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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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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 (PKG1α) 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 PKG1α 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 pre-constricted 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 inotropicvasodilator activity. In PKG1α^{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

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The authors report no conflict of interest.

inotropic action. Redox modulation of PKG1α is not a mechanism for HNO-mediated vasodilation.

Keywords

cardiovascular physiology; vasodilation; pharmacology; contractility

Introduction

Nitroxyl (HNO) is the protonated, one electron-reduced form of the signaling molecule nitric oxide (NO·)[1, 2]. Like NO·, HNO has prominent vascular effects inducing vasodilation in conduit and resistance arteries[3-7]. However, HNO differs from NO· in that tolerance does not develop with repeated exposure[8], and its effects are not suppressed by oxidative stress, but rather by administration of reducing agents such as L-cysteine[9] Exogenously administered HNO donors exhibit prominent pharmacological activity on the cardiovascular system[10-12], combining venous and arterial dilation with an augmentation of cardiac contractility and relaxation[11-17]. This net constellation of effects has triggered interest in HNO as a heart failure therapy[17], with clinical trials now underway (Clinicaltrials.gov/NCT02157506).

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[3, 6], and the stimulation of calcitonin gene related peptide (CGRP)[18]. HNO-vasodilation is blocked in vitro by the sGC antagonist [1H-[1,2,4]oxadiazolo-[4, 3-a]quinoxalin-1-one] (ODQ) [4-6, 19, 20], 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[21] and data showing this requires HNO conversion to NO· by superoxide dismutase[22]. Inhibition 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[23, 24]. Lastly, alternative mechanisms including CGRP and cysteine-42 oxidation in protein-kinase G-1α (PKG1α) that mediates cGMP-independent H_2O -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 mechanisms[29] and cGMP activation of PDE2 resulting in cAMP hydrolysis[30]. Though ODQ has no effect on HNO-stimulated inotropy in isolated ventricular myocytes[12, 17], 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 cysteines to form reversible S-S bonds or sulfinamides ($RS(O)NH_2$)[1]. In the heart, this chemistry alters phospholamban (PLN) [13, 32], sarcoplasmic reticular (SR) ATPase[33], the ryanodine

receptor[12], myosin light chain, and tropomyosin[10], resulting in enhanced Ca^{2+} 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 employed Angeli's salt (AS) that degrades into HNO but also nitrite which is itself a vasodilator[34]. Some have used IPA/NO, 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[38] that results in loss of the entire sGC protein complex. The role of PKG1α oxidation at C42 was also tested using mice with a knock-in mutation (C42S) generating a PKG1α redox-dead protein[26]. Lastly, we employed the pure HNO donor CLX-1020 that is stable at room temperature and can be administered both *in vitro* and *in vivo*[17]. 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 C57Bl6/J adult mice (Jackson Laboratories, Bar Harbor, ME), and in sGCKO and PKG^{C42S} mice and their respective littermate controls. Both genetic models have been previously described[26, 38], and develop hypertension (+ ~30 mmHg systolic pressure *vs*. controls). sGCKO mice[38] 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. PKG^{C42S} mice (provided by Phil Eaton, Kings College, London, UK) display no gastro-intestinal 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[26]. Mice aged 2-4 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 sulfonamide, Cardioxyl Pharmaceuticals Inc., NC), which chemically decomposes into HNO and an organic byproduct (CXL-1051)[17], was dissolved in 15% beta-cyclodextrin (Captisol®) in sterile water at pH 4.0 to generate a stock solution (60mM). 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/min (with 2-10 μL/min infusion rate). For the vascular ring studies, CXL-1020 was administered at doses ranging 1nM-1mM, prepared from a 100mM stock solution.

