Anchors Aweigh: Ankyrin G modulates the epithelial sodium channel in collecting duct epithelial cells
Christine A. Klemens, Michael B. Butterworth
Department of Cell Biology, University of Pittsburgh

Introduction

The Epithelial Sodium Channel (ENaC): Biosynthesis and Degradation

Fig 1. ENaC life cycle (see text for details).

- Aldosterone regulates Na⁺ transport in the kidney distal nephron by increasing ENaC translation
- ENaC is transported to the apical membrane.
- Na⁺ reabsorption from the urine is determined by the number and activity of ENaC at the membrane.
- ENaC internalization is triggered by ubiquitination by the E3 ubiquitin ligase, Nedd4-2.
- Ankyrin G (AnkG) is a novel aldosterone-regulated protein that may be interacting with ENaC at any place in this cycle.

βENaC contains an AnkG binding motif near the Nedd4-2 binding and ubiquitination site

β ENaC C-terminus

We hypothesize that AnkG modulates the interaction between Nedd4-2 and ENaC to alter the apical membrane surface density of the channel and increase Na⁺ reabsorption.

Methods

Cell lines - Fisher Rat Thyroid Cells (FRTs), mouse cortical collecting duct cells (mCCDs)

E. Phys – ENaC currents were measured in Ussing chambers using short circuit currents (Isc)

Western blot – cells were homogenized and run according to standard protocols

Biotinylation – The apical side of polarized FRTs transfected with ENaC subunits were incubated with biotin and quenched with FBS. After cell lysis, ENaC was immunoprecipitated from equal amounts of protein. Biotinylated ENaC from this concentrated fraction was then pulled down with streptavidin and run on a Western blot, and normalized to the immunoprecipitated fraction

Results

ENaC activity is altered by AnkG expression

A) Western blot showing AnkG overexpression (OE) and knockdown (KD)
B) Short-circuit current (Isc) traces of ENaC activity with AnkG OE or KD
C) Summarized ISC data for AnkG KD (n=15) and OE (n=12), ***=p<.001

Fig 3. AnkG expression impacts ENaC activity

- Total ENaC expression is increased by AnkG

A) Western blot showing α-, β-, and γENaC subunit expression from whole cell lysates
B) Summarized data from experiments similar to A. (n=3-5, **=p<0.01, *=P<0.05)

Surface βENaC is increased by AnkG

A) Western blot showing total and surface βENaC
B) AnkG OE increases apical βENaC 71% ± 26%, n=4, p-value=0.02

Fig 5. AnkG increases apical membrane βENaC

Acknowledgements

- Thanks to R. Edinger, Jackie Ho, Mike Myerburg, and the University of Pittsburgh Center for Biological Imaging
- Funding from NIH DK078917, DK079307
- American Society of Nephrology
- CBMP Teaching Fellowship

Conclusions

- βENaC has an AnkG binding motif
- ENaC current increases with AnkG over expression and decreases with AnkG knockdown
- AnkG increases expression of all three ENaC subunits
- Over expression of AnkG increases βENaC at the cell surface
- AnkG over expression does not increase activity of a mutant βENaC ENaC that cannot be ubiquitinated

AnkG OE does not impact activity of a βENaC mutant that cannot be ubiquitinated

Fig 6. AnkG OE does not increase ENaC P616L current. The P616L mutation increases ENaC current 81% ± 17% relative to WT channels; however, AnkG OE does not further increase ENaC P616L activity