Cyclic GMP-Dependent Signaling in Cardiac Myocytes

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Cyclic GMP (cGMP) and its effector kinase PKG regulate diverse cellular functions. In cardiac myocytes, cGMP is produced by soluble and particulate guanylyl cyclases (GCs), the former stimulated by nitric oxide and the latter by natriuretic peptides, and is hydrolyzed to inactive 5’-GMP by cGMP-phosphodiesterases (PDEs). cGMP-PKG modulates cardiac contractility, hypertrophy and remodeling, and exerts cardioprotection. Although early research efforts have mostly focused on cGMP synthetic pathways, recent studies have revealed that cGMP degradation controlled by PDEs plays a critical role in the physiological action of cGMP. Among several cGMP-PDEs, cGMP-specific PDE5 has been intensively investigated. Studies in experimental animal models and humans consistently demonstrate benefits from PDE5 inhibitors in various cardiac pathologies. Several clinical trials are ongoing or planned to test the efficacy of PDE5 inhibitors in human heart disease, including a large multicenter clinical trial (RELAX) led by the NIH evaluating sildenafil efficacy in heart failure with preserved ejection fraction. This review underscores the current understanding of cGMP-PKG signal regulation and its pathophysiological role in the heart, focusing on cardiac myocytes. (Circ J 2012; 76: 1819–1825)

Key Words: Cyclic GMP (cGMP); cGMP-dependent protein kinase; Myocytes; Phosphodiesterases

Cyclic GMP (cGMP) is the intracellular second messenger that mediates the action of nitric oxide (NO) and natriuretic peptides (NPs). It regulates a broad array of physiologic processes in the cardiovascular system, including cardiac contractility, vascular tone, platelet function, and cardiac and vascular remodeling. NO activates soluble guanylyl cyclase (GC) and NP activates membrane-bound GC, to produce cGMP. cGMP exerts its physiological action through cGMP-dependent protein kinase (PKG), cGMP-regulated phosphodiesterases (PDE2, PDE3) and cGMP-gated cation channels, among which PDE5 might be the primary mediator. Importantly, the cGMP signal is compartmentalized within a cell so that specific targeted proteins can be regulated by the same “generic” cGMP to exert differential physiological effects. Key players in this regulation are the cyclic nucleotide degrading PDEs that are localized to specific subcellular space with confined activity. This review highlights the cardiac regulation of cGMP signaling and its role in cardiac pathophysiology, focusing on cardiac myocytes (Figure).

C GMP Generation, Degradation, and Its Effector Kinase

Generation Via 2 Distinct Pathways

No-sGC-cGMP Synthetic Pathway NO is a gaseous signaling molecule with a half-life of several seconds. Many key biological signals of NO are mediated by cGMP, while NO also directly modifies protein function via covalent attachment of NO to reduced thiol groups, known as S-nitrosylation. NO is endogenously synthesized from L-arginine by the catalytic

Action of NO synthases (NOS), and it can be released from nitrovasodilators such as glyceryl trinitrate (nitroglycerin). There are 3 isozymes of NO synthase, including neuronal NOS (nNOS or NOS1), inducible NOS (iNOS or NOS2) and endothelial NOS (eNOS or NOS3). eNOS and nNOS are expressed constitutively and activated by Ca²⁺/calmodulin, whereas iNOS is induced by (inflammatory) stimuli and its activity is Ca²⁺ independent. eNOS is highly expressed in endothelial cells, whereas nNOS is abundant in neurons and skeletal muscle. However, many tissues express all 3 isoforms, including cardiac myocytes. NO activates soluble GC (sGC), a heterodimeric enzyme consisting of α- and β-subunits and a prosthetic heme group with a ferrous iron, and produces cGMP. This enzyme activity is critically affected by redox status: oxidation of the heme moiety on the β-subunit renders the enzyme insensitive to NO. Oxidative stress could compromise this cGMP synthetic pathway by other mechanisms as well. NO forms a reactive oxidant peroxynitrite (ONOO⁻) in the presence of superoxide (O²⁻), and NOS becomes dysfunctional (uncoupled) under oxidative stress to produce O²⁻ rather than NO.

