Cardiovascular Research 75 (2007) 303–314

Cardiovascular Research

www.elsevier.com/locate/cardiores

Review

Phosphodiesterase regulation of nitric oxide signaling

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Received 6 December 2006; received in revised form 23 February 2007; accepted 27 February 2007
Available online 2 March 2007
Time for primary review 39 days

Abstract

Nitric oxide regulation of the cardiovascular system involves both cGMP-dependent and independent mechanisms. The former directly interacts with the family of catabolic phosphodiesterases (PDEs) that control cGMP levels and thus distal effects such as protein kinase G stimulation. Growing evidence supports an important role of several PDEs, including PDE1, PDE2, and PDE5, in the regulation of cGMP in both vascular smooth muscle and cardiac myocytes. These PDEs have relatively little impact on resting function, but they can potently modulate acute contractile tone in cells stimulated by external agonists such as angiotensin or catecholamines. Regulation by PDEs is compartmentalized, with selective interactions occurring between a given source of cGMP and PDE hydrolysis. PDE1 and/or PDE5 are also reportedly up-regulated in chronic disease conditions such as atherosclerosis or cardiac pressure-load stress and heart failure as well as in response to long-term exposure to nitrates. Such up-regulation is thought to contribute to vascular and cardiac pathophysiology and to drug tolerance. Recent studies utilizing selective PDE5 inhibitors support significant cross-signaling with NO–cGMP synthetic pathways that may be particularly helpful in treating certain disease states.

Keywords: Cyclic nucleotides; Protein kinase G; cGMP; Sildenafil; Heart function

1. Introduction

Cardiovascular modulation by nitric oxide (NO) can be divided into two primary mechanisms. One depends upon NO activation of soluble guanylate cyclase (sGC) and the subsequent generation of cyclic guanosine monophosphate (cGMP), while the other is cGMP-independent and involves protein *S*-nitrosylation or nitration (reviewed in [1,2]). To a great extent, the balance between these pathways depends upon redox status that influences net NO chemistry and NO and cGMP synthetic capacity by NOS and sGC, respectively [3]. Once synthesized, cGMP regulates cellular function by binding to allosteric sites in cyclic nucleotide phosphodiesterases influencing their activity, and by stimulating protein kinase G (PKG, also cGK) [4]. By its phosphorylation of channels, receptors, kinases, and phosphatases [5], PKG serves as a primary modulator of vascular tone, and plays a key role in cell survival, endothelial permeability, and vascular homeostasis and proliferation. In the heart, PKG regulates contractile function [4], and serves as a brake to counter both acute and chronic stress responses and cardiac remodeling [6,7].

The importance of cGMP to NO signaling has naturally led to research on the catabolic enzymes that control its fate once synthesized. These proteins are members of the 21-gene family of phosphodiesterases which have been grouped into 11 different primary isoenzymes (with a total of 48 isoforms) based on substrate affinity, selectivity, and regulation mechanisms (Table 1). Of these enzymes, PDE5, PDE6, and PDE9 are highly selective for cGMP, PDE1, PDE2, and PDE11 have dual substrate affinity, and PDE3 and PDE10 are cGMP-sensitive but cAMP-selective. In the cardiovascular system, the primary cGMP–PDEs with known activity are PDE1, PDE2, and PDE5. PDE1 is a Ca^{2+}/calmodulin dependent enzyme, PDE2, a cGMP-stimulated cAMP esterase that can also hydrolyze cGMP, and PDE5 the first identified selective

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doi:10.1016/j.cardiores.2007.02.031

Table 1
The family of PDEs, their substrate specificity, tissue expression, and specific inhibitors (if known)

PDE isoenzyme	Contains GAF domain	Substrate	Km (μM), cAMP	Km (μM), GMP	Tissue expression	Specific inhibitors
1	No	Ca^{2+}/calmodulin-stimulated	1–30	3	Heart, brain, lung, smooth muscle	KS-505a IC 86340
2	Yes	cGMP-stimulated	50	50	Adrenal gland, heart, lung, liver, platelets	EHNA BAY 60-7550
3	No	cGMP-inhibited, cAMP-selective	0.2	0.3	Heart, lung, liver, platelets, adipose tissue, inflammatory cells	Cilostamide, enoxamone, milrinone
4	No	cAMP-specific	4	–	Sertoli cells, kidney, brain, liver, lung, inflammatory cells	Rolipram, roflumilast, cilomilast
5	Yes	cGMP-specific	150	1	Lung, platelets, vascular smooth muscle, heart	Sildenafil, tadalafil, vardenafil
6	Yes	cGMP-specific		60	Photoreceptor	Dipyridamole
7	No	cAMP-specific, high-affinity	0.2	–	Skeletal muscle, heart, kidney, brain, pancreas, T lymphocytes	BRL-50481
8	No	cAMP-selective	0.06	–	Testes, eye, liver, skeletal muscle, heart, kidney, ovary, brain, T lymphocytes	None
9	No	cGMP-specific		0.17	Kidney, liver, lung, brain, ?heart	BAY 73-6691
10	Yes	cGNO-sensitive, cAMP-selective	0.05	3.0	Testes, brain	None
11	Yes	cGMP-sensitive, dual specificity	0.7	0.6	Skeletal muscle, prostate, kidney, liver, pituitary and salivary glands, testes	None

cGMP esterase. An additional cGMP-selective PDE9A was recently identified [8], with an isoform (PDE9A5) expressed at low levels in heart, though its role if any remains unknown. Lastly, cGMP can inhibit PDE3, a cAMP esterase expressed in heart and vascular tissue. Among these PDEs, PDE5 has been best studied due to the existence of highly selective inhibitors, and remains the only one of the family of PDEs for which targeted inhibitors are FDA approved to treat a chronic clinical disease — erectile dysfunction and more recently pulmonary hypertension.

Fig. 1 summarizes the roles of PDEs in both vascular smooth muscle or cardiac myocytes. PDEs modulate acute stimulation cascades but can also be up-regulated in chronic conditions that may result in proliferative remodeling and desensitization to cGMP signaling. In this review, we focus on recent advances regarding the interplay between NO–cGMP synthesis and the regulation of this signaling by targeted PDEs within the cardiovascular system.

2. Modulating PDEs by NO–cGMP interaction

In addition to being hydrolyzed by PDEs, cGMP (or its target kinase PKG) can alter the catabolic activity of these enzymes for one or the other substrate (Fig. 2). A major mechanism is the binding of cGMP to tandem regulatory GAF domains, so named from the first three classes of proteins found to contain the cGMP-binding sequence (mammalian c*G*MP-binding PDEs, *Anabaena* adenylate cyclase, and *Escherichia coli F*h1A). Of the 11 member group, PDE2, 5, 6, 10, and 11 all contain such regulatory domains. Typically, binding of cGMP to a GAF domain induces a conformation change in the PDE to activate its catabolic activity for cGMP (auto-feedback mechanism — as in the case of PDE5), or for the alternative cAMP (cross-regulation mechanism, as in the case for PDE2). In ventricular myocytes, inhibition of PDE2 by *erythro*-9-(2-hydroxy-3-nonyl) adenine (EHNA) has minimal effect on basal or cAMP-stimulated L-type Ca^{2+} currents, but prevents suppression of cAMP-stimulated current by NO. A key component of the regulation appears to be mediated by the β3 adrenergic receptor [6,9], which is co-activated by the sympathetic stimulus but triggers NO and thus cGMP which in turn activates cAMP hydrolysis by PDE2. Bypassing the receptor and directly stimulating adenyl cyclase (forskolin) induces inotropy that is unaltered by PDE2 inhibition, suggesting PDE2 regulation is specifically coupled with the β-receptor. Similar effects of PDE2 on cGMP/cAMP regulation have been reported in cardiac fibroblasts [10] where NO enhanced by a donor or iNOS stimulation reduced cAMP accumulation; an effect reversed by EHNA. PDE5 has two GAF binding sites and in this instance, cGMP binding stimulates cGMP hydrolysis [11,12].

Another mechanism involves PDE phosphorylation by PKG. For PDE5, GAF binding enhances phosphorylation at Ser-92 and at rates ~10× greater for PKG than PKA [13,14]. PKG phosphorylation in turn induces positive feedback conformational changes increasing cGMP-binding affinity in the regulatory GAF domain, and enhancing cGMP catalytic activity by 50–70% [15]. Allosteric binding of cGMP to PDE5 also may serve as an intracellular storage site, providing safe harbor from catalysis and potentially releasing the pool when cGMP levels decline due to other signaling events [16,17].