In vivo Hemodynamics

Cardiac function and arterial loading were assessed by pressure-volume (PV) 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^{C42S} (n=4)] were anesthetized using an established protocol (1-2% isoflurane, followed by i.p. 750-1000 mg/kg urethane, 5-10 mg/kg etomidate, and 1-2 mg/kg morphine) [39]. Following tracheostomy, they were ventilated using 6-7 μl/g tidal volume at 130 breaths/min, and administered 12.5% human albumin (50–100 μl over 5 min) to provide modest intravascular volume expansion. The LV apex was then exposed and a 1.4-Fr PV 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 (Transonics, NY) and the hypertonic saline method[39]. Total ventricular afterload was indexed by effective arterial elastance (E_a = 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 mM NaCl, 4.7 mM KCl, 2.5 mM $CaCl_2$, 1.2 mM KH_2PO_4, 1.2 mM $MgSO_4$, 25 mM $NaHCO_3$, 7.5 mM Glucose, pH 7.4) gassed with 95% O_2 and 5% CO_2. 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 3rd order vessels of similar length were attached to a micromanipulator and force transducer under microscopic visualization, and passively stretched in 60 mM KCl, washed with Krebs buffer[41]. After equilibration (at least 60 minutes at 37 °C) in the presence of diclofenac or indomethacin (3 μM) and N-nitro-L-arginine methyl ester (L-NAME) (200 μM), rings were pre-contracted with phenylephrine (1μM; Sigma). Each experiment was conducted in parallel with aortic rings or mesenteric vessels derived from littermates of sGCKO or PKG^{C42S} mice.

PKG1α-non-reducing gel electrophoresis

Snap frozen mesentaric vessels from control and PKG^{C42S} mice were lysed in buffer containing N-ethyl maleimide (100 mM, Sigma) to prevent further thiol oxidation during protein isolation, and run in non-reducing conditions as previously described.[42] Protein concentration was determined by BCA assay (Pierce) and samples probed with primary antibodies against PKG1α (gift of Robert Blanton, Tufts University) and alpha-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*

Since *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). Example pressure-volume loops before and after CXL-1020 (Fig 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 Fig 1B and in Supplemental Table S1. The doses of CXL-1020 required to generate cardiovascular changes in intact mice were about 10-fold greater than in dog[17], but the effects (e.g. peak LV pressure: −10%; total afterload-Ea: −40%; and dP/dt_{mx}/IP: +35%) were similar ($p<0.001$ for each). Isovolumic relaxation time constant shortened by nearly 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, employing a single 500 μg/kg/min 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 (Fig 2A, 2B). By contrast, contractility rose similarly in both groups (Fig 2B, Supplemental Table S2).

To more directly test the requirement of sGC for CXL-1020 vasodilation, aortic rings were pre-constricted with phenylephrine in the presence of the pan-NOS inhibitor L-NAME and the COX-2 inhibitor diclofenac, and exposed to varying doses of CXL-1020. CXL-1020 induced dose-dependent vasodilation, with 50% reduction in pre-constriction force (IC_{50}) achieved with ~0.5 μM in control rings (Fig 3A,B). By contrast, rings from sGCKO mice showed marked insensitivity to CXL-1020, with a ~400-fold higher IC_{50}. By way of comparison, we also tested the dilator response to the prototypic HNO donor, Angeli's salt, and found a similar IC_{50} for reversing PE-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 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/min (x10 min), and an identical decline in blood pressure was observed in both groups (Fig 4A). CXL-1020 exposure had no impact on myocardial cGMP or cAMP levels in the hearts of either genotype (Fig 4B). Consistent with these *in vivo* data, aortic rings (Fig 5A) and mesenteric vessels (Fig 5B) from PKG1α^{C42S} or controls showed near identical CXL-1020 dose responses, with an IC_{50} between 1-10 μM. As a control, we applied ODQ (0.3 μM) to block sGC activity, and found this markedly blocked CXL-1020 stimulated relaxation similarly in both groups. Lastly, we directly tested if HNO stimulates formation of PKG1α dimer in mesenteric vessels. As shown in Fig 5C, whereas H_2O_2 (10 μM) enhanced PKG1α dimer, exposure to CXL-1020 showed no change over non-stimulated control. In vessels expressing PKG1α^{C42S} 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[23, 24] and concerns as to whether HNO can indeed interact with the heme in sGC as NO does[21, 22]. Additionally, 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[16]. There were alternatives such as stimulation of hyperpolarizing potassium channels[3, 6, 19], though this too has been ODQ suppressible, and release of CGRP acting via by a cAMP-dependent mechanism[16]. The latter has been recently proposed to arise by HNO generated from the interaction of NO and hydrogen sulfide (H_2S), leading to stimulation of the chemoreceptor channel TRPA1 to trigger CGRP release.[18]. Lastly, vasodilation from the oxidation of PKG1α at C42[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 indeed required and sufficient to explain HNO-vasodilation *in vivo* and *in vitro*. Use of CXL-1020 avoided ambiguities of AS which co-generates the vasodilator nitrite, or IPA/NO which donates HNO or NO depending on acid/base conditions[35]. CXL-1020 is also the only HNO donor to have been thus far administered to humans[17] where arterial vasodilation and improved LV function were observed, conferring clinical relevance to the current findings. The negative results obtained in the PKG1α^{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. Though slight residual dilation was seen at the highest dose (as in the sGSKO aorta), this most likely reflected non-specific

