Role of cGMP, NO and PDE5 in Hypertension: Review Article

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Abstract

Cyclic GMP (cGMP) is a ubiquitous intracellular second messenger that mediates a wide spectrum of physiologic processes in multiple cell types within the cardiovascular and nervous systems. Synthesis of cGMP occurs either by NO-sensitive guanylyl cyclases in response to nitric oxide or by membrane-bound guanylyl cyclases in response to natriuretic peptides and has been shown to regulate blood pressure homeostasis by influencing vascular tone, sympathetic nervous system, and sodium and water handling in the kidney. Several cGMPs degrading phosphodiesterases (PDEs), including PDE1 and PDE5, play an important role in the regulation of cGMP signaling. Recent findings revealed that increased activity of cGMP-hydrolyzing PDEs contribute to the development of hypertension. In this review, we will summarize recent research findings regarding the cGMP/PDE signaling in the vasculature which is associated with the development and maintenance of hypertension.

KeyWords:cGMP, NO,PDE5, Hypertension.

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Introduction

Nitric oxide (NO) and natriuretic peptides (NPs) are important messenger molecules. Their binding to soluble guanylyl cyclase (sGC) and particulate guanylyl cyclase (pGC), respectively, increases the formation of cGMP which is a unique second messenger. cGMP-driven activation of protein kinases, ion channels, or phosphodiesterases (PDEs) causes a broad variety of physiological responses whereas dysregulation can result in severe pathologies. Thus, understanding the mechanisms of cGMP generation and downstream signaling cascades is fundamental for the comprehension of molecular physiology and pathophysiology as well as for the development of drugs within these pathways that, as demonstrated in recent years, have already substantial therapeutic implications. An overview of sGC and pGC signaling, the physiology, pathophysiology as well as basic and clinical pharmacology of cGMP has been presented in recent monographies and reviews (1).

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The production of cGMP is increased either after stimulation of sGC by nitric oxide (NO) formed from L-arginine by NO synthases (NOS) or after activation of pGCs by specific peptides. Several effectors of cGMP have been identified, most importantly cGMP-regulated protein kinases (abbreviated as cGKs or PKGs). These kinases phosphorylate downstream target proteins which mediate cGMP effects, e.g., by decreasing the intracellular calcium concentration. cGMP signaling is abrogated by cGMP hydrolysis via PDEs and cGMP export via multidrug resistance proteins (also referred to as ABC transporters). However, these signaling cascades seem to be highly compartmentalized in individual cell types; in addition, the expression of the members of these pathways can vary between tissues and in healthy or disease states which is currently being investigated with various sensors and was also discussed in this meeting. Based on the differential expression of sGC, pGC, or regulatory PDEs, their subcellular localization and the potential crosstalk between this and other pathways, the net effects of pharmacological intervention into the cGMP cascade are difficult to predict (2).

Production of cGMP can be stimulated by organic nitrates and other NO donors, e.g., glyceryl trinitrate, sodium nitroprusside or molsidomine, which-enzymatically or non-enzymatically—release NO. NO donors are primarily used for the treatment or prevention of acute angina pectoris attacks in patients suffering from coronary heart disease, hypertensive crisis, and other emergencies requiring rapid relaxation of vascular smooth muscle cells (SMC). More recently, selective stimulators of sGC have been discovered. The first potent and selective sGC stimulator was riociguat which effectively enhances cGMP production; riociguat has been approved in 2013 for the treatment of pulmonary arterial hypertension (PAH) and chronic thromboembolic pulmonary hypertension (CTEPH). In addition, new sGC stimulators like vericiguat, praliciguat, and olinciguat are currently in phase 2 and 3 clinical development for kidney disease and chronic heart failure. Recently, the combination of sacubitril, an inhibitor of the neutral endopeptidase (NEP, neprilysin) which protects natriuretic peptides from degradation, with an angiotensin AT1 receptor antagonist (valsartan) has been introduced for the treatment of chronic heart failure (HF), especially in patients with reduced ejection fraction. Successful examples for targeting cGMP hydrolysis are PDE5 inhibitors. In 1999, the first selective PDE5 inhibitor, sildenafil, was introduced for the treatment of erectile dysfunction (ED) followed by vardenafil and tadalafil in 2003. Later on, sildenafil and tadalafil were also approved for the treatment of pulmonary hypertension, and tadalafil got approval for the treatment of symptoms of benign prostatic hyperplasia (BPH). Furthermore, various drugs targeting cGMP signaling are in clinical development for different diseases. (3).