NPs-sGC-cGMP Synthetic Pathway The NPs are a family of structurally related polypeptide hormones, including atrial NP (ANP), B-type NP (BNP) and C-type NP (CNP). ANP and BNP are derived primarily from the atria and ventricles of the heart, respectively, whereas CNP is secreted by endothelial cells. NPs bind to membrane-associated GC receptors (pGC or rGC) and produce cGMP. Among the 7 particulate GCs identified, GC-A (also called NP A receptor or NPR-A) and GC-B (also called NPR-B) are important in the cardiovascular system. GC-A is the dominant form, expressed in various tis-
sues, including cardiac myocytes, and the principal receptor for ANP and BNP, 11 GC-B, a receptor for CNP, is mainly expressed in the brain and vascular tissues, but some studies have suggested its presence and role in cardiac myocytes. 12, 13 NPs-pGMP synthesis is compromised under disease conditions such as heart failure, because of the desensitization of GC-A. 14

Degradation by PDEs
The PDEs are enzymes that hydrolyze cyclic nucleotides (cGMP, cAMP) to the inactive linear form (5'-GMP, 5'-AMP), thereby regulating both the duration and the amplitude of cyclic nucleotide signaling. PDEs comprise a 21-gene superfamily categorized into 11 families (PDE1–PDE11). 15 PDEs 1, 2, 3, 4, 5, 8 and 9 are expressed in the heart (Table) and the roles for PDEs 1, 2, 3 and 5 in cardiac cGMP regulation have been investigated. PDE2 and PDE5 harbor allosteric cGMP-binding sites, named GAF domains, located in the N-terminus portion. cGMP-binding affinity to the GAF domain is much higher than to PDE catalytic sites, and cGMP binds to the GAF domain before cyclic nucleotide catalytic action to induce the active state. 15

PDE1 PDE1 is a dual substrate PDE and is potently activated by Ca²⁺/calmodulin. 16 There are 3 isoforms (PDE1A, PDE1B and PDE1C), all expressed in cardiac myocytes. 16, 17 PDE1A and PDE1B hydrolyze cGMP with higher affinity than cAMP, whereas PDE1C, the dominant isoform in human cardiac myocytes, hydrolyzes both with similar affinity. 17 Although PDE1 is a major esterase for both cAMP and cGMP in the myocardium or cardiac myocytes in humans, 16, 18 its physiological effect, particularly in vivo, has not been fully clarified.

PDE2 PDE2 is a dual substrate esterase with similar affinity for cAMP and cGMP. 15 PDE2 is expressed in cardiac myocytes in various species, including humans, 16 and localized at the plasma membrane and Z-line of cardiac myocytes. 19 Due to the cGMP-sensitive GAF domain, the enzyme functions mainly as a cGMP-stimulated cAMP esterase and thereby a cross-talk regulator between the 2 cyclic nucleotides. 20 Through a cAMP modulating function, PDE2 regulates the β-adrenergic cardiac response in a subcellular compartment-selective manner.

PDE3 PDE3 is also a dual substrate enzyme, known as a cGMP-inhibited cAMP esterase. 15 Although both subtypes, PDE3A and PDE3B, exist in cardiac myocytes, PDE3A is the main subtype and responsible for cardiac functional change caused by PDE3 inhibition. 21 PDE3 has similar affinity for

Figure. Cardiac myocyte cGMP-PKG pathways. cGMP is synthesized by 2 pathways, NO-sGC and NP-pGMP pathways, and is under compartmentalized regulation by PDEs. cGMP-PKG regulates cardiac function, hypertrophy/remodeling and cell survival response (see text for details). cGMP, cyclic GMP; NO, nitric oxide; NP, natriuretic peptide; PDE, phosphodiesterase; PKG, cGMP-dependent protein kinase. LTCC, L-type Ca²⁺ channel; TRPC, transient receptor potential canonical; GqPCR, Gq protein coupled receptor; Ang II, angiotensin II; PE, phenylephrine; ET1, endothelin 1; β3AR, β3 adrenergic receptor; cTnI, cardiac troponin I; PLB, phospholamban; β, phosphorylation.
cAMP and cGMP, but because of the higher catalytic rate for cAMP than for cGMP. cGMP acts as a competitive inhibitor against its cAMP catalytic action.  