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 sGC-derived cGMP can induce a positive inotropic response [27, 43] due to competitive inhibition of phosphodiesterase type 3 for cAMP hydrolysis[28]. However, at higher concentrations, cGMP stimulates PKG to phosphorylate troponin I and desensitize the myofilaments to Ca^{2+} [44, 45], reducing contractility. Cyclic GMP also activates PDE2 to increase cAMP hydrolysis[30]. PKG1α 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[31]. The investigators found that in addition to vasodilation (reflected the rise in coronary flow), AS enhanced LV systolic pressure and dP/dt_{mx}, both being suppressed by ODQ. However, in isolated crystalloid-perfused hearts, increasing coronary flow can itself result in enhanced ventricular function[46, 47]. By contrast, using a constant-flow isolated heart preparation, we found hearts lacking PLN displayed identical vasodilation to AS as in controls, yet no inotropic response[13]. This is consistent with direct evidence for HNO-mediated thiol modification of PLB[32] and consequent dis-inhibition of the SR-ATPase[13]. Additional evidence against cGMP-mediated contractility from HNO is the failure of ODQ to suppress it in isolated myocytes[12, 17], and finding that HNO-inotropy is additive to β-adrenergic stimulation *in vivo* and *in vitro*, whereas the latter is blunted by NO donors [11, 12]. The present finding of similar *in vivo* HNO inotropy in the absence or presence of sGC indicates 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 very high basal Ca^{2+} cycling in mice, with 90% or more of the Ca^{2+} recycled via the SR[48] that leaves little room for HNO-derived contractile enhancement. Isolated murine myocytes display greater effects[17] but this outcome could result from the lower temperatures used that slow basal Ca^{2+} 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. Lastly, 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[49].

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 regarding its targeting in chronic disease conditions where the cyclase may become oxidized. While this can blunt NO-responsiveness, HNO appears to remain effective in such settings[50]. Lastly, the chronic influence of HNO-sGC interactions on cardiovascular disease remains unsettled. ODQ-suppressible anti-hypertrophic effects in myocytes exposed to the short-acting HNO donor IPA/NO have been reported[51]. 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 (nitroxyl) is attracting growing interest given its promising combination of vasodilator and positive inotropic effects that may benefit the failing heart. Unlike $NO^{\cdot}$, HNO-stimulated vasodilation does not induce tolerance. Furthermore, its vasorelaxant and inotropic properties are preserved in heart failure[11] and diabetes[52], both diseases that involve increased oxidative stress. The latter can suppress $NO^{\cdot}$- but not HNO-dependent signaling. The present study resolves controversies regarding mechanisms for arterial dilation and contractility enhancement by HNO by using genetic KO and KI 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 co-treatment 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.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Novelty and Significance

1) What is New?

