ANTIOXIDANTS & REDOX SIGNALING
Volume 14, Number 9, 2011

DOI: 10.1089/ars.2011.3937

Nitroxyl (HNO): A Novel Redox Signaling Molecule

Barbara K. Kemp-Harper

Abstract

Nitroxyl (HNO), the one electron reduced and protonated congener of nitric oxide, is emerging as a novel nitrogen oxide with distinct chemistry and biological actions as compared with its redox sibling. The "thiophilic" nature of HNO underlies many of its unique properties, and attention has been focused on its regulation of cellular function and therapeutic potential, particularly in the treatment of cardiovascular disease. The present Forum issue summarizes the intriguing chemistry and biology of HNO and highlights its impact in the cardiovascular and central nervous systems. Recent advances in the development of new HNO donors and their potential use as tools to study HNO signaling and therapeutic agents are discussed. Evidence is also provided for a role of HNO as a putative, endogenous regulator of vascular function. However, as highlighted in this Forum issue, the development of sensitive methods for HNO detection in a biological system is needed to conclusively prove its *in vivo* generation. As research expands in this area, it is likely that new targets and pharmacological applications of HNO will be discovered. *Antioxid. Redox Signal.* 14, 1609–1613.

Introduction

Nitric oxide ($NO^{\bullet}$) is undoubtedly the most well-recognized nitrogen oxide, with this biologically active gas playing an integral role in the control of vascular homeostasis, the immune response, cellular growth, differentiation, and neurotransmission (25). Recently, however, $NO^{\bullet}$ is having to share centre stage with its one electron reduction product, nitroxyl (HNO).

Although chemists have studied HNO since the early 1900s, significant interest in the biological actions of this nitrogen oxide has arisen only in the last decade with the discovery that HNO has unique chemical and pharmacological properties (17, 33). Indeed, the striking finding by Paolocci and colleagues that HNO, unlike $NO^{\bullet}$, serves as a positive cardiac inotrope (*via* thiol modification) (28) and has potential in the treatment of heart failure (29) has led to marked advances in the field. It is now recognized that thiol- and metallo-containing proteins serve as the primary targets for HNO and, for the most part, the facile reaction of HNO with thiols underlies its distinct biological actions. Interestingly, HNO may also be endogenously generated, and research is directed at developing sensitive detection methods for HNO *in vivo*.

The current Antioxidants and Redox Signaling Forum explores the most recent developments in the field of HNO signaling and pharmacology. The issue consists of two original articles and six reviews by many of the leaders in the HNO field. The original articles focus on the role of HNO as an endogenous regulator of vascular tone and its resistance to tolerance development with continued administration, a finding with therapeutic relevance. The review articles unravel the chemistry and biology of HNO and provide explanations for its distinct biological actions as compared with $NO^{\bullet}$. Moreover, the importance of HNO in the cardiovascular and central nervous systems (CNS) is explored, and the development of novel HNO donors and their therapeutic potential is highlighted.

Before proceeding further, it should be noted, as emphasized in this issue by Fukuto and Carrington (13), that the use of the term nitroxyl to describe HNO is ambiguous. Although this term is commonly used when describing the biological actions of HNO, such nomenclature does not describe its chemical structure and more appropriate terms are nitrosyl hydride or hydrogen oxonitrate. Moreover, nitroxides ($R_2NO^{\cdot}/R_2N^{+}\text{-}O^{-}$) are commonly referred to as HNOs, and caution should be exerted when reviewing the literature, as some reports will not be related to HNO.

HNO Chemistry and Biology

In the first review article of the Forum, Wink and colleagues (11) have eloquently compared and contrasted the chemistry of $NO^{\bullet}$ and HNO and discussed the chemical biology of HNO that underlies its unique actions. As a weak acid, HNO (pK_a 11.4) exists in its protonated form at physiological pH (2) and undergoes rapid dimerisation and dehydration to nitrous oxide. The authors highlight that the acid/base chemistry of HNO is distinct with this nitrogen oxide able to serve as a nucleophile or electrophile depending on the reaction conditions/partners. Indeed, the two main biological targets

Vascular Biology and Immunopharmacology Group, Department of Pharmacology, Monash University, Clayton, Victoria, Australia.

of HNO are thiols and metals that it oxidizes and reduces, respectively.

