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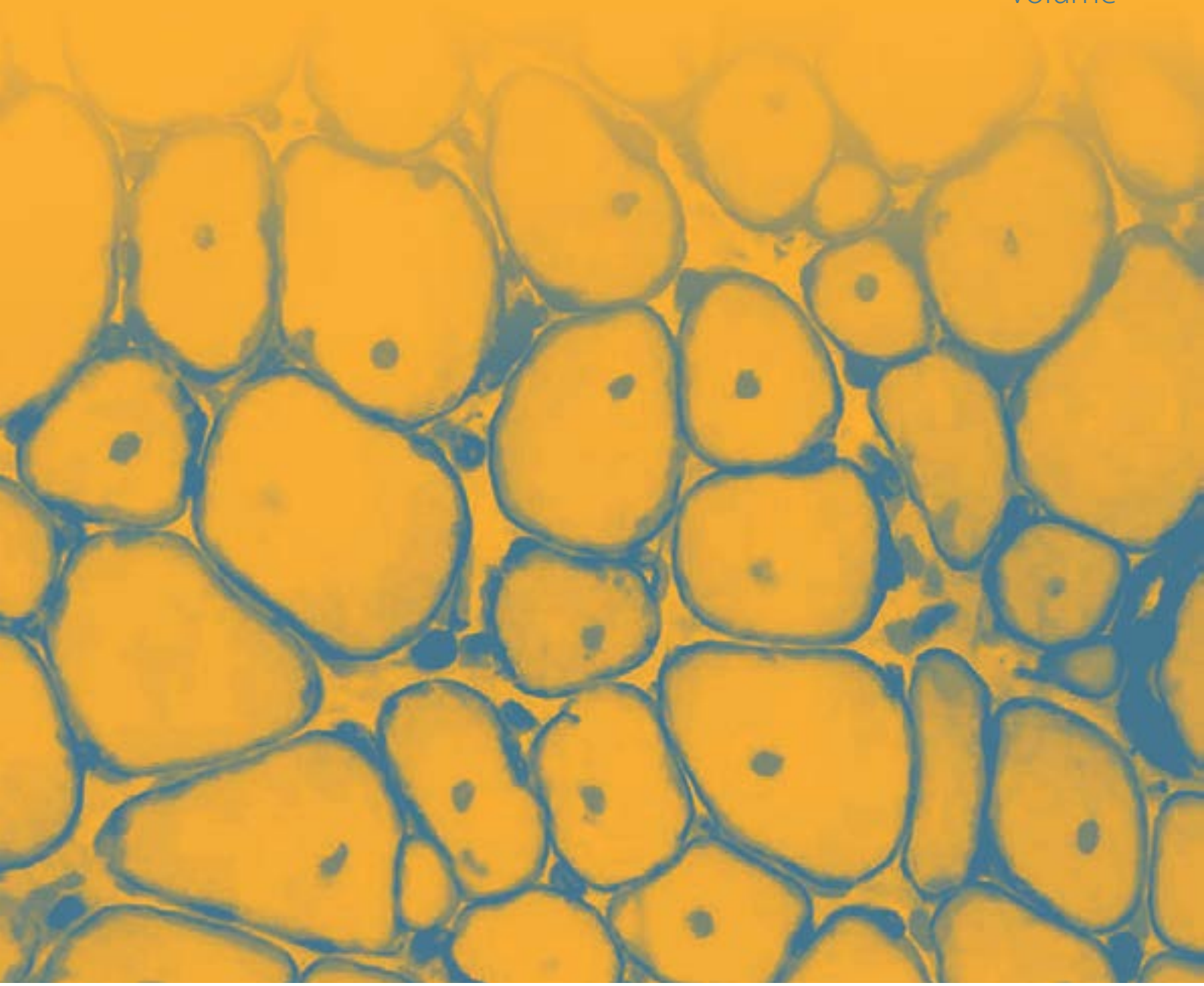

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Facultad de Ciencias Médicas; Universidad Nacional de La Plata;
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ROLE OF THE DISTAL NEPHRON ON SALT HOMEOSTASIS AND BLOOD PRESSURE REGULATION

Heeju Lim[1] and Cesar A. Romero[1]

[1] Renal Division, Department of Medicine, Emory University-School of Medicine, Atlanta, GA, USA.

