cGMP signalling: from bench to bedside

Conference on cGMP Generators, Effectors and Therapeutic Implications

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The 2nd International Conference on cGMP Generators, Effectors and Therapeutic Implications took place in Potsdam, Germany, during 10–12 June 2005, and was organized by L. Ignarro, F. Hofmann, H. Schmidt and J.P. Stasch (www.cyclicgmp.net). The next cGMP meeting will be held in Dresden, Germany, in June 2007.

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role of cGMP was unknown until the 1980s when two key discoveries were made. First, it was found that a peptide synthesized in the heart, atrial natriuretic peptide (ANP), could stimulate cGMP synthesis by binding to a transmembrane receptor, the particulate guanylyl cyclase (pGC). Second, the elusive endothelium-derived relaxing factor was identified as nitric oxide (NO), which stimulates soluble guanylyl cyclase (sGC) in smooth muscle cells to synthesize cGMP, thereby causing vasorelaxation. Subsequently, other components of cGMP metabolism and signal transduction were also identified (Fig 1). It is now known that cGMP-hydrolysing phosphodiesterases (PDEs) are responsible for cGMP breakdown, and at least three types of cGMP-binding protein transduce the cGMP signal to alter cellular function. These three types are cGMP-modulated cation channels, cGMP-dependent protein kinases (cGKs) and cGMP-regulated PDEs that degrade cAMP and/or cGMP.

Today, we know that cGMP is a second messenger that regulates processes as diverse as cellular growth and contractility, cardiovascular homeostasis, inflammation, sensory transduction, and neuronal plasticity and learning. Drugs such as the NO-releasing organic nitrates for angina pectoris or the PDE5 inhibitor sildenafil for erectile dysfunction exert their therapeutic effects through the stimulation of the cGMP system (Fig 1). After the award of the 1998 Nobel Prize to Robert F. Furchgott, Louis J. Ignarro and Ferid Murad for their discovery of the NO–cGMP pathway in the cardiovascular system, there was a renewed interest in cGMP research. However, activities in this area remained widely dispersed across several fields including cyclases, ion channels, protein kinases and PDEs, with little interaction among the scientists. To bring people and their ideas together, the 1st International Conference on cGMP was held in 2003, about half a century after the discovery of the molecule. The 2nd cGMP meeting continued and further established the focus in this area. In this report, we summarize the main concepts and the new research directions that arose at this meeting, with a focus on cGMP signalling in the mammalian cardiovascular system (Fig 1). Due to space limitations, we only briefly cover the sessions on other important areas of cGMP research, such as the role of cGMP in the central nervous system and in invertebrates.

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Introduction

Cyclic nucleotide research originated in the 1960s, when cyclic adenosine monophosphate (cAMP) and cyclic guanosine monophosphate (cGMP) were first identified as natural products (reviewed in Beavo & Brunton, 2002). The discovery of cAMP led to the formulation of the second-messenger concept in hormone signalling by Earl W. Sutherland, who was duly rewarded with the Nobel Prize in 1971. Consequently, cAMP research surged ahead, whereas cGMP attracted little attention. Indeed, the biological

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Fig 1 | Current concepts of cGMP signalling. cGMP generators (green) and effectors (red), as well as some downstream pathways and cellular functions (grey boxes) that are involved in the effects of endogenous cGMP and/or cGMP-elevating drugs (blue), are shown. The lower part shows some current (blue) as well as potential future (black) indications for drugs that modulate cGMP levels or cGMP effector pathways. BNP, B-type natriuretic peptide; cGKs, cGMP-dependent protein kinases; IRAG, IP₃ receptor-associated cGKIβ substrate; PDEs, phosphodiesterases; pGC, particulate guanylyl cyclase; sGC, soluble guanylyl cyclase; VASP, vasodilator-stimulated phosphoprotein. See text for details.

cGMP generators

Soluble guanylyl cyclases. The mammalian NO-sensitive sGC is a heterodimeric haemoprotein that exists in two isoforms, $\beta_1\alpha_1$ and $\beta_1\alpha_2$, which have similar enzymatic properties. The $\beta_1\alpha_1$ isoform predominates in most tissues except the brain, in which relatively high levels of the $\beta_1\alpha_2$ isoform are expressed (Friebe & Koesling, 2003). The prosthetic haem group of sGC is bound to the β -subunit through the axial ligand histidine 105, which is also involved in mediating NO-induced activation of the enzyme. J.P. Stasch (Wuppertal, Germany) and colleagues have shown that the invariant amino acids tyrosine 135 and arginine 139 of the β_1 -subunit also have a crucial role in the binding of the haem moiety (Schmidt *et al*, 2004). They propose that a signal transmission triad, composed of histidine 105, tyrosine 135 and arginine 139, is responsible for transducing changes in haem status and porphyrin geometry after NO binding to alter sGC catalytic activity.

