Heart Failure: A PKGarious Balancing Act

Running title: *Kass; A PKGarious balancing act*

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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, morphologic, and cellular/molecular dark forces urging the ventricle towards decompensation and ultimate demise. On the other, Jedi signaling cascades valiantly try to stave off the impending disaster. Alas, unlike the movies, the dark side often wins, and we need better-equipped counter forces to change this. Current heart failure therapies are fairly defensive; blocking neuro-humoral 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-counterin (Jedi) signaling is that related to cyclic GMP-dependent protein kinase (PKG). Cyclic GMP and its cousin cAMP are second messenger molecules involved with 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 (Epacs) to mediate chronic stress. Whereas sustained cAMP activation contributes to maladaptive stress, cGMP activation of PKG blunts these responses. cGMP is synthesized in heart muscle by two pathways – nitric oxide stimulated soluble guanylate cyclase (sGC), and a natriuretic peptide receptor-coupled cyclase (rGC). Once generated, cGMP binds to regulatory domains in PKG to activate the kinase and effect signaling. There is no known Epac equivalent for cGMP. Cyclic GMP is catabolized by members of the phosphodiesterase (PDE) super family, 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\(^1\).

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 rest conditions\textsuperscript{2, 3}, and some even questioned its role in the heart\textsuperscript{4}. However, as with an automotive brake, the influence of cGMP and PKG in hearts under stress is greater. Over the past decade, studies have shown that cGMP/PKG activation counters a broad array of acute and chronic cardiac stress responses, including those from beta-adrenergic stimulation\textsuperscript{5}, ischemic injury\textsuperscript{6}, pressure and volume overload\textsuperscript{7, 8}, and doxorubicin cardiotoxicity\textsuperscript{9}.

Methods to activate cGMP/PKG signaling have already been developed as clinical therapies, including those that enhance cGMP synthesis such as organic nitrates (e.g. isosorbide dinitrate), activate GC (e.g. cinaciguat or natriuretic peptides), and curtail cGMP-hydrolysis (e.g. sildenafil, tadalafil). All are either used or being studied in heart failure patients. PDE5 inhibitors were first thought to have negligible effects on the heart; however, research over the past decade has revealed substantial benefits in a variety of experimental and human cardiac diseases. Single-center trials testing sildenafil in dilated heart failure patients reported improvements in symptoms and exercise capacity, microvascular function, pulmonary hypertension, and cardiac morphology and function\textsuperscript{10, 11}. The RELAX trial of 225 patients, an NIH-sponsored multicenter study testing the utility of sildenafil in patients with heart failure and a preserved ejection fraction (HFpEF), completed enrollment in early 2012, and results are anticipated later this year. Another multicenter NIH trial of >2000 patients (PITCH-HF) will start shortly and test the efficacy of tadalafil in patients with heart failure and a reduced ejection fraction (HFrEF) who also have rest and/or exercise induced pulmonary hypertension. On the stimulation side, the combination of isosorbide dinitrite+hydralazine is approved for treating
HFrEF in African-Americans, direct sGC activators are currently being tested in HFrEF, and B-type and more recently CD-type natriuretic peptides are being examined as chronic therapies.

The efficacy of treatments engaging the cGMP/PKG pathway depends upon specifics of the signaling, and this itself can be modified by heart disease. For example, an NO-mimetic leverages sGC to generate cGMP. However, in vascular and cardiac disease, sGC is often subject to oxidative stress that reduces its responsiveness to NO\textsuperscript{12,13}. Direct sGC agonists that are heme-(oxidative state) independent may circumvent this limitation\textsuperscript{13}. Generating more cGMP becomes less effective if cGMP-PDEs are upregulated, and in this setting, inhibiting the appropriate PDEs becomes very useful. Neither elevated cGMP synthesis or hydrolysis is a feature of the normal heart, but it is in human HFrEF\textsuperscript{14,15} and experimental pressure-overload, supporting current efforts with PDE5 inhibition. Lastly, PKG itself needs to have something useful to do – that is a targetable pathway must exist whose modification by the kinase can offset cardiac maladaptation. Some diseases activate these pathways more than others. To date, PKG modulation of the calcineurin/NFAT signaling cascade, transient receptor potential channel 6, mitochondrial ATP-sensitive potassium channel, RhoA, regulator of G-protein signaling 2 and 4, and other factors, have all been identified as key contributors to its amelioration of cardiac disease\textsuperscript{16}. Thus, whether intrinsic cGMP/PKG signaling works as a competent Jedi knight that can be enhanced by therapeutic interventions depends upon a balancing act among factors controlling the signaling cascade.

