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Regulation of sodium transport by ENaC in the kidney

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Abstract

Purpose of review—The amiloride-sensitive epithelial sodium channel (ENaC) plays a major role in the regulation of sodium transport in the collecting duct and hence sodium balance. This review describes recent findings in the regulation of ENaC function by serine proteases in particular and other regulatory aspects.

Recent findings—Regulation of ENaC occurs at many levels (biophysical, transcriptional, post-translational modifications, assembly, membrane insertion, retrieval, recycling, degradation, etc.). Recent studies have recognized and delineated proteolytic cleavage, particularly of the α and γ subunits, as major mechanisms of activation. Release of peptide fragments from these two subunits appears to be an important aspect of activation. These proteolytic mechanisms of ENaC activation have also been demonstrated in vivo and strongly suggested in clinical circumstances, particularly the nephrotic syndrome. In the nephrotic syndrome, filtered plasminogen may be cleaved by tubular urokinase to yield plasmin which can activate ENaC. In addition to these mechanisms, regulation by ubiquitination and deubiquitination represents a pivotal process. Several important deubiquitinating enzymes have been identified as important in ENaC retention in, or recycling to, the apical membrane. New aspects of the genomic control of ENaC transcription have also been found including histone methylation.

Summary—The mechanisms of regulation of ENaC are increasingly understood to be a complex interplay of many different levels and systems. Proteolytic cleavage of α and γ subunits plays a major role in ENaC activation. This may be particularly clinically relevant in nephrotic syndrome in which plasmin may activate ENaC activity.

Keywords

Epithelial sodium channel; aldosterone; prostatic; SGK-1; plasmin

Introduction

The collecting duct is the site of final regulation of urinary sodium (Na) excretion. The importance of this regulation has been dramatically illustrated by the fact that several forms of monogenic hypertension have been associated with genetic defects targeting this site.

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Sodium transport in the collecting ducts occurs via rate limiting amiloride-sensitive epithelial sodium channels (ENaC) in series with basolateral Na-K-ATPase. ENaC is composed of a complex of three homologous subunits, α , β , and γ with the α subunit as the transporting subunit with regulatory roles for the β and γ subunits. Each subunit has a large extracellular domain in the luminal space, two transmembrane domains, and relatively small cytoplasmic domains. Aldosterone is the pivotal hormone regulating sodium transport in the collecting duct via its genomic effects on the mineralocorticoid receptor. Aldosterone induces a number of proteins, including ENaC and Na-K-ATPase, but it also has early effects on apical Na transport via stimulation of SGK1 [1]. SGK1 phosphorylates Nedd4-2, thus reducing its role in ubiquitination, retrieval, and degradation of ENaC [2]. Aldosterone may also have a number of other mechanisms to stimulate ENaC (including synthesis of subunits over a longer time scale) including increasing expression of glucocorticoid-induced leucine zipper protein (GILZ) which acts by inhibition of extracellular signal-regulated kinase (ERK) [3]. In addition to classic systemic hormones, more recent studies implicate paracrine and intracellular factors in modulating Na transport.

This review will focus on regulation of ENaC by serine proteases, and also selectively provide some update on recent studies addressing other regulatory factors involved in Na transport. ENaC has become one of the most widely investigated renal transport proteins and many aspects have been unraveled, from biophysical properties to intracellular trafficking [4]. Proteolytic processing of ENaC has been one of several areas of concentrated investigation recently [5;6]. The scope of this review does not allow recent findings related to a variety of other hormones affecting ENaC, certain other signaling pathways, and many new biophysical findings.

Protease activation of ENaC

Intense interest in serine protease effects on ENaC began when Vallet et al identified a novel serine protease called CAP1, for channel-activating protease 1, which activates ENaC [7]. CAP1 is an apical GPI anchored protein which can also be secreted in some tissues. CAP1 is the ortholog to the protein prostasin previously cloned from prostate [8;9] and is highly expressed in kidney and other epithelial tissues that express ENaC. (Subsequent reference to CAP1 herein will refer to prostasin.) Two other homologous proteins CAP2 (TMPRSS4) and CAP3 (matriptase) have been identified [10;11] and a variety of other proteases such as elastase [12;13] have been found to potentially activate ENaC. Although initial studies did not suggest actual cleavage of ENaC subunits, subsequent studies indicated critical roles for cleavage of subunits by the intracellular protease furin, and extracellular activation by prostasin (or by exogenous trypsin) [14-16]. Recently significant progress has been made in several directions regarding proteolytic activation of ENaC.

