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EDITORIAL COMMENT

Beta Testing New Roles of Cyclic Guanosine Monophosphate in Cardiac Myocyte Contractility

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Cyclic guanosine monophosphate (cGMP) functions as a key second messenger in multiple cell types and physiological processes.[1] cGMP is generated by cytosolic and receptor-associated guanylate cyclases, which are activated by nitric oxide (NO) or circulating natriuretic peptides, respectively. In the cardiovascular system, the cGMP-dependent protein kinase 1 (PKG1) functions as the principal cGMP kinase effector. Much of the early study of cGMP-PKG1 signaling in the cardiovascular system focused on the mechanisms of NO and natriuretic peptide-induced vasodilation. For example, seminal studies of PKG1 substrates within the vascular smooth muscle cell (VSMC) identified PKG1 phosphorylation of components of the VSMC contractile apparatus, such as myosin light chain phosphatase,[2] as critical factors underlying vasorelaxation.

By contrast, the recognition that PKG1 signaling directly regulates cardiac function came later. We now understand, however, that cGMP augmentation opposes pathologic remodeling in a variety of preclinical heart failure models[3] and that disruption of normal cGMP signaling in the myocardium accompanies and likely contributes to the pathogenesis of human heart failure.[1] Studies in mutant mice established that PKG1 mediates these effects globally[4] and in the cardiac myocyte (CM).[5] cGMP-augmenting drugs, including NO-generating nitrates (when combined with hydralazine), guanylate cyclase stimulation with vericiguat, and prevention of natriuretic peptide catalysis with sacubitril/valsartan, are now routinely used to treat patients with heart failure with reduced left ventricular ejection fraction.[1] Experimental studies support the idea that beneficial effects of cGMP-PKG1 signaling in heart failure arise substantially through direct functions in the CM, rather than solely through the afterload-reducing role of PKG1 in the VSMC. PKG1 phosphorylates multiple substrates in myocardial tissue, including sarcomeric proteins such as troponin I[6] and cardiac myosin binding protein C.[7] However, the full range of PKG1 myofilament substrates in the CM has not been examined. In addition, in the CM, separate local pools of cGMP have access to unique subcellular domains containing different combinations of phosphodiesterases (which catabolize cyclic nucleotides), PKG1 isoforms, and PKG1 substrates.[8] These complexities enable regulation of unique PKG1 substrates governed by separate cGMP-generating pathways. In this regard, the specific upstream regulators and signaling axes controlling the PKG1 effect on myofilaments have been unclear.

In this context, Wang et al[9] investigated in this issue of *JACC: Basic to Translational Science* the β1 adrenergic receptor (β_1AR) and how its agonism may selectively regulate PKG1 phosphorylation of CM myofilament proteins. Prior work from this group revealed that carvedilol, a β_1AR antagonist, induces PKG1 activation through a partial agonist role.[10] In the present study, the group performed unbiased phosphoproteome analysis of CMs treated with: the β_1AR agonist dobutamine; the β_1/β_2AR agonist isoproterenol; and carvedilol, a βAR antagonist that also exerts partial agonism of the β_1AR.[9] These findings revealed overlapping phosphorylated substrates between the 3 agents. However, the authors observed a unique set

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of proteins phosphorylated selectively in response to carvedilol, including myosin phosphatase target subunit 1, myosin regulatory light chain, and myosin light chain kinase. They also identified PKG1-specific phosphorylation sites on these substrates. In a series of elegant gain- and loss-of-function studies of the β_1AR, NO synthetase 3, and PKG1, the authors showed that carvedilol partial agonism of the β_1AR acts through protein kinase B2 (also termed AKT2) to phosphorylate NO synthase 3. Ultimately, this NO-induced cGMP activation of PKG1 induces increased myosin light chain phosphorylation and increased CM contractility. Notably, the β_1AR-PKG1 signaling axis exerted minimal effects on CM intracellular calcium mobilization, indicating that β_1AR-mediated PKG1 activation likely promotes positive inotropy through increased sarcomeric calcium (Ca^{2+}) sensitization, rather than through direct effects on Ca^{2+} release from the sarcoplasmic reticulum. Finally, the authors observed disruption of this signaling cascade in human and experimental failing hearts, suggesting that loss of β_1AR-mediated PKG1 myofilament phosphorylation may contribute to worsening left ventricular systolic function in heart failure.

These findings have important implications.[9] First, they provide evidence that PKG1 may, upon specific upstream activation, rapidly promote CM inotropy and thus cardiac contractility. Although PKG1 was previously known to phosphorylate cardiac myosin binding protein-C on residues expected to promote contractility,[7] the actual positive effect of PKG1 on contractility through myofilament phosphorylation had not been shown previously. Interestingly, several mouse models of PKG1 disruption developed rapid loss of left ventricular contractile function during cardiovascular stress, which suggests that loss of β_1AR-mediated PKG1 activation may contribute to these phenotypes.[4,5] In addition, these findings suggest that both carvedilol and cGMP-generating heart failure therapies described earlier may exert their therapeutic effects partially through modulation of the sarcomeric phosphoproteome.

These exciting findings should be noted with consideration of several limitations. First, although the present study[9] identifies a robust and acute positive inotropic effect of carvedilol in the mouse CM and left ventricle, such effects have not been reported in humans. It will therefore be important to learn whether this effect extends across species, as the authors appropriately acknowledge in their work. Second, this study did not evaluate the degree to which the β_1AR-PKG1-myosin light chain axis affects CM oxygen consumption, which could be important for determining the therapeutic relevance of this pathway.

This work[9] raises related mechanistic and clinical questions. First, because carvedilol is not an endogenous molecule, what is the contribution of this biased β_1AR signaling axis to normal adrenergic actions in the mammalian heart? In addition, it remains unclear how exactly biased β_1AR agonism selectively promotes myofilament phosphorylation. Do subpopulations of CM β_1ARs localize to signaling nodes, potentially mediated through binding to currently unknown scaffolding proteins? Although the current work shows a requirement of PKG1 in promoting β_1AR-mediated inotropy, PKG1 phosphorylation of other substrates such as troponin I actually opposes acute increases in CM contractility. Thus, it will be important to understand how β_1AR-regulated PKG1 activation fits into the global effects of PKG1 regulation of cardiac contractility. From the clinical perspective, to what extent does alteration of myosin regulatory light chain phosphorylation and Ca^{2+} sensitization contribute to the therapeutic effect of carvedilol in patients with heart failure, and does this novel mechanism also underlie any of the therapeutic effects of the aforementioned cGMP-generating drugs in heart failure? Finally, and as Wang et al suggest, could targeting the β_1AR in a biased fashion to promote PKG1 activation avoid other deleterious effects of chronic β_1AR stimulation previously observed in human clinical trials? Understanding these questions could have important implications for improving our therapeutic strategies in heart failure.

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