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Pathophysiology of Hypertension in the Absence of Nitric Oxide/ Cyclic GMP Signaling

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Abstract

The nitric oxide (NO)-cyclic guanosine monophosphate (cGMP) signaling system is a well-characterized modulator of cardiovascular function, in general, and blood pressure, in particular. The availability of mice mutant for key enzymes in the NO-cGMP signaling system facilitated the identification of interactions with other blood pressure modifying pathways (e.g. the renin-angiotensin-aldosterone system) and of gender-specific effects of impaired NO-cGMP signaling. In addition, recent genome-wide association studies identified blood pressure-modifying genetic variants in genes that modulate NO and cGMP levels. Together, these findings have advanced our understanding of how NO-cGMP signaling modulates blood pressure. In this review, we will summarize the results obtained with mice with disrupted NO-cGMP signaling and highlight the relevance of this pathway as a potential therapeutic target for the treatment of hypertension.

Keywords

Nitric oxide-cyclic guanosine monophosphate; NO-cGMP; Blood pressure; Hypertension; Cardiovascular function; Soluble guanylate cyclase; Nitric oxide; NO; Mutant mice; Genetic variants; Renin-angiotensin-aldosterone signaling; RAAS; Gender; S-nitrosylation; Therapeutics

Introduction: Prevalence and etiology of hypertension

High blood pressure or hypertension (HTN) is the most prevalent modifiable risk factor for cardiovascular disease, including coronary heart disease, congestive heart failure, stroke, end-stage renal disease, and peripheral vascular disease, in both women and men[1]. Over one billion people worldwide and 1 in 3 US adults have high blood pressure, with an additional 8% of US adults estimated to have undiagnosed hypertension[1]. Just as the risks associated with HTN are clear, so too are the benefits of blood pressure reduction: treatment of HTN is associated with significant reductions in cardiovascular events[2]. However, while extensive research, development of blood pressure medications, and widespread

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patient education have reduced mortality and morbidity rates associated with hypertension, it is estimated that in at least half of the US adults with HTN optimal blood pressure targets are not achieved[3].

Blood pressure is a complex heritable trait regulated by the integrated signaling from renal, neural, vascular, and hormonal inputs with multiple environmental and genetic influences[4, 5]. In the vast majority of cases, it is difficult to identify specific mechanisms that disrupt these control systems, and the etiology of hypertension remains unknown. Molecular and genetic analyses of specific, highly-heritable and rare forms of HTN point to the involvement of a small number of genes, many of which are associated with the regulation of renal salt handling[4, 6].

The concept that abnormalities in renal salt handling[7] are a major contributor to the etiology of hypertension has informed the development of treatments for HTN focused on altering dietary sodium. However, multiple clinical studies have indicated that altering salt intake does not necessarily affect the risk for the development of hypertension or mortality associated with cardiovascular disease[8]. More recently, the hypothesis that primary abnormalities of vascular smooth muscle cell (VSMC) proteins that regulate vascular tone can cause hypertension has emerged[9]. For example, ample evidence suggests that impaired vascular NO-cGMP signaling, a key pathway in regulating vascular relaxation, is associated with high blood pressure[10–12].

In this review, we will provide a brief introduction of the NO-cGMP signaling system and an overview of the blood pressure phenotypes of mouse models in which key enzymes of the NO-cGMP signaling system are mutated. We furthermore will summarize how reciprocal interaction between NO/sGC signaling and the renin-angiotensin-aldosterone system modulates blood pressure, and how recent information from population genetic studies have illustrated the clinical relevance of NO/sGC signaling in the development of hypertension. We will highlight how gender affects the impact on blood pressure of modulating the activity of the NO/sGC signaling system, and how sGC-independent effects of NO (such as S-nitrosylation) can mediate some of the cardiovascular effects of NO. Finally, we will briefly discuss available compounds that activate sGC and their development as promising therapeutic targets in the treatment of hypertension.

The nitric oxide-soluble guanylate cyclase signaling system

NO is synthesized from L-arginine by a family of three enzymes referred to as NO synthases (NOSs)[13]. Three forms of the enzyme exist: endothelial NOS (eNOS or NOS3), neuronal NOS (nNOS or NOS1), and inducible NOS (iNOS or NOS2). eNOS and nNOS are constitutively expressed, producing low levels of NO, while iNOS expression is induced by inflammatory conditions leading to the production of large amounts of NO. Upon Ca^{2+} binding, calmodulin (CaM) binds to a regulatory site on the constitutively-expressed nNOS and eNOS, triggering NO production. CaM binds irreversibly to iNOS, resulting in a continuous Ca^{2+} -independent NO production. eNOS can be activated independently of Ca^{2+} upon phosphorylation by Akt kinase in response to stimuli such as shear stress, estrogens and insulin[14]. Recently, another important *in vivo* source of bioactive NO was identified: the circulating anion nitrite (NO_2^-), previously considered a stable and inert oxidation product of NO metabolism, serves as an endocrine transporter and storage pool of NO[15].