This study demonstrates that HNO requires soluble guanylate cyclase to effect systemic vasodilation *in vivo*. It also shows potential HNO targeting to PKG1α 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 sGC, and thus are fully cGMP independent.

2) 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 sGC is the essential and required transducer of HNO-mediated vasorelaxation both in isolated vascular tissue and in the intact circulation.

3) Summary

Systemic arterial vasodilation by HNO requires sGC-dependent signaling, whereas cardiac contractility enhancement does not. HNO does not modulate vascular tone by forming an intermolecular disulfide in PKG1α at C42 to activate the kinase.

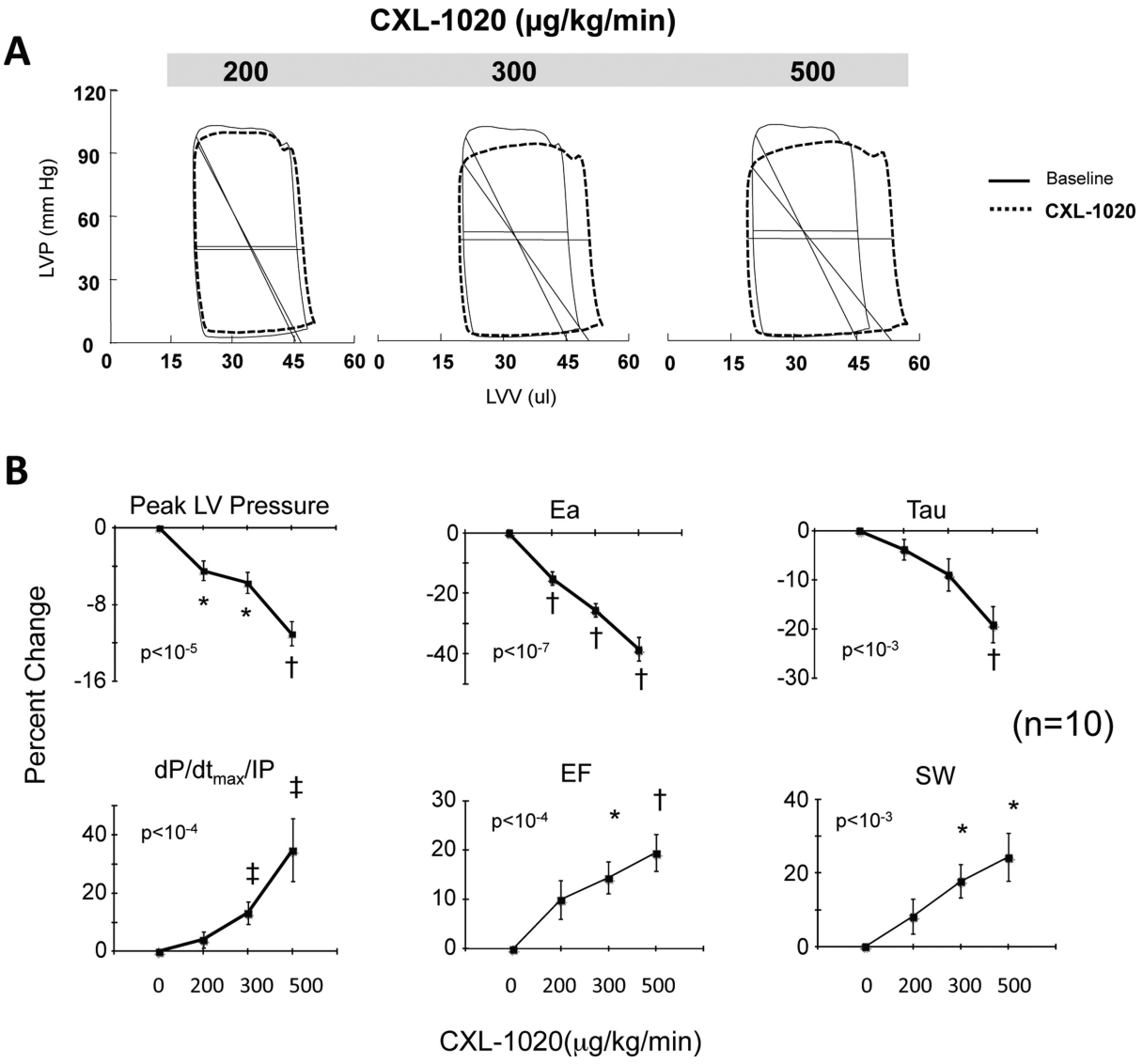

Figure 1.

