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Regulation of paracellular transport in the distal nephron

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Abstract

Purpose of review—Claudins play a major role in the regulation of paracellular electrolyte reabsorption in the kidney. This review describes recent findings of the physiological function of claudins underlying paracellular transport mechanisms for Cl^- reabsorption in the collecting duct.

Recent findings—There are two parallel mechanisms for transepithelial Cl^- reabsorption in the collecting duct that utilize the Na-driven Cl -bicarbonate exchanger (NDCBE) and the claudin-based paracellular channel. Histological studies have demonstrated the renal localization of claudin-3, -4, -7 and -8 in the collecting duct. Molecular analyses using several collecting duct cell models have come to the conclusion that claudin-4 functions as a paracellular Cl^- channel. The channel function of claudin-4 is conferred by a charged lysine residue (K65) in its extracellular loop. Claudin-8 is required for paracellular Cl^- permeation through its interaction with and recruitment of claudin-4 during tight junction assembly. Claudin-7 provides the basic barrier function to the collecting duct. Genetic ablation of claudin-7 in animals results in systemic dehydration owing to the loss of extracellular ions and fluid in the kidney.

Summary—The paracellular pathway in the collecting duct is an important route for transepithelial Cl^- reabsorption that determines the extracellular NaCl content and the blood pressure. In the collecting duct cells, claudin-4 and -8 interact to form a paracellular Cl^- channel while claudin-7 maintains the transepithelial resistance. Different subsets of the claudin family proteins fulfill diverse aspects of the tight junction function that will be fundamental to understanding the physiology of the paracellular pathway.

Keywords

claudin; collecting duct; tight junction; transepithelial resistance; chloride; hypertension

Introduction

Sodium, potassium, and chloride are the dominant ions transported by the aldosterone sensitive distal nephron (ASDN) [1–2]. Though only responsible for the reabsorption of 2–3% filtered NaCl, the ASDN plays a vital regulatory role in renal handling of NaCl, extracellular fluid volume (ECFV) control and managing blood pressure [3–4]. The ASDN comprises the distal convoluted tubule (DCT), the connecting tubule (CNT) and the collecting duct. In the DCT, Cl^- is cotransported with Na^+ by the thiazide-sensitive transporter NCC. The CNT and the collecting duct are characterized by a heterogeneous epithelium - the principal cells (PC) and intercalated cells (IC) [5]. The Cl^- transport in the CNT and the collecting duct depends on anion channels or transporters located in both PC and IC cells, dictated by two independent cellular mechanisms.

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Conflict of interest

The author declares that there is no conflict of interest.

Thiazide sensitive electroneutral transport

The Na -driven Cl -bicarbonate exchanger (NDCBE; encoded by the *Slc4a8* gene) in the apical membrane of β -IC cells absorbs Na^+ and HCO_3^- in exchange of Cl^- secretion at the molecular ratio of 1:2:1 [6]. The pendrin exchanger (encoded by the *Slc26a4* gene) located in the apical membrane of β -IC cells absorbs Cl^- in exchange of HCO_3^- secretion at the molecular ratio of 1:1 [7]. Both NDCBE and pendrin activities are sensitive to thiazide. The functional coupling between NDCBE and pendrin creates a tertiary transport mechanism to absorb a net flux of NaCl with no effect on transepithelial potential (V_{te}) [6]. Depending on physiological conditions, the NDCBE mediated NaCl reabsorption accounts for 40–70% of total NaCl reabsorption in the collecting duct [6].

Amiloride sensitive electrogenic transport

The ENaC channel expressed in the apical membrane of PC cells mediates amiloride sensitive Na^+ absorption and generates a lumen-negative V_{te} ($\sim -25\text{mV}$; Figure 1) that drives the absorption of Cl^- and the secretion of K^+ and H^+ [8–10]. K^+ is secreted by the ROMK channel in the apical membrane of the PC cell; H^+ is secreted by the H^+ -ATPase in apical membrane of the α -IC cell. Cl^- is absorbed through the tight junction between the PC cell. The level of Cl^- reabsorption must match that of Na^+ reabsorption so as to maintain luminal fluid electroneutrality, because the NDCBE-pendrin mediated transport carries no net charge. Conversely, defects in Cl^- transport will increase the magnitude of V_{te} , depolarize the luminal membrane, and subsequently inhibit ENaC . Because the tight junction creates a shunt across the epithelium of collecting duct, Cl^- transport can be rapidly switched from absorption to secretion, depending upon the direction of electrochemical gradient, which contrasts with the unidirectional transcellular pathway.