Correspondence to: cromerocba@hotmail.com

ABSTRACT

The distal nephron is constituted by the distal convoluted tubule, connecting tubule, and collecting duct. It is also known as "aldosterone sensitive distal nephron" (ASDN) because it is the anatomical substrate for the classical aldosterone effect of reabsorbing sodium and excreting potassium. This region is thought to perform the "fine-tuning" of ions and water homeostasis to balance intake levels. Consequently, this segment has significant impacts on sodium reabsorption and blood pressure regulation, illustrated by some genetic alteration effects such as Liddle syndrome, pseudohypoaldosteronism, and Pendred syndrome. In this mini-review, we explore the ASDN structure and the main channels that participate in sodium and chloride reabsorption. The epithelial sodium channel (ENaC) and the interchanger chloride-bicarbonate transporter (pendrin) are reviewed in detail as the main drivers of salt reabsorption in this segment. We also provide an introduction to crosstalk communication between the connecting tubule and the afferent arteriole as a feedback mechanism to integrate tubular sodium handling in this segment, in addition to its role in renal hemodynamics autoregulation.

Keywords: Distal nephron; ENaC; Pendrin; Connecting tubule; salt; hypertension.

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Introduction

The functional unit of the kidney, called the nephron, is responsible for the ultrafiltration of plasma and controlling which substances pass into the urine. Each human kidney contains around one million nephrons, each of which comprises vascular capillaries (the glomerulus) and a series of tubule segments where the glomerular ultrafiltrate is subjected to qualitative and quantitative changes, ultimately forming the urine. The tubule segments can be broadly classified into proximal segments and distal nephrons because of their embryological origin, thus, the proximal segments derive from the metanephric mesenchyme and the distal segments from the ureteric bud[1]. The proximal segments include the proximal tubules, the loop of Henle, the macula densa, and the first portion of the distal convoluted tubule (DCT) (figure 1). In general, the proximal segments are responsible for the majority of the quantitative changes to the ultrafiltrate as 75% of the water and 90% of solutes are reabsorbed [2]. The distal nephron includes the distal part of the DCT or DCT2, the connecting tubule (CNT), and the collecting ducts (CD). In the distal nephron, the fine-tuning of water and solute reabsorption occurs. Thus, only 3–5% of sodium chloride and 15% of water is reabsorbed in this region [2]. This distal segment is also called the "aldosterone-sensitive distal nephron" (ASDN) as typical aldosterone effects occur in this region, namely, sodium and chloride reabsorption, and potassium and proton secretion [3]. Despite the relatively small amount of water and solutes passing through it, the distal nephron segment can increase sodium and water reabsorption by a factor of between three and five times, depending on homeostasis needs[4]. Thus, this region is extremely important in determining the final sodium chloride and water volume reabsorption, and, accordingly, in controlling processes such as blood pressure regulation[5].