**PDE5**  
PDE5A is a cGMP-specific esterase, abundantly expressed in vascular smooth muscle. Although PDE5A was not originally thought to be expressed in the heart, evidence has accumulated that PDE5A protein is expressed in cardiac myocytes.  

The most recent study revealed that PDE5 represents ~20% of total cGMP-esterase activity in human cardiac myocytes, though there still remains some debate.  

PDE5 expression is upregulated in the diseased heart via an oxidant stress-dependent mechanism, and is activated by cGMP binding to the GAP domain and by PKG phosphorylation at Ser 92.  

A PDE5 inhibitor such as sildenafil occupies its catalytic site as a false substrate to increase cGMP and activate PKG, leading to enhanced activity of the enzyme, and thus the inhibitor’s efficacy.  

PDE5 is localized to the Z-band of cardiac myocytes under physiological conditions, but is diffusely distributed in disease conditions.  

**cGMP-Dependent Protein Kinase I (PKG)**  
cGMP-dependent protein kinases (PKGs) are serine/threonine kinases that mediate many of the biological effects of cGMP. PKGI and PKGII are encoded by 2 genes, and PKGI is the primary isotype in the cardiovascular system. PKGI is a homodimer of identical subunits. Each subunit has 3 functional domains: (1) an N-terminus domain containing a leucine/isoleucine zipper and auto-inhibitory/pseudosubstrate region, (2) a regulatory domain with 2 allostERIC cGMP binding sites, and (3) a catalytic domain. The N-terminus of PKGI is encoded by 2 alternative exons that produce 2 splice-variants, PKGIα and PKGIβ. This N-terminal difference provides different cGMP sensitivity (PKGIα is more sensitive to cGMP than PKGIβ by 10-fold) and protein targeting. PKGIα has a unique cysteine (Cys 43), the oxidative modification of which leads to the enzyme activation independently of cGMP. This activation mechanism operates basally to control resistance vessel tone, but its role in cardiac myocytes is unknown.  

**cGMP Signal Regulation of Cardiac Myocytes**  
**cGMP Regulation of Cardiac Contractility**  
cGMP negatively modulates contractility, accelerates relaxation and improves the stiffness of cardiac myocytes. These effects might be mediated by direct PKG phosphorylation of proteins, including cardiac troponin I, L-type Ca^{2+} channel, phospholamban and titin.  

The role of PKG in the negative inotropy induced by cGMP was demonstrated in a study of mice hearts lacking PKGI. Wegener et al reported that cGMP analogs (8-Br-cGMP, 8-pCPT-cGMP) have negative inotropic effects in control hearts but not in hearts lacking PKGI, independent of cAMP signaling.  

