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New Insights into the Role of Soluble Guanylate Cyclase in Blood Pressure Regulation

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Abstract

Purpose of review—Nitric oxide (NO) – soluble guanylate cyclase (sGC)-dependent signaling mechanisms have a profound effect on the regulation of blood pressure. In this review, we will discuss recent findings in the field which support the importance of sGC in the development of hypertension.

Recent findings—The importance of sGC in blood pressure regulation was highlighted by studies using genetically modified animal models, chemical stimulators/activators and inhibitors of the NO/sGC signaling pathway, and genetic association studies in humans. Many studies further support the role of NO/sGC in vasodilation and vascular dysfunction, which is underscored by the early clinical success of synthetic sGC stimulators for the treatment of pulmonary hypertension. Recent work has uncovered more details about the structural basis of sGC activation, enabling the development of more potent and efficient modulators of sGC activity. Finally, the mechanisms involved in the modulation of sGC by signaling gases other than NO as well as the influence of redox signaling on sGC have been the subject of several interesting studies.

Summary—sGC is fast becoming an interesting therapeutic target for the treatment of vascular dysfunction and hypertension, with novel sGC stimulating/activating compounds as promising treatment options in the clinic.

Keywords

soluble guanylate cyclase; hypertension; nitric oxide; vasodilation; redox

Introduction

Soluble guanylate cyclase (sGC) has an important and established role in a wide variety of (patho)-physiological processes, particularly in the cardiovascular system. As such, sGC is a

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potential therapeutic target in cardiovascular pathologies such as myocardial infarction [1], myocardial dysfunction associated with sepsis [2], and stroke [3]. A large body of evidence which includes many recent findings has particularly highlighted the potential role of sGC in the development of hypertension. For a more in-depth discussion of the various signaling pathways relevant to blood pressure in which sGC is involved, as well as the animal models used to study these effects we refer the reader to two very recent review articles [4*,5]. sGC is a heme-containing enzyme catalyzing the formation of cyclic guanosine monophosphate (cGMP). The literature detailing the structure and function of sGC has recently been extensively reviewed elsewhere [6*]. Nitric oxide (NO) is well-characterized as the major stimulator of sGC-dependent cGMP formation, and several synthetic compounds have been identified which can also strongly increase sGC activity. sGC stimulators act on the enzyme containing a reduced heme group and synergize with NO. sGC activators work by replacing the heme cofactor when it has been oxidized or lost from the enzyme, at which point sGC becomes NO-insensitive (Fig. 1) [7].

NO-sGC Signaling in Vasodilation

Our understanding of the role of the NO-sGC-cGMP pathway in the regulation of vasodilation is still evolving. For example, the current paradigm dictates that NO generated by endothelial NO synthase (NOS3) in the vascular endothelium activates sGC in the underlying smooth muscle cell layer. Recent data suggest however that arterial smooth muscle cells also contain functional NOS. Endothelium-denuded arterial rings have an NO/cGMP-dependent vasodilatory response to acetylcholine, but only when treated with the membrane-permeable superoxide dismutase analog tempol, [8]. These results suggest that insensitivity of endothelium-denuded vessels to acetylcholine might be the consequence of increased oxidative stress. Along the same line, aortae of spontaneously hypertensive rats were found to contain an alternative source of NO, other than NOS, which inhibits phenylephrine-induced contractions in an endothelial and sGC-dependent way [9*]. In these hypertensive vessels, which contain higher levels of nitrate and nitrite than vessels from normotensive subjects, cytochrome P450 reductase reduces nitrate to increase NO production. A role of cytochrome P450 reductase as an alternative source for NO had been proposed previously [10] and potentially functions as an endothelial mechanism to compensate for reduced NO bioavailability in the setting of hypertension. This effect is unmasked when the production of reactive oxygen species is inhibited and could explain the beneficial vascular effects of antioxidant treatments in the setting of hypertension [11,12]. In fact, NO-sGC signaling itself may counteract oxidative stress by activating antioxidant response genes via a mechanism dependent on the peroxisome proliferator-activated receptor gamma coactivator 1-alpha [13*].

An exciting new role of NO-sGC signaling was recently described in the regulation of lymphatic vessel contractility. NO generated by NOS3 in lymphatic endothelial cells is required for robust lymphatic contractions under physiological conditions [14]. A recent report indicates that, in analogy to shear stress-controlled blood vessel relaxation, flow-dependent relaxation of lymphatic vessels is also mediated by NO/sGC signaling [15*]. Together, these reports add to our already extensive knowledge of the role of NO-sGC

signaling in vasodilation and highlight the opportunity to uncover new ways by which this pathway controls vascular function.