NO has numerous targets, reacting with a variety of intracellular and extracellular molecules, typically via thiol groups or transition metal centers[16, 17]. NO's multiple targets contribute to the wide variety and often-conflicting effects of this molecule. The best-characterized target of NO in the cardiovascular system is soluble guanylate cyclase (sGC). NO stimulates sGC, an obligate heterodimer composed of an α and a β subunit, to

synthesize the intracellular second messenger cGMP[18]. Although 2 isoforms of each subunit have been identified ($\alpha 1$, $\alpha 2$, $\beta 1$, $\beta 2$), only sGC $\alpha 1\beta 1$ and sGC $\alpha 2\beta 1$ appear to function *in vivo*[19]. The N-terminal domain of the sGC $\beta 1$ subunit contains a proximal histidine ligand (His105) required for binding the heme moiety[20]. Binding of NO to the heme results in a conformational change and activation of the catalytic domain. The catalytic center of sGC is formed by the conserved C-terminal cyclase homology domains of the α and β subunit. In cardiac and aortic tissue, the sGC $\alpha 1$ subunit is expressed at higher levels than the sGC $\alpha 2$ subunit[21], suggesting that the sGC $\alpha 1\beta 1$ heterodimer is the principal cardiovascular sGC isoform. However, low levels of cGMP, generated by sGC $\alpha 2\beta 1$, are sufficient to mediate many of NO's cardiovascular effects[12, 22–27]. cGMP interacts with a variety of effector proteins, including cGMP-dependent protein kinases (PKGs), cGMP-regulated phosphodiesterases (PDEs), and ion channels. It is important to note that cGMP can also be generated by membrane guanylate cyclases (pGCs) that are activated by natriuretic peptides[28]. However, cGMP produced by sGC and by pGCs may have differential effects, possibly due to differential spatiotemporal distributions of cGMP produced by the two guanylate cyclase families [29– 31].

NO-sGC mediated regulation of blood pressure: mouse models

The role of NO-cGMP signaling in VSMC relaxation and the regulation of blood pressure is well characterized, both in humans[32, 33] and in animal models[34], and ample evidence suggests that impaired NO signaling is involved in the pathogenesis of hypertension. NO is a key regulator of smooth muscle tone, in large part by controlling sGC-mediated production of cGMP[35–37]. Increased levels of cGMP subsequently activate PKGI, which in turn phosphorylates and modulates the activity of numerous downstream targets. PKGI activation mediates a number of physiological signaling mechanisms in VSMC, including decreases in intracellular Ca^{2+} levels, membrane hyperpolarization, and inhibition of myosin light chain phosphorylation, which all contribute to vasorelaxation (Figure 1).

During the last decade, many genes involved in NO-cGMP signaling (including genes encoding NOS, sGC, and PKG subunits and/or isoforms) have been targeted by gene deletion (conventional or conditional), overexpression, or mutations in residues or domains crucial for normal enzyme function (Table 1). Hypertension is a recurring phenotype in mouse models of impaired NO-cGMP signaling. Below we will address the cardiovascular phenotype of these transgenic mouse models in greater detail.

NOS mutant mice

The crucial role of endogenously produced NO in blood pressure regulation was confirmed by the presence of hypertension in mice deficient in eNOS (eNOS^{-/-} mice). Depending on the genetic background and the method used to measure blood pressure, eNOS^{-/-} mice have blood pressures ranging from 7 to 63 mmHg higher than those in control mice (Table 1)[26, 38–43]. In contrast, blood pressure was not affected in mice deficient in nNOS[44] or iNOS[45] (Table 1). Moreover, blood pressure in mice lacking both eNOS and nNOS, or in mice deficient in all NOS isoforms, was not greater than that in eNOS-deficient mice (Table 1)[46, 47]. Together, these data suggest that eNOS is the predominate source of vaso-active NO.