The paracellular channel in the collecting duct

The presence of paracellular ionic conductance has been demonstrated by a number of elegant *ex vivo* studies using microdissected perfused collecting duct tubules [2, 11–13]. In renal epithelia, claudin, the integral membrane protein of the tight junction (TJ), confers ion selectivity to the paracellular pathway resulting in differences in TER and paracellular permeabilities [14]. The term “**paracellular channel**” has been given to a novel class of channels made of claudins and oriented perpendicular to the membrane plane and serving to join two extracellular compartments. Measurement of the paracellular permeability using cell membrane impermeable tracers reveals the size selectivity of 4–7 Å diameter for the paracellular channel [15–16]. The paracellular channel has properties of ion selectivity, pH dependence and anomalous mole fraction effects, similar to a conventional transmembrane channel [17]. Several studies have been carried out to determine claudin expression profiles in the collecting duct. Using Northern analyses and immunostaining methods, Kiuchi-Saishin et al found claudin-3, -4 and -8 expression in the collecting duct [18]. Claudin-7 has also been located in the collecting duct of porcine and rat kidneys [19], although another study described claudin-7 in the distal nephron as located primarily on the basolateral membrane [20].

The “Chloride Shunt” paradox

The term “chloride shunt” refers to paracellular Cl^- absorption across the tight junction in the collecting duct. Abnormal increases in chloride shunt conductance will cause salt retention and extracellular fluid volume expansion, while decreasing K^+ and H^+ secretion. Phenotypic manifestation of chloride shunt derangement includes hyperkalemia, hyperchloremia, hypertension, and metabolic acidosis, clinically known as the chloride shunt syndrome or the pseudohypoaldosteronism type II (PHAII) [21]. Recent genetic

studies have identified the causative genes for PHAII – the WNK kinases [22]. One of the WNKs, WNK4, is present in the tight junction of the collecting duct [22]. The PHAII related mutations in WNK4 increase paracellular Cl^- conductance in transfected MDCK kidney epithelial cells, compatible with the original hypothesis that a gain-of-function in chloride shunt conductance causes PHAII [23]. Nevertheless, the pathogenic role of chloride shunt in PHAII is paradoxical. If the paracellular conductance is dominated by the partial ionic conductance of Cl^- (as it should be for classic transmembrane Cl^- channels), then the paracellular permselectivity should be significantly anionic. This was not what Warden et al had observed [13]. The paracellular $\text{Na}:\text{Cl}$ permselectivity of the collecting duct, estimated by both dilution potentials and tracer rate coefficients, was not substantially different from the ratio of their free-water mobilities (0.65). Although the chloride shunt provides a major conductive route for transepithelial Cl^- transport, its measured permeability was several-fold lower than that carried by the electroneutral Cl/HCO_3 exchange pathway [12]. Substantial reduction of luminal Cl^- levels by Cl/HCO_3 exchanges will cause Cl^- backflux through the paracellular channel, contradicting with the proposed role of paracellular Cl^- absorption in PHAII development.

A molecular understanding of “Chloride Shunt”

Because the claudin proteins expressed in the collecting duct are now known, Hou et al employed a systematic molecular approach to identify the claudin protein responsible for chloride shunt conductance [24]. SiRNA knockdown of claudin-4 or claudin-8 in the mouse collecting duct cell, significantly decreased the paracellular Cl^- permeability (P_{Cl}) without affecting the Na^+ permeability (P_{Na}). Mutagenesis has identified a locus of amino acid (K65) in the first extracellular loop (ECL1) critical for the Cl^- permeability of claudin-4 channel [24]. K65 in claudin-4 is homologous to D65 in claudin-2, a known cation channel. The charged side chain on this conserved residue lines the channel pore and stabilizes permeating ions [25]. Claudin-8 does not make an individual channel to permeate Cl^- . Instead, it interacts with claudin-4 and recruits claudin-4 to the tight junction. In absence of claudin-8, claudin-4 demonstrates trafficking defects – mislocalization in the ER and the Golgi apparatus [24]. Piontek et al suggest a three-stage hypothesis of tight junction assembly [26]. First, claudins *cis* associate within the plane of the ER membrane into dimers, or higher oligomeric state. Second, *trans* interactions between claudins in adjacent cell membranes take place. Third, additional *cis* interactions occur elaborating tight junction strands. It is clear that the *cis*-interaction between claudin-4 and claudin-8 is required for the first stage of tight junction assembly. Knockdown of claudin-3 in the mouse collecting duct cell had no significant effect on P_{Cl} or P_{Na} , while knockdown of claudin-7 resulted in a 30% decrease in TER but no significant change in paracellular ion selectivity ($P_{\text{Cl}}/P_{\text{Na}}$), indicating a TJ barrier-function defect [24]. This is consistent with the *in vivo* findings that claudin-7 KO mouse kidneys lose extracellular ions, such as Na^+ , K^+ , and Cl^- , in a similar rate [27]. Claudin-7 KO animals, although died perinatally within 12 days after birth, had already shown severe renal defects including salt and water wasting, accompanied by extracellular volume depletion and systemic dehydration. The TJ localization of claudin-4 or claudin-8 was not affected in claudin-7 KO, ruling out the possibility that claudin-7 was part of the paracellular Cl^- channel complex [27].

