Soluble Guanylate Cyclase Is Required for Systemic Vasodilation But Not Positive Inotropy Induced by Nitroxyl in the Mouse

Guangshuo Zhu, Dieter Groneberg, Gautam Sikka, Daijiro Hori, Mark J. Ranek, Taishi Nakamura, Eiki Takimoto, Nazareno Paolocci, Dan E. Berkowitz, Andreas Friebe, David A. Kass

Abstract—Nitroxyl (HNO), the reduced and protonated form of nitric oxide (NO), confers unique physiological effects including vasorelaxation and enhanced cardiac contractility. These features have spawned current pharmaceutical development of HNO donors as heart failure therapeutics. HNO interacts with selective redox sensitive cysteines to effect signaling but is also proposed to activate soluble guanylate cyclase (sGC) in vitro to induce vasodilation and potentially enhance contractility. Here, we tested whether sGC stimulation is required for these HNO effects in vivo and if HNO also modifies a redox-sensitive cysteine (C42) in protein kinase G-1α to control vasorelaxation. Intact mice and isolated arteries lacking the sGC-β subunit (sGCKO, results in full sGC deficiency) or expressing solely a redox-dead C42S mutant protein kinase G-1α were exposed to the pure HNO donor, CXL-1020. CXL-1020 induced dose-dependent systemic vasodilation while increasing contractility in controls; however, vasodilator effects were absent in sGCKO mice whereas contractility response remained. The CXL-1020 dose reversing 50% of preconstricted force in aortic rings was ≈400-fold greater in sGCKO than controls. Cyclic-GMP and cAMP levels were unaltered in myocardium exposed to CXL-1020, despite its inotropic-vasodilator activity. In protein kinase G-1α C42S mice, CXL-1020 induced identical vasorelaxation in vivo and in isolated aortic and mesenteric vessels as in littermate controls. In both groups, dilation was near fully blocked by pharmacologically inhibiting sGC. Thus, sGC and cGMP-dependent signaling are necessary and sufficient for HNO-induced vasodilation in vivo but are not required for positive inotropic action. Redox modulation of protein kinase G-1α is not a mechanism for HNO-mediated vasodilation. (Hypertension. 2015;65:385-392. DOI: 10.1161/HYPERTENSIONAHA.114.04285.) • Online Data Supplement

Key Words: cardiotoxic agents ■ cardiovascular physiological phenomena ■ cyclic GMP-dependent protein kinases ■ guanylate cyclase ■ nitrogen oxides ■ pharmacology ■ vasodilation

Nitroxyl (HNO) is the protonated, one electron-reduced form of the signaling molecule nitric oxide (NO). Like NO, HNO has prominent vascular effects inducing vasodilation in conduit and resistance arteries. However, HNO differs from NO in that tolerance does not develop with repeated exposure, and its effects are not suppressed by oxidative stress, but rather by administration of reducing agents such as t-cysteine. Exogenously administered HNO donors exhibit prominent pharmacological activity on the cardiovascular system, combining venous and arterial dilation with an augmentation of cardiac contractility and relaxation. This net constellation of effects has triggered interest in HNO as a heart failure therapy, with clinical trials now underway. Several mechanisms for HNO-mediated vasodilation have been proposed, including NO-like activity on soluble guanylate cyclase (sGC) triggering cGMP-dependent signaling, activation of voltage and calcium-dependent potassium hyperpolarizing channels, and the stimulation of calcitonin gene-related peptide. HNO-vasodilation is blocked in vitro by the sGC antagonist [1H-[1,2,4]oxadiazolo-[4, 3-a]quinoxalin-1-one] (ODQ), which has been interpreted as supporting an sGC dependent mechanism. However, whether HNO directly interacts with sGC heme has been questioned by molecular model analysis, and data showing this requires HNO conversion to NO by superoxide dismutase. Furthermore, inhibition of vasodilation by ODQ does not guarantee sGC is solely involved. ODQ oxidizes the heme in sGC to block NO responsiveness, but it can also modify other heme-containing proteins including hemoglobin, nitric oxide synthase, and cytochrome p-450: enzymes that impact vasodilation. Last, alternative mechanisms including calcitonin gene-related peptide and cysteine-42 oxidation in protein-kinase G-1α (PKG1α),...
the latter thought responsible for cGMP-independent H2O2-induced vasodilation, could play a role.25,26