NO/sGC in Vascular Dysfunction

The NO-sGC pathway can be perturbed in many ways, including reduced NO bioavailability, reduced expression of genes encoding for NOS and/or sGC, or oxidation of sGC itself resulting in the conversion of the enzyme to an NO-insensitive state [4]. Recent data suggest that alterations in the NO-sGC signaling pathway are responsible for the disrupted control of vascular tone in aging and obese subjects. For example, postnatal rat arteries contain higher levels of NOS3 than adult vessels [16]. In the microcirculation (cremaster muscle arterioles) of diet-induced obese rats, endothelial caveolae density is decreased, which leads to improved NOS3 function and sGC-dependent vasodilation [17]. In contrast, in saphenous arteries from obese rats, increased endothelial caveolae density and caveolin-1 expression levels are associated with inhibition of NOS3 activity and impaired vasorelaxation [18]. These seemingly conflicting results most likely reflect vascular bed-specific modulation of caveolins in response to obesity. Finally, downregulation of vascular sGC expression itself appears to contribute to metabolic syndrome-induced vascular dysfunction [19].

In light of the role of sGC in vascular dysfunction, it has become a candidate target to improve the outcome of blood vessel injury or oxidative stress. Cinaciguat, a synthetic sGC activating compound, attenuates neointima formation in a rat model of vascular injury [20]. This drug also improves the nitrosative stress-induced decrease in the vasodilatory response to acetylcholine of rat aorta subjected to peroxynitrite administration [21**]. The direct sGC stimulator BAY 41-8543 and sGC activator BAY 60-2770 restore cGMP-dependent vasodilation during hemoglobin infusion [22]. The latter is especially relevant since infusion of hemoglobin-based oxygen carriers (HBOC), which are being developed as alternatives for red blood cell transfusion, cause vascular dysfunction and hypertension due to free oxyhemoglobin scavenging of NO [23]. Targeting sGC appears to be a viable approach to block at least some of the deleterious vascular side effects of HBOC transfusion.

sGC and Systemic Hypertension

Further investigation into the previously observed effect of genetic background on the hypertensive phenotype of sGC α 1-knockout mice [2,24,25] revealed the existence of genetic modifiers determining blood pressure in the setting of sGC α 1 deficiency. The mouse renin locus is strongly associated with sGC α 1-deficient hypertension, which is supported by the observation that inhibitors of the renin-angiotensin-aldosterone system (RAAS) normalize blood pressure in sGC α 1-knockout mice [26**]. In another study, sGC signaling was implicated in the upregulation of renal prorenin receptor expression in response to sodium depletion [27]. Similarly, sGC β 1 is required for the recruitment of renin-producing cells in renal afferent arterioles in response to a combination of low salt and RAAS inhibition [28]. Finally, angiotensin III-induced natriuresis was found to be dependent on sGC signaling [29]. Altogether, these studies highlight the complex interplay between sGC and RAAS signaling.

Endothelium-dependent vasorelaxation is attenuated in mesenteric arteries from sGC α 1-deficient mice [30]. This attenuation is more pronounced in hypertensive male sGC α 1-knockout mice on a 129S6/SvEvTac background than in sGC α 1-knockout mice backcrossed to the C57BL/6 background, which are normotensive [26]. These results support the hypothesis that vascular dysfunction contributes to the hypertension associated with loss of sGC α 1 in 129S6/SvEvTac mice. The pivotal role of NO-sGC-cGMP mediated vasorelaxation in the maintenance of blood pressure was also illustrated by the observation that inhibition of cGMP-dependent protein kinase 1 (PKG1), a major target of cGMP in the vasculature, with a novel cell-penetrating inhibitor peptide disrupts NO-induced vasorelaxation and increases blood pressure [31].

Intriguingly, sGC expressed in the central nervous system can have an opposite effect on blood pressure compared to its hypotensive vascular effects. For example, NO/sGC signaling in rat brain is required for the hypertensive response to administration of both cyclosporine [32] as well as orexin A [33]. Similarly, neuronal NOS-sGC signaling mediates the blood pressure and heart rate increase in response to hippocampal injection of l-glutamate [34]. This effect appears to be mostly due to cardiac sympathetic stimulation, with little impact on vascular sympathetic signaling. Furthermore, sGC is involved in baroreceptor signaling in the ventral portion of the medial prefrontal cortex, where it influences both bradycardic and tachycardic responses to changes in blood pressure [35*].

