Physiological role of NBCe2 in the regulation of electrolyte transport in the distal nephron

Donghai Wen and Steven C. Sansom
Department of Cellular/Integrative Physiology, University of Nebraska Medical Center, Omaha, Nebraska

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Wen D, Sansom SC. Physiological role of NBCe2 in the regulation of electrolyte transport in the distal nephron. Am J Physiol Renal Physiol 309: F489–F491, 2015. First published July 1, 2015; doi:10.1152/ajprenal.00192.2015.—The electrogenic Na\(^{+}\)-HCO\(_3\)^− cotransporter 2 (NBCe2) is encoded by the gene SLC4A5 and is expressed in the choroid plexus (8), liver (1), and kidney (21). In vitro electrophysiological studies showed that NBCe2 mediates Na\(^{+}\)-HCO\(_3\)^− transport with a 1:2 or 1:3 stoichiometry and plays an important role in intracellular pH regulation (12, 16). In vivo experiments also suggested that NBCe2 mediates electrogenic Na\(^{+}\)-HCO\(_3\)^− influx in the epithelial cells of the choroid plexus and plays an important role in regulating cerebrospinal fluid production and neurological function (8). The detailed localization and physiological function of NBCe2 in the kidney are still unclear. In situ hybridization has identified NBCe2 mRNA expression restricted to the aquaporin-2 (AQP2)-positive cells of the distal nephron (7). However, due to the lack of a valid antibody, the detailed subcellular localization of NBCe2 protein is uncertain. Despite the lack of details regarding NBCe2 protein expression, recent in vivo studies using targeted NBCe2 knockout mice (NBCe2-KO) have elucidated some important physiological functions of NBCe2 in the regulation of blood pressure, acid-base, and K\(^{+}\) and Ca\(^{2+}\) transport in the distal nephron.

Role of NBCe2 in Hypertension

Polymorphisms in SLC4A5/NBCe2 are associated with hypertension in humans (2, 4, 5). Groger et al. (7) found that NBCe2-KO developed hypertension associated with renal metabolic acidosis. Interestingly, induction of metabolic alkalosis with NaHCO\(_3\) feeding abolished the difference in blood pressure (BP) between wild-type (WT) and NBCe2-KO (7). In our recent study, we did not reveal a significant BP difference between WT and NBCe2-KO on a regular diet. However, we found that the NBCe2-KO had increased BP, compared with WT, only when they were on acid diets (19). This discrepancy may be due to the method of BP measurement [telemetry in Groger et al. (7) and tail-cuff in Wen et al. (19)], and/or the genetic background of these mice (mixed 129/C57BL6 in Groger et al. and C57BL6 in Wen et al.). Nevertheless, both studies demonstrated that metabolic acidosis plays an important role in the pathogenesis of hypertension in NBCe2-KO. Additional in vivo experiments showed that increased activity of the epithelial Na\(^{+}\) channel (ENaC) contributes to the hypertension of NBCe2-KO (19). Treatment with the ENaC inhibitor amiloride produced a large decrease in BP in the NBCe2-KO compared with WT. On the other hand, the Na\(^{+}\)-Cl\(^{-}\) cotransporter (NCC) inhibitor hydrochlorothiazide decreased BP in WT but not NBCe2-KO. Compared with WT, NBCe2-KO had increased ENaC\(\alpha\) expression in the renal plasma membrane and increased amiloride-sensitive Na\(^{+}\) reabsorption and K\(^{+}\) secretion. Micropuncture revealed increased luminal K\(^{+}\) concentration and decreased transepithelial potential (V\(_{\text{oc}}\)) in the connecting tubule (19). The exact mechanism of the enhanced ENaC activity in the NBCe2-KO is not understood. However, given that the plasma aldosterone concentration level is lower in NBCe2-KO (7), the activation of ENaC in NBCe2-KO is not the result of hyperaldosteronism secondary to a failure of NBCe2-mediated Na\(^{+}\) reabsorption, but rather a primary defect of ENaC-mediated Na\(^{+}\) reabsorption in the principal cells (PC). The reduced activity of NCC was probably compensatory, or secondary, to reduced aldosterone in NBCe2-KO. Studies of human subjects would be important to elucidate the role of ENaC/amiloride treatment in hypertensive patients with SLC4A5/NBCe2 polymorphisms.

Role of NBCe2 in Renal Acid-Base Regulation

Groger et al. (7) revealed that NBCe2-KO had renal metabolic acidosis as evidenced by increased urinary HCO\(_3\)^− wasting and decreased plasma HCO\(_3\)^− concentration compared with WT, although both the plasma and urine pH were similar between WT and NBCe2-KO. Our recent experiments also found that there is no difference in the plasma and urine pH between WT and NBCe2-KO when they were on a regular diet. However, after acid loading (1.5% NH\(_4\)Cl), NBCe2-KO developed renal tubular acidosis as evidenced by decreased plasma pH and HCO\(_3\)^− concentration, increased urine pH, and hyper-
chloremia (20). It is likely that increased urinary HCO₃⁻ excretion was due to upregulation of pendrin as reported by Groger et al., because we found NBCe2-KO had compensatory higher H⁺-ATPase expression in the plasma membrane of collecting ducts (20).

