Hypertrophied Right Hearts Get Two for the Price of One
Can Inhibiting Phosphodiesterase Type 5 Also Inhibit Phosphodiesterase Type 3?

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Phosphodiesterase type 5 (PDE5) was first discovered in the mid 1970s, not as a PDE but rather as a binding protein for cGMP. Shortly thereafter, it became clear that this protein also had cGMP-selective PDE activity, and cGMP-binding to domains in the N-terminus (GAF domains) regulated its enzymatic activity. PDE5 gained more attention in the mid 1980s when its role in modulating vascular tone revealed. Pharmacologists at Pfizer considered it a potential drug for hypertension or coronary heart disease, which led to the development of UK-92,480 (sildenafil), a selective and potent PDE5 inhibitor. Although the desired cardiovascular responses were unimpressive, the phase I studies revealed a now-famous side effect that was quite popular among the trial participants. This ultimately led to the development of PDE5 inhibitors for erectile dysfunction.

The plot thickened in the late 1990s when our laboratory reported that PDE5 (or PDE5A) appeared to be present and active in canine myocardium and isolated myocytes and that, in normal animals, selective PDE5 inhibition blunted β-adrenergic stimulation. Work subsequently reported by Kukreja and colleagues and Das et al revealed that PDE5 inhibition preconditioned ischemic hearts and was cytoprotective (antiapoptotic) in isolated myocytes. In mice, PDE5 inhibitors blunt adrenergic stimulation in normal myocytes, an action that depends critically on the synthesis of cGMP by nitric oxide–dependent soluble guanylate cyclase. A similar “antiadrenergic” effect was documented in a double-blinded clinical trial in healthy volunteers who showed marked blunting of dobutamine-stimulated contractility when administered sildenafil compared with placebo.

In 2005, we reported that mice administered chronic sildenafil displayed blunting of progressive pressure-overload hypertrophy with improved left ventricular function and that sildenafil also could reverse preexisting hypertrophy. Both antiadrenergic and antihypertrophic effects were coupled to a rise in the activation of cGMP-dependent protein kinase (PKG-1 or cGK-1), and inhibition of PKG-1 blocked the antiadrenergic effect of sildenafil. At the core for all these effects was cGMP/PKG-1 stimulation, which appeared important to the antiadrenergic, preconditioning, cytoprotective, and antihypertrophic influences of PDE5 inhibition.

In this issue of Circulation, Nagendran et al provide a carefully performed set of studies examining PDE5 in the right heart, specifically the hypertrophied right heart. The plot thickens even more. The study makes several intriguing observations. First, PDE5 appears virtually undetectable in the normal human right and left heart but is markedly upregulated in hypertrophied ventricles. Second, inhibiting PDE5 in normal right ventricles (RVs) or RV myocytes does nothing to contraction, but doing this in hypertrophied RVs or cells from them induces a positive inotropic response. These data were obtained using a rat model of RV hypertrophy (RVH) induced by monocrotaline-stimulated pulmonary hypertension. Remarkably, the magnitude of the contractile response to 1 μmol/L sildenafil was identical to that from 100 nmol/L isoproterenol in the hypertrophied RV, and combining isoproterenol with sildenafil yielded the same response as either alone. The authors found that cGMP-PDE activity declined and cGMP rose only in RVH hearts treated with sildenafil, that cAMP rose similarly with sildenafil or isoproterenol, and that sildenafil also blocked cAMP-PDE activity. Sildenafil did not appear to significantly increase PKG-1 activity as assessed by the phosphorylation of vascular signaling protein, although the immunoblot suggests some rise, and basal PKG-1 activity itself appeared reduced in RVH. PDE5 protein expression was present in normal rat RV, although in much lower levels than in RVH. So why the rise in cAMP with sildenafil? Nagendran et al propose that the cGMP raised by cAMP-PDE inhibition competitively inhibited another PDE, PDE3. PDE3 is a dual-substrate esterase that hydrolyzes cAMP 10 times faster than cGMP, so cAMP is its primary target. However, cGMP can bind to the catalytic site to competitively inhibit cAMP catalysis. This mechanism is proposed in the Nagendran et al study, although confirmation remains indirect. Inhibiting protein kinase A (downstream of cAMP) blunted the positive inotropic response to sildenafil, and both milrinone (PDE3 inhibitor) and sildenafil similarly blocked cAMP-PDE. The authors concluded that a PDE3-dependent inotropic effect is induced by inhibiting PDE5 in the hypertrophied RV, improving heart function in the setting of pulmonary hypertension.

