Controlling Myocyte cGMP
Phosphodiesterase 1 Joins the Fray

Eiki Takimoto

C
gMP is a central intracellular second-messenger regulating numerous cellular functions. In the cardiac myocyte, cGMP mediates effects of nitric oxide and atrial natriuretic peptide, whereas its counterpart, cAMP, mediates catecholamine signaling. Each cyclic nucleotide has a corresponding primary target protein kinase, protein kinase (PK)A for cAMP, and PKG for cGMP. PKA stimulation is associated with enhanced contractility and can stimulate growth, whereas PKG acts as a brake in the heart, capable of countering cAMP–PKA contractile stimulation and inhibiting hypertrophy. Importantly, the duration and magnitude of these signaling cascades are determined not only by generation of cyclic nucleotides, but also by their hydrolysis catalyzed by phosphodiesterases (PDEs). PDE regulation is quite potent, often suppressing an acute rise in a given cyclic nucleotide back to baseline within seconds to minutes. It is also compartmentalized within the cell, so that specific targeted proteins can be regulated by the same “generic” cyclic nucleotide. For many years, the only PDE in the crosshairs for cardiac biologists was PDE3, a principally cAMP-targeted PDE whose inhibition served as the basis for drugs such as milrinone as a heart failure therapy. However, this list was recently expanded with the recognition of PDE4 as a regulator of β-adrenergic signaling and excitation–contraction coupling, and PDE5 for its regulation of cardiac stress responses. With the study of Miller et al., in this issue of Circulation Research, we can now add PDE1 to the list of hypertrophy regulators, via its modulation of cGMP in the myocyte.

The mammalian PDEs comprise a 21 gene superfamily of enzymes grouped into 11 iso-enzymes (PDE1 to PDE11) based on sequence homology, enzymatic properties, and sensitivity to inhibitors. These isoenzymes harbor different specificities to cAMP, cGMP or both, and are also differentially expressed in a variety of tissues. PDE1, PDE2, PDE3, PDE4, PDE5, and PDE9 are expressed in the heart and among these, PDE5 and PDE9 are highly specific to cGMP (Table). Recent studies have demonstrated a role for cGMP modulation by PDE5 in the heart, although the role for PDE9 is unknown at present. PDE5 is upregulated in failing human and hypertrophied mice ventricles. PDE5 inhibitor ameliorates cardiac hypertrophy in mice, and genetic silencing of PDE5 inhibits cardiac myocyte hypertrophy in vitro. Cardiac PDE5 overexpression leads to exacerbated remodeling after myocardial infarction. Furthermore, the cardioprotective effects from PDE5 inhibitors have been reported in various animal models of cardiac pathology, including myocardial infarction, ischemia-reperfusion injury, doxorubicin cardiomyopathy, and cardiomyopathy associated with dystrophic deficiency.

PDE5 is not the only cGMP hydrolyzing PDE in myocardium, as basal activity represents ~30% of total myocardial cGMP-esterase activity in mice and dogs. Although this activity can rise with chronic pathological remodeling (eg, increasing >50% of the total cGMP esterase activity in the pressure-overloaded mouse heart), a large proportion of cGMP-esterase activity is still attributable to other PDEs. A primary candidate has been the dual substrate PDE1. Unlike PDE5, which is stimulated by cGMP binding and PKG phosphorylation, PDE1 is activated by calcium–calmodulin, thus is an appealing target for stress-stimulated regulation. Recent studies have proposed that PDE1 (particularly PDE1C) is a prominent regulator of cGMP hydrolysis in vitro in human normal and failing myocardium, raising interest in defining its role in myocytes in greater detail.

The study of Miller et al. has now revealed an important role of PDE1A in cardiac myocytes and its response to prohypertrophic stimulation. PDE1 has three primary isoforms, PDE1A–C. PDE1A and PDE1B preferentially hydrolyze cGMP with greater affinity than cAMP, whereas PDE1C hydrolyzes both cAMP and cGMP with equal affinity. Prior studies had revealed expression and in vitro enzyme activity, but to date, a physiological role of PDE1 in myocytes and intact hearts has remained unknown, because of the lack of specific inhibitors for the enzyme. Using one such inhibitor (IC86340, developed by ICOS, and unfortunately no longer available), Miller et al demonstrated an antihypertrophic effect in both isolated neonatal and adult rat cardiac myocytes stimulated with phenylephrine or isoproterenol. The IC_{50} of IC86340 shows high specificity for PDE1C (0.06 μmol/L), PDE1B (0.21 μmol/L), PDE1A (0.44 μmol/L) as compared to other myocyte PDEs (>100 μmol/L for PDE2, 3, 4, 5, 9). Though prior reports had focused on PDE1C as the dominant isoform, the present work clearly highlighted PDE1A. The antihypertrophic effect of IC 86340 was comparable to that using the PDE5 inhibitor sildenafil in neonatal rat cardiac myocytes, and intriguingly both combined resulted in a further silencing of the hypertrophic effect, suggesting different and likely compartmentalized targeted pools of cGMP and distal signaling are involved. The present study also

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showed upregulation of PDE1A expression in mice exposed to sustained neurohormones or pressure-overload. Such upregulation, as has been observed with PDE5, could itself depress cGMP, contributing pathological cardiac remodeling.

