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Cardiac Role of Cyclic-GMP Hydrolyzing Phosphodiesterase Type 5: From Experimental Models to Clinical Trials

David A. Kass

Division of Cardiology, Department of Medicine, The Johns Hopkins Medical Institutions, Ross Building, Room 858, 720 Rutland Avenue, Baltimore, MD 21205, USA

David A. Kass: dkass@jhmi.edu

Abstract

Cyclic guanosine monophosphate (cGMP) and its primary signaling kinase, protein kinase G, play an important role in counterbalancing stress remodeling in the heart. Growing evidence supports a positive impact on a variety of cardiac disease conditions from the suppression of cGMP hydrolysis. The latter is regulated by members of the phosphodiesterase (PDE) superfamily, of which cGMP-selective PDE5 has been best studied. Inhibitors such as sildenafil and tadalafil ameliorate cardiac pressure and volume overload, ischemic injury, and cardiotoxicity. Clinical trials have begun exploring their potential to benefit dilated cardiomyopathy and heart failure with a preserved ejection fraction. This review discusses recent developments in the field, highlighting basic science and clinical studies.

Keywords

Cyclic guanosine monophosphate; Protein kinase G; Cyclic GMP-dependent protein kinase; Phosphodiesterase; PDE5; PDE2; PDE1; Sildenafil; Tadalafil; heart failure; Hypertrophy; Ventricular function; Remodeling; Fibrosis; Transforming growth factor beta; Cyclic AMP; Signal transduction; RGS2; TRPC channel; Ischemia; Preconditioning; Mitochondria; Human; Genetic models

Introduction and Historical Perspective

Heart muscle must contract cyclically and continuously for the lifespan of an organism, providing the mechanical force needed to move blood about the circulation and help modulate biochemical and metabolic homeostasis. Central to these roles is its capacity to adapt to acute and chronic stress, and this involves a tangle of signaling cascades, modulators of protein modulators, and organ remodeling. In a very simplified view, these regulatory factors fall into two categories; those stimulating function and/or growth and those that blunt it. A well-accepted example of the first category is adrenergic stimulation coupled to the generation of myocardial cyclic adenosine monophosphate (cAMP). An example of the latter is cyclic guanosine monophosphate (cGMP) and its related signaling, which can serve as a counter-brake to a variety of myocardial stressors [1•, 2••]. Cyclic GMP functions by interacting with phosphodiesterases and by stimulating protein kinase G to modulate cell function. Pharmacological agents stimulating cGMP synthesis such as organo-nitrites and natriuretic peptides (NPs) are used to treat clinical heart failure, though

Correspondence to: David A. Kass, dkass@jhmi.edu.

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their perceived utility has centered on vascular and renal effects rather than direct myocardial influences.

The perspective regarding the role of cGMP-PKG signaling evolved considerably over the past 10–15 years following the identification and manipulation of hydrolytic phosphodiesterases (PDEs) that regulate cGMP once synthesized [3]. In contrast to stimulation strategies for which tissue targeting is difficult and counter-upregulation by PDEs limits net impact, PDE inhibitors work within cells, modifying selective targets. To be effective, however, there must be sufficient synthetic activity to generate cGMP in the first place. Of the 11-member superfamily of PDEs, three that hydrolyze cGMP have been identified in heart: PDE1, PDE2, and PDE5 [4•]. PDE1 and PDE2 are dual-substrate (cAMP, cGMP) enzymes. PDE1 is calcium-calmodulin dependent and provides the majority of cGMP-hydrolysis in cell-free extracts from the normal mammalian heart. PDE2 can hydrolyze cGMP [5], but most data have revealed cAMP hydrolysis as a primary function that is enhanced by cGMP binding to an N-terminus regulatory (GAF) domain [6, 7]. PDE5 is selective for cGMP catalysis and is also regulated by cGMP. Understanding the role and influence of cGMP-PDEs has been greatly enhanced by the development of selective inhibitors, though for PDE1 and 2, published data has been limited to in vitro studies.

The most widely studied cGMP-PDE in both experimental animals and humans is PDE5. PDE5 inhibitors were first developed for coronary artery disease given expression of the esterase in platelets and vascular smooth muscle. However, studies soon stumbled upon a major “side effect,” and their capacity to improve erectile function quickly became the focus of drug development. Clinical cardiovascular data focused on safety, not treatment potential, and these studies reported negligible acute impact on the heart and systemic vasculature consistent with relative low expression of PDE5 in these tissues. Normal myocardial PDE5 protein expression is very low, and early studies in isolated muscle or myocytes suggested no impact on function [8], leading to conclusions that a cardiac role was negligible.

