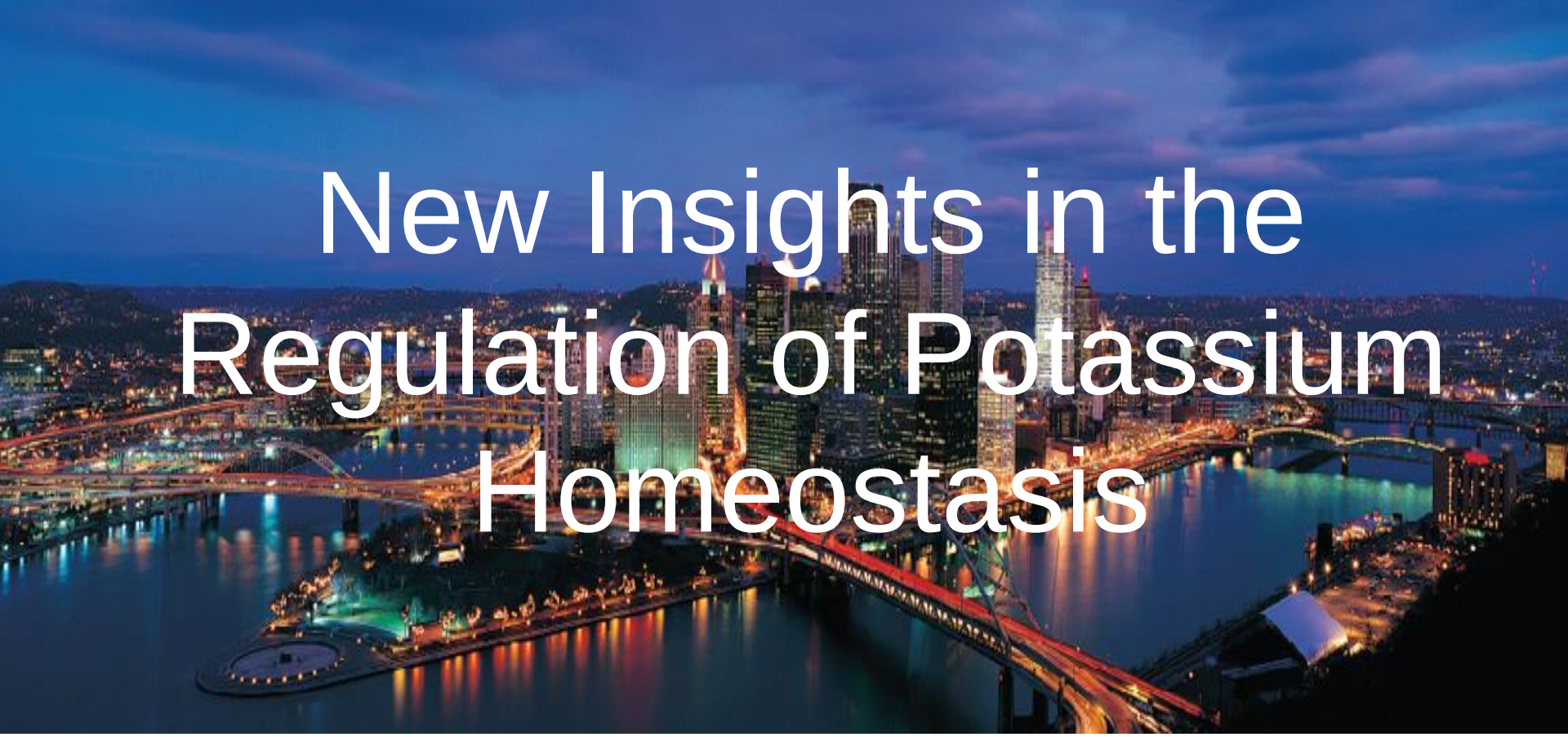

New Insights in the Regulation of Potassium Homeostasis

Thomas Kleyman, MD
Chief, Renal-Electrolyte Division
Adler Distinguished Professor of Medicine
Professor of Cell Biology and of Pharmacology and Chemical Biology
University of Pittsburgh

Claude Bernard

- "La fixité du milieu intérieur est la condition d'une vie libre et indépendante" (*"The constancy of the internal environment is the condition for a free and independent life"*)

Kidney - has a major role in fluid and electrolyte homeostasis

- K homeostasis
  - Match rates of renal K excretion to K intake

Patient

- 53 yo M admitted with cough and dyspnea
- Exam: Hypotension, basilar rales
- CXR: multilobar infiltrates
- K 4.6 : SCr 2.4
- Rx antibiotics

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Medical management in a monitored setting

Why did the patient develop hyperkalemia?

Potassium Homeostasis

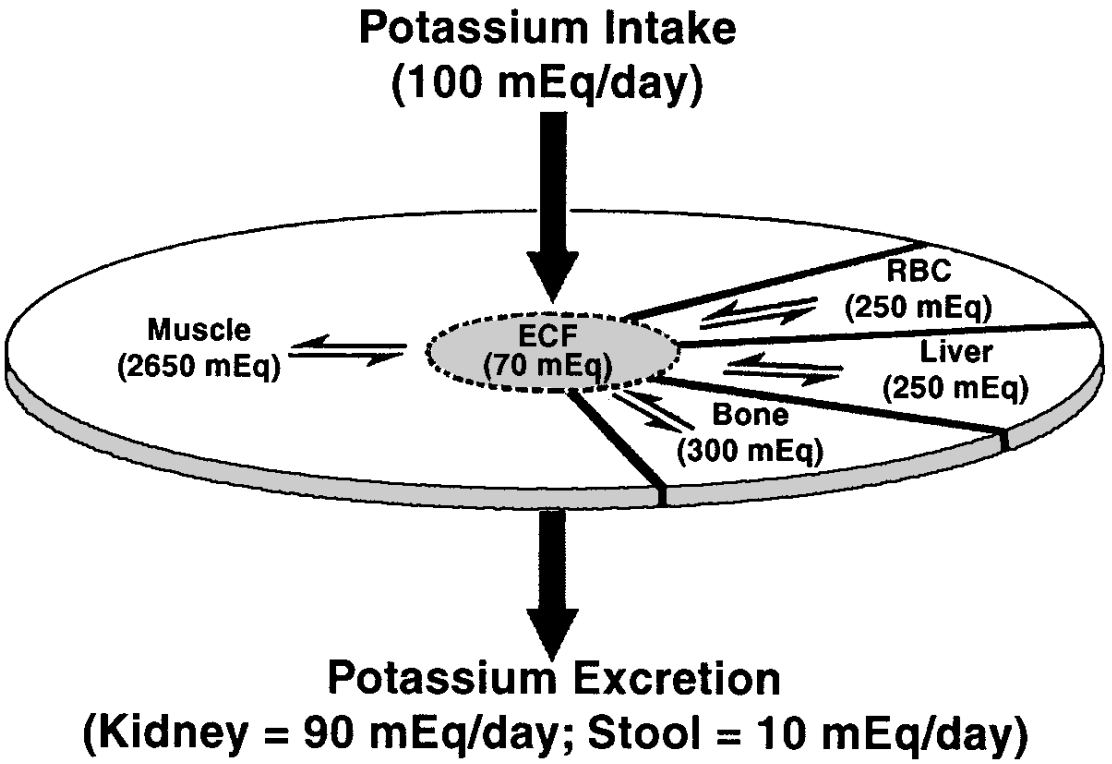
- Total body K^+ stores
  - 3,000 - 4,000 meq
- 98% intracellular
- 2% extracellular

Potassium Homeostasis

- Ingest 100 meq K^+
  - If K^+ remains in ECF
    - Increase $[K^+]$ by ~6.5 meq/L
      - ($[K^+]$ >10 meq/L)
- Maintain a constant $[K^+]_{ECF}$
  - "buffer" K^+ load

- Acute – redistribute K^+ into intracellular stores
- Chronic - renal / GI excretion

Potassium Homeostasis



Giebisch, AJP: Renal 1998

Why did this patient develop hyperkalemia?

- Increase K intake
  - oral or IV
- Shift of K from intracellular to extracellular space
  - Enhanced release of K from cells
  - Impaired cellular uptake
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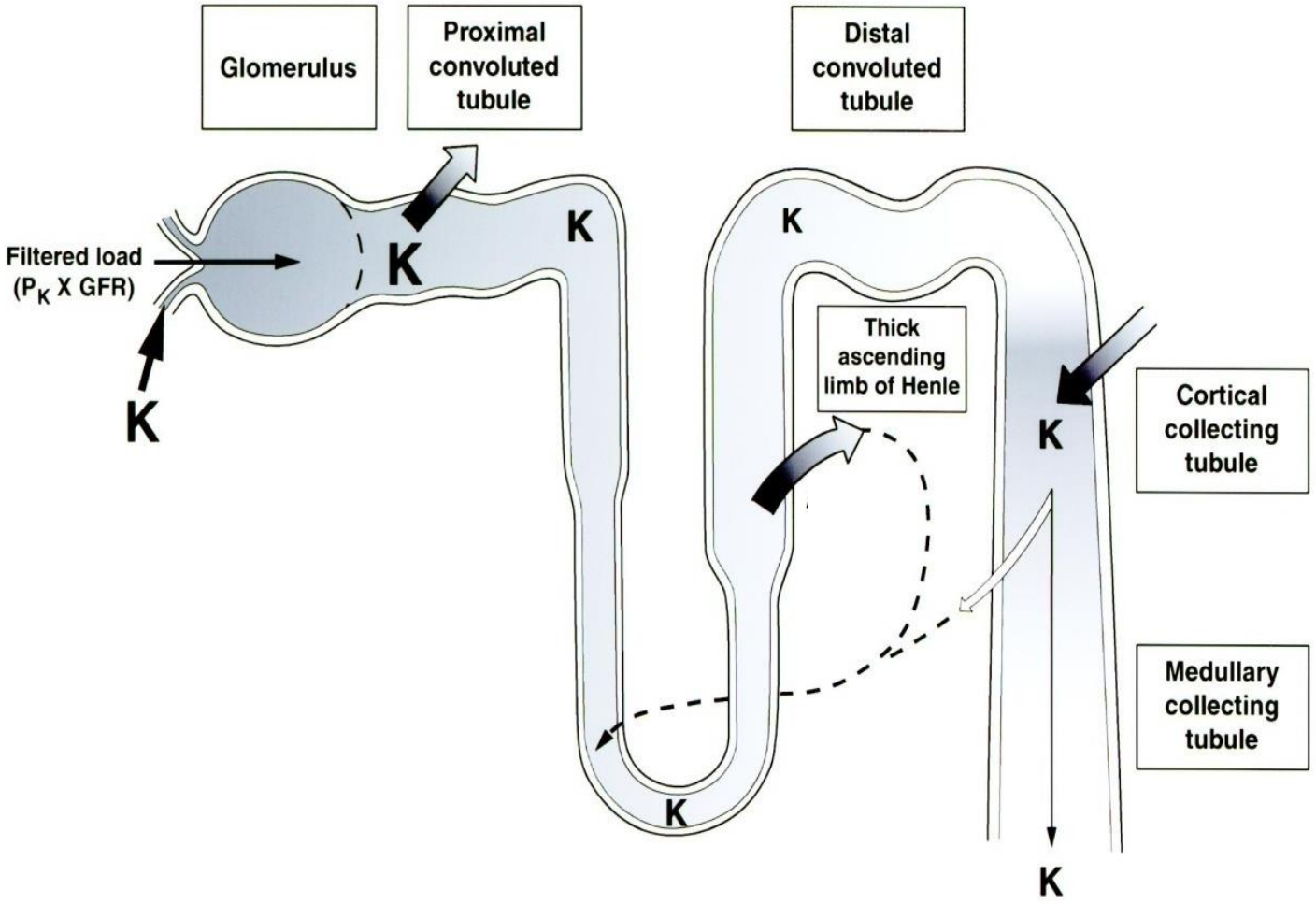
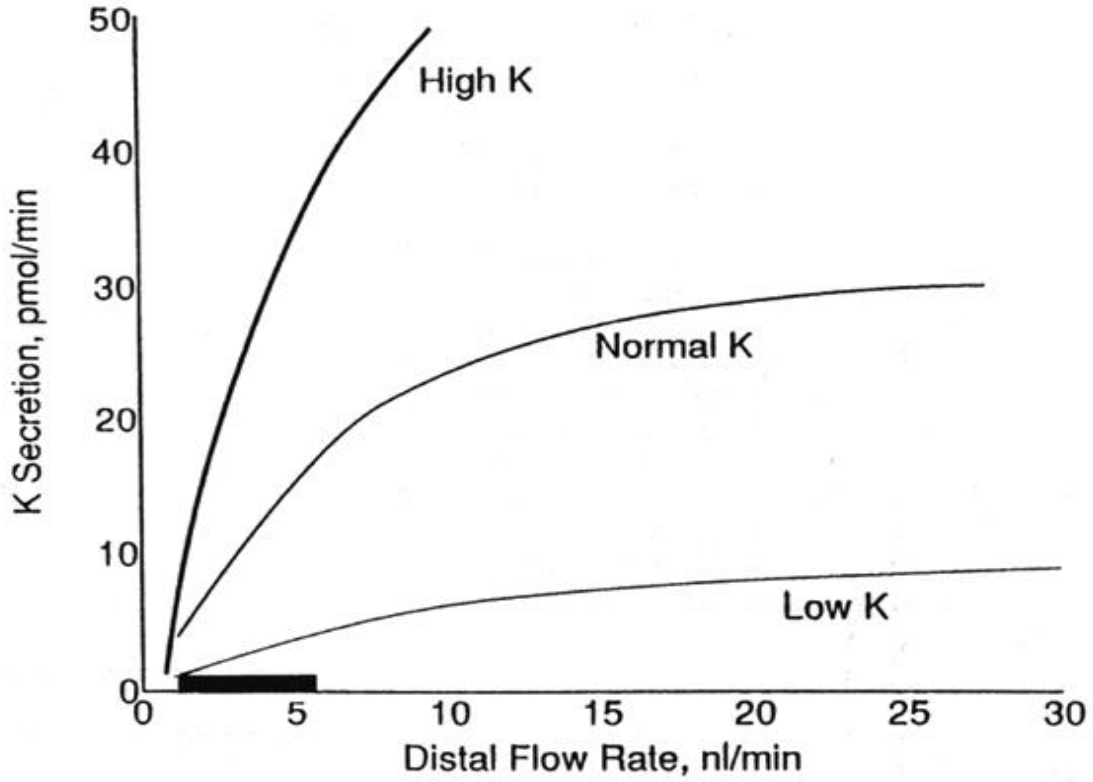
  - Renal failure (AKI, CKD, ESRD)
  - Other factors