Wink and colleagues (11) describe the nature of the interaction of HNO *versus* $NO^{\bullet}$ with thiols, which is a major distinguishing feature of these redox siblings. Thus, although $NO^{\bullet}$ requires autoxidation to induce thiol nitrosation, HNO associates with thiols rapidly and with ease, leading to their oxidation. The authors indicate that thiol modification by HNO is a two-step process, such that association with a thiol first leads to the generation of a N-hydroxysulfenamide intermediate which can react with additional thiols to form a disulfide (reversible process) or undergo rearrangement to generate a sulfinamide (irreversible process), which serves as a unique footprint for HNO activity (14, 32).

The biological consequences of HNO-thiol interactions are explored in the review by Fukuto and Carrington (13). Thus, the reactivity of HNO with thiols targets its actions to thiol-containing proteins (*i.e.*, aldehyde dehydrogenase, glyceraldehyde 3-phosphate dehydrogenase) and receptors/ion channels (*i.e.*, cardiac sarcoplasmic ryanodine receptors). The authors indicate that it is in the myocardium where the ability of HNO to modify thiol activity has gained much attention. Here HNO interacts with thiol proteins involved in sarcoplasmic reticulum (SR) Ca^{2+} release and uptake, to enhance Ca^{2+} cycling, and increase myocardial contractility, a phenomenon not observed with $NO^{\bullet}$. Both processes are reversible, indicative of disulfide or N-hydroxysulfenamide formation. Fukuto and Carrington (13) raise the interesting, although purely speculative, concept that the formation of a stable N-hydroxysulfenamide within a protein by HNO may confer specific alterations in protein function.

Intriguingly, HNO preferentially targets thiol-containing proteins, leaving cellular gluthathione levels unaltered. As discussed by Fukuto and Carrington (13), the reasons underlying such selectivity remain an enigma, particularly given the high cellular concentration of gluthathione [1–10 m*M*]. The authors put forward a number of potential explanations. First, HNO-thiol modifications may be readily reversed by biological reductants such that only a few select thiols remain oxidized by HNO. Second, irreversible (*i.e.*, sulfinamide) *versus* reversible (*i.e.*, disulfide) thiol modifications by HNO may predominate. Third, the reactivity of HNO with a thiol will depend on its pK_a and cellular location such that HNO, as an electrophile, may target nucleophilic thiolates (RS-) over sulfhydryls (11); and the hydrophobic nature of HNO may direct its action to membrane-bound molecules. From a therapeutic viewpoint, such selectivity of HNO for certain protein thiols is fortuitous, as it underlies the ability of HNO to increase myocardial contractility while maintaining cellular redox status as discussed in detail by Paolocci and colleagues (35) in this issue.

Further distinction between HNO and $NO^{\bullet}$ is evident on consideration of their interaction with metalloproteins. $NO^{\bullet}$ interacts predominantly with ferrous (Fe^{2+}) heme proteins, whereas HNO preferentially targets ferric (Fe^{3+}) heme groups (23). As discussed by Wink and colleagues (11), such interactions lead ultimately to the generation of the same end product, a ferrous nitrosyl complex (ON-Fe^{2+}-Heme). Undoubtedly, the most well-recognized heme-containing protein HNO interacts with is the $NO^{\bullet}$ receptor, soluble guanylyl cyclase (sGC). However, as explored by Fukuto and Carrington (13), whether HNO itself can directly activate sGC or first requires oxidation to $NO^{\bullet}$ remains contentious with recent studies providing evidence in support of direct (22) and indirect (39) targeting of sGC by HNO. Although further work is required to reconcile these findings, it is clear that the sGC/cGMP signaling pathway plays a predominant role in the actions of HNO in the vasculature (3). Indeed, given the preference of HNO for ferric (Fe^{3+}) *versus* ferrous (Fe^{2+}) heme groups, it is tempting to speculate that HNO may target the oxidized (Fe^{3+}), $NO^{\bullet}$-insensitive, form of sGC. However, this concept was recently refuted with HNO shown to only activate the reduced (Fe^{2+}) state of sGC (22, 39). The relative resistance of Fe^{3+}-sGC to substitution may necessitate the use of high concentrations of HNO to achieve activation. Interestingly, higher concentrations of HNO can limit sGC activity, possibly *via* oxidation of cysteine residues on the protein—an action that may serve to limit adverse effects of HNO, such as deleterious hypotension (22).