In 2001, our laboratory first reported that PDE5 inhibition blunted acute β -adrenergic contractile stimulation in the normal canine heart [9]. This study provided evidence for PDE5 expression in myocytes and activity in myocardium, while suggesting dysregulation of its signaling in HF. This was followed in 2002 by the first report that PDE5 inhibition had potent anti-ischemic effects by targeting mitochondrial K^+_{ATP} channels [10]. Yet, data finding no impact of PDE5 inhibition in myocardium [11] fueled conclusions that myocardial effects were unlikely.

Meanwhile, studies began exploring the utility of PDE5 inhibitors for the treatment of pulmonary hypertension [12], work that led to its clinical approval for this indication. Insights into myocardial roles of the enzyme began to change with the 2005 report that chronic blockade of PDE5 both suppressed and reversed pathological cardiac hypertrophy in mice subjected to pressure overload [13]. In this model, ventricular systolic load remained fixed by an aortic constriction, so myocardial benefits could not be attributed to vascular unloading. Concurrent work on ischemic injury revealed substantial cardioprotection [14] against apoptosis [15] and infarction [16], and reduced cardiotoxicity from doxorubicin, all from PDE5 inhibition [17]. The capacity for PDE5 inhibition to acutely blunt adrenergic stimulation was recapitulated in mice, where a requirement for cGMP generated by nitric oxide (NO)–soluble guanylate cyclase (sGC) was revealed [18], as well as in healthy human volunteers [19].

Quick jump to 2012, and we can see how far the field has changed [20]. Many groups now report a potent role of PDE5 in regulating cardiac remodeling in heart disease and benefits from its inhibition; small clinical trials are finding benefits in heart failure patients related to

multi-organ-targeting, including the heart; and multicenter studies in HF patients with preserved ejection fraction (eg, Phosphodiesterase-5 Inhibition to Improve Quality of Life and Exercise Capacity in Diastolic Heart Failure [RELAX]) are nearing completion, while others are about to start. This review highlights key work from the past 1–2 years that helped clarify the nature of PDE5 myocardial regulation, review the clinical trials, and discuss remaining controversies and how they might ultimately be resolved. Discussion of the other cGMP-PDEs is limited given the paucity of in vivo data.

PDE5 Molecular Biology and Regulation

PDE5A is encoded by a single gene, expressed as three splice variants (*PDE5A1-A3*) that vary in their N-termini; functional differences have not been identified. Its expression is particularly robust in vascular smooth muscle in the lung and corpus cavernosum, but is much less in systemic arteries, and even lower in the heart. Expression in cardiomyocytes in animals and humans has been reported by multiple laboratories [21–26], and while levels are low in normal hearts, they can markedly rise in heart disease [13, 21–23, 25], which may reflect oxidative stress [22]. Both the specificity of immune-reactive assays for histologic and protein assays as well as functional selectivity of inhibitors such as sildenafil for PDE5 in myocytes have been supported by gene-silencing studies [27].

PDE5A is post-translationally activated by cGMP binding to GAF regulatory domains in the N-terminus, resulting in a configuration changes to stimulate catalytic activity against cGMP by the C-terminus [28•, 29]. PDE5A is also phosphorylated by PKG (S102, human, S92 mouse), which both increases cGMP binding to GAF-A and enhances cGMP hydrolytic activity [30]. This negative feedback loop can convert to a positive one when a competitive inhibitor such as sildenafil is applied. Sildenafil acts as a false substrate with tenfold higher binding affinity at the catalytic site than cGMP. As both cGMP levels and consequently PKG activity rise, PDE5 is further triggered to hydrolyze cGMP, but this will only enhance competitive binding for the false substrate instead. None of the PDE5 inhibitors bind to the regulatory GAF domain.

PDE5 expression in normal cardiac myocytes localizes in a striated banding pattern that colocalizes with α -actinin in the z-disk [18, 22]. In this localization, PDE5 hydrolyzes a cGMP pool generated by sGC under control of NO stimulation [18], but does not impact NP-stimulated cGMP pools [31]. Inhibition of PDE5 augments nitric oxide synthase (NOS)–derived cGMP, in turn activating PKG (principally PKG1a) to phosphorylate intracellular targets. The impact of the latter depends upon the stress involved. For β -adrenergic stimulation, activation of PKG by PDE5 inhibition results in the phosphorylation of troponin I that reduces myofilament sensitivity to calcium to counter cAMP-mediated contraction [32]. This effect is absent in hearts lacking NOS-3 or with chronic NOS inhibition, and is not triggered by NP-stimulated cGMP (with or without PDE5 inhibition), highlighting compartmentation. PDE5 inhibitor–mediated PKG activation recently was linked to improved diastolic compliance in a model of hypertensive hypertrophy, linked to titin phosphorylation [33].

