Dr. Steve Marx

CaV1.2, the sarcolemmal L-type Ca2+ channel, plays a key role in cardiac excitation-contraction coupling. Abnormalities in CaV1.2 function, including increased long-opening-mode gating and blunted adrenergic responsiveness, are associated with heart failure and hypertrophy. The increased activation of CaV1.2, in turn, triggers Ca2+-responsive signaling pathways, including those affecting gene expression, which contribute to the pathogenesis of heart failure and hypertrophy. Not surprisingly, CaV1.2 is tightly regulated by components of cell type-specific macromolecular complexes that it anchors. A detailed molecular understanding of CaV1.2 regulation in myocytes has been hampered, however, by the inability to recapitulate and then dissect in heterologous expression systems key aspects of CaV1.2 function in myocytes. Our goals are to gain a better understanding of how CaV1.2 modulation by components of these macromolecular complex impacts cardiac contractility, the development of hypertrophy and heart failure, and the associated electrophysiological complications. We have developed novel tools to surmount major obstacles that have limited progress in the field, and allow us to probe molecular aspects of CaV1.2 regulation, using biochemical and electrophysiological techniques, within the context of cardiomyocytes, but with the power of a heterologous expression system. Using a transgenic (TG) approach that enables selective and reliable expression of FLAG-epitope tagged, dihydropyridine-resistant CaV1.2 channel subunits, harboring mutations at key regulatory sites or covalently linked to regulatory components, in adult cardiomyocytes and at all stages of development, we propose to determine in cardiomyocytes: (a) the role for proteolytic cleavage of the á1C C-terminus, the molecular mechanisms responsible for adrenergic regulation of CaV1.2 current and whether proteolytic cleavage is required for adrenergic regulation of Ca2+ influx in the heart; (b) the role of Ca2+/calmodulin-dependent protein kinase (CaMKII) association with, and phosphorylation of, Ca2+ channel subunits in the regulation of CaV1.2 current; and (c) whether calmodulin (CaM) associated with the C-terminus of á1C regulates channel biosynthesis in cardiomyocytes in a Ca2+-dependent manner. Using novel methodologies to isolate Ca2+ currents from the TG channels and compare these currents to endogenous channels in the same cardiomyocyte, we will determine the molecular mechanisms of adrenergic and CaMKII regulation of CaV1.2 in cardiomyocytes and define how the Ca2+-sensitivity of CaM affects CaV1.2 trafficking in cardiomyocytes, both under physiological conditions and after initiation of heart failure. The three Aims, which should provide key new understandings concerning the regulation of Ca2+ influx in cardiomyocytes, are highly relevant towards understanding cardiac pathologies and the molecular mechanisms responsible for the modulation of cardiac contractility.