The unique chemistry and biological actions of HNO just discussed have been elucidated *via* the use of HNO donor compounds that are necessary due to the rapid dimerisation of HNO. The HNO donor, Angeli's salt ($Na_2N_2O_3$), first synthesized by the Italian chemist Angelo Angeli in 1896, has been the mainstay of the field. However, its short half life (2–3 min) and concomitant release of nitrite, which itself has biological actions, confers limitations. As such, pure and longer-acting HNO donors are needed to fully characterize the physiological actions and potential toxicity of HNO and provide a platform for HNO-based therapeutics.

Advances in this area have been made with the development/modification of novel HNO donors as discussed in detail by DuMond and King (9). The authors have indicated that the development of HNO donors is based predominantly on the generation of HNO from hydroxylamine derivatives (*i.e.*, Angeli's salt, Piloty's acid, cyanamide, and diazeniumdiolates) or the decomposition of nitroso compounds (*i.e.*, acyl nitroso, acyloxy nitroso). Although secondary amine-derived diazeniumdiolates (NONOates) are well known as $NO^{\bullet}$ donors, primary amine-derived NONOates such as the isopropylamine NO-adduct can serve as HNO donors (24). The ability to modify the primary amine in this class of compounds provides the opportunity to generate HNO donors with different half lives and release kinetics. Interestingly, King and colleagues (31) have developed a new class of acyloxy nitroso-based HNO donors (blue compounds), which release HNO on ester hydrolysis. DuMond and King (9) highlighted that changes in structure of the organic group can alter the kinetics of HNO release. Although initial studies indicate that these compounds have similar cardiovascular actions as Angeli's salt (10, 31), further investigation is required as acyloxy nitroso compounds themselves react with thiols and such an action may compete with the release of HNO from these agents. It is clear that significant progress is being made in the generation of new HNO donor compounds, and these tools will strengthen research efforts in the field.

Actions of HNO in the Cardiovascular System and CNS

The next set of review articles in this Forum focus on the role of HNO in the regulation of myocardial and vascular function and its role in the CNS. Although the effects of $NO^{\bullet}$ in the cardiovascular system are attributed mainly to the activation of sGC and subsequent increase in cGMP, the facile

reaction of HNO with thiols confers actions distinct from those of its redox sibling in this setting. Indeed, as comprehensively reviewed by Paolocci and colleagues (35), there is no better place to look for the unique pharmacological profile of HNO than in the heart.

In 2001, Paolocci and colleagues (28) identified an ability of HNO to increase myocardial contractility (positive inotropy) and hasten relaxation (positive lusitropy)–an effect not observed with $NO^{\bullet}$. Importantly, such positive inotropic effects of HNO are preserved in the setting of acute experimental heart failure (29) and this property, coupled with its unloading action (*via* vasodilatation), make HNO a promising agent for the treatment of heart failure.

Thiol modification by HNO underpins its cardiostimulatory action. As discussed by Paolocci and colleagues (35), the excitation-contraction machinery of the myocardium is a prime target for HNO, as these structures contain cysteine residues (redox switches) that are susceptible to redox modification. Specifically, HNO activates cardiac ryanodine receptors (RyR2), *via* oxidation of cysteine thiols, leading to Ca^{2+} release from the SR (4, 34). In addition, HNO can target thiol groups on the SR Ca^{2+}-ATPase (SERCA2a) (19) and its regulatory partner, phospholamban (12), to stimulate Ca^{2+} uptake into the SR as well as sensitize myofilaments to Ca^{2+} (8). These actions of HNO on SR Ca^{2+} cycling and myofilament function lead to an increase in contractile force and account, to a large extent, for its cardioprotective properties.

The authors also discuss the protective (27) and detrimental (21) actions of the HNO donor, Angeli's salt, which been reported in the setting of myocardial ischemia/reperfusion (I/R) injury. Although HNO protects against I/R injury when administered preischemia, it aggravates injury when given at the time of reperfusion and the mechanisms underlying such opposing actions remain to be elucidated.

Complementary to its positive inotropic actions, HNO also serves as a vasodilator and anti-aggregatory agent. Our understanding of the vasoprotective actions of HNO have been expanded over the past few years as reviewed by Bullen and colleagues (3). Similar to $NO^{\bullet}$, HNO elicits vasodilatation predominantly *via* activation of sGC, yet it can also signal *via* distinct pathways including the release of calcitonin gene-related peptide and the activation of K^+ channels (K_v, K_{ATP}).

