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Nitric Oxide-cGMP Signaling in Hypertension
Current and Future Options for Pharmacotherapy

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Abstract—For the treatment of systemic hypertension, pharmacological intervention in nitric oxide-cyclic guanosine monophosphate signaling is a well-explored but unexploited option. In this review, we present the identified drug targets, including oxidases, mitochondria, soluble guanylyl cyclase, phosphodiesterase 1 and 5, and protein kinase G, important compounds that modulate them, and the current status of (pre)clinical development. The mode of action of these compounds is discussed, and based upon this, the clinical opportunities. We conclude that drugs that directly target the enzymes of the nitric oxide-cyclic guanosine monophosphate cascade are currently the most promising compounds, but that none of these compounds is under investigation as a treatment option for systemic hypertension.

Key Words: hypertension ■ mitochondria ■ nitric oxide ■ oxidoreductase ■ soluble guanylyl cyclase

Hypertension is a major clinical risk factor for cardiovascular disease, the leading cause of disability and death in developed societies.[1] As a consequence, antihypertensive treatment is expected to deliver a major contribution to healthcare. Current treatments are, however, challenged by a lower than expected protective cardiovascular effect.[2] The currently used antihypertensive drugs either have a diuretic effect through blockade of sodium/chloride transport in the renal tubule or are blocking the vasoconstriction to calcium, epinephrine/norepinephrine, angiotensin II or endothelin, or block the formation of these vasoconstrictors. Thus, 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 (Figure 1), 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.[3] 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).[4] Upon stimulation of G-protein coupled receptors that increase Ca^{2+} in endothelial cells, eNOS is phosphorylated at Ser1177. Subsequently, with tetrahydrobiopterin (BH_4) 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.[5] NO is oxidized to NO_2^-, and subsequently to NO_3^-.[6,7] NO_3^- can be reduced back to NO_2^- by commensal bacteria in the gastrointestinal tract and by xanthine oxidoreductase.[7] NO_2^-, in turn, can be reduced back to NO (Figure 1) 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.[7] Thus, an NO_2^--NO_3^--NO cycle is created, the role of which in vasodilatation and hypertension has recently been extensively reviewed.[7]

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.[8] PDEs (phosphodiesterases) that degrade cGMP are present in VSMCs to participate in tonus control.[4] 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.[4]

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

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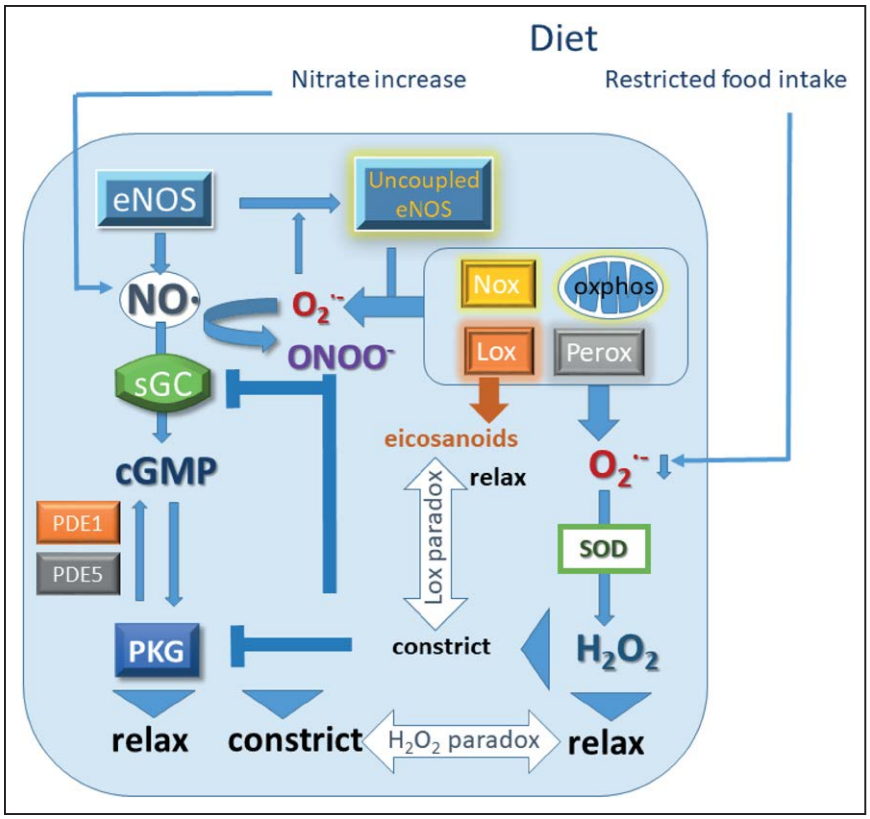

Figure 1. Overview of the nitric oxide (NO)-cyclic guanosine monophosphate signaling cascade and inhibitory oxidative pathways in the vascular wall. cGMP indicates cyclic guanosine monophosphate; eNOS, endothelial nitric oxide synthase; Lox, lipoxygenase; Nox, NADPH oxidase; Oxphos, oxidative phosphorylation; PDE, phosphodiesterase; Perox, peroxidase; PKG, protein kinase G; sGC, soluble guanylyl cyclase; and SOD, superoxide dismutase.

cGMP metabolism by PDE can occur, as will be addressed in detail below. The current review will focus on intervention in this signaling cascade. The role of oxidative stress will be shortly summarized, with reference to comprehensive review articles.

