Potential Role of Serine Proteases in Modulating Renal Sodium Transport in vivo

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Kidney • Renal tubule • Sodium • Protease • Epithelial sodium channel • Protease-activated receptor

Abstract

The maintenance of sodium (Na⁺) homeostasis is an essential function of the kidney. It is achieved by a variety of transport processes localized all along the highly specialised segments of the nephron. Impairment of these transport mechanisms, and thereby Na⁺ handling, is associated with disturbed Na⁺ and water balance, leading to hypertension and oedema. This review focuses on the novel regulation of sodium reabsorption by serine proteases acting along the entire nephron.

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Sodium (Na⁺) Transport and Its Regulation along the Nephron

Sodium reabsorption occurs throughout the length of the nephron (fig. 1) via both paracellular (between the cells) and transcellular (across cells) pathways (fig. 2–4). Transcellular Na⁺ entry across the apical membrane is

carried out by sodium transporters (cotransporters, exchangers, and ion channels) expressed in each nephron segment; Na⁺ extrusion is achieved across the basolateral membrane by the Na⁺,K⁺-ATPase pump present along the entire nephron. Sodium uptake is mediated predominantly by the Na⁺-H⁺ exchanger NHE3 in the proximal tubule (fig. 2), by the Na⁺,K⁺-2Cl⁻ cotransporter NKCC2 in the thick ascending limb (TAL) of Henle's loop (fig. 3), and by the thiazide-sensitive Na⁺-Cl⁻ cotransporter NCC in the distal convoluted tubule. The connecting tubule and collecting duct play an important role in the fine regulation of Na⁺ handling. In principal cells, Na⁺ reabsorption is mediated by a selective and amiloride-sensitive electrogenic epithelial ion channel, ENaC (fig. 4). Also, recently in the collecting duct intercalated cells an ENaC-independent, thiazide-sensitive, electroneutral Na⁺ reabsorptive mechanism has been described, suggesting a parallel action of Na⁺-dependent Cl⁻/HCO₃⁻ exchange (NDCBE/SLC4A8) with Na⁺-independent Cl⁻/HCO₃⁻ exchange (pendrin/SLC26A4) (fig. 4) [1].

The importance of Na⁺ transporting proteins in the control of Na⁺ balance and blood pressure is clearly demonstrated by genetic disorders of NKCC2 (Bartter's syndrome) and NCC (Gitelman's syndrome), in which loss of function mutations in these genes is associated with in-

Fig. 1. Sodium reabsorption along the nephron.

creased renal Na^+ losses and a low blood pressure, and mutations of *ENaC*, where loss of function also causes increased Na^+ wasting and a low blood pressure (pseudohypoaldosteronism type 1a, PHA-1), and gain of function the opposite with hypertension (Liddle's syndrome) (fig. 1).

Apical Na^+ entry is limiting for transepithelial Na^+ transport; it is regulated by various factors (hormones, and intra- and extracellular non-hormonal signalling factors) acting on Na^+ transporters at several levels: mRNA and protein synthesis, protein degradation, intracellular protein trafficking, its surface expression, and its activity. For example, the steroid hormone aldosterone increases the activity of NCC and ENaC in the aldosterone-sensitive distal nephron via induction of serum- and glucocorticoid-inducible kinase, SGK1 [2, 3]. The action of SGK1 on ENaC appears to be mediated through a Nedd4-2 (Neural precursor cell-expressed, developmentally down-regulated 4-2)-dependent mechanism. SGK1 phosphorylates and sequesters the E3 ubiquitin ligase Nedd4-2, which promotes ubiquitination-dependent targeting of ENaC for endocytosis at the plasma membrane and intracellular degradation [4]. WNK (with no lysine) kinases comprise a regulatory system that controls several Na^+ transport proteins (NKCC2, NCC, ENaC). For example, aldosterone-induced SGK1 phosphorylates WNK4 and inactivates the inhibitory effects of WNK4 on NCC, resulting in enhanced Na^+ reabsorption in dis-

tal tubular cells [3]; in collecting duct cells, WNK1 and WNK4 stimulate SGK1 leading to activation of ENaC [5]. The action of SGK1 on ENaC can also be mediated by a Nedd4-2-independent mechanism: Diakov and Korbmayer [6] have demonstrated a novel pathway of ENaC activation by SGK1 involving phosphorylation of a serine residue of its C terminus. This finding suggests that SGK1 may directly contribute to aldosterone-induced activation of ENaC channels already present in the plasma membrane.