Renal Potassium Handling



Urinary K^+ excretion is determined by K^+ secretion in the distal nephron.
Malnic, Klose and Giebisch: AJP 1964, 1966

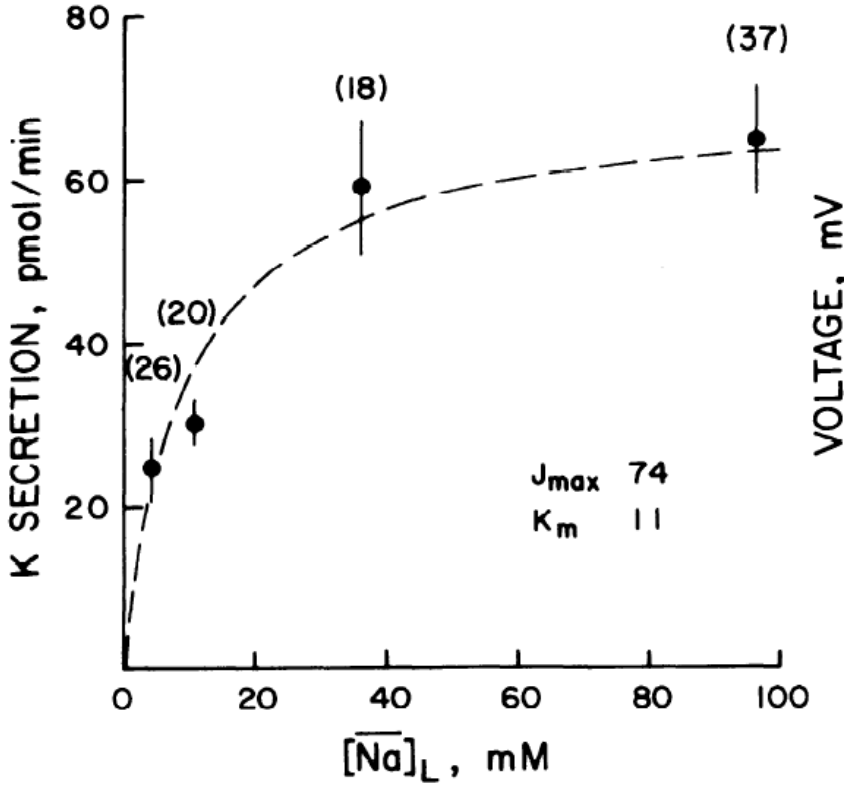
Flow and increases in dietary K^+ activates K^+ secretion in the ASDN



Khuri et al AJP 1975

Luminal Na^+ is required for renal K^+ secretion

Effect saturates at a luminal [Na^+] of ~40 mM



Good et al AJP 1984

Why does an increase in tubular flow enhance renal K^+ secretion

- Increases in distal tubular flow usually accompanied with increases luminal [Na^+].
- Increases in ENaC-dependent Na^+ absorption leads to luminal hyperpolarization and a favorable **electrical gradient** for K^+ secretion.
- Enhanced sodium entry from the lumen stimulates the basolateral **Na,K-ATPase**, enhancing K^+ uptake into cells and luminal K^+ secretion.
- Increases in distal tubular flow limits the increase in luminal K^+, maintaining a favorable **chemical gradient** for K^+ secretion.

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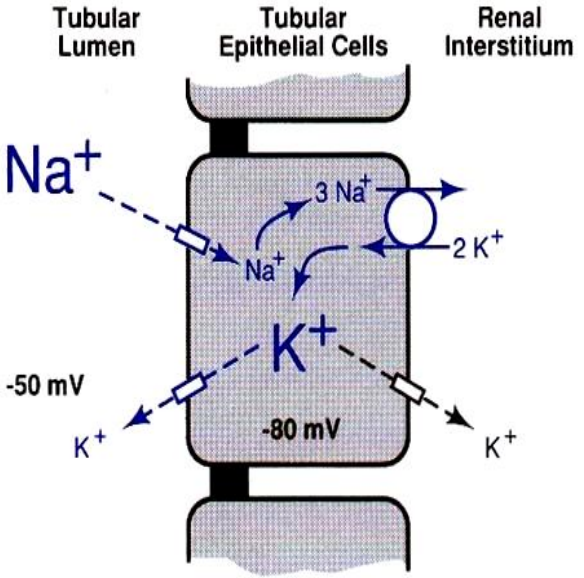
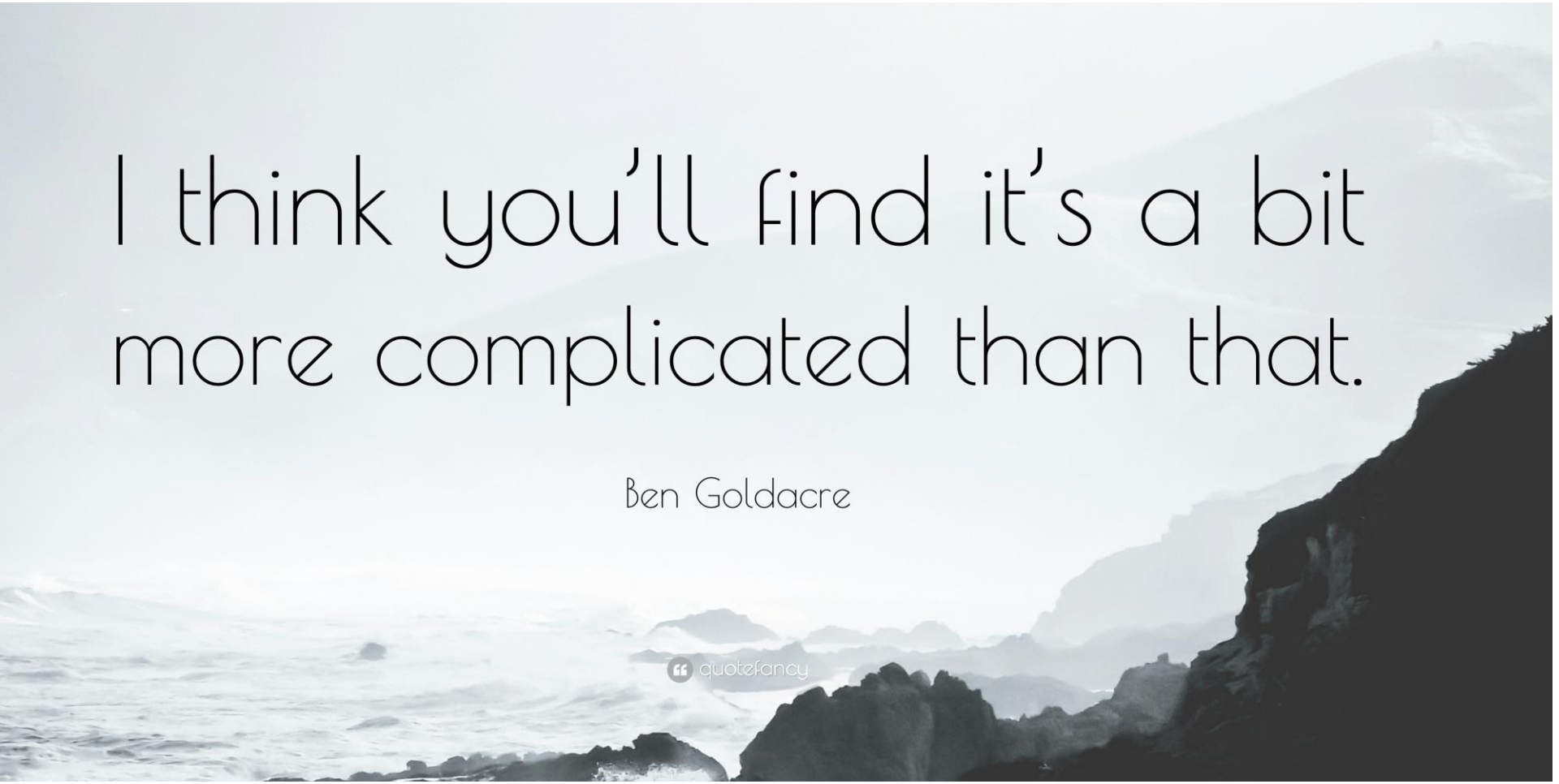
Epithelial Na^+ Channels (ENaCs)

- Na^+ entry generates a favorable electrical gradient for K^+ secretion
- Na^+ extrusion via the Na/K-ATPase - favorable chemical gradient for K^+ secretion
- ENaC is required for K^+ secretion in the ASDN



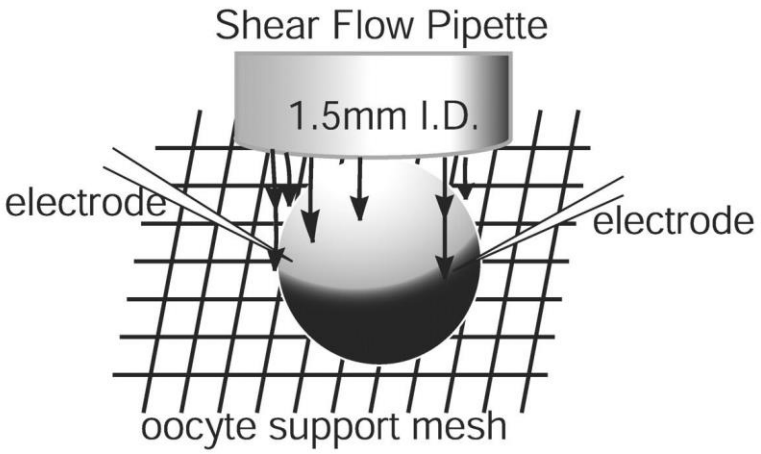

I think you'll find it's a bit more complicated than that.
Ben Goldacre
quotefancy

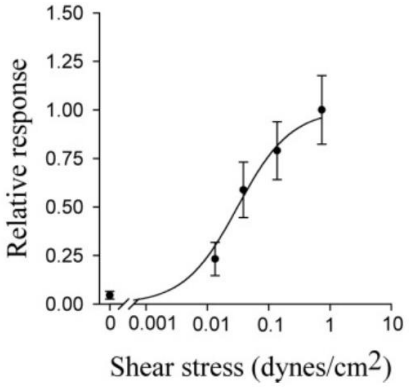
Increases in tubular flow in the ASDN directly activates ENaC, and indirectly activates a K^+ secretory channel (BK) in intercalated cells

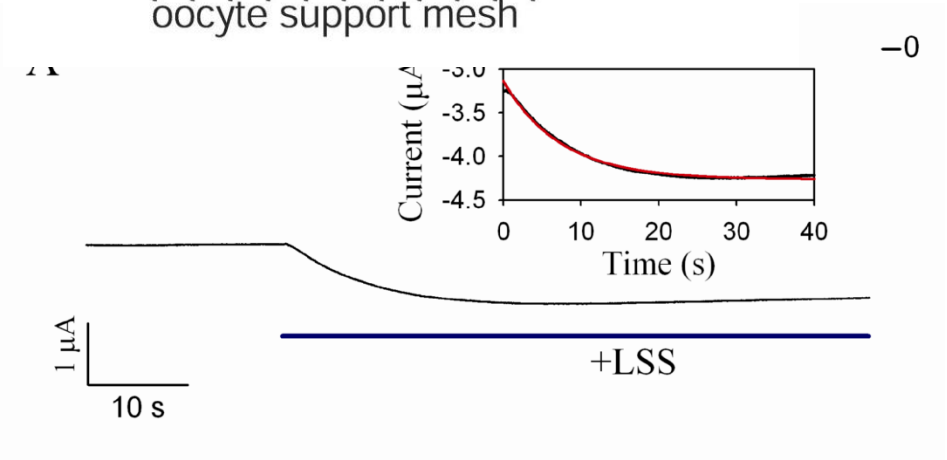
Activate epithelial Na^+ channels and renal K^+ secretion

