Phosphodiesterase Type 5: Expanding Roles in Cardiovascular Regulation

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Abstract—Phosphodiesterase type 5A (PDE5A) selectively hydrolyzes cyclic GMP. Inhibitors of PDE5A such as sildenafil are widely used to treat erectile dysfunction, but growing evidence supports important roles for the enzyme in both the vasculature and heart. In disorders such as cardiac failure, PDE5A upregulation may contribute to a decline in cGMP and protein kinase G signaling, exacerbating dysfunction. PDE5A plays an important role in the pulmonary vasculature where its inhibition benefits patients with pulmonary hypertension. In the heart, PDE5A signaling appears compartmentalized, and its inhibition is cardioprotective against ischemia-reperfusion and anthracycline toxicity, blunts acute adrenergic contractile stimulation, and can suppress chronic hypertrophy and dysfunction attributable to pressure-overload. In this review, we discuss the molecular biology, pharmacology, and physiology of PDE5A, mechanisms of vascular and cardiac regulation, and recent evidence supporting the utility of selective PDE5A inhibition for the treatment of cardiovascular disorders. (Circ Res. 2007;101:1084-1095.)

Key Words: blood vessels ■ cardiac myocytes ■ cardiovascular physiology ■ phosphodiesterase type 5 inhibitor ■ pressure overload ■ protein kinase G ■ reperfusion injury

The cyclic nucleotides cAMP and cGMP both play central roles in cardiovascular regulation, influencing function, gene expression, and morphology. cAMP mediates many of the effects of epinephrine on the heart and other tissues, whereas cGMP is a mediator of nitric oxide and atrial natriuretic peptide action. The study of cyclic nucleotide phosphodiesterases began shortly after the discovery of these intracellular messengers, though medicinal use of phosphodiesterase inhibitors goes back many centuries to early Chinese medicine because caffeine, theophylline, and ginseng are all PDE inhibitors. In the Western literature, Salter is credited with first noting the benefits of a strong coffee or tea for patients with asthma. But it was Sutherland and Rall in the late 1950’s who first identified cAMP as a second messenger and showed caffeine to be an agent that weakly inhibited its degradation. This revelation was followed shortly thereafter by Ashman’s report of cyclic guanosine monophosphate, and soon investigators were hunting for enzymes that specifically hydrolyzed the different cyclic nucleotides.

Initially, the phosphodiesterases were arbitrarily divided into those selectively targeting cGMP, cAMP, or being
activated by calcium-calmodulin. Eventually, 11 different PDE isozyme families (PDE1-PDE11) were identified.\textsuperscript{16,17} Each family has a highly conserved catalytic domain near the C terminus; however, there are enough structural differences among the domains that selective inhibitors have been identifiable for almost all of the individual PDE families. For most of the PDEs one or more regulatory N-terminal domains have also been identified. Of the 11 families, what we now know as PDE5 and PDE6 were the first to be found functionally specific for cGMP.

PDE5 received relatively little attention until studies using zaprinast, which was known to inhibit cGMP-PDE (subsequently shown to be PDE5A), revealed its role in regulating smooth muscle tone. By the mid 1980’s, pharmacologists at Pfizer research laboratories in Sandwich, England working on an antihypertension drug development program speculated that a selective cGMP PDE inhibitor might relax arteries and lower blood pressure by augmenting intracellular cGMP. After screening for agents that inhibited cGMP hydrolysis but not cAMP hydrolysis the investigators identified a cGMP PDE selective agent, UK-92 480. Because this type of PDE activity was present in vascular smooth muscle\textsuperscript{18} as well as platelets\textsuperscript{19} (where its inhibition reduced aggregation), the first clinical target was angina pectoris. Clinical testing in normal volunteers was disappointing, revealing little cardiovascular impact. However, some “side-effects” were reported at higher doses, including penile erections. Around the same time, Kukreja and colleagues\textsuperscript{23,24} presented evidence that PDE5 inhibition provided cardiac ischemic preconditioning, whereas Kiss and colleagues showed it regulated acute adrenergic-stimulated contractility\textsuperscript{25–27} and suppressed chronic hypertrophic stress remodeling to pressure-overload.\textsuperscript{28} This and other work\textsuperscript{29–33} has expanded interest in the cardiovascular potential for PDE5 inhibitors. In this review, we discuss the molecular biology and regulation of PDE5, its pharmacologic modulation by selective inhibitors, and its emerging role as a regulator of acute and chronic cardiac stress responses and response signaling.