NO Donors

Various compounds that release NO are available for clinical use. In hypertensive emergencies, SNP (sodium nitroprusside), given intravenously, is used to rapidly decrease blood pressure. Organic nitrates are instead used to relieve angina pectoris. The use of these NO donors for hypertension is limited by their physicochemical properties (short-term efficacy) and the occurrence of tolerance, caused by inhibition of the nitrate bioactivators (aldehyde dehydrogenase for nitroglycerin and pentaerithrityl tetranitrate, and cytochrome p450 for isosorbide-5-mononitrate and isosorbide dinitrate), and pseudotolerance pathways caused by hormonal counterregulation.[9]

NO Signaling in Relation to Risk Factors

On a more chronic term, intervention in risk factors for cardiovascular disease is widely applied and can act, in part, through improvement of NO—cGMP signaling. As reviewed by Daiber et al[10], the classical cardiovascular risk factors hypertension, dyslipidemia, obesity, diabetes mellitus, smoking and aging, but also sex-related factors (menopause, estrogen-use), air pollution, mental stress, sleep disturbance through noise exposure, and alcohol abuse are all associated with diminished vasodilator function in humans.[10,11] Lifestyle interventions, such as a healthy diet, exercise, weight reduction, and smoking cessation are first-step therapies. For smoking, nicotine delivery systems and vaping products with nicotine, such as e-cigarettes, have been developed in the hope that these would have less negative effect on health and may aid in cessation of smoking cigarettes. Nevertheless, flavoring products in vaping fluids scavenge NO in cultured endothelial cells, although this does not necessarily translate into decreased vasodilation in vivo.[12,13] Also, e-cigarette constituents lead to oxidative stress and cell death in cultured endothelial cells, which could lead to lower levels of NO, although the effects of smoking tobacco cigarettes are generally more pronounced.[14,15] Systolic and diastolic blood pressure have been reported to change differentially during the acute use of e-cigarettes. Tobacco cigarettes generally increase both systolic and diastolic pressure, varying from 1 to 8 mm Hg,[16] while e-cigarettes either affect both, or diastolic pressure only (≈7 mm Hg).[17] In vivo effects of e-cigarettes on endothelial NO—cGMP and the relation to blood pressure have not been studied yet in parallel. However, recent studies demonstrated that flow-mediated dilation and NO bioavailability in the forearm were decreased shortly after vaping, and implicated NOX (NADPH oxidase) 2, an enzyme that decreases NO bioavailability due to superoxide production as a potential cause.[18,19] In mice, acetylcholine-induced vasodilation is diminished, and reactive oxygen species (ROS) production is increased from 3 days after vaping. This is absent in NOX2 knockout mice, indicating that oxidative stress due to NOX2 activation underlies the vaping-induced endothelial dysfunction.[18] Macitentan, an antagonist for the vasoconstrictor hormone ET-1 (endothelin-1), and bepridil, an activator of the transcription factor Forkhead box O3 that induces antioxidant gene expression, blocked the effect of vaping-induced endothelial dysfunction.[18] Decreased NO availability due to NOX activation, ET-1 and Forkhead box O3 have also been implicated in noise-induced hypertension, with an important role for inflammation.[11,20] Therefore,

macitentan and bepridil might also be applicable in this condition. With respect to dietary interventions, nitrate (NO_3^-) and nitrite (NO_2^-) may be of benefit (Figure 1). Beet juice combines the presence of antioxidants with a high amount of NO_3^-. NO_3^- is converted to NO_2^- by bacteria in the gastrointestinal tract.[6] In short-lasting studies, beet juice has shown to lower blood pressure and to enhance vasodilation. Also, supplementation of NO_3^- and NO_2^- exerted antihypertensive effects in various hypertensive rat models and in human hypertension. For further reading on this topic and an overview of clinical trials, we refer to a recent article.[21]

Reduced NO Bioavailabity Due to Oxidative Stress and eNOS Uncoupling

Decrease of bioactive NO by superoxide occurs under the formation of peroxynitrite ($ONOO^-$; Figure 1).[22,23] Superoxide can be produced by a number of enzymes, of which NOX, LOX (lipoxygenase), XO (xanthine oxidase), and peroxidase have been explored as drug targets. Inhibition of NOX has received the widest attention.[23–29] Based on specificity, the existing NOX inhibitors can be subdivided into 3 generations (Figure 2).[30,31] Only the third-generation compounds are orally available and sufficiently specific to be used in the clinic, although the potentially antihypertensive NOX inhibitor setanaxib did not meet the clinical end point of a reduction in albuminuria in diabetic nephropathy in a phase II study.[30–33] Several other third-generation NOX inhibitors are now under preclinical investigation, and results in hypertensive models are awaited.[34,35] Another approach is the induction of a decrease of NOX gene expression. G36 is an orally available compound that reduces NOX1 transcription through antagonism of GPER (G-protein-coupled estrogen receptor). It prevents angiotensin II-induced hypertension and preserves endothelial vasodilation in rats.[36,37] XO forms NO from nitrite but also produces O_2^- and H_2O_2 as a waste product during purine degradation.[38] As such it might not be an optimal drug target to improve NO signaling.[39] Classical purinergic XO inhibitors, allopurinol and oxypurinol, originally developed to treat gout, have been reported to improve endothelial function and to reduce blood pressure, but display variable efficacy and cause renal damage.[38] The nonpurine-like XO inhibitor febuxostat mildly reduced blood pressure in rats and humans and improved acetylcholine-induced vasodilation in spontaneously hypertensive rats (SHR), but unfortunately, its use resulted in increased cardiovascular mortality in patients with gout and cardiovascular disease.[40–43] The lipoxygenase 12/15-LOX catalytically consumes NO during metabolism of polyunsaturated fatty acids and produces $O_2^{\cdot -}$.[44] LOX inhibition was proposed as a treatment in hypertension, however, LOX (eg, 15-LOX), is also involved in the production of endothelial vasodilator eicosanoids, limiting it as a target to treat hypertension.[45,46] Myeloperoxidase is released from polymorphonuclear neutrophils and has the capacity to infiltrate endothelial cells. It consumes NO as a substrate during formation of oxidants, for example, HOCl, that in turn oxidizes NO and its precursor L-arginine.[47–50] Peroxidase inhibition, therefore, would be a way to upregulate NO.

As an alternative to get rid of surplus superoxides and related oxidative substances pharmacological stimulation of genes coding for antioxidant enzymes has been attempted. Nrf2 (nuclear factor erythroid 2-related factor 2) is a transcription factor that plays a major role in expression of antioxidant genes.[51] The Nrf2-activators bardoxolone methyl and its analogs RTA 405 and dh404 have been tested clinically, but after initially showing renoprotection, a recent clinical trial in patients with end-stage diabetic kidney disease with bardoxolone methyl was halted prematurely because it increased blood pressure and heart failure-induced mortality.[52,53] This may relate to Nrf2-induced upregulation of renin-angiotensin system (RAS) components,[54] which might have counteracted the RAS-blocking therapy in the enrolled patients.[52] Therefore, Nrf2-activation does not seem useful in hypertension in patients who are on RAS inhibition, a well-known antihypertensive and cardiac, vascular and renal protective treatment. It is, however, still an open question if higher dosages of RAS-inhibiting drugs might overcome this problem.

