The pathologically hypertrophied and failing heart is a battlefield in a war that would make even George Lucas proud. On the one side, you have hemodynamic, neurohormonal, morphological, and cellular/molecular dark forces urging the ventricle toward decompensation and ultimate demise. On the other, Jedi signaling cascades try valiantly to stave off the impending disaster. Alas, unlike the movies, the dark side often wins, and we need better-equipped counterforces to change this. Current heart failure therapies are fairly defensive—blocking neurohumoral stimuli and hemodynamic overload. However, adaptive/offensive strategies are advancing, including those aimed at enhancing metabolism, vascular supply, and cell regeneration, and those activating molecular signaling to counter maladaptation.

An example of disease-countering (Jedi) signaling is that related to cyclic GMP-dependent protein kinase (PKG). cGMP and its cousin cAMP are second-messenger molecules involved in a broad array of cell signaling. In the heart, the role of cAMP is firmly established, with localized signaling targeting protein kinase A to modulate excitation–contraction coupling and metabolism, and exchange proteins activated by cAMP (Ekacs) to mediate chronic stress. Although sustained cAMP activation contributes to maladaptive stress, cGMP activation of PKG blunts these responses. cGMP is synthesized in heart muscle by 2 pathways—nitric oxide-stimulated soluble guanylyl cyclase (sGC) and a natriuretic peptide receptor-coupled cyclase. Once generated, cGMP binds to regulatory domains in PKG to activate the kinase and to affect signaling. There is no known Epac equivalent for cGMP. cGMP is catalyzed by members of the phosphodiesterase (PDE) superfamily, and by its binding to regulatory (PDE2 and PDE5) or catalytic (PDE3) domains in several of these proteins; cGMP also regulates its own hydrolysis and that of cAMP.

Unlike in the vasculature, where resting cGMP and corresponding PKG activity contribute to vascular tone and endothelial function, basal activity of this signaling cascade in cardiac myocytes is very low. PKG knock-down or loss-of-function studies have found minimal impact on either cardiac morphology or function under resting conditions, and some even questioned its role in the heart. However, similar to an automotive brake, the influence of cGMP and PKG in hearts under stress is greater. During the past decade, studies have shown that cGMP/PKG activation counters a broad array of acute and chronic cardiac stress responses, including those from β-adrenergic stimulation, ischemic injury, pressure and volume overload, and doxorubicin cardiotoxicity.

Methods to activate cGMP/PKG signaling have already been developed as clinical therapies, including those that enhance cGMP synthesis, such as organic nitrates (eg, isosorbide dinitrate), activate guanylate cyclase (eg, cinaciguat or natriuretic peptides), and curtail cGMP hydrolysis (eg, sildenafil, tadalafil). All are either used or being studied in patients with heart failure. PDE5 inhibitors were first thought to have negligible effects on the heart; however, research during the past decade has revealed substantial benefits in a variety of experimental and human cardiac diseases. Results of single-center trials testing sildenafil in patients with dilated heart failure indicated improvements in symptoms and exercise capacity, microvascular function, pulmonary hypertension, and cardiac morphology and function. The Phosphodiesterase-5 Inhibition to Improve Quality of Life And Exerci...
feature of the normal heart, but it is in human HFrEF and experimental pressure overload, supporting current efforts with PDE5 inhibition. Last, PKG itself needs to have something useful to do; that is, a targetable pathway must exist in which modification by the kinase can offset cardiac maladaptation. Some diseases activate these pathways more than others. To date, PKG modulation of the calcineurin/nuclear factor of activated T-cells signaling cascade, transient receptor potential channel 6, mitochondrial ATP-sensitive potassium channel, Ras homolog gene family, member A, regulator of G-protein signaling 2 and 4, and other factors have all been identified as key contributors to its amelioration of cardiac disease. Thus, whether intrinsic cGMP/PKG signaling works as a competent Jedi knight that can be enhanced by therapeutic interventions depends on a balancing act among factors controlling the signaling cascade.

In the current issue of Circulation, van Heerebeek et al present provocative new data regarding this balance, and in particular show how things go awry in HFrEF. The data set is unique—left ventricular endocardial biopsies from nearly 150 patients with nonischemic HFrEF, HFrEF, or aortic stenosis (the latter obtained at surgery from the left ventricular outflow tract). The measurements include passive tension in isolated cardiac myocytes and assays to assess various levels, activity, and posttranslational modifications of proteins involved with the NO/natriuretic peptide–cGMP/PKG cascade. As these investigators reported previously, maximal passive myocyte stiffness is higher in HFrEF than HFrEF (in the new study, they find it higher than in aortic stenosis [AS] patients as well). Their earlier study showed this disparity could be eliminated by incubation with PKA, and in the current research they find a similar result using PKG, which suggests a deficit of PKG activity in HFrEF that is supported more directly by several kinase assays. With regard to why PKG activity might be less in HFrEF, the investigators find cGMP is also much reduced. This correlates with lower pro-brain natriuretic peptide expression; cGMP/PKG expression was similarly reduced in AS patients whose PKG activity is much higher. PDE5 protein expression appears similar among groups. Rather, van Heerebeek et al highlight greater oxidative/nitrosative stress in HFrEF, suggesting reduced NO-stimulated cGMP synthesis as the culprit.