The authors also explore the possibility that the vasoprotective actions of HNO may be preserved under pathophysiological conditions associated with increased reactive oxygen species generation and compromised $NO^{\bullet}$ signaling. Thus, unlike traditional $NO^{\bullet}$ donors, such as the organic nitrate glyceryl trinitrate (GTN), HNO is resistant to scavenging by superoxide and does not develop tolerance after acute administration *in vitro* (16). Moreover, in an original article in this Forum issue, Irvine *et al.* (15) demonstrate that chronic *in vivo* administration of the HNO donor Angeli's salt, in rats, does neither lead to tolerance to its vasodilatory actions (*in vivo* or *ex vivo*) nor cause cross-tolerance to GTN. Such findings are of clinical relevance, suggesting that HNO donors may be of use in patients resistant to the effects of GTN.

Bullen *et al.* (3) highlight that there is a paucity of information on the effects of HNO on cellular growth and proliferation and its potential anti-inflammatory actions. Although a recent study has identified an ability of the HNO donor, isopropylamine NO-adduct, to inhibit vascular smooth muscle and endothelial cell proliferation (36) and preliminary evidence suggests that HNO is able to limit vascular NADPH-oxidase derived superoxide generation, further investigation is needed to fully elucidate the cytoprotective armoury of HNO.

Although much attention has been afforded to the actions of HNO in the cardiovascular system, evidence is emerging that HNO also modulates the CNS, as comprehensively reviewed by Donzelli and colleagues (6). The authors indicate that HNO may target thiol groups to modulate neuronal function, including stimulation of *N*-methyl-D-aspartate receptors (18) and, hypothetically, neuronal RyR to evoke excitoxicity and calcium-induced calcium release, respectively. Such actions of HNO, together with its potential to impair energy metabolism, may be detrimental in the CNS and high concentrations of the HNO donor Angeli's salt have been shown to induce neurotoxicity (motor neuron injury, reduced striatial dopamine) (37). In addition, Donzelli and colleagues discuss their recent finding that systemic administration of Angeli's salt increased infarct size in an animal model of ischemic stroke (5). Taken together, these studies indicate that HNO may have adverse effects in the CNS, yet further work is required to determine whether such neurotoxic actions are observed with lower doses of HNO donors, which have been shown to be cardioprotective, if they are dependent on the route of administration and nature of the HNO donor used and the model of neurotoxicity employed.

Endogenous Generation of HNO

Although the chemistry and pharmacology of HNO is being rapidly unraveled, a major question remains unanswered—is HNO generated endogenously? Despite the identification of a number of potential biosynthetic pathways for HNO, the lack of direct detection methods for this nitrogen oxide has precluded definitive proof for its production *in vivo.*

Nevertheless, the role of HNO as an endogenous signaling molecule can be inferred from pharmacological studies that employ well-characterized tools (*i.e.*, thiols and $NO^{\bullet}$ scavengers) to discriminate between HNO and $NO^{\bullet}$. A number of reviews in this Forum issue have addressed the concept of putative endogenous HNO formation from NO synthase (NOS)-dependent and -independent sources in the myocardium (35), vasculature (3), and brain (6). Bullen *et al.* (3) indicated that in the vasculature, HNO may serve as an endothelium-derived relaxing factor working in concert with $NO^{\bullet}$ to cause vasorelaxation of conduit and resistance arteries (1). Further support for this idea is provided by the original article by Yuill *et al.* (38) in this issue, in which the authors have identified an ability of both exogenous and endothelial-derived HNO to initiate spreading vasodilatation in isolated, pressurized rat small mesenteric arteries. Such a phenomenon occurs as a consequence of HNO-mediated vascular smooth muscle hyperpolarization and suggests a physiological role for HNO in maintaining tissue perfusion. Importantly, spreading vasodilatation is not observed with $NO^{\bullet}$, further highlighting the distinct actions of these redox siblings. Evidence also suggests that HNO may serve as a nitregic transmitter (7, 20), and Donzelli and colleagues (6) speculated that the generation of HNO from neuronal NOS during cerebral ischemia may contribute to CNS injury, a concept which remains to be proven.

The detection of HNO remains a challenge for the field. However, with the recent development of small-molecule, metal-based fluorescent probes specific for HNO (30), we are

now hopefully one step closer to determining whether HNO is an endogenous signaling partner of NO•.

