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The Role of Sodium, Calcium, and Magnesium Transport in the Renal Distal Tubule

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Abstract：The renal distal tubule, which includes the early distal tubule (i.e., the distal convoluted tubule) and the late distal tubule (i.e., the connecting segment and initial collecting tubule), plays an important role in regulating ion homeostasis, body fluid volume, and blood pressure. These tubular segments regionally express many kinds of ion channels and transporters permeable to Na^+, Ca^{2+}, and Mg^{2+}, which are exquisitely tuned by several regulatory mechanisms. Recent molecular biological and electrophysiological studies revealed the physiological functions and regulatory mechanisms of Na^+, Ca^{2+}, and Mg^{2+} transports in the distal tubule. This short review presents the recent advance in the understanding of the roles of the distal tubule in regulating cation metabolism.

Key words：**Renal distal tubule, Ion channel, Ion transporter, Mg^{2+} transport**

Introduction

The renal distal tubule is composed of the distal convoluted tubule (DCT), the connecting tubule (CNT), and initial part of collecting tubule (ICT; Fig. 1).[1] Furthermore, the DCT in rodents and humans, but not in rabbits, is divided into the early (DCT1) and late segments (DCT2). Both these DCT segments contain a uniform population of principal cells.[1)–3)] On the other hand, the CNT includes both principal cells and two types of intercalated cells.[1)–3)] Many kinds of ion channels and transporters (for Na^+, Ca^{2+}, Mg^{2+}, and so on) regulated by individual molecular mechanisms are regionally expressed in the renal distal tubule (see Fig. 1).[2)–6)] Consequently, the distal tubule exquisitely tunes cation metabolism. This article reviews the functional characteristics and molecular mechanisms of cation transports in the distal tubule.

Na^+ transport in the distal tubule

Na^+ transport in the distal tubule is important for Na^+ reabsorption via the renal epithelia, resulting in controlling Na^+ homeostasis, body fluid volume, and blood pressure. As shown in Figure 2A, a two–step process is involved in the Na^+ transport in the distal tubule; 1) Luminal Na^+ enters into the tubular cell through Na^+ transporters in the apical membrane. 2) Intracellular Na^+ is excreted into the vessel side via Na^+,K^+–ATPase (NKA) in the basolateral membrane. The major apical Na^+ transporters of the DCT and CNT are the thiazide–sensitive Na^+–Cl^- cotransporter (NCC) and the amiloride–sensitive epithelial Na^+ channel (ENaC), respectively.[1)4)6)] The NCC is a member of the solute carrier family (SLC12A3) and its loss–of–function mutation causes Gitelman's syndrome, an autosomal recessive tubulopathy characterized by mild renal Na^+ wasting, hypocalciuria, hypomagnesemia, and hypokalemic alkalosis.[7)] On the other hand, the NCC activity requires appropri-

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ate Cl^- excretion across the basolateral membrane (Fig. 2A), which is physiologically mediated by ClC-K2 (the rodent ortholog of human ClC-Kb).[8)9)] Thiazide diuretics specifically inhibit NCC, thus resulting in an enhanced renal Na^+ excretion. Furthermore, thiazide diuretics are known to affect the Ca^{2+} and Mg^{2+} balance, inducing hypocalciuria and hypomagnesemia, respectively.[10)] Consequently, thiazides are frequently used in the treatment of idiopathic hypercalciuria and nephrolithiasis, as