**Early Studies on PDE5**

The initial discovery and characterization of PDE5 was first performed largely in the context of its being an unidentified binding protein for cGMP and not as a PDE.\textsuperscript{34} The original descriptions noted a high-affinity cGMP-binding protein present in lung and platelets that did not comigrate with cGMP-dependent protein kinase (cGK) activity on fractionation by DEAE cellulose chromatography. At the time cGK was the only known high-affinity binding protein for cGMP. These early studies noted that the same fractions contained a cGMP-PDE activity but did not definitively conclude that this PDE molecule actually contained the high-affinity cGMP binding site because of the multiplicity of PDE isozymes and other proteins that comigrated in the same region. However, within a few years this cGMP-binding activity was further characterized from platelets\textsuperscript{35} and lung\textsuperscript{36} and shown likely to be attributable to sites on a cGMP-specific PDE. Later of course, several different PDEs, including PDE5, were found to contain high-affinity cGMP binding sites that we now call GAF domains. For many years what we now call PDE5 was variously called the cGMP-binding protein PDE (cG-BPP) or the cGMP-binding cGMP-specific PDE. The acronym cGMP B-PDE, cGMP-BPDE, PDE-V, and PDE5 have all been used to describe this enzyme. Nevertheless, because other PDEs also were known that contained high affinity cGMP-binding sites, including what are now known as PDE2s and PDE6s, there was continuing confusion in the scientific and patent literature regarding the molecular identity of the cGMP-specific PDE(s).

The inhibitor selectivity characteristics of PDE5 were very similar to those previously described for rod and cone photoreceptor PDEs, leading many investigators to presume that PDE5 might be just a splicing or alternative start variant of the already well-known light-sensitive, cGMP-selective PDEs found in the retina of the eye (now known as PDE6s). Both enzyme families selectively used cGMP compared with cAMP as substrate (at low substrate levels), both bound cGMP with high affinity, both were inhibited by many of the same agents, and both had a very similar size. However, differences in Vmax activities and the lack of regulation of PDE5 by light or G proteins suggested that they might be different PDEs. It was not until the cloning of the PDE5 cDNA\textsuperscript{37} and its comparison to the previously published sequences of PDE6\textsuperscript{38} and of PDE2\textsuperscript{39} that it was entirely clear PDE5 was a distinct gene product from these two structurally similar PDEs.

**Molecular Structure**

**cGMP Binding**

The high affinity cGMP binding sites on PDE5 are now known to be on the N-terminal regulatory GAF-A domain of the enzyme (Figure 1A). The structural basis for high-affinity cGMP binding to PDE5A was solved when it was found to have two highly homologous GAF domains (GAF-A and GAF-B) that are very similar to known GAF domains on PDE2 and PDE6. However, in contrast with PDE2, high-affinity cGMP binding occurs only to the GAF-A domain (kDa \( \approx 40 \text{ nmol/L} \)) of PDE5.\textsuperscript{40} Cyclic nucleotide binding to this domain is \( \approx 100\)-fold selective for cGMP over cAMP. As might be expected, the GAF-A domain of PDE5 is most homologous to the cGMP-binding GAF-B domain of PDE2A. It also bears substantial structural homology with the GAF-A domains of PDE6s that also bind cGMP. Mutation analysis based on homology models to the crystal structure of the PDE2A
detected to date. Although some differences exist in regulation of the first common exon for all the isoforms. PDE5A2 is the most widely expressed. It is thought by many investigators that under usual physiologic conditions the GAF-A domain is likely to be occupied by cGMP, PKA can also phosphorylate this site. This phosphorylation then stabilizes the increased catalytic activity by enhancing the affinity of cGMP binding to the GAF-A domain. It is postulated that this mechanism provides the cell with a method for prolonging the activation of PDE5 in a feedback loop initiated by cGMP synthesis. Perhaps more importantly, this also implies less than full saturation of the site under basal conditions of cGMP. Phosphorylation of PDE5 by PKG has been demonstrated in vivo. By both of these mechanisms, ie, allosteric binding and cGMP-dependent phosphorylation, activators of guanylyl cyclases can promote a feed-forward activation of the enzyme to blunt the amplitude and duration of the cGMP rise. An important correlate is that when a PDE5 inhibitor is present, these same mechanisms result in enhanced binding affinity of the catalytic site to the inhibitor, perhaps enhancing and prolonging its efficacy. It is presumed this also provides a molecular mechanism for rapid cGMP oscillations to occur in response to rapid changes in NO or ANP, though it remains unclear how this could operate given the very high affinities measured for cGMP binding in vitro. It seems likely that another layer of regulation of cGMP binding affinity exists but has yet to be elucidated.

