The effects of nitric oxide and 8-iso-prostaglandin F2α on chloride absorption in cortical thick ascending limb

Tae-Hwan Kwon

Department of Biochemistry and Cell Biology, Kyungpook National University School of Medicine, Daegu, Republic of Korea

Introduction

In this issue of *Kidney Research and Clinical Practice*, Cabral et al. [1] studied the effects of nitric oxide (NO) on chloride absorption ($J_{\text{Cl}}$) in the isolated perfused cortical thick ascending limb (cTAL) of rabbit kidneys. They also examined the effects of 8-iso-prostaglandin F2α (8-iso-PGF2α), an isoprostane produced by the nonenzymatic peroxidation of arachidonic acid in membrane phospholipids, on $J_{\text{Cl}}$ in the presence of NO donor treatment. They concluded that 1) NO significantly decreases $J_{\text{Cl}}$ in the cTAL, which is reversed by 8-iso-PGF2α; 2) 8-iso-PGF2α stimulates $J_{\text{Cl}}$ via a cyclic adenosine 3'5'-monophosphate (cAMP)-dependent mechanism despite the presence of NO donor treatment; and 3) 8-iso-PGF2α requires protein kinase A (PKA) activity to reverse the NO-induced inhibition of $J_{\text{Cl}}$. The study found that the effects of 8-iso-PGF2α on sodium reabsorption in cTAL prevailed over the natriuretic effects of NO and that activation of PKA was required for such interaction. Thus, it is likely that sodium retention may prevail over sodium excretion in clinical conditions associated with an increased 8-iso-PGF2α level in plasma and urine, such as several chronic inflammatory and metabolic diseases, including coronary heart disease, hypertension, diabetes mellitus, obesity, hypercholesterolemia, and non-alcoholic fatty liver disease.

Expression of sodium (co)transporters in renal tubule

Renal tubular sodium and water reabsorption depend on active sodium transport through sodium transporters and osmotic water transport through aquaporins expressed in the renal tubular epithelial cells [2,3]. The proximal tubule reabsorbs the majority of the filtered sodium and water through glomerular ultrafiltration. The electrochemical gradient driving the reabsorption is generated by the pumping function of the sodium-potassium adenosine triphosphatase (Na/K-ATPase). The Na/K-ATPase is expressed basolaterally in all renal tubular segments, where it pumps three Na+ ions out of the cells and two K+ ions into the cells. In the proximal tubule, sodium is reabsorbed through the apically expressed Na/H exchanger (Na/H exchanger type 3 [NHE3]; solute carrier family 9, isoform A3 [SLC9A3]) and basolaterally expressed Na/K-ATPase and Na-HCO3 cotransporter (electrogenic NBC1 encoded by the gene SLC4A4). The sodium-glucose cotransporters...
(SGLT-2; SLC5A2) and type II Na-Pi cotransporters (NaPi-2; mainly NPT2a [SLC34A1] and NPT2c [SLC34A3]) are also expressed apically in the proximal tubule and play a role in sodium reabsorption, in addition to glucose or phosphate transport, respectively.

Establishing and maintaining a hyperosmotic medullary interstitium is a prerequisite to urine concentration. The loop of Henle generates a high medullary osmolality by driving countercurrent multiplication, which is mediated by active NaCl reabsorption (Fig. 1). In the medullary thick ascending limb (mTAL), the apically expressed Na-K-2Cl cotransporter (NKCC2; rat type 1 bumetanide-sensitive cotransporter [BSC-1]; solute carrier family 12 member 1 [SLC12A1]) and NHE3, as well as basolaterally expressed Na/K-ATPase, are the components mediating sodium reabsorption. In addition, the apical potassium channel (Kir1.1 or renal outer medullary potassium channel) and basolateral chloride channels (ClC-kb) also play a role in the reabsorption of sodium and chloride in the mTAL. Urinary dilution in the tubular lumen is further mediated by NaCl absorption in the cTAL and distal convoluted tubule (DCT). Micropuncture studies have shown that the DCT receives approximately 4% to 20% of the filtered sodium, of which it reabsorbs approximately 80% to 90%, primarily mediated by the sodium-chloride cotransporter (NCC, thiazide-sensitive cotransporter [TSC], solute carrier family 12 member A3 [SLC12A3]). The following collecting duct (CD) is the renal tubular segment for the fine regulation of sodium reabsorption and excretion into the urine, where the epithelial sodium channel (ENaC) and Na/K-ATPase are involved. ENaC is apically expressed in the late DCT, connecting tubule, and CD in the kidney tubule, where the regulation of ENaC controls extracellular fluid (ECF) volume and blood pressure.