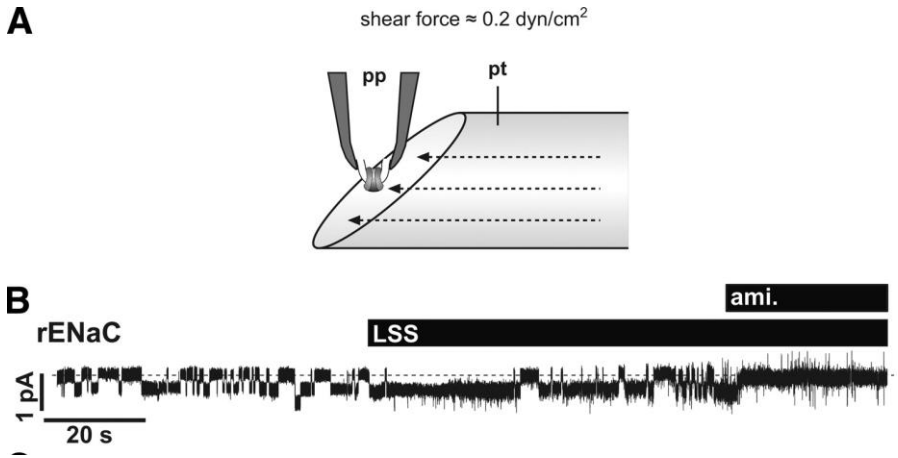
- Aldosterone
- Dietary K
- **Flow**

Activation of ENaC by shear stress





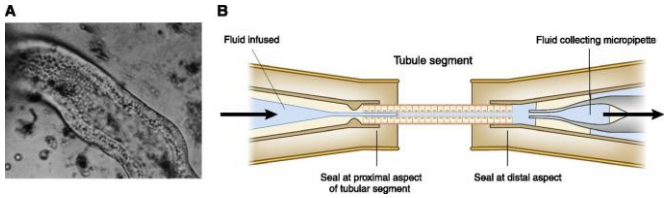




Carattino et al. JBC 2004

Althaus… Fronius, FASEB J. 2007

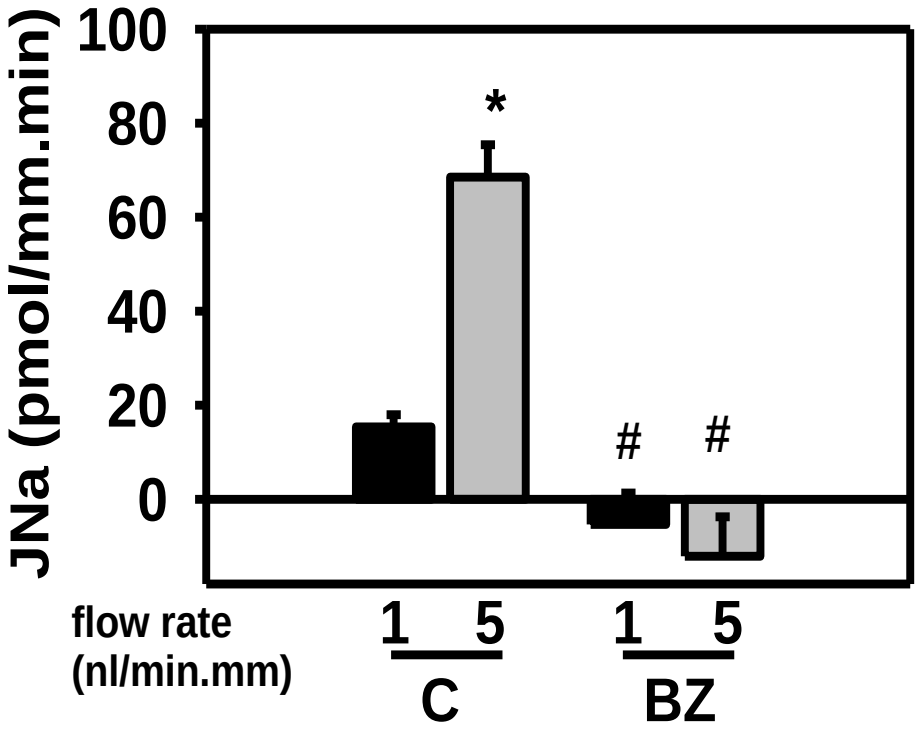
Basal and flow-induced K^+ secretion are dependent on Na^+ absorption

