is derived, that modulates contractile response to the highly

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oxidative and nitrosative stress of sepsis. This idea is wholly consistent with the notion that NOS is colocalized with the proteins which it regulates (“stimulus-coupled regulation of S-nitrosylation within the confines of the signaling module”) a concept which is well summarized in a recent review by Drs Hare and Stamler.10 It is also consistent with the idea that the NOS3TG is in fact confined to the SR? There is some evidence that the reductase domain of eNOS may in fact be a binding partner for the RYR and might thereby be responsible for the nitrosylation of the RYR.15 With regard to the RYR receptor, it is now well established that its function is sensitive to ROS and RNS. On the other hand nitrosylation of a single thiol by physiologic concentrations of NO leads to channel activation and normally regulated function.8 The identification of the subcellular compartmentalization of NOS3TG by the investigators would go a long way to help explain the interesting pathophysiologic findings.

A further intriguing finding by the investigators relates to the observation that inhibition of XO by allopurinol before LPS stimulation significantly attenuates LPS dependent decreases in [Ca²⁺]i and depressed Ca²⁺ sensitivity in LPS treated WT mice. This suggests that the source of the ROS is at least in part XO. This finding is interesting in light of the known interaction between NO and XO in general and in the cardiac myocyte specifically. For example, cardiac myocyte NOS-1 colocalizes with XO in the SR and NOS-1 dependent NO prevents XO-dependent ROS production.16 Indeed XO has been implicated in the pathophysiology of a number of cardiovascular diseases associated with oxidative stress. The mechanism by which NO may prevent XO-dependent ROS production remains unclear. XO, a molybdenum containing enzyme, expressed as a 150 kDa homodimer, produces superoxide in the process of purine metabolism. The enzyme has two forms: XO and xanthine dehydrogenase (XDH), the former, resulting from either irreversible proteolytic cleavage or reversible oxidation of sulfhydryl residues of XDH. It is interesting to speculate whether redox sensitive nitrosylation (by NO produced from the NOS3TG) of XDH sulfhydryl residues may prevent oxidation of these residues and thus prevent irreversible activation of XO from XDH. This is even more intriguing given the idea that DTT, a sulfhydryl reducing reagent restores Ca²⁺ sensitivity in LPS treated mice. It should also be considered that irreversible proteolytic cleavage of XDH to XO rather than the reversible conversion may be the mechanism of activation. This is supported by the

Subcellular location of myocyte NOS isoforms as well as potential site of the NOS3TG and proteins that may be altered by NOS3TG-mediated NO-dependent nitrosylation. They may thereby be "protected" from the profound oxidative and nitrosative stress induced by sepsis. Redox dependent signaling represents a continuum from physiologic concentration of NO and ROS that can reversibly alter and modulate function of myocyte proteins (S-nitrosylation) to severe nitrosative and oxidative stress that may irreversibly alter protein function resulting in pathophysiologic disease processes. LPS stimulates cytokine production, cytokine receptor (CRs) activation resulting in a transcriptional upregulation of NOS2 and high levels of NO production. NOS3TG could potentially S-nitrosylate the rymodine 2 receptor channel (RYR) at a single site preserving channel function and attenuating sepsis dependent reductions in [Ca²⁺]i and contractile function. S-nitrosylation may also inhibit protein phosphatase 1 (PP1) activity maintaining phospholamban phosphorylation and thereby enhancing diastolic function and SR Ca²⁺ loading. In addition nitrosylation of contractile myofilaments may preserve Ca²⁺ sensitivity and diastolic function. NOS3TG may also S-nitrosylate xanthine oxidase (XO) thereby inhibiting its activity and reducing oxidative stress. NOS3TG dependent-NO may also protect mitochondria and preserve mechanon-energetics. LTCC (L-type Ca²⁺ channel); (+), positively modulate; (-), negatively modulate; Dashed arrow, inhibit; solid arrow, facilitate.

LPS

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produced O₂

decreased, NOS3 uncouples to the monomeric form and derived O₂

to atherosclerosis because of a decrease in uncoupled eNOS

that generates NO from L-arginine. When exposed to an

hypertrophy and heart failure. NOS3 exists as a homodimer

relates to eNOS uncoupling and ROS generation in patho-

production in a sepsis model is at first glance inconsistent

LPS.

dependent ROS production in cardiac myocytes following

DTT or the serine protease inhibitor would inhibit XO

enhance contractility and protect against the development of atherosclerosis.

mitochondrially driven programmed cell death.7

It is emerging evidence that phosphatases may be regulated by S-nitrosothiols such that nitrosylation leads to loss of activity.24 This would tend to preserve and enhance PLB phosphorylation which would in turn enhance SERCA activity. This would support the findings of enhanced diastolic (and ultimately systolic) function. Furthermore SERCA can be regulated directly in a redox-sensitive fashion by S-nitrosylation in a manner analogous to RYR2.

One cannot fail to mention the possible role of the NOS3TG in the preservation of mitochondrial function and mechano-energetics in the septic heart. Suffice to say that the pathogenetic mechanism of mitochondrial dysfunction in sepsis is complex but central to the syndrome. Reactive nitrogen and oxygen species can directly inhibit mitochondrial respiration by competing with oxygen in binding to the cytochromes of the electron transport chain. It is emerging that nitrosylation of discrete proteins in the mitochondria might regulate function in addition to regulating/inhibiting mitochondrial driven programmed cell death.7

The cellular NO/redox balance is profoundly perturbed by sepsis. It should be remembered however that this balance is not only regulated by enzymes that produce reactive oxygen and nitrogen species but those endogenous antioxidant systems that are activated in response to the stress. Although those systems that are important in the inactivation of ROS have been well investigated, it has only recently been discovered that regulation of nitrosative stress is critical to the survival of the organism, because mice deficient in the enzyme GSNO reductase, an ancient enzyme which is conserved across most species have a markedly increased mortality when exposed to a profound nitrosative stress such as sepsis.26

Thus, an emerging theme with regard to this work is that NO/redox disequilibrium contributes to specific alterations in the nitrosylation status of proteins that are involved in excitation-contraction coupling in the sepsis models. Although a candidate approach to identifying altered proteins will be informative, a proteomic/nitrosoproteomic approach might identify further candidates that are modified and could explain the effects of sepsis on these proteins and thereby the whole organism. Furthermore the identification of proteins that are “protected” by NOS3TG overexpression may lead to the development of other therapeutic strategies that could ultimately defend the heart against sepsis-induced myocardial depression.

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References


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