Acetylcholine (ACh)-induced vasorelaxation was blocked in aortic and carotid artery rings and in the pulmonary vasculature of eNOS^{-/-} mice, indicating that eNOS is required for ACh-induced vasodilation[38, 48, 49] and suggesting that the hypertension associated with the lack of endothelial NO can primarily be attributed to increased vascular resistance. However, in small resistance arterioles, that are thought to primarily control systemic blood pressure, other endothelium-derived vasodilators (such as prostacyclin and endothelium-

derived hyperpolarizing factor (EDHF)) appear to compensate for the loss of eNOS. For example, in mesenteric arteries of eNOS^{-/-} mice, ACh-induced relaxation was preserved. Together, these findings suggest that, although NO is a central mediator of vasorelaxation and blood pressure control, other endothelial vasodilators (such as EDHF or prostacyclins) [50, 51] can mediate vasorelaxation in small resistance vessels in the absence of eNOS-derived NO.

sGC mutant mice

Increasing evidence, both from genetic association studies and from work in mutant mice, illustrates the central role for sGC in the regulation of blood pressure. In sGC α 1^{-/-} mice, basal and NO-stimulated cGMP production was significantly reduced in the cardiovascular system[22, 23, 25]. As a result, the ability of endothelial-derived NO to induce vasorelaxation was impaired[23, 25, 52], highlighting the importance of sGC α 1 β 1-derived cGMP in the relaxation of vascular smooth muscle by NO. Nevertheless, NO donor compounds could still modulate hemodynamic parameters in sGC α 1^{-/-} mice, albeit to a lesser extent[22, 53], most likely reflecting the ability of sGC α 2 β 1 to modulate the hemodynamic effects of NO in the absence of sGC α 1 β 1. Interestingly, varying blood pressure phenotypes have been reported in mice deficient in the sGC α 1 subunit of sGC (sGC α 1^{-/-} mice), ranging from a normotensive phenotype[53] to an increase in blood pressure of 7 mmHg[25] to 22 mmHg[22] (Table 1). Differences in genetic background and gender likely contribute to this discrepancy: male but not female sGC α 1^{-/-} mice on a 129S6 (S6) background (sGC α 1^{-/-}S6) develop hypertension[22], and no hypertension was observed in either male or female sGC α 1^{-/-} mice on the C57BL/6 (B6) background (sGC α 1^{-/-}B6)[53]. These findings suggest the existence of genetic modifiers of the hypertensive effects associated with sGC α 1-deficiency (see below).

The importance of sGC in regulating blood pressure and vascular tone was further supported by the observation that blood pressure was higher in mice deficient in sGC β 1 (sGC β 1^{-/-} mice) than in WT mice[12] (Table 1). Similarly, smooth muscle cell-specific sGC β 1^{-/-} mice developed hypertension[34] (Table 1). In addition, in sGC β 1^{-/-} mice, administration of NO donor compounds did not reduce blood pressure and the vasodilatory effect of NO in isolated aortic rings was abrogated[12], suggesting that NO-inducible sGC activity is required for NO to mediate its vasorelaxing effects in these vessels. The observed differences in phenotype between sGC α 1^{-/-} and sGC β 1^{-/-} mice suggest that sGC α 1 accounts for only part of the sGC-dependent vasorelaxing effects of NO in mice. In sGC α 1^{-/-} mice, NO stimulation likely leads to a small increase in cGMP production, attributable to sGC α 2 β 1. This low level of sGC activity appears to be sufficient to mediate NO-induced vasorelaxation in sGC α 1^{-/-} mice. On the other hand, deletion of the sGC α 2 subunit was associated with only a slight reduction in sGC enzyme activity in aortic tissue, and did not appear to influence vasorelaxation in response to NO-donor compounds[25] (Table 1). The relatively minor impact of sGC α 2-deficiency (in the context of intact sGC α 1 β 1 activity) on the ability of NO to mediate vasorelaxation is most likely due to the fact that sGC α 2 β 1 is expressed at low levels in the vasculature[21]. Additional studies in available sGC mutant mice and recently developed mice expressing only the heme-free form of sGC (apo-sGC) (Table 1)[54] are required to further characterize the role of sGC in maintaining cardiovascular homeostasis.

PKG mutant mice

PKG1 is generally accepted to be the principal mediator of the hemodynamic effects of NO-cGMP signaling. VSMCs express two alternative splice variants of PKG1, α and β , both of which mediate VSMC relaxation[55, 56]. Dilation of both large conductance and small resistance arteries in response to NO/cGMP signaling are severely impaired in mice

deficient for both PKG1 isoforms (PKG1^{-/-}), indicating that PKG1 mediates, at least in part, the vasorelaxant effects of NO and other cGMP-elevating agents[57, 55, 58, 59].