A third type of functional modulation by cGMP occurs at the catalytic site. PDE3 binds both cAMP and cGMP at its catalytic site at high affinity and similar Km (see Table 1), but has a V_{max} for cAMP that is 10 times greater which confers catalytic specificity. However, cGMP can function as a competitive inhibitor of PDE3 hydrolysis [18], and at low levels of cGMP, the effect is to enhance cAMP and its downstream effects. For cardiac myocytes, this results in increased L-type Ca^{2+} current [19,20] and contractility [20–22]. This is opposite to what is observed at higher levels of cGMP

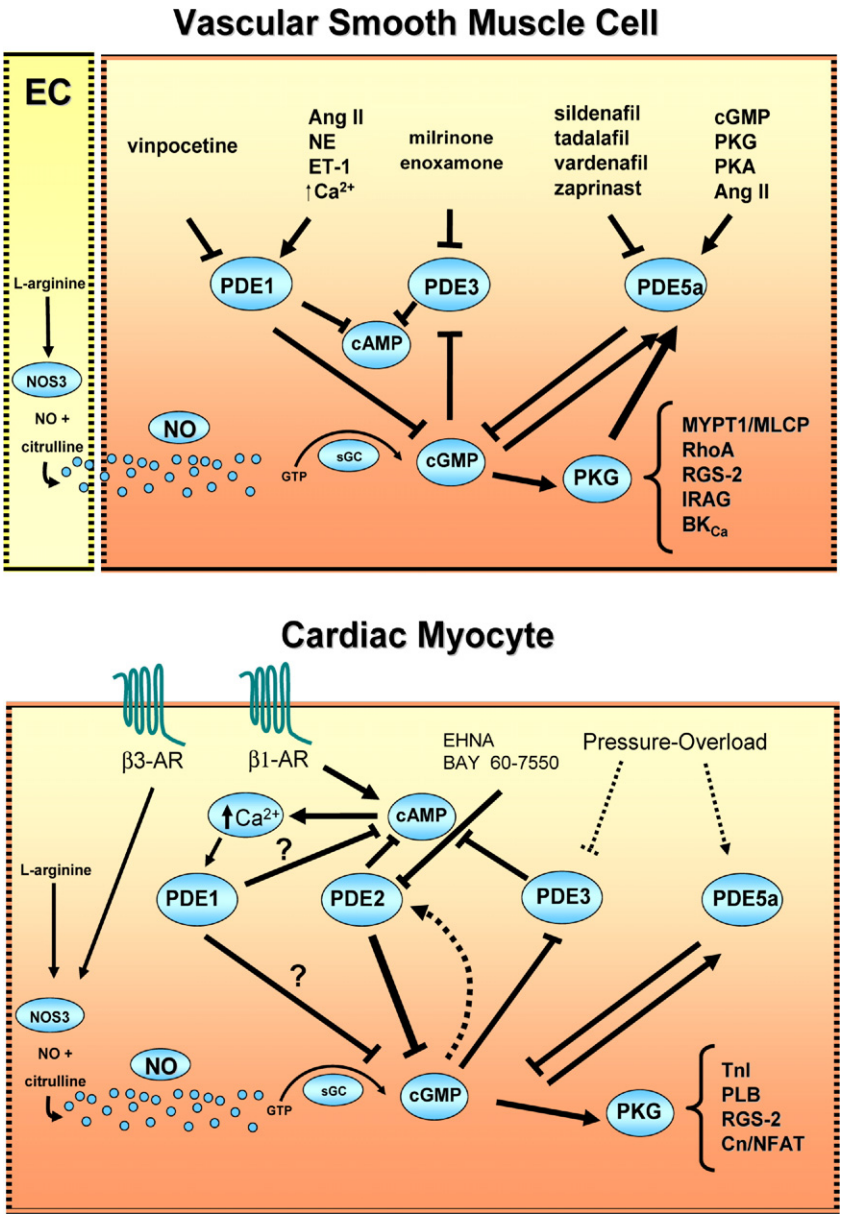

Fig. 1. Schematics of PDE regulation of NOS–NO generated cGMP in a vascular smooth muscle cell and cardiac myocyte. Small molecule inhibitors and signal-activators for each PDE are shown at the top. *Smooth muscle cell*: NOS3-derived NO diffuses from neighboring endothelial cells (EC) interacting with soluble guanylate cyclase (cGC) to convert GTP to cGMP. cGMP can be hydrolyzed by PDE1 in the presence of Ca^{2+}/calmodulin (Ca/CM) stimulation, and may also hydrolyze cAMP. PDE3 hydrolyzes primarily cAMP, but this can be inhibited competitively by cGMP. PDE5 selectively hydrolyzes cGMP, and cGMP and its distal effecter kinase — protein kinase G (PKG) also activate the enzyme. Activation of PKG results in phosphorylation of myosin light chain phosphatases (MLCP), RhoA, regulator of g-protein signaling (RGS-2), inositol 1,4,5-trisphosphate receptor-associated PKG substrate; IRAG, and calcium-sensitive potassium channels (BKc_a) that serve to reduce smooth muscle tone. *Cardiac myocyte*: NO possibly derived from several intracellular NOS isoforms (eNOS being coupled to β3 adrenergic receptors) stimulates cGMP synthesis. PDE regulation is similar to that in vascular smooth muscle, with the addition of PDE2, which increases cAMP hydrolysis when stimulated by cGMP, but may also hydrolyze cGMP. Agonists and small molecule inhibitors would be similar for those shown in the upper panel. Chronic stimulation by pressure over-load lowers PDE3 but increases PDE5 activity to alter the balance of cAMP/cGMP regulation.

stimulation, which blunts contractility coupled to the activation of PKG and subsequent phosphorylation of troponin I [23,24].

3. Modulating NO–cGMP signaling by PDEs

In the cardiovascular system, cGMP hydrolysis is thought to be accomplished by PDE1, PDE2, and PDE5. PDE1 contains an auto-inhibitory domain which maintains low activity in the absence of Ca^{2+}, and neighboring calmodulin binding domains that restore full activation in the presence of Ca^{2+}-calmodulin [25]. An intriguing feature of PDE1 is its activation by specific Ca^{2+} pools entering from the extracellular space [26] suggesting compartmentation, though this remains to be clarified in vascular smooth muscle or cardiomyocytes. PDE1 has three primary isoforms (a, b, c) that are

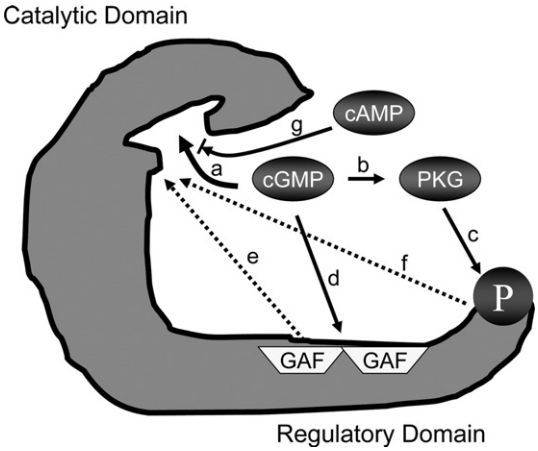

Fig. 2. Schematic of mechanisms by which cGMP/PKG interacts with a PDE to influence its catalytic activity. Diagram shows PDE with GAF binding domains, such as PDE2 or PDE5. (a) cGMP can interact with the PDE in the catalytic domain to undergo hydrolysis to GMP; (b) cGMP can activate PKG which in turn can phosphorylate (c) the N-terminus to (f) enhance catalytic activity; (d) cGMP can bind to regulatory GAF domains that facilitate phosphorylation by PKG and (e) also enhance PDE catalytic activity; and (g) cGMP binding at the catalytic site can impact PDE hydrolysis for cAMP (as the case for PDE3).

all expressed in vascular smooth muscle. It is stimulated by norepinephrine, angiotensin II, and endothelin-1 by their elevation of intracellular calcium, and this serves to lower cGMP levels and augment vasoconstriction [27]. Inhibition of PDE1 with vinpocetine, an often used but not very specific inhibitor, has little effect on basal cGMP or cAMP in pulmonary vascular tissue, but enhances NO-stimulated dilation suggesting an interaction with NO-derived cGMP [28]. Chronic up-regulation of PDE1 has been associated with nitrate tolerance [29] and vascular proliferation [30,31]. Although PDE1 is strongly expressed in the heart [32], its physiologic role and indeed even precise cell of origin remains unclear [33]. Lack of genetic models targeting PDE1 as well as selective inhibitors has limited such research to date.

PDE2 is not a primary PDE in vascular smooth muscle, but is expressed in cardiac myocytes, and recent data supports its role in the targeted regulation of cGMP and cAMP. In rat myocytes, cGMP synthesis was assayed by a sarcolemmal membrane targeted olfactory cGMP-gated channel current [34]. The current was enhanced more by natriuretic peptide (NP) than NO donors, and PDE2 inhibition potentiated this current with both stimuli. However, the functional role of this modulation remains unknown, as PDE2 inhibition alone has little influence on resting myocyte contraction [35]. It is also unclear if PDE2 preferentially catabolizes cGMP or cAMP (or both simultaneously), and under what conditions. Though PDE2 hydrolyzes cGMP when this is the primary substrate [34], it appears to target cAMP when there is co-generation of cGMP and cAMP [35]. The development of novel and selective PDE2 inhibitors, such as BAY 60-7550 [34], and advances in fluorescent imaging methods may help resolve some of these questions.

PDE5 was first identified as a cGMP-binding protein in lung tissue, and only later was it revealed to have cGMP-hydrolytic activity. It has since been shown to play a key role in vascular smooth muscle tone particularly in the venous system of the corpus cavernosum and the pulmonary vasculature. Protein expression and activity are also well documented in the cerebellum, stomach, small and large intestine, bladder, and platelets [36]. Early physiologic studies explored the role of PDE5 using the inhibitor zaprinast; which also has significant cross-reactivity with PDE1 as well. However, in the late 1980's, highly selective and potent PDE5 inhibitors such as sildenafil and tadalafil were developed, and this greatly improved our understanding of this PDE.