Recent studies suggest that proteolytic activation of ENaC results from proteolytic release of inhibitory peptides from within the α and γ subunits (figure 1). Carattino et al first reported that cleavage of the α subunit by furin at two sites released a 26-amino acid tract, leading to the activation of ENaC [17]. Bruns et al [18], following the work of Hughey et al [16] on the cleavage sites for the γ subunit by furin, found that the γ subunit was further cleaved by prostasin at a site distal to the furin cleavage site. Cleavage of the γ subunit by furin and prostasin released a 43-residue tract leading to an increase of ENaC activity. A synthetic peptide, representing the cleaved tract from γ subunit, inhibited ENaC activity [18]. Most recent studies suggest that the γ subunit cleavage has the dominant role in activation of ENaC. Carattino et al [19] reported that in *Xenopus* oocytes expressing ENaC subunits, prostasin fully activated ENaC even with mutation of furin cleavage sites in the α subunit, whereas the activation of ENaC by prostasin was blocked by the mutation of furin and prostasin cleavage sites in the γ subunit. Deletion of the γ subunit inhibitory tract fully activated ENaC despite the mutation of furin cleavage sites

in the α subunit or the presence of α subunit inhibitory tract. When the γ subunit inhibitory tract was removed, the cleavage of α subunit was required to fully active sodium channels. Removal of the γ subunit inhibitory tract reduced the response of ENaC activity to α subunit 26-residue inhibitory tract. These results suggested that proteolytic cleavage of the γ subunit has a major role in the regulation of the ENaC activity. Smaller peptide sequences from both α and γ inhibitory tracts are sufficient for channel inhibition. Diakov et al [20] also found that the γ subunit was critical for regulation by proteases, but in a manner that involves both gating of active channels and more importantly recruitment of “near silent channels”. The γ subunit is also critical for elastase and CAP2 activation of ENaC [21][22]. This seeming importance of the γ subunit does contrast with other studies which demonstrated the importance of α subunit cleavage by furin [15][13][23].

In terms of regulation of these processes, aldosterone and volume status alter prostasin and the abundance of cleaved subunits as discussed below. SGK1, discussed below, may also regulate γ ENaC cleavage [24]. Also Synder’s group has found that the rate of proteolytic cleavage and activation of ENaC are reduced by increased levels of intracellular Na (“Na feedback inhibition”) and altered also by apical membrane resident time and by Nedd4, the E3 ubiquitin ligase that targets ENaC for degradation [25;26][27]. Deubiquitylation also alters proteolytic cleavage of ENaC [28]. Other aspects of regulation are discussed below.

In addition to the proteases mentioned above, two other proteases have recently been found to have intriguing possible pathophysiologic relevance: tissue kallikrein and plasmin (discussed below). Kallikrein is known to be secreted into the urinary space and to have significant relevance to sodium balance and blood pressure control. Picard et al [29] recently demonstrated that luminal tissue kallikrein (TK) activates Na current in cortical collecting duct principal cells microperfused in vitro. The 70 KD cleaved fragment of γ ENaC subunit was absent in TK knockout mouse, but it reappeared when incubated with TK. These results strongly suggest that TK might activate sodium transport via the cleavage of γ ENaC subunit. Plasmin and its possible role in nephrotic syndrome is discussed below.

In contrast to the above studies that implicate direct channel cleavage by serine proteases, some studies have shown activation of ENaC can occur without active proteolysis [30][31]. Also, indirect activation of ENaC by proteases, rather than by proteolytic cleavage, may also contribute to channel activation [32]; protease activated receptors present in the kidney could be important in this regard. Several investigators have proposed the possibility of proteolytic cascades with multiple components [6]. Another aspect of serine protease action on distal nephron transport is that protease inhibitors increase paracellular permeability and proteases decrease permeability [11][33].