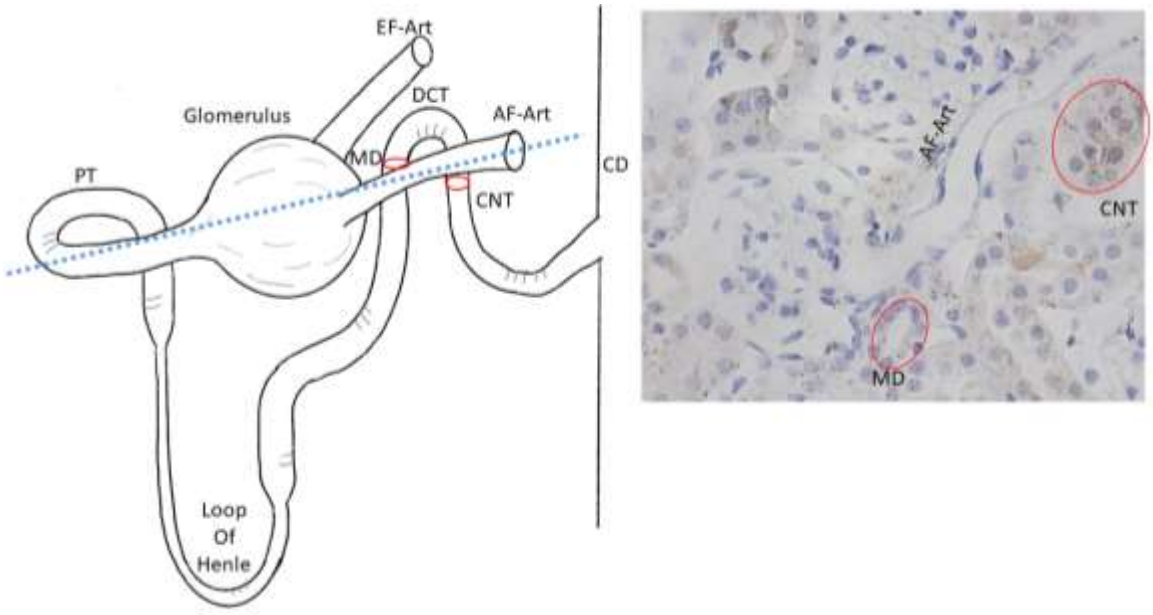

Figure 1. The Nephron. Schematic representation of a isolated nephron and its segments. The capillaries are surrounded by the Bowman's capsule constituting the glomerulus where ultrafiltration occurs. The ultrafiltrate pass through the proximal tubule (PT) and the loop of Henle where a massive amount of solutes and water are reabsorbed. After the macula densa (MD) the Distal convoluted tubule, connecting tubule and collecting duct are in charge of the fine tuning of salt and water reabsorption. Notice the close interaction between the MD and the CNT with the afferent arteriole (Af-Art), where different crosstalk communications occur between the tubule and the vascular bed. The dash-line represents a imaginary cut section plane represented in figure 1B. Figure 1B represent an histologic section of the kidney. The macula densa (MD) is in close relation with the afferent arteriole (Af-Art). A closed tubule next to the Af-art may represent a downstream CNT.

Cell types and transporters in the distal nephron

Typically, aldosterone-sensitive distal nephrons have two main cell types [3]: the principal cells (PC) which express aquaporin-2 and the epithelial sodium channel (ENaC) as cell markers, and the intercalated cells (IC) that express H^+-ATPase[6]. PC starts to appear in the last part of the DCT (i.e. DCT2) and are the main type of cells in the CNT and CD (80%), while intercalated cells form the minority of cells under normal circumstances [7]. In general, PCs participate in sodium and water reabsorption and potassium secretion, whereas ICs participate in acid or bicarbonate secretion and chloride reabsorption. There are three subtypes of IC, namely, the type-A, type-B, and non-A, non-B intercalated cells[5]. These three subtypes have different functions, and their relative frequency occurrence is related to the in-situ physiological status, which can lead to a greater tendency to secrete acid or bases (figure 2). All intercalated cells are characterized by the "family" cell marker H^+-ATPase, however, the localization and addition of other markers can help us to identify the different subtypes [6]. Type-A ICs express H^+-ATPase in the apical membrane since their principal function is to secrete acid (hydrogen ions). Additionally, Type-A ICs express anion exchanger 1 (AE1) in the basolateral side; this protein can interchange bicarbonate and chloride ions by passing bicarbonate into the interstitial space and absorbing a chloride anion to the cytosol (figure 2). Type-B ICs express H^+-ATPase on their basolateral side and express the Cl^-/HCO_3^- exchanger, named pendrin, on their surface. The main role of type-B ICs is to secrete HCO_3^- and reabsorb Cl^-. As discussed later, the chloride reabsorption performed by pendrin is of extreme importance in both sodium reabsorption and facilitating systemic hypertension. Finally, the non-A, non-B IC has an intermediate phenotype where H^+-ATPase and pendrin are both expressed at the apical membrane; although less is known about these cells, they appear to function as reserve cells to differentiate the "mature phenotype" type-A or type-B IC according to homeostasis needs[5].