PDE5 appears to interact closely with NO–sGC generated cGMP as the effectiveness of PDE5 inhibitors are generally blocked by NOS inhibitors such as L-NAME. While hypoxia-induced pulmonary hypertension is substantially ablated by sildenafil, the drug has little effect in mice lacking NOS3 (eNOS) [37]. Pharmacologic inhibition of NOS suppresses the vasodilator effects of PDE5 *in vitro* [38,39] and *in vivo* [40], and PDE5 [40] inhibitors are less effective in disorders associated with reduced NOS activity, such as diabetes [41]. In experimental diabetic rats, the blunted improvement of erectile function by sildenafil is restored by eNOS gene transfer into the corpus cavernosum [41]. As already noted, auto-activation of PDE5 by cGMP generated by NO plays an important feedback role in NO signaling. For example, exogenous administration of NO to both platelets or aortic tissue triggers an initial marked rise and then rapid decline in cellular cGMP, with the latter attributed to cGMP–PDE5 activation [42]. NO may also activate PDE5 by direct protein *S*-nitrosylation, though this remains to be verified.

4. PDE5 and the heart

The interplay between NO–sGC generated cGMP and PDE5 hydrolysis has recently been shown to be important in the heart as well. NO triggers differing responses in myocyte contraction and relaxation depending upon its concentration, intracellular redox, concomitant stimulation by agonists of cAMP, and other factors (reviewed in [3,43]). Its ability to suppress basal and β-adrenergic stimulated contraction, observed across many species including human [23,44,45], has been generally linked to its generation of cGMP [23,44]. There remains some controversy regarding the role of NOS (and NO) in adrenergic regulation, with some studies showing negligible effects [46] and others finding enhanced contraction [6,47] in mice lacking eNOS, the dominant isoform. Overall, however, most evidence supports a role of this pathway as a negative modulator of cardiac stress responses.

Given that blocking PDE5 hydrolysis should also increase cGMP, one might anticipate a similar result with agents such as sildenafil. However, initial studies examining myocardial PDE5 gene and protein expression [48,49] and enzyme activity found them to be quite low, leading to a conclusion that the enzyme was not physiologically important in the

heart [50–52]. Functional data suggested this too. For example, Cremers et al. [53] found no effect of 10 μM sildenafil (a relatively high dose) on either basal or isoprenaline stimulated function in human papillary muscle strips. This may in part have been due to the use of mostly end-stage failing heart tissue, as a canine study had found PDE5 inhibition failed to suppress dobutamine stimulated contractility in failing hearts, but did so in normal hearts [54]. Another functional study [52] reporting no effects examined tissue under non-stimulated conditions.

Subsequent investigations have found both detectable PDE5 expression and protein in isolated myocytes, and revealed direct cardiac effects induced by PDE5 inhibitors [6,54–56]. PDE5 inhibition has little effect on basal contractility, but suppresses acute β-adrenergic stimulation in dog [54], mouse [6], and human heart [57] (Fig. 3A). In mouse myocytes, this effect is unaccompanied by alterations in whole cell Ca^{2+} transients [6] consistent with reduced myofilament calcium sensitivity as seen with NO [23]. Others, however, found a PKG-dependent decline in L-type Ca^{2+}-channel currents, though the agent used, zaprinast, is a less specific inhibitor [58]. The anti-adrenergic effect of sildenafil is prevented by ODQ, an inhibitor of sGC [6], supporting a key role of NO–sGC generated cGMP, and by PKG inhibition (*i.e.* Rp-8br-PET-cGMPs) [59]. Among the potential mechanisms for the negative adrenergic effect troponin I phosphorylation at S23 and S24, which is observed with NO stimulation [23], results in myofilament Ca^{2+}-desensitization.

PDE5 inhibition has also been shown to suppress and reverse pressure over-load induced ventricular hypertrophy [7], attenuate apoptosis [60] and reduce post-ischemic dysfunction [61] in mice, and these effects have been observed at the level of the cardiac myocyte. Importantly, these effects also appear to be critically coupled to the nitric oxide synthase. In the case of cardioprotection to ischemia–reperfusion injury, studies have shown that this is coupled to the generation of NO by both eNOS and iNOS, working by the activation of protein kinase C and extracellular response kinase via opening of mitochondrial ATP-sensitive channels [60]. PDE5 inhibitors also attenuate cell death due to necrosis and apoptosis by an NO-stimulated increase in the ratio of Bcl2/Bax [61].

5. Specificity of PDE5 and NO–sGC interaction

As PDE5 hydrolyzes cGMP, and myocyte cGMP can be generated by both NO and natriuretic peptides, one can ask whether the source of cGMP matters. Recent studies performed in adult rat myocytes support selective interaction between PDE5 and NO-stimulated cGMP [34]. Functional

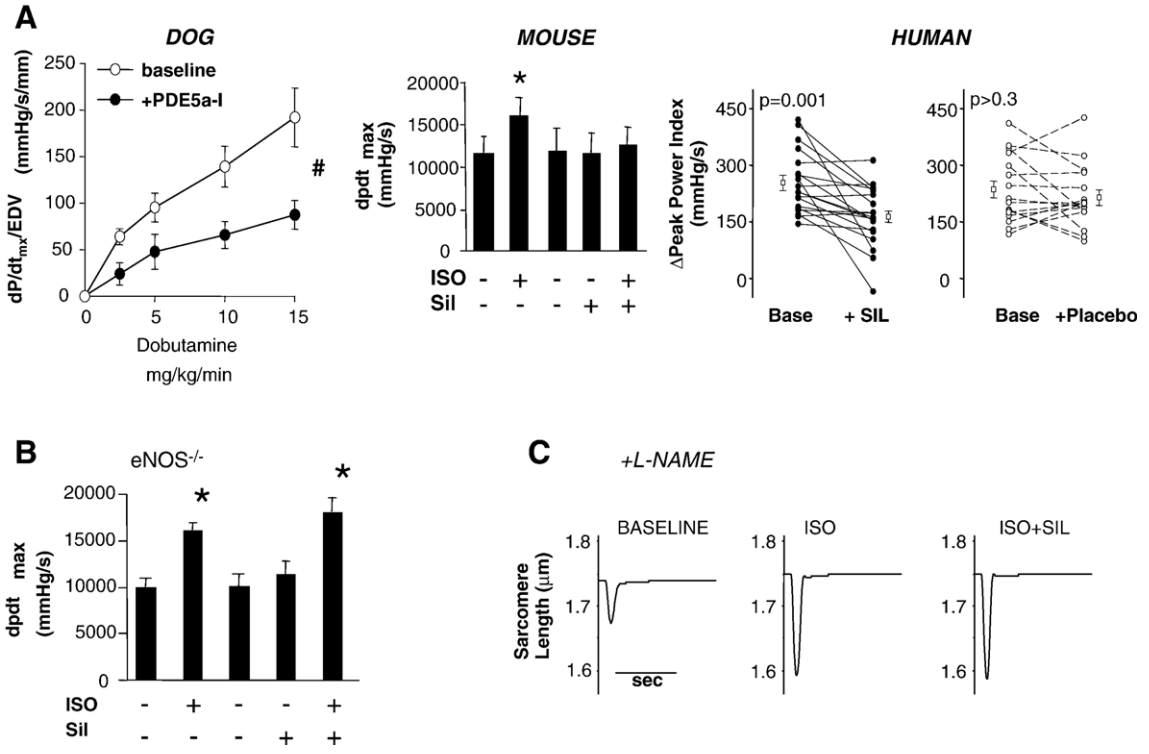

Fig. 3. A) Reduction of β-adrenergic stimulated contractility by selective PDE5a inhibitor in the dog (from Senzaki et al. [54]), mouse (from Takimoto et al. [6]), and human (from Borlaug et al. [57]). In dog, a dose response to dobutamine is shown and is blunted by a PDE5a inhibitor (EMD x), in mouse, the positive response to isoproterenol (ISO) is blunted, while in human, the change in maximal ventricular power index in response to dobutamine is shown in subjects before and after receiving either 100 mg sildenafil, or placebo. B) Suppression of β-adrenergic stimulation by sildenafil does not occur in mice lacking eNOS (from Takimoto [62]). C) β-adrenergic stimulation is not suppressed by sildenafil in primary isolated mouse myocytes pre-treated with 5 μM L-NAME to block NOS (modified from Takimoto et al. [59]).

evidence for a specific interaction between NOS-dependent cGMP generation and PDE5 modulation of myocardial β-adrenergic signaling has also been observed [6]. Mice lacking eNOS (NOS3) or those with NOS inhibited by L-NAME display no impact of sildenafil PDE5 inhibitors on cardiac chamber (Fig. 3B) or myocyte (Fig. 3C) β-adrenergic contractile stimulation. In preliminary studies, chronic catecholamine or pressure over-load induced hypertrophy in eNOS$^{-/-}$ mice was also found not to be blunted by PDE5 inhibition [62].