PDE5 and Myocardial Disease: Experimental Studies

Over the past 10 years, studies have explored the potential for PDE5 regulation to participate serve as a therapeutic target in a broad range of myocardial diseases. Initial work focused on ischemia/reperfusion injury and cardioprotection (see Kukreja et al. [34••] for recent review). In this condition, PDE5 inhibition stimulates PKG to activate extracellular response kinase (ERK1/2) that in turn activates glycogen synthase kinase 3 β resulting in cytoprotection due to mitochondrial K_{ATP} channel opening and stimulation of Bcl-2 [14]. Mitochondrial-dependent protection is also thought to be important to PDE5-inhibitor

benefits in countering doxorubicin toxicity [35]. Importantly, cardioprotection is observed without interference with the chemotherapeutic efficacy of doxorubicin. PDE5 inhibition also induces preconditioning against ischemic injury via similar mechanisms [36]. Sustained PDE5 inhibition has been used to counter chronic hypoxia, resulting in enhanced tolerance to ischemia/reperfusion (I/R) injury in the heart via a mechanism coupled to enhanced NOS3 and Akt activation [37]. In vitro preconditioning of adipose tissue-derived stem cells with sildenafil or gene-targeted PDE5 knockdown recently was found to enhance cell engraftment in infarcted ventricles, improving chamber function, reducing fibrosis, and enhancing vascular density [38]. In this sense, the effect likely mediated via PKG activity may be similar to other proposed methods to enhance stem cell replication and survival, such as the upregulation of Pim-1 [39].

PDE5 modulation of left ventricular hypertrophy involves chronic stimuli, and again, PKG activation appears the primary effector. One target is suppression of Gq-protein-coupled receptor signaling by PKG activation of regulator of G-coupled signaling 2 and 4 (RGS2/4) [40, 41••]. RGS2 and RGS4 are guanosine triphosphatase (GTP-ase) accelerators that catalyze restoration of the G-protein heterotrimer (inactive form) to reverse receptor-agonism. Recent studies have shown PKG also directly phosphorylates a member of the transient potential receptor canonical channel family, TRPC6 [42•, 43•]. In studies in both vascular and cardiac muscle, PKG activation by NP or PDE5 inhibition resulted in TRPC6 phosphorylation, suppressing this nonselective ion channel's Ca^{2+} conductance. This in turn led to a decline in Ca^{2+} -calmodulin-dependent calcineurin activation, blunting activation of nuclear factor of activated T-cells (NFAT), and thus, hypertrophy. Hypertrophic stimulation in myocytes expressing mutated forms of TRPC6 in which PKG-targeted sites are silenced is enhanced, whereas a phosphomimetic form blunts this signaling [43•]. Another target is the small GTP-binding protein RhoA, which is a PKG substrate. Phosphorylation reduces RhoA activation for Rho-kinase, and this too appears to contribute to antihypertrophic efficacy [44].

Beneficial effects on the dilated – volume overloaded – failing heart have been less studied, but new evidence supports utility here as well. Using a rat mitral regurgitation model, chronic sildenafil treatment led to smaller left ventricles and enhanced systolic function, reduced myocardial fibrosis and apoptosis, and improved exercise capacity [45].

The central role of myocyte PKG activation to stress-remodeling, including that linked to PDE5 regulation, was recently questioned [46], but two studies using genetic loss-of-function models support this role. In one, Zhang et al. [47••] generated mice with tetracycline-sensitive myocyte-targeted PDE5 expression and showed that upregulation of myocyte PDE5 depressed PKG activity and in turn worsened maladaptive responses to pressure overload. They further showed that upon induction, lowering myocyte PDE5 expression, and thereby raising PKG activity, subsequently reversed hypertrophy/dysfunction. Intriguingly, manipulation of solely myocyte PDE5, and thereby PKG, impacted not only myocyte function and hypertrophy, but also interstitial fibrosis. In 2012, Kuhn and colleagues [48] studied mice with myocyte-targeted PKG1 α/β knockdown, and also reported worse cardiac remodeling and fibrosis in hearts subjected to pressure overload or angiotensin-II infusion.