Gene Structure/Splicing
Only one gene for PDE5 has been discovered, PDE5A. However, 3 variants of the PDE5A mRNA and protein have been identified. PDE5A1, 5A2, and 5A3 differ at their N-terminal regions, and all 3 have unique first exons followed by a common sequence of 823 amino acids (Figure 1B). The order of the first exons from the 5 end in the gene is PDE5A1, PDE5A3, and PDE5A2. A promoter has been characterized upstream of the PDE5A1 first exon, and an alternative intronic promoter was also found upstream of the PDE5A2 first exon. PDE5A3 seems to be regulated by the same promoter as PDE5A1, although it has a separate first exon. Transcription from the PDE5A2 promoter is positively regulated by both cAMP and cGMP. Mutation analysis suggests that AP2 and Sp1 elements may be most responsible for the cyclic nucleotide responsiveness, although cAMP response elements (CREs) are also present. It is assumed, but has not yet been clearly shown, that the different promoters for the PDE5 isoforms allow physiologically relevant differential control of PDE5 gene expression thereby providing a mechanism for longer-term feedback regulation than the allosteric and phosphorylation mechanisms described earlier.

Cellular Distribution and Subcellular Localization
PDE5A is generally considered to be a cytosolic protein. In rodents, relatively high levels of PDE5A mRNA have been localized to vascular smooth muscle, heart, placenta, skeletal muscle, pancreas, brain, liver, several gastrointestinal tissues, and lung. Because PDE5 is highly expressed in the smooth muscle of all vascular beds and because all tissues have blood vessels, it often has been difficult to determine...
which cell types within a tissue contain this PDE. One assumes where very high levels of mRNA are found, PDE5 is in the major cell type of a tissue. For example the highest levels of PDE5A mRNA are found in the cerebellum, kidney, and pancreas, whereas PDE5A3 expression is also seen in lung. A small amount can be seen in normal heart, whereas PDE5A5 variants have been detected at varying levels in a wide variety of tissues, whereas PDE5A3 expression is apparently regulated as PDE5A1 and PDE5A2 variant mRNAs have been detected at varying levels in various studies. The expression of different PDE5A variant mRNAs has been shown to increase with pressure-induced hypertrophy. It has a longer t1/2 (17.5 hours). They also vary somewhat in tissue and activity against other PDEs (PDE6 in retina, PDE1 in heart and vessels for sildenafil and vardenafil; PDE11 for tadalafil; reviewed in64). Many experimental studies have reported in cardiac fibroblasts where NO enhanced by a donor or iNOS stimulation reduced cAMP accumulation; an effect reversed by EHNA. The role of cGMP modulated by cAMP-stimulated current and contraction by nitric oxide.66 Similar effects of PDE2 on cGMP/cAMP regulation were reported in cardiac fibroblasts where NO enhanced by a donor or iNOS stimulation reduced cAMP accumulation; an effect reversed by EHNA.67 The role of cGMP modulated by PDE5 in altering PDE2 function remains unknown. Another cGMP role is competitive binding to the catalytic site of PDE5. This enzyme binds both cAMP and cGMP at high affinity and similar Ks, but has a Vmax for cAMP 10× greater conferring catalytic specificity. At low levels, cGMP may inhibit PDE5 hydrolysis60 to enhance cAMP which influences myocyte contractility,69 and also plays a role in platelet aggregation70 and endothelial cell permeability.71 This action has not been generally observed in most studies of PDE5 inhibition in either vascular tissue or the heart; however,
Figure 2. Schematic diagram of putative regulation of vascular smooth muscle contraction by PDE5A. cGMP is either generated by soluble guanylate cyclase (sGC) triggered by nitric oxide (NO), nitroglycerin (NTG), or NO donors (eg, sodium nitroprusside, SNP), or from natriuretic peptide receptor (NPR) coupled receptor guanylate cyclase (rGC). The synthesized cGMP likely resides in different pools that may be more or less targeted by inhibitory PDEs. They active protein kinase G (PKG) which in turn targets Ca2+ channels, RhoA which regulates Rho kinase (ROCK), RGS2, and myosin phosphatase targeting subunit (MYPT). The latter results in dephosphorylation of myosin light chain kinase (MLCK). Receptor-coupled G-protein signaling by angiotensin, endothelin, and β2-adrenergic receptor (AT1, ET1, α-AR) is shown coupled to transient receptor potential channels (TRPC) which conduct specific Ca2+ pools coupled to growth transcriptional regulation. PDE5A activation depresses PKG stimulation enhancing tone and proliferative responses, whereas PDE5A inhibition would do the reverse.