As highlighted by M. Marletta (Berkeley, CA, USA), sGC belongs to a recently identified family of prokaryotic and eukaryotic H-NOX (haem nitric oxide/oxygen-binding) proteins. In contrast to many other haemoproteins that bind both NO and O₂, the H-NOX domain of the sGC is selective for NO. On the basis of the recent crystal structure of a bacterial O₂-binding H-NOX domain, Marletta's group has shown that a single tyrosine is required for O₂ binding (Boon *et al*, 2005). Given that mammalian sGC lacks such a tyrosine in its hydrophobic haem pocket, O₂ binding is limited.

The ability of sGC to distinguish between NO and O₂ is central to selective biological responses to NO in an aerobic environment.

Evidence is emerging that sGC might not reside solely in the cytoplasm and can translocate to the plasma membrane after activation (Aguillo *et al*, 2005). In the light of these findings, there is an urgent need to identify endogenous modulators of sGC such as Ca²⁺, ATP, protein kinases and other interacting proteins. NO binding and sGC activity might be regulated by several allosteric interactions with substrate and reaction products as well as NO itself, as reported by Marletta (ATP, GTP and NO), J. Garthwaite (London, UK; ATP and Ca²⁺), and D. Koesling (Bochum, Germany; GTP, Mg²⁺, cGMP and PP_i). Molecular chaperones might also have a role. A. Beuve (Newark, NJ, USA) provided evidence that the heat-shock protein 70 (Hsp70)–Hsp90 chaperone machinery might be involved not only in sGC maturation, but also in its transport to intracellular compartments and to the plasma membrane.

So far, the elucidation of the functional role of sGC *in vivo* has been limited due to the lack of transgenic mouse models. However, two presentations at the meeting started to address this issue. Koesling and co-workers generated null mutants for the α_1 -, α_2 - as well as β_1 -subunits, and the group of P. Brouckaert (Ghent, Belgium) established a mouse line lacking functional α_1 -subunits. Koesling's $\alpha_1^{-/-}$ and $\alpha_2^{-/-}$ mice seemed viable and fertile with no overt defects or behavioural phenotypes. Interestingly,

Brouckaert's male, but not female, α_1 -mutants developed systemic hypertension around 12–14 weeks of age, which implies an age- and gender-specific role for cGMP signalling in blood pressure control. The relatively mild phenotypes of $\alpha_1^{-/-}$ and $\alpha_2^{-/-}$ mice suggest that, at least in certain tissues, the $\alpha_1\beta_1$ and $\alpha_2\beta_1$ isoforms can compensate for each other. By contrast, deletion of the β_1 -subunit, the dimerizing partner for both α -subunits, resulted in strongly impaired vasorelaxation and platelet responses after NO stimulation. About 70% of the β_1 -knockout mice died directly after birth, and the remaining 30% died before six weeks of age, most likely due to severe gastrointestinal abnormalities. These phenotypes are strikingly similar to those of cGK type I null mutants, and together these findings confirm the essential role of the sGC–cGMP–cGKI pathway in mediating many NO effects *in vivo*.

Particulate guanylyl cyclases. pGCs constitute a family of at least seven plasma membrane receptors (GC-A to GC-G) that have an extracellular ligand-binding domain, a single transmembrane region and an intracellular cyclase domain (Kuhn, 2003). GC-A binds ANP and B-type natriuretic peptide (BNP) and mediates their hypotensive and cardioprotective actions; GC-B is activated by C-type natriuretic peptide and regulates bone growth; GC-C mediates the effects of guanylin and uroguanylin, as well as heat-stable enterotoxins, on intestinal electrolyte and water transport.