A) Example of pressure-volume loops in control mice exposed to incremental doses of the pure HNO donor, CXL-1020. There is a gradual decline in ventricular afterload indexed by Ea (diagonal line). B) Summary data (n=10) for dose-dependent changes in ventricular endsystolic pressure (LV-ESP), effective arterial elastance (Ea), LV contractility (dP/dt_{max}/IP) and relaxation time constant (Tau-l), 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, †≤0.001, ‡p≤0.03 *vs*. baseline (Bonferroni corrected for multiple comparisons).

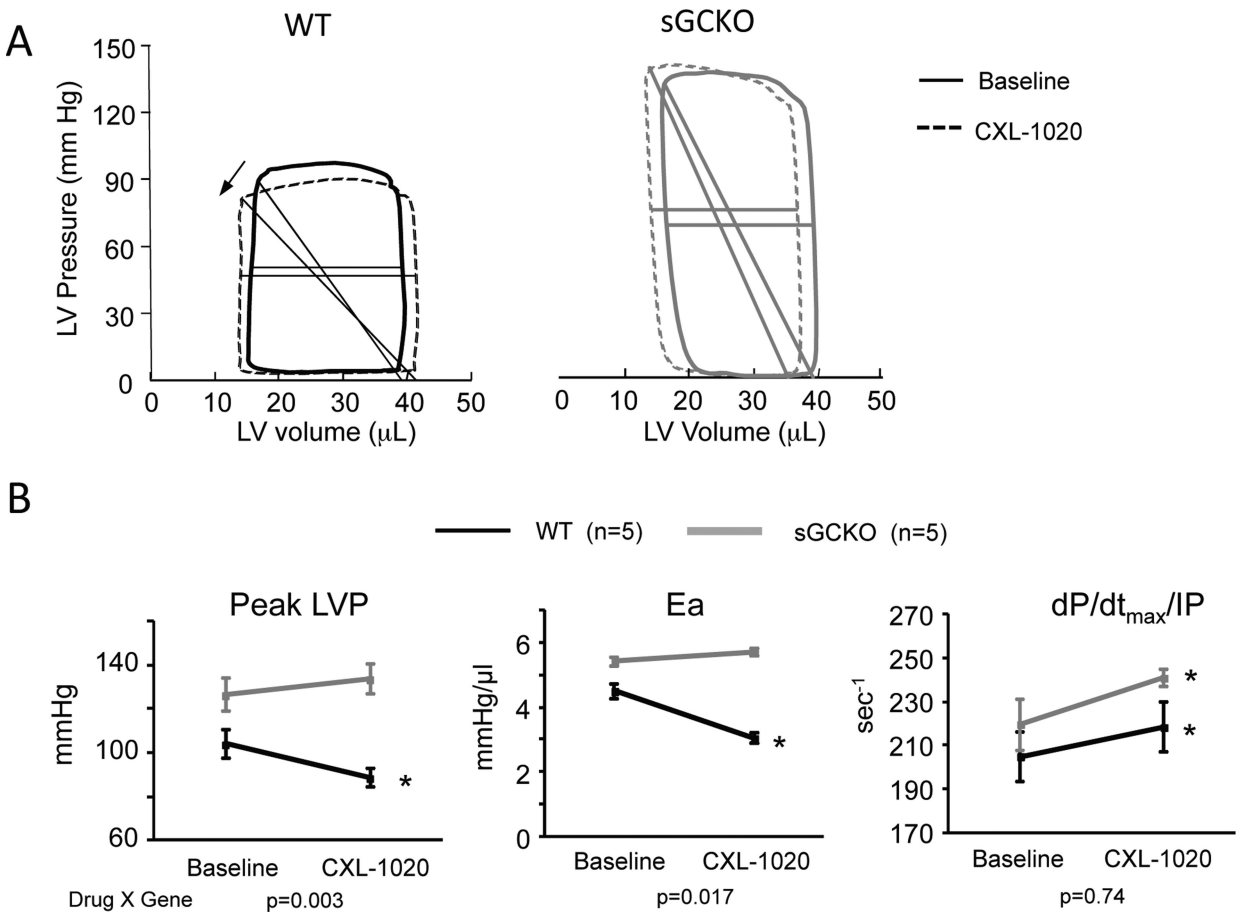

Figure 2.

A) Example PV loops from WT-littermate control and GC-KO mice, before and after exposure to 500 μg/kg/min CXL-1020. The HNO donor reduced ventricular afterload in controls (arrow notes decline in Ea), but not in mice lacking sGC. B) Summary data for these studies (n=5 per group). SBP – systolic blood pressure; Ea – effective arterial elastance. P-values below are for interaction term of two-way ANOVA, with dose and genotype as the two groups. * p≤0.05 *vs.* baseline.