Vascular Modulation by PDE5

The role of PKG in regulating vascular tone and growth have been recently reviewed74,75 and are briefly discussed here. Figure 2 shows a schematic for the pathways involved. Smooth muscle relaxation is thought to occur by several mechanisms including decreasing intracellular free calcium concentration, reducing calcium sensitization, and regulating thin filament proteins. Calcium is required for myosin light chain phosphorylation and smooth muscle contraction, and PKG lowers calcium by activating calcium pumps, inhibiting voltage-gated Ca2+ channels, and inhibiting receptor/G protein–coupled Ca2+ activation. Additional targets may be calcium-activated potassium channels (BKCa)76–78 and phospholamban,79 though recent knock-out model studies have questioned their role in vivo.80,81 PKG also phosphorylates a protein associated with the IP3 receptor (IP3Receptor-Associated-PKG substrate, IRAG) to reduce SR Ca2+ release.82–84

PKG activation also reduces Ca2+ sensitivity by modulating myosin light chain phosphorylation, and through this pathway, PDE5 inhibitors may suppress RhoA–Rho Kinase (ROCK) stimulation signaling.85–88 Agonist-induced smooth muscle contraction can activate RhoA leading to ROCK-dependent threonine phosphorylation of myosin phosphatase targeting subunit, MYTP.89,90 This inhibits myosin light chain phosphatase (MLCP) activity enhancing MLCP phosphorylation. PKG-dependent phosphorylation of MYTP at S695 prevents ROCK-dependent phosphorylation of the protein, thereby activating myosin light chain phosphatase.91–93 PKG may also directly inhibit RhoA94,95 by direct phosphorylation at serine-188, but evidence for this is conflicting.96,97 Reduced RhoA/ROCK activation may contribute to the observation that PDE5 inhibition increases NOS activity.98 Inhibition of receptor/G protein–stimulated tone is also suppressed by PKG interaction and activation of regulatory of G-coupled signaling 2 (RGS2),99 and this too likely plays a role in PDE5 inhibitory effects.

PDE5 and Cardiac Function

Despite early evidence of myocardial PDE5 gene expression,90,91 protein synthesis and enzyme activity are rather low compared with lung, and have been traditionally thought to be physiologically insignificant.100,101 Some early functional analysis suggested this as well. For example, Cremers et al102 found no effect of 10−7–10 μmol/L sildenafil (IC50 of purified protein is ~10 nmol/L) on basal or isoprenaline stimulated function in human papillary muscle strips. However, they mostly used end-stage failing heart tissue, which might be relevant as a canine study found PDE5 inhibition had negligible effects on basal or dobutamine stimulated contractility in failing but not normal hearts.103 Another functional study101 reporting no effects examined tissue only under basal conditions where cyclase tone is low.