There are also controversies surrounding the role of sGC in mediating HNO cardiac contractility. Low levels of cGMP stimulate myocyte contractility by impairing cAMP hydrolysis by phosphodiesterase type 3 (PDE3),27,28 whereas higher levels blunt contractility by PKG1α-dependent mechanisms29 and cGMP activation of PDE2 resulting in cAMP hydrolysis.30 Although ODQ has no effect on HNO-stimulated inotropy in isolated ventricular myocytes,11,12 it reportedly blunts HNO inotropy in isolated rat hearts.31 Yet, unlike NO, HNO donors do not inhibit β-adrenergic stimulated contractility.11,12 This has suggested different mechanisms, most notably HNO modification of selective cysteine to form reversible S–S bonds or sulfinamides (RS(O)OH).1 In the heart, this chemistry alters phospholamban,13,32 sarcoplasmic reticular ATPase,33 the rymyodine receptor,25 myosin light chain, and tropomyosin,31 resulting in enhanced Ca2+ cycling and myofilament sensitivity.

Critically, no prior work has tested whether sGC is required for HNO effects in vivo as the compounds to inhibit sGC or quench NO cannot be administered in the intact animal, and genetic deletion studies have not been performed. Furthermore, prior HNO studies have mostly used Angeli’s salt (AS) that degrades into HNO but also nitrite which is itself a vasodilator.34 Some have used isopropylamine NONOate, which is a pH-dependent HNO donor,35 while others acyloxy-nitroso compounds that are limited to in vitro use.36,37 Here, we performed studies in mice genetically lacking the sGCβ1 subunit (sGCKO) that results in loss of the entire sGC protein complex. The role of PKG1α oxidation at C42 was also studied using only the C42S-mutant PKG1α.26 Last, we used the pure HNO donor CLX-1020 that is stable at room temperature and can be administered both in vitro and in vivo.38,39 We find both vasodilator and inotropic effects of HNO are observed in control mice, but vasodilation is absent while positive inotropy persists in sGCKO mice. By contrast, HNO-induced dilation is unaltered by expressing solely the C42S-mutant PKG1α.

Methods

Mouse Models

Studies were performed in C57Bl/6J adult mice (Jackson Laboratories, Bar Harbor, ME) and in sGCKO and PKG1α−/− mice and their respective littermate controls. Both genetic models have been previously described26,30 and develop hypertension (+ ≈30 mm Hg systolic pressure versus controls), sGCKO mice develop gastrointestinal dysmotility and consequent early lethality but can survive by using a fiber-free diet. These mice were maintained and studied at the University of Würzburg to avoid trauma from shipping. PKG1α−/− mice (provided by Phil Eaton, Kings College, London, UK) display no gastrointestinal dysfunction and live a normal lifespan. Isolated and in vivo resistance vessels in these mice dilate in response to cGMP stimuli but show reduced responsiveness to hydrogen peroxide.38 Mice aged 2 to 4 months were used in the study. The protocols were approved by the Animal Care and Use Committee of the Johns Hopkins Medical Institutions or University of Würzburg.

Pharmaceuticals

CXL-1020 (2-Methylsulfonyl benzene N-hydroxy salanomide, Cardioxyl Pharmaceuticals Inc, NC), which chemically decomposes into HNO and an organic byproduct (CXL-1051),37 was dissolved in 15% β-cyclodextrin (Capitol) in sterile water at pH 4.0 to generate a stock solution (60 mmol/L). For in vivo intravenous administration, CXL-1020 was diluted in 0.9% NaCl and infused at incremental doses of 200, 300, or 500 μg/kg per minute (with 2–10 μL/min infusion rate). For the vascular ring studies, CXL-1020 was administered at doses ranging 1 mmol/L to 1 mmol/L, prepared from a 100 mmol/L stock solution.