Protease inhibitors and TGF- β 1

Pharmacologic inhibitors of serine proteases reduce Na transport, but importantly an endogenous inhibitor of prostasin, protease nexin 1 (PN-1) has been identified [34]. PN-1 is a member of the serpin family (serine protease inhibitor) and is an endogenous inhibitor for α -thrombin, plasmin, and plasminogen activators. PN-1 decreases sodium current by ENaC in vitro. And knockdown of PN-1 gene expression increases baseline sodium current in M-1 cells. Furthermore prostasin and PN-1 appear to be reciprocally regulated by aldosterone (increased prostasin and decreased PN-1) and TGF- β (decreased prostasin and increased PN-1) [34]. However, as with many hormones, the actions of TGF- β 1 are also more complicated than a single mechanism: TGF- β 1 significantly increases the amount of I κ B α , and may inhibit prostasin promoter activity through the induction of I κ B α and subsequent inhibition of c-Rel activity in M-1 cells [35]. But activation of the NF κ B pathway inhibits SGK1 expression [36;37], a key modulator of ENaC as discussed below. Edinger et al [37] also showed that

Nedd-4 function and phosphorylation by IKKB are required for IKKB regulation of ENaC activity. In another study, TGF- β 1 inhibited ENaC activity through smad-4 and α ENaC mRNA expression reduction in mpkCCDcl4 mouse collecting duct cells. Removal of N-terminus of Smad4 not only abolished the inhibitory effects of Smad4 on sodium current and α ENaC mRNA expression, but also prevented the inhibition of sodium current by TGF- β 1 [38]. These studies suggest that TGF- β 1 regulates sodium transport by a variety of mechanisms whose relative importance is yet to be determined.

The synthetic serine protease inhibitor nafamostat mesilate was shown to reduce renal sodium reabsorption and prostatic excretion in vivo [39]. More recently, Maekawa et al [40] demonstrated that an orally active synthetic serine protease inhibitor, camostat mesilate, decreased Na transport in vitro and blood pressure in Dahl salt-sensitive rats fed with high-salt diet. Proteinuria and renal function were also improved. This study raises the possibility that protease inhibitors could represent a potential new class of antihypertensive agent with renoprotective effects.

In vivo evidence of proteolytic cleavage of ENaC subunits

Although much of the above data is in vitro, substantial evidence supports these proteolytic mechanisms in vivo. Masilamani et al. [41] first demonstrated a shift in the molecular weight of γ ENaC from 85 KD to 70 KD with elevated circulating aldosterone. Subsequent studies by the Frindt and Palmer group, among others, have significantly expanded our understanding of the in vivo events [42-45]; these studies have combined biochemical information (western blots etc.) with physiologic data (whole cell patch clamp Na currents) from intact tubules from rats. The studies have demonstrated that salt deprivation and/or aldosterone increase the cleaved form of the α and γ subunits, that these changes can occur rapidly (hours) and correlate with Na conductance, and that these mechanisms are present in medullary collecting ducts as well as cortical collecting ducts [43-45]. The most recent of these studies have been able to demonstrate that apical surface membrane subunits increase with aldosterone or salt depletion, and decrease with salt repletion [42]; also the Na currents were not able to be further activated by addition of trypsin in tubules from salt depleted rats in contrast to those from salt replete rats [42]. Aldosterone and/or salt depletion both increase expression of the subunits at the membrane and increase their activity via cleavage. Increased glycosylation of the β subunit was also seen with salt depletion [42]. In addition to these and other studies of intact tubules [29;46], Nesterov et al [47] also showed by whole cell patch-clamp data that trypsin increases amiloride-sensitive sodium current in microdissected distal tubules of mice on low and normal salt diets. The stimulatory effect of trypsin on sodium current was blocked by pretreatment with a protease inhibitor.

Initial clinical studies indicated that urinary prostatic is elevated in patients with hyperaldosteronism [48]. Recent clinical studies have suggested more generally that urinary prostatic may serve as an in vivo marker of activation of ENaC [49], correlate with urinary aldosterone [50], and increase with pressure natriuresis [51]. Another study suggested that genetic polymorphisms in prostatic may be correlated with hypertension [52]. All of these clinical studies are intriguing but will need confirmation.