Open questions

Recent advances in claudin biology have shed novel molecular insights into the function of chloride shunt in the kidney. What remain to be defined for chloride shunt are its pathophysiological roles in salt, volume and blood pressure regulation. The traditional view of chloride shunt coupling transepithelial Cl^- absorption with Na^+ absorption in the collecting duct is now being challenged by two lines of evidence. (1) Transepithelial Cl^-

absorption persists in absence of the electrogenic amiloride-sensitive Na^+ absorption and depends on the thiazide-sensitive NDCBE [6]. (2) The amiloride-sensitive Cl^- absorption can be blocked by bumetanide, the NKCC1 inhibitor or in NKCC1 KO animals [28]. While a large number of studies have been carried out to investigate claudin functions *in vitro* using cultured epithelial cells, no conclusive data are available for claudin functions *in vivo* in the collecting ducts of live animals. Because claudin-4 and claudin-8 are expressed in most epithelia of the body [29], the constitutive KO strategy will generate claudin KO phenotypes confounded by its extrarenal functions, which may also lead to perinatal death or developmental defects as observed in claudin-7 KO mice [27]. The ultimate generation of claudin KO mouse models will require a conditional KO strategy using the collecting duct specific promoters. The collecting duct segments from mouse kidneys can be directly perfused to determine the biophysical properties of the paracellular Cl^- channel. The difficulty will be how to distinguish paracellular from transcellular Cl^- conductance. There are several useful criteria: (1) Recording can be carried out in the presence of transcellular Cl^- transport blocker. The NDCBE-pendrin pathway is sensitive to thiazide [6]; AE1 is sensitive to DIDS [30]; Cl^- channels are inhibited by the typical Cl^- channel blocker DPC [31]. (2) A linear current-voltage (I–V) relationship of the R_{te} indicates a predominant paracellular conductance, because the transmembrane carriers such as Cl^- channels and transporters have limited capacity to conduct current and thus have a nonlinear I–V curve. (3) Symmetrical dilution potentials independent of the polarity of applied NaCl electrochemical gradient reflect a predominant paracellular pathway, because transcellular Cl^- conductance is always unidirectional. The clinical significance of paracellular channels in the collecting duct remains to be determined. There has been no mutation found in claudin-4, -7 or -8 that can be associated with human renal disorders. In fact, a genetic heterogeneity study of pseudohypoaldosteronism type I (PHA-I) has excluded claudin-8 as a candidate gene [32]. Although *wnk4* has been shown to modulate paracellular Cl^- permeability in cultured renal epithelial cells [23], transgenic mouse models harboring the PHA-II mutations reveal no difference in paracellular ion selectivity of the collecting duct [33]. A novel genetic assay – genome-wide association study (GWAS) may provide useful hints of susceptible claudin genes in common renal diseases such as hypertension, chronic renal failure and kidney stone [34].

Conclusion

The concept of paracellular Cl^- shunt in the collecting duct of the kidney is known for decades. Recent evidence from both *in vitro* and *in vivo* studies has revealed important molecular components of the paracellular Cl^- transport pathway. Physiological and clinical analyses of the paracellular channels in the collecting duct will unravel novel mechanisms involved in renal salt handling and blood pressure regulation.

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Bullet points

- Chloride transport in the collecting duct is handled by the thiazide-sensitive electroneutral and amiloride-sensitive electrogenic pathways
- The paracellular channel provides a mechanism to couple Cl^- reabsorption with Na^+ reabsorption in the collecting duct
- The paracellular Cl^- channel in the collecting duct is made of claudin-4 and claudin-8
- Claudin-7 is critical for the barrier function of the collecting duct epithelia against a non-selective loss of ions and solutes

Figure 1.

Ion transport mechanism in the collecting duct. The membrane voltage (V_m) trace depicts the virtual measurement by an electrode that is pushed from the basolateral side through the cell to the luminal side. In this example, the basolateral membrane voltage is -70 mV and the luminal membrane voltage is -45 mV, resulting in a transepithelial V_{te} of -25 mV with respect to the basolateral side. V_{te} drives Cl^- transport through the paracellular channel. The ENaC mediated salt reabsorption is sensitive to amiloride; the NDCBE-pendrin mediated salt reabsorption is sensitive to thiazide.