cGMP downstream targets structure and function:

GMP signaling is conveyed by several effectors including PKG, cGMP-cgated ion channels and phosphodiesterases. A major cGMP effector is PKG-I, which plays a key role in the regulation of smooth vascular and cardiac function. PKG-I is a homodimeric enzyme that is expressed as two isoforms, PKG-Iα and Iβ. After activation, PKG-I signals through phosphorylation of various proteins in a tissue-specific manner. Research over the past decade has established that PKG-I may also be activated by oxidizing agents such as hydrogen peroxide. The in vivo relevance of this cGMP-independent redox activation of PKG-I is being increasingly recognized (4).

The pathophysiological relevance of oxidative stress on NO/sGC signaling and the impact on disease progression is still poorly understood. However, recently and also presented on the last cGMP meeting in 2017, it was shown that vascular SMC express a reductase (CYB5R3) turning back oxidized sGC into the native form. More recently, it has been demonstrated that knock out of CYB5R3 in vascular SMC is linked to a hypertensive phenotype by reducing NO-mediated sGC stimulation (5).

Besides sGC, other signaling molecules in the NO and NP pathway may be very sensitive to oxidative stress, too. At the cGMP conference in Mainz, two talks focused on redox regulation of PKG-Iα. First, nitroxyl (HNO) donors, which hold therapeutic promise as vasorelaxants, were shown to induce an interdisulfide bond involving Cys42 of two PKG-Iα subunits and an intradisulfide bond between Cys117 and Cys195 in the cGMP-binding site of PKG-Iα (FriederikeCuello, Hamburg). Both disulfides were shown to enhance the kinase activity, thereby inducing vasorelaxation (6).

The second talk investigated the molecular basis of the constitutive activation of PKG-Iα by oxidizing agents. Data from an in vitro assay suggest that constitutive activation of PKG-Iα is not mediated via Cys42, but rather through oxyacid formation at Cys117. This mechanism could be particularly relevant to PKG anchored near hydrogen peroxide sources or under pathophysiological conditions associated with high intracellular concentrations of hydrogen peroxide (7).

Another research group recently determined the crystal structure of a PKG-Iβ holoenyzme complex at 2.3 Å that enabled to visualize the regulatory and catalytic domain interface of the inhibited state. A key finding was that the regulatory domain of each monomer binds the catalytic domain of the other monomer, giving rise to inhibition in trans. This structure suggests that activated and inhibited states of PKG-Iβ are stabilized by domain–domain contacts that either expose or occlude the active site (7).

cGMP PDE structure and function:

The importance of cGMP for various body functions requires a tight control of its intracellular concentration. Phosphodiesterases (PDEs) are responsible for breakdown of cyclic nucleotides. In mammals, twenty-one PDE genes have been identified, which are classified into at least 11 families (PDE1 to PDE11), each with distinct substrate specificity, regulatory properties, and tissue distribution. According to preferences for cyclic nucleotides, cGMP-specific PDEs (PDE 5, 6, and 9) and cAMP-specific PDEs (PDE 4, 7, and 8) show strong specificities for either cGMP or cAMP as substrate, whereas dual-specificity PDEs (PDE 1, 2, 3, 10, and 11) may hydrolyze both cyclic nucleotides. PDE5, PDE6, and PDE9 are of particular importance for the field since they very efficiently hydrolyze sGC- and pGC-produced cGMP (9). In addition, PDE2 and PDE3, whose activity may be modulated by cGMP, are also involved in NO and NP signaling. It was also demonstrated that PDEs are able to cleave non-canonical cNMPs (in particular, cCMP and cUMP) in addition to hydrolyzing the canonical cNMPs, cAMP, and cGMP (10) which may broaden the therapeutic impact of PDE inhibitors in the future. In recent years, it turned out that PDE inhibition provides effective treatment approaches in various diseases (8).