Role of plasmin in nephrotic syndrome

Two recent studies have demonstrated that plasmin activation of ENaC may contribute to Na retention in nephrotic syndrome. Passero et al [53] showed that plasmin activated Na current in oocyte expressing ENaC by cleaving lysine 194 in the γ subunit. Also plasminogen and plasmin were found in the urine of obese ZSF1 rats, but not in control rats. Svenningsen et al [54] found that urine of nephrotic rats showed a 10 fold increase of serine protease activity compared with that of control rats. Moreover, urine of nephrotic rats activated Na current which

was blocked by aprotinin. Plasmin was identified as the primary serine protease in the nephrotic urine. In oocytes expressing ENaC subunits, sodium current was activated by combination of plasminogen and urokinase-type plasminogen activator (uPA), but not by plasminogen or uPA alone, suggesting that plasmin is important for ENaC activation. Furthermore, the combination of plasminogen and uPA also generated a 67 KD cleaved fragment of the γ ENaC subunit. In addition, urine of nephrotic patients was also shown to stimulate Na current which was blocked by amiloride or plasmin inhibitors. These two important studies suggest that plasminogen filtered in the nephrotic urine may be cleaved by tubule urokinase to generate plasmin which can then activate ENaC by cleavage of the γ subunit. This provides a possible mechanism of sodium retention in diseases with significant proteinuria.

Regulation of ENaC activity by membrane trafficking

The importance of ENaC insertion into the apical membrane, retrieval, and recycling have been recognized since shortly after the cloning of ENaC subunits and their identification as the genes mutated in Liddle's syndrome. Significant progress continues in this area [4;55-57], but the scope here will be limited to selected recent findings (figure 2). The mechanisms that govern subunit synthesis, assembly, and insertion in the membrane (although most is degraded prior to insertion) have been investigated but still not completely understood [55]; but more attention has focused on retrieval and subsequent events. Basically, Nedd4-2 ubiquitinates surface subunits by binding to PPxY motifs in ENaC subunits [27;58], initiating their retrieval from the apical membrane to endosomes, to be either degraded in lysosomes or proteosomes, or recycled to the apical membrane. After ubiquitination, ENaC is probably retrieved from the membrane by clathrin mediated pathways predominantly. Epsin and PIP₂ play significant roles in this process [59;60]. The importance of Nedd4-2 in regulating ENaC and hence blood pressure was recently confirmed with a Nedd4-2 knockout mouse model that exhibited salt sensitive hypertension and increased expression levels of all three ENaC subunits [61].

Aldosterone induces the PI3K-dependent kinase SGK1, which is now identified as a central modulator of the ubiquitination process by Nedd4-2. SGK1 phosphorylates Nedd4-2, inducing its interaction with 14-3-3 proteins and decreasing its interaction with ENaC. In a negative feedback manner, phosphorylated Nedd4-2 ubiquitinates and leads to degradation of SGK1. SGK1 also directly activates α ENaC [62]. SGK1 also has a variety of other actions as discussed elsewhere. (A 5' variant alternate transcript of SGK1 that encodes a Sgk1 isoform, Sgk1_i2 may be most efficient in regulating ENaC [63].) Despite the central role in regulation of ENaC, SGK1 knockout animals have a surprisingly mild phenotype and aldosterone is still able to activate ENaC [24;64]. Other signaling pathways also interact with Nedd4 binding. A Raf-1–MAPK/ERK kinase pathway phosphorylates ENaC, increasing its association with Nedd4, and decreasing activity, e.g. [65-67]. GILZ1, another aldosterone induced protein, disrupts the Raf-1/ ERK pathway, stimulating ENaC. Recently Pearce's group have not only shown synergistic actions, but also physical interaction, between the GILZ1 and SGK1 pathways, specifically a complex of ENaC, Nedd4-2, and Raf-1 [68;69]. The G protein coupled kinase GRK2 also impinges at the intersection of Nedd4-2 interaction with ENaC [70]. The phosphatidylinositol 3'-kinase (PI3K) pathway (recently reviewed [71]) not only stimulates SGK1, but both PIP₃ and PIP₂ directly affect ENaC gating. A variety of other kinases including Akt, WNK1, I κ B kinase- β , and AMP-activated kinase also affect ENaC activity indirectly [37;72-74].