Exogenous NO exerts a negative inotropic effect, which is mediated by cGMP-PKG-induced reduction in myofilament calcium responsiveness. Layland et al demonstrated that a NO donor induces negative inotropic effects and accelerates relaxation, without affecting the calcium transient, but with increased troponin I phosphorylation, in rat ventricular myocytes. These effects were abrogated by soluble GC inhibitor or PKG inhibitor. Endogenous NO has cGMP-dependent and cGMP-independent effects, depending on the source. Studies have revealed that NO from eNOS is coupled to the cGMP-PKG signal pathway, regulating cardiac function, whereas NO from nNOS directly modifies the ryanodine receptor (RyR) by S-nitrosylation and affects cardiac function. NO-cGMP derived from eNOS is functionally coupled to PDE5, modulating cardiac β-adrenergic stimulated contractility. PDE5 inhibition suppresses β-adrenergic receptor-stimulated cardiac contractility in wild-type mice hearts/myocytes, an effect that is abrogated in the presence of PKG inhibitor, but is absent in hearts/myocytes lacking eNOS or β3 adrenergic receptor (β3AR) coupled to eNOS. This antiadrenergic effect might be attributable to troponin I phosphorylation by PKG and/or inhibition of the L-type Ca^{2+} channel (phosphorylation by PKG). The antiadrenergic response to PDE5 inhibitor is also observed in humans. Importantly, normal subcellular localization of PDE5 to the Z-band is the key to this regulation. The antiadrenergic effect of PDE5 inhibition is absent in failing canine hearts/myocytes in which PDE5 is diffusely distributed.  

Importantly, cGMP derived from pGC (GCA) by exogenous ANP has no effect on cardiac systolic function or the β-adrenergic contractility response, suggesting the existence of compartmentalized regulation. On the other hand, cardiac diastolic function is modified by the NP-pGC pathway similarly to the NO-cGMP pathway. Exogenous BNP enhances the speed and extent of ventricular relaxation in normal and heart failure dogs. The PDE5 inhibitor, sildenafil, alone or in combination with BNP, increases left ventricular diastolic properties (distensibility) in both normal and old hypertensive dogs. This might be at least partially attributable to phosphorylation of titin, a sarcomeric “spring” that regulates myocardial passive stiffness. Reduction of titin-stiffness by cGMP-PKG was also demonstrated in the human myocardium.  

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**Table. PDEs in the Heart**  

<table>
<thead>
<tr>
<th>PDE isoenzyme</th>
<th>Km (μM)</th>
<th>Heart expression</th>
</tr>
</thead>
<tbody>
<tr>
<td>PDE1</td>
<td>cAMP: 73–120, PDE1B: 10–24, PDE1C: 0.3–1.2</td>
<td>Yes</td>
</tr>
<tr>
<td>PDE2</td>
<td>cGMP-stimulated, Dual specificity</td>
<td>30–50</td>
</tr>
<tr>
<td>PDE3</td>
<td>cGMP-inhibited, Dual specificity</td>
<td>0.02–0.15</td>
</tr>
<tr>
<td>PDE4</td>
<td>cAMP-specific</td>
<td>2.9–10</td>
</tr>
<tr>
<td>PDE5</td>
<td>cAMP-specific</td>
<td>–</td>
</tr>
<tr>
<td>PDE8</td>
<td>cAMP-specific</td>
<td>0.15</td>
</tr>
<tr>
<td>PDE9</td>
<td>cGMP-specific</td>
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</table>

PDEs, phosphodiesterases.
cGMP affects cAMP signaling via cross-talk regulation by cGMP-regulated PDEs (PDE2 or PDE3), and modulates cardiac function. Using a real-time cyclic nucleotide imaging technique, Mongillo et al. reported that catecholamine stimulates cGMP via β3AR coupled to eNOS, and activates PDE2-cAMP hydrolysis, attenuating localized accumulation of cAMP. A more recent study has suggested that PDE2 activity is confined to the PKA RII isoforms residing compartment that is functionally coupled to phospholamban or troponin I phosphorylation. cGMP-cAMP cross-talk involving PDE3 was implicated in pathological right hearts. In a rat model of right ventricular hypertrophy, acute PDE5 inhibition increased myocardial cAMP (as well as cGMP) and cardiac contraction, associated with reduced PDE3 activity. A similar positive inotropic effect by PDE5 inhibition was observed in human failing right hearts.