PDE5 does not appear to acutely alter cGMP generated by the NP-signaling pathway. In studies using the cGMP-channel reporter [34], NP-stimulated cGMP was unaltered by concomitant PDE5 inhibition. Unlike an NO donor or PDE5 inhibitor, administration of ANP alone does not blunt β-stimulated contractility (Fig. 4A), and even when combined with a PDE5 inhibitor, the anti-adrenergic effect is the same as with PDE5 inhibition alone [59]. Furthermore, loss of adrenergic modulation by PDE5 following acute NOS inhibition is not rescued by co-administration of ANP [59]. These results support separate compartments regulating acute NP versus NO-stimulated cGMP, with the latter being the target of PDE5 hydrolysis.

Intriguingly, both cGMP and PKG activation vary in opposite directions depending on the stimulation pathway. With NP stimulation, myocardial cGMP rises substantially, whereas it is little changed by PDE5 inhibition (alone or with concomitant β-stimulation) [59]. Activation of myocardial PKG follows the opposite pattern, rising with PDE5 inhibition but displaying little change with NP stimulation (Fig. 4B), and this, rather than the cGMP change, correlates with the anti-adrenergic effect. These findings strongly support compartmentalized signaling not only of cGMP generation and hydrolysis, but also of PKG activation and targeting. This likely underlies differential effects of NP versus NO and PDE5 inhibitor modulation of adrenergic stimulation. Whether such separation applies to chronic stimuli, or stimulation from other pathways and loading stress, remains to be determined.

6. PDE5 myocyte localization and role of NO

The observation that sildenafil has no anti-adrenergic effect in eNOS$^{-/-}$ mice is intriguing, in that gene and protein expression levels for PDE5, and *in vitro* PDE5 activity appear similar between controls and knock-out hearts [6]. Earlier studies in the failing canine heart first revealed that the myocyte localization of PDE5 was another feature that could be altered [54]. As shown in Fig. 5A, PDE5 normally appears in a banding pattern within the myocyte, but this becomes quite diffuse in cells from a failing heart which also

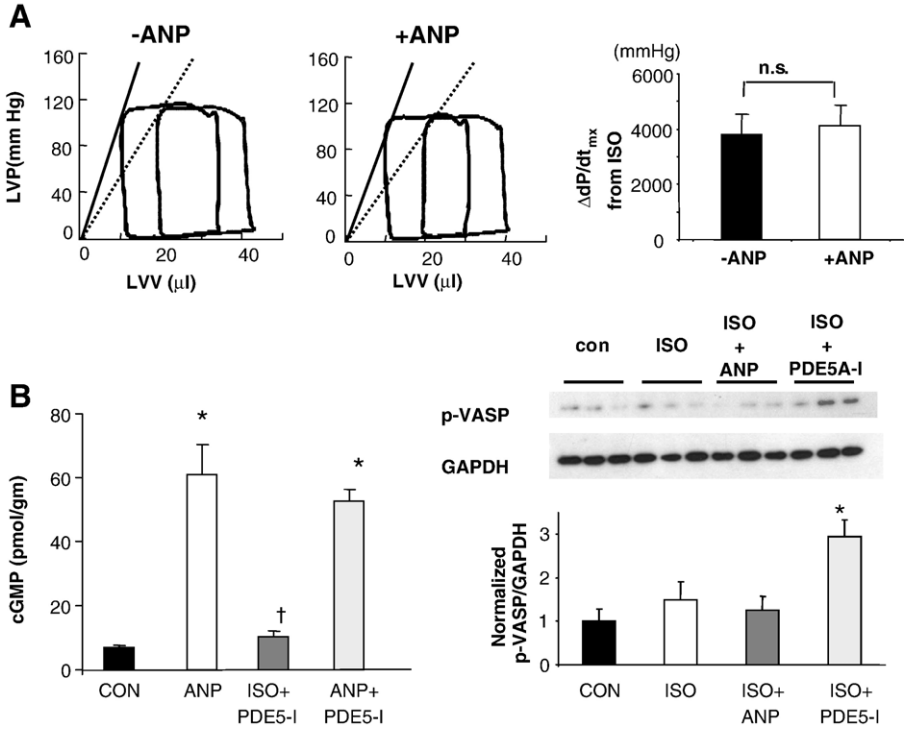

Fig. 4. A) A-type natriuretic peptide (ANP) does not suppress acute β-adrenergic stimulated contractility. *In vivo* LV pressure–volume loops and end-systolic PV relations are shown for a heart pre (-) and post (—) exposure to isoproterenol (ISO) in the presence or absence of ANP infusion. Increases in contractility were the same despite ANP stimulation. B) Left panel: Change in myocardial cGMP in response to ANP versus PDE5 inhibition. While PDE5 inhibition suppressed the ISO response and ANP did not, the latter was associated with a marked rise in measurable cGMP while the former led to very little change. Right panel: *In vivo* activation of PKG based on s239 phosphorylated vasodilator stimulator protein (VASP). pVASP increased with PDE5 inhibition, but was not altered by ANP. This is opposite to the changes observed in cGMP, but in a direction consistent with the regulation of β-adrenergic stimulation. From Takimoto et al. [59]. These data support strong compartmentation of cGMP/PKG signaling.

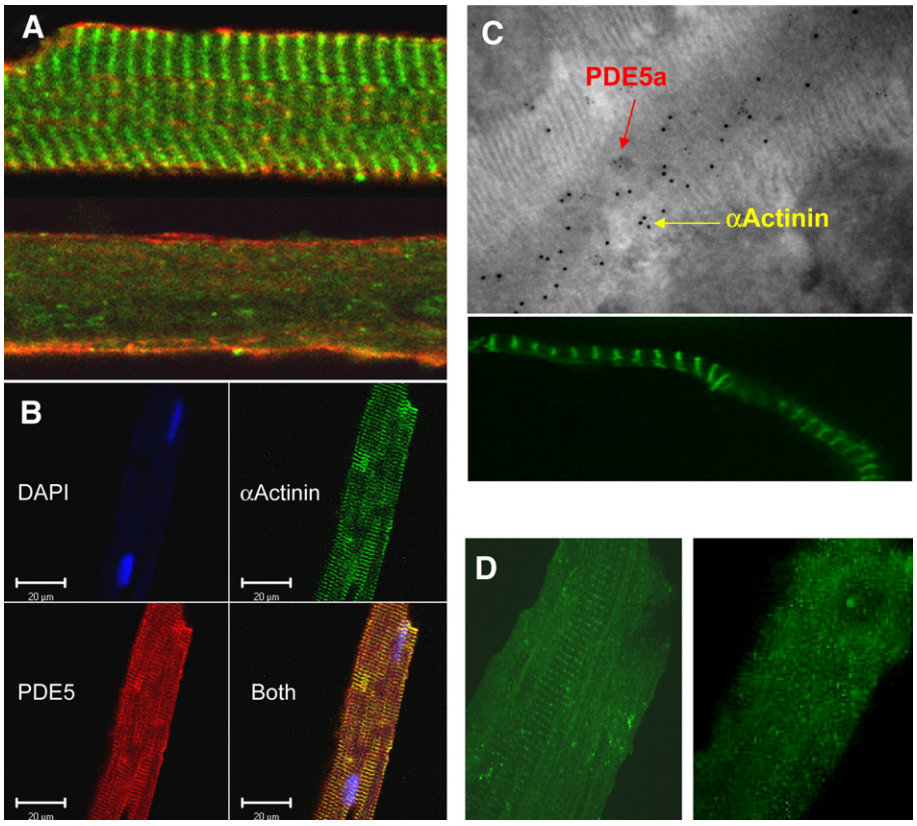

Fig. 5. A) PDE5a confocal fluorescent immuno-histochemistry shows striated pattern in normal myocyte from canine heart (upper), but a diffuse pattern in cells obtained form hearts with dilated cardiomyopathy in canine model (from Senzaki et al. [54]). B) Co-localization of PDE5 with α-actinin in adult myocyte from C57/Bl6 mouse. Nuclear staining (DAPI) is also shown. C) Localization of PDE5a (small particles) to z-band in the sarcomere revealed by immuno-electron microscopy, with larger particles co-staining for α-actinin. Lower panel shows isolated myofibril stained for PDE5a, revealing striated banding pattern. D) Diffuse PDE5a myocyte distribution in cells from mice genetically lacking eNOS (left), or animals exposed to NOS inhibition (L-NAME) for 2 weeks prior to cell isolation (right).

lacked PDE5 inhibitor anti-adrenergic effects. The functional role of the re-localization was unclear in the canine study, but better elucidated in subsequent studies contrasting mice with and without eNOS. Myocytes from normal mice also have PDE5 co-localized in a banding pattern with α-actinin (Fig. 5B), with further evidence that this is indeed in z-bands provided by staining of isolated myofibrils, and immuno-electron microscopy (Fig. 5C). In mice lacking eNOS or exposed to two weeks of L-NAME, this pattern also becomes diffuse (Fig. 5D), as in the failing canine heart. Importantly, localization was restored by re-expressing eNOS in the knock-out heart [6].