PKG1^{-/-} mice are hypertensive early in life, but blood pressure normalizes by 8 weeks of age[57, 60] (Table 1). However, PKG1 deficiency is associated with severe gastro-intestinal dysfunction and mortality greater than 50% by 6 weeks of age. Therefore, it is conceivable that non-cardiovascular effects of interfering with NO-cGMP signaling confound the blood pressure recordings obtained at 8 weeks. To avoid the confounding effects of the increased morbidity and mortality associated with complete loss of PKG1 activity, a mouse strain was generated which expresses a mutant PKG1 α leucine zipper (LZ) (LZM mice[11]) (Table 1). The LZ mediates the interaction of PKG with its downstream targets RhoA and myosin light chain phosphatase (MLCP) (Figure 1). LZM mice express a PKG1 α protein with preserved catalytic activity but which is incapable of making LZ-dependent interactions with these PKG1 α target proteins. Homozygous LZM mice displayed a marked age-dependent hypertension, which developed at 10–12 weeks of age[11]. ACh-dependent relaxations were attenuated but not abolished in isolated large conductance arteries and resistance arteries from LZM mice. Together, these findings suggest that LZ-dependent PKG1 α effects are not the only mechanisms by which PKG1 mediates the vasorelaxing effect of NO[11]. Recently it was shown that PKG1 is also a direct mediator of NO-independent EDHF-dependent vasorelaxation via its direct activation by H₂O₂ (Table 1)[61]. Other studies have indicated that PKG1 inhibits vasoconstrictor-induced Ca²⁺ release from the sarcoplasmic reticulum (SR) in several ways: 1) by inhibiting G protein signaling via activation of the regulator of G protein signaling-2[60], 2) by inhibition of inositol 1,4,5-triphosphate (IP₃) production by direct phosphorylation of phospholipase C[62], and 3) by inhibition of the IP₃ receptor via the IP₃ receptor-associated PKG substrate[63]. In addition, PKG1-mediated inhibition of phospholamban increases Ca²⁺ reuptake into the SR via sarco/endoplasmic reticulum ATPase[64]. Finally, PKG1 activation has also been shown to inhibit Ca²⁺ entry into VSMC directly by phosphorylation of the L-type Ca²⁺ channel[65] and indirectly by activation of the big conductance Ca²⁺-activated K⁺ channel[66] and the ATP-sensitive K⁺ channel[67], leading to membrane hyperpolarization and subsequent inhibition of the voltage-dependent L-type Ca²⁺ channel (Figure 1). In summary, PKG1 is an important mediator of both NO-dependent and NO-independent vasodilation via its association with and phosphorylation of downstream targets that eventually lead to a multitude of signaling cascades involved in inhibition of contraction and activation of vasorelaxation.

Taken together, these studies using genetically modified mice underscore the important role of NO-sGC-PKG1 signaling in blood pressure control. Disrupting this signaling cascade at any point leads to impaired vasodilation, which in most cases is associated with a hypertensive phenotype.

NO/sGC signaling and its reciprocal interaction with the renin-angiotensin-aldosterone system

Multiple signaling mechanisms regulate blood pressure. Both NO-cGMP signaling and activity of the renin-angiotensin-aldosterone system (RAAS) play a central role in homeostatic control of systemic blood pressure[7, 68]. Increasing evidence suggests that NO-cGMP signaling regulates activity of the RAAS and vice versa. Different reports have suggested that NO-cGMP signaling either decreases or increases RAAS activity[69]. For example, plasma renin concentration (PRC) was elevated in eNOS^{-/-} mice[39], suggesting that NO-cGMP inhibits renin secretion. NO also downregulates the synthesis of angiotensin I-converting enzyme (ACE) in the endothelium[70], as well as the expression of the angiotensin II (Ang II) type 1 receptor (AT1R) in VSMC, attenuating both Ang II production as well as its downstream actions[71, 72]. Other studies indicated that NO-cGMP

signaling can stimulate renin secretion[73] and aldosterone production[74]. Together, these data illustrate that, although the interaction between the NO-cGMP signaling system and the RAAS remains controversial, there is evidence that NO-cGMP signaling modulates RAAS activity.