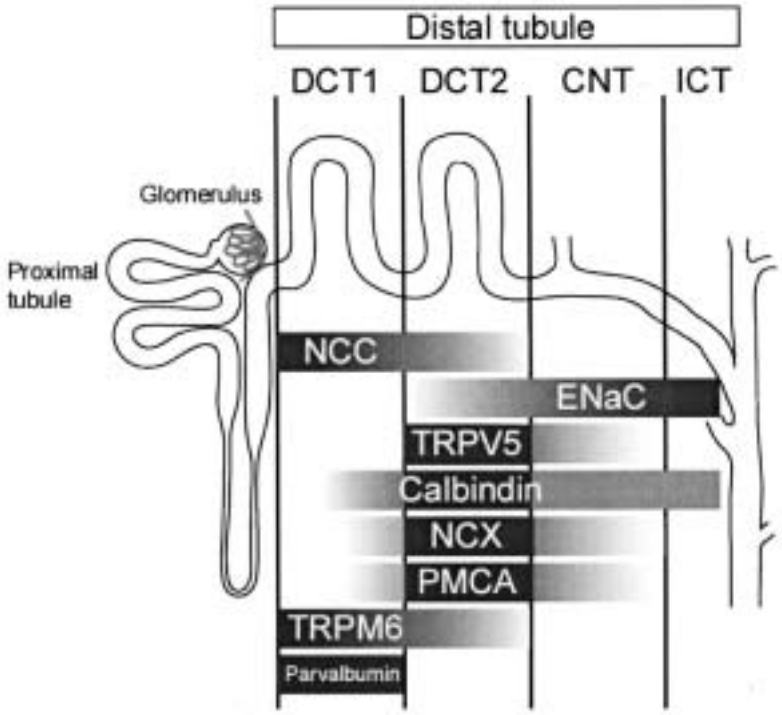

Fig. 1. Distributions of Na^+, Ca^{2+}, and Mg^{2+} transporters in the distal tubule. Shadings of bars indicate relative changes of the abundance of these transporters along the segments. Abreviations are exhibited in the text.

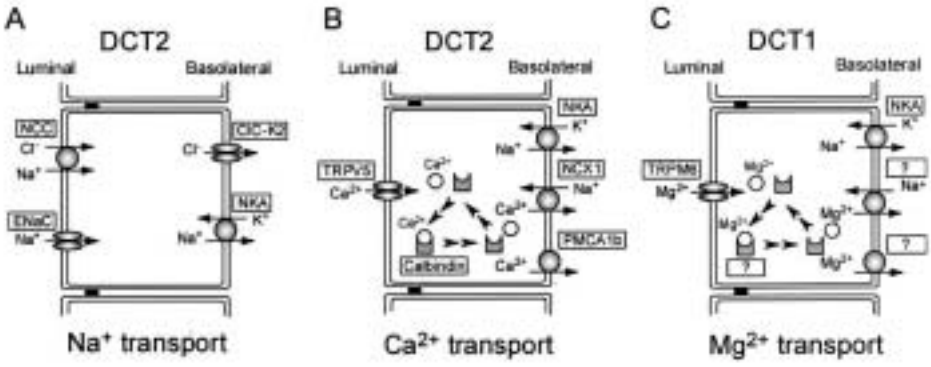

Fig. 2. Schematic diagrams of ion transports in the distal tubule. (A) Na^+ transport of the distal tubule is mediated by apical Na^+ transporters and the basolateral Na^+, K^+-ATPase (NKA). Na^+ entry in the apical membrane of DCT and CNT are mainly mediated by the thiazide-sensitive NCC and ENaC, respectively. (B) Ca^{2+} transport of the distal tubule is mainly mediated by apical TRPV5 channel and basolateral NCX1. (C) Mg^{2+} entry of the DCT is meditated by apical TRPM6 channel. On the other hand, basolateral Mg^{2+} excretion is assumed to be mediated by Na^+/Mg^{2+} exchanger and Mg^{2+}-ATPase.

well as in the treatment of arterial hypertension.[10]

The ENaC is formed by the combination of three structurally related subunits, the α-, β- and γ-subunits.[11)-13)] Functional ENaC exhibits high sodium selectivity (>1/20) with a small conductance (<5 pS) and is inhibited by submicromolar amiloride.[13)] ENaC is hormonally upregulated by aldosterone and vasopressin.[13)-15)] The ENaC activity is also controlled by several intra- and extracellular factors such as proteases (prostasin and elastase), the extracellular and intracellular Na^+ concentration, and mechanical stress.[13)16)-18)] These findings raise the possibility that ENaC is a sensor and modulator for renal Na^+ metabolism. Gain-of-function mutations in the β- and γ- subunits of ENaC have been identified in patients with Liddle's syndrome, a disorder characterized by volume expansion and hypertension.[19)20)] In contrast, its loss-of-function mutations have been identified in patients with type I pseudohypoaldosteronism, a disorder characterized by volume depletion, hypotension, and hyperkalemia.[21)] These findings confirm that ENaC plays a critical role in the regulation of body fluid volume and blood pressure in humans. However, ENaC has actually been dismissed as a target for antihypertensive treatment, because of the weak efficacy of the ENaC antagonist, amiloride.