Whereas PDE5 inhibitors represent an approved and established treatment paradigm ranging from erectile dysfunction, pulmonary arterial hypertension to benign prostatic hyperplasia, PDE9 inhibitors are still not available for patients. In preclinical studies, PDE9 inhibitors enhanced the performance of animals in various cognition tasks in models of Alzheimer's disease (AD), schizophrenia, and Huntington's disease (11).

Nitric Oxide-cGMP Signaling in Hypertension:

It is an intriguing concept to stimulate the production of messenger molecules leading to active vasorelaxation, most notably nitric oxide (NO)-cyclic guanosine monophosphate (cGMP) signaling, to decrease total peripheral resistance and blood pressure. Apart from inducing vasorelaxation, NO—cGMP signaling is also known to reduce blood pressure through other mechanisms (12).

First, it decreases renin release, which lowers production of the blood pressure increasing hormone angiotensin II. Second, it increases natriuresis through modulation of various sodium transporters, for example, sodium–hydrogen exchanger 3, epithelial sodium channel, and Na-K-Cl cotransporter 2, in the tubules. However, in this review, the focus will be on vascular NO—cGMP signaling. The main source of vascular NO is eNOS (endothelial NO-synthase) (13).

Upon stimulation of G-protein coupled receptors that increase Ca2+ in endothelial cells, eNOS is phosphorylated at Ser1177. Subsequently, with tetrahydrobiopterin (BH4) as co-factor, eNOS monomers form dimers, and electrons donated by NADPH are transferred to the N-terminal catalytic center, which results in the release of the NO moiety from the amino acid L-arginine (14).

NO is oxidized to NO2−, and subsequently to NO3−. NO3− can be reduced back to NO2− by commensal bacteria in the gastrointestinal tract and by xanthine oxidoreductase. NO2−, in turn, can be reduced back to NO by various nitrite reductases under low pH and oxygen levels, representing a possible mechanism for blood pressure lowering during metabolic acidosis. BP is usually increased during acute hypoxia. Thus, an NO2−-NO3−-NO cycle is created, the role of which in vasodilatation and hypertension has been extensively reviewed (15).

NO diffuses to vascular smooth muscle cells (VSMCs) where it binds sGC (soluble guanylyl cyclase). NO-bound sGC produces cGMP, a key

regulator of vascular tone. The rise in intracellular cGMP in VSMCs triggers activation of PKG (protein kinase G) and subsequent phosphorylation of VASP (vasodilator-stimulated phosphoprotein) at serine 239. Thereupon, contracted, phosphorylated actin-myosin is dephosphorylated by myosin light chain phosphatase, resulting in relaxation. PDEs (phosphodiesterases) that degrade cGMP are present in VSMCs to participate in tonus control. Next to NO—cGMP signaling endothelium-derived eicosanoids are active to cause vasodilation through cyclic adenosine monophosphate (cAMP) release (prostaglandins), or through endothelium-dependent hyperpolarziation (EDH) of VSMCs (13).

In hypertension, NO—cGMP signaling can be reduced as a consequence of decreased bioavailability of NO due to superoxide. More directly in the NO signaling cascade, decreased NO production due to reduced eNOS levels or uncoupling, reduced cGMP production by sGC, decreased PKG activity, and increased cGMP metabolism by PDE can occur (13).