The physiologic importance of PDE5 z-band localization has been tested in two ways. In the gene-transfer study where eNOS was re-expressed, and PDE5 re-localized to z-bands, the anti-adrenergic effect of PDE5 inhibition also recovered [6]. In a second test, the capacity of an NO donor to circumvent absent or inactivated eNOS was tested (Fig. 6). Hearts with NOS acutely inhibited by L-NAME lose the anti-adrenergic effect from PDE5 inhibition, but localization of PDE5 within the cell is unaltered. In such hearts, pre-administration of an NO donor restored an anti-adrenergic effect. In contrast, mice lacking eNOS had both loss of activity and re-distributed

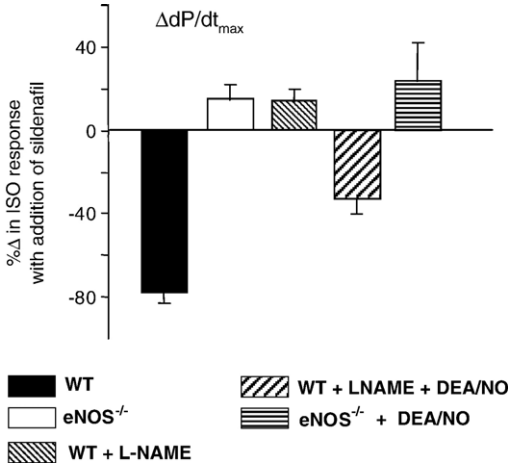

Fig. 6. Percent reduction of isoproterenol stimulated contractility by sildenafil in intact mouse hearts studied under various conditions. L-NAME was used to acutely inhibit NOS, which does not result in a change in the cellular localization of PDE5. Acute NOS inhibition prevented sildenafil from acting to suppress ISO-stimulated contractility, but this was partially reversed by an NO donor (DEA/NO). However, this did not restore anti-adrenergic activity of sildenafil in eNOS$^{-/-}$ hearts. Modified from Takimoto [62].

PDE5. In such hearts, co-administration of the NO donor failed to recover anti-adrenergic effects of sildenafil. Thus, localization of PDE5 to z-bands appears important at least for its capacity to regulate adrenergic contractile stimulation.

PKG binding sites have been revealed in the sarcomere which lends support to the notion that a cGMP generator and catabolic enzyme system may indeed be targeted to this substructure. TnT was shown to bind to a leucine zipper motif of PKG, and this binding is central to facilitating PKG phosphorylation of TnI, resulting in myofilament desensitization to calcium [63]. Little is known about G-kinase anchoring proteins — the presumed mirror of the family of A kinase-anchoring protein (AKAPs) that bind and locally target cAMP–PKA signaling [64]. Intriguingly, one report has suggested that myosin itself may be a GKAP [65]. Localized binding of PKG would logically couple to a local PDE, much as cAMP–PKA–PDE complexes are organized. To date, virtually nothing is known about how such targeting may occur, and in particular, how and why PDE5 moves from its location to influence its cellular regulatory potency. The obligate requirement for NOS-derived NO and/or cGMP suggests cGMP binding and/or PKG activation are key to this localization, perhaps by providing tonic enzyme activation *via* the regulatory domain. Ongoing research is attempting to elucidate these mechanisms.

7. PDE up-regulation: role in NO tolerance and disease pathophysiology

In addition to acute modulation of cardiovascular function, PDEs can be differentially regulated in chronic conditions and thereby contribute to pathophysiology. For example, PDE3 is down-regulated in failing and hypertrophied hearts, and the consequent increase in cAMP and its associated signaling is thought to worsen the pathophysiology [66]. Both PDE1 and PDE5 appear up-regulated in several chronic conditions and this also likely contributes to the disease. For example PDE1a (in rat) and PDE1c (in human) increase in smooth muscle cells in a proliferative state [30,31]. This is observed in neonatal and adult atherosclerotic vessels [30], and the suppression of PDE1 blunted the associated smooth muscle proliferation. Nagel et al. [31] recently showed PDE1A translocates to the nucleus in proliferating vascular smooth muscle cells, and this was central to its activation of cell growth. Prevention by RNA interference led to G1 arrest and induction of apoptosis in proliferating cells. In cells in a non-proliferative contractile state, PDE1a was cytosolic and its inhibition had no effect on apoptosis but altered its regulation of myosin light chain phosphorylation. These effects appeared primarily related to its hydrolysis of cGMP [31].

Another example of chronic PDE up-regulation is the response of both PDE1a and PDE5 to sustained cellular stimulation by NO resulting in NO tolerance. Rats exposed to 3 days of continuous nitroglycerin display a 2.3-fold rise in PDE1A1, and the accompanying nitrate tolerance is partially restored by vinpocetine, a PDE1 inhibitor [29]. Induction of both PDE1a [29] and PDE5a [67] by exposure to angiotensin II is thought to contribute to reduced cGMP/PKG signaling and be a mechanism for exacerbated hypertension and vascular proliferation. PDE5 activity is also enhanced by chronic nitrate exposure, and its inhibition reverses nitrate tolerance [68,69].

PDE5 up-regulation at expression and activity levels is also being observed in disease conditions such as pulmonary hypertension [70] and congestive heart failure [71], and is increasingly thought to underlie reduced responsiveness to NP or NO as well as an enhanced responsiveness to PDE5 inhibitors in affected tissues. A potential mechanism is that increased cGMP, perhaps from NP stimulation, activates the PDE5 promoter as a feedback mechanism [72,73]. PDE5 inhibitors are particularly potent in patients with pulmonary hypertension [74] and congestive heart failure [75], and this potency is consistent with up-regulation of PDE5 activity in central venous, pulmonary, and arterial vasculatures, and in the kidney (Fig. 7A) [71]. In addition to nitrate tolerance, PDE5 up-regulation appears to impact cGMP-stimulated signaling *via* NP pathways. Exposure to chronic PDE5 inhibitors improves acute renal responsiveness to NP in a canine model of cardiac failure [76]. While sildenafil has negligible effects on normal dogs, it exhibits similarly potent

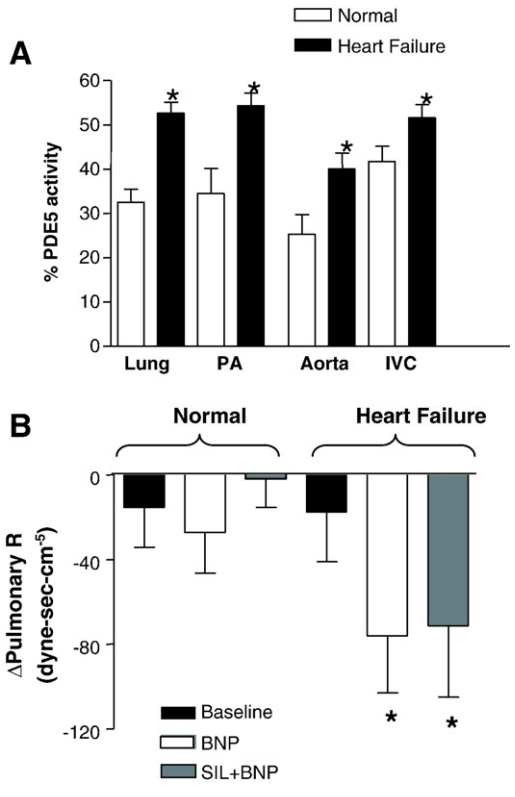

Fig. 7. A) Increased PDE5 activity in lung, pulmonary artery (PA), aorta, and inferior vena cava (IVC) in dogs with tachypacing-induced heart failure. B) Enhanced central pulmonary vasodilator activity of PDE5 inhibition (sildenafil) in dogs with heart failure versus controls. Change in pulmonary vascular resistance (PVR) is shown in response to BNP, sildenafil (SIL), and both combined. Modified from Forfia et al. [71].

pulmonary vasodilator effects to BNP in animals with heart failure, vasodilation, and enhances the impact of acute BNP stimulation (Fig. 7B) [71].

8. PDE5 and cardiac stress remodeling

Both NO–sGC–cGMP and NP–pGC–cGMP attenuate cardiac hypertrophy in neonatal myocytes [77]. Activation of PKG by NO and cGMP, or constitutively activated kinase suppresses cardiac myocyte hypertrophy in conjunction with inhibiting the calcineurin–NFAT pathway [78,79]. However, while ANP suppresses angiotensin II stimulated hypertrophy of neonatal myocytes, inhibiting PKG did not prevent this effect suggesting alternative pathways exist [80]. Genetic ablation of the A-type NP receptor or receptor guanylate cyclase produces modest cardiac hypertrophy at baseline and accelerates hypertrophic responses to pressure over-load [81–83]. NPRA null hearts have enhanced baseline calcineurin–NFAT activity and their spontaneous hypertrophy is blunted by inhibition of calcineurin with chronic FK506 treatment [84]. Cardiac over-expression of constitutively active receptor guanulate cyclase in myocytes prevents modest pressure-load induced hypertrophy [85].

In mice subjected to chronic pressure over-load, PDE5 activity increases, and its inhibition has been shown to attenuate cardiac hypertrophy despite sustained systolic loading. This occurs with improved cardiac function, reduced fibrosis, and the deactivation of multiple signaling cascades including calcineurin, MAP kinase, and Akt [7]. Unlike prior work with NP-dependent cascades, the hypertrophic stimulus employed in these studies was marked (>150% increase in LV mass, studied over a 9-week period), and the PDE5 inhibitor effect substantial. This suggests that more potent targeted signaling may be generated by preventing catabolism rather than enhancing cGMP synthesis.

