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Selectively Targeting Gi Signaling in Normal and Dysfunctional Myocardium

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One of the salient characteristics of the failing heart is an up-regulation of the alpha-subunit of the adenylyl cyclase (AC) inhibitory heterotrimeric G protein, Gi, both at the protein and transcript level. This increase in Gi can contribute to the loss of contractile function in HF especially through beta-adrenergic receptors (BetaAR). For BetaARs the up-regulation of Gi is one of several derangements leading to an overall dampened and desensitized signaling system. However, this upregulation of Gi protein and transcript levels has also been correlated to improved survival and decreased apoptosis in myocytes *in vitro* in response to chronic BetaAR signaling. Therefore, the *in vivo* beneficial or maladaptive contribution of this upregulation of Gi is presently unknown. The goal of the present work is to specifically target and inhibit intracellular Gi signaling in myocytes to define more precisely the role of Gi up-regulation in the ischemic heart. To this end we have constructed a minigene encoding a peptide inhibitor of Gi signaling. This peptide, which we term GiCT, is comprised of the carboxyl-terminal 63 amino acids of Galphai$_2$ and represents the region of Gi that interacts specifically with the intracellular domains of activated G protein-coupled receptors (GPCRs). We have created a transgenic mouse model with inducible cardiac expression of GiCT using the α-myosin heavy chain (αMHC) promoter in a Tet-Off regulated expression system (Tg-GiCT mice). At baseline, these Tg-GiCT mice display a physiological and structural phenotype consist with that of non-transgenic littermate control (NLC) mice as assessed by echocardiography, hemodynamics, and histology. However, when subjected to stress in the form of myocardial ischemia followed by reperfusion, Tg-GiCT mice demonstrate a significant increase in myocardial infarct size as compared to NLC mice, with unchanged areas at risk. Furthermore, Tg-GiCT mice demonstrate a dramatic increase in myocardial apoptosis in response to ischemia / reperfusion injury as compared to NLC mice.

Figure 1 Selectivity of GiCT minigene for blockade of Gi signals compared to Gs and Gq. Cos-7 cells were transfected with β1AR, α1AR, or empty vector in addition to control or GiCT plasmid. The cells were then stimulated with 10μM isoproterenol, phenylephrine, or lysophosphatidic acid to assess responses through Gs, Gq, or Gi pathways through p42/44 ERK phosphorylation.

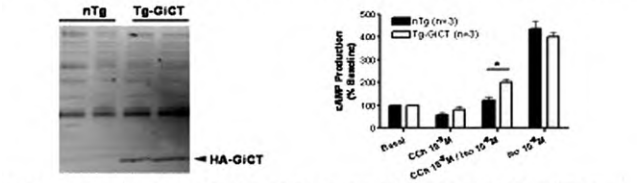

Figure 2: Expression of GiCT transgene at protein Levels, and demonstration of biochemical phenotype in myocytes isolated from Tg-GiCT animals. Tg-GiCT animals demonstrate reduced adenylyl cyclase inhibitory signaling through carbachol which is a Gi coupled agonist. Carbachol pretreatment dramatically reduces the cAMP accumulation induced by isoproterenol stimulation which signals through Gs. GiCT blocks the adenylyl cyclase inhibitory input from carbachol pretreatment.