PDE5 and cGMP Signaling in The Vasculature:

Since the discovery of NO, the role of the NO/cGMP signaling cascade regulating vascular tone has been demonstrated in many studies. An impairment of this pathway results in vascular dysfunction and hypertension. Beside a decreased NO bioavailability, increased activity of cGMP-degrading PDEs in vascular smooth muscle cells is the main cause for vascular dysfunction characterized by exaggerated vasoconstrictor response and impaired NO-dependent vasodilation. Vascular dysfunction alters vascular tone and contributes to development of hypertension. Until now, the circumstances leading to an activation of either PDE1 or PDE5 are still not known. In vascular smooth muscle cells (VSMC), augmentation of intracellular Ca2+ levels induced by angiotensin II have been shown to increase PDE1 expression (16).

In line with this, PDE1-dependent reduction of cGMP availability has been shown to contribute to the increased vasoconstrictor response observed in chronic angiotensin II infusion. Interestingly, PDE1 more than PDE5 seem to play also an important role in age-dependent vascular dysfunction. In mice with defective nucleotide excision repair gene (Ercc1d/−), a mouse model which develops severe age-related vascular dysfunction, inhibition of both PDE1 and PDE5 restored vascular dysfunction. In addition, senescent human VSMC have elevated PDE1A, PDE1C and PDE5 mRNA levels. Moreover, a single nucleotide polymorphism in PDE1A was associated with elevated diastolic blood pressure and vascular hypertrophy suggesting a role of the PDE1A isoform in human vascular function (17).

On the other side, the studies have shown a higher contribution of PDE5 to vascular dysfunction observed in experimental hypertension. In renovascular hypertension, an initially increased NO/cGMP signaling leads to cGMP-dependent PDE5 activation which in turn enhances cGMP degradation and promotes vascular dysfunction (18).

In support of this concept, inhibition of the PDE5 by sildenafil increased renal cGMP content, restored renal and mesenteric vascular function, and reduced blood pressure in renovascular hypertension. Beside the direct effects of PDE5 inhibition on vascular function, sildenafil also seems to exert its effect on blood pressure by reducing angiotensin II levels and restoring the baroreflex in renovascular hypertension (19).

Cyclic GMP Signaling Influences Natriuresis and Hypertension:

In the past few years, various studies highlighted the effect of cGMP on fluid and salt transport within the nephron. Under hypertensive blood pressure conditions, cGMP signaling plays an important role in regulating pressure natriuresis by affecting the solute transport of several sodium channels in the proximal tubule, the thick ascending limb, and the collecting duct like the Na+/K+-ATPase, the type 3 Na+/H+ exchanger (NHE3), the Na+/K+/2Cl− cotransport (NKCC), and the epithelial sodium channel (eNaC), respectively (20).

In the proximal tubule, cGMP has been shown to increase phosphorylation of Src-kinase and initiate thereby signaling events that are capable of reducing the surface expression of apical NHE3 and basolateral Na+/K+-ATPase leading to increased pressure natriuresis (21).

The relevance of cGMP signaling in regulating pressure natriuresis was confirmed in a study showing that a polymorphism in the cGMP-dependent protein kinase I was associated with salt-sensitive hypertension and impaired pressure

natriuresis after salt load in humans. These cGMP-related polymorphisms resulted in a loss of pressure natriuresis control in a Src-Na+/K+-ATPase dependent manner (22).

In line with these human data, Kemp and colleagues have shown that selective activation of the angiotensin II type 2 (AT2) receptor induced the internalization/inactivation of Na+/K+-ATPase and NHE3 in proximal tubule leading to pressure natriuresis and attenuated blood pressure response in experimental hypertension. Again, these effects seemed to be mediated by a NO/cGMP-dependent mechanism which involved the signaling kinases ERK1/2 and Src (23).

However, it should be noted that this finding indicate that the effect of the NO/cGMP signaling cascade on proximal tubule transport seemed to be species dependent and dependent on the circumstances how cGMP is generated. Thus, in contrast to the results seen in mice, Shirai et al. could demonstrate that angiotensin II activates NHE3, and the basolateral $Na+-HCO3-$ co-transporter through a NO/cGMP-dependent mechanism in human proximal tubular cells (24).

