Nitroxyl, Redox Switches, Cardiac Myofilaments, and Heart Failure

A Prequel to Novel Therapeutics?

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Heart failure (HF) remains to be the leading cause of death in developed countries, and the prevalence of HF worldwide continues to escalate. Despite enormous efforts made to improve our understanding of the molecular mechanisms of HF and to develop effective therapies, HF remains to be a therapeutic challenge. One hallmark of HF is altered cardiac myofilament responsiveness to intracellular Ca\(^{2+}\), resulting in depressed contraction. Although increasing intracellular Ca\(^{2+}\) is a possible strategy for strengthening contraction, currently available inotropic agents, such as β-agonists and phosphodiesterase inhibitors, which raise intracellular cAMP levels can lead to deleterious side effects when given as a long-term therapy. Recently, promising inotropic agents targeting novel mechanisms have been developed, including (1) nitroxyl (HNO) released from HNO donors, (2) istaroxime, an inhibitor of Na\(^+/\)K\(^+-\)ATPase and an activator of the sarcoplasmic reticulum Ca\(^{2+}\) pump (SERCA), (3) cardiac myosin activator, and (4) ryanodine receptor stabilizer. In this issue, Gao et al report that HNO can increase myofilament Ca\(^{2+}\) responsiveness by inducing disulfide bonds between key cysteine residues in the myofilament, suggesting that HNO donors may improve cardiac function in patients with HF without the possible deleterious consequences of continued Ca\(^{2+}\) manipulation (Figure).

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In the past few years, HNO has emerged as a novel therapy for treating cardiovascular diseases. HNO is a 1-electron–reduced and protonated sibling of the gaseous signaling molecule NO. Although the therapeutic use of NO as a nitrovasodilator in the treatment of cardiovascular disorders has been documented for over a century, the pharmacological and therapeutic potential of HNO has been overlooked until recently. HNO seems to have distinct chemical and physiological properties and unique signaling mechanisms from those of NO. HNO is a weak acid (pKa 11.5), indicating that HNO and not NO\(^-\) is the predominant species at physiological pH. One of the most biologically significant and facile reactions of HNO is with thiols on cysteine forming N-hydroxysulfenamide intermediates, which can lead to formation of sulfamides or disulfides in the presence of an additional free thiol (Figure, A). Importantly, actions of HNO can be compartmentalized by the location of the molecular target and the pKa of a given cysteine, making HNO an attractive pharmacological agent.

The positive inotropic effect of HNO has generated great interest clinically. Early studies in a canine model by Paolocci et al suggested that HNO has both positive inotropic and lusitropic properties, which significantly improve left ventricular contractility and lower cardiac preload and diastolic pressure. Paolocci et al determined that the mechanisms of these beneficial inotropic and lusitropic effects are apparently independent of cGMP-dependent signaling pathways. Instead, because of its thiophilic nature, HNO may interact with redox switches in key components of the cardiac electromechanical machinery regulating myocardial function. Tocchetti et al reported that HNO can improve cardiac function by a direct interaction of HNO with SERCA2a and ryanodine receptor 2 leading to increased Ca\(^{2+}\) uptake and release from sarcoplasmic reticulum, supporting the idea that HNO/thiolate interactions enhance the activity of Ca\(^{2+}\)-handling proteins. Furthermore, Dai et al found that HNO increased force development in rat cardiac muscle, and the effects of HNO on myofilaments were reversed by treatment with the reducing agent, dithiothreitol, suggesting possible involvement of disulfides. Nonetheless, the nature and sites of potential HNO-induced modifications in myofilaments have remained elusive until now.

Here, Gao et al elegantly elucidated the effects of HNO modification of cardiac myofilament proteins and provide detailed characterization of the reversible cysteine modifications of the proteins involved (Figure, B). They found that HNO can have direct effects on myofilament proteins by increasing both maximum force ($F_{\text{max}}$) and Ca\(^{2+}\) sensitivity of force in intact and skinned cardiac muscles. Importantly, they have used mass spectrometry (MS) coupled with a modified biotin switch assay to locate the crucial cysteines involved in forming the disulfides: Cys257 in actin subdomain 4 with Cys190 in tropomyosin; Cys81 in myosin light chain 1 (MLC1) with Cys37 in the myosin heavy chain’s head region, which accounted for increased myofilament responsiveness to Ca\(^{2+}\) (Figure, B). Interestingly, HNO only increased Ca\(^{2+}\) sensitivity and not $F_{\text{max}}$ in skeletal muscle, which lacks the Cys81 in MLC1, indicating that MLC1 Cys81 is the critical residue in...
Despite proposals for mechanisms, the role of disulfides as thiol-based redox switches remains poorly defined. Determination of disulfide effects on cardiac myofilaments is important to gain comprehensive understanding of NCA-released HNO in diseased myocardium and animal models are not yet known. Nonetheless, Gao et al1 used a modified biotin switch assay combined with bottom-up MS for detection, capture, and identification of HNO-modified proteins. They have confidently identified a total of 12 sites of HNO-induced disulfides in key myofilament proteins, such as actin, tropomyosin, myosin heavy chain, MLC1, α-actinin, myosin binding protein C, and troponin C and further characterized 2 disulfides between actin-tropomyosin and myosin heavy chain-MLC1. One potential caveat of their method is the incomplete sequence coverage by a bottom-up MS approach, suggesting the possibility that other HNO-induced modification sites are yet to be identified.

Another important finding of this work is that HNO effects on myofilament contractility can differ dramatically with different HNO donors.3 Because of the transitory nature of HNO, HNO must be generated from donor compounds in situ. The commonly available HNO donors include Angeli’s salt (AS, Na₃N₂O₃), Piloty’s acid (PhSO₂NHOH) and its derivatives.5,7 Despite its short half-life and the well-known downside of corelease of NO₂⁻, AS remained the most widely used HNO donor until recently.7 In 2006, Sha et al21 synthesized a new class of HNO donors, acetyloxy nitroso compounds such as 1-nitrosocyclohexyl acetate (NCA), which releases HNO upon cleavage of the ester bond.7 Gao et al3 compared 2 HNO donors, AS and NCA, and found that AS increased Fₘₘₐₓ but not Ca²⁺ sensitivity, in contrast to NCA which increased both. In addition, some of the disulfide sites mapped differ between AS and NCA treatments. The authors reasoned that the distinct physiological effects of AS and NCA could be because of their different release kinetics: the rapid release of HNO by AS may result in simultaneous conversion of thiolos to N-hydroxysulfenamides, thus preventing the formation of disulfide due to the lack of free thiols, whereas the slower HNO release by NCA favors the generation of N-hydroxysulfenamide intermediates sequentially so that other free thiols have the chance to form disulfide before being converted to sulfimide.3 Therefore, NCA, the new class of HNO donor, will likely exceed the performance of previously characterized HNO donors on cardiac contractility.

A remaining important question is the possibility of an endogenous source of HNO, ie, can HNO serve as an endogenous signaling molecule?9,6 Despite proposals for mechanisms for the endogenous production of HNO, no mechanism has been verified to date mainly because of the lack of evidence for HNO in vivo.4 Also, given that experiments in the present study were performed in intact and skinned muscles from healthy myocardium, the potential functional consequences of NCA-released HNO in diseased myocardium and animal models are not yet known. Nonetheless, Gao et al1 provide direct evidence for HNO-induced disulfide formation in myofilaments, which increases cardiac contractility, further defining the mechanism of HNO as an inotropic agent. This new feature of HNO, combined with previously known cardiovascular
effects, points to HNO donors, particularly NCA, as attractive pharmacological agents for the treatment of HF.

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References

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