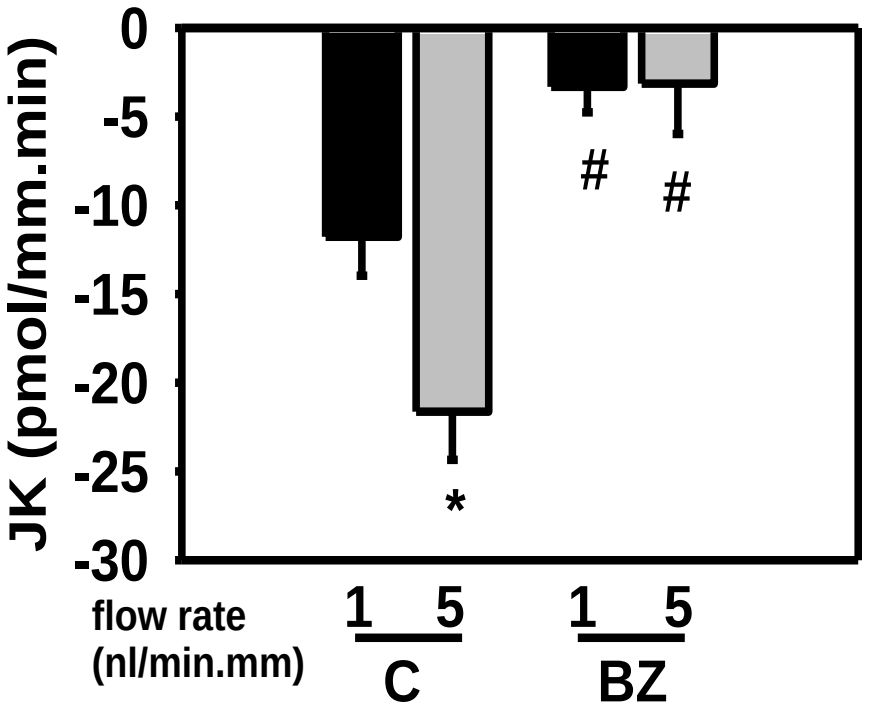


Hoenig and Zeidel, CJASN 2014

Na

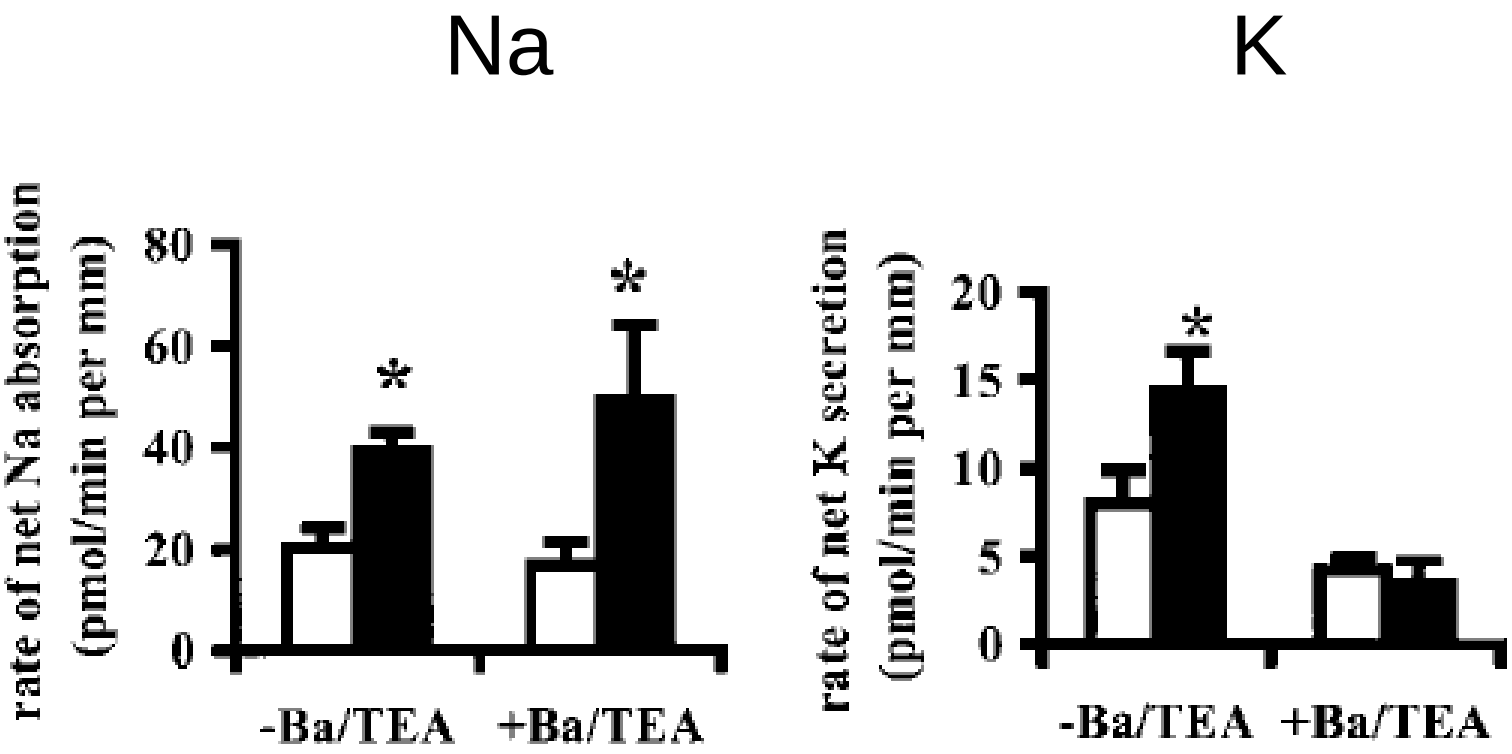
K





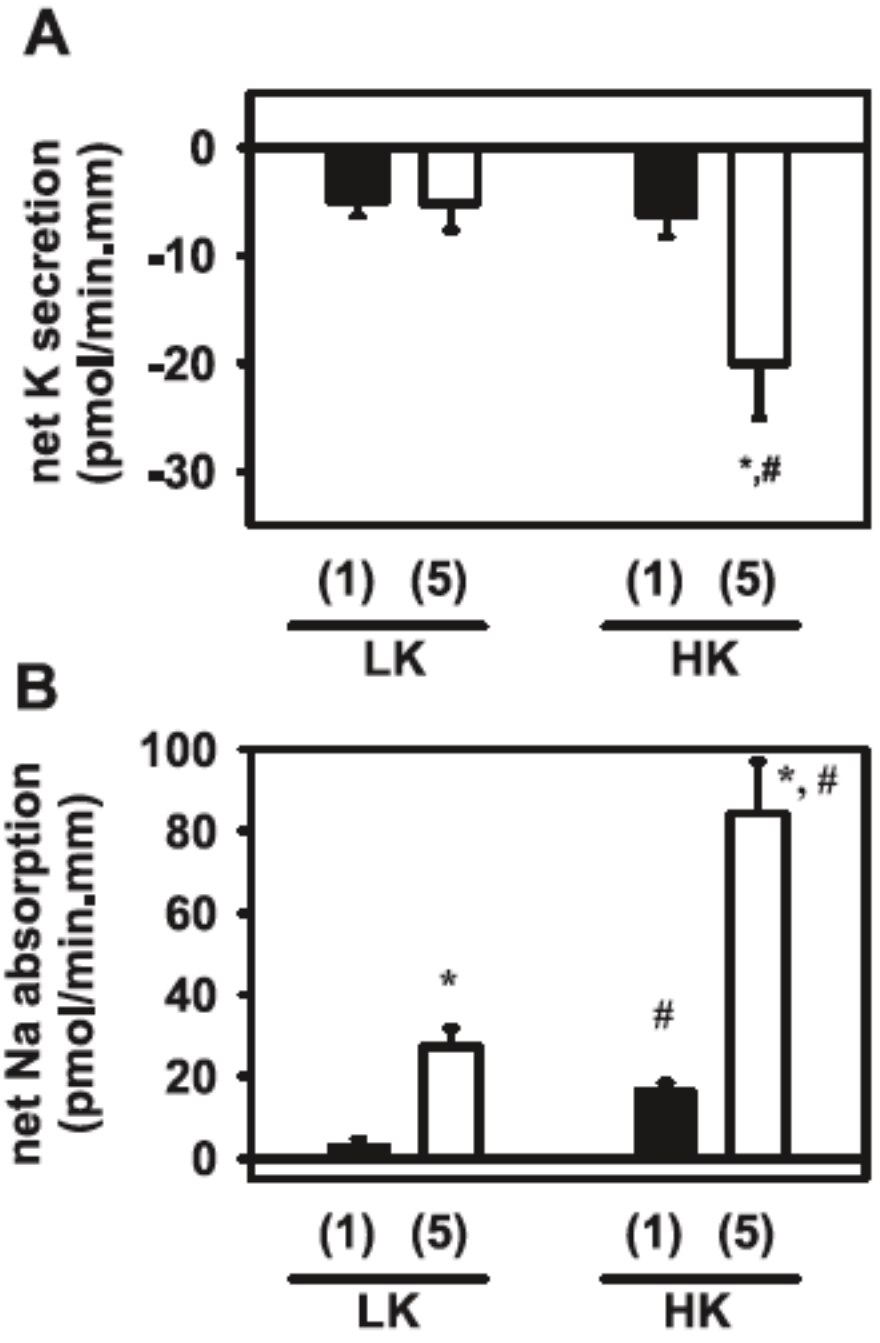
Morimoto et al, AJP 2006

Na^+ absorption is not dependent on K^+ secretion



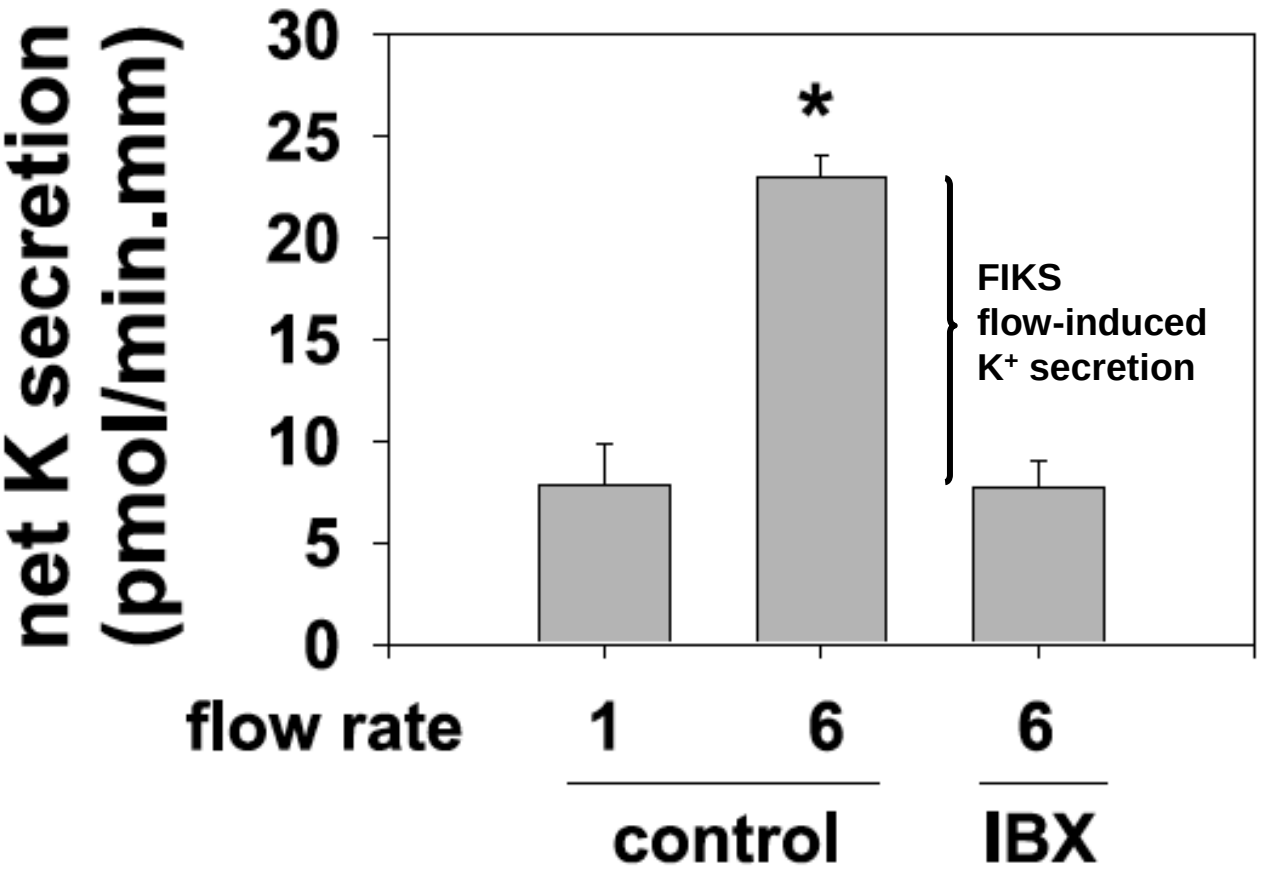
Satlin et al: AJP 2001

Effects of dietary K on renal K secretion



Najjar AJP 2005

The flow-induced component of K^+ secretion is blocked by Iberiotoxin, a selective inhibitor of BK channels



Woda et al. Am J Physiol, 285: F629, 2003

BK channels

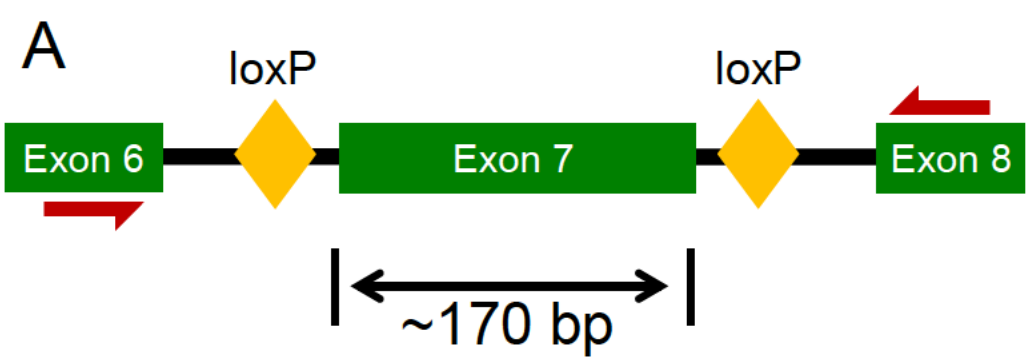
- Large conductance Ca^{2+} activated K^{+} channels
- pore-forming α subunit which undergoes extensive alternative splicing that modifies
  - Ca^{2+} and voltage sensitivity
  - regulated by phosphorylation and other signaling cascades
  - cell surface expression
- regulatory β subunit
- regulatory γ subunit (LRRCs)
  - Allows BK channels to open at near-physiological Ca^{2+} and membrane voltage in non-excitable cells
- Inhibited by Iberiotoxin (IBX)

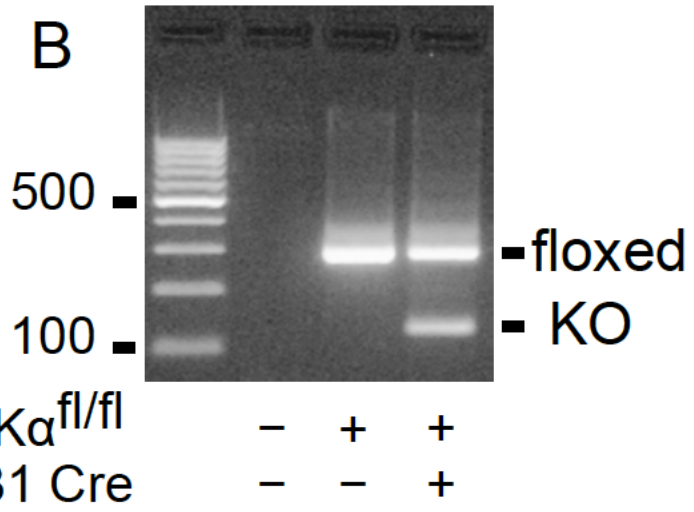
BK channels

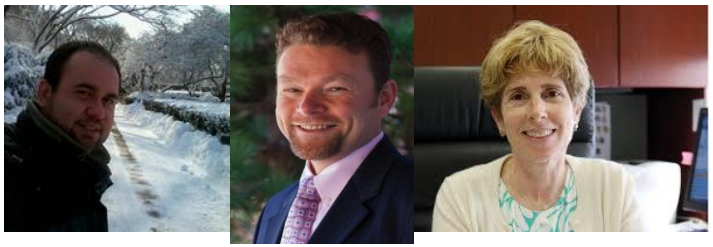
- Functional expression in both intercalated cells (ICs) and principal cells (PCs)
  - IC>PC
- Immunolocalization
  - Initial results suggested that BK expression is primarily in ICs
    - Increased IC expression with a high K^+ diet
  - Later studies suggest that BK is expressed in the primary cilium in PCs

Are BK channels in intercalated cells responsible for flow-induced K^+ secretion?