In addition to BNP, which is used as a diagnostic and therapeutic tool (see below), there is much interest in the cardiovascular actions of ANP. It has been hypothesized that lowering of blood pressure by ANP is mainly related to a reduction in plasma volume rather than to direct vasorelaxation. To analyse the relative importance of renal versus extrarenal actions, M. Kuhn (Würzburg, Germany) and colleagues have generated endothelium-specific knockout mice for the ANP receptor, GC-A (Sabrane *et al*, 2005). These mouse mutants display mild but significant systemic hypertension, hypervolemia and cardiac hypertrophy. These phenotypes did not result from changes in renal excretion or vasodilation but from reduced vascular permeability to plasma protein. Thus, ANP-induced increases of endothelial permeability contribute to its ability to lower arterial blood pressure.

cGMP effectors

cGMP-dependent protein kinases. The cGKs are attractive candidates as mediators of cGMP signalling (Feil *et al*, 2003). Mammals have three cGKs: the membrane-bound cGK type II and the cytosolic cGK type I (cGKI) that has two isoforms, cGKI α and cGKI β . The cGK type II mediates effects of pGC-derived cGMP on intestinal electrolyte transport and bone formation, whereas the cGKI transduces many effects of sGC-derived cGMP on cardiovascular homeostasis.

To explore the role of cGKI in the regulation of vascular tone and blood pressure, the group of M. Mendelsohn (Boston, MA, USA) generated a new mouse line that expresses a mutated cGKI α isoform that is unable to interact with the regulatory subunit of myosin phosphatase. These cGKI α mutants had altered growth properties of vascular smooth muscle cells (VSMCs) and age-dependent hypertension and cardiac hypertrophy evident at eight weeks. These results provide *in vivo* evidence that cGKI α dilates vessels that have resistance through the activation of VSMC myosin phosphatase and dephosphorylation of the myosin light chain. Another model of cGKI signalling proposes a specific interaction of the cGKI β isoform

with the IRAG protein (IP $_3$ receptor-associated cGKI β substrate), which results in the inhibition of intracellular Ca $^{2+}$ release. J. Schlossmann (Munich, Germany) and colleagues generated a mouse line that expresses a mutated IRAG protein that is unable to interact with the inositol 1,4,5-trisphosphate (IP $_3$) receptor (Geiselhöringer *et al*, 2004). IRAG-mutant mice showed impaired relaxation of the aorta and colon, and gastrointestinal abnormalities but no hypertension, which supports a role for the cGKI β –IRAG–Ca $^{2+}$ pathway in the regulation of smooth muscle and intestinal function but not blood pressure. In addition, IRAG seems to be involved in the anti-platelet effects of exogenously supplied NO, but not in thrombus formation in the absence of exogenous NO. U. Walter (Würzburg, Germany) described a new role for another cGKI substrate, the vasodilator-stimulated phosphoprotein (VASP), in platelet function. The adhesion of VASP $^{-/-}$ platelets to endothelial cells *in vivo* was enhanced significantly after vascular injury and was unresponsive to exogenous NO (Massberg *et al*, 2004), suggesting that platelet inhibition under pathophysiological conditions involves the cGKI–VASP pathway.

The role of cGMP in the regulation of cell growth and phenotype remains a complex issue. R. Feil (Tübingen, Germany) and colleagues showed that the cGMP–cGKI pathway stimulates growth in primary VSMCs but inhibits growth in subcultured cells *in vitro*. The traditional view that cGKI inhibits vascular proliferation *in vivo* could not be confirmed by the analysis of conventional and smooth-muscle-specific cGKI-knockout mice. These data indicate that cGKI does not affect restenosis after mechanical vessel injury and might even promote atherosclerosis and angiogenesis. Thus, it is unlikely that the vasoprotective effects reported for some cGMP-elevating agents are mediated by vascular cGKI signalling. In an effort to understand the regulation of VSMC phenotype by cGMP, T. Lincoln (Mobile, AL, USA) studied the effect of cGKI overexpression on gene expression in subcultured VSMCs. He presented evidence that cGKI stimulates sumoylation of the transcription factor Elk1, thereby resulting in de-repression of smooth-muscle-specific promoters.

An important problem in the functional analysis of cGKs and other cGMP effectors is the lack of highly selective agonists and inhibitors. W. Dostmann (Burlington, VT, USA) has developed membrane-permeable peptides, such as DT-2, that inhibit cGK catalytic activity *in vitro*. *In vivo*, administration of DT-2 or of the proteolytically stable analogue (D)DT-2 (consisting of D-amino acids) induced hypertension in mice, suggesting that cGKI is indeed involved in blood pressure control. However, before firm conclusions can be drawn, the *in vivo* specificity and potential toxicity of high doses of these peptides should be tested in a cGKI-deficient background.