Both the PC and IC express potassium channels that promote potassium excretion. Thus, PCs express the renal outer medullary potassium channel (ROMK); this potassium channel, as discussed below, is coupled to the electrogenic ENaC transport. Additionally, PCs and ICs both express voltage-gated potassium channels, known as BK channels [8].

ENAC and extracellular volume effects

ENaC is an important sodium channel in principal cells and plays a significant role in the fine-tuning of sodium reabsorption at the end of the nephron. Due to its localization, and because it is the target of several hormones and paracrine factors, ENaC is crucial to extracellular volume regulation.

ENaC is a heterotrimer ion channel with high conductance for sodium. Three subunits form the main components of the channel: alpha, beta, and gamma[9]. A fourth subunit, delta, has been described in some epithelia as partially replacing the alpha subunit, however, its expression is very weak in the kidney and this subunit is not present in rodents[10]. The expression of the three subunits is necessary for full activity of the channel [9]. ENaC total activity depends on both the density of channels at the cytoplasmatic membrane and the open probability. These two variables are regulated in parallel, however, in certain circumstances the surface density and open activity are regulated separately. To be fully active at the surface membrane, the ENaC requires proteolytic cleavage on the alpha and/or gamma subunits in the extracellular loops. Different proteases such as furin and plasmin can increase the open probability by cleaving these subunits [11].