Another controversy developed around the selectivity of drug treatments for PDE5, as sildenafil's IC_{50} for PDE1 is only about 200x that for PDE5, and fewer trials have used tadalafil (> 1000x selective for PDE5). Given that PDE1 is the more highly expressed PDE in heart and cell-free in vitro data shows it underlies a majority of cGMP-esterase activity, the concern for a potential “off target” deserves attention [49]. Though in vitro gene-deletion studies have supported specificity and selectivity for PDE5, attempts to generate in vivo

PDE5 knockout models have been repeatedly unsuccessful. However, a new study [50] used small interfering RNA (siRNA) against PDE5 delivered intramyocardially by adenovirus and found improved cardiac remodeling postinfarction, with enhanced cGMP/PKG signaling. Furthermore, studies have utilized the more selective tadalafil reporting similar efficacy. Importantly, PDE1 also hydrolyzes cAMP, and the relative role of this function over cGMP regulation in vivo and in the diseased as well as normal heart remains unknown.

One question raised by the more recent research is why PDE5 regulation appears meaningful in the heart, whereas prior studies found expression and physiologic impact to be negligible. A major factor is the condition under study. Resting cGMP generation, corresponding PKG activity, and PDE5 expression in the normal heart are all very low, and this was primarily the setting in the prior work. PDE5 would have minimal impact in this condition. However, with sustained cardiac stress, cGMP rises in response to NP stimuli, and equally importantly, PDE5 expression also increases. As a result, inhibiting PDE5 can more greatly enhance cGMP and, in turn, activate PKG. Enhanced PDE5 expression/activity in experimental models is also observed [13] and has been linked to oxidative stress [22], and recent data have confirmed upregulation in multiple studies of human heart failure and hypertrophy [26, 51•, 52].

PDE5 and Human Heart Disease

One of the notable features of research into PDE5 and cardiac disease is that translation of experimental findings to the clinic could be accelerated by the pre-existence of multiple highly selective orally active inhibitors. Starting with studies in the mid 2000s, investigators such as Semigran [53, 54] in Boston and Guazzi [55, 56, 57••] in Milan began reporting on the efficacy of short-term sildenafil on exercise capacity and pulmonary vascular pressures in patients with dilated cardiomyopathy. The impact on the pulmonary vasculature in patients with secondary pulmonary artery hypertension (PAH) has been striking, with far greater reductions in pressure and resistance than observed with primary PAH. Exercise improvement coupled to longer-term sildenafil therapy has now been reported by several groups and linked to enhancement of systemic microvascular perfusion in skeletal muscle as well as improved ventilation [55, 58].

Two new studies have provided evidence that chronic PDE5 inhibition can also impact the left ventricle. In one, Guazzi and colleagues [59] studied 45 patients with dilated heart failure, providing them with sildenafil (50 mg 3x/d) or placebo for 12 months. Left ventricular function assessed by echo Doppler was slightly but significantly improved with active therapy without concomitant changes in systemic arterial pressure. Giannetta et al. [60] performed a study in 59 diabetic men with evidence of modest hypertrophy but no systolic dysfunction. Three months of sildenafil therapy improved cardiac function (torsion and strain), and reduced the mass/volume ratio over placebo. Intriguingly, transforming growth factor beta (TGF β) markers were also improved, something we previously observed in our mouse hypertrophy studies. Other new experimental data have identified enhanced antioxidant protein such as glutathione S-transferase Kappa-1 with attenuation of the oxidized to reduced glutathione ratio in a mouse model of diabetes (db/db) [61]. The potential use of PDE5 inhibition to reverse remodel hypertrophied ventricles due to aortic stenosis was recently tested in a single-dose safety trial [62]. Here, 20 patients with compensated aortic stenosis (AS), with a mean ejection fraction (EF) of 60%, received a single oral dose of 40 or 80 mg sildenafil. The drug was well tolerated; systemic pressure changes were small (11%), and wedge pressure declined by nearly 20%.

Exploration of the clinical utility of PDE5 inhibition has not been restricted to acquired heart disease, but also has been recently examined in congenital heart disease. In a study of 27

children or young adults (mean age 15 years) referred for the Fontan procedure, Goldberg et al. [63] administered sildenafil (20 mg tid) for 6 weeks and documented improvement in myocardial function and estimated cardiac output. Another study examined 28 adult patients with Eisenmenger syndrome and showed 6-week treatment with tadalafil reduced pulmonary but unaltered systemic resistance, and enhanced exercise capacity.

