# PEROXYNITRITE, PUMPS AND PERIVASCULAR ADIPOSE TISSUE: STUDIES ACROSS THE PHYSIOLOGICAL SPECTRUM

Matthew Reifenberger

Dr. Mark Milanick, Dissertation Supervisor

## ABSTRACT

Peroxynitrite ($ONOO^-$) is a reactive nitrogen species produced when nitric oxide (NO) and superoxide ($O_2^-$) react. In vivo studies suggest that reactive oxygen species and perhaps peroxynitrite can influence Na,K-ATPase (Na pump) function. However, the direct effects of peroxynitrite on Na,K-ATPase function remain unknown. We show that a single bolus addition of peroxynitrite inhibit purified renal Na, K – ATPase activity with an $IC_{50}$ of 107 ± 9 μM. Peroxynitrite treatment produced 3-nitrotyrosine residues on the α, β and FXYD subunits of the Na pump and also modified cysteine residues. Taken together these results show that peroxynitrite is a potent inhibitor of Na,K-ATPase activity and that peroxynitrite can induce specific amino acid modifications to the pump. We also investigated if the Na pump was a "target" of peroxynitrite in vivo under cellular conditions. Preliminary evidence suggests that LLC-PK1 cells do contain nitrated Na pumps and that tissue from sedentary high fat fed pigs contain nitrated proteins.

A denitrase activity capable of modifying 3-nitrotyrosine back to tyrosine would have implications for cell signaling/repair mechanisms as well as overall 3-nitrotyrosine levels. The red blood cell, due to its lack of nucleus and long life span, represents an ideal cell type that may or may not contain a denitrase activity. Shown here are results of just two experiments that might suggest a denitrase activity in RBCs. However, other experiments (not shown) seemed to lack

any denitrase activity and ultimately the results from all experiments neither clearly demonstrated nor ruled out an obvious denitrase activity in red blood cells.

Also, presented here is a study investigating some basic aspects of Na pump functioning. Specifically, we demonstrate that terbium is a non-competitive inhibitor of rubidium uptake suggesting it does not bind to the outside transport site of the Na pump. In contrast we show that chrysoidine competes with sodium and potassium for ATPase activity suggesting it binds to the inside transport site. Together these results support that chrysoidine, but not terbium, might be a useful probe for the transport site. We also show that the outside transport site is very specific for monovalent cations over divalent cations.

Also, presented here is a study investigating the effects of perivascular adipose tissue on coronary artery reactivity and the influence of diet and exercise. Results from this study suggest that perivascular adipose tissue blunts contraction induced by endothelin-1 in coronary arteries from normal fat and high fat fed pigs. While exercise abolished this effect normal fat fed pigs, exercise did not alter the anti-contractile effect in the high fat fed pigs.