Once ubiquitinated, ENaC is retrieved into endosomal compartments and much undergoes degradation in lysosomes. However recent studies indicate that deubiquitination occurs constitutively, maintaining ENaC at the surface and playing a role in recycling subunits from sorting endosomes to the surface. Recently, three deubiquitinating enzymes (DUBs) have been identified to influence ENaC. Butterworth et al [75] found a ubiquitin C-terminal hydrolase

(UCH) isoform L3 was the predominant deubiquitinating enzyme in CCD cells. Fakitsas et al [76] showed that aldosterone increased expression of ubiquitin-specific protein 2-45 (Usp2-45) in mouse CCD. Usp2-45 increased ENaC activity and surface expression and has also been found to increase cleavage of α and γ ENaC subunits [28]. ENaC surface expression may be reciprocally regulated by deubiquitylation by DUBs and ubiquitylation by Nedd4-2. Another DUB, USP10, is induced by vasopressin and enhances ENaC activity by deubiquitinating sorting nexin 3 instead of ENaC subunits directly [77].

After deubiquitination, ENaC subunits may be recycled from certain endosomes back to the apical surface; a variety of Rab GTPases may be involved [4;78]. Recently, Martel et al. [79] identified melanophilin as an aldosterone induced protein that may be involved in ENaC trafficking. Fusion of ENaC containing vesicles with the plasma membrane likely involves lipid rafts and various SNARE components [4;80].

Recent findings related to genomic regulation of ENaC

Although most of the past focus has been on up-regulation of Na transport by aldosterone's genomic actions, several recent findings have highlighted negative genomic effects by various factors. Zhang et al [81] found that Dot1a (a histone methyltransferase) represses α ENaC transcription by histone methylation but that aldosterone, via SGK-1 phosphorylation of its binding partner AF9, down-regulates Dot1a [82][83]. Naray-Fejes-Toth [84] found another negative regulator of β and γ ENaC expression, promyelocytic leukemia zinc finger (PLZF) protein which is a transcriptional repressor. Overexpression of PLZF inhibited sodium current and mRNA expression of β and γ ENaC subunits in collecting duct cells.

Structural features of ENaC

ENaC is a member of a large gene family which also contains the acid sensing ion channels (ASIC) of the vertebrate nervous system. Recently the crystal structure of chicken ASIC1 was reported [85], leading to intense study for insights into the structural/functional correlates for ENaC [86][87]. Although there are significant differences between the two ion channels, this breakthrough will likely lead to significant insight into the structure of ENaC.

Conclusion

ENaC activity is regulated at many different levels. Two systems have recently received particular attention: activation by proteolytic cleavage of the α and γ subunits, and retrieval and recycling of ENaC by ubiquitination and deubiquitination.

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Figure 1.

Model of proteolytic cleavage sites in α and γ ENaC subunits by furin, proctasin, neutrophil and pancreatic elastase, plasmin, CAP2 and trypsin. Adapted from refs. ⁵ and ⁶. The dashed line is shown to illustrate possible inhibitory fragments cleaved from these two subunits for ENaC activation.

Figure 2.

Model of the regulation of Na transport in collecting duct principal cells. Ubiquitin ligase Nedd4-2 and deubiquitinase Usp2-45 reciprocally regulate ubiquitination and recycling of ENaC. Aldosterone regulates gene expression of ENaC subunits via Sgk1-induced phosphorylation of Af9 and PLZF-dependent repression. Sgk1 regulates ENaC function via inhibition of Nedd4-2 dependent ubiquitination. Aldosterone and TGF- β 1 reciprocally regulate ENaC function via antagonistic influence on PN-1 expression. MR, mineralocorticoid receptor; PN-1, protease nexin 1; U, ubiquitin; Sgk1, Serum- and glucocorticoid-inducible kinase 1; PLZF, promyelocytic leukaemia zinc finger protein; Dot1a, disruptor of telomeric silencing alternative splice variant a; Af9, ALL1-fused gene from chromosome 9.