Generated and analyzed an intercalated cell BK α subunit KO mouse



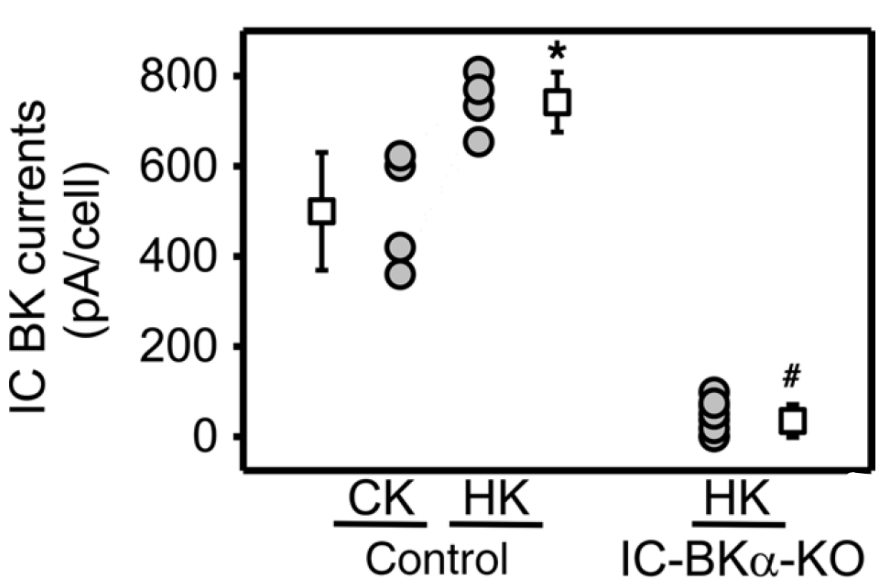


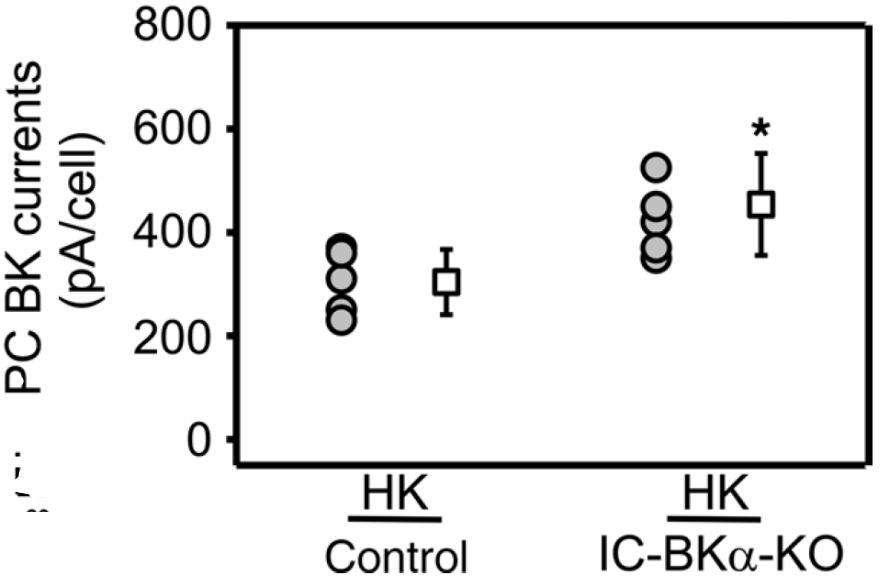


Carrisoza R et al, JCI Insight 2020

BK channels in intercalated cells are responsible for flow-induced K^+ secretion

Intercalated Cell BK channel KO





Carrisoza R et al, JCI Insight 2020

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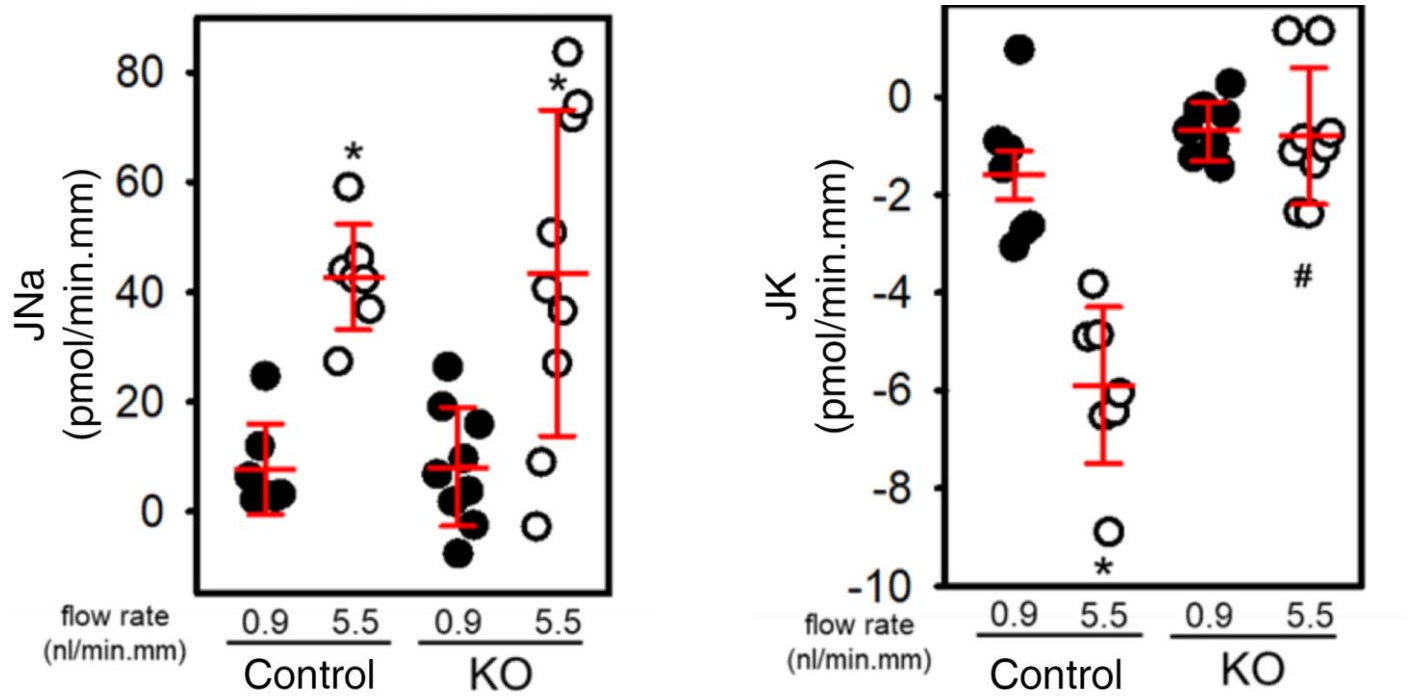
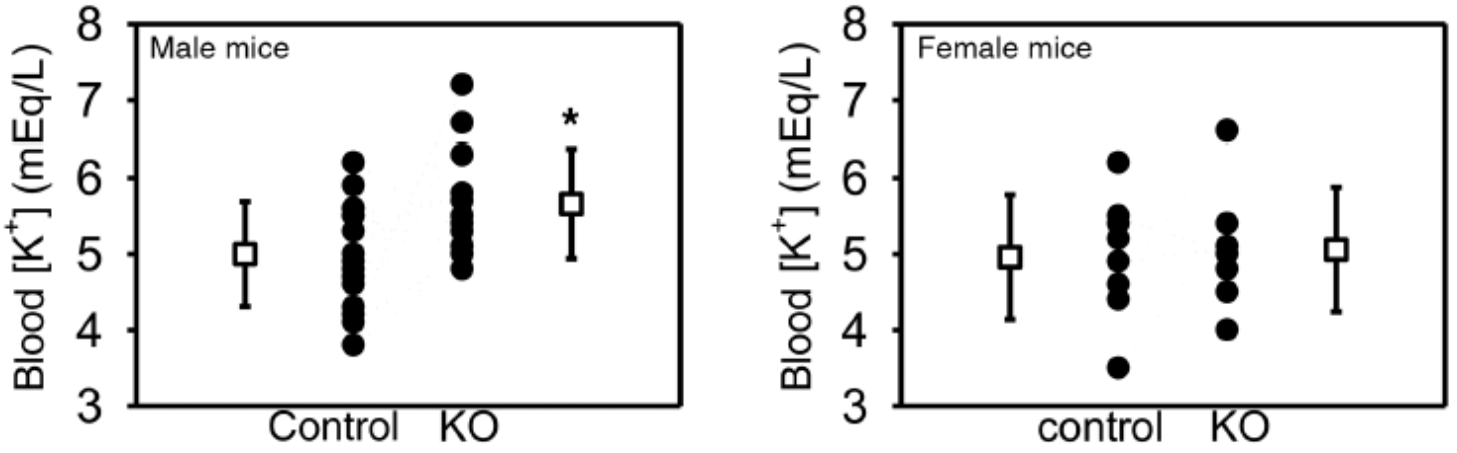


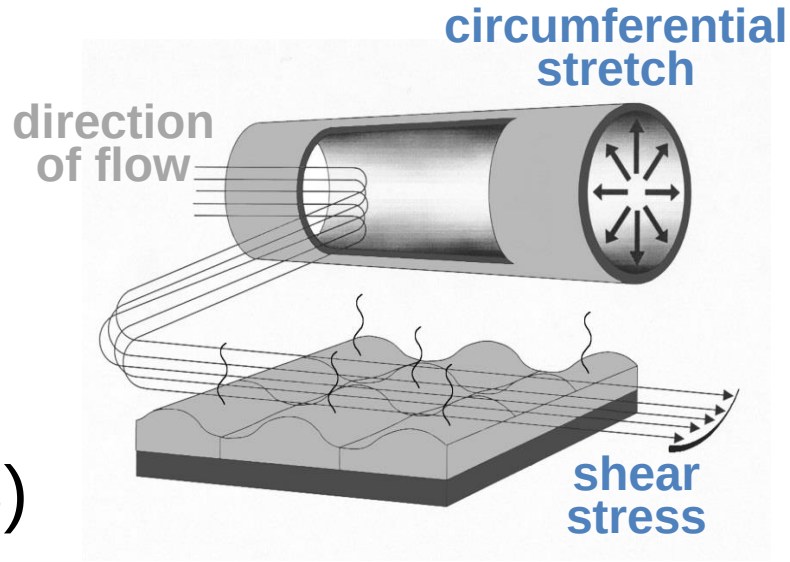
Carrisoza R et al, JCI Insight 2020

Male IC BK KO mice on a high K^+ diet have a higher blood [K^+] than controls



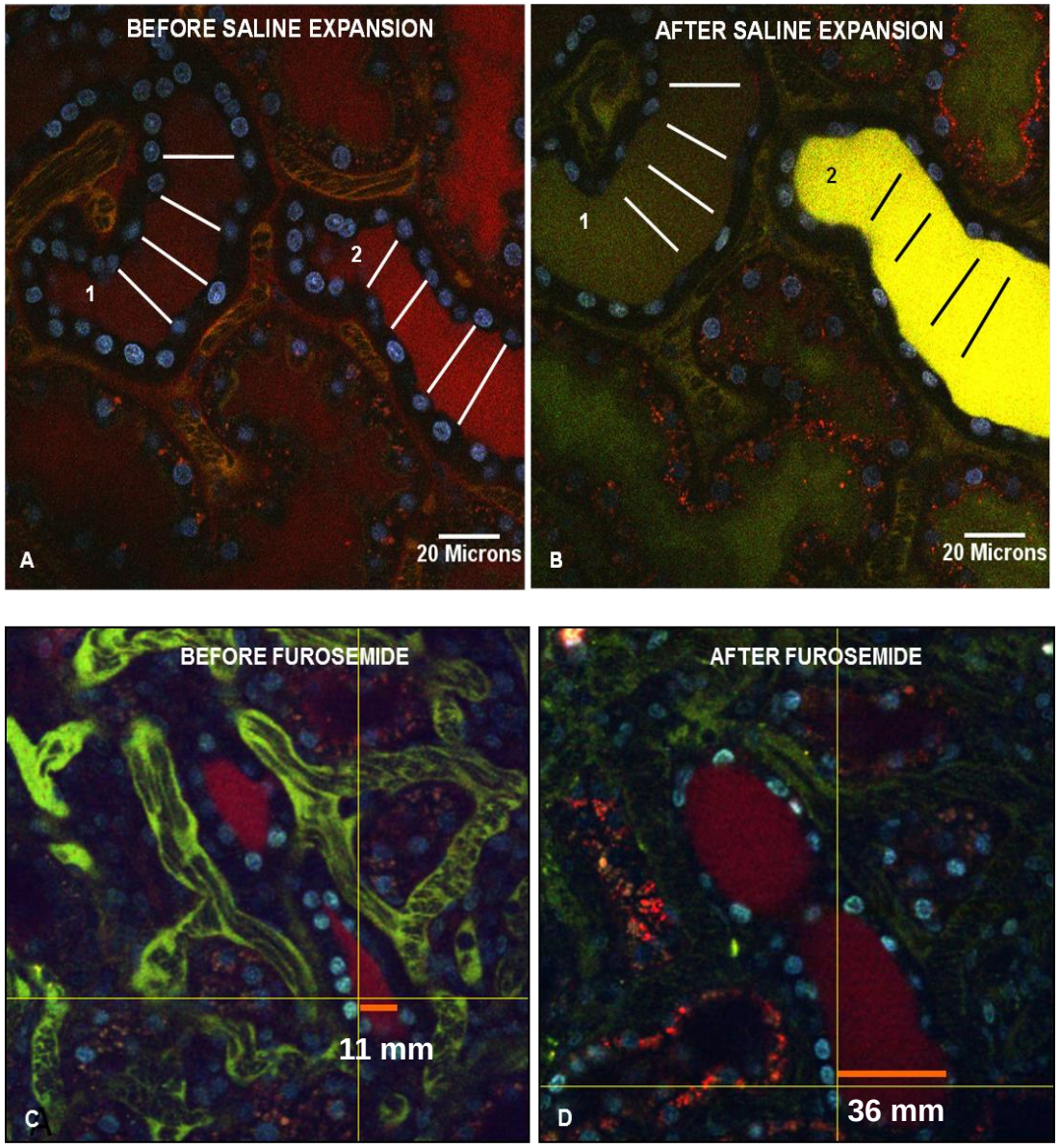
Epithelial cells are exposed to mechanical forces in ASDN segments

- drag and torque on the primary cilia of principal cells (PCs) and microvilli/microplicae of intercalated cells (ICs).
- fluid shear stress (FSS) parallel to the tubule wall.
- circumferential stretch (CS) perpendicular to the tubule wall.



Liu W et al: AJP Renal Physiol, 2003; Weinbaum et al: AJP Renal Physiol, 2010

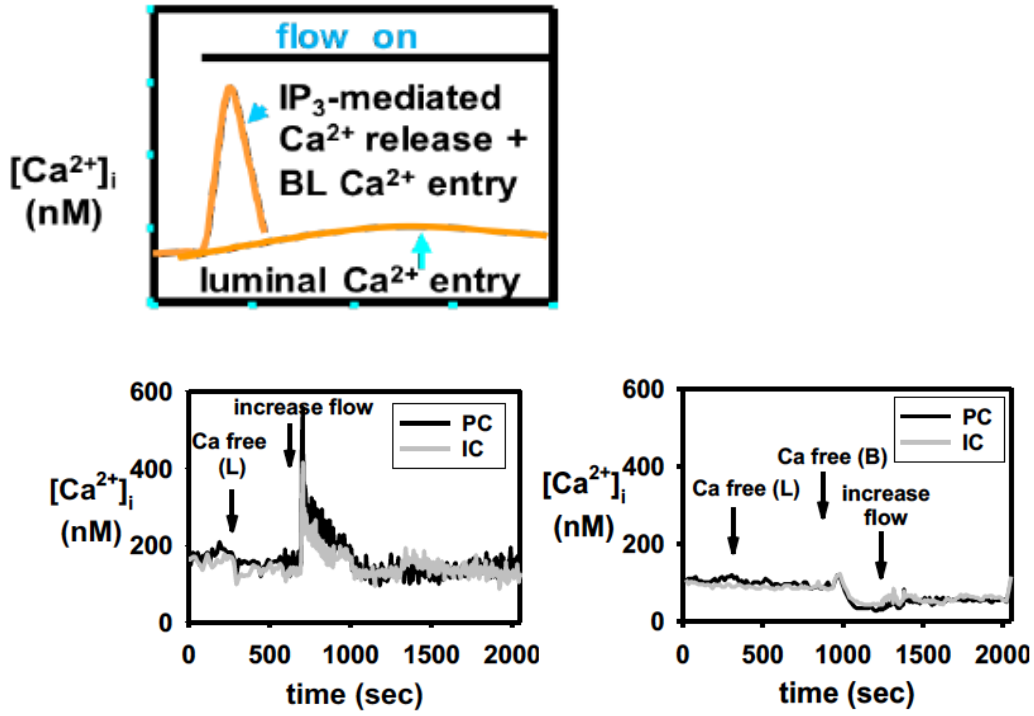
Two photon imaging of superficial rat kidney pre/post volume expansion or furosemide



Carrisoza-Gaytan R et al: AJP Renal Physiol., 2014

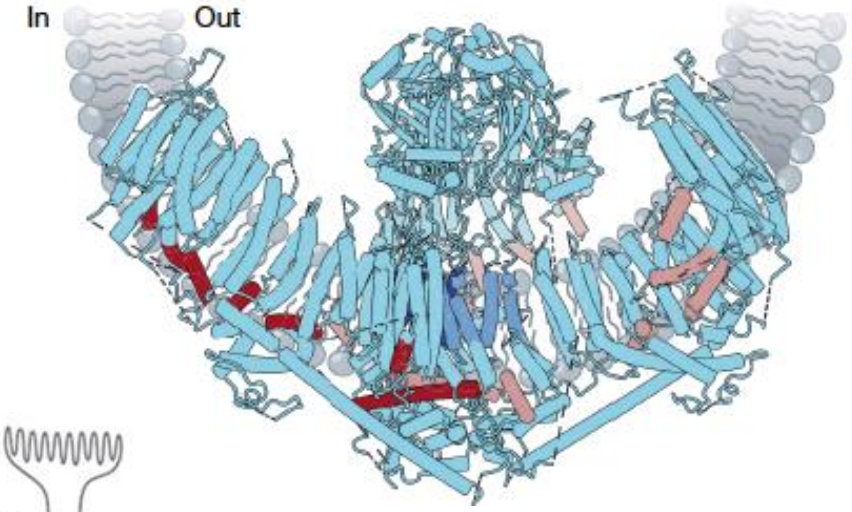
How do intercalated cells “sense” increases in tubular flow/volume?