Other novel mechanisms influencing Na^+ transport include the serine proteases. This review focuses on Na^+ transport regulation along the nephron by serine proteases. Serine protease regulation of Na^+ reabsorption can be achieved by direct cleavage of a Na^+ transporting protein (e.g. ENaC in the cortical collecting duct, CCD) or through an interaction with protease-activated receptors (e.g. PAR-2 in the TAL), or by modulating paracellular permeability.

Serine Proteases in the Kidney

Serine proteases are proteolytic enzymes present in viruses, bacteria and eukaryotes, and they are involved in the regulation of several physiological processes, including blood coagulation, digestion, and inflammation [7]. They are characterized by a catalytic triad motif composed of serine, histidine and aspartate residues that are required for enzymatic activity. Serine proteases are classified and grouped on the basis of their structural and functional similarities (MEROPS database, <http://merops.sanger.ac.uk>). In the kidney, serine proteases can act intracellularly, such as the ubiquitously expressed furin, or extracellularly. The membrane-bound serine proteases are anchored either via a carboxy-terminal transmembrane domain (type I), a carboxy-terminal hydrophobic region that functions as a signal for membrane attachment via a glycosyl-phosphatidylinositol linkage (GPI-anchored), or via an amino-terminal transmembrane domain (type II).

Serine proteases such as plasmin and secreted CAP1 (channel-activating protease), and kallikrein are also present in urine [8–10]. In the kidney, the GPI-anchored serine protease CAP1 is expressed in the proximal tubule, the cortical TAL and the CCD [11]. The type II transmembrane serine protease CAP3 is expressed at the basolateral plasma membrane of ductal epithelial cells of the kidney mainly in the distal collecting ducts more than in the proximal collecting ducts [12], and the serine

Fig. 2. Proximal convoluted tubule sodium transport.

Fig. 3. TAL of Henle's loop sodium transport.

Fig. 4. Collecting duct sodium transport.

exopeptidase dipeptidyl-peptidase IV (DPPIV; CD26), which degrades the insulin-stimulating hormone glucagon-like peptide-1 (GLP-1), is one of the major brush-border membrane proteins of the proximal tubules [13].

Effect of Serine Proteases on Na⁺ Transporting Proteins of the Brush Border Membrane of the Proximal Tubule (see fig. 2)

Only a few studies have described the interaction of proteases with Na⁺ transport along the proximal tubule. In 1992, Sabolic et al. [14] showed that, when applied externally, several proteases can cause significant inhibition of NHE3 in apical brush border membrane (BBM)

vesicles prepared from rat renal cortex, although not trypsin. However, when applied to the cytoplasmic membrane surface, trypsin activated NHE3 in BBM vesicles prepared from rabbit renal cortex by cleaving an inhibitory site on the exchanger corresponding to the PKA regulatory binding site [15].

A key aspect of the regulation by proteases of Na⁺ transport in the proximal tubule seems to be an association between DPPIV and NHE3. DPPIV is a plasma membrane anchored ecto-protease also known as the leukocyte antigen CD26; it is expressed in a variety of epithelial and non-epithelial cells [16]. The kidney is a main site of expression of DPPIV, where it constitutes up to 14% of total membrane protein [17]. It is a highly specific serine protease with catalytic activity, but it can also

function as a binding protein [18, 19]. When associated with NHE3, the resulting oligomeric complex is exclusively distributed in microvilli of the BBM of the proximal tubule, where NHE3 is also active [13]. Conversely, when NHE3 is associated with megalin in intermicrovillar coated pits it is inactive [13]. The association with DPPIV seems to regulate directly or indirectly NHE3 activity by controlling its surface expression and/or activity. Girardi et al. [20] have shown that inhibition of DPPIV catalytic activity in the proximal tubule of rats in vivo inhibits NHE3 activity by redistributing the exchanger from microvillar membranes to the intermicrovillar microdomain of the brush border.

