Characterizing the Role of Ser670 Phosphorylation in Non-canonical Mechanisms of GRK2 Inhibition in Heart Failure

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Background and Significance: Heart failure (HF) is characterized by aberrant cardiac beta-adrenergic receptor (β-AR) signaling, leading to upregulation of GPCR kinase 2 (GRK2) and subsequent phosphorylation and desensitization of β-ARs. A peptide inhibitor of GRK2, comprised of the last 194 carboxyl-terminal amino acids of GRK2 (βARKct), has been shown to bind to the G protein beta-gamma subunits, preventing GRK2 binding and β-AR desensitization. Overexpression of βARKct attenuates HF and improves outcomes in animal models. Emerging evidence indicates that following oxidative stress, mitogen-activated protein kinases (MAPKs) phosphorylate the Ser670 (S670) residue of GRK2, which induces GRK2 binding to Hsp90 and localization to mitochondria, where pro-death pathways are initiated. As S670 is also found in βARKct it may prevent endogenous GRK2 accumulation in the mitochondria. We hypothesize that βARKct-mediated cardioprotection in HF is due primarily to mitochondrial GRK2 blockade by βARKct. To test this notion, our lab has generated a cardiac-specific mutant βARKct-S670A transgenic mouse harboring a Ser-to-Ala mutation at the S670 residue that prevents Hsp90 binding and allows for endogenous GRK2 to continue to translocate to the mitochondria upon ischemic injury, while retaining βARKct in the cytosol to act on β-AR signaling pathways.

Methods: In vivo hemodynamic analysis was performed to assess cardiac function in βARKct and βARKct-S670A transgenic mice and respective normal littermate controls (NLCs). Additionally, intracellular cyclic AMP (cAMP) levels in AC16 cardiomyocytes transfected with βARKct or βARKct-S670A, and βARKct-S670D plasmids were quantified in response to increasing doses of isoproterenol.

Results: Early hemodynamic analysis of the βARKct-S670A mice has demonstrated increased baseline contractility in βARKct-S670A mice compared to NLC mice, indicating comparable cardioprotective effects to βARKct mice lacking the Ser-to-Ala mutation, which are mediated by canonical pathways. In vitro cAMP quantification revealed AC16s transfected with βARKct, βARKct-S670A, and βARKct-S670D plasmids increased cAMP accumulation in response to increasing doses of isoproterenol compared to control.

Conclusion: Studies characterizing the mitochondrial pathways involved in HF rescue following ischemic injury are ongoing. These findings will identify new mechanistic information of GRK2 inhibition for HF, and elucidate a new method for attenuating mitochondrial dysfunction during HF.

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