The impact of sGC α 1-deficiency on blood pressure is dramatically impacted by the genetic background of the mice studied. sGC α 1^{-/-}S6 but not sGC α 1^{-/-}B6 develop hypertension, offering an opportunity to consider the existence of genetic modifiers of blood pressure in sGC α 1^{-/-} mice. Linkage analysis in sGC α 1^{-/-} mice revealed a quantitative trait locus (QTL) for blood pressure on chromosome 1 (designated *Hsgcq10*), syntenic with previously identified hypertension-related QTLs in the human and rat genome[10]. Within this region, renin was identified to be a strong biological candidate modifier gene of hypertension. The role of renin in the hypertension associated with impaired NO-cGMP signaling was confirmed by the observation that interfering with activity of the RAAS with a renin inhibitor, an ACE inhibitor, or an aldosterone receptor antagonist, normalized blood pressure in male sGC α 1^{-/-}S6 mice[10]. In addition, an ACE inhibitor attenuated the vascular dysfunction in conductance and resistance arteries isolated from sGC α 1^{-/-}S6 mice[10]. Taken together, these results suggest that at least part of the increased blood pressure seen in eNOS^{-/-} (see above) and sGC α 1^{-/-}S6 mice compared to WT mice could be due to higher activity of the RAAS in the mutant mice. These observations illustrate the intricate interplay between the RAAS and NO-cGMP signaling and highlight the interaction between two well-known blood pressure regulating pathways. Further characterization of how established blood pressure-modifying signaling mechanisms (such as NO-cGMP and RAAS signaling) reciprocally interact to modulate blood pressure (e.g. by studying the mutant mice listed in Table 1), is anticipated to improve our understanding of the role of those signaling pathways in blood pressure regulation, which may result in the identification of novel strategies to treat hypertension.

Role of sGC in the genetic control of HTN

Animal, population, and epidemiological studies have demonstrated that genetic factors contribute to the pathogenesis of essential hypertension, a polygenic trait influenced by both autosomal and sex-linked genes. Dozens of distinct genetic variants that are associated with blood pressure have been identified in GWAS and in candidate gene association studies[5, 32, 75–78]. In humans, an estimated 60% of blood pressure variation is attributable to additive genetic factors[77].

Among the common variant loci identified to date, 4 are involved in the NO/NP-sGC/pGC-cGMP system highlighting a central role of cGMP in blood pressure regulation. No other pathway includes more than 1 locus with a recognized common variant. Variants were identified in or near the genes encoding eNOS (NOS3)[79–82], sGC (GUCY1A3 and GUCY1B3)[32], and cardiac NP genes (NPPA and NPPB)[78, 83] and their clearance receptor (NPR3)[32, 76, 83, 84]. The GUCY1A3-GUCY1B3 variant that was identified in 200,000 individuals of European ancestry (designated rs13139571) is located in an intron of GUCY1A3 and is associated with a significant increase of 0.3 mmHg in systolic and diastolic blood pressure[32]. Understanding the mechanisms by which these genetic variants (e.g. in genes encoding sGC subunits) contribute to the development of hypertension may not only improve our understanding of the etiology of hypertension but may also identify novel therapeutic targets for the treatment of hypertension.

Gender-specific cardiovascular effects of impaired NO-cGMP signaling

Epidemiologic studies have identified male gender as an important risk factor for developing hypertension[85, 86]. However, after the onset of menopause, women are exposed to a

unique set of risks and are at higher risk for developing hypertension than are men[87]. Several studies suggest that NO-cGMP signaling has a greater role in regulating blood pressure in male than in female mice. Although blood pressure was elevated in both genders in an independently generated eNOS^{-/-} strain[39], eNOS^{-/-} mice generated by Huang *et al.* [38] were reported to display hypertension in males only[88]. Similarly, in mice lacking both cyclooxygenase-1 (COX-1) and eNOS, only male mice were hypertensive, and ACh could relax resistance arteries only in female but not in male mice. These results were interpreted to suggest that EDHF is the principal endothelial-derived relaxing factor in females, while in males NO and prostacyclin are the predominant vasorelaxing mediators[88].

Male but not female sGC α_1 ^{-/-}S6 mice develop hypertension[22]. Orchiectomy or treatment with an androgen receptor antagonist prevented hypertension in male sGC α_1 ^{-/-}S6 mice, and chronic testosterone treatment increased blood pressure in ovariectomized female sGC α_1 ^{-/-}S6 mice[22]. Downstream of cGMP, PKG1 deficiency also increased blood pressure in male but not female mice[57, 58]. Together, these data illustrate that NO-cGMP signaling has gender-specific and testosterone-dependent cardiovascular effects. The exact mechanism underlying this gender-specific role of NO-cGMP signaling on cardiovascular function remains to be elucidated but may importantly impact the clinical development of compounds that modulate activity of the NO-cGMP signaling system, possibly in a gender-specific manner.