Finally, ROS have been reported to affect eNOS directly. Superoxide and peroxynitrite both oxidize BH_4 to BH_2, which can compete for eNOS binding.[5] Depletion of BH_4 combined with reduced binding leads to decreased eNOS dimerization and electron transport. This so-called uncoupling of eNOS forces the enzyme to produce superoxide instead of NO, leading to decreased vasodilation and increased inflammation, thereby contributing to hypertension.[5,55] Both NOX and vascular peroxidase 1 have been implicated in eNOS uncoupling.[26,56] Evidence indicated that uncoupling of eNOS underlies the hypertension in rodents exposed to either angiotensin II or deoxycorticosterone acetate-salt, and co-factor supplementation has been suggested as a possible remedy.[5]

NO and Mitochondrial Respiration

Mitochondria interact with NO-cGMP signaling in at least 3 ways: (1) mitochondrial superoxide scavenges NO, (2) mitochondrial arginase II competes with eNOS for L-arginine, and (3) NO regulates mitochondrial respiration.

The formation of free radicals like superoxide by mitochondria is due to spillover of electrons in the respiratory chain during the process of oxidative phosphorylation (Figure 3). This makes the respiratory chain a potential drug target. 6-Chromanol-derived SUL (Sulfateq) compounds preserve mitochondrial membrane potential by activating complex I and IV of the chain, thus keeping up ATP production during respiratory chain-disturbing conditions like ischemia, cooling, warming, and metabolic diseases.[57,58] For instance, SUL-121 diminished diabetes mellitus-induced kidney damage by upregulating SOD2 (superoxide dismutase 2), and the same is true for SUL-109.[57–59] SOD2 is an enzyme that is involved in conversion of O_2^- into H_2O_2.[29,60] H_2O_2 is a mediator of O_2^- signaling and serves as a vasodilator, but, paradoxically, also as a vasoconstrictor and apoptosis inducer, depending on the vessel type.[29,61,62] Despite the involvement of SOD2, it is not clear whether the protective effect of SUL-121 arises from increased NO availability due to lowered oxidative stress, the beneficial signaling properties of H_2O_2, or other mechanisms. SUL compounds mimic the gasotransmitter hydrogen sulphide at complex IV,[63] creating a hypometabolic state, which should reduce ROS while preserving ATP synthesis (Figure 3). Hydrogen

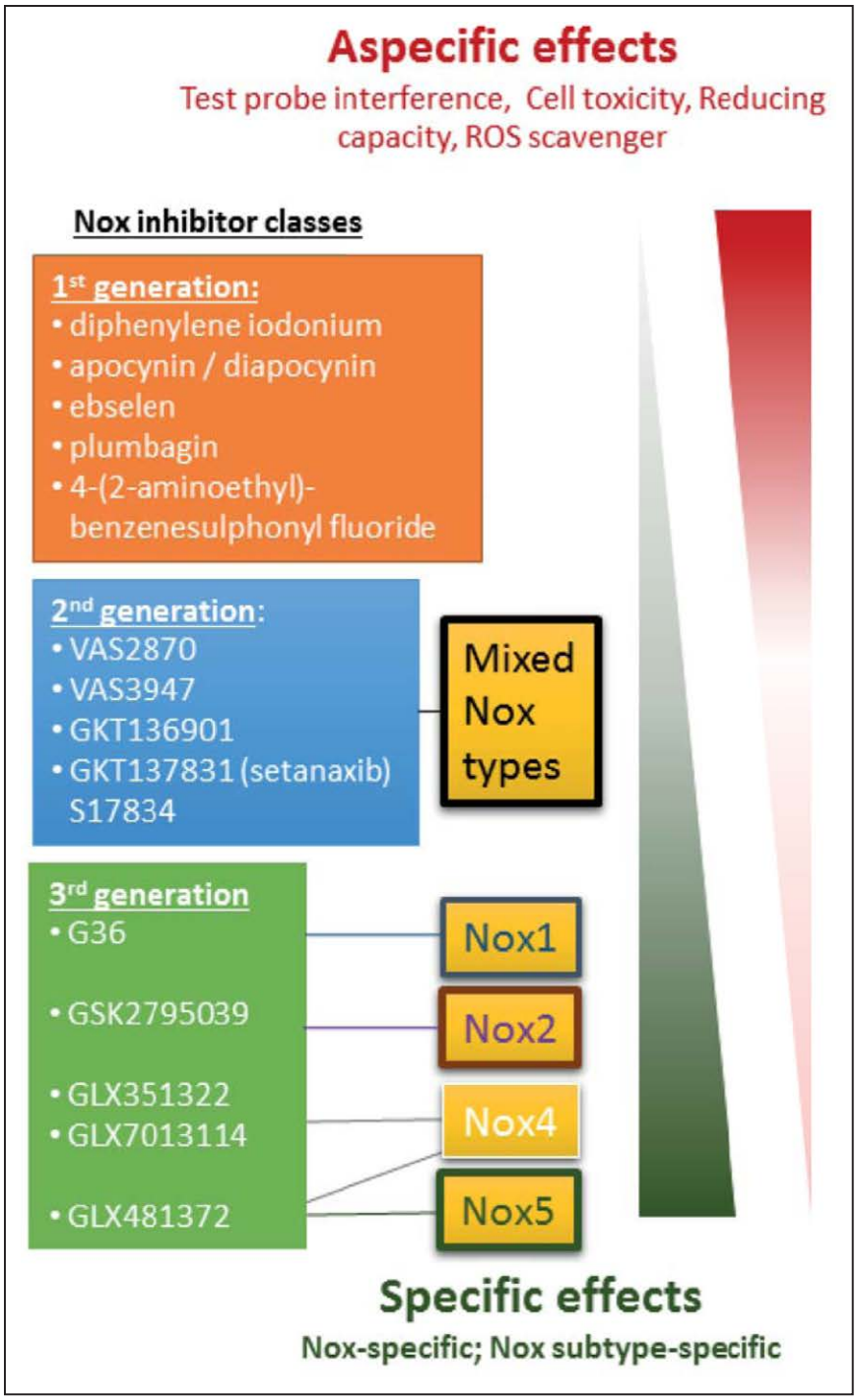

Figure 2. Overview of 3 generations of Nox (NADPH oxidase) inhibitors in order of specificity (**top**: low specificity; **bottom**: high specificity). No clinical trials are currently being undertaken with these compounds. ROS indicates reactive oxygen species.

