K⁺ Rich Diet during Angiotensin II Hypertension Reduces Renal Na-Cl Cotransporter and Phosphorylation, but not Blood Pressure

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Introduction: Experimental angiotensin II infusion hypertension

- Used in thousands of studies
- CNS, blood vessels, immune cells and kidney are all implicated

Hypertensive stimuli
ANG II, salt, stress, aldosterone
CNS inflammation and oxidative stress

Vasoconstriction
Vascular hypertrophy

Sympathetic outflow

T cell

Pre-hypertension - “Barotrauma”
Neoantigen formation and presentation by dendritic cells

Severe hypertension

Sodium
Volume Retention

Background: Region specific regulation of Na\(^+\) transporters during AngII-hypertension in rats:
distal transporter activation
proximal and loop transporter inhibition

Nguyen and McDonough, Hypertension 2015

AngII infusion:
- In the distal convoluted tubule: doubles Na-Cl cotransporter (NCC) and its activated form NCC-P, the target of thiazide diuretics.
- In the cortical collecting duct: Increases ENaC subunits both full length (FL) and cleaved (CL) activated form.
Background: a 3 hr 2% K⁺ meal: reduces NCC-total and NCC-P → increases Na⁺ delivery to CCD ENaC which drives K⁺ secretion

*Rengarajan, ...Youn, McDonough (AJP Renal, 2014)*

**Aim 1:** Can a 3 hr K⁺ rich meal lower NCC and NCC-P abundance during Ang II hypertension?
Aim 1.
Infuse male Sprague Dawley rats with AngII for 14 days, then provide a K⁺ rich (2% K⁺) meal for 3 hours.

- Days 0-14: Sham or Ang II (400ng/kg/min; by osmotic mini-pump)
- Day 13 3pm: Overnight fast in metabolic cage
- Day 14 3-hr meal (7-10am)
  - Sham + 0% K⁺ = Control
  - Ang II + 0% K⁺ = A0K
  - Ang II + 2% K⁺ = A2K
- Day 14 10am: Euthanize Blood Urine Kidneys
The K⁺ rich meal does not reduce NCC or NCC-P in Ang II infused rats

Questions:

- Does ENaC activation drive potassium depletion during AngII infusion?
- Does K⁺ loss activate NCC-P to limit Na⁺ delivery to ENaC in order to reduce K⁺ secretion?
AngII infusion → K⁺ deficiency and kaliuresis vs. controls (measured during o/n fast)

- Lower plasma [K⁺] in AngII vs. control rats.
- Increased urine K⁺ excretion in Ang II vs. control rats.

In AngII infused rats, 3 hr K⁺ rich meal raises plasma [K⁺] but not urine K⁺ excretion

- Plasma [K⁺] rises ~ 1 mM after 2K⁺ meal.
- Urine K⁺ does not increase after 2K⁺ meal.
- **This is evidence for K⁺ depletion:** ingested K⁺ is used to replenish stores and normalize plasma [K⁺]

Results suggest that NCC-P is elevated to limit Na⁺ reabsorption by ENaC to reduce K⁺ secretion and loss during Ang II treatment.
Aim 2:
Test the hypothesis that preventing K\(^+\) depletion by doubling dietary K\(^+\) will blunt the rise in NCC, NCC-P and blood pressure during Ang II infusion

- Sham surgery + 1% K\(^+\) diet = Control
- Ang II (400 ng/kg/min):
  - Ang II + 1% K\(^+\) = A1K
  - Ang II + 2% K\(^+\) = A2K

Measure BP by tail cuff

Day 0-14

Day 13
3pm:
Overnight in metabolic cages to collect urine

Day 14
10am:
Euthanize:
Blood
Urine
Kidneys
Doubling dietary K⁺ during AngII infusion:
- doubled urine K⁺ excretion
- Did not raise plasma [K⁺]
- Increased plasma aldosterone
- Did not significantly lower blood pressure
As hypothesis predicts, doubling dietary K\(^+\) prevents NCC activation

Control              AngII+1%K diet      AngII+2%K diet

NCC

250kD  
130kD

1.00 ± 0.08  1.59 ± 0.07*  0.91 ± 0.04 #

NCCpT53

1.00 ± 0.13  2.83 ± 0.44*  1.62 ± 0.24 #

NCCpS71

1.00 ± 0.12  2.86 ± 0.50*  1.34 ± 0.14 #

*: p<0.05 vs. Control; #: p<0.05 vs. A1K
Summary, conclusions, relevance

- Ang II infusion leads to K⁺ deficient state, likely due to ENaC activation.
- K⁺ deficient state activates NCC to reduce Na⁺ delivery to ENaC to limit K⁺ secretion.
- Doubling dietary K⁺ prevents NCC activation during Ang II infusion, thus, stimulation of NCC appears secondary to K⁺ deficiency.
- During AngII infusion, animals become hypokalemic. Investigators should consider a K⁺ enriched diet to prevent K⁺ dependent phenotypes.
- This study illustrates the cardiovascular benefits of a K⁺ rich diet to suppress NCC activity, analogous to the effects of a blood pressure lowering thiazide diuretic (without the side effects).
Thank you!

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