These reports further add to the existing data indicating that sGC plays an important role in blood pressure control, both by direct effects on vascular smooth muscle function as well as via neuronal mechanisms.

sGC in the Lung and its Role in the Pulmonary Vasculature

Increasing evidence highlights the role of sGC in both lung morphology and function. For example, newborn mice lacking the sGC α 1 isoform have reduced lung volume and small airway structures. Moreover, during hyperoxic injury, alveolarization is impaired in sGC α 1-deficient mice, an observation that is associated with decreased smooth muscle cell differentiation [36]. From a functional perspective, the pharmacologic increase of cGMP, either via inhibition of its breakdown or stimulation of its production, has gained considerable importance in the treatment of pulmonary arterial hypertension. This was recently the subject of a comprehensive review article [37*].

Recent animal studies illustrate that sGC stimulation, e.g. with BAY 41-8543, can completely reverse hypoxic pulmonary vasoconstriction in pigs [38] and reduce pulmonary vascular resistance and right ventricular remodeling in a rat model of pulmonary embolism [39]. Sodium nitrite therapy also improves the outcome in monocrotaline-induced pulmonary hypertension in the rat via sGC activation after conversion of nitrite to vasoactive NO [40]. On the other hand, stimulation of sGC by the riociguat-related stimulator BAY 41-2272, with or without inhibition of cGMP phosphodiesterase by sildenafil administration, fails to prevent right ventricle hypertrophy after pulmonary trunk banding in rats [41]. A likely explanation for the discrepancy between these studies is that the surgical procedure performed in the latter study directly induces right ventricular hypertrophy, while the other

studies rely on models of primary pulmonary vascular dysfunction. Together, the results from these studies suggest that stimulation of NO-cGMP signaling improves pulmonary vascular dysfunction but does not have a direct beneficial effect on right ventricular hypertrophy. Surprisingly, the authors even observed increased mortality in the banded animals treated with BAY 41-2272 [41]. The detrimental effect of sGC stimulation in this model is most likely the result of arterial vasodilation leading to hypotension and/or increases in the workload of the failing right ventricle due to the baroreflex response or increased venous return.

Lastly, chronic thrombin exposure was found to reduce expression of NOS3 and sGC and increase PDE5 in lung endothelial cells, likely contributing to NO-cGMP depletion in pulmonary arterial hypertension [42]. These studies confirm the important contribution of NO/cGMP signaling to pulmonary vasorelaxation, which is already being exploited as a pharmacological target to treat pulmonary vascular dysfunction and pulmonary hypertension, as discussed below.

Development and Characterization of New sGC Stimulating and Activating Drugs

The synthetic heme-dependent sGC stimulator BAY 41-2272 and heme-independent sGC activator cinaciguat (BAY 58-2667) were originally identified using high-throughput screening of large chemical libraries [7]. Characterization of chemical modifications of these compounds [43] resulted in the identification of more stable and potent variants (e.g. BAY 63-2521, also known as riociguat, an analog of BAY 41-2272). Recently, new variants of the BAY 41-2272 parent compound have been synthesized with potentially even more improved physicochemical and pharmacokinetic properties [44].

New sGC structural data is currently becoming available [45*], enabling more targeted strategies to develop novel sGC modulating compounds [46]. Characterization of the crystal structure of the human sGC heterodimeric catalytic domains confirmed the existence of a pseudo-symmetrical site analogous to adenylate cyclase. This observation could potentially lead to the development of novel sGC modulators working in a similar way as forskolin does in the case of adenylate cyclase [47]. Structural studies can also explain the different effects of the various activators and stimulators on sGC activity. For example, resolving the crystal structure of the binding of the sGC activator BAY 60-2770 to an NO-sensitive heme domain homologous to sGC demonstrated that BAY 60-2770 shows a slightly more efficient conformational shift of the heme-binding domain than its parent compound cinaciguat, explaining its marginally more potent stimulation of sGC activity [48*]. And finally, the vasodilatory molecule 1-nitro-2-phenylethane was recently characterized as a novel heme-dependent sGC stimulator, distinct from the previously discovered riociguat-related compounds [49]. All these compounds hold great therapeutic potential in a variety of cardiovascular and other defects related to sGC dysfunction.