More recent studies, however, reported evidence of both detectable PDE5 mRNA and protein expression in isolated myocytes, myocyte physiologic effects (at ranges of 0.1 to 1 μmol/L for sildenafil, and 50 nmol/L for tadalafil25), and...
whole heart effects of PDE5 inhibitors (at 20 to 50 nmol/L free plasma concentration). As found for erectile function or vasodilation, PDE5 inhibition had little effect on basal cardiac function, but suppressed acute beta-adrenergic stimulation in dog, mouse, and human heart. This is relevant because such stimulation was shown to increase cGMP generation, thereby providing the setting where PKG activation could act as a brake to contractile stimulation. Figure 3 shows a schematic for various putative PDE5 signaling pathways in the cardiac myocyte. The effects on basal function have been minimal across species. Whole cell Ca\(^{2+}\) transient was unaltered consistent with reduced myofilament calcium sensitivity similar to that with NO, though unlike NO, contractile effects were negligible in the basal state. Another study reported declines in L-type Ca\(^{2+}\)-channel currents, though the species used (guinea pig) differed, and agent (zaproprast) a less specific PDE5 inhibitor.

The antiadrenergic effect of sildenafil was prevented by ODQ, an inhibitor of soluble guanylate cyclase (sGC), and was absent in myocytes lacking eNOS or with NOS inhibited by L-NAME supporting a key role of NO-sGC generated cGMP. The effect in otherwise normal myocytes appears dependent on PKG activity which may relate to S23, S24 phosphorylation of TnI, which results in myofilament Ca\(^{2+}\)-desensitization.

Whereas an absence of eNOS prevented PDE5 inhibitory modulation of \(\beta\)-adrenergic stimulation, both myocardial and myocyte gene and protein expression and in vitro PDE5 activity were intriguingly similar between eNOS\(^{-/-}\) and WT myocytes and whole heart. However, the intracellular localization of PDE5 was different, shifting from a normal z-band localization (recently further confirmed by immunoelectron microscopy) to a more diffuse cytosolic distribution analogous to that previously reported in canine heart failure. Interestingly, the latter condition was also one wherein PDE5 inhibition did not suppress \(\beta\)-adrenergic stimulation. Restoration of eNOS to the null background by gene transfer restored both PDE5 z-band localization and antiadrenergic effects from PDE5 inhibition supporting a mechanistic link.

### Myocyte Compartmentation of PDE5-cGMP Interaction

Growing evidence supports an important role of compartmentation in cyclic nucleotide–PDE modulation, and this appears to apply to PDE5 as well. Adult myocytes transfected with a cGMP-sensitive Ca\(^{2+}\) reporter channel (olfactory protein) recorded increased membrane cGMP induced by natriuretic peptide stimulation that was totally unchanged by concomitant PDE5 inhibition. This contrasted to the signal generated by NO donors, which sildenafil enhanced.

Other evidence for compartmentation stems from studies comparing PDE5 inhibition versus natriuretic peptide stimulation effects on \(\beta\)-stimulated contraction; with the former but not the latter suppressing the response in mice and dogs. This correlated with PKG activation observed by myocardial phosphorylation of VASP but not with levels of cGMP which rose far more with NP stimulation. Whether chronically augmented NP stimulation as occurs in heart disease conditions may lead to a cGMP pool susceptible to PDE5 hydrolysis, or conversely, whether upregulation of PDE5 in chronic disease influences solely NOS-sGC but not NP-receptor guanylate cyclase derived cGMP, remains to be determined.

Compartmentation of cAMP-PKA involves the family of anchoring proteins (AKAPs) that bind and locally target this signaling. Far less is known about PKG equivalents (ie, GKAPs). Indeed whether such an equivalent even exists is unclear; however, a number of PKG binding and interacting proteins have been revealed. One is troponin T which binds PKG via a leucine zipper and this appears central to facilitating PKG-phosphorylation of TnI that desensitizes the myofilament to calcium. Another is myosin itself though the role of this interaction remains unknown. Potential binding partners underlying PDE5 localization or migration under different conditions remain unknown. The obligate requirement for NOS-derived NO or cGMP suggests cGMP binding or PKG activation maybe key perhaps by providing tonic enzyme activation at the regulatory domain. Analogous compartmentation of PDE5-cGMP regulation in vascular smooth muscle or endothelial cells has not yet been reported.