Future Directions

Over the last decade, significant advances have been made in our understanding of the chemistry and biology of HNO. It is becoming increasingly evident that the thiol reactivity of HNO underlies much of its unique pharmacology as compared with NO•. From a therapeutic perspective, HNO-induced thiol modification confers potential in the use of HNO donors in the treatment of alcoholism (*via* inhibition of aldehyde dehydrogenase) and heart failure. Moreover, the vasoprotective actions of HNO, coupled with its resistance to tolerance development, indicate that these compounds may be utilized in the treatment of vascular dysfunction associated with angina and atherothrombotic syndromes. Interestingly, in this issue, Wink and colleagues (11) also highlighted a potential use of HNO donors in the treatment of cancer. Thus, at high millimolar concentrations, HNO can lead to DNA double-strand breaks and cellular toxicity. In addition, HNO inhibits breast and neuroblastoma cancer proliferation and limits blood vessel density in tumors (26), possibly *via* HNO-mediated inhibition of glycolysis (*via* glyceraldehyde 3-phosphate dehydrogenase inhibition) and DNA repair (*via* poly (ADP-ribose) polymerase inhibition). Although such actions of HNO are desirable in cancer therapy, it will be necessary to limit the potential cytotoxic effects of HNO (*i.e.*, *via* dose) in the treatment of other pathophysiological conditions. This is of particular relevance in light of the findings that HNO can, under certain circumstances, aggravate I/R injury in the myocardium and brain. As such, it is imperative that future studies fully elucidate the potential toxicological effects of HNO and delineate the impact of dose, mode, and timing of administration, model (*in vitro vs. in vivo*), and species utilized in such actions.

Currently, the field of HNO research is faced with a number of burning questions. Is HNO an endogenously generated species? How does HNO selectively target certain thiols/thiol proteins? Are the actions of HNO sustained under redox-altered conditions? Does HNO influence energy production or ROS generation? What impact does HNO have outside the cardiovascular system? Excitingly, with advances being made in the development of pure, long-lasting HNO donors and sensitive detection methods for HNO in the intact cell, it is anticipated that many of these questions will be answered in the near future.

HNO is no longer the forgotten redox sibling of NO•. Its unique chemistry and pharmacology has captured the interest of researchers in the field and focused attention on its potential role as an endogenous regulator of cellular signaling and therapeutic agent. I wish to thank all the authors for their valuable contributions to this Forum issue; it has provided an authoritative insight into the area of HNO research.

Acknowledgments

Dr. Barbara Kemp-Harper is supported by a grant from the National Health and Medical Research Council, Australia (Grant ID: 606556).