Clinical Trials using sGC Stimulators and Activators

Considering the ample evidence in the literature demonstrating an important role for sGC in vascular function, sGC stimulators and activators are actively being developed for their clinical use, especially for the treatment of pulmonary hypertension. The phase III CHEST and PATENT clinical trials illustrated the efficacy of the sGC stimulator riociguat for the treatment of chronic thromboembolic pulmonary hypertension [50**] and pulmonary arterial hypertension [51**,52], respectively. Similarly, in the phase IIb LEPHT trial [53], 2 mg daily doses of riociguat improve the cardiac index and reduce pulmonary and systemic vascular resistance in patients with pulmonary hypertension caused by systolic left ventricular dysfunction [54,55]. A pilot trial in patients with pulmonary hypertension associated with interstitial lung disease showed that riociguat improves cardiac output and pulmonary vascular resistance [56].

sGC activation is also being considered for the treatment of cardiomyopathy. Cinaciguat reduced right heart preload in a phase IIb study of patients with acute decompensated heart failure [57*]. Interestingly however, this trial was stopped prematurely because of the high incidence of hypotension at higher doses of the drug. Although not unexpected, the systemic side effects of modulating sGC activity confirm the profound effect of sGC stimulation on blood pressure regulation, undoubtedly (but likely not prohibitively) complicating the clinical development of sGC activators and stimulators.

Activation of sGC by Non-NO Gasotransmitters

Carbon monoxide (CO) was previously described as an alternative activator of sGC. More recently however, the CO releasing molecule CORM-3 was shown to stimulate endothelium-dependent aortic relaxation via increased NO production rather than by a direct effect on sGC. Another major mechanism leading to CORM-3-induced aortic relaxation involves direct activation of Ca^{2+} -induced K^{+} channels, with only a marginal role for sGC [58,59]. Interestingly, CORM-3 relaxed precontracted aortae from both wild-type and spontaneously hypertensive rats to the same extent [59]. CORM-3 has also been used to study the effects of CO on tubuloglomerular feedback (TGF), a renal autoregulatory mechanism that matches the glomerular filtration rate with sodium excretion, thereby keeping the plasma volume within a narrow range. In this system, adding CORM-3 to isolated perfused rabbit macula densa reduced TGF via stimulation of cGMP production by sGC [60,61]. Taken together, these results indicate that CO has both sGC-dependent and -independent vasodilating and renal effects.

1-Nitrosocyclohexyl acetate, a novel long-acting nitroxyl (HNO) donor relaxes aortic vessels in a sGC-dependent manner [62]. In contrast to NO, binding of the HNO donor to the sGC heme iron is unable to cause cleavage of the heme iron-histidine bond [63], suggesting that the HNO donor activates sGC in an alternative manner. Interestingly, and in contrast to NO, HNO can activate sGC even under conditions of oxidative stress [64*]. For example, the HNO donor Angeli's salt relaxes aortae from angiotensin II-treated mice to the same extent as control vessels, while the response to acetylcholine was significantly decreased in the former [65].

In spite of extensive evidence showing that the heme cofactor of sGC does not bind molecular oxygen (O₂) [6], a provocative study has suggested that O₂ can directly stimulate sGC activity via an at this point unknown mechanism. O₂-induced activation of sGC activity possibly constitutes the mechanism of hypoxic vasodilation of precontracted vascular smooth muscle, due to the release of O₂ from red blood cells [66*]. While NO, CO, and HNO stimulate sGC by binding the heme cofactor, the putative effect of O₂ is somewhat unlikely to be the result of a similar interaction with heme, considering numerous previous studies of the characteristics of sGC-bound heme. Instead, it is conceivable that a redox-sensitive modulation of sGC, as discussed in the following section, might be involved.

Redox Modulation of sGC

In addition to the classical regulation of sGC activity via interactions with the heme cofactor, sGC activity can be modulated by redox-sensitive mechanisms (Fig. 1). For example, chronic angiotensin II infusion in rats is associated with S-nitrosylation of sGC, resulting in decreased vascular NO-induced cGMP production and hypertension. Mutation of the cysteine residue at position 516 in sGC α 1 to alanine restores NO sensitivity in angiotensin II-treated cells [67**]. This study confirms that increased nitrosative stress, leading to sGC desensitization [48,68,69], can cause vascular dysfunction associated with hypertension. S-nitrosylation has also been suggested to be responsible for the NO insensitivity of sGC containing an oxidized (Fe³⁺) heme cofactor. Oxidation of the heme iron induces a conformational change in the enzyme which reportedly enables heme-assisted S-nitrosylation of two cysteine residues in the β 1 subunit, rendering the enzyme NO insensitive [70*].