Another intriguing target of PKG that may influence hypertrophy is the small GTP-binding protein RhoA and its activation of Rho-kinase (ROCK) (reviewed in [86]). Here, there are also potential different influences of NO versus ANP stimulation pathways. ROCK suppresses NOS activity by several mechanisms, including inhibiting its activation by Akt (*via* inhibition of phosphoinositol 3-kinase), reducing eNOS mRNA stability (an effect also imparted by activated RhoA), and enhancing oxidant stress to reduce NOS function [86]. Inhibition of RhoA with targeted inhibitors and statins improves eNOS activity [87–89], while NO *via* PKG stimulation inhibits RhoA activation by phosphorylation at S188 [90]. Thus, this pathway can both modulate NO signaling and be modulated by the same. Fig. 8 displays evidence for this cross-talk in chronically pressure over-loaded (TAC) hearts exposed to PDE5 inhibition. RhoA and ROCK2 were

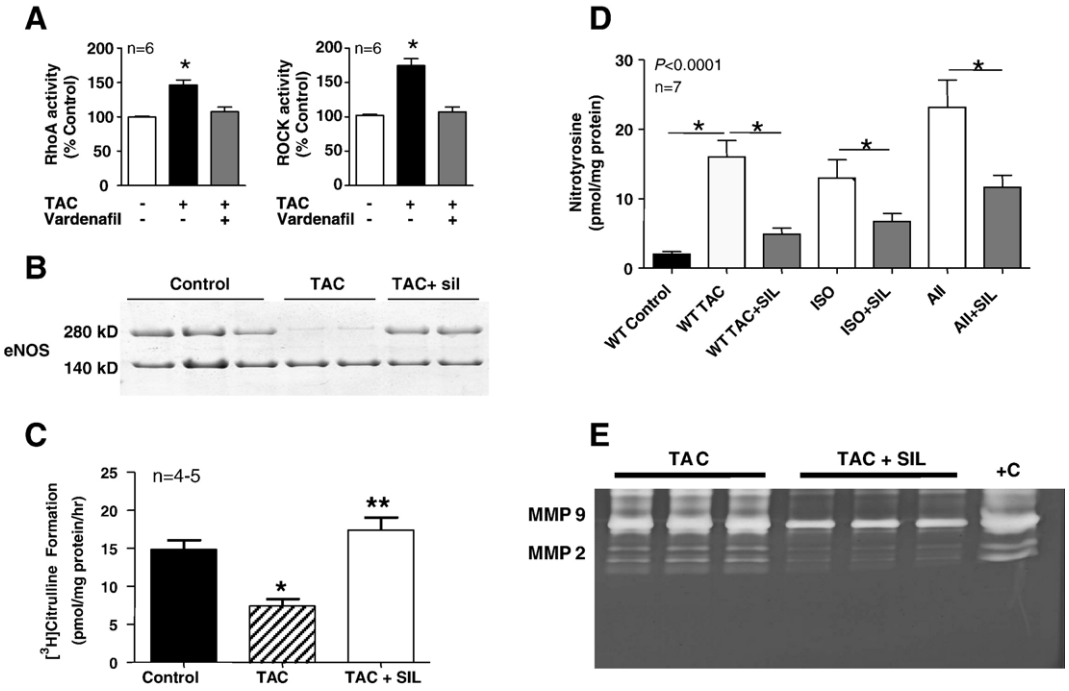

Fig. 8. A) PDE5 inhibitor, vardenafil, suppresses increase in RhoA and total Rho-kinase (ROCK) activity in myocardium exposed to chronic pressure over-load by trans-aortic constriction (TAC). Activity was assessed in whole heart lysates with a commercial colormetric assay (Cytoskeleton, Inc, Denver, CO and Cyclex, Nagano, Japan, respectively) performed using manufacturer's specifications. 96 well assay plates were read on a Spectramax M5 plate reader. B) TAC results in eNOS uncoupling demonstrated by reduction of dimer (280 kD). This is improved by co-treatment with the PDE5 inhibitor sildenafil (Sil). C) NOS activity assessed by arginine to citruline conversion is reduced by 3 weeks of TAC, and restored by co-treatment with SIL. D) Increase in protein nitration assessed by nitrotyrosine Elisa assay with TAC, and 2-week ISO or angiotensin II (AII) infusion is reduced by co-treatment with sildenafil. E) Zymogram showing activation of metalloproteinase 2 and 9 by TAC which is reduced by co-treatment with sildenafil.

up-regulated by TAC, and this was suppressed by the PDE5a inhibitor, vardenafil. This was accompanied by reduction in hypertrophy, but also by improvement in NOS coupling, reduction in nitrotyrosine, and reduction in activated metalloproteinases, all suggesting improvement in nitroso–redox balance. Whether this can be accomplished by NP stimulation is unclear, as to date we are aware of no data showing suppression of RhoA by this pathway. It also remains to be determined if compartmentalized separation of NP–cGMP signaling from PDE5 hydrolysis that is observed acutely will translate to more chronic exposure as in heart failure conditions.

9. Conclusion

Expanding appreciation of the cellular mechanisms and interactions between cGMP-targeted PDEs and NO–sGC or NP–pGC signaling has revealed a complex but strategically important role played by these hydrolytic enzymes. General recurring themes are their compartmentation to locally control cGMP–PKG signaling, their up-regulation in response to proliferative stresses and ultimately their contribution to sustaining these stresses and the pathophysiologic remodeling that ensues. Development of genetically targeted knockout mice has proven somewhat difficult and remains scant in this field. Selective inhibitors have also been difficult to come by, though new developments for PDE2 and PDE9 will help considerably, and efforts are continuing for targeted PDE1 inhibitors. With such tools, we should be better able to elucidate the role these PDEs play in cardiovascular health and disease.

Acknowledgements

This study was supported by National Institute of Health Grants AG18324, HL47511 (DAK), American Heart Association Scientist Development Awards (HCC, ET), The Peter Belfer Laboratory Endowment and Abraham and Virginia Weiss Professorship (DAK).

References

[1] Bian K, Ke Y, Kamisaki Y, Murad F. Proteomic modification by nitric oxide. J Pharm Sci 2006;101:271–9.
[2] Hess DT, Matsumoto A, Kim SO, Marshall HE, Stamler JS. Protein *S*-nitrosylation: purview and parameters. Nat Rev Mol Cell Biol 2005;6:150–66.
[3] Zimmet JM, Hare JM. Nitroso–redox interactions in the cardiovascular system. Circulation 2006;114:1531–44.
[4] Hofmann F, Feil R, Kleppisch T, Schlossmann J. Function of cGMP-dependent protein kinases as revealed by gene deletion. Physiol Rev 2006;86:1–23.
[5] Lincoln TM, Dey N, Sellak H. Invited review: cGMP-dependent protein kinase signaling mechanisms in smooth muscle: from the regulation of tone to gene expression. J Appl Physiol 2001;91:1421–30.
[6] Takimoto E, Champion HC, Belardi D, Moslehi J, Mongillo M, Mergia E, et al. cGMP catabolism by phosphodiesterase 5A regulates cardiac adrenergic stimulation by NOS3-dependent mechanism. Circ Res 2005;96:100–9.
[7] Takimoto E, Champion HC, Li M, Belardi D, Ren S, Rodriguez ER, et al. Chronic inhibition of cyclic GMP phosphodiesterase 5A prevents and reverses cardiac hypertrophy. Nat Med 2005;11:214–22.
[8] Wang P, Wu P, Egan RW, Billah MM. Identification and characterization of a new human type 9 cGMP-specific phosphodiesterase splice variant (PDE9A5). Differential tissue distribution and subcellular localization of PDE9A variants. Gene 2003;314:15–27.
[9] Zaccolo M. Phosphodiesterases and compartmentalized cAMP signalling in the heart. Eur J Cell Biol 2006;85:693–7.
[10] Gustafsson AB, Brunton LL. Attenuation of cAMP accumulation in adult rat cardiac fibroblasts by IL-1beta and NO: role of cGMP-stimulated PDE2. Am J Physiol Cell Physiol 2002;283:C463–71.
[11] Rybalkin SD, Rybalkina IG, Shimizu-Albergine M, Tang XB, Beavo JA. PDE5 is converted to an activated state upon cGMP binding to the GAF A domain. EMBO J 2003;22:469–78.
[12] Okada D, Asakawa S. Allosteric activation of cGMP-specific, cGMP-binding phosphodiesterase (PDE5) by cGMP. Biochemistry 2002;41:9672–9.
[13] Thomas MK, Francis SH, Corbin JD. Substrate- and kinase-directed regulation of phosphorylation of a cGMP-binding phosphodiesterase by cGMP. J Biol Chem 1990;265:14971–8.
[14] Turko IV, Francis SH, Corbin JD. Binding of cGMP to both allosteric sites of cGMP-binding cGMP-specific phosphodiesterase (PDE5) is required for its phosphorylation. Biochem J 1998;329(Pt 3): 505–10.
[15] Corbin JD, Turko IV, Beasley A, Francis SH. Phosphorylation of phosphodiesterase-5 by cyclic nucleotide-dependent protein kinase alters its catalytic and allosteric cGMP-binding activities. Eur J Biochem 2000;267:2760–7.
[16] Gopal VK, Francis SH, Corbin JD. Allosteric sites of phosphodiesterase-5 (PDE5). A potential role in negative feedback regulation of cGMP signaling in corpus cavernosum. Eur J Biochem 2001;268:3304–12.
[17] Kotera J, Francis SH, Grimes KA, Rouse A, Blount MA, Corbin JD. Allosteric sites of phosphodiesterase-5 sequester cyclic GMP. Front Biosci 2004;9:378–86.
[18] Leroy MJ, Degerman E, Taira M, Murata T, Wang LH, Movsesian MA, et al. Characterization of two recombinant PDE3 (cGMP-inhibited cyclic nucleotide phosphodiesterase) isoforms, RcGIP1 and HcGIP2, expressed in NIH 3006 murine fibroblasts and Sf9 insect cells. Biochemistry 1996;35:10194–202.
[19] Kirstein M, Rivet-Bastide M, Hatem S, Benardeau A, Mercadier JJ, Fischmeister R. Nitric oxide regulates the calcium current in isolated human atrial myocytes [see comments]. J Clin Invest 1995;95:794–802.
[20] Kojda G, Kottenberg K. Regulation of basal myocardial function by NO. Cardiovasc Res 1999;41:514–23.
[21] Vila-Petroff MG, Younes A, Egan J, Lakatta EG, Sollott SJ. Activation of distinct cAMP-dependent and cGMP-dependent pathways by nitric oxide in cardiac myocytes. Circ Res 1999;84:1020–31.
[22] Vandecasteele G, Verde I, Rucker-Martin C, Donzeau-Gouge P, Fischmeister R. Cyclic GMP regulation of the L-type Ca(2+) channel current in human atrial myocytes. J Physiol 2001;533:329–40.
[23] Layland J, Li JM, Shah AM. Role of cyclic GMP-dependent protein kinase in the contractile response to exogenous nitric oxide in rat cardiac myocytes. J Physiol 2002;540:457–67.
[24] Shah AM, Spurgeon HA, Sollott SJ, Talo A, Lakatta EG. 8-bromo-cGMP reduces the myofilament response to Ca2+ in intact cardiac myocytes. Circ Res 1994;74:970–8.
[25] Sonnenburg WK, Seger D, Kwak KS, Huang J, Charbonneau H, Beavo JA. Identification of inhibitory and calmodulin-binding domains of the PDE1A1 and PDE1A2 calmodulin-stimulated cyclic nucleotide phosphodiesterases. J Biol Chem 1995;270:30989–1000.
[26] Goraya TA, Cooper DM. Ca2+-calmodulin-dependent phosphodiesterase (PDE1): current perspectives. Cell Signal 2005;17:789–97.
[27] Hagiwara M, Endo T, Hidaka H. Effects of vinpocetine on cyclic nucleotide metabolism in vascular smooth muscle. Biochem Pharmacol 1984;33:453–7.
[28] Evgenov OV, Busch CJ, Evgenov NV, Liu R, Petersen B, Falkowski GE, et al. Inhibition of phosphodiesterase 1 augments the pulmonary