**Role of NBCe2 in Renal K⁺ and Ca²⁺ Transport**

Our recent study revealed no significant differences in the urinary K⁺ and Ca²⁺ excretion rates between WT and NBCe2-KO on regular diets. However, after acid loading, NBCe2-KO exhibited hypercalciuria and developed hypokalemia associated with increased urinary K⁺ wasting compared with WT. Molecular experiments showed NBCe2 mRNA expression in the connecting tubule segment (20), which is the critical segment for renal K⁺/Ca²⁺ transport (3, 6). Together with the finding that NBCe2-KO has increased ENaC expression/activity and decreased luminal Vₑ in the connecting tubule, we conclude that the hyperkaliuria and hypercalciuria in the NBCe2-KO are caused by overactivation of ENaC-mediated Na⁺ reabsorption.

**Summary**

The detailed subcellular localization of NBCe2 is not well established. However, NBCe2 should be a basolateral transporter like its close family member, the electroneutral Na⁺-/HCO₃⁻ cotransporter 1 (NBCn1) of the thick ascending limb (9), where it buffers the cell during acidosis. In the basolateral membrane, NBCe2 has access to high plasma HCO₃⁻ concentration of 20–25 mM that would help drive the HCO₃⁻ concentration of the distal tubule lumen is very low in acidicifying conditions. The cell model in A depicts the role of NBCe2 in the principal cells (PC) during acidic conditions in wild-type (WT) mice. NBCe2 is activated to buffer the increased H⁺ concentration during acidosis. Cellular hyperpolarization from 1Na⁺/2HCO₃⁻ cell entry inhibits voltage-gated BK-α/β1 and reduces the driving force for both ENaC- and KCN-dependent Na⁺ reabsorption and accounting for the increased transepithelial potential (Vₑ) in the connecting tubule in acidosis (19). With less K⁺ secretion and more recycled across the basolateral membrane, the positive movement of Na⁺ is countered by electronegative Cl⁻ transport through the paracellular pathway (11), and less through the pendrin Cl⁻/HCO₃⁻ exchanger of β-intercalated cells (IC), thereby preserving HCO₃⁻. The cell model in B illustrates a hypothetical explanation for the increased epithelial Na channel (ENaC)-mediated Na⁺ reabsorption and HCO₃⁻ secretion (increased urine pH) found in knockout mice (KO). In the absence of NBCe2 in acidosis (or when NBCe2 is turned off in alkalosis), ENaC activity is enhanced, as the driving force for K⁺ secretion is enhanced with a depolarized cell potential and fully activated Na⁺/K⁺-ATPase. Increased K⁺ secretion allows relatively less electrical Cl⁻ reabsorption via the paracellular pathway and more pendrin-mediated Cl⁻ reabsorption in exchange for secreted HCO₃⁻. The result is enhanced HCO₃⁻ loss in acidic conditions or necessary HCO₃⁻ secretion by the β-IC during alkalotic conditions.

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**Fig. 1. Hypothetical cell model depicting role of the electrogenic Na⁺–HCO₃⁻ cotransporter 2 (NBCe2) in distal nephron cells.** The cell model in A depicts the role of NBCe2 in the principal cells (PC) during acidic conditions in wild-type (WT) mice. NBCe2 is activated to buffer the increased H⁺ concentration during acidosis. Cellular hyperpolarization from 1Na⁺/2HCO₃⁻ cell entry inhibits voltage-gated BK-α/β1 and reduces the driving force for both ENaC- and BK-α/β1-mediated K⁺ secretion, thereby increasing K⁺ concentration. The enhanced Na⁺ influx reduces Na⁺/K⁺-ATPase activity, thereby increasing K⁺ secretion, and accounting for the increased transepithelial potential (Vₑ) in the connecting tubule in acidosis (19). With less K⁺ secretion and more recycled across the basolateral membrane, the positive movement of Na⁺ is countered by electronegative Cl⁻ transport through the paracellular pathway (11), and less through the pendrin Cl⁻/HCO₃⁻ exchanger of β-intercalated cells (IC), thereby preserving HCO₃⁻. The cell model in B illustrates a hypothetical explanation for the increased epithelial Na channel (ENaC)-mediated Na⁺ reabsorption and HCO₃⁻ secretion (increased urine pH) found in knockout mice (KO). In the absence of NBCe2 in acidosis (or when NBCe2 is turned off in alkalosis), ENaC activity is enhanced, as the driving force for K⁺ secretion is enhanced with a depolarized cell potential and fully activated Na⁺/K⁺-ATPase. Increased K⁺ secretion allows relatively less electrical Cl⁻ reabsorption via the paracellular pathway and more pendrin-mediated Cl⁻ reabsorption in exchange for secreted HCO₃⁻. The result is enhanced HCO₃⁻ loss in acidic conditions or necessary HCO₃⁻ secretion by the β-IC during alkalotic conditions.
the nonremodeled β-IC. The linked NBCe2 inhibition of ENaC-pendrin may be a redundant or “insurance” mechanism that turns off the Cl⁻/HCO₃⁻ exchange in the nonremodeled cells.

Therefore, the role of the NBCe2 in the PC is to prevent the exchange of reabsorbed NaCl for secreted HCO₃⁻ in acidic conditions. A recent study has suggested that intracellular Cl⁻ concentration may also regulate NBCe2 activity through the GXXXP motif (14). Thus a feedback system may exist between Cl⁻ sensing and NBCe2 function in the distal nephron to regulate electrolyte homeostasis.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

Author contributions: D.W. and S.C.S. edited and revised manuscript; D.W. and S.C.S. approved final version of manuscript.

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