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Intracellular chloride: a regulator of transepithelial transport in the distal nephron

Aylin R. Rodan^{1,‡}

¹ Department of Internal Medicine, Division of Nephrology and Hypertension and Molecular Medicine Program, University of Utah, and Medical Service, Veterans Affairs Salt Lake City Health Care System, Salt Lake City, UT

Abstract

Purpose of review—This review focuses on the role of intracellular chloride in regulating transepithelial ion transport in the distal convoluted tubule (DCT) in response to perturbations in plasma homeostasis.

Recent findings—Low dietary potassium increases the phosphorylation and activity of the sodium chloride cotransporter (NCC) in the DCT, and vice versa, affecting sodium-dependent potassium secretion in the downstream aldosterone-sensitive distal nephron. In cells, NCC phosphorylation is increased by lowering of intracellular chloride, via activation of the chloride-sensitive WNK (With No Lysine)-SPAK/OSR1 (Ste20-related proline/alanine rich kinase/oxidative stress response) kinase cascade. *In vivo* studies have demonstrated pathway activation in the kidney in response to low dietary potassium. A possible mechanism is lowering of DCT intracellular chloride in response to low potassium due to parallel basolateral potassium and chloride channels. Recent studies support a role for these channels in the response of NCC to varying potassium. Studies examining chloride-insensitive WNK mutants, in the *Drosophila* renal tubule and in the mouse, lend further support to a role for chloride in regulating WNK activity and transepithelial ion transport. Caveats, alternatives and future directions are also discussed.

Summary—Chloride sensing by WNK kinase provides a mechanism to allow coupling of extracellular potassium with NCC phosphorylation and activity to maintain potassium homeostasis.

Keywords

hypokalemia; hyperkalemia; ion transport; hypertension

Introduction

The kidney plays an essential role in maintaining homeostasis of serum electrolytes. Potassium concentration, for example, is maintained between 3.5 – 5 mmol/L, and even

[‡]Corresponding author: Aylin Rodan, M.D., Ph.D., Current address: Department of Internal Medicine, Division of Nephrology and Hypertension, University of Utah Molecular Medicine Program, 15 North 2030 East, Bldg. 533, Room 2420A, Salt Lake City, UT 84112, USA, Phone: 801-587-7929, Fax: 801-585-0701, aylin.rodan@hsc.utah.edu.

Conflicts of interest
None.

small deviations from normal (including within the laboratory-defined “normal range”) are associated with morbidity and mortality [1–6, 7*,8*,9*,10*,11,12]. Furthermore, a potassium-deficient diet is associated with higher blood pressures and hypertension [13,14], and there is an inverse association between potassium intake and death [15], indicating the importance of homeostatic mechanisms that maintain normal serum potassium concentrations. The kidney excretes ~90% of daily ingested potassium [16], and therefore is central to potassium homeostasis. This article will review the role of intracellular chloride in regulating transepithelial ion transport in the distal convoluted tubule (DCT) to achieve potassium homeostasis.

Dietary potassium modulates phosphorylation of the sodium chloride cotransporter

A major mechanism for potassium secretion by the aldosterone-sensitive distal nephron (ASDN) is the exchange of sodium ions for potassium. Therefore, increasing distal delivery of sodium facilitates renal potassium secretion [16], and it has long been appreciated that potassium has natriuretic (and blood pressure-lowering) effects [17–19]. Potassium loading decreases sodium reabsorption by the proximal tubule [20,21*] and thick ascending limb [22–26]. The DCT is also upstream of the ASDN and therefore positioned to influence ASDN sodium delivery. Genetic mutations influencing DCT sodium reabsorption result in hypokalemia [27,28] or hyperkalemia [29], suggesting an important role for DCT transport in the regulation of serum potassium concentrations.