sulphide-releasing compounds, for example, GYY4137, improve NO signaling and lower blood pressure in rats,[64] but the blood pressure effect appears to be K_{ATP} channel activation- rather than cGMP-mediated. As discussed below, H_2O_2 can mediate vasodilation through hyperpolarization, in which potassium channels are involved. Thus, it is well possible that H_2O_2-mediated vasodilation is an important complementary mechanism for blood pressure reduction induced by SUL compounds. The (R) enantiomer of SUL-121, SUL-150, also lowers blood pressure, but this is at least partly due to its function as an α-adrenergic receptor antagonist.[65]

The antioxidant MitoQ, selectively accumulating in mitochondria, and the SOD2 mimic mitoTEMPO, both exert beneficial effects in hypertensive animals.[66,67] Yet, none of this has resulted in a clinical follow-up.

Mitochondrial adaptation (Figure 3) involves inner membrane stabilization, fusion (=the joining of mitochondria to restore function) and inhibition of fission (=mitochondrion splitting).[68] Elamipretide stabilizes cardiolipin, an important component of the mitochondrial inner membrane.[69] As a consequence, it restored eNOS levels and endothelium-dependent vasodilation in metabolic syndrome models, yet without affecting blood pressure.[69,70] The fission inhibitor Mdivi-1 has

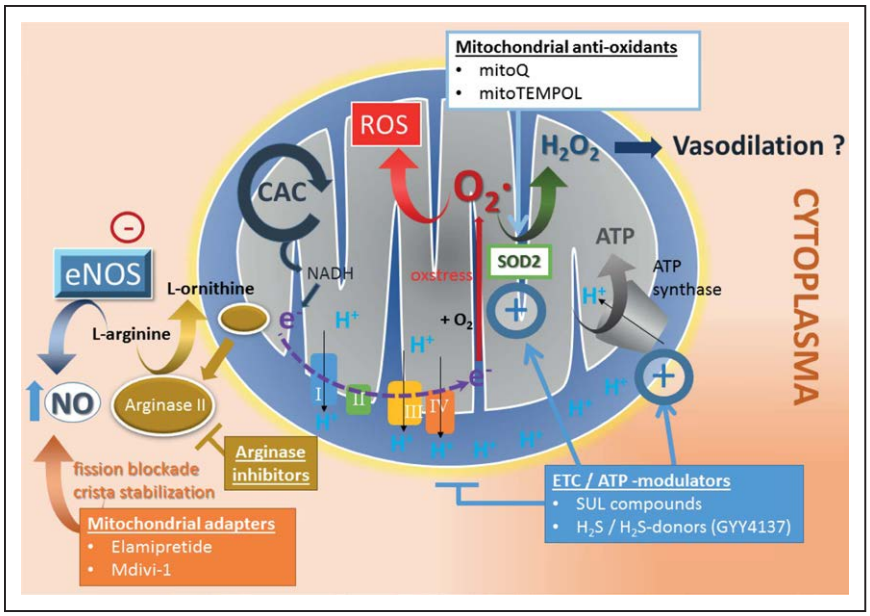

Figure 3. Interaction of mitochondrial metabolism with vascular nitric oxide (NO) signaling and vasodilation. Four drug categories (rectangular boxes, underlined text) are of potential interest in hypertension. CAC indicates citric acid cycle; eNOS, endothelial NO-synthase; ETC, electron transport chain; PPP, pentose phosphate pathway; ROS, reactive oxygen species; SOD2, superoxide dismutase 2; and SUL, Sulfateq.

been reported to lower blood pressure and to suppress inflammation in SHR.[71]

Arginase II synthesizes L-ornithine from L-arginine. L-ornithine is needed for the production of spermidine and L-proline, that contribute to cell division and collagen formation, respectively. In endothelial cells, arginase II is capable of translocating from mitochondria to the cytoplasm. Since this prevents NO formation from L-arginine by eNOS (Figure 3),[72] it may cause vasoconstriction contributing to hypertension.[73,74] Arginase II also upregulates mitochondrial [Ca^{2+}] at the cost of cytosolic [Ca^{2+}], thereby lowering eNOS activity.[75] Thus, arginase II inhibitors would enhance eNOS activity both by increasing L-arginine availability and by upregulating cytosolic [Ca^{2+}]. The classical inhibitors *S*-(2-boronoethyl)-L-cysteine, N^G-Hydroxy-L-arginine, and 2(S)-amino-6-boronohexanoic acid are not under clinical development, and newer compounds, for example, cinnamides, are still in their infancy of development.[76,77]

Finally, NO inhibits the respiratory chain through its binding to the heme group in cytochrome c oxidase (complex IV) and by formation of peroxynitrite due to its reaction with superoxide.[78] This process involves the incorporation of both mitochondrial NOS and nitrite reductases that generate NO from nitrites into the mitochondria. Although respiratory chain suppression may result in apoptosis in cardiovascular and renal tissue, thus potentially indirectly contributing to hypertension, there are currently no pharmacological strategies to target this mechanism. Importantly, mitochondrial NO does not result in cGMP signaling.