**cGMP Regulation of Cardiac Hypertrophy and Remodeling**

The heart develops hypertrophy in response to pathological stress. Although cardiac hypertrophy provides the initial functional compensation response to stress, it evolves into maladaptive remodeling/dilation with functional de-compensation, and can result in overt failure. cGMP signaling plays a “brake”-like role (negative regulator) in the cardiac hypertrophy remodeling process. Gq-agonist-induced hypertrophy in cultured cardiac myocytes is suppressed by ANP, NO, cGMP or PKG. Although an early study using conventional PKGI null animals with vascular re-introduction of PKGβ failed to demonstrate a significant role for PKGI in cardiac hypertrophy or remodeling, this could be partly because their pressure-load protocol was not severe enough to induce the pathological signal activation that cGMP-PKG targets. The 2 most recent studies clearly demonstrated the role of PKGI as a key negative regulator of cardiac hypertrophy and remodeling. Mice with reduced myocyte PKG activity by cardiac myocyte PDE5 overexpression develop exacerbated hypertrophy and remodeling in response to pressure overload, and normalizing PKG activity by turning off PDE5 overexpression ameliorates the once established remodeling. Mice with cardiac-specific deletion of PKGI revealed worse remodeling to angiotensin II or pressure overload. Studies of animals with a modified NP-cGMP pathway have shown consistent results. Mice lacking NPPA throughout the body show arterial hypertension with a disproportionate degree of cardiac hypertrophy, and mice with cardiac-specific NPPA deletion develop exaggerated hypertrophy in response to pressure overload. Contrastly, cardiac overexpression of constitutive active GC inhibits pressure-overload hypertrophy.

Most importantly, small molecule cGMP-PDE inhibitors ameliorate hypertrophy and remodeling. We first reported that the PDE5 inhibitor, sildenafil, activates myocardial PKG and ameliorates cardiac hypertrophy/remodeling in a mouse model of pressure overload. Sildenafil efficacy in volume overload remodeling was recently demonstrated in a rat model of chronic mitral valve regurgitation. Miller et al reported that a PDE1 inhibitor (IC86340) has antihypertrophic effects in cultured cardiac myocytes exposed to Gq-agonist and in chronic isoproterenol-induced hypertrophy in mice. PDE1 and PDE5 inhibitors show additive antihypertrophic effects, suggesting that these enzymes do not hydrolyze identical intracellular cGMP pools.

Several mechanisms have been suggested for the antihypertrophic/remodeling effects of the cGMP signals. The cardiac hypertrophic response involves activation of various intracellular signaling cascades. Gq-coupled signaling, and in particular, calcineurin, plays a major role in this process. Fiedler et al first demonstrated in cultured myocytes that PKG deactivates calcineurin in via inhibition of the L-type Ca2+ channel, blunting the cellular hypertrophy response. Recently, studies have implicated a role for transient receptor potential canonical (TRPC) 6, a receptor-operated Ca2+ channel, in this regulation. PKG phosphorylates TRPC6 at Thr 69, 70 and Ser 322, and suppresses its conductance, resulting in inhibition of calcineurin-NFAT signaling. However, calcineurin inhibition is not the sole mechanism, as mice lacking calcineurin Aβ can still develop moderate cardiac hypertrophy in response to pressure overload that is inhibited by PDE5 inhibitor. Studies have demonstrated a central role for Regulator of G protein Signaling (RGS2 and RGS4) in the cGMP-mediated anti-hypertrophy/remodeling mechanism. RGS proteins de-activate G protein-coupled signaling via GTPase activating properties. PKGIa phosphorylates activates RGS2 and RGS4, and terminates the Gq signal, inhibiting hypertrophy. We reported that PKG activation by PDE5 inhibitor fails to de-activate multiple hypertrophy signaling cascades in hearts lacking RGS2, resulting in a lack of the antihypertrophic/remodeling effect, and Tokudome et al reported that RGS4 overexpression ameliorates cardiac spontaneous hypertrophy in NPRa (GCa) null animals. Recent study suggested that PKG-RGS2 is also involved in the ANP-GCa antihypertrophy.

Lastly, it is worthwhile mentioning the differential response between the right and left heart to PDE5 inhibitor in hypertrophy/remodeling. Interestingly, PDE5 inhibitor fails to exert a direct antihypertrophic effect on right heart hypertrophy induced by surgically induced pressure overload. This difference might be attributable to fundamental anatomical, biological and physiological differences between the right and left sides of the heart.