sGC-independent effects of NO: role of protein S-nitrosylation in cardiovascular function

An alternative pathway by which NO can affect the vasculature is the direct modification of protein thiols, resulting in the formation of S-nitrosothiols. Although NO-induced VSMC relaxation was reported to be abolished in mice lacking sGC β_1 , this observation does not preclude the possibility that NO has other, non-sGC dependent effects on blood pressure, since only isolated aortic rings were examined in this study[12]. S-nitrosylation might play a more prominent role in smaller resistance vessels, which have a larger contribution to determining systemic vascular resistance and blood pressure than do large conductance arteries. In support of this hypothesis, mice deficient for S-nitrosoglutathione reductase (GSNOR, an enzyme promoting protein denitrosylation) were found to be in a persistent state of systemic vasodilation[89] and became hypotensive when anesthetized[90]. Combined with the observation that GSNOR inhibition causes relaxation of murine aortic rings[91], these results suggest that increased levels of protein S-nitrosylation are associated with improved vasodilation and lower blood pressure. Multiple possible targets of S-nitrosylation have been identified in smooth muscle, including structural and contractile proteins[92, 93]. For example, the direct activation of VSMC Ca²⁺-dependent K⁺ channels by S-nitrosylation[94] was proposed as a potential mechanism of cGMP-independent vasorelaxation[95]. Interestingly, sGC itself has also been identified as a target for S-nitrosylation. S-nitrosylation of two cysteines, Cys243 in sGC α and Cys122 in sGC β , leads to desensitization of sGC to activation by binding of NO to its heme cofactor[96]. This might represent a mechanism responsible for the development of tolerance to NO.

sGC as a therapeutic target in hypertension

Several mechanisms, including genetic variation (see above) and oxidative stress[97] can modulate NO-cGMP signaling. Cardiovascular diseases, including hypertension, are often associated with oxidative stress caused by increased endogenous production of reactive oxygen species[98]. These strong oxidants not only scavenge NO, reducing its bioavailability[99, 100], but also oxidize sGC leading to loss of the heme cofactor, rendering

sGC unresponsive to NO stimulation[97, 101, 102]. The suggestion that the level of heme-oxidized or apo-sGC is elevated in disease conditions[97, 103] highlights the physiological relevance of oxidative modification of sGC. Therefore, compounds that can activate sGC via an NO-independent mechanism hold great therapeutic potential for the treatment of cardiovascular diseases[100, 104, 105], including hypertension[106].

Two classes of compounds have been developed that can directly stimulate and increase cGMP formation in a setting where the use of classical NO donors may be problematic because of the development of tolerance or when sGC is oxidized: heme-dependent NO-independent sGC stimulators and heme-independent NO-independent sGC activators[104]. Preclinical studies confirmed the ability of the first class of heme-dependent NO-independent stimulators of sGC (YC-1, BAY 41-2272, BAY 41-8543, BAY 63-2521 or riociguat) to lower systemic and pulmonary arterial pressure, relax isolated vessels in a variety of organ systems, and reverse the cardiovascular complications associated with hypertension[106–112].

The second class of NO-independent, heme-independent sGC activators, only activates the oxidized and/or heme-deficient sGC enzyme, which cannot be stimulated by the first class of heme-dependent stimulators[97, 113, 114]. The sGC activator HMR1766 (ataciguat) did not affect blood pressure in humans or animals[115], although this compound did have beneficial effects in chronic renal disease[116]. In contrast, BAY 58-2667 (cinaciguat) and BAY 60-2770 were shown to be potent vasodilators, lowering systemic blood pressure in preclinical trials[117–119]. Cinaciguat reduced oxidative stress and attenuated cardiac dysfunction in a rat model of myocardial infarction[120]. In addition, cinaciguat improved cardiac contractility, coronary blood flow, and endothelium-dependent vasodilation to acetylcholine when given prior to cardiac ischemia-reperfusion[121]. In a Phase I clinical trial, cinaciguat exhibited dose-dependent hemodynamic effects on diastolic blood pressure, mean arterial blood pressure and heart rate. In addition, cinaciguat reduced both preload and after load in the heart[122]. In a phase II study in patients suffering from acute decompensated heart failure, cinaciguat significantly reduced systemic vascular resistance[123]. Together, these results illustrate the clinical potential of sGC stimulators and activators as modulators of blood pressure. Together with other compounds that modulate NO-cGMP signaling (e.g. PDE5 inhibitors), sGC activators and stimulators may provide an opportunity to manipulate the cGMP system and treat hypertension.

