Increased calpain-mediated cleavage of the cardiac Na\(^+\)-Ca\(^{2+}\) exchanger 1 (NCX1) in the heart failure

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The cardiac Na\(^+\)/Ca\(^{2+}\) exchanger 1 (NCX1) is an important regulator of intracellular Ca\(^{2+}\) homeostasis and cardiac function. Its exchange activity is tightly regulated by extracellular and intracellular cations, signaling molecules, hormones and peptides. Calpain is a ubiquitously expressed Ca\(^{2+}\) dependent cysteine protease shown to be activated after Ca\(^{2+}\) overload. An increased activity of both NCX1 and calpain has been associated with pathophysiological conditions in heart diseases such as hypertension, heart failure, ischemia/reperfusion injury, diabetic heart disease and myocardial stunning. We and others have previously shown that calpain cleaves endogenous NCX1 into a 75 kDa proteolytic fragment with a yet unknown function. Thus we hypothesized that calpain might be an important regulator of NCX1 activity during development of heart failure.

Our data show that NCX1 and calpain coimmunoprecipitate in the rat left ventricle, suggesting an interaction between these two proteins in the heart. Moreover, we show that both fulllength NCX1 and the 75 kDa proteolytic NCX1 fragment were upregulated 1.5-fold and 3.1-fold, respectively, in the failing left ventricle of rats after chronic pressure-overload induced by aortic banding for six weeks, indicating a role for NCX1 proteolysis in development of heart failure. This was paralleled by increased protein kinase Ca (PKCa) proteolysis, a known substrate for calpain, implying increased calpain activity in our cardiac disease model. To elucidate the mechanism for NCX1 proteolysis in heart disease, mass spectroscopy will be performed to identify the specific calpain cleavage site. Full length clones of NCX1 and calpain and various deletion mutants thereof have been generated and are currently expressed in HEK293 cells for mapping studies of the interaction and cleavage site. For functional analysis, the 75 kDa NCX1 fragment will be cloned and expressed in the HL-1 cardiomyocyte cell line. In conclusion, we here show increased levels of a 75 kDa proteolytic fragment of NCX1 in the pressure-overloaded heart. Thus, our results suggest a role for calpain-mediated cleavage of NCX1 in heart failure.