Ca^{2+} transport in the distal tubule

Ca^{2+} homeostasis in the body is tightly controlled by three mechanisms, including bone resorption and formation, intestinal absorption, and renal reabsorption. In the kidney, approximately 85% of filtered Ca^{2+} in the glomerulus passively enters the proximal tubule and the thick ascending limb. The Ca^{2+} absorption in these segments is not systemically regulated. On the other hand, Ca^{2+} transport mediated by the transient receptor potential vanilloid 5 (TRPV5) and Na^+/Ca^{2+} exchanger 1 (NCX1) in the DCT and CNT are finely regulated, thus finally resulting in the control of the renal Ca^{2+} excretion.[5)22)23)]

Ca^{2+} transport of the distal tubule is a three-step process; 1) Ca^{2+} entry is primarily mediated by the TRPV5 channel in the apical membrane. 2) Entered Ca^{2+} binds an intracellular carrier protein, calbindin-D_{28k}, and it diffuses to the basolateral membrane. 3) Ca^{2+} excretion is mediated by NCX1 and the plasma membrane Ca^{2+}-ATPase type 1b (PMCA1b) in the basolateral membrane (Fig. 2B).[5)22)] The TRPV5, a member of the TRP channel superfamily, works as an epithelial Ca^{2+} channel.[22)23)] Calbindin-D_{28k}, a vitamin D_3-dependent Ca^{2+} binding protein, is definitely expressed in the principal cells of the distal tubule and plays a role in facilitating the intracellular Ca^{2+} diffusion in the tubular cells.[24)] In addition, calbindin-D_{28k} translocates to the TRPV5 and directly associates with the channel protein under the low intracellular Ca^{2+} conditions.[25)] NCX1 exchanges Ca^{2+} and Na^+ in a 1:3 stoichiometric ratio and accounts for 70% of the Ca^{2+} excretion in the basolateral membrane of the DCT and CNT.[5)26)27)] The benzyloxyphenyl derivatives (KB-R7943, SEA0400, SN-6, and YM-244769) have recently been developed as specific NCX inhibitors.[28)] However, the effects of NCX inhibitors in physiological renal function have not been examined in detail. The Ca^{2+} reabsorption in the kidney is all under the control of the calciotropic hormones that are released upon a demand for Ca^{2+}. These calciotropic hormones affect systemic Ca^{2+} reabsorption, which is controlled by TRPV5, calbindin-D_{28K}, NCX1, and PMCA1b.[29)]

Mg^{2+} transport in the distal tubule

Mg^{2+} is abundant in the body and an important divalent cation in biological systems such as cellular energy metabolism, ion channel activity, and enzyme activity. Mg^{2+} homeostasis primarily depends on the balance between intestinal absorption and renal excretion. Intestinal absorption mainly occurs in the small intestine via active transcellular and paracellular passive transport. In the kidney, ~80% of the total serum Mg^{2+} is filtered in the glomeruli with >95% being reabsorbed along the nephron. Although only 5-10% of the filtered Mg^{2+} is reabsorbed in the DCT, this segment exquisitely regulates renal Mg^{2+} excretion.[6)29)-31)]

