Dr. Konstantinos Drosatos [mentor: Dr. Ira Goldberg]

"he objective of this proposal is to understand the involvement of cardiac toxic lipids such as ceramide (Cer) and diacylglycerol (DAG) in the impairment of beta-adrenergic receptor (b-AR) function via activation of PKC. Both cardiac Cer and DAG are increased upon long chain fatty acid (LCFA) uptake that occurs in obesity and diabetes. We have observed that three animal models, which express LpL-GPI, PPARg and ACS in the heart and are characterized by cardiolipotoxicity, display impaired contractile and lusitropic responses to catecholamine administration prior to the development of heart failure. Membrane bound-protein kinase C-alpha (PKCa) and PKC-delta (PKCd) are increased in the MHC-LpL-GPI mice. Palmitate (PA) treatment of a cardiomyocyte-derived cell line increased DAG and Cer and reduced b-AR responsiveness to isoproterenol This was reproduced in adult rat primary cardiomyocytes and could be reversed by supplementation of PA with oleic acid. We show that impaired b-AR function is associated with increased intracellular accumulation of non-polar lipids and increased activity of PKCa and PKCd. PA treatment also reduced membrane b-AR density as shown by ligand binding assays and mobility of GFP-tagged b-AR. This effect was abrogated upon PKC inhibition. Both Cer and DAG reproduced the toxic effects of PA. Pharmacological and genetic inhibition of PKCa and PKCd blunted the effect of PA in b-AR insensitivity. Based on these observations it is my hypothesis that DAG and also Cer, lead to cardiac b-AR insensitivity via activation of PKCa and PKCd. To address this hypothesis, I propose the following specific aims: To define the relative contribution of DAG and Cer in b-AR insensitivity. This specific aim will be pursued by modulation of DAG or Cer in cells. For this purpose, I will treat cardiomyocytes with PA and will use recombinant adenovirus-mediated gene transfer to modulate proteins that affect DAG or Cer content. Treatments that will reduce DAG or Cer levels are expected to improve b-AR function. 2. To test whether inhibition of PKC signaling will abrogate defective b-AR function in cardiac lipotoxicity. To pursue this specific aim I will generate mice expressing cardiomyocyte-specific GPI-anchored Lipoprotein Lipase (LpLGPI) on a PKCa deficiency (MHC-LpLGPI +/+ x Prkca-/-) or PKCd deficiency (MHC-LpLGPI +/+ x Prkcd-/-) background. Inactivation of PKCa or PKCd in the MHC-LpLGPI animal model is expected to improve b-AR sensitivity and heart function."