Phosphodiesterases. Cyclic nucleotide PDEs that either hydrolyse cGMP and/or are regulated by cGMP are important modulators of the spatiotemporal dynamics of cGMP and cAMP signals (Rybalkin *et al*, 2003). J. Beavo (Seattle, WA, USA) introduced the mammalian PDE superfamily, which consists of 11 gene families comprising at least 21 genes that encode perhaps more than 100 protein variants. All PDEs have significant similarity in their catalytic region but differ in their regulatory domains. Several PDEs are allosterically regulated by the binding of cGMP to GAF domains (found in cGMP-regulated PDEs, several adenylyl cyclases and FhlA), which may form new targets for

cGMP-modulating drugs. Beavo and J. Schultz (Tübingen, Germany) discussed recent studies that indicate fundamental differences in the overall structure of prokaryotic and eukaryotic GAF domains. In addition, Beavo highlighted that cGMP-elevating agents can produce changes in cAMP levels through stimulation or inhibition of cAMP-PDEs. Such cross-talk with the cAMP system might present a physiologically important mechanism of cGMP signalling that is independent of effectors such as cGKs. Last, but not least, Beavo promoted the idea that, in many systems, cyclic nucleotides act as physiological 'brakes' on cellular functions, and the expression of PDEs can be regulated individually as a mechanism to tighten or to release these brakes.

cGMP-elevating drugs: innovative approaches

Aberrant NO-sGC-cGMP signalling and an associated loss in potency of NO-based therapeutics is observed in several disease states such as hypertension, atherosclerosis and diabetes, in which oxidative stress has a central role. Such NO-sGC-cGMP dysfunction can arise as a consequence of reactive-oxygen-species-mediated decreases in NO bioavailability and an impairment of sGC activity through the oxidation of its prosthetic haem moiety. Excitingly, a new generation of NO-independent sGC activators has been developed. These drugs stimulate cGMP synthesis and target sGC in its NO-sensitive, ferrous (Fe^{2+}) state, its NO-insensitive, oxidized ferric (Fe^{3+}) state, or its haem-free state. Such compounds include BAY 41-2272 (Fe^{2+}), HMR1766 (Fe^{3+}) and BAY 58-2667 (Fe^{3+} and haem-free), of which the latter two have the potential to target diseased tissue (containing oxidized sGC).

The therapeutic potential of BAY 41-2272 in the setting of persistent pulmonary hypertension in the newborn (PPHN) was highlighted by S. Abman (Denver, CO, USA). Although inhaled NO represents an important advance in the treatment of this condition, up to 40% of patients remain refractory to this therapy. In a model of severe PPHN in fetal sheep, BAY 41-2272 and sildenafil were found to cause pulmonary vasodilation *in utero* despite a loss in acetylcholine-induced vasodilation (Deruelle *et al.*, 2005). Importantly, at birth, BAY 41-2272 and sildenafil caused a marked reduction in pulmonary vascular resistance and augmented the pulmonary vasodilator response to inhaled NO. Similar beneficial effects of BAY 41-2272 were observed by O. Evgenov (Boston, MA, USA) and colleagues in lambs with acute pulmonary hypertension induced by the thromboxane analogue U-46619 (Evgenov *et al.*, 2004). Interestingly, unlike the systemic effects of BAY 41-2272, its pulmonary actions seem to be independent of endogenous NO generation. These findings suggest that direct pharmacological stimulation of sGC either alone or in combination with inhaled NO could provide a new approach for the treatment of pulmonary hypertension.

HMR1766 stimulates the ferric (Fe^{3+}) form of sGC and U. Schindler (Frankfurt, Germany) showed that chronic treatment with HMR1766 reduced atherosclerotic plaque formation and endothelial dysfunction in ApoE^{-/-} mice, normalized platelet activation and restored endothelial and vascular function in type-1 diabetic rats. Similarly, HMR1766 attenuated endothelial dysfunction and increased survival in aged spontaneously hypertensive rats, and improved vascular remodelling, haemodynamics and mortality in rats with monocrotaline-induced pulmonary hypertension. These findings highlight the ability of the new sGC activators to exert anti-remodelling effects and suggest that targeting of

oxidized sGC might not only relieve symptoms acutely, but also result in disease regression.