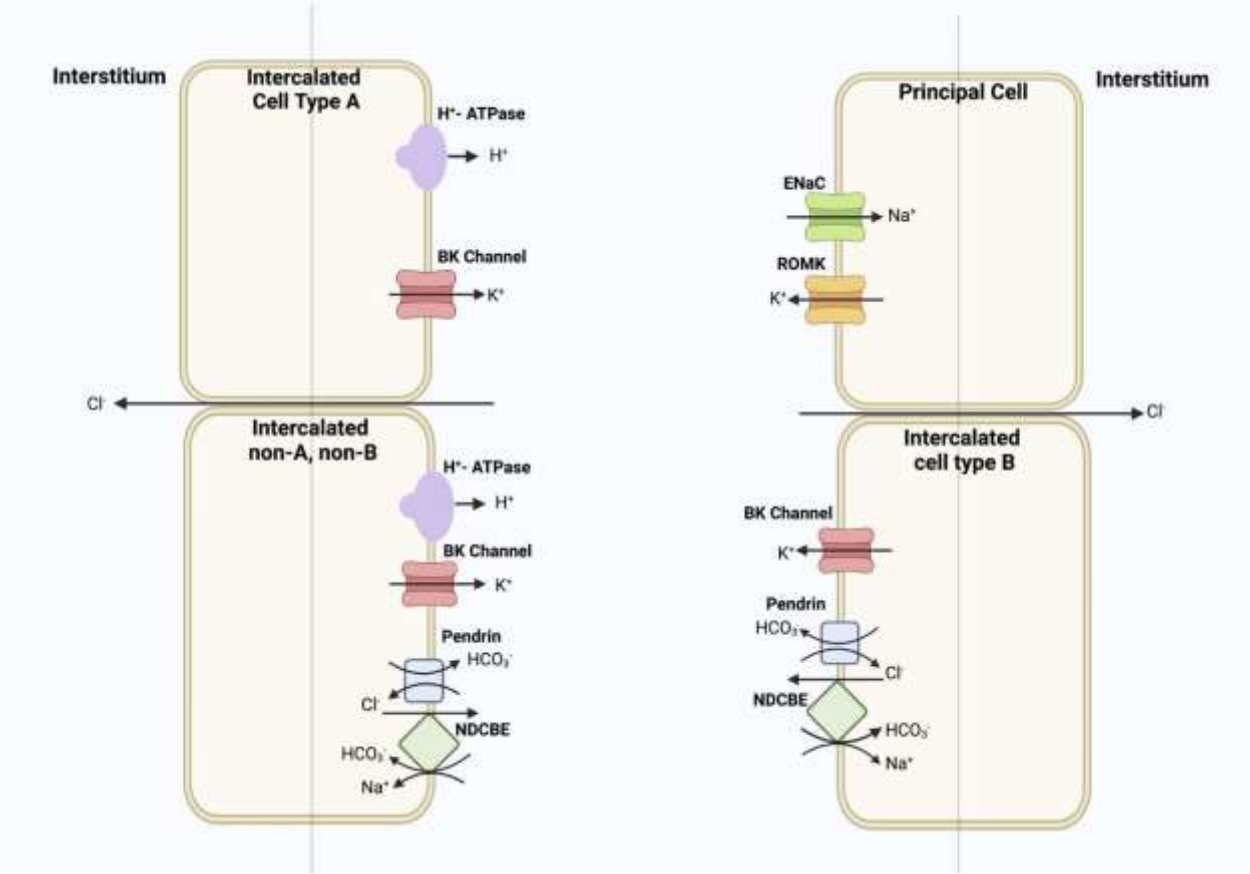

Figure 2. Distal nephron transporters. Principal cells express the epithelial sodium channel (ENaC), that is responsible for a significant reabsorption of sodium. ENaC is an electrogenic transporter that facilitate the excretion of potassium by ROMK. There are three subtypes of IC, namely, the type-A, type-B, and non-A, non-B intercalated cells. All intercalated cells are express the cell marker H+-ATPase. Type-A ICs express H+-ATPase in the apical membrane. Type-B ICs express H+-ATPase on their basolateral side and express the Cl-/HCO3- exchanger, named pendrin, on their surface. The main role of type-B ICs is to secrete HCO3- and reabsorb Cl-. Additionally, Type-B express the sodium-dependent chloride bicarbonate exchanger (NDCBE) channel (SLC4A8) in the surface. The non-A, non-B IC has an intermediate phenotype where H+-ATPase and pendrin are both expressed at the apical membrane; Both the PC and IC express potassium channels that promote potassium excretion. The renal outer medullary potassium channel (ROMK) in PC; Additionally, PCs and ICs both express voltage-gated potassium channels, known as BK channels.

However, ENaC surface density may also decrease through endocytosis and degradation. Nedd4-2 protein is an ubiquitin ligase that ubiquitinates ENaC; this process induces internalization and ulterior degradation, thus decreasing ENaC activity [12, 13].

ENaC is a target of several endocrine, paracrine, and autocrine factors that affect its function. The classic example of endocrine regulation on ENaC is mediated by the renin-angiotensin-aldosterone system. Aldosterone, through the mineralocorticoid receptor increases ENaC surface density by a factor of more than three times, however, it also increases the open probability[14, 15]; Aldosterone has some slow and rapid effects; while the slow effects relate to increased expression of the ENaC subunits, the rapid effects tend to be more strongly related to its surface regulatory pathways[16].

Angiotensin II stimulates ENaC through a combination of increasing aldosterone and mineralocorticoid receptor-independent effects. This aldosterone-independent effect is exacerbated most in DCT2/CNT compared to CCD [17].