References

1. Andrews KL, Irvine JC, Tare M, Apostolopoulos J, Favaloro JL, Triggle CR, and Kemp-Harper BK. A role for nitroxyl (HNO) as an endothelium-derived relaxing and hyperpolarizing factor in resistance arteries. *Br J Pharmacol* 157: 540–550, 2009.
2. Bartberger MD, Liu W, Ford E, Miranda K, Switzer CH, and Fukuto JM. The reduction potential of nitric oxide (NO) and it importance to NO biochemistry. *Proc Natl Acad Sci USA* 99: 10958–10963, 2002.
3. Bullen ML, Miller AA, Andrews KL, Irvine JC, Ritchie RH, Sobey CG, and Kemp-Harper BK. Nitroxyl (HNO) as a vasoprotective signaling molecule. *Antioxid Redox Signal* 14: 1675–1686, 2011.
4. Cheong E, Tumbev V, Abramson J, Salama G, and Stoyanovsky DA. Nitroxyl triggers Ca^{2+} release from skeletal and cardiac sarcoplasmic reticulum by oxidizing ryanodine receptors. *Cell Calcium* 37: 87–96, 2005.
5. Choe CU, Lewerenz J, Fischer G, Uliasz TF, Espey MG, Hummel FC, King SB, Schwedhelm E, Boger RH, Gerloff C, Hewett SJ, Magnus T, and Donzelli S. Nitroxyl exacerbates ischemic cerebral injury and oxidative neurotoxicity. *J Neurochem* 110: 1766–1773, 2009.
6. Choe CU, Lewerenz J, Gerloff C, Magnus T, and Donzelli S. Nitroxyl in the central nervous system. *Antioxid Redox Signal* 14: 1699–1711, 2011.
7. Costa G, Labadia A, Triguero D, Jimenez E, and Garcia-Pascual A. Nitrergic relaxation in urethral smooth muscle: involvement of potassium channels and alternative redox forms of NO. *Naunyn Schmiedebergs Arch Pharmacol* 364: 516–523, 2001.
8. Dai T, Tian Y, Tocchetti CG, Katori T, Murphy AM, Kass DA, Paolocci N, and Gao WD. Nitroxyl increases force development in rat cardiac muscle. *J Physiol* 580: 951–950, 2007.
9. DuMond JF and King SB. The chemistry of nitroxyl-releasing compounds. *Antioxid Redox Signal* 14: 1637–1648, 2011.
10. El-Armouche A, Wahab A, Wittkopper K, Schulze T, Bottcher F, Pohlmann L, King SB, DuMond JF, Gerloff C, Boger RH, Eschenhagen T, Carrier L, and Donzelli S. The new HNO donor, 1-nitrosocyclohexyl acetate, increases contractile force in normal and beta-adrenergically desensitised ventricular myocytes. *Biochem Biophys Res Commun* 4022: 340–344, 2010
11. Flores-Santana W, Salmon DJ, Donzelli S, Switzer CH, Basudhar D, Ridnour L, Cheng R, Glynn SA, Paolocci N, Fukuto JM, Miranda KM, and Wink DA. The specificity of nitroxyl chemistry is unique among nitrogen oxides in biological systems. *Antioxid Redox Signal* 14: 1659–1674, 2011.
12. Froehlich JP, Mahaney JE, Keceli G, Pavlos CM, Goldstein R, Redwood AJ, Sumbilla C, Lee DI, Tocchetti CG, Kass DA, Paolocci N, and Toscano JP. Phospholamban thiols play a central role in activation of the cardiac muscle sarcoplasmic reticulum calcium pump by nitroxyl. *Biochemistry* 47: 13150–13152, 2008
13. Fukuto JM and Carrington SJ. HNO signaling mechanisms. *Antioxid Redox Signal* 14: 1649–1657, 2011.
14. Hoffman MD, Walsh GM, Rogalski JC, and Kast J. Identification of nitroxyl-induced modifications in human platelet proteins using a novel mass spectrometric detection method. *Mol Cell Proteomics* 8: 887–903, 2009.
15. Irvine JC, Kemp-Harper BK, and Widdop RE. Chronic administration of the HNO donor Angeli's salt does not lead to tolerance, cross-tolerance, or endothelial dysfunction: comparison with GTN and DEA/NO. *Antioxid Redox Signal* 14: 1615–1624, 2011.