BAY 58-2667 mimics the spatial structure of the haem moiety and competes at high concentrations with haem for the anchoring residues tyrosine 135 and arginine 139 (Schmidt *et al.*, 2004). It has the remarkable ability to stimulate sGC more potently once the haem group has been oxidized or removed. Stasch together with H. Schmidt (Melbourne, Vic, Australia) and colleagues have now shown that endogenously generated oxidants, such as peroxynitrite, enhance the sGC-stimulating effects of BAY 58-2667 in isolated endothelial cells and blood vessels. BAY 58-2667 was found to be a more potent vasodilator in aortae from atherosclerotic ApoE^{-/-} mice, as well as in mesenteric arteries from patients with type-2 diabetes compared with matched controls. Thus, the ability of BAY 58-2667 to target oxidized sGC provides a new approach for the selective dilation of diseased blood vessels and treatment of cardiovascular disease. Moreover, in the setting of chronic hypoxia-induced pulmonary hypertension in mice, A. Ghofrani (Giessen, Germany) and colleagues showed that BAY 58-2667 can reverse the associated neomuscularization of pulmonary vessels and cardiac hypertrophy. In addition, G. Boerrigter (Rochester, MN, USA) showed that in experimental heart failure in dogs, BAY 58-2667 potentially reduces pre- and afterload, increases cardiac output and preserves renal function without activation of the renin-angiotensin system.

The pGC activator BNP is a diagnostic and prognostic marker for heart failure (McKie & Burnett, 2005). Cardiac secretion of BNP increases with the progression of heart failure with plasma concentrations of >100 pg/ml used as a biomarker for the disease. Moderate elevations in BNP (20–100 pg/ml) could serve as a prognostic marker for other cardiovascular pathologies, such as atrial fibrillation and coronary artery disease. The therapeutic relevance of natriuretic peptides was highlighted by J. Burnett (Rochester, MN, USA), who introduced a new generation of orally active BNP analogues that have potential in the treatment of heart failure.

It is becoming increasingly evident that PDE5 inhibitors have clinical applications beyond the treatment of erectile dysfunction. Thus, in addition to Abman's data in the setting of pulmonary hypertension (see above), D. Kass (Baltimore, MD, USA) showed that sildenafil markedly reduced the development of cardiac hypertrophy in response to pressure overload and reversed pre-established cardiac enlargement and fibrosis in mice (Takimoto *et al.*, 2005). Interestingly, the potent anti-hypertrophic effect of sildenafil occurred without an apparent increase in total myocardial cGMP content, but cGKI activity was enhanced. Kass proposed that the anti-hypertrophic cGMP pathway is localized to a distinct compartment of the stressed cardiomyocyte that sildenafil targets to elevate cGMP and exert its beneficial effects. PDE inhibition might also represent a new therapeutic strategy to enhance learning and memory. Using a rat model of hepatic encephalopathy, V. Felipo (Valencia, Spain) provided evidence that impaired cGMP signalling and learning under pathological conditions can be restored by chronic administration of sildenafil. Finally, M. Hendrix (Wuppertal, Germany) introduced two new PDE inhibitors (BAY 60-7550 for PDE2 and BAY 73-6691 for PDE9) that showed memory-enhancing effects in rodents. These PDE inhibitors are promising candidate drugs for the treatment of cognition disorders such as Alzheimer's disease.

Conclusions

After years of focus on NO, we are now entering an exciting new era with regard to cGMP research. Recent investigations have revealed unexpected signalling and regulation events downstream of NO. Structural studies have identified amino acids that are crucial in conferring the selectivity of sGC for NO over O₂ and its ability to bind haem. The widely held view that sGC is a purely cytosolic enzyme and lacks partners is changing. There is emerging evidence that it can translocate to the plasma membrane and can be found in signalling complexes with other proteins, in which it is subject to regulation through allosteric interactions and post-translational modifications. The analysis of conventional and conditional mouse mutants for sGCs, pGCs, cGKs and cGK substrates has revealed a key role for cGMP signalling in the regulation of cardiovascular homeostasis including smooth muscle relaxation, platelet inhibition, and vascular growth and differentiation, and we are beginning to understand the respective molecular mechanisms downstream of cGMP (Fig 1). Two new therapeutic approaches were highlighted at this meeting. First, chronic treatment with PDE5 inhibitors or NO-independent sGC activators might not only normalize cardiovascular function, but also exert anti-remodelling effects in disease states such as atherosclerosis, pulmonary and systemic hypertension and cardiac hypertrophy. Second, novel NO-independent activators of sGC, such as BAY 58-2667, have the unprecedented ability to target oxidized sGC and thus to selectively dilate the diseased versus non-diseased vasculature. Such sGC activators also have the potential to be diagnostic aids for detecting oxidative stress or blood vessels at risk of vascular complications. Clearly, the biochemical, genetic and pharmacological data presented at this meeting have provided important steps forwards in our understanding of the basic mechanisms and therapeutic potential of cGMP signalling.