Conclusion

The studies described in this review illustrate how knowledge of the role of NO-cGMP signaling in the regulation of blood pressure has advanced over the last decade and demonstrate that the presence of a functional NO-cGMP signaling system is imperative for the maintenance of blood pressure homeostasis. Studies in animal models and human data illustrate that disruption of the NO-cGMP signaling cascade (e.g. genetically, pharmacologically, or in response to increased oxidative stress) leads to the development of hypertension. The identification of genetic variants in key NO-cGMP modulating enzymes and the further development and investigation of animal models of impaired NO-cGMP signaling will undoubtedly facilitate our further understanding of the role of NO-cGMP signaling and its interacting pathways in the development of hypertension.

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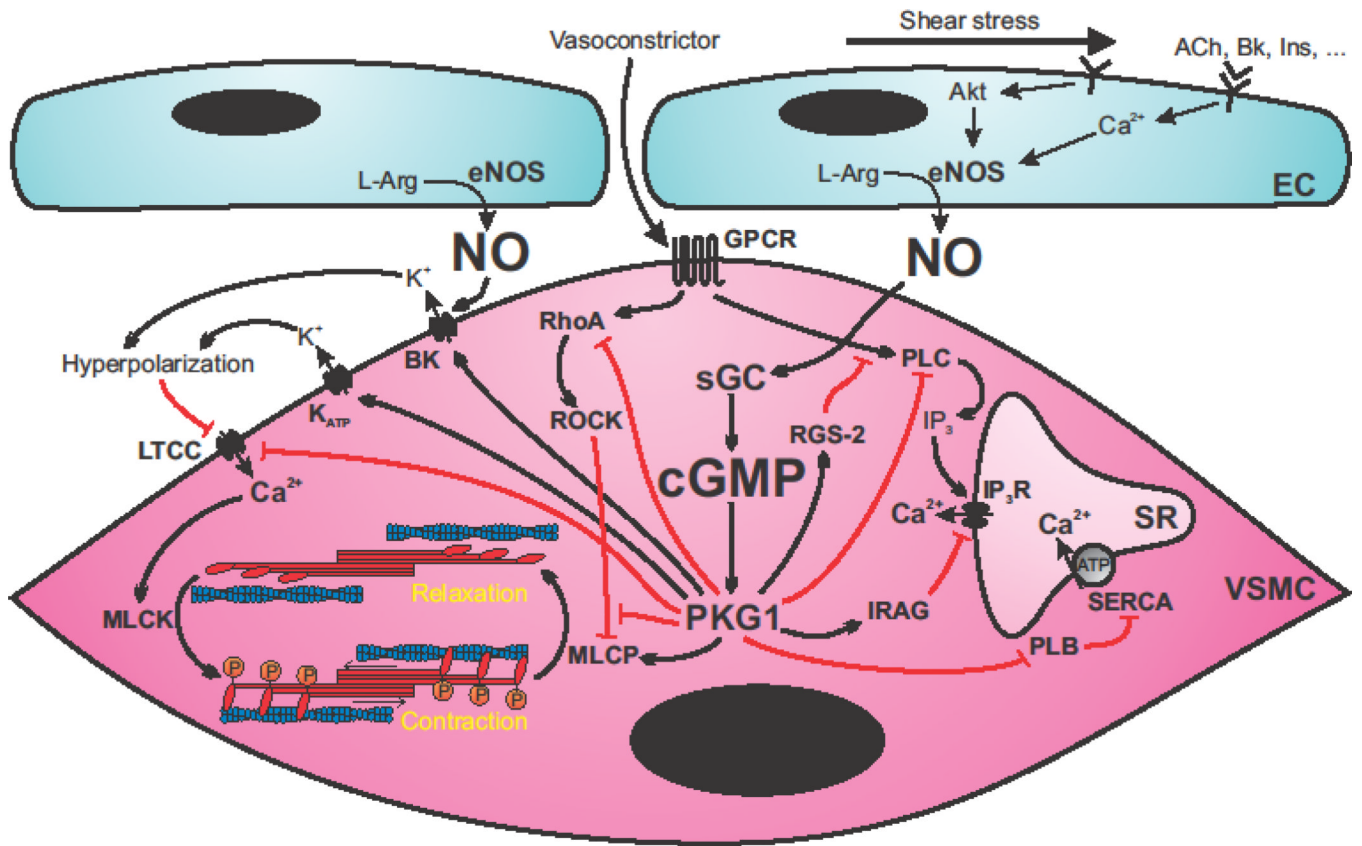