16. Irvine JC, Favaloro JL, Widdop RE, and Kemp-Harper BK. Nitroxyl anion donor, Angeli's salt, does not develop tolerance in rat isolated aortae. *Hypertension* 49: 885–892, 2007.
17. Irvine JC, Ritchie RH, Favaloro JL, Andrews KL, Widdop RE, and Kemp-Harper BK. Nitroxyl (HNO): the Cinderella of the nitric oxide story. *Trends Pharmacol Sci* 29: 601–608, 2008.
18. Kim WK, Choi YB, Rayudu PV, Das P, Asaad W, Arnelle DR, Stamler JS, and Lipton SA. Attenuation of NMDA receptor activity and neurotoxicity by nitroxyl anion, NO^-. *Neuron* 24: 461–469, 1999.
19. Lancel S, Zhang J, Evangelista A, Trucillo MP, Tong X, Siwik DA, Cohen RA, and Colucci WS. Nitroxyl activates SERCA in cardiac myocytes via glutathiolation of cysteine 674. *Circ Res* 104: 720–723, 2009.
20. Li CG, Karagiannis J, and Rand MJ. Comparison of the redox forms of nitrogen monoxide with the nitrergic transmitter in the rat anococcygeus muscle. *Br J Pharmacol* 127: 826–834, 1999.
21. Ma XL, Gao F, Liu GL, Lopez BL, Christopher TA, Fukuto JM, Wink DA, and Feelisch M. Opposite effects of nitric oxide and nitroxyl on postischemic myocardial injury. *Proc Natl Acad Sci USA* 96: 14617–14622, 1999.
22. Miller TW, Cherney MM, Lee AJ, Francoleon NE, Farmer PJ, King SB, Hobbs AJ, Miranda KM, Burstyn JN, and Fukuto JM. The effects of nitroxyl (HNO) on soluble guanylate cyclase activity: interactions at ferrous heme and cysteine thiols. *J Biol Chem* 284: 21788–21796, 2009.
23. Miranda KM, Nims RW, Thomas DD, Espey MG, Citrin D, Bartberger MD, Paolocci N, Fukuto JM, Feelisch M, and Wink DA. Comparison of the reactivity of nitric oxide and nitroxyl with heme proteins. A chemical discussion of the differential biological effects of these redox related products of NOS. *J Inorg Biochem* 93: 52–60, 2003.
24. Miranda KM, Katori T, Torres de Holding CL, Thomas L, Ridnour LA, McLendon WJ, Cologna SM, Dutton AS, Champion HC, Mancardi D, Tocchetti CG, Saavedra JE, Keefer LK, Houk KN, Fukuto JM, Kass DA, Paolocci N, and Wink DA. Comparison of the NO and HNO donating properties of diazeniumdiolates: primary amine adducts release HNO in vivo. *J Med Chem* 48: 8220–8228, 2005.
25. Moncada S and Higgs EA. The discovery of nitric oxide and its role in vascular biology. *Br J Pharmacol* 147: S193–S201, 2006.
26. Norris AJ, Sartippour MR, Lu M, Park T, Rao JY, Jackson MI, Fukuto JM, and Brooks MN. Nitroxyl inhibits breast tumor growth and angiogenesis. *Int J Cancer* 122: 1905–1910, 2008.
27. Pagliaro P, Mancardi D, Rastaldo R, Penna C, Gattullo D, Miranda KM, Feelisch M, Wink DA, Kass DA, and Paolocci N. Nitroxyl affords thiol-sensitive myocardial protective effects akin to early preconditioning. *Free Radic Biol Med* 34: 33–43, 2003.
28. Paolocci N, Saavedra WF, Miranda KM, Martignani C, Isoda T, Hare JM, Espey MG, Fukuto JM, Feelisch M, Wink DA, and Kass DA. Nitroxyl anion exerts redox-sensitive positive cardiac inotropy *in vivo* by calcitonin gene-related peptide signaling. *Proc Natl Acad Sci USA* 98: 10463–10468, 2001.
29. Paolocci N, Katori T, Champion HC, St. John ME, Miranda K, Fukuto JM, Wink DA, and Kass DA. Positive iontropic and lusitropic effects of HNO/NO- in failing hearts: Independence from β-adrenergic signalling. *Proc Natl Acad Sci USA* 100: 5537–5542, 2003.
30. Rosenthal J and Lippard SJ. Direct detection of nitroxyl in aqueous solution using a tripodal copper(II) BODIPY complex. *J Am Chem Soc* 132: 5536–5537, 2010.
31. Sha X, Isbell TS, Patel RP, Day CS, and King SB. Hydrolysis of acyloxy nitroso compounds yields nitroxyl (HNO). *J Am Chem Soc* 128: 9687–9692, 2006.
32. Shen B and English AM. Mass spectrometric analysis of nitroxyl-mediated protein modification: comparison of products formed with free and protein-based cysteines. *Biochemistry* 44: 14030–14044, 2005.
33. Switzer CH, Flores-Santana W, Mancardi D, Donzelli S, Basudhar D, Ridnour LA, Miranda KM, Fukuto JM, Paolocci N, and Wink DA. The emergence of nitroxyl (HNO) as a pharmacological agent. *Biochim Biophys Acta* 1787: 835–840, 2009.
34. Tocchetti CG, Wang W, Froehlich JP, Huke S, Aon MA, Wilson GM, Benedetto GD, O'Rourke B, Gao WD, Wink DA, Toscano JP, Zaccolo M, Bers DM, Valdivia HH, Cheng H, Kass DA, and Paolocci N. Nitroxyl improves cellular heart function by directly enhancing cardiac sarcoplasmic reticulum Ca^{2+} cycling. *Circ Res* 100: 96–104, 2007.
35. Tocchetti CG, Stanley BA, Murray CI, Sivakumaran V, Donzelli S, Mancardi D, Pagliaro P, Gao WD, van Eyk J, Kass DA, Wink DA, and Paolocci N. Playing with cardiac "redox switches": the "HNO way" to modulate cardiac function. *Antioxid Redox Signal* 14: 1687–1698, 2011.
36. Tsihlis ND, Murar J, Kapadia MR, Ahanchi SS, Oustwani CS, Saavedra JE, Keefer LK, and Kibbe MR. Isopropylamine NONOate (IPA/NO) moderates neointimal hyperplasia following vascular injury. *J Vasc Surg* 51: 1248–1259, 2010.
37. Vaananen AJ, Moed M, Tuominen RK, Helkamaa TH, Wiksten M, Liesi P, Chiueh CC, and Rauhala P. Angeli's salt induces neurotoxicity in dopaminergic neurons *in vivo* and *in vitro*. *Free Radic Biol Med* 37: 381–389, 2003.
38. Yuill KH, Yarova P, Kemp-Harper BK, Garland CJ, and Dora KA. A novel role for HNO in local and spreading vasodilatation in rat mesenteric resistance arteries. *Antioxid Redox Signal* 14: 1625–1635, 2011.
39. Zeller A, Wenzl MV, Beretta M, Stessel H, Russwurm M, Koesling D, Schmidt K, and Mayer B. Mechanisms underlying acivation of soluble guanylate cyclase by the HNO donor Angeli's Salt. *Mol Pharmacol* 76: 1115–1122, 2009.