In Vivo Hemodynamics

Cardiac function and arterial loading were assessed by pressure–volume analyses, using a miniature micromanometer/conductance catheter (Millar, Inc) as described previously.39 Briefly, mice (C57Bl/6 controls [n=10], sGCKO and littermate controls, [n=5 each group], and PKG1α−/− [n=4]) were anesthetized using an established protocol (1%–2% isoflurane, followed by IP 750–1000 mg/kg urethane, 5–10 mg/kg etomide, and 1–2 mg/kg morphine).39 After tracheotomy, they were ventilated using 6 to 7 μL/g tidal volume at 130 breaths/min and administered 12.5% human albumin (50–100 μL over 5 minutes) to provide modest intravascular volume expansion. The left ventricular apex was then exposed, and a 1.4-F pressure–volume catheter (SPR 839; Millar Instruments Inc) was advanced through the apex to lie along the longitudinal axis. Data were measured at steady state with each dose of CXL-1020, allowing sufficient time to establish steady state responses. The volume signal was calibrated using ultrasound-aortic flow (Transonic, NY) and the hypertonic saline method.39 Total ventricular afterload was indexed by effective arterial elastance (Ea=ventricular end-systolic pressure/stroke volume) and by total systemic arterial resistance. Ventricular contractility was determined by peak rate of pressure rise normalized to instantaneous developed pressure (dP/dt max/IP), and relaxation by a logistic-model time constant.40 Analysis used custom software (WinPVAN) developed in our laboratory.

Isolated Vascular Rings

Direct vasodilator effects of CXL-1020 were tested in aortic rings or mesenteric vessels using tissue bath force-transducer systems. Animals were euthanized with an overdose of isoflurane, and thoracic aorta or mesenteric vessels excised, cleaned from connective tissue, and placed in Krebs–Henseleit solution (118 mmol/L NaCl, 4.7 mmol/L KCl, 2.5 mmol/L CaCl2, 1.2 mmol/L KH2PO4, 1.2 mmol/L MgSO4, 25 mmol/L NaHCO3, 7.5 mmol/L glucose, pH 7.4) gassed with 95% O2, and 5% CO2. Aortic rings (2–3 mm) were mounted in a myograph 700 (Danish Myo Technology, Aarhus, Denmark) set to a rest tension of 5 mN. Mesenteric third order vessels of similar length were attached to a micromanipulator and force transducer under microscopic visualization, and passively stretched in 60 mmol/L KCl, washed with Krebs buffer.41 After equilibration (260 minutes at 37°C) in the presence of diclofenac or indomethacin (3 mmol/L) and N-nitro-l-arginine methyl ester (200 mmol/L), rings were precontracted with phenylephrine (1 μmol/L, Sigma). Each experiment was conducted in parallel with aortic rings or mesenteric vessels derived from littermates of sGCKO or PKG1α−/− mice.

PKG1α-Nonreducing Gel Electrophoresis

Snap frozen mesenteric vessels from control and PKG1α−/− mice were lysed in buffer containing N-ethyl maleimide (100 mmol/L, Sigma) to prevent further thiol oxidation during protein isolation and run in nonreducing conditions as previously described.42 Protein concentration was determined by BCA (bovine serum albumin) assay, and samples probed with primary antibodies against PKG1α (gift of Robert Blanton, Tufts University) and α-tubulin (loading control; Cell Signaling Technology). Antibody binding was imaged and analyzed using an infrared system (Odyssey, Licor).

Cyclic Nucleotide Assay

Ventricular myocardium was homogenized in 6% trichloroacetic acid, centrifuged, and extracted with water-saturated ether. The aqueous phase was then transferred and vacuum dried, and the pellet resuspended in sodium acetate buffer for cGMP or cAMP immunoassay (Amersham Pharmacia Biotech) following manufacturers instruction.
Statistical Analysis
Dose-dependent effects were tested by multiple regression analysis, with a dummy variable encoding each mouse. For single-dose analysis testing genotype-drug treatment interaction, a 2-way analysis of variance was used. Analysis was performed using Systat 11.0 software.

Results

CXL-1020 Induces Combined Inotropy and Vasodilation in the Mouse Heart In Vivo
Because in vivo cardiovascular effects of any HNO donor have not been previously reported in mice, we first established this by a dose response to CXL-1020. The HNO donor displayed both dose-dependent arterial vasodilation and positive cardiac contractility and enhanced relaxation (Figure 1). For example, pressure–volume loops before and after CXL-1020 (Figure 1A) reveal dose-dependent reductions in total ventricular afterload (E_a, slope of diagonal line in each loop) consistent with a decline in systemic resistance. Summary data for the various loop-derived parameters are provided in Figure 1B and in Table S1 in the online-only Data Supplement. The doses of CXL-1020 required to generate cardiovascular changes in intact mice were ≈10-fold greater than in dog, but the effects (eg, peak