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REFERENCES

- Aguillo L, Garcia-Dorado D, Escalona N, Ruiz-Meana M, Mirabet M, Inserte J, Soler-Soler J (2005) Membrane association of nitric oxide-sensitive guanylyl cyclase in cardiomyocytes. *Cardiovasc Res* **68**: 65–74
- Beavo JA, Brunton LL (2002) Cyclic nucleotide research—still expanding after half a century. *Nat Rev Mol Cell Biol* **3**: 710–718
- Boon EM, Huang SH, Marletta MA (2005) A molecular basis for NO selectivity in soluble guanylate cyclase. *Nat Chem Biol* **1**: 53–59
- Deruelle P, Grover TR, Abman SH (2005) Pulmonary vascular effects of nitric oxide-cGMP augmentation in a model of chronic pulmonary hypertension in fetal and neonatal sheep. *Am J Physiol Lung Cell Mol Physiol* **289**: L798–L806
- Evgenov OV, Ichinose F, Evgenov NV, Gnath MJ, Falkowski GE, Chang Y, Bloch KD, Zapol WM (2004) Soluble guanylate cyclase activator reverses acute pulmonary hypertension and augments the pulmonary vasodilator response to inhaled nitric oxide in awake lambs. *Circulation* **110**: 2253–2259
- Feil R, Lohmann SM, de Jonge H, Walter U, Hofmann F (2003) Cyclic GMP-dependent protein kinases and the cardiovascular system: insights from genetically modified mice. *Circ Res* **93**: 907–916
- Friebe A, Koesling D (2003) Regulation of nitric oxide-sensitive guanylyl cyclase. *Circ Res* **93**: 96–105
- Geiselhöringer A *et al.* (2004) IRAG is essential for relaxation of receptor-triggered smooth muscle contraction by cGMP kinase. *EMBO J* **23**: 4222–4231
- Kuhn M (2003) Structure, regulation, and function of mammalian membrane guanylyl cyclase receptors, with a focus on guanylyl cyclase-A. *Circ Res* **93**: 700–709
- Massberg S *et al.* (2004) Enhanced in vivo platelet adhesion in vasodilator-stimulated phosphoprotein (VASP)-deficient mice. *Blood* **103**: 136–142
- McKie PM, Burnett JC, Jr (2005) B-type natriuretic peptide as a biomarker beyond heart failure: speculations and opportunities. *Mayo Clin Proc* **80**: 1029–1036
- Rybalkin SD, Yan C, Bornfeldt KE, Beavo JA (2003) Cyclic GMP phosphodiesterases and regulation of smooth muscle function. *Circ Res* **93**: 280–291
- Sabrane K, Kruse MN, Fabritz L, Zetsche B, Mitko D, Skryabin BV, Zwiener M, Baba HA, Yanagisawa M, Kuhn M (2005) Vascular endothelium is critically involved in the hypotensive and hypovolemic actions of atrial natriuretic peptide. *J Clin Invest* **115**: 1666–1674
- Schmidt PM, Schramm M, Schroder H, Wunder F, Stasch JP (2004) Identification of residues crucially involved in the binding of the haem moiety of soluble guanylate cyclase. *J Biol Chem* **279**: 3025–3032
- Takimoto E, Champion HC, Li M, Belardi D, Ren S, Rodriguez ER, Bedja D, Gabrielson KL, Wang Y, Kass DA (2005) Chronic inhibition of cyclic GMP phosphodiesterase 5A prevents and reverses cardiac hypertrophy. *Nat Med* **11**: 214–222

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