Pendrin as a Novel Target for Diuretic Therapy

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Key Words

Kidney • Salt absorption • Distal nephron

Abstract

The $\text{Cl}^-/\text{HCO}_3^-$ exchanger pendrin (SLC26A4, PDS) and the thiazide-sensitive NaCl cotransporter NCC (SLC12A3) are expressed on the apical membranes of distal nephron segments and mediate salt absorption, with pendrin working in tandem with the epithelial Na channel (ENaC) and NCC working by itself. Pendrin is expressed on the apical membrane of intercalated cells in late distal convoluted tubule (DCT), connecting tubule (CNT) and the cortical collecting duct (CCD) whereas the thiazide-sensitive NaCl cotransporter NCC is primarily detected on the apical membrane of DCT cells. Recent studies indicate that pendrin expression is increased in kidneys of NCC knockout mice, raising the possibility that pendrin and NCC can compensate for loss of the other by increasing their expression and activity. Current investigations in our laboratories demonstrate that pendrin plays an important role in compensatory salt absorption in response to the loop diuretics and the thiazide derivatives. These studies

further demonstrate that whereas single deletion of pendrin or NCC does not cause salt wasting in mutant mice under baseline conditions, double knockout of pendrin and NCC causes profound polyuria and polydipsia, along with salt wasting under basal conditions. As a result, animals develop significant dehydration. We propose that pharmacologic inhibition of pendrin and NCC can provide a novel and strong diuretic regimen for patients with fluid overload, including those with congestive heart failure, nephrotic syndrome or renal failure.

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Introduction

Kidney plays a crucial role in the regulation of vascular volume and arterial pressure predominantly through the reabsorption of filtered sodium and chloride and via highly specialized transporters within different nephron segments. The systemic balance of sodium and chloride is achieved in coordination with the regulation of water reabsorption in distinct nephron sites. The reabsorption and maintenance of sodium and water homeostasis are under the influence of several hormones,

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including aldosterone, angiotensin II, antidiuretic hormone and atrial natriuretic factor. These hormones regulate the activity of sodium and water transporters and allow the kidney to adjust salt and water excretion to the need of the body, and thus control the vascular volume and blood pressure.

Several nephron segments are actively involved in the reabsorption of filtered sodium and chloride using both transcellular as well as paracellular transport pathways. In this article, we will predominantly discuss the role of salt absorbing transporters in the distal nephron (thick ascending limb, distal convoluted tubule and collecting duct) in systemic salt and fluid homeostasis. We will specifically discuss the role of pendrin and sodium chloride cotransporter NCC in salt absorption in the distal nephron and the potential salt wasting effect of pendrin inhibition in fluid overloaded state and in response to loop diuretic. We will further discuss the potential therapeutic effect of combined inhibition of NCC and pendrin in pathophysiologic states presenting with fluid overload.

Segmental organization of renal tubular transport

The thick ascending limb (TAL) of loop of Henle reabsorbs ~15% of filtered load of NaCl mostly through the transcellular pathway involving several transport proteins as shown in Fig. 1 (reviewed in [1]). NaCl enters the TAL cells from the apical membrane via $\text{Na}^+\text{-K}^+\text{-2Cl}^-$ cotransporter called NKCC2, regulated by vasopressin, and works as a secondary active system energized by the chemical gradient of Na^+ generated and maintained by the basolateral primary active and ATP-dependent $\text{Na}^+\text{-K}^+\text{-ATPase}$ [2]. The basolateral exit of Na^+ is mediated via the $\text{Na}^+\text{-K}^+\text{-ATPase}$ pump in exchange for K^+ , whereas Cl^- leaves the cells through a chloride channel called CLC-KB (Fig. 1) [2, 3]. In the lumen side of the TAL, K^+ recycles back into the urine through a K^+ channel (ROMK), allowing more NaCl reabsorption via NKCC2 (Fig. 1). Na^+ is also absorbed via the paracellular pathway due to the lumen-positive voltage created by K^+ recycling through ROMK in the luminal membrane and back into urine [4, 5]. The apical membrane of the TAL exhibits a unique characteristic of being impermeable to water and highly permeable to NaCl, both of these features are crucial to the urinary concentrating and diluting mechanisms, and thus the regulation of water and salt metabolism.

Fig. 1. Schematic diagram showing a cell model of the thick ascending limb with its different transport pathways involved in the transcellular reabsorption of NaCl as well as the paracellular transfer of Na^+ activated by the luminal positive voltage.

