Phosphodiesterase-5 and Retargeting of Subcellular cGMP Signaling During Pathological Hypertrophy

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Both cAMP and cGMP are critical intracellular second messengers regulating fundamental physiological processes in the myocardium, from acute contraction/relaxation to chronic gene expression, cell growth and apoptosis, and cardiac structural remodeling. cAMP is synthesized by adenylate cyclases on activation of G-protein–coupled receptors. cGMP is generated from the cytosolic purine nucleotide GTP by guanylyl cyclases (GCs) with Mg$^{2+}$ or Mn$^{2+}$ as cofactors. Two isoforms of GCs exist in vertebrate cells and tissues: a nitric oxide (NO)–sensitive cytosolic or soluble GC (sGC) and atrial natriuretic peptides (NP)–activated, plasma membrane–bound, particulate GC (pGC).1 Once produced, the effects of cGMP occur through 3 main groups of cellular target molecules: cGMP-dependent protein kinases (PKGs), cGMP-gated cation channels, and phosphodiesterases (PDEs). PDEs are metallohydrolases that catalyze the breakdown of cAMP or cGMP into the inactive 5’-AMP, thus modulating the duration and intensity of their intracellular response. PDEs have 11 families (PDE1–PDE11) that are encoded by 21 different genes. More than 80 enzyme variants are generated from multiple promoters and as a consequence of alternative splicing.2 PDE1 through PDE3, PDE10, and PDE11 are dual-specificity esterases because they hydrolyze both cAMP and cGMP; PDE4, PDE7, and PDE8 specifically degrade cAMP; and PDE5, PDE6, and PDE9 hydrolyze cGMP.3 The NH$_2$-terminal portion of the PDE enzyme may undergo phosphorylation/dephosphorylation events, binding of Ca$^{2+}$/calmodulin, and allosteric binding of cGMP and can mediate interactions with other proteins. PDE1, PDE3, PDE4, and PDE5 contain phosphorylation sites for various kinases. PDE1 also contains Ca$^{2+}$/calmodulin binding sites, and stimuli that increase or decrease intracellular Ca$^{2+}$ hereby profoundly affecting its activity. PDE2, PDE6, and PDE9 contain allosteric binding sites for cGMP called GAF. The binding of cGMP to GAFB in PDE2 activates the enzyme, whereas the binding of cGMP to GAFA in PDE5 favors PKG-mediated phosphorylation and activation of the enzyme.2 Phosphorylation of PDE5 by PKG serves to increase its cGMP affinity and represents an alternative mode of regulatory feedback inhibition within the cGMP/PKG signaling cascade, thus normalizing levels of cGMP (reviewed elsewhere4). The activation of PKG phosphorylates numerous intracellular proteins that in turn regulate many primary physiological functions such as modulator of vascular tone, vasorelaxation in vascular smooth muscle, endothelial permeability, and cell differentiation and proliferation.3 The PDEs can serve in different functional compartments in cells because they are not colocalized with each other. They are also distributed inside the cell at critical sites and thus regulate local cAMP dynamics in space and time. Such compartmentalization of signaling components allows the extracellular signal to propagate inside the cell along defined and specific pathways within the network.4

In recent years, there has been tremendous interest in discovering the new clinical uses of PDE5 inhibitors in cardiovascular protection (reviewed previously5,6). Today, close to 100 clinical trials with PDE5 inhibitors focusing on the potential cardiovascular benefits (http://www.clinicaltrials.gov) have been completed or are ongoing. Several preclinical studies have demonstrated that PDE5 inhibitors, including sildenafil, have a powerful protective effect against ischemia/reperfusion injury.5–7 Mechanistically, sildenafil protects the heart against ischemia/reperfusion injury through increased expression of NO synthase (NOS)2/NOS3, activation of PKG, PKG-dependent hydrogen sulfide generation, and phosphorylation of glycogen synthase kinase-3β, which is considered a master switch immediately proximal to mitochondrial permeability transition pore and the end effector of cardioprotection8 (Figure 1). Treatment with sildenafil before doxorubicin administration inhibited cardiomyocyte apoptosis, preserved mitochondrial membrane potential and myofibrillar integrity, and prevented left ventricular (LV) dysfunction and ST-segment prolongation. Similarly, tadalafil improved LV function and prevented cardiomyocyte apoptosis in doxorubicin-induced cardiomyopathy through mechanisms involving upregulation of cGMP, PKG activity, and manganese superoxide dismutase levels without interfering with the chemotherapeutic benefits of doxorubicin.9 Pretreatment with sildenafil or PDE5 gene silencing improved survival of adipose-derived stem cells in vitro and improved their impact on cardiac function, fibrosis, cardiomyocyte apoptosis, and vascular density in the postinfarcted LV.8 Moreover, PKG1α gene transfer in adult cardiomyocytes enhanced their resistance to ischemic injury.9 Raising cGMP in the heart by activating pGC with NP at reperfusion also reduced infarct size in rabbits (reviewed elsewhere6). Cinnaciguat (BAY 58-2667), a novel NO-independent activator of sGC that induces cGMP generation, protects against...
ischemia/reperfusion injury through PKG-dependent generation of H₂S in cardiomyocytes and heart.¹⁰