NO and DNA Damage Response-Related Aging and Hypertension

DNA damage in mice that display accelerated aging due to genetic deletion of the DNA repair enzyme ERCC1 (*Ercc1*$^{\Delta/-}$ mice), leads to premature vascular aging and hypertension. This involves reduced NO-cGMP signaling,[79–82] due to a combination of decreased eNOS expression and phosphorylation at Ser1177, increased ROS and increased PDE 1 and 5 activity.[79,80] How this intrinsic process of aging prompted by DNA damage leads to decreased eNOS and increased PDE expression remains to be clarified. Interestingly, a well-known antiaging intervention, dietary caloric restriction (a 10%–30% reduction in food intake), not only increased longevity and health in *Ercc1*$^{\Delta/-}$ mice, but also restored NO signaling, possibly via ROS suppression (Figure 1).[81,82] Unrepaired DNA damage normally results in the recruitment of protective mechanisms, the so-called survival response.[83] This includes Nrf2-regulated antioxidant pathways, hypothetically allowing eNOS to keep its coupled NO-producing state as ROS are decreased. However, as discussed, Nrf2-activation also upregulates RAS components.[54] Nfr2 is already activated in *Ercc1*$^{\Delta/-}$ mice that are fed ad lib, and RAS blockade with losartan could not restore NO—cGMP signaling.[81] Therefore, Nrf2—RAS signaling does not appear to play a role in the decreased NO—cGMP signaling, or in the effect of dietary caloric restriction, in the vasculature of *Ercc1*$^{\Delta/-}$ mice. An alternative pharmacological strategy to improve vascular aging in *Ercc1*$^{\Delta/-}$ mice is PDE inhibition (see below).[4]

Stimulators and Activators of sGC

The recognition that nitrates were not suitable for the chronic treatment of hypertension led to attempts to exploit downstream targets of NO. In VSMCs, NO binds sGC to form cGMP. Importantly, NO binds at the enzyme's heme group, which contains Fe^{2+}. O_2^- and peroxynitrite oxidize sGC, leading to Fe^{3+} formation and eventually to loss of the heme group, both leading to inactivation of sGC (Figure 4).[84] On this basis, the discovery of sGC stimulators and activators could overcome dysfunctional sGC signaling.[84] Stimulators bind sGC directly, allosterically, outside the heme group, augmenting the catalytic activity to form cGMP (Figure 4). This can take place both NO-independently and in synergy with endogenous NO by stabilizing the NO binding to the sGC heme group. Activators also bind and stimulate sGC in an NO-independent manner. They bind the heme-NO binding pocket, and thus, unlike stimulators, activate the oxidized and heme-free form of sGC

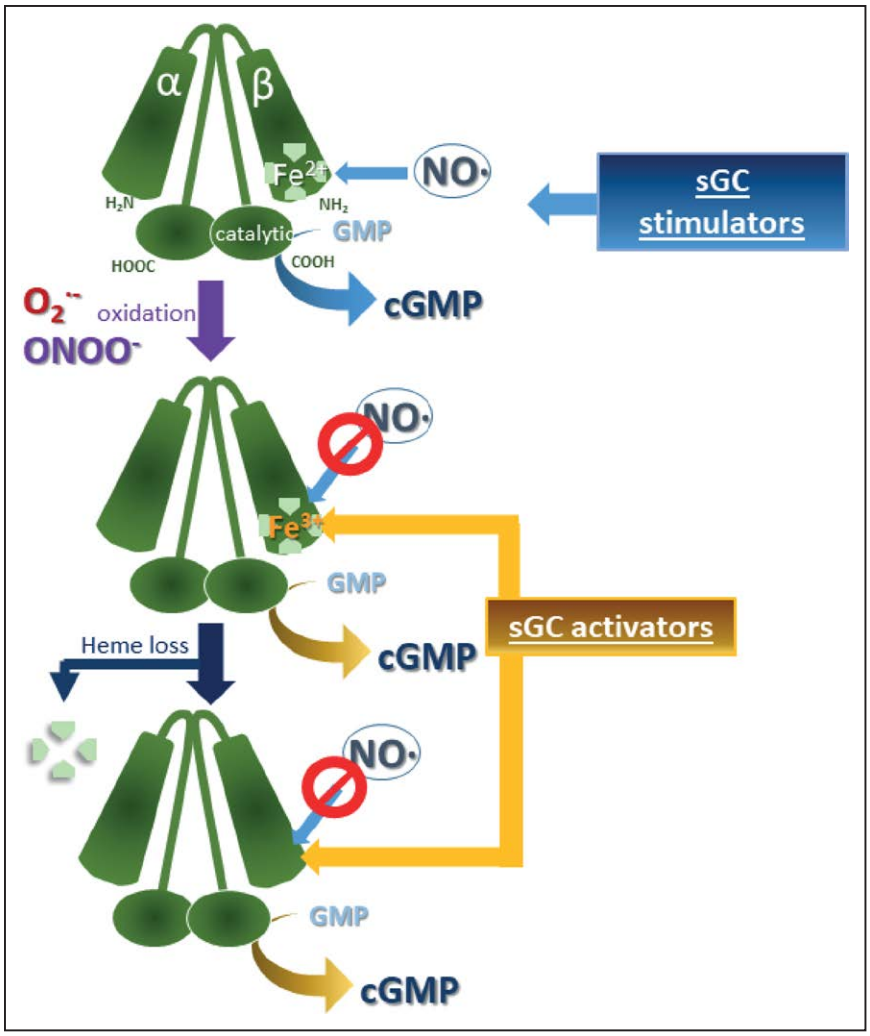

Figure 4. sGC consists of heterodimers formed of α and β subunits, the latter containing a ferrous heme nitric oxide (NO) binding site. Binding of NO activates the catalytic part. Oxidation leads to loss of NO binding, which can be substituted by sGC (soluble guanylyl cyclase) activators. sGC Stimulators act through allosteric binding outside the heme group. Adapted from Sandner et al.[84] cGMP indicates cyclic guanosine monophosphate.

(Figure 4). In summary, stimulators work NO-independently, as well as in an additive manner with NO, and are heme-dependent, while activators work NO-independently, additive with NO, and are heme-independent.

Despite the clear molecular mechanistic distinction of both drug classes, the World Health Organization assigned them the same suffix: ciguates. Partly, this could be due to the limited understanding of the (patho) physiological role of oxidized and heme-free sGC, in particular of the spatio-temporal pattern of the underlying oxidative stress—which might be variable in different diseases and disease causes. This makes it difficult to predict the treatment potential and efficacy of sGC stimulators and activators. In addition, it is not known if activators and stimulators have a differential efficacy with respect to their treatment effects; head-to-head comparisons have not been made for their diverse applications, and the first oral activators have just reached phase 2 studies. The Table summarizes important cardiovascular applications of these drugs.