vasodilator response to inhaled nitric oxide in awake lambs with acute pulmonary hypertension. Am J Physiol Lung Cell Mol Physiol 2006;290:L723–9.
[29] Kim D, Rybalkin SD, Pi X, Wang Y, Zhang C, Munzel T, et al. Upregulation of phosphodiesterase 1A1 expression is associated with the development of nitrate tolerance. Circulation 2001;104:2338–43.
[30] Rybalkin SD, Bornfeldt KE, Sonnenburg WK, Rybalkina IG, Kwak KS, Hanson K, et al. Calmodulin-stimulated cyclic nucleotide phosphodiesterase (PDE1C) is induced in human arterial smooth muscle cells of the synthetic, proliferative phenotype. J Clin Invest 1997;100: 2611–21.
[31] Nagel DJ, Aizawa T, Jeon KI, Liu W, Mohan A, Wei H, et al. Role of nuclear Ca2+/calmodulin-stimulated phosphodiesterase 1A in vascular smooth muscle cell growth and survival. Circ Res 2006;98:777–84.
[32] Yanaka N, Kurosawa Y, Minami K, Kawai E, Omori K. cGMP-phosphodiesterase activity is up-regulated in response to pressure overload of rat ventricles. Biosci Biotechnol Biochem 2003;67:973–9.
[33] Sonnenburg WK, Rybalkin SD, Bornfeldt KE, Kwak KS, Rybalkina IG, Beavo JA. Identification, quantitation, and cellular localization of PDE1 calmodulin-stimulated cyclic nucleotide phosphodiesterases. Methods 1998;14:3–19.
[34] Castro LR, Verde I, Cooper DM, Fischmeister R. Cyclic guanosine monophosphate compartmentation in rat cardiac myocytes. Circulation 2006;113:2221–8.
[35] Mongillo M, Tocchetti CG, Terrin A, Lissandron V, Cheung YF, Dostmann WR, et al. Compartmentalized phosphodiesterase-2 activity blunts beta-adrenergic cardiac inotropy via an NO/cGMP-dependent pathway. Circ Res 2006;98:226–34.
[36] Lin CS, Lin G, Xin ZC, Lue TF. Expression, distribution and regulation of phosphodiesterase 5. Curr Pharm Des 2006;12:3439–57.
[37] Zhao L, Mason NA, Morrell NW, Kojonazarov B, Sadykov A, Maripov A, et al. Sildenafil inhibits hypoxia-induced pulmonary hypertension. Circulation 2001;104:424–8.
[38] Shukla N, Jones R, Persad R, Angelini GD, Jeremy JY. Effect of sildenafil citrate and a nitric oxide donating sildenafil derivative, NCX 911, on cavernosal relaxation and superoxide formation in hypercholesterolaemic rabbits. Eur J Pharmacol 2005;517:224–31.
[39] Takagi M, Mochida H, Noto T, Yano K, Inoue H, Ikeo T, et al. Pharmacological profile of T-1032, a novel specific phosphodiesterase type 5 inhibitor, in isolated rat aorta and rabbit corpus cavernosum. Eur J Pharmacol 2001;411:161–8.
[40] Weimann J, Ullrich R, Hromi J, Fujino Y, Clark MW, Bloch KD, et al. Sildenafil is a pulmonary vasodilator in awake lambs with acute pulmonary hypertension. Anesthesiology 2000;92:1702–12.
[41] Bivalacqua TJ, Usta MF, Champion HC, Leungwattanakij S, Dabisch PA, McNamara DB, et al. Effect of combination endothelial nitric oxide synthase gene therapy and sildenafil on erectile function in diabetic rats. Int J Impot Res 2004;16:21–9.
[42] Mullershausen F, Russwurm M, Thompson WJ, Liu L, Koesling D, Friebe A. Rapid nitric oxide-induced desensitization of the cGMP response is caused by increased activity of phosphodiesterase type 5 paralleled by phosphorylation of the enzyme. J Cell Biol 2001;155:271–8.
[43] Massion PB, Feron O, Dessy C, Balligand JL. Nitric oxide and cardiac function: ten years after, and continuing. Circ Res 2003;93:388–98.
[44] Flesch M, Kilter H, Cremers B, Lenz O, Sudkamp M, Kuhn-Regnier F, et al. Acute effects of nitric oxide and cyclic GMP on human myocardial contractility. J Pharmacol Exp Ther 1997;281:1340–9.
[45] Hare JM, Loh E, Creager MA, Colucci WS. Nitric oxide inhibits the positive inotropic response to beta-adrenergic stimulation in humans with left ventricular dysfunction. Circulation 1995;92:2198–203.
[46] Vandecasteele G, Eschenhagen T, Scholz H, Stein B, Verde I, Fischmeister R. Muscarinic and beta-adrenergic regulation of heart rate, force of contraction and calcium current is preserved in mice lacking endothelial nitric oxide synthase. Nat Med 1999;5:331–4.
[47] Barouch LA, Harrison RW, Skaf MW, Rosas GO, Cappola TP, Kobeissi ZA, et al. Nitric oxide regulates the heart by spatial confinement of nitric oxide synthase isoforms. Nature 2002;416:337–9.
[48] Stacey P, Rulten S, Dapling A, Phillips SC. Molecular cloning and expression of human cGMP-binding cGMP-specific phosphodiesterase (PDE5). Biochem Biophys Res Commun 1998;247:249–54.
[49] Loughney K, Hill TR, Florio VA, Uher L, Rosman GJ, Wolda SL, et al. Isolation and characterization of cDNAs encoding PDE5A, a human cGMP-binding, cGMP-specific 3′,5′-cyclic nucleotide phosphodiesterase. Gene 1998;216:139–47.
[50] Wallis RM, Corbin JD, Francis SH, Ellis P. Tissue distribution of phosphodiesterase families and the effects of sildenafil on tissue cyclic nucleotides, platelet function, and the contractile responses of trabeculae carneae and aortic rings in vitro. Am J Cardiol 1999;83:3C–12C.
[51] Reffelmann T, Kloner RA. Therapeutic potential of phosphodiesterase 5 inhibition for cardiovascular disease. Circulation 2003;108:239–44.
[52] Corbin J, Rannels S, Neal D, Chang P, Grimes K, Beasley A, et al. Sildenafil citrate does not affect cardiac contractility in human or dog heart. Curr Med Res Opin 2003;19:747–52.
[53] Cremers B, Scheler M, Maack C, Wendler O, Schafers HJ, Sudkamp M, et al. Effects of sildenafil (viagra) on human myocardial contractility, in vitro arrhythmias, and tension of internal mammaria arteries and saphenous veins. J Cardiovasc Pharmacol 2003;41:734–43.
[54] Senzaki H, Smith CJ, Juang GJ, Isoda T, Mayer SP, Ohler A, et al. Cardiac phosphodiesterase 5 (cGMP-specific) modulates beta-adrenergic signaling in vivo and is down-regulated in heart failure. FASEB J 2001;15:1718–26.
[55] Chen Y, Traverse JH, Hou M, Li Y, Du R, Bache RJ. Effect of PDE5 inhibition on coronary hemodynamics in pacing-induced heart failure. Am J Physiol Heart Circ Physiol 2003;284:H1513–20.
[56] Das A, Xi L, Kukreja RC. Phosphodiesterase-5 inhibitor sildenafil preconditions adult cardiac myocytes against necrosis and apoptosis. Essential role of nitric oxide signaling. J Biol Chem 2005;280: 12944–55.
[57] Borlaug BA, Melenovsky V, Marhin T, Fitzgerald P, Kass DA. Sildenafil inhibits beta-adrenergic-stimulated cardiac contractility in humans. Circulation 2005;112:2642–9.
[58] Ziolo MT, Lewandowski SJ, Smith JM, Romano FD, Wahler GM. Inhibition of cyclic GMP hydrolysis with zaprinast reduces basal and cyclic AMP-elevated L-type calcium current in guinea-pig ventricular myocytes. Br J Pharmacol 2003;138:986–94.
[59] Takimoto E, Belardi D, Tocchetti CG, Vahebi S, Cormaci G, Ketner EA, et al. Compartmentation of cardiac beta-adrenergic inotropy modulation by phosphodiesterase 5. Circulation 2007;115:2159–67.
[60] Das A, Xi L, Kukreja RC. Phosphodiesterase-5 inhibitor, sildenafil preconditions adult cardiac myocytes against necrosis and apoptosis: essential role of NO signaling. J Biol Chem 2005;280:12944–55.
[61] Fisher PW, Salloum F, Das A, Hyder H, Kukreja RC. Phosphodiesterase-5 inhibition with sildenafil attenuates cardiomyocyte apoptosis and left ventricular dysfunction in a chronic model of doxorubicin cardiotoxicity. Circulation 2005;111:1601–10.
[62] Takimoto E, Kass DA. Nitric oxide synthase-3 is essential for anti-hypertrophic effect of chronic phosphodiesterase 5a inhibition. Circulation 2005;112(suppl II):II-148.
[63] Yuasa K, Michibata H, Omori K, Yanaka N. A novel interaction of cGMP-dependent protein kinase I with troponin T. J Biol Chem 1999;274:37429–34.
[64] McConnachie G, Langeberg LK, Scott JD. AKAP signaling complexes: getting to the heart of the matter. Trends Mol Med 2006;12: 317–23.
[65] Vo NK, Gettemy JM, Coghlan VM. Identification of cGMP-dependent protein kinase anchoring proteins (GKAPs). Biochem Biophys Res Commun 1998;246:831–5.
[66] Ding B, Abe J, Wei H, Huang Q, Walsh RA, Molina CA, et al. Functional role of phosphodiesterase 3 in cardiomyocyte apoptosis: implication in heart failure. Circulation 2005;111:2469–76.
[67] Kim D, Aizawa T, Wei H, Pi X, Rybalkin SD, Berk BC, et al. Angiotensin II increases phosphodiesterase 5A expression in vascular smooth muscle cells: a mechanism by which angiotensin II antagonizes cGMP signaling. J Mol Cell Cardiol 2005;38:175–84.