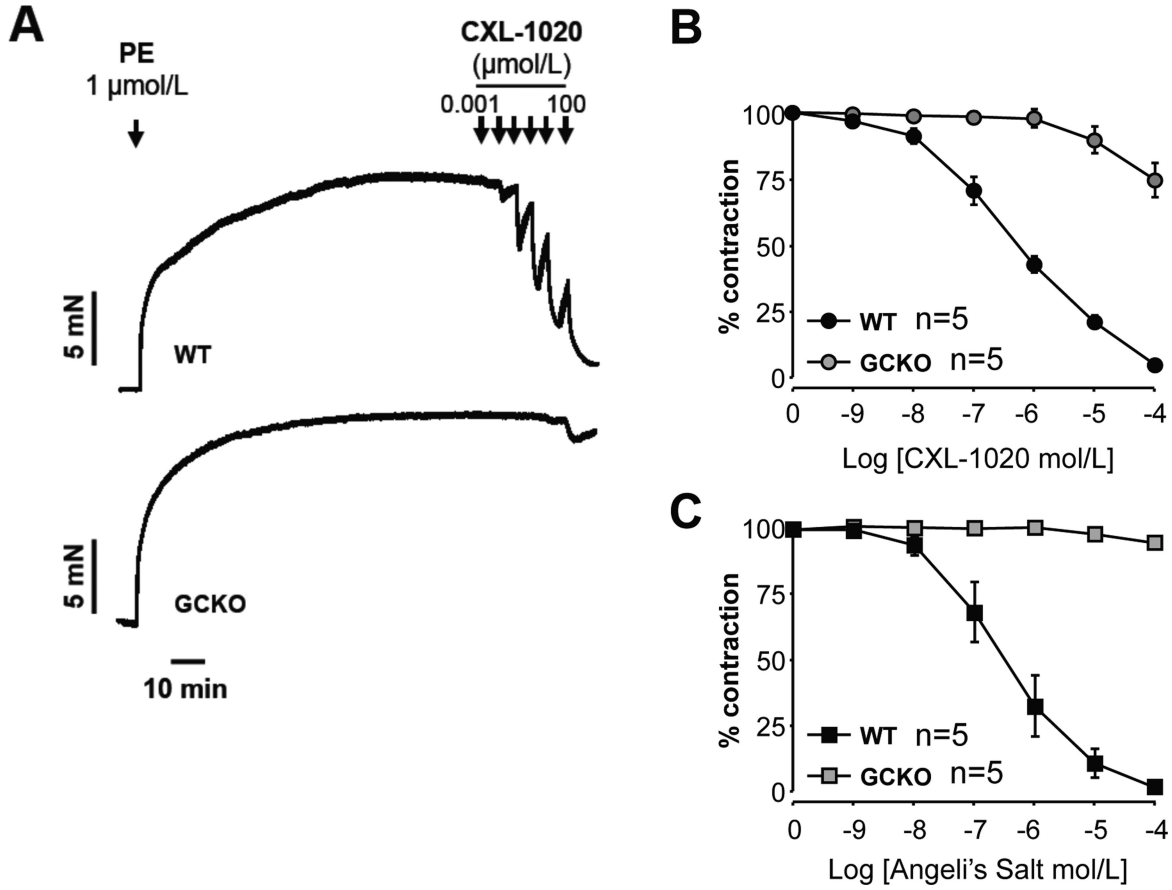

Figure 3.

A) Dose-dependent vasorelaxation of isolated aortic rings from control and GCKO mice. Reversal of phenylephrine (PE) induced constriction was produced by CXL-1020 at more than 100-fold lower concentrations in controls than in the GCKO animals. B) Summary results for CXL-1020 dose-response relations (n=5 for each group). C) Summary results for Angeli's salt dose-response relations (n=5 for each group).