Modulation of Na⁺ Transport by Serine Protease-Induced Proteolysis of Na⁺ Transporting Proteins (see fig. 4)

Serine protease regulation of Na⁺ transport implicates cleavage of Na⁺ transporting proteins. Proteolytic activation of the Na⁺ channel ENaC in the aldosterone-sensitive distal nephron has a key role in optimising channel activity (Po, open probability). ENaC is composed of three homologous subunits α , β and γ . Each subunit has short cytosolic N- and C-terminal domains, two transmembrane domains separated by a large extracellular region, which is the target for proteolysis.

Furin

Furin is a proprotein convertase that resides mainly in the *trans*-Golgi network, where it processes target proteins passing through the biosynthetic pathway, including ENaC. Furin cleaves the α ENaC subunit at two sites in the extracellular domain, releasing a 26-residue inhibitory fragment, resulting in ENaC channel activation [21]. However, furin cleaves the γ -subunit in the ectodomain at a single site only [22], whereas full activation of the channel requires the release of a second inhibitory domain from the γ -subunit, indicating that a second distal cleavage of the γ -subunit is produced by other serine proteases (e.g. CAP1, kallikrein or plasmin).

CAP1/Prostasin

CAP1, a GPI-anchored serine protease, also called prostasin, is encoded by *Prss8* gene (protease serine S1 family member 8). CAP1 is the first 'channel-activating protease' isolated from a mouse cortical collecting duct cell line [11] and activates ENaC in the *Xenopus* oocyte expression system [11], and in cortical collecting duct cell

lines [8, 23], by modulating channel gating; silencing of the CAP1/prostasin gene reduced sodium transport in a cortical collecting duct cell line [23]. Mechanisms by which CAP1 affects ENaC activity are complex. Bruns et al. [22] have identified a CAP1 cleavage site in the γ -subunit of ENaC at a tetrabasic site distal to the furin cleavage site. Cleavage of the γ -subunit by furin and CAP1 release a 43-residue inhibitory peptide, leading to full activation of the ENaC channel [22].

CAP2 and CAP3

CAP2 (TMPRSS4) and CAP3 (matriptase), the gene products of *Prss4* and *Prss14*, respectively, are type II transmembrane serine proteases originally isolated from a mouse cortical collecting duct cell line. Both increase ENaC activity by enhancing channel open probability in the *Xenopus* oocyte expression system [24]. CAP2 has been shown to activate ENaC by cleaving the γ -subunit of rat ENaC at position R138, a site distal to the furin site [25]. In intestinal Caco-2 cells, Friis et al. [26] have shown a new basolateral prostasin activation process. Even if prostasin is mainly located on the apical plasma membrane, a small fraction can be found on the basolateral plasma membrane co-localizing with matriptase. Basolateral prostasin can be activated by matriptase, endocytosed and transcytosed to the apical plasma membrane, where it accumulates.

Plasmin

The serine protease plasmin has also been shown to directly cleave and activate ENaC; extracellular plasmin application cleaves the γ -subunit of ENaC at a site distal to the furin site and activates Na⁺ currents in *Xenopus* oocytes [9]. In a mouse cortical collecting duct cell line, Svenningsen et al. [27] demonstrated that at high concentrations plasmin directly activates ENaC, while at low concentrations plasmin interacts with CAP1, leading to γ -subunit cleavage. Nephrotic urine from human and rats activates ENaC currents in mouse collecting duct cells, and plasmin was identified by mass spectrometry as the dominant serine protease responsible for this effect [28].

Kallikrein

Tissue kallikrein (TK) is a serine protease synthesized in the renal connecting tubule cells and secreted into the tubular fluid at late distal nephron segments. In isolated mouse cortical collecting ducts microperfused in vitro, luminal TK was shown to activate ENaC [10]. Studies with TK knockout mice suggest that TK may activate

ENaC by cleavage of its γ -subunit in the kidney [10]. Besides TK's potential role in Na^+ homeostasis, it has also been reported to control acid-base and potassium transport in CCD intercalated cells by directly inhibiting $\text{HCO}_3^-/\text{Cl}^-$ exchange [29] and (colonic) H^+/K^+ ATPase, respectively [30].

