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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, 4Nephropathy 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 and synthesized in the human renal system. While several factors contribute to the aggregation of these stones, elucidating the role of ion 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 midgut tubule (MT), isolate transport via dPrestin—the fly Slc26a6 (CaOx) exchanger—was studied using both electrophysiology and MT dissection with CaOx birefringence assays. Here, the fly model serves as an recapitulates renal oxalate function. In addition to dPrestin, the mammalian cilare transporter NaDC1 (Indy) was developed to have a protein-protein interaction with Slc26a6 such that oxalate transport is increased above normal [Ghana et al.] and further pursued in this study with the fly system. In order to more faithfully control the perfusion of the fly MT in these studies, a toner transfer microfluidic device was developed to better assess renal function via a range of genetically encoded pH and voltage sensors. Preliminary results from ex-vivo MT CaOx assays reveal an increase in crystal count with dPrestin and Indy knockdown (RNAi) alone, however statistically insignificant. Electrophysiology experiments demonstrate the co-expression of dPrestin and dINDY in Xanupa oxyx increase oxalate transport, with possible voltage-dependent activity. This work investigates the mechanisms of CaOx formation in the renal system via two transporters. Further work includes developing a fully functional microfluidic platform for assessing the formation of CaOx in a physiologically accurate renal tubule system.

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) in MT perfusion and secretion assays.

Results

1) Ex-vivo 1 hour 10mM Oxalate MT tubule bath:

2) Preliminary Electrophysiology Experiments:

Microfluidics

Fig. 3: Protocol for MT CaOx crystallization studies.

Fig. 4: Voltage Clamp method.

Fig. 5: Physiological process of CaOx kidney stone formation in the fly, along with sequence.

Relevance of NaDC1 (Indy) & Slc26a6 (dPrestin):

• Rapid CaOx stone formation
• Genetic Manipulation: Use of the UAS/GAL4 promoter/driver with corresponding RNAi of interest

Fig. 2: (A) Function of Prestin stitches. (B) Proposed protein interaction between dPrestin and INDI in the fly.

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

Fig. 7: (A) WT midgut tubule birefringence imaging with CaOx stones. (B) dPrestin knockdown (MT) crystal imaging. (C) dINDY knockdown (MT) analysis.

Fig. 8: (A) Crystals counted over 600 μm2. (B) Average crystal size analysis. Statistical analysis done with 1-way ANOVA.

Background

Kidney Stone Formation (Nephrolithiasis) & Drosophila Model:

- Rapid CaOx stone formation
- Genetic Manipulation: Use of the UAS/GAL4 promoter/driver with corresponding RNAi of interest

Fig. 1: Kidney Stone Formation (Nephrolithiasis) & Drosophila Model:

Fig. 3: Protocol (adapted from Easley, et al.) for microfluidic device. (B) Equation for controlling depth of etch.

Fig. 4: Tonic prestin, in applications in assessing renal function in vitro and with ex-vivo tissue.

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

Conclusions

1) Fly MT CaOx Birefringence Assays: crystal decreases with either dPrestin or Indy 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 coexpression of dINDY + dPrestin, however dINDY is thought to be non-electrogenic based on past experiments.

- 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 vitro and with ex-vivo tissue

• Greater applications in drug delivery

References


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