The sodium chloride-reabsorbing transporter on the apical membrane of DCT epithelial cells is the sodium chloride cotransporter (NCC) [30,31]. NCC phosphorylation increases its activity [32,33]. In rodents, acute potassium administration, via oral or intravenous routes [34,35*,36–38], results in rapid dephosphorylation of NCC, as does acute elevation in plasma potassium due to amiloride administration [39**]. Feeding rodents high potassium diet over several days results in decreased total, phosphorylated and surface NCC abundance [40–42,43*,44], while low potassium diet results in increased total and phosphorylated NCC [45–48]. Importantly, the effects of low potassium on NCC upregulation persist in mice and humans concomitantly fed high sodium, ie, the typical “Western” diet [49,50]. These data indicate that low potassium states result in NCC activation, and vice versa. Indeed, there is an inverse linear association between plasma potassium concentration and phosphorylated NCC levels in mice [51], and a similar association is observed in DCT cells in kidney slices bathed in varying potassium baths for 30 minutes [36], suggesting a direct effect of potassium on DCT epithelial cells.

Chloride regulates SLC12 cotransporters

NCC is an SLC12 cation-coupled chloride cotransporter that is closely related to the sodium-potassium-2-chloride cotransporters (NKCCs) [52]. John Russell and colleagues, working with the internally dialyzed squid giant axon preparation, showed that NKCC was activated by lowering of intracellular chloride concentrations [53–55]. This relationship was also demonstrated in salivary gland acinar cells [56] and in a transporting epithelium, the shark rectal gland, and was associated with increased transporter phosphorylation [57]. In

cultured cells and *Xenopus* oocytes, maneuvers resulting in lowering of intracellular chloride, such as hypotonic low chloride bathing medium or expression of chloride-excreting transporters, stimulate the phosphorylation and activity of NCC, NKCC1 and NKCC2 [32,33,58,59].

Role of basolateral potassium and chloride channels in the DCT

In a secretory epithelium like the shark rectal gland, hormonal stimulation of apical chloride exit lowers intracellular chloride, providing a mechanism for increasing basolateral NKCC1 phosphorylation and activation [60]. On the DCT basolateral membrane, potassium entering through the sodium/potassium-ATPase is recycled through a potassium channel consisting of a heterotetramer of Kir4.1, encoded by *KCNJ10*, and Kir5.1, encoded by *KCNJ16* [61–63]. In parallel, Clc-Kb/2 channels, together with the β -subunit barttin, allow chloride exit across the basolateral membrane [64,65] (Figure 1). This provides a potential mechanism to link changes in extracellular potassium to changes in intracellular chloride. A decrease in plasma extracellular potassium will increase the driving force for potassium exit across the basolateral membrane, hyperpolarizing the membrane potential, and increasing the driving force for basolateral chloride exit. This could lower intracellular chloride, increasing NCC phosphorylation and activity.

A number of observations support the model that the activity of Kir4.1/5.1 and Clc-Kb/2 (with barttin) influences NCC phosphorylation and activity and serum potassium concentrations. Human mutations in *KCNJ10*, *CLCKB* or *BSND* (encoding barttin) result in hypokalemia [66–69]. In mice, low potassium diet increases the activity of Kir4.1/5.1 channels on the basolateral membrane of DCT, resulting in hyperpolarization of the DCT basolateral membrane and increased total and phosphorylated NCC expression and activity. High potassium diet has the opposite effects, and low and high potassium diet effects were abolished in mice with nephron knockout or knockdown of Kir4.1 [70**,71**,72*]. Loss of Kir4.1 also results in loss of NPPB (5-Nitro-2-(3-phenylpropylamino)benzoic acid)-sensitive chloride currents [71**,73], and the changes in potassium current in response to low and high potassium diets are paralleled by changes in chloride currents [70**], suggesting coupling between the basolateral potassium and chloride conductances. Mice carrying a hypomorphic mutation in *Bsnd* are hypokalemic, and, unlike wild-type mice, there was no increase in total or phosphorylated NCC in response to low potassium diet in the mutant mice, supporting a role for basolateral chloride efflux in this process [74*]. The *Bsnd* mutant mice were also protected from the hypertensive effect of a high sodium/low potassium diet [74*].