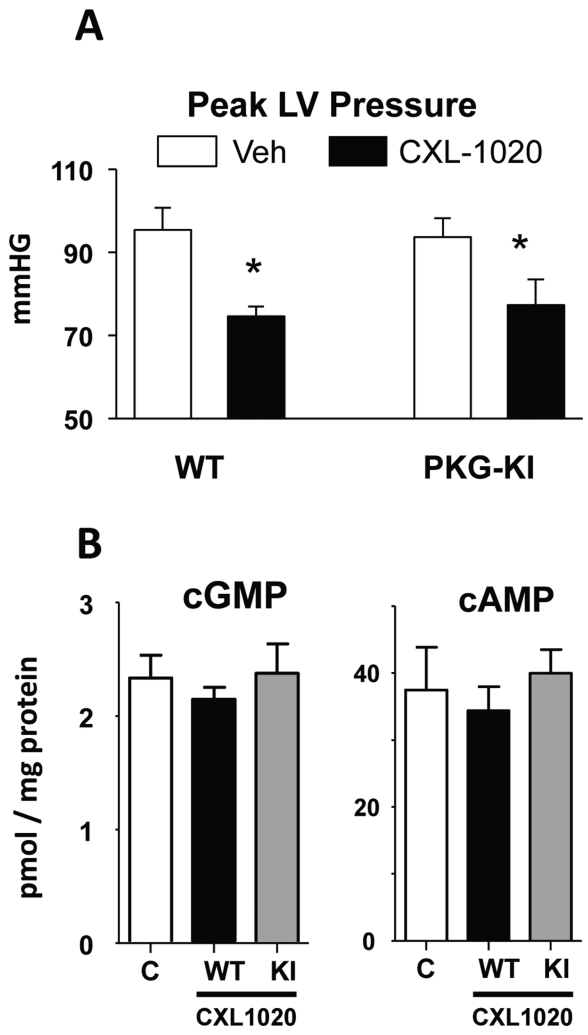

Figure 4.

A) Peak left ventricular pressure declines similarly in mice with a knock-in redox-dead PKG1α^{C42S} mutation as in littermate controls (n=4 for each group); *p<0.05. B) Myocardial cGMP and cAMP levels measured in control myocardium, and after 10 minute exposure to HNO (500 μg/kg/min CLX-1020). Neither cyclic nucleotide was altered by CXL-1020 exposure.