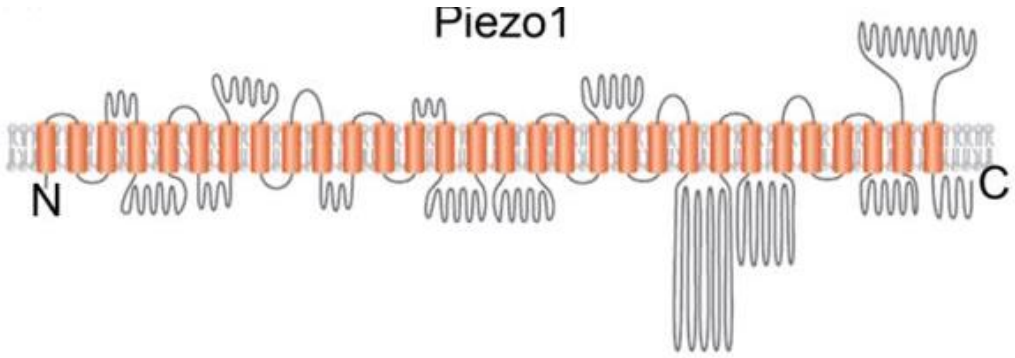
- An acute increase in tubular fluid flow rate in microperfused CCDs increases tubular diameter by 15-20%

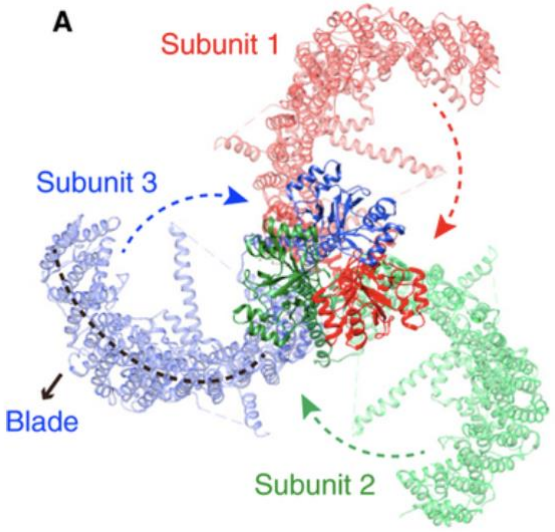


Mechanosensing Ion Channels

- Piezo1 and 2
- TMEM 63
- Trp
- ENaC

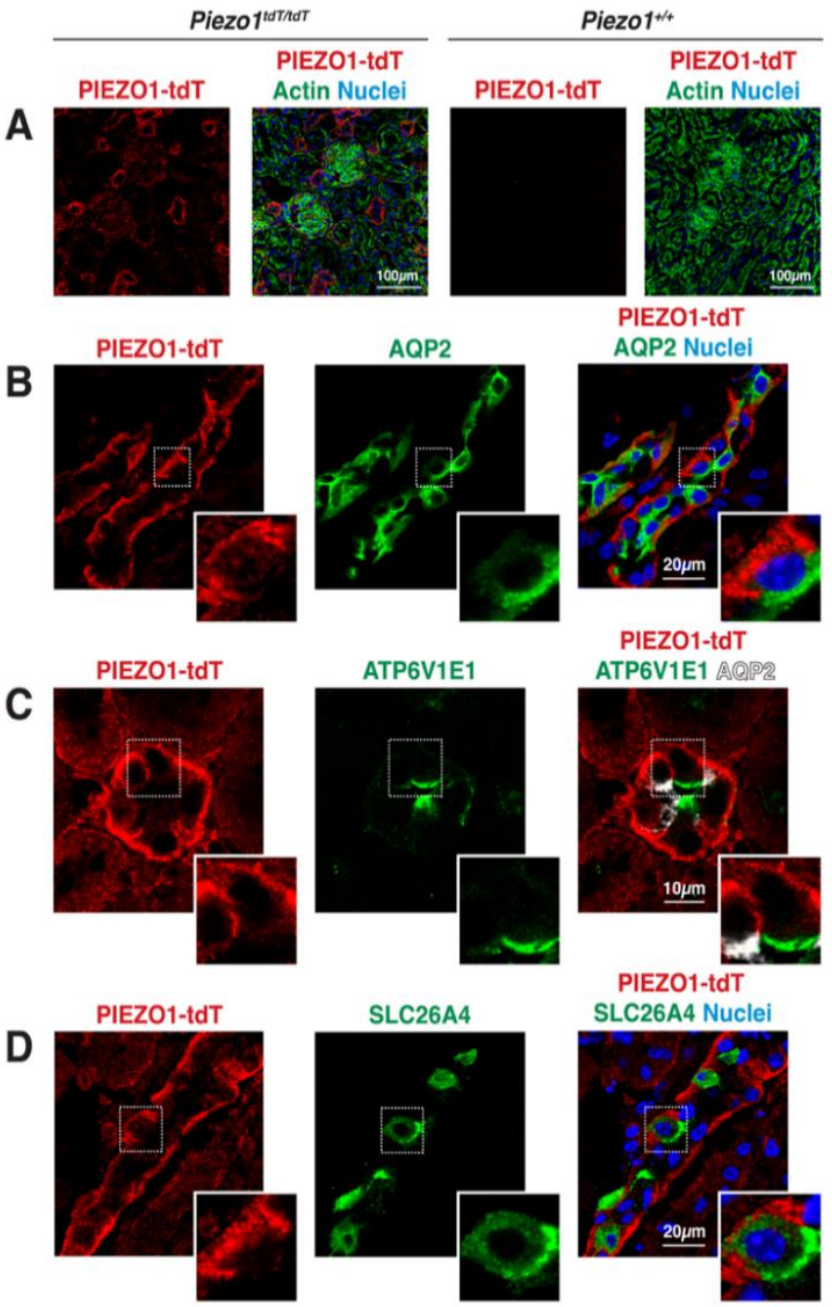






Liang et al Curr Biol 2018
Gu et al Mol Neurobiol 2013
Kefauver et al Nature 2020

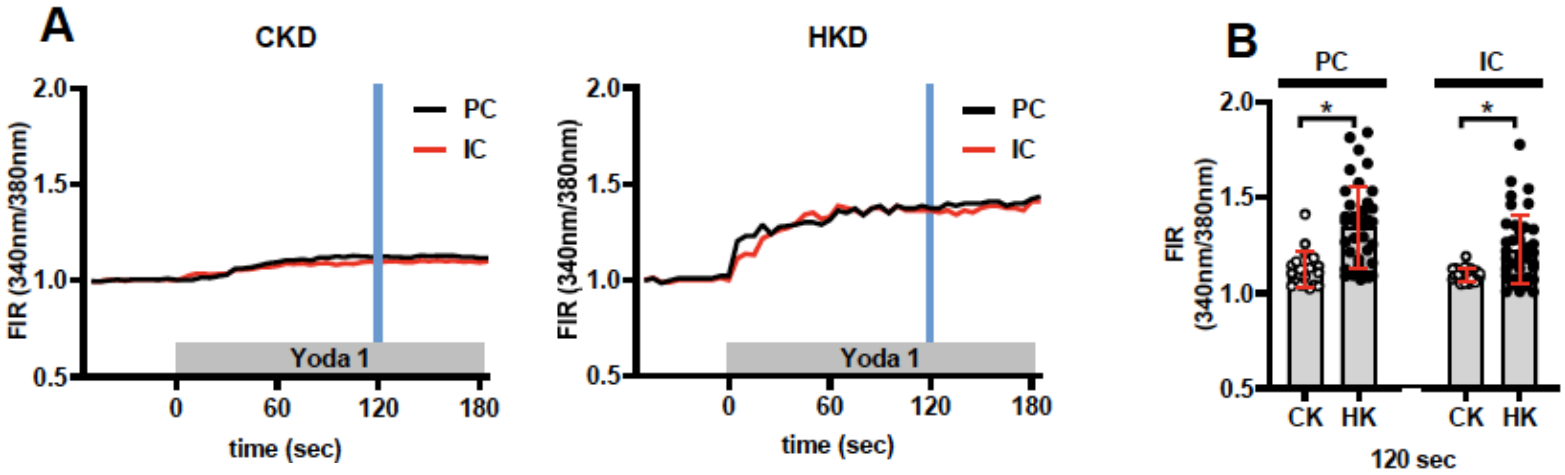
Piezo 1 channels have a basolateral localization in ICs and PCs



Dalghi et al AJP 2019

Are Piezo1 channels in intercalated cells responsible for flow-induced increases in intracellular [Ca^{2+}] and BK-mediated K^+ secretion?

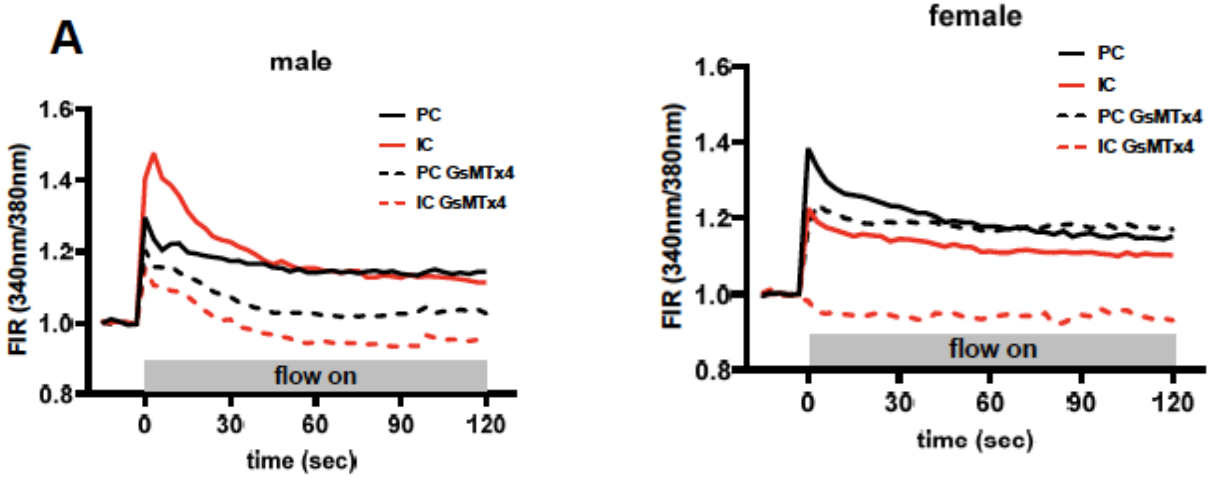
Effects of Yoda 1 (Piezo1 activator) on $[Ca^{2+}]_i$ in CCDs



Carrisoza R et al, manuscript under review

Are Piezo1 channels in intercalated cells responsible for flow-induced increases in intracellular [Ca^{2+}] and BK-mediated K^+ secretion?

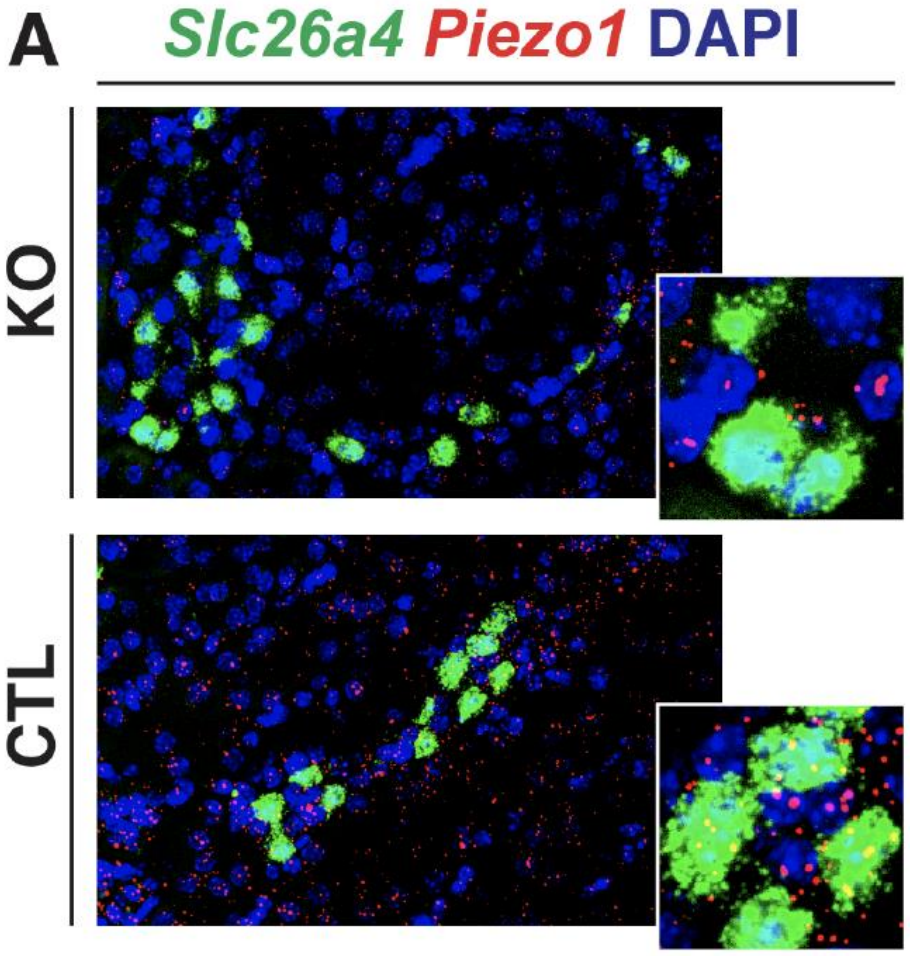
Effects of GsMTx4 (Piezo inhibitor) on flow-induced increases in $[Ca^{2+}]_i$ in CCDs

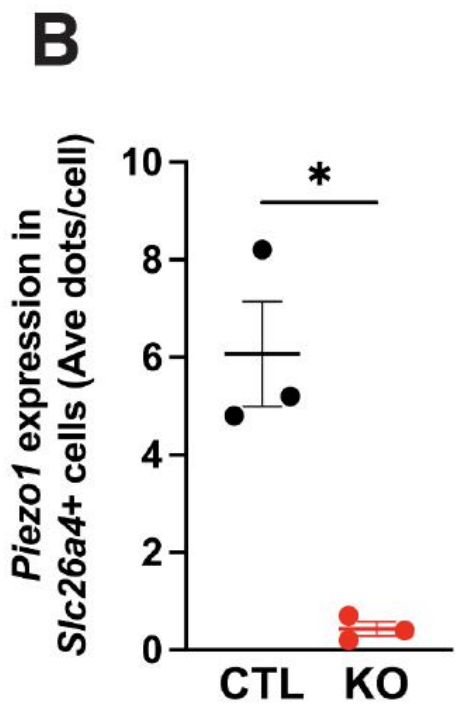


Are Piezo1 channels in intercalated cells responsible for flow-induced increases in intracellular [Ca^{2+}] and BK-mediated K^+ secretion?

