Studies in Transgenic Rabbits with Cardiac-Specific Phospholamban Over-expression

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Purpose: A hallmark characteristic of human and experimental heart failure is increased diastolic Ca2+ levels in the cardiomyocyte. Phospholamban (PLN) inhibits uptake of calcium into the SR during diastole by inhibiting the SR calcium pump, SERCA2. Phosphorylation of PLN relieves this inhibition. A secondary means of removing calcium during diastole occurs through the sodium-calcium exchanger (NCX) in the plasma membrane. In PLN knockout mice, ablation of PLN was associated with dramatic increases in Ca2+-cycling and contractile parameters. In contrast to the mouse studies, either ablation or over-expression of PLN in humans was associated with dilated cardiomyopathy, indicating that PLN may play a more critical role in humans. The goal of using the rabbit model is to gain a better understanding of the role of PLN in humans because the rabbit heart more closely mimics human cardiac calcium cycling than the mouse heart. We currently have two lines of transgenic rabbits, one with wild type PLN over-expression (PLN-WT OE) and one with mutant PLN over-expression (PLN-16D17D OE) in the heart.

Hypothesis and Aims: The PLN-WT is expected to result in increased inhibition of SERCA2 due to the increased PLN/SERCA2 ratio, while the 16D17D mutant PLN, which mimics the constitutively phosphorylated PLN form, should not inhibit SERCA2 activity. The aims of this study were to determine the levels of PLN, SERCA2, and NCX expression in the transgenic rabbit hearts and compare to the expression levels in the non-transgenic (NTG) rabbit hearts.

Methods: Homogenized cardiac tissue samples of the rabbit transgenic and wild-type models were prepared and electrophoresed. Gels were transferred to nitrocellulose membranes and detection of SERCA2, PLN and NCX bands were detected using specific primary and secondary antibodies. Protein levels were quantified by the intensity of the bands and normalized to GAPDH levels, a protein whose concentration remains constant in the failing cardiomyocyte.

Results:

- SERCA2 levels: NTG = 1.0, PLN-WT OE (N=3) = 1.01 ± 0.03, PLN16D17D OE (N=3) = 0.90 ± 0.07
- PLN levels: NTG = 1.0, PLN-WT OE (N=3) = 1.34 ± 0.10, PLN16D17D OE (N=3) = 1.43 ± 0.13
- NCX levels: NTG = 1.0, PLN-WT OE (N=2) = 0.86 ± 0.06, PLN16D17D OE (N=2) = 0.89 ± 0.10.

Conclusions:

- There were increased levels of PLN expression in the transgenic rabbit hearts, which was expected.
- The levels of PLN expression in the transgenic rabbits correlated with an expected shift in functional experiments that measured SR Ca uptake in the heart tissue.
- Significant compensatory alterations in other proteins involved in cardiac calcium cycling were not observed in rabbit hearts with this level of PLN overexpression.