Serum- and glucocorticoid-regulated kinase (SGK1) is a serine-threonine kinase that upregulates ENaC by inhibiting Nedd4-2 and favoring increased surface density of ENaC[18]. Notably, aldosterone and insulin stimulate SGK1, which tends to favor the retention of sodium [18].
In addition to protease activation other stimulus can activate ENaC. Thus ions, phospholipids, and mechanical forces can modify the ENaC open probability. High extracellular sodium (>100 mM) can also inhibit ENaC by a mechanism called Na^+ self-inhibition [19]. Na^+ self-inhibition is an important mechanism to prevent exaggerated sodium absorption, however, it is dramatically modified by regulatory proteases which tend to make ENaC less sensitive to the effects of Na^+ self-inhibition. Intracellular phospholipids can increase ENaC open probability without changes in channel density; this lipid regulation is a key feature of recently described ENaC regulation mechanisms by other proteases such as MARCK or MARCKS-like protein (MLP) [20, 21]. Finally, ENaC is mechanosensitive and is activated by shear stress [22]. Thus, an increase in tubular flow tends to increase ENaC activity, however, other mechanisms associated with the natriuresis (such as Na^+ self-inhibition) or endocrine factor may limit this activation.
The role of ENaC in sodium reabsorption, extracellular volume expansion, and blood pressure homeostasis is illustrated by some pathologic states where the channel or its associated regulatory pathway are altered.
Thus, increases in the functional variant of ENaC are responsible for Liddle syndrome, where hypertension and hypokalemia are present in young people [23]. The ENaC variant present in most Liddle syndrome cases affects the region where ENaC interacts with Nedd4-2, thus, increasing the apical density of the channel[24]. The electrical gradient generated by Na^+ reabsorption is also responsible for K^+ secretion by ROMK to maintain electroneutrality. The massive potassium excretion is responsible of the hypokalemia observed in most patients, although not in all of them [23]. Other mutations of ENaC have been described, however, it is not yet clear whether these can be linked to other forms of hypertension [25].
Alternatively, the loss of some functional variants of ENaC is responsible for pseudohypoaldosteronism type I, where patients present with hypotension, hypovolemia, hyponatremia, and hyperkalemia despite the presence of high levels of aldosterone and glucocorticoids in the plasma[26].
These scenarios clearly illustrate the role of ENaC and the distal nephron on extracellular volume and blood pressure regulation. Additionally, as we will discuss later, ENaC participates in renal blood flow regulation, linking the intratubular status to the renal blood supply.

The role of chloride in salt and blood pressure homeostasis

For many years, a great deal of emphasis has been placed on the effect of sodium on extracellular volume expansion and blood pressure. However, there is evidence showing that if chloride anions are replaced in a saline solution, for example by citrate or bicarbonate, the effects on extracellular volume are diminished[27]. Therefore, various studies have considered the impact of renal chloride and its associated effects on sodium absorption.
Pendrin is a chloride/bicarbonate interchange transporter, present in both type-B and non-A, non-B intercalated cells[28]. Pendrin is responsible for a significant part of chloride absorption and HCO_3^- excretion in the distal nephron, and was originally understood to participate primarily in acid-base disorders [29]; thus, pendrin is highly active in metabolic alkalosis and decreases in metabolic acidosis. In addition, pendrin has a significant role in salt reabsorption and shares some of the regulatory pathways of ENaC, such as angiotensin II and aldosterone [30, 31]. The role of pendrin on salt reabsorption has been demonstrated in several acute and chronic experiments. The acute genetic ablation of pendrin, by pharmacological promoter manipulation, decreased blood pressure in around 10 mmHg [32]. However, in the long term, these animals displayed compensations in terms of blood pressure; pendrin knockout mice are normotensive in

comparison with wild-type mice, and only show defects in salt reabsorption when another stimulus is introduced. Thus, in pendrin knockout mice, the exogenous administration of aldosterone prevented the blood pressure elevation and water retention observed in wild-type animals where pendrin is highly activated compared with vehicle-infused animals[33]. Notably, the lack of hypertension is also accompanied by urinary loss of chloride and sodium[33]. Due to the impairment of bicarbonate secretion, these knockout animals are more prone to developing metabolic alkalosis. As stated above, chloride absorption is necessary for sodium reabsorption, thus, some researchers have explored the role of pendrin in sodium absorption. Despite not being a Na^+ transporter, the absence of pendrin tends to decrease sodium absorption. Mechanistic studies have shown that the decreased sodium absorption in absence of pendrin is, at least in part, due to decreased ENaC activity in principal cells[33]. The mechanism controlling ENaC inhibition in the absence of pendrin is not currently known, however, there seems to be some paracrine communication between intercalated and principal cells. In this regard, intratubular bicarbonate or pH levels may affect ENaC activity[34]. Additionally, ATP secretion from intercalated cells due to reduced H^+-ATPase action may also inhibit ENaC activity[5].