There are two multicenter trials of PDE5 inhibition in heart failure funded by the National Institutes of Health. One is the RELAX trial, which is testing 6 months of therapy in patients with heart failure with preserved ejection fraction. The primary outcome is an improvement in exercise function (metabolic stress testing), but various secondary neurohormonal and cardiac function/structure end points also will be evaluated. The study completed enrollment in March 2012, and results are due later this year. Another NIH multicenter trial, PITCH-HF, was recently initiated, and will study tadalafil in patients with dilated HF. These trials will more robustly test the efficacy of PDE5 inhibitors in larger patient populations at multiple centers.

Other PDEs to Consider?

As noted, cardiac cGMP-PDE activity is dominated by PDE1, not PDE5, and the role of PDE1 may well be important. There is some controversy over the precise isoform involved (PDE1a or PDE1c), but chronic data using small molecule or genetic inhibition of either remain lacking. PDE1 inhibitors have been tested in cell studies in vitro, where they blunt hypertrophic signaling [64] and fibroblast collagen synthesis [65]. A short-duration administration model found one inhibitor, IC86340, blocked isoproterenol hyperstimulation [64]. Interestingly, inhibition of PDE1 and PDE5 appears additive, suggesting they target different cGMP pools. A potential limitation to clinical use of PDE1 inhibitors are its regulation of vascular tone and heart rate, and impact on vascular remodeling (reviewed by Chan and Yan [66]). Thus, clinical responses may be a bit more complex and vary with the disease.

Conclusion

Less than 10 years ago, PDE5 inhibitors were well established for erectile dysfunction, Viagra became among if not the most widely recognized drug name worldwide, and few had considered the heart as a second potential target. Today, re-purposing of this drug class for heart disease seems ever more feasible. Clearly, definitive clinical trials are needed, as is research to better understand the biology of this and other cGMP regulators. If proven successful, the clinical utility of PDE5-inhibition to heart failure would reflect a rare example of mouse-to-man translation, and importantly provide a new approach to brake maladaptive remodeling, this time from inside the cell.

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Fig. 1.

Cardiac myocyte signaling coupled to phosphodiesterase (PDE) 5. Cyclic GMP (cGMP) is generated by two mechanisms: a nitric oxide synthase (NOS)-soluble guanylate cyclase (sGC) coupled pathway, and membrane natriuretic receptor-guanylate cyclase (NPR-A/B, rGC) pathway. These two pools of cGMP interact with different effectors – namely other PDEs that regulate both cGMP and cyclic adenosine monophosphate (cAMP), and protein kinase G (PKG). PDE5 is a cGMP-selective PDE that is also activated by cGMP binding to the enzyme as well as PKG phosphorylation of the enzyme. sGC-generated cGMP can both augment and reduce cAMP in the cell by inhibiting PDE3 and activating PDE2, respectively. Inhibiting PDE5 also augments cGMP from this pool, and while this has been proposed to modulate PDE3 in the right ventricle [58], this has not been observed for the left ventricle. PDE5-modulated cGMP pools do not appear to impact cAMP-dependent signaling in normal adult myocytes [23]. Natriuretic peptide receptor (NPR)-coupled cGMP does not normally interact with PDE5; indeed the precise PDE responsible for modulating this pool remains unclear.

Cardiac stress regulation by PDE5 inhibition is primarily controlled by PKG targeting, and PDE5 inhibition enhances PKG signaling to stimulate these pathways. Shown on the left side are sarcomere proteins, sarcoplasmic reticulum (SR) calcium handling proteins (PLB [phospholamban]), mitochondrial signaling involving ATP-sensitive potassium channels (K_{ATP}), mitogen activated kinases (MAPK), calcineurin (Cn), Rho-activated kinase (ROCK), protein kinase Ca (PKCα) their regulation of nuclear transcription factors (nuclear factor of activated T-cells [NFAT], myocyte enhancer factor-2 [MEF2], SRF, and GATA4). Upstream, these activators are blunted by PKG due to direct inhibition of transient receptor potential channel 6 (TRPC6), and regulator of G-coupled signaling 2 and –4 (RGS2/4) coupled inhibition of Gq-coupled receptors.

At1 angiotensin I; *Et1* endothelin 1; *α-AR* α-adrenergic receptor).