**GENE DELIVERY TO MODIFY VASCULAR SMOOTH MUSCLE CALCIUM CHANNEL LOCALIZATION AND FUNCTION**

**J.D. Marsh**, S. Telemaque, M. Fausther, J.A. Dranoff

University of Arkansas for Medical Sciences, Little Rock, AR, USA

Objectives: Develop dominant-negative L-type calcium (Ca) channel beta subunit genes that interdict normal protein trafficking of Ca channels and thus decrease calcium entry/release in vascular smooth muscle (VSM) cells. A long term goal is to develop gene delivery approaches to treat hypertension.

Background: L-type Ca channels play a central role in regulating Ca entry in VSM and thus vascular tone and blood pressure; Ca channels are upregulated in hypertension. Ca channel beta subunits function as chaperones of the pore-forming alpha1c (a1c) subunit, escorting the a1c subunit to the cell membrane. Perturbing protein trafficking may change the Ca dynamics of the cell.

Methods: Truncated beta subunit genes were developed, coupled to a green fluorescent protein (GFP) reporter gene, and cloned into adenoviral (Ad) vectors. VSM cells (A7r5) were infected with the Ad vectors and protein trafficking assessed by fluorescence microscopy and immunoblots. Fura-Red was used to assess intracellular free Ca response to agonists.

Results: Dominant-negative beta subunits caused a shift from cytosol to the membrane fraction of the a1c subunit and also the a2delta subunit. Of note, overexpression of wild-type beta subunits markedly enhanced total cell expression of the IP3 receptor, as well as expression of the a1c subunit. Ca transients were depressed by dominant negative beta subunits.

Conclusion: An Ad vector can deliver specifically to VSM dominant-negative Ca channel beta subunits that perturb calcium channel subunit localization and function.