Figure 1. A, Example of pressure-volume loops in control mice exposed to incremental doses of the pure nitroxyl donor, CXL-1020. There is a gradual decline in ventricular afterload indexed by E_a (diagonal line). B, Summary data (n=10) for dose-dependent changes in ventricular end-systolic pressure (LV-ESP), effective arterial elastance (E_a), LV contractility (dP/dt_{max}/IP), relaxation time constant (Tau), and integrated function (ejection fraction [EF] and stroke work [SW]). Data are shown as percent change from baseline. P values in figure are for multiple regression analysis of variable vs dose that also included a dummy variable for each mouse. *P≤0.01, †P≤0.001, ‡P≤0.03 vs baseline (Bonferroni corrected for multiple comparisons).

Figure 2. A, Example pressure-volume loops from wild-type (WT)-littermate control and sGCKO mice, before and after exposure to 500 μg/kg per minute CXL-1020. The nitroxyl donor reduced ventricular afterload in controls (arrow notes decline in E_a), but not in mice lacking soluble guanylate cyclase. B, Summary data for these studies (n=5 per group). P values below are for interaction term of 2-way ANOVA, with dose and genotype as the 2 groups. *P≤0.05 vs baseline. E_a indicates effective arterial elastance; and SBP, systolic blood pressure.
left ventricular pressure: −10%; total afterload−Ea: −40%; and dP/dt max/IP: +35%) were similar (P<0.001 for each). Isovolumic relaxation time constant shortened by ≈20%. The net effect of these changes was a near 20% rise in ejection fraction and stroke work.

CXL-1020 Induces Inotropy But Not Vasodilation in sGCKO Mice

We next tested if sGC activation is required for HNO-induced arterial vasodilation, cardiac inotropy, or both. sGCKO mice and their littermate controls were studied using the same in vivo pressure–volume analysis, utilizing a single 500 μg/kg per minute dose of CXL-1020 based on the dose-response data. CLX-1020 lowered systolic blood pressure and Ea in littermate controls, but both changes were absent in sGCKO mice (Figure 2A and 2B). By contrast, contractility rose similarly in both groups (Figure 2B; Table S2). To more directly test the requirement of sGC for CXL-1020 vasodilation, aortic rings were preconstricted with phenylephrine in the presence of the pan-NOS inhibitor N-nitro-L-arginine methyl ester and the COX-2 (prostaglandin-endoperoxide synthase 2) inhibitor diclofenac and exposed to varying doses of CXL-1020. CXL-1020 induced dose-dependent vasodilation, with 50% reduction in preconstriction force (IC50) achieved with ≈0.5 μmol/L in control rings (Figure 3A and 3B). By contrast, rings from sGCKO mice showed marked insensitivity to CXL-1020, with a ≈400-fold higher IC50. By way of comparison, we also tested the dilator response to the prototypic HNO donor, AS, and found a similar IC50 for reversing phenylephrine constriction in control rings and negligible effect in sGCKO rings (Figure 3C).

CXL-1020 Vasodilation Is Not Mediated by PKG1α-C42-Oxidation

The data from sGCKO mice indicated that sGC was required for HNO vasodilation; however, as some mild dilation was observed at the highest CXL-1020 doses in vascular rings, we further tested if HNO might directly alter PKG1α at its redox sensitive C42 residue to induce cGMP-independent vasorelaxation. Mice expressing solely wild-type PKG1α or the C42S mutation were administered CXL-1020 at 500 μg/kg per minute (×10 minute), and an identical decline in blood pressure was observed in both groups (Figure 4A). CXL-1020 exposure had no impact on myocardial cGMP or cAMP levels in the hearts of either genotype (Figure 4B). Consistent with these in vivo data, aortic rings (Figure 5A) and mesenteric vessels (Figure 5B) from PKG1αC42S or controls showed near identical CXL-1020 dose responses, with an IC50 between 1 and 10 μmol/L. As a control, we applied ODQ (0.3 μmol/L) to block sGC activity and found this markedly blocked CXL-1020–stimulated relaxation similarly in both groups. Last, we directly tested if HNO stimulates formation of PKG1α dimer in mesenteric vessels. As shown in Figure 5C, whereas H2O2 (10 μmol/L) enhanced PKG1α dimer, exposure to CXL-1020 showed no change over nonstimulated control. In vessels expressing PKG1αC42S, the dimer signal was negligible. Collectively, these data show that HNO does not modify PKG1α-C42 to induce vasorelaxation.