### PDE5 Upregulation in Cardiovascular Disease

In a variety of chronic cardiovascular diseases, cGMP rises often in response to sustained activation of natriuretic peptides, and growing evidence supports PDE5 upregulation occurs as well perhaps as a countering mechanism. Increased levels may relate to cGMP/PKG effects on transcription and posttranslational activation. Upregulation has been reported in pulmonary hypertension, congestive heart failure, and right ventricular hypertrophy. Angiotensin II (AII) stimulation of VSM responds with rapid induction of PDE5A that augments the AII response by reducing cGMP/PKG signaling. This may exacerbate hypertension and vascular proliferation in diseases involving renin/angiotensin stimulation. PDE5 also increases in vascular rings and the venous circulation of rats chronically administered nitroglycerin, suggesting a role in nitrate tolerance. Inhibition of PDE5 reversed this tolerance in affected vessels.

A correlate of PDE5 upregulation in disease conditions such as heart failure is that effects from its inhibition can become more important. For example this has been observed in canine failure models, with enhanced vasodilation in the central venous, pulmonary, arterial, and renal vasculatures. Exposure to chronic PDE5 inhibition improved the acute renal responsiveness to NP in canine heart failure. In another study, acute sildenafil infusion minimally affected normal dogs, but triggered pulmonary vasodilation similar to that from B-type NP in animals with heart failure. This was accompanied by increased PDE5 activity in lung vascular tissue from the failing heart animals. A marker of NP-desensitization is the circulating ratio of plasma BNP/cGMP which rose markedly with heart failure yet declined to control levels with administration of a PDE5 inhibitor, and administration of exogenous NP did not alter this ratio. Genetic models of PDE5 overexpression are under development to further test the role of such upregulation more directly.
Lastly, PDE5A expression is reportedly increased in human and animal models of right ventricular hypertrophy. Surprisingly, this did not translate to enhanced PKG signaling when PDE5A inhibitors were applied. Rather, Nagendran et al. observed a marked rise in cAMP (similar to that from isoproterenol) and a positive inotropic effect with PDE5A inhibition. They proposed that cGMP augmented by PDE5A inhibition leads to competitive inhibition of PDE3A, thereby elevating cAMP. These studies used acute infusions and stand in contrast to data in normal hearts where PKG-dependent signaling prevails. However, the 2 mechanisms are not mutually exclusive, and more research is needed to determine which predominates under which condition.

**PDE5 and Ischemic Cardioprotection**

PDE5 inhibition was first shown to protect hearts against ischemia-reperfusion (IR) injury by Ockaili et al., who used sildenafil (0.7 mg/kg i.v.) provided 30 minutes or 3-hour reperfusion. These investigators found a 68% and 41% reduction in infarct size with oral treatment. Protection was abolished by coadministration of the mitochondrial KATP channel inhibitor, 5-hydroxydecanoate, suggesting a role for this channel. The acute or delayed phase treatment, respectively. Similar findings were obtained with oral treatment. Protection was abolished by coadministration of the mitochondrial KATP channel inhibitor, 5-hydroxydecanoate, suggesting a role for this channel. The beneficial effect was recapitulated in mice exposed to sustained pressure overload, and pharmacologic inhibition of PKC also prevented IR protective effects.

Such translocation of PKC also prevented IR protective effects. Such translocation from cytosol to the sarcolemmal membrane (PKC-ζ) has been shown to protect against IR injury by Ockaili et al., who used sildenafil (0.7 mg/kg i.v.) provided 30 minutes or 24 hours before 30-minute ischemia and 3-hour reperfusion. These investigators found a 68% and 41% reduction in infarct size with oral treatment. Protection was abolished by coadministration of the mitochondrial KATP channel inhibitor, 5-hydroxydecanoate, suggesting a role for this channel. The acute or delayed phase treatment, respectively. Similar findings were obtained with oral treatment. Protection was abolished by coadministration of the mitochondrial KATP channel inhibitor, 5-hydroxydecanoate, suggesting a role for this channel. The beneficial effect was recapitulated in mice exposed to sustained pressure overload, and pharmacologic inhibition of PKC also prevented IR protective effects.