Preclinical Data With sGC Stimulators and Activators

The sGC stimulators and activators BAY 41-8543, BAY 58-2667 (cinaciguat), HMR-1766, and YC-1 increase cGMP and relax blood vessels from different animal species.[85–88] sGC stimulators also relax aortas from nitrate-tolerant rats.[85] In addition, relaxation of coronary arteries, without any effect on left ventricular pressure and heart rate, was demonstrated for these drugs in the Langendorff preparation. Increase of coronary blood flow was reported for the sGC stimulators riociguat (BAY 63-2521) and vericiguat (BAY 1021189).[89,90]

The relaxation of blood vessels induced by sGC stimulators and activators translates into blood pressure decreases in a broad range of animal models including dogs, normotensive rats, and hypertensive rats (SHR and chemically and genetically induced models).[89–95] It is important to note that the effects of sGC stimulators in these preclinical models were dose-dependent, and that there was no tachyphylaxis, thus avoiding the tolerance problems seen for nitrates.[89,95] These drugs additionally induced cardiac and vascular antifibrotic effects, offered renal protection, and decreased mortality in stroke-prone SHR rats, and rat models of cardiovascular diseases including models with a hypertensive, diabetic, or metabolic disease background, implying a role in heart failure but also other cardiovascular diseases.[96–101] In line with that, praliciguat showed efficacy in a rat cardiorenal model of heart failure with reduced ejection fraction.[93]

Clinical Data With sGC Stimulators and Activators

The only drug registered up to now is the sGC stimulator (riociguat, Adempas). It is approved for the treatment of pulmonary arterial hypertension and chronic thromboembolic

Table. Selection of Frequently Used sGC Stimulators and sGC Activators and Their Actual Development Status

	International Nonproprietary Name	Indication(s)/ Highest Development Level	Status
sGC stimulators			
BAY 41-8543	Unknown	Vasorelaxation, also in nitrate-tolerant conditions. Increases coronary perfusion and reduces coronary perfusion pressure in rat.[88,95]	Preclinical tool
BAY 41-2272	Unknown	Vasorelaxation and blood pressure decrease in SHR.[134]	Preclinical tool
YC-1	Lificiguat	Vasorelaxation in normotensive and hypertensive models.[86,87]	Preclinical tool
BAY 60-4552	Nelociguat	ED/Phase 2[135]	Completed
BAY 63-2521	Riociguat	Blood pressure reduction[89]; PAH/phase 3[136]; CTEPH/phase 3.[137]	Completed, approved, marketed as Adempas
BAY 102-1189	Vericiguat	Chronic heart failure (HFrEF)/phase 3.[102]	Completed
		Chronic heart failure (HFpEF)/phase 2 b, tolerability.[138]	
IW-1973	Praliciguat	Blood pressure-lowering in hypertensive and diabetic nephropathy models, and hypertensive humans.[93,106,107,139]	Preclinical tool
		Chronic heart failure (HFpEF)/phase 2.[140]	Ongoing
sGC activators			
HMR-1766	Ataciguat	PAD/phase 2.[141]	Completed
BAY 58-2667	Cinaciguat	ADHF/phase 2.[103,104]	Completed
BAY 60-2770	Unknown	Vasorelaxation pulmonary hypertension model.[142,143]	Preclinical tool
BI 704704	Unknown	Blood pressure lowering and renal protection in ZSF1 rats with diabetic nephropathy.[143]	Preclinical tool

ADHF indicates acute decompensated heart failure; CTEPH, chronic thromboembolic pulmonary hypertension; ED, erectile dysfunction; HFpEF, heart failure with preserved ejection fraction; HfrEF, heart failure with reduced ejection fraction; PAD, peripheral arterial occlusive disease; PAH, pulmonary arterial hypertension; sGC, soluble guanylyl cyclase; SHR, spontaneously hypertensive rat; and ZSF1, is a cross between a female Zucker Diabetic Fatty rat and a male Spontaneously Hypertension Heart Failure rat.

pulmonary hypertension. A recent study with vericiguat in patient with heart failure and reduced ejection fraction showed a reduced incidence of death from cardiovascular causes or hospitalization for heart failure versus placebo.[102] The difference favoring vericiguat appeared already after 3 months of treatment. Symptomatic hypotension was more common in the vericiguat group (P=0.12). Early studies in acute decompensated heart failure patients with the sGC activator cinaciguat also demonstrated a pronounced blood pressure-lowering effect.[103–105] Although this led to a premature stop of a trial with cinaciguat, these data support the potential usefulness of ciguats in hypertension.

Phase 1 studies in healthy subjects recently revealed the potential of praliciguat as a blood pressure-lowering agent.[106] In a subsequent phase, 2A clinical trial study in hypertensive patients with type 2 diabetes mellitus praliciguat lowered 24 hour mean arterial pressure by 5 mm Hg, with a greater effect in those with a baseline mean arterial pressure >92 mm Hg.[107] Further clinical trials in a broad range of patients with hypertension still have to be performed. Despite the preclinical evidence and the clinical results, currently, no sGC stimulator and sGC activator is in development for the treatment of hypertension. This is at a first glance surprising, since the mode of action of sGC stimulators and sGC activators is completely different from and complementary to approved antihypertensive drugs.

PDE Inhibitors

PDEs inactivate the second messengers cGMP and cAMP. This large group of enzymes consists of 11 distinctive families or subtypes.[4] The PDE1-3 and PDE10-11 families are dual-substrate PDEs, that is, they are able to hydrolyze both cAMP and cGMP, while PDE4, −7 and −8 are cAMP-specific and PDE5, −6, and −9 are cGMP-specific. PDEs display a specific tissue distribution which is family- and subtype-dependent. PDEs in VSMC are logical targets for the treatment of hypertension.[108] Here the 2 most abundant cGMP-hydrolyzing PDEs are PDE1, which is Ca^{2+}-calmodulin-dependent, and PDE5 (Figure 5). PDE1 has 3 subtypes A, B, and C, and among them, PDE1A and PDE1B prefer cGMP over cAMP.[109] The relative importance of PDE1 and 5 depends on the (patho) physiological conditions, that is, in VSMC of the quiescent, contractile phenotype and (healthy) conditions featured by relatively low [Ca^{2+}] PDE5 is commanding, while PDE1 presumably has a dominant role under disease conditions, such as in high [Ca^{2+}] conditions and in proliferative VSMC (Figure 5).[110,111] Thus

