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Jessica Lin

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Assessing dPrestin & NaDC1 (Indy) Interaction on Calcium Oxalate Crystal Formation in a Drosophila Model of Kidney Stones

Jessica Lin1,2,5, Jacob Anderson2,5, Adam Rossano2,5, Thomas Burghardt2,5, Michael F. Romero2,3,4,5
1Washington University in St. Louis, St. Louis, MO, USA, 2Physiology & Biomedical Engineering, 3O'Brien Urology Research Center; 4Nephrology and Hypertension, 5Mayo Clinic College of Medicine, Rochester, MN, USA

Abstract

Calcium oxalate (CaOx) crystals are one of the most common constituents in kidney stones found in the human renal system. Several factors contribute to the aggregation of these stones, elucidating the role of anion transporter activity leads to a better understanding of this phenomenon. Using a Drosophila model to study the formation and inhibition of CaOx crystals in the fly model, transport anion exchange is observed using both electrophysiology and CaOx birefringence assays. Here, the fly model suffices as it recapitulates renal oxalate function. In addition to dPrestin, the mammalian citrate transporter NaDC1 (Indy) (Karnik et al.) and further pursued in this study with dINDY knockdown (O et al.) and INDY knockdown. Use of the UAS/GAL4 promoter/driver with corresponding RNAi of interest.

Methods

1) Drosophila Genetics: 3 pairs of autosomes, 1 pair of sex chromosomes
- Use of the UAS/GAL4 promoter/driver with corresponding RNAi of interest.

2) Electrophysiology Experiments: By holding the membrane voltage at a controlled value, the kinetics and morphology of the induced currents through a particular ion transporter can be observed and analyzed.

3) Microfluidic Fabrication: prototype created via toner-transfer method to solve problems related to the current limitations (separating apical and basolateral sides of the tubule for more physiologically relevant conditions).

Results

1) Ex vivo 10mM Oxalate MT Tubule Bath:

Fig. 4: Crystal count of wild type (WT), dPrestin knockdown fly MT crystal imaging, (C) INPHY knockdown MT analysis.

Fig. 5: Average crystal size analysis. Statistical analysis done with 1-way ANOVA.

Conclusions

1) Fly MT CaOx Birefringence Assays: crystal decreases with either dPrestin or INPHY knockdown (RNAi) alone.

2) Electrophysiology: still need to identify transporter activity of interaction between Slc26a6 & NaDC1. Preliminary data shows a decrease in oxalate transport with expression of INPHY + dPrestin, however dINPHY is thought to be non-electrogenic based on past experiments.

3) Microfluidics (Future Direction): Many applications with pH and voltage sensors, cell culture, secretion assays
- Develop fully functional microfluidic device for variety of applications in assessing renal function in vivo and ex vivo tissue.
- Greater applications in drug delivery.

Microfluidics

Fig. 6b: Microfluidic design for cell culture and platform for modeling physiological renal system.

References


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Fig. 10: "Assessing dPrestin & NaDC1 (Indy) Interaction on Calcium Oxalate Crystal Formation in a Drosophila Model of Kidney Stones."