**Cardiac Protection by cGMP Signaling: Ischemic Injury and Cardiomyopathy**

The cGMP-PKG pathway has been implicated in cardiac protection against cell death-inducing stress, such as ischemic injury and doxorubicin toxicity. NO, BNP and 8Br-cGMP all ameliorate cell death in cultured cardiac myocytes exposed to ischemia-reoxygenation, and mice lacking PKGI develop a larger infarct than controls after ischemia-reperfusion. On the other hand, NPs also limit the infarct size from ischemia-reperfusion injury in animal models, and in vivo and ex vivo studies have revealed that NO donors limit infarct size and improve post-ischemic functional recovery. Such beneficial effects of NO, however, were not consistently observed, which might be attributable to impaired bioavailability of NO because of reactive oxygen species generated during early reperfusion. Inhibition of NOS during ischemic preconditioning or postconditioning abolishes its cardiac protective effects, suggesting the essential role of NOS-cGMP-PKG in the cardiac protection conferred by preconditioning or post-conditioning. On the other hand, NPs also limit the infarct size from ischemia-reperfusion injury in animal models and in humans. Kitakaze et al reported that patients with acute myocardial infarction who were given carperitide (recombinant human ANP) as an adjunct to reperfusion therapy had smaller infarcts, fewer reperfusion injuries and better clinical outcomes than controls. Recent studies have revealed potent cardioprotection by PDE5 inhibitors. The infarct-limiting effect of PDE5 inhibitor in myocardial ischemia-reperfusion injury has been reported in mouse, rat and rabbit hearts.

The underlying mechanisms are complex, involving mitochondrial KATP channel (mitoKATP) regulation and stress responsive (survival) signaling cascades. Ischemia-reperfusion induces mitochondrial permeability transition (MPT) pore...
formation that induces a permeability change of mitochondria, leading to cell death. Ischemic preconditioning potently inhibits this process, which is mediated by transient opening of mitochondrial permeability transition pore (mPTP). Opening the mPTP partially compensates the membrane potential (ΔΨm), which enables additional protons to be pumped out to form an H+ gradient for both ATP synthesis and Ca2+ transport. Costea et al reported the mechanism that links PKG to mPTP and MPT. PKG increases opening of mPTP by PKCε via indirect activation. Rapid influx of K+ increases the matrix pH and increases H:O2 production from complex I, to further activate PKCε coupled to MPT, resulting in MPT inhibition. In addition to direct PKG mitochondrial regulation, other mechanisms might be at play in cGMP-PKG mediated cardioprotection. Phosphorylation of ERK and glycogen synthase kinase 3β (GSK3β) and upregulation of anti-apoptotic bcl2 were reported in ischemia re-perfused hearts treated with PDE5 inhibitor. Although ERK activation lies upstream of this regulation, mitochondrial effects might be directly mediated by GSK3β inactivation (phosphorylation) and upregulated bcl2, the former inhibiting MPT pore formation and the latter inhibiting mitochondrial apoptosis. Fiedler et al reported PKG inhibition of pro-apoptotic p38 MAPK. Ischemia-reperfusion activates p38 via TAB1 (TAK1 binding protein), independently of upstream kinases MKK3 or 6, inducing apoptosis. PKGα physically associates with p38 MAPK and prevents TAB1 interaction, resulting in reduced p38 activation and ischemia-reperfusion injury. Another protective mechanism involves sarcolemmal Na+/K+ ATPase regulation. Madhani et al reported that sildenafil or PKG phosphorylates phospholemman and activates Na+/K+ ATPase, to limit Na+ overload and thus Ca2+ overload during reperfusion.