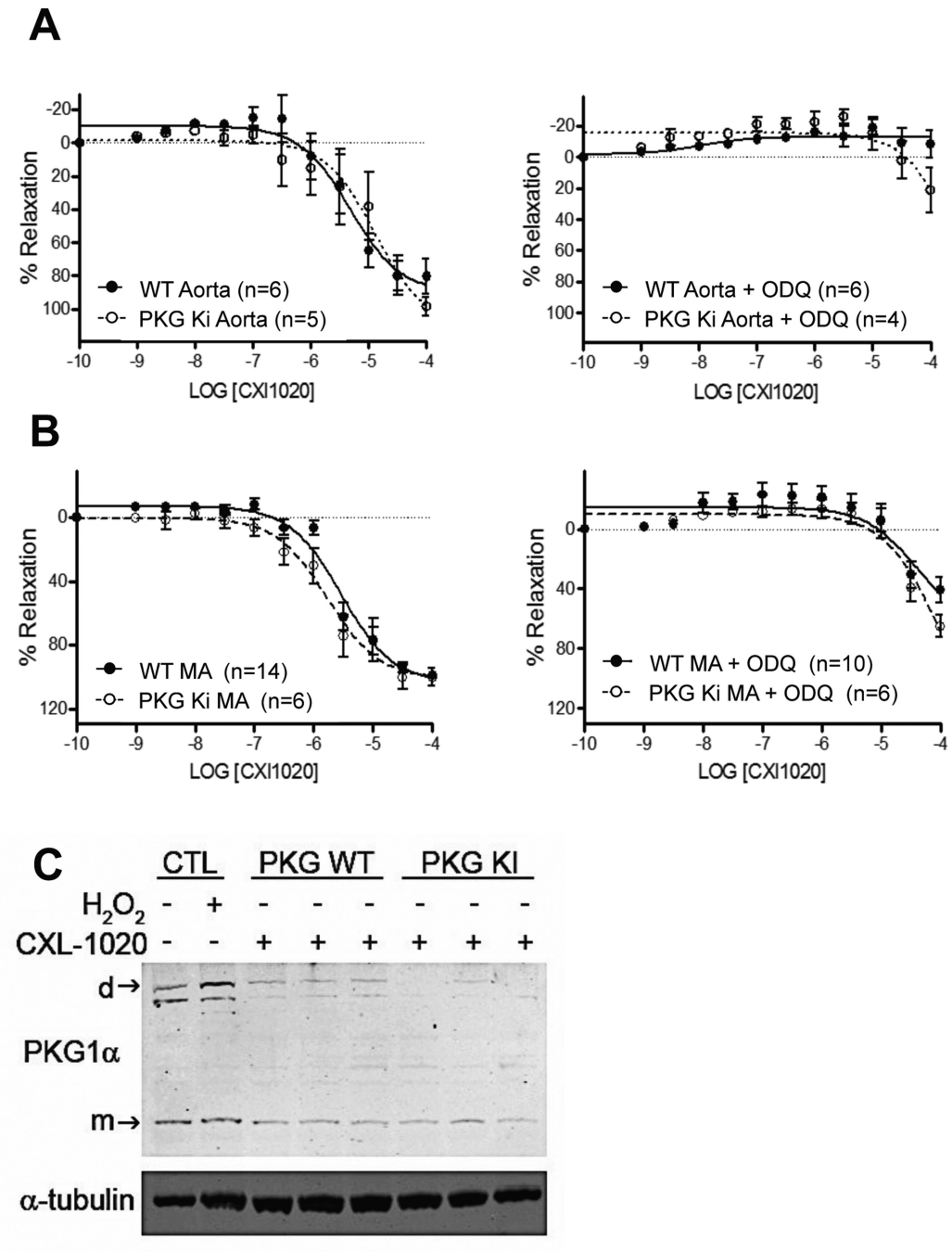

Figure 5.

Dose-dependent reversal of PE-induced vasoconstriction in mice with WT *vs*. redox-dead (C42S) PKG1α (n=4-16/group). The absence of PKG1α redox sensitivity did not alter CXL-1020 vasodilation dose-sensitivity in both A) conduit (aorta) and B) resistance (B) (mesenteric) arteries. In both cases, application of ODQ suppressed CXL-1020 vasodilation. C) Non-reducing gel electrophoresis for PKG1α monomer and dimer in response to H_2O_2

(positive control) versus CXL-1020 (the latter tested in both wild type (WT) and PKG1α^{C42S} mutant mesenteric arteries. CXL-1020 did not stimulate dimer formation.