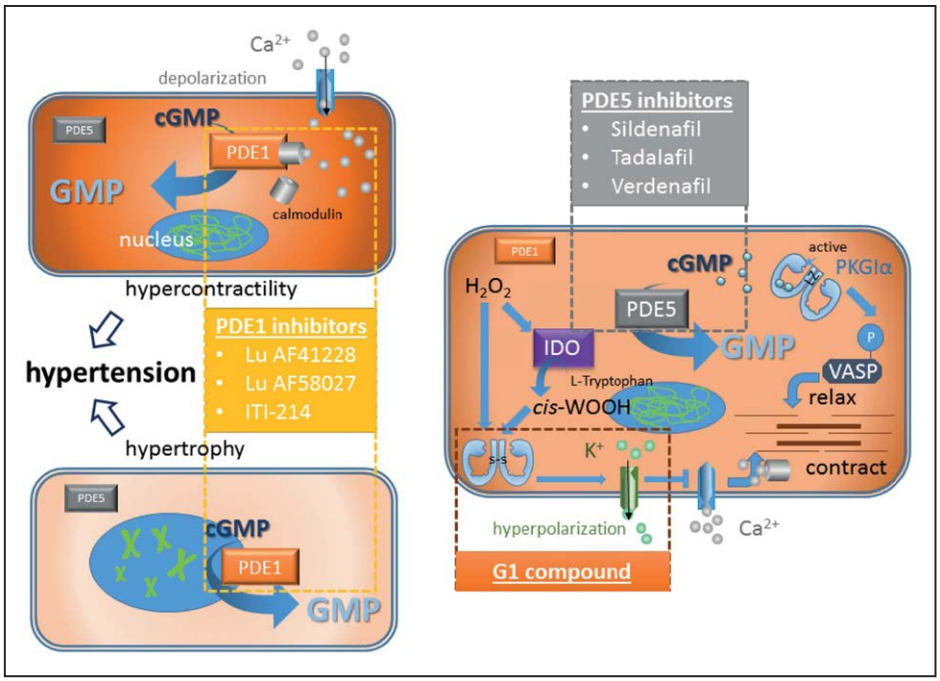

Figure 5. PDE (phosphodiesterase) and PKG (protein kinase G) signaling in vascular smooth muscle. Drug classes that decrease blood pressure through these pathways are in boxes and underlined. cGMP indicates cyclic guanosine monophosphate; IDO, indoleamine 2,3-dioxygenase 1; LZ, leucine zipper; S-S, disulfide bridge; and VASP, vasodilator-stimulated phosphoprotein.

PDE1 and PDE5 represent 2 distinct drug targets, and their inhibition has been tested in various models.

Laursen et al[109] evaluated the vasodilatory and blood pressure-lowering effect of 2 PDE1 selective inhibitors (Lu AF41228 and Lu AF58027) in Wistar rats. Both compounds reduced blood pressure within 60 minutes upon intravenous infusion in anesthetized rats, while Lu AF41228 also decreased blood pressure when given orally to conscious animals. PDE1A, PDE1B, and PDE1C mRNA was found in aorta, lung, heart, and brain, while mesenteric small arteries only showed PDE1A and PDE1B mRNA. Mesenteric arteries relaxed dose-dependently to Lu AF41228 and Lu AF58027, and this effect disappeared after endothelium removal or during blockade of eNOS with N w-Nitro-L-arginine methyl ester HCl. PDE1A null mice display low blood pressure, further supporting the possible role of PDE1 inhibition in blood pressure reduction.[112] The PDE1 inhibitor ITI-214 is in clinical development in patients with neurodegenerative disease and heart failure (www.intracellulartherapies.com). No long-term blood pressure studies have been performed in animals, nor have blood pressure effects been reported in humans thus far.

PDE5 inhibitors have been extensively used in clinical conditions, most notably erectile dysfunction (sildenafil) and pulmonary hypertension (sildenafil, vardenafil, and tadalafil). Short-term PDE5 inhibitor treatment (bolus injection or up to 1 week intake) induced mild vasodilator effects in healthy volunteers and a small additive blood pressure-lowering effect in patients already taking other antihypertensive drugs.[108,113,114] The modesty of this effect might have various causes. First, the other antihypertensive drugs (in particular RAS inhibitors) may already have upregulated the NO pathway. In addition, angiotensin II is capable of upregulating PDE1 and 5, for example, in resistance arteries.[115–117] This implies that RAS blockade would lower PDEs, thus reducing the potential efficacy of PDE inhibition. Unfortunately, the blood pressure-lowering effect of PDE5 inhibition seems to wane off over time. For instance, when comparing the PDE5 inhibitor UK-357 903 to the ACE inhibitor enalapril in SHR, the former was effective on day 1 only, while enalapril did not lose efficacy over multiple days.[118] Nevertheless, the effects of UK-357 903 on vascular conductance and plasma cGMP levels remained present. In contrast, Yaguas et al[119] observed blood pressure-lowering effects of sildenafil in SHR after 2 months of treatment only, and this effect remained present during continued treatment up to 6 months. These data imply that there may be some resistance to PDE5 inhibition in the vasculature, especially on the short-term. Whether this is due to feedback via upregulation of PDE1, or any adaptation of downstream signaling, needs to be further addressed. It is worthy to mention that despite its mild effect on blood pressure, PDE5 inhibition exerts protective effects on endothelial cells and VSMC, by increasing NO bioavailability and reducing oxidative stress.[119,120] For these reasons, PDE5 inhibition is currently used in pulmonary hypertension, in which also PDE1 inhibition has shown promise.[121]

Effects in the aging vasculature might be of particular interest for PDE1 and 5 inhibitors. Vascular aging is a leading factor for hypertension development. VSMC from accelerated aging mice with increased blood pressure and human senescent VSMC showed elevated level of PDE1A, PDE1C, and PDE5 expression, and human genetic studies confirmed an association between PDE1A and diastolic blood pressure.[79,80,122] These observations warrant the exploration of chronic PDE1 inhibition in models of aging.