Mg^{2+} transport in the DCT is assumed to be three-step process; 1) Mg^{2+} entry is primarily mediated by the transient receptor potential melastatin 6 (TRPM6) channel in the apical membrane. 2) Entered Mg^{2+} binds an intracellular carrier protein

and diffuses into the basolateral membrane. 3) Mg^{2+} excretion may be mediated by a Na^+/Mg^{2+} exchanger and Mg^{2+}–ATPase in the basolateral membrane (Fig. 2C).[6)29)] TRPM6, a member of the TRP channel superfamily, exhibits strong outward rectification with a high affinity for Mg^{2+}.[32)] Human mutations in TRPM6 are responsible for hypomagnesemia with secondary hypocalcemia.[33)34)] Parvalbumin and calbindin–D_{28K} are assumed to be intracellular Mg^{2+} binding proteins, since these proteins are co–localized with TRPM6 in the DCT.[32)] On the other hand, Mg^{2+} transporters in the basolateral membrane have not been identified. Na^+/Mg^{2+} exchangers have been shown to be inhibited by quinidine and imipramine.[6)] However, there is little information about the renal actions of these inhibitors. Recent molecular biological and electrophysiological analyses found several functional Mg^{2+} transporters in an immortalized DCT cell line (Table 1).[35)–47)] Although these transporters are considered to be involved in Mg^{2+} influx or efflux in the DCT, further studies are necessary to clarify their physiological functions.

Conclusions

Numerous studies have revealed that several ion channels and transporters permeable to Na^+, Ca^{2+} and Mg^{2+} are regionally expressed in the renal distal tubule and these ion transports may play an important role in regulating ion homeostasis, body fluid volume, and blood pressure. However, the

Tabel 1. Main features of molecularly identified Mg^{2+} transporters in the kidney

Transporter	Cells/Tissue Distribution	Comments	Ref.
ACDP2	Ubiquitous, mRNA highest in brain and kidney	Gene upregulated in hypomagnesimic condition	35)
HIP14	MDCT, MDCK	Protein and Gene upregulated in hypomagnesimic condition ; HIP like protein (HIP14L) also carried Mg^{2+} and upregulated in hypomagnesiumic condition	36)
MagT1	Ubiquitous, mRNA highest in Liver, followed by Heart and Kidney	Protein and Gene upregulated in hypomagnesimic condition	37)
MMgT1	Ubiquitous, mRNA highest in Heart, followed by Kidney	Protein and Gene upregulated in hypomagnesimic condition	38)
MMgT2	Ubiquitous, mRNA highest in Kidney, followed by Brain	Gene upregulated in hypomagnesimic condition	38)
Mrs2	Ubiquitous, Inner mitochondrial	Regulates mitochondrial membrane potential	39)
NIPA family	Ubiquitous	Protein and Gene upregulated in hypomagnesimic condition ; NIPA1–4 are Mg^{2+} transporter ; NIPA2 is most selective Mg^{2+} transporter	40)41)
Paracellin–1 (Claudin 16)	Tick ascending limb (TAL)	Tight junction protein that regulates paracellular Mg^{2+} transport in the TAL ; mutations cause hypomagnesemia with hypercalciuria and nephrocalcinosis	42)
SLC41A1	Ubiquitous, mRNA highest in Heart and Testis	Related to bacterial MgtE transporter family ; Gene upregulated in hypomagnesimic condition	43)
SLC41A2	Kidney, MDCT, Immune lineage cells	Related to bacterial MgtE transporter family ; Overexpression in $TRPM7^{-/-}$ cells can partially compensates for their requirement in supplemental Mg^{2+}	44)
TRPM6	Mainly in Intestine, Kidney, Lung	Mutations cause hypomagnesemia with secondary hypocalceia(HSH), leading to seizures and death unless supplemented with Mg^{2+} ; associates with TRPM7	32)
TRPM7	Ubiquitous	Deficiency is lethal, negatively regulated by intracellular Mg^{2+}, regulated by angiotensin II, aldosterone, bradykinin, stretch, and osmotic gradient	45)

Information is based on data from Touyz[46)] and Schmitz et al.[47)] ACDP, ancient conserved domain protein ; HIP, huntingtin–interacting protein ; MagT, magnesium transporter ; MMgT, membrane magnesium transporter ; NIPA, noninprinted in Prader–Willi/Angelman ; SLC, solute carrier ; TRPM, transient receptor potential melastatin.

molecular mechanisms of basolateral Mg^{2+} transports in the distal tubule still remain to be elucidated. Further work is therefore required, especially to define the potential role of Mg^{2+} transport, in both experimental models and human diseases.

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