Finally, cellular reducing conditions [71] and hypoxic conditions in pulmonary arteries [72*] decrease sGC dimerization and activity. On the other hand, interaction of thiol oxidized sGC with protein disulfide-isomerase also inhibits NO stimulation of sGC activity [73]. Together, these results suggest that optimal sGC activity depends on a delicate balance in the redox environment.

Conclusion

The recent data reviewed in this paper reaffirm the central role of sGC signaling in cardiovascular function and blood pressure regulation. Together, these findings bolster the concept of sGC as a viable drug target for the treatment of systemic and pulmonary hypertension, and associated cardiovascular pathologies. sGC modulating compounds have already entered the final phases of clinical testing as pulmonary hypertension drugs. New modulators of sGC are being developed, and new biochemical ways to regulate sGC activity, e.g. by modifying the cellular redox environment, have recently been discovered, paving the way for novel therapeutic strategies and further clinical development of sGC stimulating and activating compounds.

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* of special interest

** of outstanding interest

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Key Points

- sGC is a central mediator of blood pressure regulation predominantly via its effects on vascular function.
- Dysfunction in the NO-sGC-cGMP signaling cascade is implicated in cardiovascular disease, particularly systemic and pulmonary hypertension.
- Synthetic compounds stimulating or activating sGC activity have important clinical potential.
- sGC activity is sensitive to the redox environment.

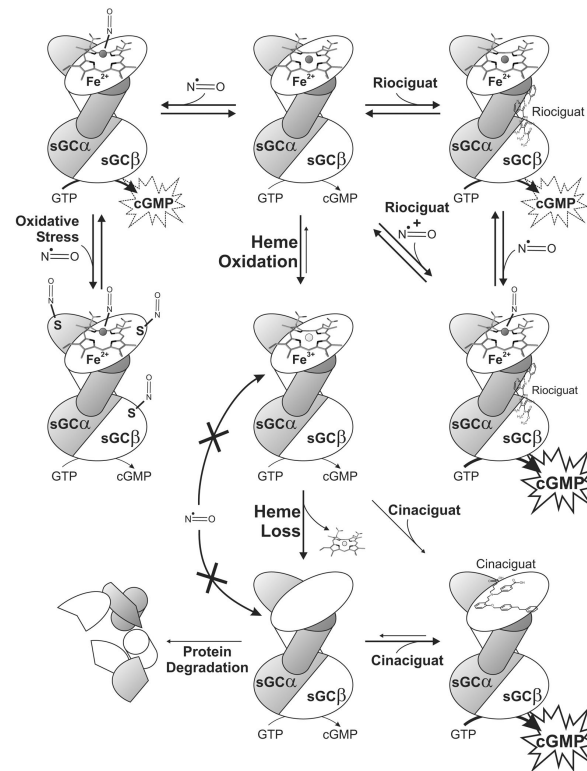


Figure 1.
Regulation of sGC activity.

In the absence of any stimulating or activating factors, sGC converts guanosine triphosphate (GTP) to cGMP at a low basal catalytic rate (center, top line). sGC containing a prosthetic heme group with reduced iron (Fe^{2+}) can be stimulated by NO and by heme-dependent sGC stimulators such as riociguat. NO and riociguat act synergistically to increase sGC activity several hundred-fold. Sustained NO stimulation and/or the presence of oxidative stress can lead to S-nitrosylation of sGC, which makes the enzyme insensitive to NO stimulation. Direct oxidation of the heme iron to the Fe^{3+} state also prohibits stimulation by NO as well as by the heme-dependent sGC stimulators. Heme oxidation can lead to loss of the heme cofactor from sGC, which renders the enzyme prone to ubiquitination and proteasomal degradation. A new class of sGC modulators known as the sGC activators (e.g. cinaciguat) selectively activates heme-oxidized and heme-free sGC, protecting it from degradation and increasing sGC activity by mimicking the NO-heme complex in the heme-binding pocket of sGC.