Address correspondence to:
Dr. Barbara K. Kemp-Harper
Vascular Biology and Immunopharmacology Group
Department of Pharmacology
Monash University
Clayton VIC 3800
Australia

E-mail: barbara.kemp@monash.edu

Date of first submission to ARS Central, February 6, 2011; date of acceptance, February 7, 2011.

Abbreviations Used

CNS = central nervous system
GTN = glyceryl trinitrate
HNO = nitroxyl
I/R = ischemia/reperfusion
$NO^•$ = nitric oxide
NOS = nitric oxide synthase
RyR = ryanodine receptor
SERCA = SR Ca^{2+}-ATPase
sGC = soluble guanylyl cyclase
SR = sarcoplasmic reticulum

This article has been cited by:

1. C.H. Leo, A. Joshi, J.L. Hart, O.L. Woodman. 2012. Endothelium-dependent nitroxyl-mediated relaxation is resistant to superoxide anion scavenging and preserved in diabetic rat aorta. *Pharmacological Research* **66**:5, 383-391. [CrossRef]
2. Meredith R. Cline, Tyler A. Chavez, John P. Toscano. 2012. Oxidation of N-Hydroxy-L-Arginine by Hypochlorous Acid to Form Nitroxyl (HNO). *Journal of Inorganic Biochemistry* . [CrossRef]
3. Kevin M. Nash, Antal Rockenbauer, Frederick A. Villamena. 2012. Reactive Nitrogen Species Reactivities with Nitrones: Theoretical and Experimental Studies. *Chemical Research in Toxicology* **25**:8, 1581-1597. [CrossRef]
4. Narufumi Kitamura, Tatsuhiro Hiraoka, Kazuo Tanaka, Yoshiki Chujo. 2012. Reduced glutathione-resisting 19F NMR sensors for detecting HNO. *Bioorganic & Medicinal Chemistry* **20**:15, 4668-4674. [CrossRef]
5. María Elena Knott, Damián Dorfman, Mónica S. Chianelli, Daniel A. Sáenz. 2012. Effect of Angeli's salt on the glutamate/glutamine cycle activity and on glutamate excitotoxicity in the hamster retina. *Neurochemistry International* **61**:1, 7-15. [CrossRef]
6. Chris R. Triggle, Samson Mathews Samuel, Shalini Ravishankar, Isra Marei, Gnanapragasam Arunachalam, Hong Ding. 2012. The endothelium: influencing vascular smooth muscle in many ways. *Canadian Journal of Physiology and Pharmacology* **90**:6, 713-738. [CrossRef]
7. Gizem Keceli, John P. Toscano. 2012. Reactivity of Nitroxyl-Derived Sulfinamides. *Biochemistry* 120514141935001. [CrossRef]
8. Daryl A. Guthrie, Nam Y. Kim, Maxime A. Siegler, Cathy D. Moore, John P. Toscano. 2012. Development of N -Substituted Hydroxylamines as Efficient Nitroxyl (HNO) Donors. *Journal of the American Chemical Society* 120117130646001. [CrossRef]
9. Mar#a Elena Knott, Dami#n Dorfman, M#nica S. Chianelli, Daniel A. S#enz. 2012. Effect of angeli#s salt on the glutamate/glutamine cycle activity and on glutamate excitotoxicity in the hamster retina. *Neurochemistry International* . [CrossRef]
10. Art D. Sutton, Morgan Williamson, Hilary Weismiller, John P. Toscano. 2011. Optimization of HNO Production from N , O - bis -Acylated Hydroxylamine Derivatives. *Organic Letters* 111223133321004. [CrossRef]