The long-term inhibition of PDE5 with sildenafil suppressed evolving disease and reversed preexisting hypertrophy, interstitial fibrosis, and chamber and myocyte dysfunction in mice.¹¹ The antihypertrophic effects coexisted with PKG activation, and its targets included regulator of G protein–coupled signaling-2, as well as calcineurin-NFAT–transient receptor potential channel 6, one of the nonsensitive and non–voltage-gated ion channels that convey signaling information linked to a broad range of sensory inputs (reviewed previously¹²). However, another study questioned the antihypertrophic role of PKG because its deletion in cardiomyocytes did not affect the development of hypertrophy induced by transaortic constriction or long-term infusion of isoproterenol in mice.¹³ In the hypertrophied right ventricular myocardium, PDE5 is upregulated, PKG activity is inhibited, and cGMP is preferentially shifted to inhibition of PDE3.¹⁴ This leads to an increase in cAMP, protein kinase A activation, increased intracellular calcium, and increased contractility. The increased PDE5 expression predisposed mice to adverse LV remodeling after myocardial infarction. LV systolic dysfunction and diastolic dysfunction were more marked in PDE5 transgenic mice with cardiomyocyte-specific overexpression of PDE5 than in wild-type mice, associated with enhanced hypertrophy and reduced contractile function in isolated cardiomyocytes from remote myocardium.¹⁵ Long-term treatment with sildenafil immediately after myocardial infarction or beginning 3 days after myocardial infarction attenuated ischemic cardiomyopathy,¹⁶ suggesting that PDE5 inhibition may be a promising therapeutic tool for patients with heart failure. Interestingly, PKG activation with sildenafil was also associated with the inhibition of Rho kinase.¹⁶ In normal mice, PDE5 made up ~22% of the LV cGMP hydrolytic activity; the percentage was ~43% in failing mouse hearts. In contrast, PDE5 made up ~5% of the total cGMP hydrolytic activity in LV from normal and failing human hearts, the vast majority of which was attributable to PDE1. This large difference in the relative levels of PDE5 in mouse and human myocardium raises some questions as to whether PDE5 inhibition is as likely to be as beneficial in humans as in mouse models.¹⁷

NO also induces cardioprotection, particularly during acute ischemia/reperfusion injury, through several protection strategies such as the ischemic and pharmacological preconditioning (reviewed previously¹⁸). However, if NOS becomes uncoupled, the formation of reactive oxygen species in combination with low NO bioavailability predisposes the heart to damage during pathophysiological conditions. Accordingly, NO-derived cGMP declines in part as a result of sGC oxidation, whereas the NP-derived cGMP rises in the failing heart, thereby contributing to the pathophysiology. It was shown that PDE5 hydrolyzed cGMP coupled to NOS3-derived NO but not the NP-stimulated pGC.¹⁹

In this issue of Circulation, Zhang and colleagues²⁰ hypothesized that PDE5 substrate specificity is retargeted from cytosolic to particulate cGMP. To test this idea, the authors performed very elegant studies using mice with cardiac myocyte–inducible PDE5 overexpression (P⁵⁺) that were crossed to those lacking NOS3 (N³⁻). Each of these models, ie, P⁵⁺, N³⁻, and P⁵⁺/N³⁻, were then subjected to transaortic constriction–induced hypertrophy. As summarized in Figure 2, P⁵⁺ hearts developed worse dysfunction and hypertrophy and enhanced stimulation by NP. The N³⁻ hearts were protected, displaying no dilation but concentric hypertrophy, less net increase in LV mass, and less myocyte enlargement, which essentially confirmed previous findings.²⁰ Interestingly, the protective effect in N³⁻ hearts was lost in double-crosed (P⁵⁺/N³⁻) mice with LVs developing marked dilatation and dysfunction. PDE5 expression, which was localized mostly in sarcomere in P⁵⁺ hearts, was redistributed in a diffuse pattern, suggesting its retargeting from sGC to pGC (ie, NP-stimulated cGMP) in P⁵⁺/N⁻ mice. However, PKG activation was preserved in N³⁻ after transaortic constriction despite a diffuse PDE5 distribution, which was likely due to persistent low levels of PDE5 activation in these hearts. The
retargeting was also confirmed by an ANP-stimulated rise in cGMP that was blunted more in P5⁺ hearts. Moreover, the potential protective effects from NP signaling were blunted by PDE5 upregulation, thereby worsening maladaptive remodeling but also increasing the therapeutic impact from PDE5 inhibition. Although the superoxide production was high in the P5⁺ hearts after transaortic constriction, its levels were low in the P5⁺/N3⁻ mice, raising questions about its potential cause-and-effect relationship in maladaptive hypertrophy in the double-crossed mice.

The authors should be complimented for reporting this interesting paradigm of functional retargeting of PDE5 from 1 compartment to another. The approach of crossing PDE5⁺ with the N3⁻ mice is innovative in answering this question. As suggested by the authors, such retargeting of the esterase may play a key role in the pathophysiological consequences of the increased PDE5 expression observed in experimental and human heart disease and contribute to the ameliorative effects of its inhibition in heart diseases in which NOS and sGC activity are impaired. Nevertheless, it is not clear whether PDE5 targeting would occur similarly in the NOS2 knockout mice, which also seem to display much less hypertrophy, dilation, fibrosis, and dysfunction in response to transaortic constriction. The identification of proteins involved with PDE5 migration in adult cardiomyocytes is going to be challenging in these studies because of the multitude of protein interactions and their roles in localization. Regardless, it would be interesting to see whether PDE5 retargeting/migration occurs in acute models of myocardial injury, including ischemia/reperfusion, stunning, or infarction. In addition, PDE5 retargeting could potentially play a role in the setting of cardioprotection induced by ischemic preconditioning or postconditioning and pharmacological protective agents, including atrial/brain NP or PDE5 inhibitors, which also appear to use sGC or pGC for generation of the cGMP pool and subsequent activation of PKG. Overall, the present study has set the stage for future research investigations in this important area that might aid in further understanding of the role of PDE5 in cardiac pathophysiology and help optimize therapies based on cGMP-dependent signaling.

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Disclosures
None.

References

Figure 2. Summary of experimental approach and results. PDE5 indicates phosphodiesterase-5; ROS, reactive oxygen species; and PKG, protein kinase G.


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