In the current issue of *Circulation*, van Heerebeek\textsuperscript{17} and colleagues present provocative new data regarding this balance, and in particular show how things go awry in HFpEF. The data set is unique: left ventricular endocardial biopsies from nearly 150 patients with non-ischemic HFrEF, HFpEF, or aortic stenosis (the latter obtained at surgery from the LV outflow tract). The
measurements include passive tension in isolated cardiac myocytes, and assays to assess various levels, activity, and post-translational modifications of proteins involved with the NO/NP-cGMP-PKG cascade. As these investigators have previously reported, maximal passive myocyte stiffness was higher in HFpEF than HFrEF (or AS). Their earlier study showed this disparity could be eliminated by incubation with PKA, and in the current research, they found a similar result using PKG. This suggested a deficit of PKG activity in HFpEF that was more directly supported by several kinase assays. As to why PKG activity might be lower in HFpEF, the investigators found cGMP was also much reduced. Though correlated with lower pro-BNP expression, the latter was also observed in AS patients who had higher PKG activity. PDE5 protein expression appeared similar among groups. Rather, the authors highlighted greater oxidative/nitrosative stress in HFpEF, suggesting reduced NO-stimulated cGMP synthesis as the culprit.

The authors also performed sub-group analysis, examining myocyte passive stiffness, PKG activity, and cGMP levels in each group with or without diabetes mellitus (DM). HFpEF patients had the highest stiffness regardless of DM status. However, unlike DM- patients, both HFpEF and AS with DM+ had similarly reduced PKG activity, raising questions about its role. Furthermore, despite low PKG activity in AS-DM+, these subjects had higher cGMP levels unlike HFpEF-DM+ patients. The investigators have previously shown that DM exacerbates myocyte passive stiffness in AS patients, and this seems true in the current analysis though it was not mentioned. PKG activity also appeared to be about half in DM-AS versus AS alone, though significance not noted.

The van Heerebeek study poses an intriguing new explanation for HFpEF pathophysiology. Reduced PKG activity in stressed myocardium would be anticipated to
exacerbate the pathophysiology of HFpEF, 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 to pressure-overload model, while reducing PDE5 expression (increasing PKG activity) did the opposite\(^2\). Though a prior genetic loss-of function model concluded PKG was unimportant to chronic cardiac stress remodeling\(^4\), more recent studies\(^2\), including one from some of the same investigators\(^3\) employing a more direct genetic approach to suppress PKG in myocytes, support an important protective role.

The finding of similar improvement in myocyte stiffness in HFpEF (and HFrEF) to PKA (prior data\(^1\)) 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 prior work raised questions about the use of beta-blockade in HFpEF, given its suppression of PKA. Rather than further stimulate PKA, however, the current study suggests a similar benefit can be obtained by cGMP/PKG stimulation. The authors speculated that oxidation of titin may also play a role in HFpEF, though the rescue of stiffening by PKG alone in vitro argues for phosphorylation as a dominant mechanism.

The data also raise intriguing questions as to how one might increase PKG activity to treat HFpEF. Though PDE5 immunohistochemistry staining was similar among the groups, others have reported substantial upregulation of PDE5 expression in HFrEF compared to low levels in normal controls\(^14,15\). 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 due to post-translational changes and alterations in protein localization and function (e.g. hydrolysis of cGMP generated by sGC versus rGC)\(^20\). Lastly, the LV biopsy analysis reflects
limited sampling, while prior studies supporting PDE5 upregulation have examined tissue from explanted hearts.

Relatively low BNP was observed in HFpEF myocardium, and while this may or may not have contributed to the decline in cGMP, NP stimulation would still seem a reasonable therapeutic option. The mechanism favored by the authors was that NO-ROS interactions rose in HFpEF reflected by enhanced tissue nitrotyrosine, presumably reflecting diminished NOS-NO-cGMP synthesis. This is interesting, though still speculative. Specifically, confirmation that NOS-derived NO and cGMP were indeed more prominently suppressed (and ROS reciprocally enhanced) in HFpEF was not demonstrated. The nitrotyrosine assay has limitations, one being a lack of specificity for peroxinitrite formation. While the authors suggest that a higher prevalence of DM may have contributed to redox imbalance in HFpEF, their sub-set analysis found similar depression of cGMP and PKG activity in HFpEF with or without DM. It would have been interesting to see nitrotyrosine data for these two sub-groups. Other studies have found ROS activation and ROS/NO imbalance in HFrEF models, indeed this has been suggested as pertinent to such patients who respond to isosorbide dinitrate+hydralazine. If HFpEF 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 rise. The recent promising results from PDE5 inhibitor trials in HFrEF, and evidence of potency in HFpEF as well are moving this field forward, with pivotal clinical trials now underway. To date, natriuretic peptide therapy has been used sub-acutely – given the need for intravenous administration. Whether BNP or newer more stable and potent NPs are beneficial
when used chronically remains to be tested. Lastly, novel methods to improve NOS activity and/or sGC generation of cGMP are moving forward. Understanding how to best use these approaches will require appreciation of the balance between cGMP synthesis, hydrolysis, and PKG targeting. The Paulus laboratory and colleagues are to be congratulated for providing us valuable new insights in this regard.

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**References:**


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