Until now, the responsible phosphodiesterase which is controlling cGMP availability and thereby proximal tubular transport is not known. However, other data have shown that chronic PDE5 inhibition increased expression of cGMP-dependent protein kinase I in proximal tubule cells and thereby attenuated the development of renal fibrosis in a mouse model of unilateral urethral obstruction (25).

Similar to the proximal tubule, cGMP signaling regulates blood pressure by influencing sodium and water transport in the distal tubule [64]. In the thick ascending limb, cGMP exerts its natriuretic effect by inhibiting cAMP-mediated trafficking of Na-K-2Cl co-transporter (NKCC2) through the activation of the cGMP-stimulated PDE2 which lowers cAMP levels (26).

Beside PDE2, the data examining the effect of NO on NKCC2 function suggest that PDE5 activation reduces cGMP availability and thereby increases sodium reabsorption via the NKCC2 in angiotensin II-dependent hypertension. In addition, inhibition of PDE5 increases cGMP levels and restores the inhibitory effect of NO on NKCC2 in the thick ascending limb. The source of NO mediating this effect can be of quite different origins. Thus, it has been shown that luminal flow-induced shear stress stimulates NO generation and thereby inhibits NKCC2 (27).

Conclusion:

The pathogenesis of hypertension is a multifactorial process. Decrease of cGMP signaling in the blood vessels contributes to the development and maintenance of hypertension. Among the causes that decreased cGMP availability, enhanced degradation by PDE5 plays a central role. Thus, PDE5 inhibition leads to blood pressure reduction via vasodilation and increased pressure natriuresis by affecting several sodium transporters within the kidney.

REFERENCES

1. **Sandner P, Zimmer DP, Milne GT et al.** Soluble guanylate cyclase stimulators and activators. Handb Exp Pharmacol.2019. https://doi.org/10.1007/164_2018_197
2. **Friebe A, Sandner P &Schmidtko A. cGMP:** A unique 2nd messenger molecule–recent developments in cGMP research and development. Naunyn-schmiedeberg's Archives of Pharmacology.2020, 393(2), 287-302.
3. **Krishnan SM, Kraehling JR, Eitner F et al.** The impact of the nitric oxide (NO)/soluble guanylyl cyclase (sGC) signaling cascade on kidney health and disease: a preclinical perspective. Int J Mol Sci. 2018: 9:19(6).
4. **Hofmann F. A** concise discussion of the regulatory role of cGMP kinase I in cardiac physiology and pathology. Basic Res Cardiol. 2018: 113(4):31
5. **Durgin BG, Hahn SA, Schmidt HM et al.** Loss of smooth muscle CYB5R3 amplifies angiotensin II-induced hypertension by increasing sGC heme oxidation. JCI Insight.2019;4(19)
6. **Donzelli S, Goetz M, Schmidt K et al.** Role of PDE9 in cognition. Adv Neurobiol. 2017:17:231–254
7. **Sheehe JL, Bonev AD, Schmoker AM et al.** Oxidation of cysteine 117 stimulates constitutive activation of the type Iα cGMP-dependent protein kinase. J Biol Chem.2018: 293(43):16791–16802
8. **Baillie GS, Tejeda GS, Kelly MP.** Therapeutic targeting of 3′,5′-cyclic nucleotide phosphodiesterases: inhibition and beyond. Nat Rev Drug Discov. 2019: 18(10):770–796
9. **Lee DI, Zhu G, Sasaki T, Cho GS et al.** Phosphodiesterase 9A controls nitric-oxide-independent cGMP and hypertrophic heart disease. Nature.2015. 519(7544):472–476
10. **Schneider EH, Seifert R.** Inactivation of non-canonical cyclic nucleotides: hydrolysis and transport. Handb Exp Pharmacol. 2017: 238:169–205.
11. **Dorner-Ciossek C, Kroker KS, Rosenbrock H.** of PDE9 in cognition. Adv Neurobiol.2017: 17:231–254
12. **Mergia E, Stegbauer J.** Role of phosphodiesterase 5 and cyclic GMP in hypertension.CurrHypertens Rep. 2016; 18:39. doi: 10.1007/s11906-016-0646-5.
13. **Golshiri K, AtaeiAtaabadi E, Portilla Fernandez EC et al.** The importance of the nitric oxide-cgmp pathway in age-related cardiovascular disease: focus on phosphodiesterase-1 and soluble guanylate cyclase.Basic Clin Pharmacol Toxicol.2020; 127:67–80.
14. **Li Q, Youn JY, Cai H.** Mechanisms and consequences of endothelial nitric oxide synthase dysfunction in hypertension.J Hypertens.2015; 33:1128–1136
15. **Bailey JC, Feelisch M, Horowitz JD et al.** Pharmacology and therapeutic role of inorganic nitrite and nitrate in vasodilatation.Pharmacol Ther.2014; 144:303–320.
16. **Groneberg D, Konig P, Wirth A et al. (2010).** Smooth muscle-specific deletion of nitric oxide-sensitive guanylyl cyclase is sufficient to induce hypertension in mice. Circulation.2010;121(3):401–9.
17. **Bautista Nino PK, Durik M, Danser AH et al.** Phosphodiesterase 1 regulation is a key mechanism in vascular aging. Clin Sci (Lond).2015;129(12):1061–75.
18. **Cavalcanti CO, Alves RR, de Oliveira AL et al.** Inhibition of PDE5 restores depressed baroreflex sensitivity in renovascular hypertensive