**PDE5 Modulation of Cardiac Stress Remodeling**

Cyclic GMP/PKG activation by NO or NP or by constitutively activated PKG expression attenuates hypertrophy in neonatal myocytes in conjunction with inhibition of the calcineurin-NFAT pathway. However, although ANP suppresses angiotensin II–stimulated hypertrophy of neonatal myocytes, the role of PKG in this response remains unclear. Chronic PDE5A inhibition elevated myocardial PKG activity in mice exposed to sustained pressure overload, which was accompanied by attenuation of cardiac and myocyte hypertrophy, interstitial fibrosis, improved cardiac function, and the deactivation of various hypertrophy signaling cascades including calcineurin, ERK-MAP kinase, and Akt.
This effect was independent of net ventricular load. Others have found PDE5 inhibition suppresses hypertrophy induced by sustained catecholamine stimulation\textsuperscript{31} and improves function and limits postinfarct remodeling,\textsuperscript{32} the latter attributed to activation of phosphoglycerate kinase-1 and subsequent blunting of sodium-hydrogen exchanger function.\textsuperscript{32}

Genetic ablation of the A-type NP receptor (NPRA) in mice produces modest cardiac hypertrophy at baseline and worsens the hypertrophic response to pressure overload,\textsuperscript{133-135} whereas cardiac overexpression of constitutively active pGC prevents modest pressure-load induced hypertrophy.\textsuperscript{136} It remains unclear whether more severe stress accompanied by chamber dilation is also prevented by this pathway. Intriguingly, the NPRA null heart has enhanced baseline calcineurin-NFAT activity and its spontaneous development of hypertrophy is blunted by calcineurin inhibition with FK506.\textsuperscript{137} This suggests that ANP/pGC/cGMP can regulate calcineurin as well as NO/sGC/cGMP.

The molecular mechanisms by which PDE5A inhibition produces antihypertrophic effects remain unclear, particularly in the intact animal, but there are several possibilities. One is inhibition of RhoA/ROCK.\textsuperscript{60} Mice lacking ROCK-1 display reduced hypertrophic responses to pressure-overload, though inhibition of apoptosis and suppression of reactive fibrosis appear to dominate the response.\textsuperscript{138} ROCK suppresses NOS activity by inhibiting its activation by Akt and reducing eNOS mRNA stability (an effect also imparted by activated RhoA), enhancing oxidant stress to reduce NOS function.\textsuperscript{139} As previously noted, PKG can interfere with this pathway, though the importance of this to cardiac pathophysiology remains to be settled. Another potential mechanism is PKG-activation of the regulator of G-coupled signaling RGS2. In smooth muscle, PKG-phosphorylation of RGS2 and protein-binding via a leucine zipper motif leads to its translocation to the sarcosomal membrane, where the Go\(_\gamma\) tri-meric complex is reassembled to suppress signaling.\textsuperscript{99} This pathway likely plays a role in hypertension,\textsuperscript{99} and recent studies have shown it may be similarly important in suppressing cardiomyocyte hypertrophic stimulation.\textsuperscript{140,141} Ongoing studies are examining the potential importance of this signaling to the PDE5 modulation of pressure overload.

As with prior paradigms, PDE5A modulation of cardiac and vascular stress responses requires sufficient cyclic activity, PDE5A needs to be expressed and active, and cGMP/PKG (and perhaps cAMP as well) need to have targetable pathway(s) costimulated. This could mean that diseases with insufficient cGMP synthesis because of nitric oxide synthase deficiency, PDE5A dysregulation,\textsuperscript{26} or lack of activation of strategic pathways that PKG targets, may not respond to PDE5 inhibition. Many of these conditions remain to be tested. Lastly, it should be noted that reversal of hypertrophy in intact animals may stem from nonmyocyte effects of PDE5 inhibitors as well. This includes systemic vascular, renal, and pulmonary vascular changes, potentially hormone and cytokine release from different cell types, myofibroblasts, antifibrotic activity,\textsuperscript{142} and modulation of inflammatory responses. All need to be investigated in this context.