[68] MacPherson JD, Gillespie TD, Dunkerley HA, Maurice DH, Bennett BM. Inhibition of phosphodiesterase 5 selectively reverses nitrate tolerance in the venous circulation. J Pharmacol Exp Ther 2006;317: 188–95.
[69] Rybalkin SD, Yan C, Bornfeldt KE, Beavo JA. Cyclic GMP phosphodiesterases and regulation of smooth muscle function. Circ Res 2003;93:280–91.
[70] Corbin JD, Beasley A, Blount MA, Francis SH. High lung PDE5: a strong basis for treating pulmonary hypertension with PDE5 inhibitors. Biochem Biophys Res Commun 2005;334:930–8.
[71] Forfia PR, Lee M, Tunin RS, Mahmud M, Champion HC, Kass DA. Acute PDE5 inhibition mimics hemodynamic effects of B-type natriuretic peptide and potentiates B-type natriuretic peptide effects in failing but not normal canine heart. J Am Coll Cardiol 2007;49: 1079–88.
[72] Lin CS, Chow S, Lau A, Tu R, Lue TF. Regulation of human PDE5A2 intronic promoter by cAMP and cGMP: identification of a critical Sp1-binding site. Biochem Biophys Res Commun 2001;280:693–9.
[73] Lin CS, Chow S, Lau A, Tu R, Lue TF. Identification and regulation of human PDE5A gene promoter. Biochem Biophys Res Commun 2001;80:684–92.
[74] Galie N, Ghofrani HA, Torbicki A, Barst RJ, Rubin LJ, Badesch D, et al. Sildenafil citrate therapy for pulmonary arterial hypertension. N Engl J Med 2005;353:2148–4157.
[75] Lewis GD, Semigran MJ. The emerging role for type 5 phosphodiesterase inhibition in heart failure. Curr Heart Fail Rep 2006;3:123–8.
[76] Chen HH, Huntley BK, Schirger JA, Cataliotti A, Burnett Jr JC. Maximizing the renal cyclic 3′–5′-guanosine monophosphate system with type V phosphodiesterase inhibition and exogenous natriuretic peptide: a novel strategy to improve renal function in experimental overt heart failure. J Am Soc Nephrol 2006;17:2742–7.
[77] Calderone A, Thaik CM, Takahashi N, Chang DL, Colucci WS. Nitric oxide, atrial natriuretic peptide, and cyclic GMP inhibit the growth-promoting effects of norepinephrine in cardiac myocytes and fibroblasts. J Clin Invest 1998;101:812–8.
[78] Fiedler B, Lohmann SM, Smolenski A, Linnemuller S, Pieske B, Schroder F, et al. Inhibition of calcineurin–NFAT hypertrophy signaling by cGMP-dependent protein kinase type I in cardiac myocytes. Proc Natl Acad Sci U S A 2002;99:11363–8.
[79] Wollert KC, Fiedler B, Gambaryan S, Smolenski A, Heineke J, Butt E, et al. Gene transfer of cGMP-dependent protein kinase I enhances the antihypertrophic effects of nitric oxide in cardiomyocytes. Hypertension 2002;39:87–92.
[80] Rosenkranz AC, Woods RL, Dusting GJ, Ritchie RH. Antihypertrophic actions of the natriuretic peptides in adult rat cardiomyocytes: importance of cyclic GMP. Cardiovasc Res 2003;57:515–22.
[81] Holtwick R, van Eickels M, Skryabin BV, Baba HA, Bubikat A, Begrow F, et al. Pressure-independent cardiac hypertrophy in mice with cardiomyocyte-restricted inactivation of the atrial natriuretic peptide receptor guanylyl cyclase-A. J Clin Invest 2003;111:1399–407.
[82] Kishimoto I, Rossi K, Garbers DL. A genetic model provides evidence that the receptor for atrial natriuretic peptide (guanylyl cyclase-A) inhibits cardiac ventricular myocyte hypertrophy. Proc Natl Acad Sci U S A 2001;98:2703–6.
[83] Knowles JW, Esposito G, Mao L, Hagaman JR, Fox JE, Smithies O, et al. Pressure-independent enhancement of cardiac hypertrophy in natriuretic peptide receptor A-deficient mice. J Clin Invest 2001;107:975–84.
[84] Tokudome T, Horio T, Kishimoto I, Soeki T, Mori K, Kawano Y, et al. Calcineurin-nuclear factor of activated T cells pathway-dependent cardiac remodeling in mice deficient in guanylyl cyclase A, a receptor for atrial and brain natriuretic peptides. Circulation 2005;111:3095–104.
[85] Zahabi A, Picard S, Fortin N, Reudelhuber TL, Deschepper CF. Expression of constitutively active guanylate cyclase in cardiomyocytes inhibits the hypertrophic effects of isoproterenol and aortic constriction on mouse hearts. J Biol Chem 2003;278:B47694–9.
[86] Noma K, Oyama N, Liao JK. Physiological role of ROCKs in the cardiovascular system. Am J Physiol Cell Physiol 2006;290:C661–8.
[87] Wolfrum S, Dendorfer A, Rikitake Y, Stalker TJ, Gong Y, Scalia R, et al. Inhibition of Rho-kinase leads to rapid activation of phosphatidylinositol 3-kinase/protein kinase Akt and cardiovascular protection. Arterioscler Thromb Vasc Biol 2004;24:1842–7.
[88] Nohria A, Grunert ME, Rikitake Y, Noma K, Prsic A, Ganz P, et al. Rho kinase inhibition improves endothelial function in human subjects with coronary artery disease. Circ Res 2006;99:1426–32.
[89] Rikitake Y, Liao JK. Rho GTPases, statins, and nitric oxide. Circ Res 2005;97:1232–5.
[90] Ellerbroek SM, Wennerberg K, Burridge K. Serine phosphorylation negatively regulates RhoA in vivo. J Biol Chem 2003;278:19023–31.