WNK kinases as chloride sensors

The increased phosphorylation of NCC observed in low potassium conditions implies activation of a kinase cascade. NCC, together with NKCC1 and NKCC2, is regulated by phosphorylation of conserved N-terminal serines and threonines by the WNK (With No Lysine)-SPAK/OSR1 (Ste20-related proline/alanine rich kinase/oxidative stress response) kinase cascade [33,75–79]. SPAK and OSR1 are paralogous Ste20 kinases that are phosphorylated and activated by all WNK isoforms, although WNK4 and SPAK dominate in

the DCT [29]. Bathing cultured cells in hypotonic low chloride medium results in activation of WNK1 and SPAK/OSR1, NCC phosphorylation, and increased NCC transport activity [33,80].

Chloride regulates WNK-SPAK/OSR1 phosphorylation of NCC

Chloride binds directly to the WNK1 kinase active site, as observed by X-ray crystallography, and inhibits the autophosphorylation required for WNK activation [81]. Mutating the chloride-binding Leu 369 in WNK1 decreases the inhibitory effect of chloride on WNK1 autophosphorylation *in vitro* [81], and introducing this mutation into WNK1, or the homologous mutation into WNK4, in HEK293 cells or *Xenopus* oocytes resulted in increased NCC phosphorylation and transport activity [51,82]. This implies that a decrease in intracellular chloride relieves inhibition of WNKs to allow SPAK/OSR1 activation and NCC phosphorylation and activation. Interestingly, SPAK autophosphorylation is also inhibited by increased chloride concentration *in vitro* [83]; whether the mechanism is similar to WNKs has not been determined.

As a chloride-sensitive signaling cassette, the WNK-SPAK/OSR1 kinase cascade could mediate changes in NCC phosphorylation and activity in response to altered potassium and consequent changes in DCT intracellular chloride, as first proposed by Terker *et al.* (Figure 1) [49]. In COS7 cells, there was an inverse association between bathing medium potassium concentration and OSR1 phosphorylation [84], mirroring the relationship between serum potassium and NCC phosphorylation in mice [51]. Bathing HEK293 cells in low potassium medium, which decreased intracellular chloride, also increased SPAK/OSR1 and NCC phosphorylation in a WNK1 and WNK3-dependent manner. Potassium channel inhibition with barium, or expression of mutant Kir4.1 or CLC-K2, blocked the effect, while expression of chloride-insensitive WNK1 mimicked the effect of low potassium and conferred insensitivity to changes in bath potassium [49]. In an *ex vivo* kidney slice preparation, 30 minutes of low potassium bath increased phosphorylated SPAK/OSR1 in physiological chloride concentrations (110 mM) but not in low chloride (5 mM) conditions [36].

Effects of low and high potassium diets on WNK signaling

Studies have demonstrated increased SPAK [47,49,50], apical localization of OSR1 in the DCT [47], or increased SPAK/OSR1 phosphorylation [46,47] in mice fed low potassium diets. WNK4 was also increased in mice fed a low potassium diet [49], and there was no increase in total NCC in response to low potassium diet in *WNK4* knockout mice [46]. A recent study corroborated this finding, demonstrating that there was no increase in either total or phosphorylated NCC in response to low potassium diet in *WNK4* knockout mice, nor an increase in NCC activity (as measured by thiazide-induced natriuresis), despite preserved ability to increase NCC phosphorylation and activity in response to increased sodium delivery to the DCT via furosemide administration. This indicates a specific role for WNK4 in regulating NCC in response to low dietary potassium [85**]. Similarly, mice in which SPAK was knocked out together with nephron-specific OSR1 knockout had either blunted [49] or absent [86] increase in total or phosphorylated NCC in response to low potassium diet.