Generated and analyzed an intercalated cell (IC)-specific Piezo1 KO mouse

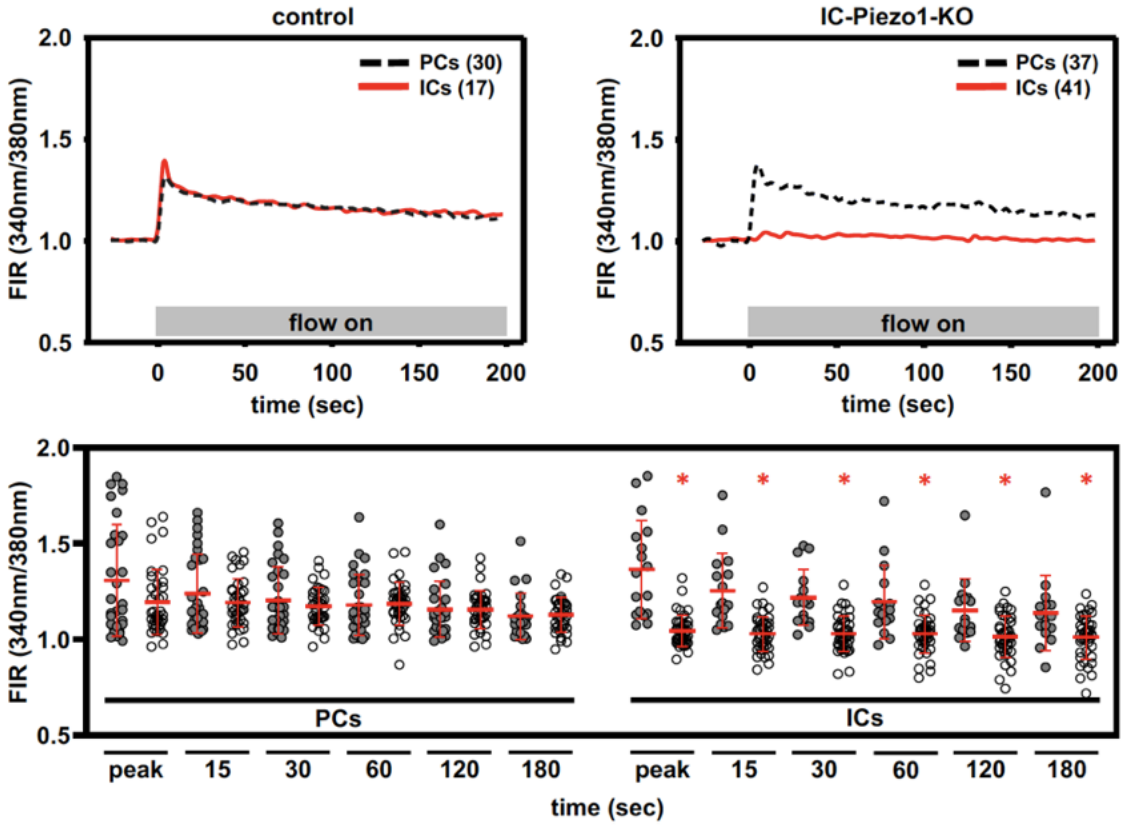
IC-Piezo1-KO



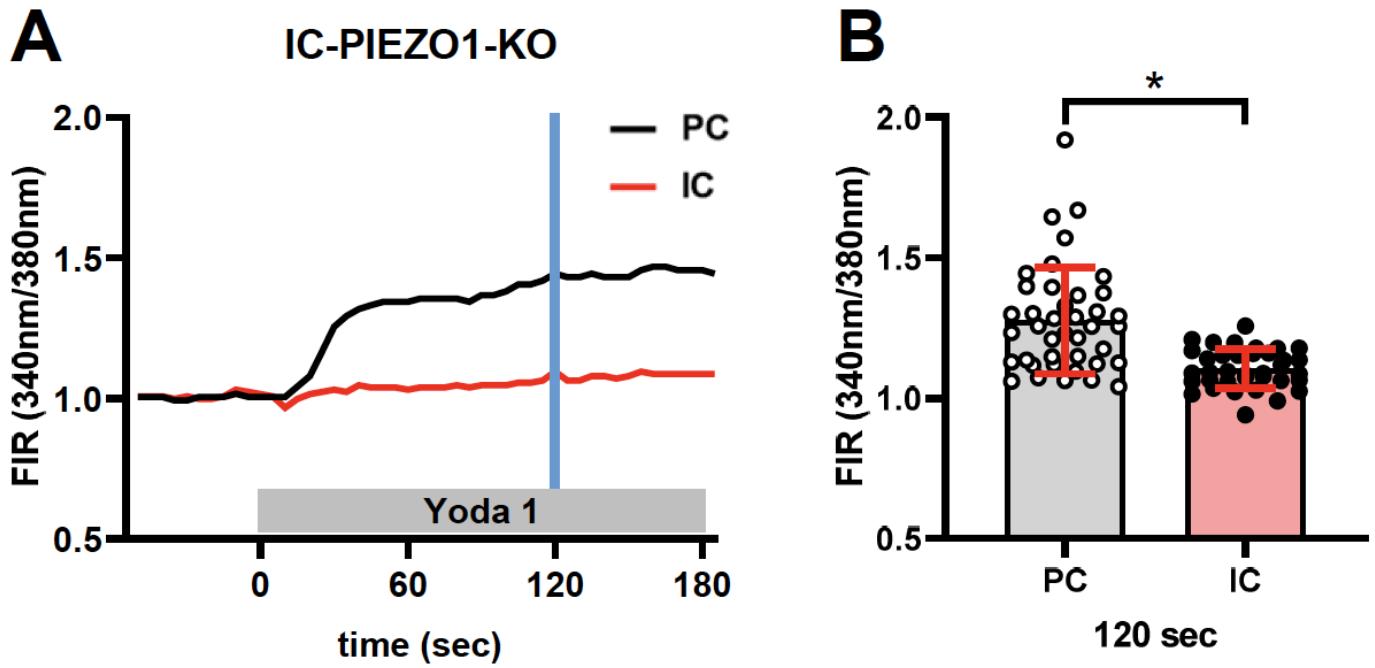


RNAscope

IC Piezo1 KO lacks flow-dependent increase in [Ca^{2+}] in ICs

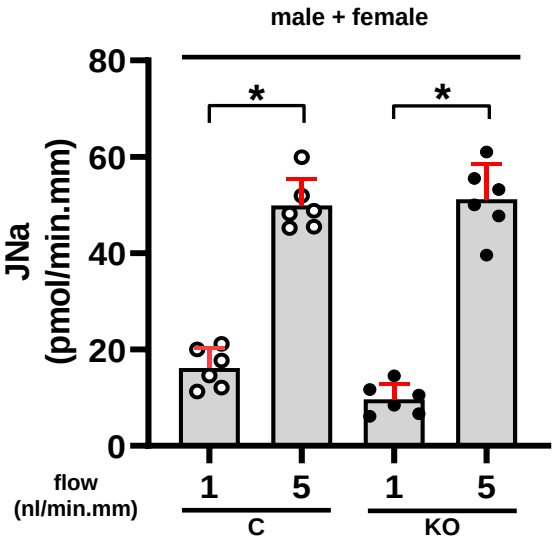


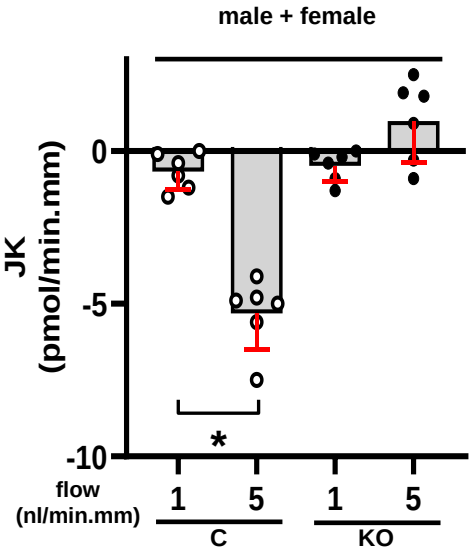
IC Piezo1 KO lack Yoda1 (Piezo1 agonist)-dependent increase in [Ca^{2+}] in ICs



Mice on a high K diet

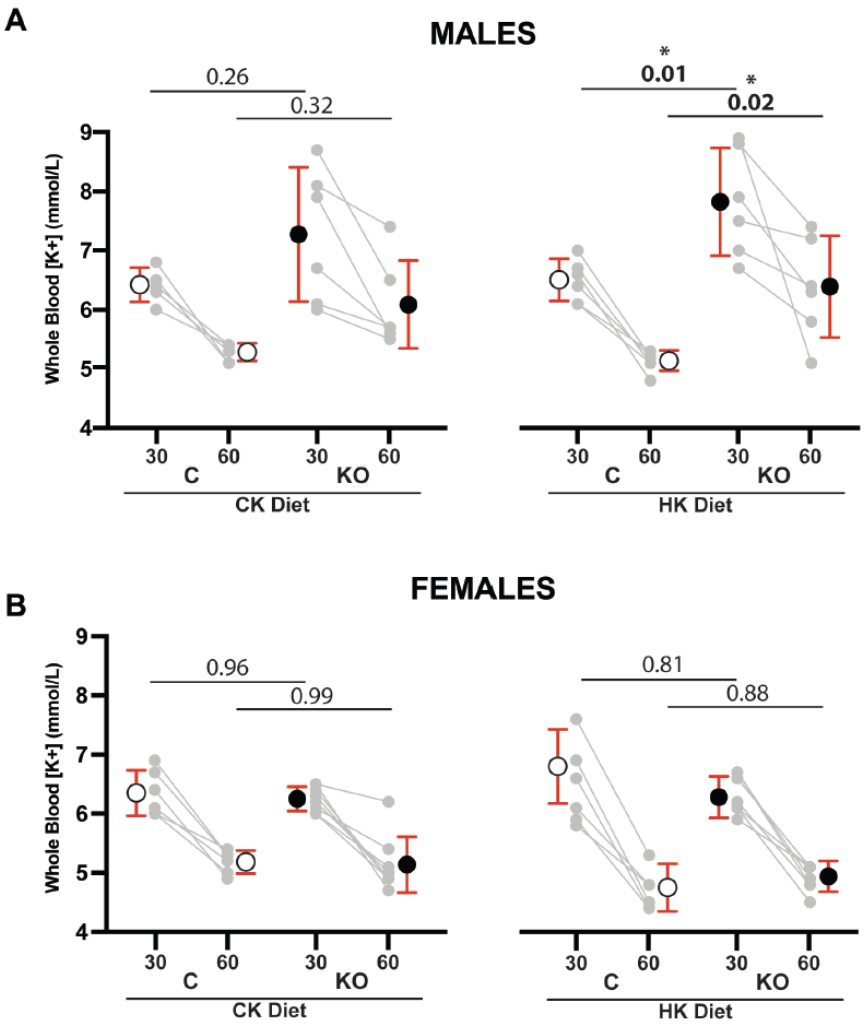
IC Piezo1 KO lacks flow-dependent K^+ secretion in isolated perfused CCDs