On the contrary, the genetic overexpression of pendrin induces hypertension in mice when they are exposed to high salt diets. Such mice showed a positive chloride and sodium balance in comparison to wild-type animals[35].

Although the aforementioned studies provide insights into pendrin's role in salt and blood pressure regulation, less, however, is known about pendrin regulation. Similar to ENaC, pendrin is regulated by angiotensin II, aldosterone, and Nedd4-2. Angiotensin II, through the AT1(a) receptor, induces pendrin cell surface expression, and, in doing so, increases Cl^- absorption[30]. Aldosterone, through mineralocorticoid receptor-dependent and -independent effects, increases pendrin expression and surface density, independent of the K levels induced by aldosterone[31]. Thus, in animals infused with angiotensin II or aldosterone, the absence of pendrin minimizes blood pressure increase.

The genetic ablation of Nedd4-2 tends to increase pendrin expression and blood pressure. The blood pressure elevation observed in Nedd4-2 knockout mice was notably prevented in mice with the concomitant absence of pendrin, demonstrating a key role of pendrin in blood pressure regulation[36].

A less well-studied mechanism associated with NaCl reabsorption in the aldosterone-sensitive nephron is dependent on the sodium-dependent chloride bicarbonate exchanger (NDCBE) channel (SLC4A8). NDCBE in intercalated cells may be responsible for up to ~40-50% of NaCl reabsorption in the distal nephron and is thiazide-sensitive. NDCBE is an electroneutral exchanger that reabsorbs sodium and bicarbonate and secretes chloride that is eventually re-reabsorbed paracellularly by claudins[5, 37].

In summary, these data demonstrate that the role of distal nephron on salt homeostasis is not restricted to ENaC on principal cells but also to intercalated cells.

Connecting tubule regulate renal blood flow and sodium excretion

Distal nephron includes the CNT, which has the particular characteristic of being in physical contact with the afferent arteriole. A special crosstalk communication, therefore, occurs between the CNT and the afferent arteriole that forms part of the kidneys' autoregulatory mechanisms (figure 1).

The kidneys have autoregulatory mechanisms that modulate the afferent arteriole tone, for instance, the capillary pressure and the glomerular filtration[38]. In addition to local autoregulatory mechanisms present in the majority of the body's vascular bed, such as myogenic response and paracrine/autocrine signaling, the kidney has two intrinsic mechanisms known as tubule-glomerular feedback (TGF) and connecting tubule-glomerular feedback (CNTGF)[38].