A recent study in the *Drosophila* renal tubule measured acute changes in intracellular chloride and WNK activity in transporting tubules. In this epithelium, WNK-SPAK/OSR1 signaling regulates transport through a basolateral NKCC [87,88*]. Hypotonic bathing medium (in which both potassium and chloride concentrations are lowered) stimulates transport in a WNK-SPAK/OSR1-NKCC-dependent manner [87]. Under these conditions, intracellular chloride, measured using a transgenic sensor, decreased, with a nadir at 30–60 minutes, and WNK activity increased during the same timeframe [88*], supporting the hypothesis that acute changes in intracellular chloride can influence WNK activity, and, importantly, transepithelial ion transport [87]. Like the DCT, the basolateral membrane of the *Drosophila* renal epithelium has both potassium and chloride conductances [89], and manipulation of bath potassium, without changes in tonicity or extracellular chloride, was sufficient to result in changes in WNK activity, with low potassium bath stimulating WNK activity and vice versa [88*]. Furthermore, expression of chloride-insensitive WNK, together with the scaffold protein Mo25 (Mouse protein 25)/Cab39 (calcium-binding protein 39), was sufficient to increase transepithelial ion transport in standard bathing conditions, indicating a role for chloride regulation of WNK in a transporting renal epithelium [88*].

A recent paper similarly examined the effect of chloride-insensitive WNK4 on potassium homeostasis in the mouse. WNK4 is the dominant WNK paralog regulating NCC in the DCT [90,91], and is the most sensitive to chloride *in vitro* and in cellular studies [51,82]. Chen *et al.* generated chloride-insensitive WNK4 knockin mice, which were hyperkalemic, with decreased fractional excretion of potassium, and hypertensive. The mice had increased phosphorylated SPAK/OSR1 and total and phosphorylated NCC expression and activity, which did not increase further with low potassium diet (Chen JC, Lo YF, Lin YW, Lin SH, Huang CL, Cheng CJ, unpublished data). These findings support the model that the effects of low potassium diet on NCC phosphorylation and activity are mediated by chloride regulation of WNK4 activity.

Whether the effects of acute or chronic potassium administration on NCC dephosphorylation are mediated by the WNK-SPAK/OSR1 pathway is controversial. In the *Drosophila* renal epithelium, high potassium bath decreased tubule WNK activity acutely, although whether this is mediated by chloride is unknown. In rodent tissue, NCC dephosphorylation has been observed in the absence of changes in SPAK/OSR1 phosphorylation, in the presence of low or high extracellular chloride, and in the presence of chloride channel blockers [34,36,92*]. In the chloride-insensitive WNK4 mice, there was blunted natriuresis in the first 6 hours of a high-potassium diet, but total and phosphorylated NCC expression, and thiazide-sensitive sodium excretion, were suppressed in both wild-type and mutant mice, suggesting that the effect of high potassium diet on NCC is independent of WNK4 chloride sensing (Chen *et al.*, unpublished data). Activation of phosphatases could explain this effect [92*,93,94]. On the other hand, rapid NCC dephosphorylation after potassium loading by gavage failed to occur in the chloride-insensitive WNK4 mutant mice, with a greater rise in plasma potassium after gavage (Chen *et al.*, unpublished data). Further research is required to clarify the roles of chloride-regulated WNK signaling, phosphatases, and other mechanisms in potassium-induced NCC dephosphorylation.

Caveats and alternatives

Modeling studies have suggested that changes in proximal tubule and/or thick ascending limb sodium reabsorption may be quantitatively more important than changes in DCT sodium reabsorption in influencing ASDN potassium secretion [21*,43*]. Furthermore, although initial modeling studies supported the idea that changes in NCC phosphorylation could be driven by changes in intracellular chloride [49], a more recent study challenged this concept, with the caveat that changes in intracellular chloride were predicted rather than measured [39**]. Thus, measurement of intracellular chloride in the DCT with varying perturbations in systemic potassium would be useful, but are difficult to perform *in vivo*; *ex vivo* measurements could be complicated if tubules are analyzed in conditions in which they are no longer transporting, due to altered driving forces for chloride movement across apical and basolateral membranes.