Discussion

The first question tested by the present study was whether sGC signaling is required for HNO-mediated vasodilation in vivo. Despite prior work conducted in vitro, this was far from a forgone conclusion given the potential for ODQ off-target effects and concerns as to whether HNO can indeed
interact with the heme in sGC as NO does.\textsuperscript{21,22} In addition, our prior conscious animal data had found arterial and venous blood cGMP failed to rise after AS infusion whereas equal vasodilating doses of an NO donor augmented levels.\textsuperscript{16} There were alternatives such as stimulation of hyperpolarizing potassium channels,\textsuperscript{3,6,19} although this too has been ODQ suppres-sible, and release of calcitonin gene-related peptide acting via by a cAMP-dependent mechanism.\textsuperscript{16} The latter has been recently proposed to arise by HNO generated from the interaction of NO and hydrogen sulfide, leading to stimulation of the chemoreceptor channel TRPA1 (transient receptor potential cation channel subfamily A, member 1) to trigger calcitonin gene-related peptide release.\textsuperscript{18} Last, vasodilation from the oxidation of PKG1\(\alpha\) at C42\textsuperscript{25,26} raised a possibility that HNO could also target this residue and reduce vascular tone while bypassing sGC.

By using a gene targeting approach, we isolated the role of sGC and found it is required and sufficient to explain HNO vasodilation in vivo and in vitro. Use of CXL-1020 avoided ambiguities of AS which cogenerates the vasodilator nitrite, or isopropylamine NONOate which donates HNO or NO depending on acid/base conditions.\textsuperscript{35} CXL-1020 is also the only HNO donor to have been thus far administered to humans\textsuperscript{17} where arterial vasodilation and improved left ventricular function were observed, conferring clinical relevance to the current findings. The negative results obtained in the PKG1\(\alpha^{C42S}\) mutant mice indicate that this redox modification does not contribute to HNO vasodilation. The control data in Figure 5 shows that CXL-1020 counters vasoconstriction with similar potency in both aorta and mesenteric arteries, both responses being largely blocked by ODQ. Although slight residual dilation was seen at the highest dose (as in the sGCKO aorta), this most likely reflected nonspecific effects at this concentration. We did not study mesenteric arteries from sGCKO mice, but the failure of CXL-1020 to vasodilate in vivo indicated that resistance vessel targeting was central.

The second major question we addressed was whether sGC is required for acute positive inotropic effects of HNO, as prior studies had also led to some conflicting results. Low levels of sGC-derived cGMP can induce a positive inotropic response\textsuperscript{27,43} because of competitive inhibition of PDE3 for cAMP hydrolysis.\textsuperscript{28} However, at higher concentrations, cGMP stimulates PKG to phosphorylate troponin I and desensitize the myofilaments to Ca\textsuperscript{2+},\textsuperscript{44,45} reducing contractility. Cyclic GMP also activates PDE2 to increase cAMP hydrolysis.\textsuperscript{30} PKG1\(\alpha^{C42S}\) stimulation has not been linked to positive cardiac inotropy.

Given that sGC was required for HNO-stimulated vasodilation, some modulation of contraction seemed possible and indeed was recently reported in a study performed in isolated rat hearts perfused with constant-pressure crystalloid buffer.\textsuperscript{34} The investigators found that in addition to vasodilation (reflected the rise in coronary flow), AS enhanced left ventricular systolic pressure and \(dP/dt_{max}\), both being suppressed by ODQ. However, in isolated