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These results are intriguing, although they appear at odds with a fair amount of existing data. PDE5 inhibition has been examined in multiple cell systems, including vascular smooth muscle, heart, and corpus cavernosum, and these studies have not reported marked increases in cAMP or evidence of cAMP-PDE blockade.\textsuperscript{18,19} One argument posed in the study by Nagendran et al is that the lack of a positive isotropic effect in normal hearts is due to a lack of PDE5 expression. However, PDE5 is certainly expressed in these other tissues, yet cross-talk between PDE5 and PDE3 does not appear or is a minor factor. In the study by Nagendran et al, such cross-talk would appear to dominate, with little cGMP stimulating PKG-1. Prior studies performed in human, dog, and mouse showing minimal basal effects of PDE5 inhibition but potent suppression of \( \beta \)-adrenergic costimulation\textsuperscript{10,13} or sildenafil-induced cytoprotection\textsuperscript{10,11} are inconsistent with a complete lack of expression or activity in the normal heart. Isolated mouse and rat myocytes have been studied and reveal functional responses to PDE5 inhibitors consistent with cGMP/PKG-1 activation.\textsuperscript{12,20} As recently reported, cGMP increases little with a PDE5 inhibitor in normal hearts and appears to exist in a subcellular compartment.\textsuperscript{12,15} We have found little cGMP change with acute PDE5 inhibition, in marked contrast to stimulation with a natriuretic peptide, yet only the former acutely modulates adrenergic contractile response.\textsuperscript{15} If anything, one might expect upregulation of PDE5 as observed with RVH to potentiate cGMP-coupled cascades when an inhibitor is applied. Cardiac inotropic modulation by cGMP (and nitric oxide) is known to be dose dependent; at low concentrations, the inhibition of PDE3 and augmentation of contractility by suppressing cAMP hydrolysis are observed, whereas at higher concentrations, the effects are negative as a result of activation of PKG-1.\textsuperscript{21} In the left ventricle, chronic hypertrophy was accompanied by a rise in both PKG-1 activity, particularly in hearts cotreated with sildenafil, and in the activity of PDE5 itself.\textsuperscript{14} In addition, cAMP was found not to be enhanced by sildenafil treatment. Several factors may explain these discrepancies. First, the right heart could be very different from other tissues and the left ventricle, where sildenafil primarily stimulates cGMP–PKG-1–dependent signaling. However, I would guess that this is not the case. Again, the similarity between sildenafil- and isoproterenol-induced inotropy in the study by Nagendran et al is striking yet stands as unique among existing literature. It is possible that with chronic hypertrophy, cGMP generated by PDE5 inhibition is more effective at blocking PDE3 and that such cross-talk is indeed enhanced. This would be important and needs to be confirmed in other models such as those involving the left ventricle. The study used MY-5445, an early PDE5 inhibitor that is less selective and potent than newer agents, and although sildenafil was used in some experiments, dose responses (or plasma levels for the oral treatment study) were not tested. Although sildenafil is pretty selective, it can inhibit other PDEs at higher concentrations.

Is PDE5 normally present in the human heart? To answer this question, we must await more evidence. The present analysis is based on immunohistological staining and in situ polymerase chain reaction, but this may not be enough. Antibodies are polyclonal and vary in their efficacy and specificity for various assays, and better reagents remain needed. We and others have found PDE5 in specific compartments within the myocyte (specifically at z bands),\textsuperscript{11,12} and this appears important to its modulation of adrenergic signaling. Careful enzyme activity and protein chemistry measured in isolated myocytes, data from a wider clinical population, and testing with better and more specific antibodies will be important. The physiological results that have been reported in humans in a randomized placebo-controlled trial\textsuperscript{13} are difficult to interpret if there is no PDE5 in the normal human heart.

Besides the potential impact on understanding PDE5 regulation in the heart itself, the present results may have implications for the use PDE5 inhibitors in treating pulmonary hypertension. PDE5 has long been known to be highly expressed in the lung vasculature, even more than in the corpus cavernosum, so it was not surprising that years after its clinical introduction for erectile dysfunction, these agents were tested and found beneficial for pulmonary hypertension as well.\textsuperscript{22} RVH and enlargement are common in this disease, and it is in this context that the present data should be considered. The key question is whether stimulating cAMP synthesis by inhibiting PDE3, albeit as a consequence of blocking PDE5, would be beneficial in this disease.

The story of PDE3 inhibitors is complex, but their use in left heart failure has largely been abandoned because studies with drugs such as milrinone and amrinone showed acute hemodynamic benefits but chronic toxicity with increased mortality.\textsuperscript{23} Recent efforts to reexamine PDE3 inhibitors in heart failure patients cotreated with a \( \beta \)-blocker did not reproduce earlier negative results but also did not generate a positive result either. Experimental studies have shown that chronic PDE3 diminution can stimulate apoptosis and worsen heart failure.\textsuperscript{24} Nagendran et al\textsuperscript{26} speculate that this is not pertinent as long as the left ventricle is not hypertrophied because PDE5 expression will be minimal. However, this assumes that adverse effects from PDE3 inhibition and increased myocardial cAMP are relevant only to the left ventricle and that the RV will somehow be different. That seems unlikely. Given that existing clinical data with PDE5 inhibitors in patients with RVH and pulmonary hypertension indicate that the treatment is beneficial and tolerated, either PDE3 inhibition is good for the RV, the effect does not actually occur in humans, or the current acute rat data are less applicable if PDE5 is inhibited chronically. Perhaps it is in this setting that PKG-1 stimulation is more manifest. Clearly, more studies, including direct analysis of contractile effects of PDE5 inhibitors in RVH patients, are needed to sort this out. A few years back, no one thought that PDE5 had much to do with the heart at all. Now, it seems as if it may be an important regulator for contraction and stress remodeling pathways. Here is a safe prediction: The plot will continue to thicken.

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References


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