These mechanisms include communication between the tubules (epithelial cells) and the arterioles, taking advantage of the physical contact that exists between the macula densa (thick ascending limb) and the connecting tubule with the afferent arteriole (figure 1).
At the macula densa, sodium is detected by the NKCC2 channel and ATP-adenosine is released, inducing vasoconstriction of the afferent arteriole when high sodium levels are detected. Tubulo-glomerular feedback is mainly oriented to decrease the sodium filtration by decreasing glomerular pressure after afferent arteriole vasoconstriction; this mechanism, therefore, tends to maintain sodium levels or avoid the exaggerated loss of sodium[38].
On the other hand, CNTGF is a vasodilator mechanism that is triggered when high amounts of sodium are detected in the connecting tubule, inducing afferent arteriole vasodilation[39]. CNTGF is a positive feedback mechanism; thus, the induced vasodilator response tends to increase glomerular pressure and sodium filtration, thereby increasing sodium load to the connecting tubule and increasing vasodilation. This mechanism is thought to be oriented to favor sodium excretion in an environment of volume expansion[39].
CNTGF occurs in the principal cells of CNT. When high sodium is detected by ENaC, the principal cells release prostaglandins and epoxyeicosatrienoic acids that induce afferent arteriole dilation. It appears that half of the vasodilation effect is mediated by prostaglandin E2, through the EP4 receptor, and the other half is mediated by the epoxyeicosatrienoic acids[40]. Several physiological situations show high CNTGF activity when sodium excretion is increased, such as in high salt diets, obesity, and in the remnant kidney after nephrectomy[39].
As stated above, as opposed to CNTGF, TGF tends to increase vasoconstriction and retains sodium, however, the stimulus is the same in both instances, i.e., the presence of a high amount of sodium. Although it may seem counterintuitive that the same stimulus (in different parts of the nephron) can induce such opposite responses, considering the physiological situation may help in understanding the nature of this mechanism. TGF acts to prevent acute sodium loss, however, if we acquire a significant sodium load, more sodium then needs to be excreted. If only the TGF mechanism was taking place, every time that the amount of sodium increased, TGF would prevent its excretion and sodium and water would be retained, however, this is not the case in our daily life. Thus, when sodium needs to be excreted, an adaptation occurs called TGF resetting, where the amount of sodium required for TGF activation becomes much higher. Thus, TGF resetting allows the nephron to lose more sodium. The mechanism behind TGF resetting is not well-understood, but CNTGF can explain TGF resetting, at least in part. When CNTGF is activated, the vasoconstriction induced by TGF is much less than when CNTGF is inhibited; thus, the amount of sodium that is needed to induce the same vasoconstriction through TGF is increased. This is known as the TGF-CNTGF interaction and appears to have an important effect on modulating sodium excretion according to the body's needs[39].
Hypothetically, if CNTGF is absent, more TGF activation with sodium retention and a subsequent increase in blood pressure can be predicted. In spontaneously hypertensive rats, used as a genetic model of hypertension, CNTGF is almost absent and TGF response is greatly exaggerated; thus, the absence of CNTGF may at least partially explain the hypertension observed in those rats. In our lab, preliminary work has also demonstrated that CNTGF inhibition may increase blood pressure[41].

Conclusion

The aldosterone-sensitive distal nephron is extremely important in determining the final reabsorption of sodium chloride and water volume, and, therefore, in processes such as blood pressure regulation.
This segment provides the anatomical substrate for fine-tuning of sodium and potassium excretion in addition to acid-base regulation and water reabsorption. In this review, we focused

primarily on salt homeostasis, with potassium, acid-base, and water regulation to be addressed in another chapter.

The distal nephrons with their different cell subtypes and interrelated channels, such as ENaC, pendrin, and NDCBE, are crucial for sodium chloride homeostasis, blood flow regulation, and, for instance, in blood pressure regulation. Many endocrine and paracrine factors as well as their pharmacological manipulation, such as the RAAS system, target this important segment of the nephron.

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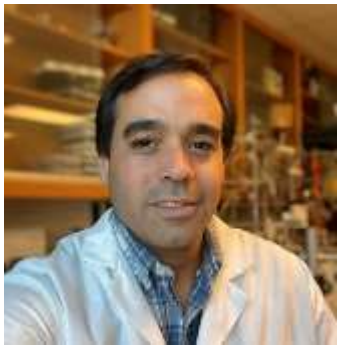
ABOUT AUTHORS



Cesar Romero MD, PhD is assistant professor at Renal Division, Emory University School of Medicine in Atlanta, USA. He coordinates the Integrative and Translational Research (ITR) in Hypertension group where several aspects of hypertension are explored, including renal mechanism of hypertension and blood pressure control. A complete list of contribution can be found: https://www.ncbi.nlm.nih.gov/myncbi/cesar.romero.1/bibliography/public/

Heeju Lim is a fourth-year undergraduate student at Emory University College of Arts and Sciences in Atlanta, GA. She is pursuing her Bachelor of Science degree as a Biology major and religion minor.