rats. Front Physiol.2016;7:15.

19. **Dias AT, Rodrigues BP, Porto ML et al.** Sildenafil ameliorates oxidative stress and DNA damage in the stenotic kidneys in mice with renovascular hypertension. J Transl Med.2014;12:35.
20. **Ramseyer VD, Ortiz PA, Carretero OA et al.** Angiotensin II-mediated hypertension impairs nitric oxide-induced NKCC2 inhibition in thick ascending limbs. Am J Physiol Ren Physiol.2016.
21. **Nascimento NR, Kemp BA, Howell NL et al.** Role of SRC family kinase in extracellular renal cyclic guanosine 3′,5′-monophosphate - and pressure-induced natriuresis. Hypertension.2011; 58(1):107–13.
22. **Citterio L, Ferrandi M, DelliCarpini S et al.** cGMP-dependent protein kinase 1 polymorphisms underlie renal sodium handling impairment. Hypertension.2013;62(6):1027–33.
23. **Kemp BA, Howell NL, Gildea JJ et al.** AT(2) receptor activation induces natriuresis and lowers blood pressure. Circ Res.2014;115(3):388–99.
24. **Shirai A, Yamazaki O, Horita S et al.** Angiotensin II dose-dependently stimulates human renal proximal tubule transport by the nitric oxide/guanosine 3′,5′-cyclic monophosphate pathway. J Am Soc Nephrol.2014;25(7):1523–32.
25. **Cui W, Maimaitiyiming H, Qi X et al.** Increasing cGMP-dependent protein kinase activity attenuates unilateral ureteral obstruction-induced renal fibrosis. Am J Physiol Ren Physiol.2014; 306(9):F996–1007.
26. **Ares GR, Caceres P, Alvarez-Leefmans FJ et al.** cGMP decreases surface NKCC2 levels in the thick ascending limb: role of phosphodiesterase 2 (PDE2) Am J Physiol Ren Physiol.2008;295(4):F877–87.
27. **Cabral PD, Garvin JL.** Luminal flow regulates NO and O2(−) along the nephron. Am J Physiol Ren Physiol.2011;300(5):F1047–53.