Distal tubule remodeling also influences sodium reabsorption and potassium secretion. Maneuvers which increase sodium delivery to the DCT or connecting tubule (CNT) result in parallel changes in ultrastructure and transport capacity [95–97], while DCT atrophy occurs in mice lacking NCC, SPAK or Kir4.1 [98,99,100**,101]. In mice expressing constitutively active SPAK in the early DCT (DCT1) [102**], which results in increased NCC expression and activity, DCT1 undergoes hypertrophy, while the CNT, quantitatively the most important segment for potassium secretion [103,104], atrophies [102**]. The effects of hydrochlorothiazide on tubule morphology and the timecourse of natriuresis and kaliuresis suggested that these structural changes explain renal potassium retention and hyperkalemia [102**]. Three days of low potassium diet itself is sufficient to induce hyperplasia in multiple nephron segments, including DCT1 [100**]. In light of these results, it would be interesting to determine whether there are morphological changes in *WNK4* knockout or chloride-insensitive *WNK4* knockin mice that could explain altered potassium homeostasis in these animals, rather than the inability of *WNK4* to respond to acute changes in intracellular chloride concentration.

As discussed above, potassium also influences sodium chloride reabsorption, via NKCC2, in the thick ascending limb, and NKCC2 is regulated by WNK-SPAK/OSR1 signaling, although other kinases also contribute to NKCC2 phosphorylation [26,78,105–107,108*,109]. However, studies have shown variable effects on NKCC2 in response to potassium loading [34,43*,110*], and there was no difference in total or phosphorylated NKCC2 in the chloride-insensitive *WNK4* mutant mice in whole-kidney lysates (Chen *et al.*, unpublished data). Thus, whether intracellular chloride could regulate WNK-SPAK/OSR1-mediated NKCC2 phosphorylation and activity in response to changes in potassium is, so far, unclear, and other mechanisms for NKCC2 regulation (for example, membrane trafficking) could also play a role [111,112]. Interestingly, while Kir4.1/5.1 is the sole potassium conductance on the basolateral membrane of the DCT, it coexists with other potassium channels on the basolateral thick ascending limb [113]. This may make the DCT more sensitive to hormonal regulators of Kir4.1/5.1, such as bradykinin or angiotensin II [114,115].

Conclusion and Future Directions

Substantial evidence supports a role for chloride-regulated WNK signaling in mediating changes in NCC activity in response to perturbations in potassium homeostasis, particularly low potassium diet. WNK paralogs interact [116], influencing WNK activity [117]. A recent study demonstrated that co-expression in *Xenopus* oocytes of a WNK1 isoform lacking the kinase domain, KS-WNK1, increases WNK4 chloride sensitivity [118**]. Further studies are needed to understand the effects of low or high potassium diets on WNK heteromers. Furthermore, perturbations in potassium homeostasis result in the formation of KS-WNK1-dependent “WNK bodies,” consisting of KS-WNK1, WNK4, SPAK, and OSR1, in cultured cells and mouse and human DCT [49,101,119–121,122*,123*]. Whether and how WNK bodies interact with chloride regulation of WNKs remains unknown. Future studies should further elucidate the elegant mechanisms by which the kidney achieves potassium homeostasis, with important implications for maintaining normokalemia and normotension.

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Key points

- A low potassium diet is associated with higher blood pressure and increased mortality, and both hypo- and hyperkalemia are associated with morbidity and mortality in human populations.
- Low potassium diet increases the phosphorylation and activity of the sodium chloride-reabsorbing sodium chloride cotransporter in the distal convoluted tubule through the activation of the WNK (With No Lysine)-SPAK/OSR1 (Ste20-related proline/alanine rich kinase/oxidative stress response) kinase cascade.
- Basolateral potassium and chloride channels in the distal convoluted tubule provide a mechanism for lowering of intracellular chloride in response to low potassium diet.
- Relief of chloride inhibition of WNK allows pathway activation by low potassium diet.

Figure 1.

Changes in plasma potassium regulate NCC phosphorylation and activity through chloride regulation of WNK pathway activity. A model of the DCT epithelial cell is shown, including apical NCC and the chloride/WNK4/SPAK kinase pathway regulating it. Mo25 may also play a cooperative role in pathway activation, although this has not been demonstrated in the DCT thus far. Sodium/potassium-ATPase, the heteromeric Kir4.1/Kir5.1 potassium channel, and Clc-Kb/2 chloride channel (with barttin) are on the basolateral membrane, and provide a mechanism to lower intracellular chloride concentration and increase WNK4/SPAK activity and NCC phosphorylation and activity when plasma potassium concentration is low. See text for further details.