### Clinical Targeting of PDE5

The most widely known and established use for PDE5 inhibitors is for erectile dysfunction, and this has been well reviewed elsewhere.\textsuperscript{63,64,143} Here, we focus on both existing and potential uses for cardiovascular disease. Oral sildenafil lowers pulmonary artery pressures, increases cardiac output, and reduces pulmonary vascular resistance \(\approx 20\% \sim 30\%\) when combined with inhaled nitric oxide). This occurs with little change in systemic blood pressure, suggesting pulmonary vascular selectivity. In patients with left heart disease and pulmonary hypertension, sildenafil is also now used to assess pulmonary vascular reactivity. The Sildenafil Use in Pulmonary Arterial Hypertension (SUPER) trial reported on 278 function class II-IV patients with PAH, who were randomized to placebo or sildenafil at 20, 40, or 80 mg 3 \times daily for 12 weeks. The 6-minute walk test (primary end point) improved by 45 meters with active treatment and invasive hemodynamics found a fall in mean pulmonary artery pressure and resistance and rise in cardiac output. Intriguingly, there did not appear to be a clear dose response relationship. Chronic treatment was well tolerated, with the most frequent side effects being headache, dyspepsia, flushing, and diarrhea. More recently, sildenafil has been tested to treat PAH from porto-pulmonary,\textsuperscript{145,146} sickle cell,\textsuperscript{147} and Gaucher disease.\textsuperscript{148} In these small noncontrolled studies, sildenafil appears to benefit patients, but confirmation awaits larger trials.

Another potential clinical target is congestive heart failure accompanied by increased pulmonary vascular resistance. This is an independent risk factor for a poor outcome\textsuperscript{149} and an impediment to initiating therapies such as heart transplantation.\textsuperscript{150} Sildenafil can acutely lower PVR,\textsuperscript{151,152} improve endothelium-dependent flow-mediated dilation,\textsuperscript{153} and generate more uniform skeletal muscle perfusion during exercise.\textsuperscript{154} Lewis et al\textsuperscript{29} recently reported that sildenafil (50 mg po) improves exercise capacity, reducing pulmonary pressures and resistance while augmenting cardiac output during exercise, and also improving right heart ejection and ventilatory efficiency.

Lastly, PDE5A inhibition is being tested for its impact on another form of heart failure without dilation and with a preserved ejection fraction (also referred to as diastolic heart failure). Affected patients are typically more elderly, of female sex, and often have a history of hypertension and left ventricular hypertrophy.\textsuperscript{155,156} Nearly 40\% have pulmonary hypertension, and renal function and vascular volume homeostasis is often compromised. Given the potential of PDE5A inhibition to target all of these abnormalities concomitantly, interest has grown to test such therapy in these patients. The NIH will fund a multi-center trial (RELAX) which is expected to commence later this year and will test the efficacy of chronic sildenafil to treat heart failure patients with a preserved ejection fraction. The primary end point is metabolic exercise capacity, and a number of clinical and physiologic secondary end points will be examined, including ventricular mass and function. This will represent the largest and first multi-center trial examining the utility of a PDE5A inhibitor for heart failure.

### Summary

Though interest in PDE5A inhibitors started in the cardiovascular arena, it took nearly 15 years and a urological side-trip before
returning to this focus. However, there is growing excitement that these agents may provide a number of beneficial effects for both heart and arteries. Despite recent rapid progress, many questions remain to be answered. In particular, how are the cardiac effects being generated, what conditions will they most likely be applicable to, and why? Why does it apparently take much higher levels of sildenafil to demonstrate direct effects in isolated cardiomyocytes that it does for the in vivo effects? Does the apparent compartmentation of cGMP-hydrolysis stay the same in the failure state, does this vary depending on the tissue, and what would this imply for combinations of natriuretic peptide or soluble guanylate cyclase stimulation and PDE5A modulation? What controls PDE5A translocation in myocytes and how does it impact compartmentalization regulation? How does PDE5A regulation change in pathophysiologic states? Under which conditions does upregulation occur, and does this only impact certain pools of cGMP? Does cGMP enhanced by PDE5 inhibition target alternative pathways in normal or diseased hearts and vessels? If one inhibits PDE5 long enough, is there any alternative pathway that is upregulated as a countermeasure? The existing wide-spread use of safe and effective PDE5 inhibitors has meant key questions related to clinical efficacy have been readily amenable to study. With the renewed interest and new genetic models, we can look forward to major strides in the basic molecular physiology as well.

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Disclosures
None.

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