TEXAS TECH UNIVERSITY
HEALTH SCIENCES CENTER™

Graduate School *of* Biomedical Sciences

Dissertation Defense

"Leak" currents in normal and hyperaldosteronism-associated Na/K pumps

Presented by:

Dylan Meyer

Ph.D. Candidate
Translational Neuroscience and Pharmacology

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ABSTRACT: The Na^+,K^+-ATPase (or Na/K pump) actively exports 3 Na^+ ions and imports 2 K^+ ions across the plasma membrane using energy harnessed from ATP hydrolysis. In doing so, the Na/K pump establishes gradients of Na^+ and K^+ across the plasma membrane that are essential for cellular excitability and secondary active transport. The Na/K pump's canonical $3Na^+$:$2K^+$ exchange means that active Na^+/K^+ transport generates an outward (hyperpolarizing) current. A lesser-known, non-canonical function of the wild-type Na/K pump is as a H^+ importer; this occurs in the absence of external Na^+ and K^+ and is exacerbated by negative membrane voltage and extracellular acidity. Inward currents were also detected in hyperaldosteronism-associated (HA) mutant Na/K pumps within aldosterone-producing adenoma (APA) cells, but these inward currents occur when external Na^+ and K^+ are present. A reported membrane depolarization of APA cells harboring mutant Na/K pumps was attributed to the abnormal inward currents, but our initial experiments suggested that they are too small to profoundly affect membrane voltage. I used inside-out patch clamp, two-electrode voltage clamp, radioactive ion uptake, and radioactive ligand binding assays to determine the mechanism of inward H^+ current through wild-type pumps and the pathophysiological relevance and mechanism(s) of abnormal inward currents through HA mutant pumps. The results indicate that wild-type inward H^+ current occurs in the E2P (outward-facing, phosphorylated) conformation with ATP bound. Abnormal inward currents through HA mutant pumps are carried by Na^+ and H^+ and some traverse a different pathway than wild-type inward H^+ current. Turnover rate measurements revealed that abnormal inward currents through two mutants have similar rates as the wild-type outward current, suggesting that they do not profoundly affect the membrane voltage. Moreover, two HA mutants lack abnormal inward current but generate canonical outward current with reduced ion affinities that impede Na^+/K^+ transport at physiological [Na^+] and [K^+] concentrations. This establishes that inward current through HA mutant pumps is not required for hyperaldosteronism development and points to loss of function as the common mechanism for how these mutant pumps contribute to illness.

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