Serine Protease Modulation of Na^+ Homeostasis through Cleavage of Protease-Activated Receptors (see fig. 3)

Another way in which serine proteases may regulate Na^+ transport is to act on protease-activated receptors (PARs). PARs are a family of G-protein-coupled receptors with four members (PAR-1–4). Specific serine proteases cleave PARs within the extracellular domain to expose a tethered ligand domain that binds and activates the cleaved receptors, initiating an intracellular signalling cascade [31]. PAR-2 is expressed in the renal cortex [32]. In vivo, in normal conditions, it is mainly located within the blood vessel wall, whereas in IgA nephropathy there is a considerable increase in PAR-2 protein expression, particularly at the level of the proximal tubule [33]. In vitro, PAR-2 can also be found at the basolateral membrane of the isolated cortical TAL and in cultured collecting duct cells [31, 34]. Several serine proteases regulate PAR-2, such as trypsin, kallikrein, and elastase [31]. PAR-2-activated Na^+ transport by serine protease has been described in cortical TAL microperfused in vitro through regulation of Na^+/K^+ -ATPase [34].

Serine Protease Modulation of Sodium Homeostasis through Paracellular Permeability Regulation (see fig. 3, 4)

In addition to modulating transcellular Na^+ transport in the kidney, serine proteases have also been reported to regulate paracellular permeability. The tight junctions constitute the main barrier in epithelia to the passive movement of electrolytes and macromolecules via the paracellular pathway. In mouse CCD cells, prostasin has been shown to be an important regulator of tight junction paracellular permeability [35, 36], and it is more likely that prostasin is required for the development of transepithelial electrical resistance in renal collecting duct epithelium [37]. Moreover, PAR-2 activation has been shown to increase paracellular permeability to Na^+ in rat cortical TAL microperfused in vitro [34]. Furthermore, a

link between serine proteases and tight junction structure has been described in other tissues: a model of skin-specific CAP1/Prss8-deficient mouse exhibited impaired function of tight junctions [38], and genetic deletion of matriptase in the intestine was associated with an increased paracellular permeability through an effect on claudin-2 expression [39].

Serine Proteases in Na^+ Homeostasis in vivo and in Disease

The question arises whether protease-mediated Na^+ transport in the kidney is physiologically or pathologically relevant and important in vivo. Changes in renal serine protease activity might disturb Na^+ balance and therefore blood pressure control. Indeed, altered serine protease levels in human urine, such urinary kallikrein [40, 41], CAP1/prostasin [8, 42], and plasmin [28], have been associated with hypertension and kidney diseases with disturbed Na^+ and water balance like the nephrotic syndrome.

In vivo studies in rats have shown that increasing the levels of aldosterone (by a salt-restricted diet or by aldosterone infusion), an important regulator of Na^+ reabsorption, stimulated urinary excretion of CAP1/prostasin, and induced hypertension [8]. Urinary kallikrein excretion has also been reported to be increased by aldosterone increase and decreased following adrenalectomy in rats [43]. Moreover, aldosterone stimulation in rats increased expression of proteolytic fragments of α ENaC and γ ENaC subunits in kidneys, corresponding, presumably, to cleavage of the α -subunit by furin and the γ -subunit by both furin and a second serine protease [44–46].

Plasmin is present in the urine of patients with the nephrotic syndrome, but not under normal conditions [28]. In proteinuric disease, plasminogen crosses the glomerular filtration barrier and can be converted to plasmin by tubular fluid urokinase. Plasmin-induced activation of ENaC may contribute to Na^+ retention, extracellular volume expansion and hypertension, all of which can be observed in the nephrotic syndrome.

Recently, Maekawa et al. [23] have shown that camostat mesilate, a synthetic serine protease inhibitor, directly inhibited CAP1/prostasin activity and decreased Na^+ transport in a cortical collecting duct cell line in vitro; oral administration of camostat mesilate to Dahl salt-sensitive rats fed a high salt diet, a model of salt-sensitive hypertension, decreased blood pressure and renal injury [23].

Conclusions

The evidence that serine proteases can activate ENaC is clear-cut, but whether this occurs to any significant extent in vivo is still not known. Moreover, how the production, release and control of these enzymes occur is also unknown. Further studies addressing the role of these proteases in Na⁺ homeostasis in vivo and at different nephron

sites is necessary to determine their physiological relevance, but then also what physiological or pathological factors can affect their synthesis, release and activity.

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