The cardioprotective effects of cGMP signaling has been demonstrated in some types of cardiomyopathy. Doxorubicin (Dox), an anticancer drug, has cardiac toxicity that ultimately leads to cardiomyopathy. PDE5 inhibitor (sildenafil or tadalafil) improved cardiac function and survival in a rodent model of Dox-cardiomyopathy by attenuating oxidative stress and apoptosis, which might be attributable to upregulation of bcl2 and mitochondrial superoxide dismutase (MnSOD). A genetic mouse model of Duchenne muscular dystrophy lacking dystrophin (mdx) develops cardiomyopathy as do human muscular dystrophy patients. Enhancing cGMP signal by cardiac overexpression of sGC or by PDE5 inhibitor, sildenafil, ameliorates the cardiac phenotype of mdx, including cardiac contractile performance, metabolic status and sarcolemmal integrity via mechanisms that might involve inhibition of MPT formation. PDE5 inhibitor is effective also in diabetic cardiomyopathy. Tadalafil improves cardiac function in leptin-deficient mice, an animal model for type II diabetes, which is associated with an altered proteomic profile of cytoskeletal rearrangement and redox regulation.

**Conclusion**

**cGMP Signal Modification as Therapy for Human Heart Disease**

Accumulating evidence suggests that cGMP-PKG signaling plays a cardioprotective role against pathological stress. Among several strategies to enhance this signaling, PDE5 inhibition has been gaining great attention, with 3 inhibitors (sildenafil, vardenafil and tadalafil) already in clinical use to treat erectile dysfunction and pulmonary hypertension. Recent small human studies revealed beneficial cardioprotective effects from chronic sildenafil treatment, while it also provides acute hemodynamic benefits. Guazzi et al reported that 1-year sildenafil treatment improved cardiac function and exercise performance, associated with reverse remodeling of left atrial volume index and LV mass index, in heart failure patients (New York Heart Association class II–III). Giannetta et al reported anti-remodeling effects and improved cardiac kinetics from 3 months sildenafil treatment in diabetic cardiomyopathy patients. These results suggest that PDE5 inhibition is a promising new approach to treating various human heart diseases. A large placebo-controlled multicenter trial (RELAX, NCT00763867) led by NIH is ongoing to test sildenafil efficacy in heart failure patients with preserved ejection fraction. A single center study is currently testing sildenafil efficacy in cardiomyopathy with Duchenne and Becker muscular dystrophies (REVERS-DBMD, NCT01168908). These studies will soon provide more information on the clinical benefit of PDE5 inhibitor treatment in human heart disease. On the other hand, BNP (nesiritide) was introduced into clinical practice in 2001 for early relief of dyspnea in acute heart failure patients, because of its vasodilatory properties. However, recent evaluation in a large randomized trial showed minimal benefit and an increased risk of hypotension. The efficacy of combined use of tadalafil and nesiritide is being tested in subclinical heart failure patients, in whom the expected benefit is renal function improvement rather than a direct cardiac effect (NCT01544998). Considering that the enzymes for cGMP synthesis, including NOS, sGC and GC-A, could be "uncoupled" from cGMP synthesis under chronic stress conditions, stimulation of sGC with a redox-sensitive activator and/or co-coupling of NO with tetrahydrobiopterin (BH4) might have intriguing potential as a strategy to enhance cGMP signaling other than inhibiting cGMP degradation.

In summary, the cardiac cGMP system plays a key role in protection against various cardiac pathologies, including hypertrophy, heart failure, ischemic injury and cardiomyopathy. PDE5 inhibitors, in particular, are promising therapeutic modality to enhance this signal, and efficacy has been demonstrated in human heart failure and diabetic cardiomyopathy. Ongoing and future large-scale clinical trials will determine whether the therapy will have long-term beneficial effects on cardiac remodeling and a reduction in major cardiac events. NP (nesiritide) failed to show benefit in acute decompensated heart failure, but its cardioprotective effect, particularly against ischemic injury, warrants further investigation.

**Acknowledgments**

This work was supported by National Institute of Health (ROI 993432) and American Heart Association (11GRNT7700071).

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