Figure 1. Nitric oxide (NO) - cGMP signaling in endothelium-dependent relaxation of vascular smooth muscle cells (VSMC)

Endothelial cells (EC) respond to agonist stimulation by increasing intracellular Ca²⁺ concentration and activity of endothelial nitric oxide (NO) synthase (eNOS). Shear stress also leads to eNOS activation via Akt-dependent phosphorylation. NO diffuses into adjacent VSMC and activates soluble guanylate cyclase (sGC), leading to increased cGMP production and protein kinase G I (PKG1) activation. PKGI activation promotes vasorelaxation via multiple mechanisms. PKGI interferes with vasoconstrictor signaling via G protein-coupled receptors (GPCR) by activating regulator of G protein signaling-2 (RGS-2), inhibiting phospholipase C (PLC), and stimulating inositol triphosphate (IP₃) receptor-associated PKG substrate (IRAG), which ultimately leads to inhibition of Ca²⁺ release from the sarcoplasmic reticulum (SR) via the IP₃ receptor (IP₃R). Phosphorylation by PKGI also inhibits phospholamban (PLB), leading to increased Ca²⁺ reuptake into the SR by sarco/endoplasmic reticulum ATPase (SERCA). On the other hand, PKGI interferes with RhoA and rho-associated protein kinase (ROCK)-dependent inhibition of myosin light chain phosphatase (MLCP). Together with a direct activation of MLCP, this leads to increased dephosphorylation of the regulatory light chain of myosin, which promotes vasorelaxation by blocking actin-myosin interaction. PKGI also reduces extracellular Ca²⁺ influx directly by inhibiting the L-type Ca²⁺ channel (LTCC). Activation of the large conductance Ca²⁺-activated K⁺ channel (BK), which can also be directly stimulated by NO, and the ATP-dependent K⁺ channel (K_{ATP}) cause VSMC hyperpolarization leading to inhibition of the LTCC. ACh: acetylcholine, Bk: bradykinin, Ins: insulin, L-Arg: L-Arginine, MLCK: myosin light chain kinase.

Table 1

Overview of the different mutant mouse strains in NO-cGMP signaling that have been generated, including their effect on blood pressure regulation.

Mutant	Genotype	Blood Pressure Phenotype	Ref
nNOS ^{-/-}	nNOS β splice variant present	Normotensive	[124]
nNOS ^{-/-}	Null mutation	Normotensive	[125]
eNOS ^{-/-}	Null mutation	Hypertensive	[38,39,126,127]''
iNOS ^{-/-}	Null mutation	Normotensive	[128–130]''
n/iNOS ^{-/-}	nNOS β splice variant present	Normotensive	[131,132]''
n/eNOS ^{-/-}	nNOS β splice variant present	Hypertensive	'[131–133]
e/iNOS ^{-/-}	nNOS β splice variant present	Hypertensive	[131,132]'
e/i/nNOS ^{-/-}	nNOS β splice variant present	Hypertensive	[132]
nNOS transgenic	Conditional myocardial overexpression	Normotensive	[134,135]
	Brain overexpression	Normotensive	[136]
eNOS transgenic	Myocardial overexpression	Normotensive	[137,138]
	Endothelial overexpression	Hypotensive	[139,140]'
iNOS transgenic	Conditional myocardial overexpression	Normotensive	[141]
	Myocardial overexpression	Normotensive	[142]
	Pancreatic β cell overexpression	Normotensive	[143]
sGC α 1 ^{-/-}	Null mutation	Mild Hypertensive	[25]
sGC α 1 ^{-/-}	Truncated inactive protein	Normotensive on C57Bl/6 Hypertensive on 129S6	[22]
sGC α 2 ^{-/-}	Null mutation	Normotensive	[25]
sGC β 1 ^{-/-}	Null mutation	Hypertensive	[12]
sGC β 1 ^{H105F}	NO-insensitive mutant protein	Hypertensive	[54]
PKG1 ^{-/-}	Null mutation	Hypertensive at 6W Normotensive at \geq 8W	[57]
PKG1 SM α rescue	PKG1 α only in SMC of PKG1 ^{-/-}	Normotensive	[55]
PKG1 SM β rescue	PKG1 β only in SMC of PKG1 ^{-/-}	Normotensive	[55]
PKG-LZM	Mutant protein	Hypertensive at \geq 12W	[11]
PKG1 α ^{C42S}	H ₂ O ₂ -insensitive mutant protein	Hypertensive	[61]
PKG2 ^{-/-}	Null mutation	Normotensive	[144]
PDE5 transgenic	Myocardial overexpression	Normotensive	[145]