The distal convoluted tubule (DCT) reabsorbs ~10% of filtered NaCl mainly via a transcellular mechanism which involves the apical $\text{Na}^+\text{-Cl}^-$ cotransporter (NCC), and mediates the entry of NaCl in the DCT cells as depicted in Fig. 2 (reviewed in [6, 7]). NCC is a secondary active transporter, dependent on the chemical gradient of Na^+ created by $\text{Na}^+\text{-K}^+\text{-ATPase}$, and regulated by aldosterone and **with-no-lysine (K)** kinases (WNK1 and WNK4) pathways [8-11]. The basolateral exit of NaCl in the DCT involves a mechanism that is identical to that of the TAL cells, and is mediated via $\text{Na}^+\text{-K}^+\text{-ATPase}$ in conjunction with a chloride channel (CLC-KB) as shown in Fig. 2. In the distal portion of DCT, CNT and cortical collecting duct (CCD), Na^+ is reabsorbed through the apical membrane via ENaC, which generates a lumen negative voltage, which in turn promotes K^+ secretion in the lumen via ROMK as depicted in Fig. 2 [12-14]. The action of ROMK in the DCT and CCD is responsible for the daily obligatory losses of K^+ and thus dictates the level of K^+ in the blood [15-17].

In the connecting tubule (CNT) and CCD, around 4% of filtered NaCl reabsorption is mediated by a rather

Fig. 2. Schematic diagram showing a cell model of the distal convoluted tubule (DCT) with its different transport pathways involved in the transcellular reabsorption of NaCl. Early DCT expresses only NCC whereas late DCT expresses both NCC and ENaC in the apical membrane.

Fig. 3. Schematic diagram showing cell types in both cortical collecting duct (CCD) and connecting tubules (CNT) with different transport pathways involved in transcellular reabsorption of NaCl. ENaC is expressed on the apical membrane of principal cells and pendrin (PDS) is expressed on the apical membrane of B-intercalated cells.

more complex mechanism involving coordinated and parallel activities of two transport processes expressed in the apical membranes of two different cell types as shown in Fig. 3. In this process, the $\text{Cl}^-/\text{HCO}_3^-$ exchanger pendrin (SLC26A4) expressed on the apical membrane of B-type of intercalated cells absorbs Cl^- in exchange for HCO_3^- , which is secreted in the lumen of late DCT, CNT and CCD tubules [18, 19]. In B-type of intercalated cells, chloride exits the cell through the basolateral membrane via CLC-3 chloride channel [20]. In parallel, Na^+ reabsorption is mediated via ENaC, which is expressed on the apical membrane of principal cells along CNT and CCD. The epithelial sodium channel ENaC activity is energized by the inward gradient of Na^+ created by the basolateral Na^+/K^+ -ATPase activity (Reviewed in [21, 22]). The reabsorption of Na^+ via ENaC generates a lumen negative voltage which activates the secretion of K^+ via K^+ channel ROMK. Pendrin is sensitive to changes in acid-base status [23, 24], and both pendrin and ENaC expression and activities are regulated by aldosterone [25-28] and vasopressin [29-31].

Pendrin and diuresis

In conditions associated with impaired fluid balance such as congestive heart failure, nephrotic syndrome, renal failure, and liver cirrhosis, loop diuretics such as furosemide have been used in conjunction with thiazide derivatives to inhibit NaCl reabsorption in the distal nephron and increase salt excretion in order to reduce fluid retention. The schematic diagram in Fig. 4 indicates that furosemide, a loop diuretic, inhibits the thick ascending limb apical $\text{Na}^+/\text{K}^+/\text{2Cl}^-$ cotransporter (NKCC2), whereas thiazide diuretics such as hydrochlorothiazide or its derivatives block the apical NaCl cotransporter (NCC) in the distal convoluted tubule. This combined inhibition of salt absorption in two distinct nephron sites significantly increases salt excretion. Interestingly, the PDS/ENaC functional complex is actively involved in NaCl reabsorption in the CNT and CCD as described above, raising the possibility that it might interfere with the diuretic effects of both loop and thiazide diuretics by blunting their impact on salt and fluid excretion.

Fig. 4. Schematic diagram of various nephron segments, including TAL and DCT along with transport proteins targeted by furosemide (NKCC2) or thiazides (NCC). G, glomerulus; PCT, proximal convoluted tubule; PST, proximal straight tubule; TAL, thick ascending limb; DCT, distal convoluted tubule; CCD, cortical collecting tubule; OMCD, outer medullary collecting duct; IMCD, inner medullary collecting duct.

