

**Title:** Aldosterone Upregulates NKCC1 Membrane Proteins and Voltage-Gated Potassium Currents as a Modulator.

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**Introduction:** NKCC1 co-transporter protein facilitates transport of Na<sup>+</sup>, K<sup>+</sup> and Cl<sup>-</sup> into cells. It is present in many parts of the body e.g., kidney, heart, ear, brain, skeletal muscle and hippocampus, and plays an important role in maintaining ionic balance. Also, NKCC1 – located in marginal cells of the cochlear lateral wall, helps to maintain potassium rich endolymph in the inner ear, critical to maintain normal hearing. Age-linked mis-regulation of Na<sup>+</sup> and K<sup>+</sup> ions could result from decreases in NKCC1 levels in the aging cochlea. Recently, we have shown that treatment with aldosterone (ALD) – a natural occurring agonist for NKCC1, reduces age-related decline of NKCC1 in the lateral wall of the cochlea in aging CBA/CaJ mice. Here, we investigated the underlying *in-vitro* cellular mechanisms of ALD treatment for NKCC1 protein, gene and activity levels.

**Methods:** *Cell Culture:* SH-SY5Y neural cell lines were used for *in-vitro* experiments. Cells were cultured in DMEM: F12 media at 37°C, 5% CO<sub>2</sub>. For molecular biology experiments, cells were treated with ALD. *Western Blotting:* Western blot analysis were performed for total cell lysates and membrane fractions. A cellular fractions kit from Cell Signaling (#9038) was used to separate different cellular fractions: nucleus, cytoplasm and membrane fractions. *RT-PCR:* Quantitative RT-PCR experiments were performed using SsoFast EvaGreen supermix. *In-Vitro Electrophysiology:* *In-vitro* whole-cell voltage-clamp electrophysiology experiments were performed using a 700B Multiclamp system.

**Results and Discussion:** An increase in NKCC1 protein expression was observed in response to ALD treatments (1 nM to 10 μM) for both total lysate and cell membrane fractions. No β-actin was observed for membrane fractions as it is a structural protein, not located in the membrane. Total lysate concentration was used to achieve equal loading for membrane fractions. ALD treatment had no effects on the mRNA levels, indicating that ALD-induced protein levels were not associated with increases in RNA levels, i.e., the changes were post-translational. For *in-vitro* whole-cell voltage clamp experiments, an increase in outward potassium currents was observed with application of 1 μM ALD. This increase in outward potassium currents was sensitive to tetraethyl ammonium (TEA)– a K<sup>+</sup> channel blocker; as well as bumetanide– a specific NKCC1 activity blocker. Together, these findings indicate that NKCC1 channels are important biological mechanisms for modulation of ALD-induced outward potassium currents; and that ALD can play a role in cochlear therapeutics.

**Conclusions:** This study elucidates the possible molecular mechanisms ALD exert during the long-term treatment of mice.

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Poster or Podium

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COI Disclosure: JPW, XX, BD and RDF have a patent relating ALD to inner ear therapies for age-related hearing loss.