The study by Miller et al. leaves open the underlying mechanisms by which PDE1 inhibition resulted in suppression of hypertrophy. However, some of the same pathways already reported from natriuretic peptide stimulation or PDE5 inhibition could play a role. These include inhibition of Gq-coupled signaling and in particular inhibition of the calcineurin-NFAT pathway by PKG. The key regulator appears to be regulator of G protein signaling (RGS): both RGS2 and RGS4. These proteins are GTPase accelerators, functioning as negative regulators of Gq activation. Mice lacking RGS2 have exacerbated hypertrophic response to pressure-overload that is not inhibited by sildenafil. Overexpression of RGS4 in mice lacking natriuretic peptide receptor type A rescues the hypertrophic phenotype in the latter coupled to calcineurin-NFAT inhibition. The present study examined responses to isoproterenol which can stimulate Cn-NFAT pathway but does not replicate Gq signaling presented by pressure overload. More studies will be needed.

Another unexplored question is whether and how different pools of cGMP are targeted in localized subcellular pools by PDE1 versus PDE5. Such compartmentation would be consistent with what is now recognized to be a general feature of PDE regulation. Compartmentalized cGMP regulation in myocytes has already been demonstrated for PDE5 and another cGMP targeting (and dual esterase) PDE2. For example, nitric oxide–stimulated soluble guanylyl cyclase generates cGMP that is hydrolyzed by PDE5 localized at Z-bands of adult cardiac myocytes, and inhibiting PDE5 blunts acute β-adrenergic responses coupled to enhanced PKG activation. In contrast, atrial natriuretic peptide stimulation of the receptor guanylyl cyclase generates cGMP that has no impact on β-adrenergic responses and appears targeted more by PDE2 at the sarcolemmal membrane. We do not yet know where PDE1 is localized intracellularly, whether it is differentially coupled to the two cGMP synthetic pathways, whether it modulates acute adrenergic stimulation, and/or whether it targets different or overlapping pools of cGMP to that by PDE5. All are intriguing questions to pursue.

There are some controversies at present regarding which PDE, PDE5, or PDE1, is likely to be more dominant in human hearts for cGMP regulation. The discussions remain largely speculative, based purely on in vitro enzyme assays, with no functional data regarding PDE1 and some data regarding PDE5. Because signaling via PDEs is likely compartmentalized, activity measured in a test tube from cell extracts may not reflect the in vivo activity. The only human functional data to date with a cGMP–PDE has been with sildenafil in normal volunteers, wherein Borlaug et al showed that acute PDE5 inhibition suppresses dobutamine stimulated contractility, just as observed in dogs and in mice. The role of PDE5 inhibition in ameliorating pathological heart disease is currently being tested in the RELAX trial (NCT00763867), an NIH-sponsored multicenter trial of sildenafil for treating heart failure with preserved ejection fraction. The major limitation of PDE1 studies to date has been the very limited availability of selective inhibitors (none are commercially available). As noted, the drug used by Miller et al has been available to some at very limited quantities and there is little left. New agents in amounts that facilitate whole animal and even human studies are needed.

In summary, we can add PDE1 to PDE5 as a likely important regulator of cGMP-hydrolysis and mediator of cardiac hypertrophy. Both appear to contribute as regulatory “brakes” to pathological remodeling, although the relative role and targeted signaling which may be species dependent, remains to be sorted out. The potential to combine both inhibitors as a therapy is intriguing and worthy of further studies. Future in vivo studies using both genetic manipulation of each cGMP-hydrolyzing PDEs and use of chronic selective suppression will hopefully provide needed insights.

### Table. PDEs in the Heart

<table>
<thead>
<tr>
<th>PDE Isoenzyme</th>
<th>Characteristic</th>
<th>cAMP, ( K_{m} (\mu\text{mol/L}) )</th>
<th>cGMP, ( K_{m} (\mu\text{mol/L}) )</th>
<th>Heart Expression</th>
</tr>
</thead>
<tbody>
<tr>
<td>PDE1</td>
<td>Ca(^{2+})/CaM–regulated, dual specificity</td>
<td>73–120</td>
<td>2.6–5</td>
<td>Yes</td>
</tr>
<tr>
<td>PDE1A</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PDE1B</td>
<td></td>
<td>10–24</td>
<td>1.2–5.9</td>
<td></td>
</tr>
<tr>
<td>PDE1C</td>
<td></td>
<td>0.3–1.2</td>
<td>0.6–2.2</td>
<td></td>
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<tr>
<td>PDE2</td>
<td>cGMP-stimulated, dual specificity</td>
<td>30–50</td>
<td>10–30</td>
<td>Yes</td>
</tr>
<tr>
<td>PDE3</td>
<td>cGMP-inhibited, dual specificity</td>
<td>0.02–0.15</td>
<td>0.18</td>
<td>Yes</td>
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<tr>
<td>PDE4</td>
<td>cAMP-specific</td>
<td>2.9–10</td>
<td></td>
<td></td>
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<tr>
<td>PDE5</td>
<td>cGMP-specific</td>
<td>1–6.2</td>
<td></td>
<td>Yes</td>
</tr>
<tr>
<td>PDE9</td>
<td>cGMP-specific</td>
<td>0.17–0.39</td>
<td></td>
<td>?</td>
</tr>
</tbody>
</table>

### Non-standard Abbreviations and Acronyms

- PDE: phosphodiesterase
- PK: protein kinase
- RGS: regulator of G protein signaling
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References

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