Fig. 5. Schematic diagram of the nephron showing the action sites for thiazides, inhibitors of NCC, and a new diuretic which can inhibit pendrin.

It is well known that the furosemide and thiazide combination therapy is more efficient in causing salt and fluid excretion than furosemide alone [32]. Thiazide therapy alone is far less potent than furosemide as a diuretic agent. Thiazide specifically inhibits NCC and a wealth of studies demonstrates that NCC deletion in mouse or inactivating mutation in human does not cause significant salt wasting under baseline conditions [33]. This suggests that either the DCT, and thus NCC, is not handling a significant amount of salt reabsorption, or that other NaCl absorbing transporters such as PDS/ENaC functional complex in the CNT and CCD are rather activated and thus compensate for the inhibition or deletion of NCC in the DCT. In support of this possibility, recent studies from our laboratory [34] demonstrated that pendrin is significantly upregulated in kidneys of NCC knockout mice.

The PDS gene deletion in mouse or inactivating mutations in human does not lead to excessive salt and fluid wasting or changes in blood pressure under baseline conditions [32-34]. These results have been interpreted to indicate that pendrin might be only active under salt depleted states. However, treatment of PDS knockout mice with thiazide results in a more significant diuresis

than their wild type littermates, as shown by increased urine volume and decreased urine osmolality [34]. The role of pendrin in compensatory salt absorption was further supported in mice treated with furosemide: pendrin KO mice demonstrated a more profound diuresis and weight loss relative to their wild-type littermates [34]. These results correlate very nicely with studies demonstrating that furosemide treatment of rats increased the abundance [35] and trafficking of pendrin to the plasma membrane of B-type IC [36]. Taken together, these findings support a compensatory role for PDS/ENaC functional complex in response to the deletion or inhibition of NCC or the inhibition of the TAL NKCC2 by furosemide.

Our results demonstrate that thiazide treatment in NCC KO mice does not cause additional diuresis above what is observed under baseline conditions in the mutant animals, strongly suggesting that the diuretic effect of thiazide is exclusively due to the inhibition of NCC [34]. This latter observation may be at conflict with a recent publication indicating that thiazide might increase salt excretion in NCC KO mice [37]. The authors concluded that thiazides might inhibit the sodium dependent chloride/bicarbonate exchanger (NDCBE) functioning in

conjunction with pendrin [37]. One impediment to this conclusion is the non physiologic concentration of thiazide that was used to inhibit NDCBE [37]. The authors used a pharmacological rather than a physiological concentration of thiazide (1mM) to inhibit NDBCE *in vitro*, casting doubt on the specificity of the thiazide effect. Further, while NDCBE activity was optimally observed in sodium depleted wild type mice, there was no study showing enhanced salt excretion in sodium depleted NDCBE KO mice [37].

Published reports indicate that single deletion of pendrin or NCC does not cause any salt wasting under baseline condition [33, 38, 39]. These studies indicate that mice with single deletion of pendrin or NCC develop hypotension only under salt restricted conditions, raising the possibility that these two transporters are only active in salt depleted states. We hypothesized that pendrin and NCC are active under baseline conditions but compensate for the loss of each other, therefore minimizing the amount of salt that is wasted in pendrin or NCC KO mice. To test our hypothesis, we generated mice with double deletion of pendrin and NCC by crossing pendrin KO and NCC KO mice with each other. The results are startling and show profound polyuria,

polydipsia, and decreased urine osmolality along with a sharp increase in urinary salt excretion in double pendrin/NCC KO mice, when compared to wild-type, NCC KO or PDS KO mice (American Society of Nephrology annual meeting, Philadelphia, Nov. 2011). Double Pendrin/NCC KO mice show profound dehydration along with significant renal failure subsequent to reduced vascular volume (ASN, Philadelphia, Nov. 2011; Manuscript in preparation).

Taken together these data clearly indicate that pendrin plays an important role in compensatory salt absorption in response to furosemide or thiazide therapy. Further, pendrin compensates for the loss of NCC, as shown by profound diuresis and salt wasting in double pendrin/NCC KO mice. We propose that pendrin inhibition could be a novel target of diuretic therapy which in conjunction with thiazides could provide a powerful diuretic regimen, as indicated in the Fig. 5.

Acknowledgements

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