**Regulation of sodium (co)transporters**

The regulation of renal sodium transporters is importantly involved in controlling sodium balance and ECF volume [2]. The NHE3 mediates a major fraction of the transcellular sodium and bicarbonate reabsorption. The proximal convoluted tubules from NHE3-deficient mice exhibited a significant reduction in fluid and HCO₃⁻ reabsorption by 69% and 61%, respectively. The findings indicate that NHE3 is importantly involved in sodium, fluid, and bicarbonate reabsorption in the proximal tubule. NKCC2 expression in the TAL plays a significant role in the urinary concentration mechanism. An increase in the delivery of NaCl to the loop of Henle by chronic oral saline loading or vasopressin treatment upregulates NKCC2 expression. In contrast, hypercalcemia or hypokalemia is associated with decreased NKCC2 expression associated with polyuria. Since the vasopressin V2 receptor is coupled with the activation of adenylyl cyclase, vasopressin-induced upregulation of NKCC2 is likely to be a result of an elevated intracellular cAMP level. The reduction in the intracellular concentration of chloride activates NKCC2 by phosphorylation, which requires the interaction of WNK3 and SPAK [4]. ENaC is regulated by the

![Figure 1. Main sodium and chloride transporters expressed in the thick ascending limb (TAL).](image-url)
adrenal mineralocorticoid, vasopressin, and insulin, which increase the apical permeability of the CD to sodium. The importance of ENaC in ECF volume regulation has been demonstrated as the basis of the pathogenesis of Liddle’s syndrome as well as type I pseudohypoaldosteronism.

The renin-angiotensin-aldosterone system plays a critical role in the regulation of renal sodium and water metabolism. Aldosterone increases sodium reabsorption in part by increasing the NCC expression in the DCT cells and the α-subunit of the ENaC (α-ENaC) in the CD principal cells. In contrast, the administration of spironolactone, a mineralocorticoid receptor antagonist, substantially decreases the expression of the NCC and the α-ENaC. Moreover, angiotensin II (Ang II) has known effects on the regulation of renal hemodynamics and glomerular filtration rate, as well as direct effects on the renal tubule. Increased NHE3 expression in the proximal tubule brush border and mTAL cells was observed in response to Ang II treatment [5]. Renal sodium transporters play a critical role in renal sodium handling, and dysregulation could be the underlying mechanism for clinical conditions with altered urine concentration and/or deranged renal sodium excretion and ECF volume.

**The action of renal nitric oxide on sodium and chloride transport**

Renal NO increases the urinary excretion of water and solutes by inhibiting tubular sodium reabsorption [6]. The effects of NO on sodium reabsorption in the proximal tubule are associated with decreased activity of apical Na-H exchange and basolateral Na/K-ATPase. In TAL, as Cabral et al. [1] demonstrated in this issue, exogenous and endogenous NO, acting as an autacoid, decreases sodium and chloride absorption, which could be mediated by the inhibition of NKCC2 and NHE3. Interestingly, a previous study demonstrated the reduced ability of NO to inhibit sodium transport in TAL in Dahl salt-sensitive rats, which might account for the salt sensitivity of blood pressure in this strain [7]. In the CD, NO affects tubular sodium reabsorption by inhibiting amiloride-sensitive ENaC. Accordingly, mice with CD-specific knockout of NO synthase 1 (NOS1) have salt-sensitive hypertension associated with impaired urinary sodium excretion [8]. Nephron-specific disruption of NOS3 in mice also results in hypertension and impaired urinary sodium excretion [9]. In addition, NO inhibits vasopressin-induced osmotic water permeability in the cortical CD. The effects may be due to the cyclic guanosine 3′,5′-monophosphate (cGMP)-dependent protein kinase-mediated decrease in vasopressin-stimulated cAMP content [10].