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure5.png}
\caption{Dose-dependent reversal of phenylephrine-induced vasoconstriction in mice with wild-type (WT) vs redox-dead (C42S) protein kinase G-1\(\alpha\) (PKG1\(\alpha\); \(n=4–16/\text{group}\). The absence of PKG1\(\alpha\) redox sensitivity did not alter CXL-1020 vasodilation dose-sensitivity in both (A) conduit (aorta) and (B) resistance (mesenteric) arteries. In both cases, application of 1H-[1,2,4]oxadiazolo-[4,3-a]quinoxalin-1-one (ODQ) suppressed CXL-1020 vasodilation. C, Nonreducing gel electrophoresis for PKG1\(\alpha\) monomer and dimer in response to H\textsubscript{2}O\textsubscript{2} (positive control) vs CXL-1020 (the latter tested in both WT and PKG1\(\alpha^{C42S}\) mutant mesenteric arteries). CXL-1020 did not stimulate dimer formation.}
\end{figure}
crystalloid-perfused hearts, increasing coronary flow can itself result in enhanced ventricular function. By contrast, using a constant-flow isolated heart preparation, we found hearts lacking phospholamban displayed identical vasodilation to AS as in controls, yet no inotropic response. This is consistent with direct evidence for HNO-mediated thiol modification of phospholamban and consequent dis-inhibition of the sarcoplasmic reticulum ATPase.

Additional evidence against cGMP-mediated contractility from HNO is the failure of ODQ to suppress it in isolated myocytes, and finding that HNO-inotropy is additive to β-adrenergic stimulation in vivo and in vitro, whereas the latter is blunted by NO donors. The present finding of similar in vivo HNO inotropy in the absence or presence of sGC indicates that these alternative mechanisms are central, confirming a dichotomy for sGC dependent (vessels) and independent (heart) effects.

Our study has several limitations. One is that both inotropic and lusitropic responses to HNO were modest in the in vivo mouse heart, making it more difficult to assess cardiac responses. Given the small changes in inotropy, we relied on dP/dt max/IP as the index that could be most reliably measured from multiple steady state cycles. The difference between mouse and canine or human dose responses may stem from high basal Ca2+ cycling in mice, with 90% or more of the Ca2+ recycled via the sarcoplasmic reticulum that leaves little room for HNO-derived contractile enhancement. Isolated murine myocytes display greater effects, but this outcome could result from the lower temperatures used that slow basal Ca2+ cycling kinetics and removal from intrinsic adrenergic stimulation. Species differences in circulating or intracellular thiols may also impact the HNO response and concentration needed for physiological effects. Last, we did not specifically examine the role of α1/β1 versus α2/β1 forms of sGC, as the sGCKO mouse deletes both. This contrasts to mice lacking solely α1 or α2 subunits, where compensatory effects from the other isoform are observed.

In conclusion, we show that sGC activation is required and sufficient to explain arterial vasodilation from a pure HNO donor but is not necessary for HNO positive inotropy. Redox targeting of HNO to C42 in PKG1α does not play a significant role to its vasomotor regulation. The importance of sGC to vascular modulation raises questions about its targeting in chronic disease conditions where the cyclase may become oxidized. Although this can blunt NO responsiveness, HNO seems to remain effective in such settings. Last, the chronic influence of HNO–sGC interactions on cardiovascular disease remains unsettled. ODQ-suppressible antihypertrophic effects in myocytes exposed to the short-acting HNO donor isopropylamine NONOate have been reported. These findings need to be confirmed using a stable and much longer acting pure HNO donor combined with sGC gene deletion and then further tested in vivo to identify their translational potential.

Perspectives

The physiological biochemistry of HNO is attracting growing interest given its promising combination of vasodilator and positive inotropic effects that may benefit the failing heart. Unlike NO, HNO-stimulated vasodilation does not induce tolerance. Furthermore, its vasorelaxant and inotropic properties are preserved in heart failure and diabetes mellitus, both diseases that involve increased oxidative stress. The latter can suppress NO but not HNO-dependent signaling. This study resolves controversies about mechanisms for arterial dilation and contractility enhancement by HNO by using genetic knockout and knockin mouse models to provide unambiguous support for sGC but not PKG1α dependence for vasomotor responses. Their lack of influence on contractility modulation means that varying cGMP/PKG signaling as occurs with cotreatment by natriuretic peptides or PDE5 inhibitors is unlikely to impact HNO-mediated inotropy. In addition, reduced sGC functionality that might accompany cardiac disease is unlikely to impact the positive contractility efficacy from HNO donors.

Acknowledgments

We thank Philip Eaton for generously providing the PKG C42S KI mice and Cardioxyl Pharmaceuticals Inc for providing CXL-1020.