The authors also perform subgroup analysis, examining myocyte passive stiffness, PKG activity, and cGMP levels in each group with or without diabetes mellitus (DM). Patients with HFrEF have the highest stiffness regardless of DM status. However, unlike DM– patients, both HFrEF and AS with DM + similarly reduce PKG activity, raising questions about its role. Furthermore, despite low PKG activity in patients with AS and DM, these subjects have greater cGMP levels unlike patients with HFrEF and DM. These investigators have shown previously that DM exacerbates myocyte passive stiffness in patients with AS, and this seems true in the current analysis, although it is not mentioned. PKG activity also appears to be about half in DM-AS versus AS alone, although significance is not noted.

The current study by van Heerebeek et al poses an intriguing new explanation for HFrEF pathophysiology. Reduced PKG activity in stressed myocardium would be anticipated to exacerbate the pathophysiology of HFrEF, not only through effects on passive properties but also on remodeling and on systole. In mice harboring myocyte-targeted and controllable PDE5 expression, upregulation (lowered PKG activity) worsened hypertrophy, function, and fibrosis in a pressure overload model; reducing PDE5 expression (increasing PKG activity) did the opposite. Although a prior genetic loss-of-function model concluded PKG was unimportant to chronic cardiac stress remodeling, more recent studies, including one from some of the same investigators using a more direct genetic approach to suppress PKG in myocytes, support an important protective role.

The finding of similar improvement in myocyte stiffness in HFrEF (and HFrEF) to PKA (prior data) and now to PKG suggests the residue targets may be similar. Titin, for example, serves as a molecular spring, and both PKG and PKA modify the same residues in the variable N2b region that reduce its stiffness constant. The previous work raised questions about the use of β-blockade in HFrEF, given its suppression of PKA. Rather than stimulate PKA further, however, the current study suggests a similar benefit can be obtained by cGMP/PKG stimulation. van Heerebeek et al speculate that oxidation of titin may also play a role in HFrEF, although the rescue of stiffening by PKG alone in vitro argues for phosphorylation as a dominant mechanism.

The data also raise intriguing questions regarding how one might increase PKG activity to treat HFrEF. Although PDE5 immunohistochemical staining is similar among the groups in the current study, others have reported substantial upregulation of PDE5 expression in HFrEF compared with low levels in normal control subjects. Thus, the current data could be consistent with upregulation in all groups. PDE5 activity was not assessed, and expression does not always reflect enzyme activity as a result of posttranslational changes and alterations in protein localization and function (eg, hydrolysis of cGMP generated by sGC versus natriuretic peptide receptor-coupled cyclase). Last, the left ventricular biopsy analysis reflects limited sampling, whereas prior studies supporting PDE5 upregulation have examined tissue from explanted hearts.

Relatively low pro-brain natriuretic peptide is observed in HFrEF myocardium, and although this may or may not have contributed to the decline in cGMP, natriuretic peptide stimulation still seems a reasonable therapeutic option. The mechanism favored by van Heerebeek et al is that NO-reactive oxygen species (ROS) interactions rise in HFrEF, as reflected by enhanced tissue nitrotyrosine, presumably reflecting diminished nitric oxide synthase-NO-cGMP synthesis. This is interesting, although still speculative. Specifically, confirmation that nitric oxide synthase-derived NO and cGMP were indeed suppressed more prominently (and ROS enhanced reciprocally) in HFrEF was not demonstrated. The nitrotyrosine assay has limitations, one being a lack of specificity for peroxinitrite formation. Although the authors suggest that a greater prevalence of DM may have contributed to redox imbalance in HFrEF, their subset analysis found a similar depression of cGMP and PKG activity in HFrEF with or without DM. It would have been interesting to see nitrotyrosine data for these 2 subgroups. Other studies have found ROS activation and ROS/NO imbalance in
HFrEF models; indeed, this has been suggested as pertinent to patients who respond to isosorbide dinitrate and hydralazine. If HfPfEF reflects greater NO-ROS imbalance, then perhaps direct heme-independent sGC activators such as cinaciguat would be a better choice, given likely redox changes in sGC that blunt its NO response.

As more and more therapeutic avenues become available to modulate cGMP/PKG signaling, interest in this pathway and ways to leverage it for treating heart disease will continue to increase. The recent promising results from PDE5 inhibitor trials in HFrEF, and evidence of potency in HfPfEF as well, are moving this field forward, with pivotal clinical trials now underway. To date, natriuretic peptide therapy has been used subacutely, given the need for intravenous administration. Whether brain natriuretic peptide or newer, more stable and potent natriuretic peptides are beneficial when used chronically remains to be tested. Last, novel methods to improve nitric oxide synthase activity or sGC generation of cGMP are moving forward. Understanding how to use these approaches best requires appreciation of the balance between cGMP synthesis, hydrolysis, and PKG targeting. The Paulus Laboratory and colleagues are to be congratulated for providing us with valuable new insights in this regard.

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References


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David A. Kass

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