Protein Kinase G Iα Activators

In conduit vessels, NO is most relevant for relaxation, while in resistance vessels EDH is prominent. This makes EDH an attractive target for the treatment of hypertension. NO-mediated relaxation depends on PKG type I in VSMC. The *PKGI* gene expresses as 2 separate isoforms (α and β), which are splice variants of the same gene and differ only in sequence of their N-terminal leucine zipper interaction domain.[123] They occur as homodimers, and their leucine zipper domains, apart from holding the monomers together, mediate PKG binding to specific substrates. Upon binding of cGMP, auto-inhibitory domains disinhibit phosphorylation activity of the PKGI catalytic region, thus phosphorylation-activating VASP (Figure 5).

EDH-mediated relaxant pathways display great diversity, and among its stimulators are *S*-nitrosothiols and H_2O_2.[124,125] Interestingly, through oxidization, H_2O_2 is capable of activating PKGIα in a cGMP-independent manner, inducing the formation of a disulfide bond between the cysteine 42 (C42) residues of 2 adjacent chains in PKGIα homodimers (Figure 5).[126] The linked PKGIα homodimers phosphorylate large-conductance Ca^{2+}-dependent K^+ (BK_{Ca}) channels, thus leading to hyperpolarization. By screening a large set of electrophilic small molecules, Burgoyne et al[126] discovered a PKGIα activator, G1.[127] G1 relaxed arteries to the same degree as cinaciguat, yet without affecting mean arterial pressure in vivo, due to a compensatory heart rate increase. In angiotensin II-infused mice, G1 did lower mean arterial pressure, although the effect waned off gradually. This is reminiscent of the tolerance phenomenon and is suggestive for counterregulatory mechanisms like inactivation of oxidized PKGIα by thioredoxin. In a subsequent study, the same investigators reported that resveratrol, normally considered an antioxidant, was also capable of inducing PKGIα oxidation.[128] Consequently, like G1, it relaxed preconstricted arteries and lowered blood pressure in the angiotensin II-infused mice. Stanley et al[129] reported that the enzyme indoleamine 2,3-dioxygenase 1 in the presence of H_2O_2 generates yet another PKGIα activator, (2S,3aR,8aR)-3a-hydroperoxy-1,2,3,3a,8,8a-hexahydropyrrolo[2,3-b] indole-2-carboxylic acid from L-tryptophan. Furthermore, Feelisch et al[130] observed that nitrite, although generally believed to result in NO-dependent effects, lowered blood pressure by binding to the haem moiety of catalase, thus inhibiting H_2O_2 decomposition and also facilitating PKGIα activation.

Thus, the PKGIα activator field is rapidly evolving. Yet, there still are several caveats. Cys42 may not be the only cysteine residue that can be oxidized to activate: Cys117 seems to be another.[131] Most studies agree on a role for endothelial small- and intermediate conductance Ca^{2+}-dependent K^+ channels in EDH, while activation of BK_{Ca} is less well accepted.[124] In fact, PKG inhibition with KT5823 blocked the relaxant effects of G1 in mesenteric arteries by only 50%,[127] while *S*-nitrosothiol-induced EDH did not involve PKGIα activation at all.[132] Moreover, the sGC activator BAY412272 still evoked vasodilation during sGC inhibition, by activating Na^+-K^+-ATPase, implying that even these drugs may induce EDH.[125] Clearly, future studies should investigate what non-PKG mechanisms are activated by PKGIα activators, and how sGC activators might exert EDH. Since G1 prevented VASP phosphorylation by the stable cGMP analog 8-Br-cGMP,[127] it is possible that the cGMP-activated PKGIα-VASP pathway and the oxidized PKGIα-BK_{Ca} pathway are mutually exclusive. If so, we need to know what exactly determines their respective contributions in conduit versus resistance vessels, since PKG occurs at both sites. Ultimately, this will tell us to what degree PKGIα activator truly would be universal vasodilators.

Summary and Perspectives

Currently, no antihypertensive drugs that are directed at improvement of NO—cGMP signaling are in clinical use. To predict which approach is most likely to succeed, a number of aspects needs to be considered.

First, LOX and XO inhibition do not appear to be an option due to the promiscuous behavior towards substrates, leading to formation of substances that both oppose and support vasodilation through NO. Myeloperoxidase and vascular peroxidase 1 appear to be more promising targets, but clinically applicable inhibitors have not been developed yet. NADPH oxidase has had overwhelming attention the past 3 decades, but disappointingly, no clinical drugs are in sight yet. Thus, oxidases seem to be on a hold.

Mitochondrial compounds are intriguing newcomers. There is a profound interaction between NO and mitochondria. Although this interaction remains to be further evaluated, Nrf2 stimulators are inappropriate in hypertension since they activate the RAS, whereas compounds that affect nutrient sensing and the electron transfer chain, for example, modified 6-chromanols such as SUL compounds, are interesting to further explore.

Much closer to the clinic are the compounds that affect the enzymes of the NO-cGMP signaling cascade, sGC, PDE, and PKG, through a direct allosteric interaction. Still far from the clinic are compounds that facilitate PKGIα homodimerization. In addition, they will lead to hyperpolarization of cells rather than specific myosin-actin rearrangement through VASP, thus missing the specific, beneficial cGMP effects. PDE5 inhibition is a clinically feasible strategy, but does not appear to find its way to systemic hypertension. After initial fear for dangerous side effect in several cardiovascular conditions by the end of the 1990s, studies in patients with hypertension were started with a 15-year delay, but the last publication was released more than 10 years ago.[133] PDE1 inhibition is an attractive novel option since it very specifically targets the cGMP pool in VSMC. Of note, the specific PDE1 inhibitor ITI-214 is well-tolerated by humans and is in a phase 2 clinical development for neurodegenerative disease.[4] Since PDE1 appears to have a very specific and differential function in contractile versus proliferating VSMC, PDE1 inhibitors might more specifically exert vascular protection in hypertension in comparison to the other compounds presented here. sGC activators and stimulators are already in clinical use or in clinical development and could be tested in hypertensive disorders on the very short notice. These compounds may combine blood pressure-lowering effects with outcome benefits in cardiovascular diseases.

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