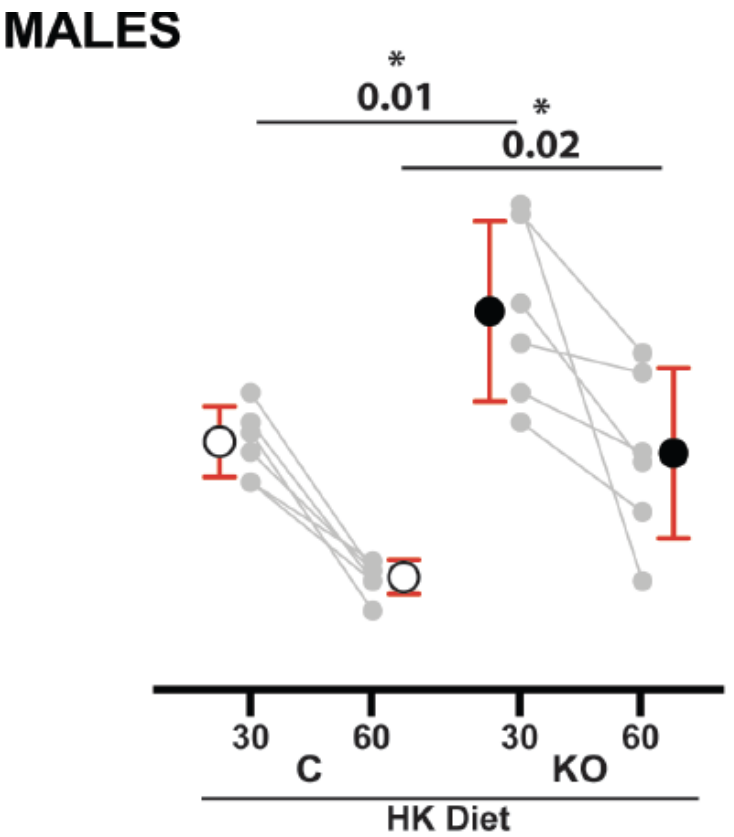




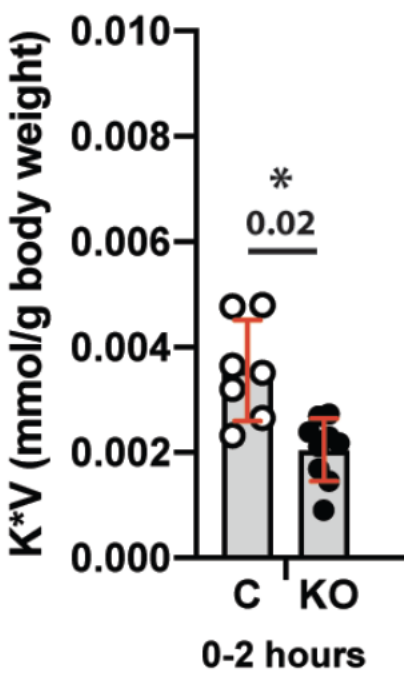
Unpublished

Male IC Piezo1 KO mice on a high K^+ diet have a higher blood [K^+] in response to an acute K^+ load

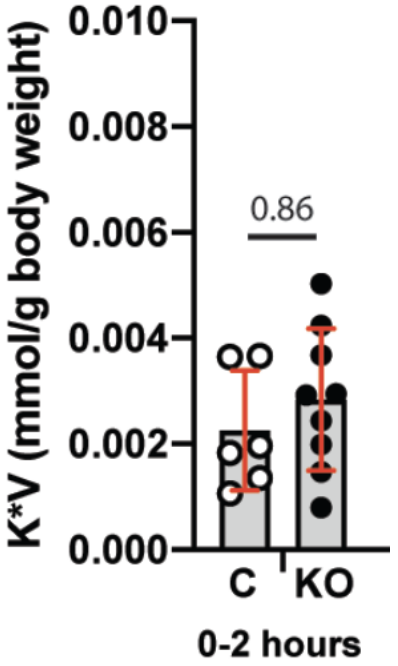




Male IC Piezo1 KO mice exhibit a reduction in urinary K^+ excretion in response to acute volume expansion



Males



Females

Summary

- A knockout of Piezo1 in intercalated cells prevents flow-dependent increases in intracellular Ca^{2+} in intercalated cells and flow-induced K^+ secretion in the ASDN

- **Piezo1 is required for flow-induced K^+ secretion in ICs mediated by BK channels**

Patient

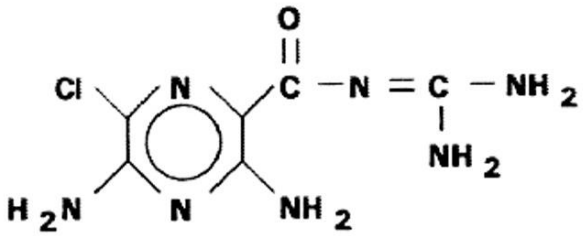
- 53 yo M admitted with cough and dyspnea
- Exam: Hypotension, oral thrush, basilar rales
- CXR: multilobar infiltrates
- K 4.6 : SCr 2.4
- Rx high dose trimethoprim and sulfamethoxazole
- Day 5: K 5.5 meq/L
- Day 9: SCr 3.1; K 7.9 meq/L
- Urine K 14 meq/L

Inhibit epithelial Na^+ channels and renal K^+ secretion

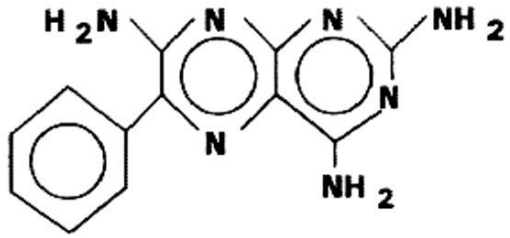
- Pituitary/Adrenal insufficiency
- Hypoaldosteronism
  - Heparin, ACEI, ARB
- Mineralocorticoid receptor
  - Mutations
  - Blockers (Spironolactone)
- ENaC blockers
  - Amiloride / Triamterene
- ENaC loss of function mutations

Is trimethoprim an ENaC inhibitor?

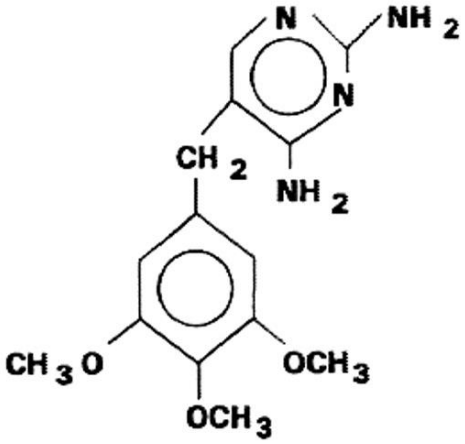
Amiloride



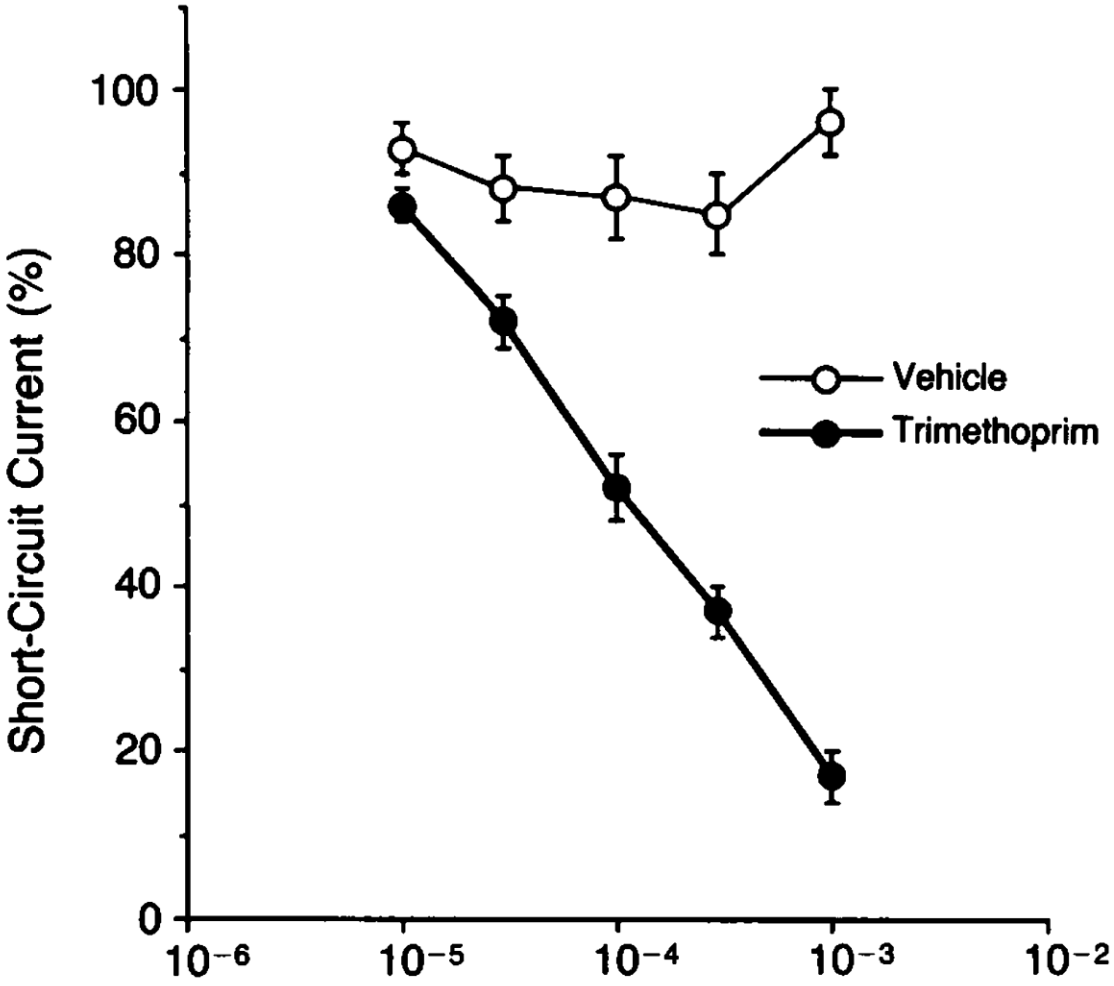
Triamterene



Trimethoprim



Trimethoprim Inhibits ENaC



Choi et al. NEJM 1993

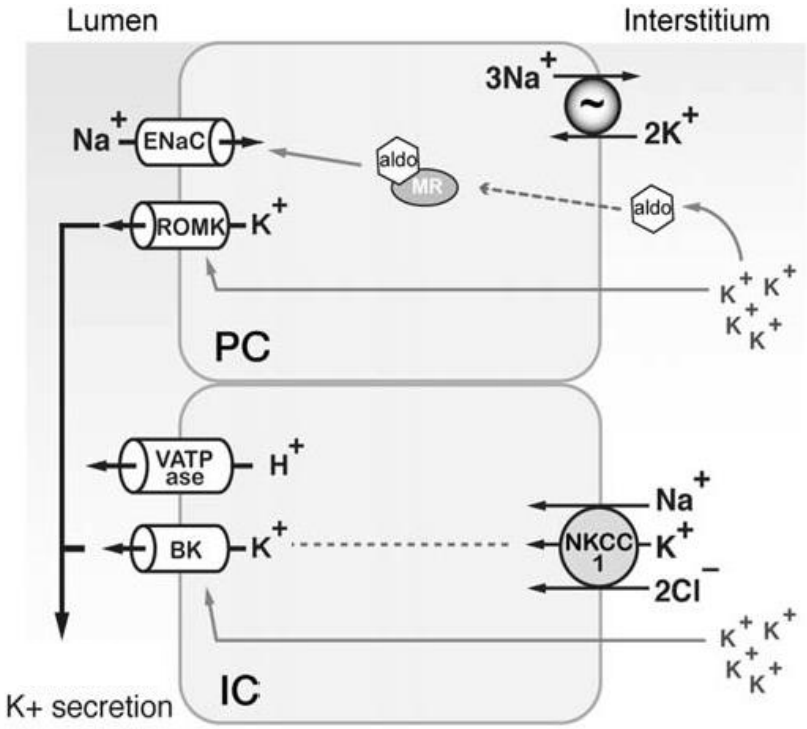
Trimethoprim is a K^+ sparing diuretic

- Trimethoprim inhibits ENaC
- Half maximal inhibition = 120 μM

- Trimethoprim 320 mg/d
  - double strength bactrim twice a day
- Mean urinary trimethoprim concentrations of 200 μM

Renal Potassium Handling

- Principal Cells
  - ROMK channels
    - baseline K^+ secretion
  - BK channels
    - Role?
- Intercalated Cells
  - BK channels
    - flow activated K^+ secretion



Boyd-Shiwarski and Subramanya, Curr Opin Nephrol Hypertens 2017

Why does an increase in tubular flow enhance renal K^+ secretion?

- Increases in tubular flow in the ASDN:
  - Activates ENaC
  - Activates Piezo1 leading to an increase in intracellular [Ca^{2+}]
  - The increase in intracellular [Ca^{2+}] activates BK channels in intercalated cells

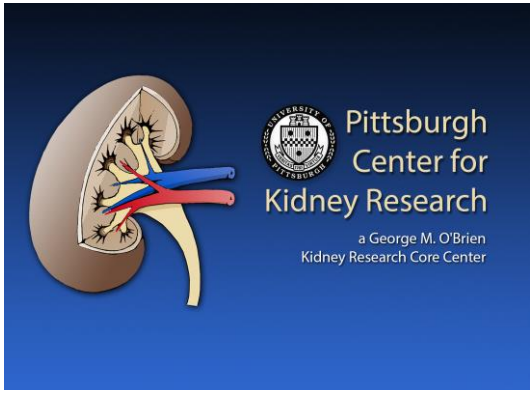
It's more than distal Na^+ delivery

Pittsburgh
Evan Ray
Stephanie Mutchler
Andrew Nickerson
Allison Marciszyn
Gerry Apodaca
Marienela Dalghi

Mount Sinai
Lisa Satlin
Rolando Carrazosa
Francisco Carattino

Utah
Don Kohan

NY Medical College
WenHui Wang
Peng Wu



Funding
NIDDK