**The action of renal 8-iso-prostaglandin-F2α on sodium and chloride transport**

Cabral et al. [11] previously demonstrated that 8-iso-PGF2α stimulates sodium and chloride transport in cTAL via a PKA-dependent mechanism. The administration of 8-iso-PGF2α to the lumen of the isolated cTALs increased JCl by 54%, and adding it to the bath enhanced Jcl by 35%. In contrast, the 8-iso-PGF2α-induced increase in Jcl was significantly diminished by adding furosemide, an inhibitor of NKCC2. Since 8-iso-PGF2α, a product of non-enzymatic peroxidation of arachidonic acid, increases in plasma and urine in disease conditions such as hypertension, chronic kidney disease, and liver cirrhosis, the high levels of 8-iso-PGF2α could contribute to NaCl retention.

**Summary**

In this issue of *Kidney Research and Clinical Practice*, Cabral et al. [1] further reported that 8-iso-PGF2α could override NO’s natriuretic effects in the cTAL. This finding suggests that sodium retention may prevail over sodium excretion in the renal tubule in clinical conditions, particularly when they are associated with an increased 8-iso-PGF2α level and blunted NO production. Further studies are warranted to elucidate the effects of 8-iso-PGF2α on the phosphorylation and intracellular trafficking of NKCC2 and the expression of other ion transporters expressed in TAL and different renal tubular segments.

**Conflicts of interest**

The author has no conflicts of interest to declare.

**Funding**

This work was supported by grants from the Korea Health Technology R&D Project through the Korea Health Indus-
try Development Institute (KHIDI), funded by the Ministry of Health & Welfare, Korea (HI15C0001).

ORCID

Tae-Hwan Kwon, https://orcid.org/0000-0002-1561-6508

References

1. Cabral PD, Silva GH, Baigorria ST, Juncos LI, Ajayi EI, Garcia NH. Nitric oxide-inhibited chloride transport in cortical thick ascending limbs is reversed by 8-isoprostaglandin-F2. Kidney Res Clin Pract 2022;41:699–706.
2. Knepper MA, Brooks HL. Regulation of the sodium transporters NHE3, NKCC2 and NCC in the kidney. Curr Opin Nephrol Hypertens 2001;10:655–659.
3. Knepper MA, Kwon TH, Nielsen S. Molecular physiology of water balance. N Engl J Med 2015;372:1349–1358.
4. Ponce-Coria J, San-Cristobal P, Kahle KT, et al. Regulation of NKCC2 by a chloride-sensing mechanism involving the WNK3 and SPAK kinases. Proc Natl Acad Sci U S A 2008;105:8458–8463.
5. Kwon TH, Nielsen J, Kim YH, Knepper MA, Frøkiaer J, Nielsen S. Regulation of sodium transporters in the thick ascending limb of rat kidney: response to angiotensin II. Am J Physiol Renal Physiol 2003;285:F152–F165.
6. Lee J. Nitric oxide in the kidney: its physiological role and pathophysiological implications. Electrolyte Blood Press 2008;6:27–34.
7. García NH, Plato CF, Stoes BA, Garvin JL. Nitric oxide-induced inhibition of transport by thick ascending limbs from Dahl salt-sensitive rats. Hypertension 1999;34:508–513.
8. Hyndman KA, Boesen EI, Elmarakby AA, et al. Renal collecting duct NOS1 maintains fluid-electrolyte homeostasis and blood pressure. Hypertension 2013;62:91–98.
9. Gao Y, Stuart D, Takahishi T, Kohan DE. Nephron-specific disruption of nitric oxide synthase 3 causes hypertension and impaired salt excretion. J Am Heart Assoc 2018;7:e009236.
10. Garcia NH, Stoes BA, Carretero OA, Garvin JL. Mechanism of the nitric oxide-induced blockade of collecting duct water permeability. Hypertension 1996;27(3 Pt 2):679–683.
11. Cabral PD, Silva GB, Baigorria ST, Juncos LA, Juncos LI, García NH. 8-iso-prostaglandin-F2α stimulates chloride transport in thick ascending limbs: role of cAMP and protein kinase A. Am J Physiol Renal Physiol 2010;299:F1396–F1400.