Sources of Funding

This study was supported by grants from the National Institute of Health (HL-119012, and HL093432), Deutsche Forschungsgemeinschaft (Fr 1725/1–5), a research grant from Cardioxyl Inc, and by the Japan Heart Foundation/Bayer Yakultin Research Grant Abroad (D. Hori).

Disclosures

Drs Paolocci and Kass are founders and scientific advisors to Cardioxyl, Inc. The other authors report no conflicts.

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Novelty and Significance

What Is New?

- This study demonstrates that nitroxyl (HNO) requires soluble guanylate cyclase to effect systemic vasodilation in vivo. It also shows potential HNO targeting to protein kinase G-1α itself does not play a role in this vasomotor modulation. By contrast, positive inotropic and lusitropic actions exerted by HNO donors in vivo do not require the presence of soluble guanylate cyclase, and thus are fully cGMP independent.

What Is Relevant?

- The mechanisms by which HNO impacts cardiac inotropy have been well studied at the molecular level, but data have remained limited with respect to its modulation of arterial tone, particularly in vivo. The current data provide definite evidence that soluble guanylate cyclase is the essential and required transducer of HNO-mediated vasorelaxation both in isolated vascular tissue and in the intact circulation.

Summary

Systemic arterial vasodilation by HNO requires soluble guanylate cyclase–dependent signaling whereas cardiac contractility enhancement does not. HNO does not modulate vascular tone by forming an intermolecular disulfide in protein kinase G-1α at C42 to activate the kinase.
Soluble Guanylate Cyclase Is Required for Systemic Vasodilation But Not Positive Inotropy Induced by Nitrotyl in the Mouse

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Hypertension. 2015;65:385-392; originally published online December 1, 2014;
doi: 10.1161/HYPERTENSIONAHA.114.04285

Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0194-911X. Online ISSN: 1524-4563

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SOLUBLE GUANYLATE CYCLASE IS REQUIRED FOR SYSTEMIC VASODILATION BUT NOT POSITIVE INOTROPY INDUCED BY NITROXYL (HNO) in the MOUSE

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Supplemental Tables : S1 and S2
Supplemental Table S1

Dose-dependent effect of CXL-1020 in the normal (C57Bl/6J) mouse heart.

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<td>LVP es (mmHg)</td>
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<td>182.7±10.2†</td>
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</table>

Data are mean ± SEM, n=10 in each group. P value in table is for drug effect in repeated measures analysis of co-variance, *P< 0.05; † p<0.01; ‡p<0.001 versus baseline by paired Student’s t-test with Bonferoni correction for multiple comparisons.
Supplemental Table S2

Effect of 500 mg/kg/min of CXL-1020 in sGC-knock out mice (GCKO) and littermate controls (WT).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>WT Baseline</th>
<th>WT CXL-1020</th>
<th>P</th>
<th>GCKO Baseline</th>
<th>GCKO CXL-1020</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart Rate (min⁻¹)</td>
<td>606±0.0</td>
<td>606±0.0</td>
<td>1.00</td>
<td>606±0.0</td>
<td>603.6±2.2</td>
</tr>
<tr>
<td>LVPmax (mmHg)</td>
<td>103.8±6.2</td>
<td>88.3±4.3</td>
<td>0.00</td>
<td>126.2±7.7</td>
<td>133.4±6.4</td>
</tr>
<tr>
<td>LVPes (mmHg)</td>
<td>97.1±6.9</td>
<td>78.4±3.6</td>
<td>0.00</td>
<td>116.4±5.6</td>
<td>121.1±6.9</td>
</tr>
<tr>
<td>SV (µl)</td>
<td>21.5±0.7</td>
<td>26.1±1.9</td>
<td>0.01</td>
<td>21.6±0.9</td>
<td>22.1±1.0</td>
</tr>
<tr>
<td>EF (%)</td>
<td>55±1</td>
<td>63±2</td>
<td>0.00</td>
<td>58±3</td>
<td>60±2</td>
</tr>
<tr>
<td>dP/dtmax/IP (sec⁻¹)</td>
<td>204.7±11.7</td>
<td>218.5±11.3</td>
<td>0.00</td>
<td>219.5±11.8</td>
<td>240.9±4.02</td>
</tr>
</tbody>
</table>

Data are mean ± SEM, n=6 in each group. P values are for paired Student’s t-test versus within each group.