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Regulation of the Epithelial Na+ Channel by Peptidases

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Abstract

Recent investigations point to an important role for peptidases in regulating transcellular ion transport by the epithelial Na^+ channel, ENaC. Several peptidases, including furins and proteasomal hydrolases, modulate ENaC maturation and disposal. More idiosyncratically, apical Na^+ transport by ENaC in polarized epithelia of kidney, airway, and gut is stimulated constitutively by one or more trypsin-family serine peptidases, as revealed by inhibition of amiloride-sensitive Na^+ transport by broad-spectrum antipeptidases, including aprotinin and bikunin/SPINT2. *In vitro*, the transporting activity of aprotinin-suppressed ENaC can be restored by exposure to trypsin. The prototypical channel-activating peptidase (CAP) is a type 1 membrane-anchored tryptic peptidase first identified in *Xenopus* kidney cells. Frog CAP1 strongly upregulates Na^+ transport when coexpressed with ENaC in oocytes. The amphibian enzyme's apparent mammalian orthologue is prostasin, otherwise known as CAP1, which is coexpressed with ENaC in a variety of epithelia. In airway cells, prostasin is the major basal regulator of ENaC activity, as suggested by inhibition and knockdown experiments. Other candidate regulators of mature ENaC include CAP2/TMPRSS4 and CAP3/matriptase (also known as membrane-type serine protease 1/ST14). Mammalian CAPs are potential targets for treatment of ENaC-mediated Na^+ hyperabsorption by the airway in cystic fibrosis (CF) and by the kidney in hypertension. CAPs can be important for mammalian development, as indicated by embryonic lethality in mice with null mutations of CAP1/prostasin. Mice with selectively knocked out expression of CAP1/prostasin in the epidermis and mice with globally knocked out expression of CAP3/matriptase exhibit phenotypically similar defects in skin barrier function and neonatal death from dehydration. In rats, transgenic overexpression of human prostasin disturbs salt balance and causes hypertension. Thus, several converging lines of evidence indicate that ENaC function is regulated by peptidases, and that such regulation is critical for embryonic development and adult function of organs such as skin, kidney, and lung.

I. Introduction

Many epithelia form high-resistance barriers to Na^+ and other ionic solutes. Control and modulation of the passage of Na^+ across these barriers is critical for development and proper functioning of a variety of organs before, during, and after birth. Such epithelia use transcellular flux of Na^+ to control the movement of water, which migrates passively across and between cells to equalize inequalities in concentrations of Na^+ and other solutes. Movement of water in response to modulation of transepithelial Na^+ flux is important in processes as diverse as removing liquid from neonatal mammalian lung on exiting the womb, maintaining blood pressure and volume, preventing excessive electrolyte loss via sweating, and maintaining

optimal hydration of the luminal surface of wet and ciliated mucosa. Failure to regulate Na^+ flux can cause or contribute to a variety of serious diseases, including respiratory distress syndrome, high or low blood pressure, and cystic fibrosis (CF). Although several Na^+ channels have been described, probably the most important for regulating Na^+ and water flux across high-resistance epithelia of airway, alveoli, bladder, kidney, and distal colon is the apical epithelial Na^+ channel, ENaC, which allows Na^+ to pass into the cell following electrochemical gradients established by extrusion of Na^+ by a baso-lateral membrane pump (Na^+, K^+-ATPase). Mutations in ENaC genes cause diseases associated with channel overactivity [e.g., Liddle's syndrome (Shimkets *et al.*, 1994)] as well as with loss of function [e.g., pseudo-hypoaldosteronism (Chang *et al.*, 1996)]. Given the critical contributions of ENaC to organ homeostasis and the ever-changing dynamics of water and salt intake and loss, it is no surprise that ENaC function is subject to regulation by a variety of "accessory factors" originating from epithelial cells (Huang *et al.*, 2004; Snyder, 2005; Stutts *et al.*, 1995; Thomas and Itani, 2004; Voilley *et al.*, 1997; Warnock, 1999). In the past decade, investigators in several laboratories recognized and explored an initially surprising but potent modulation of ENaC function by peptidases, which is the focus of this review.

II. ENaC Regulation by Peptidases: *In Vitro* and Biochemical Evidence

A. Inhibition of Na^+ Transport in Epithelial Monolayers by Antipeptidases

A seminal observation suggesting that ENaC is regulated by endogenous serine peptidases was made in a frog line of kidney cells (*Xenopus* A6) (Vallet *et al.*, 1997). Amiloride-sensitive Na^+ uptake in these cells was partially blocked by apical exposure to the broad-spectrum inhibitor of serine peptidases, aprotinin, and was restored in aprotinin-treated cells by application of trypsin. This suggested the presence of an ENaC-activating serine peptidase in the apical membrane. Vallet and colleagues then used an expression-cloning strategy to identify a candidate "channel-activating peptidase 1" (CAP1), which augmented ENaC activity when coexpressed with ENaC in oocytes. Similar responses to apical aprotinin and trypsin were noted in M-1 cells derived from mouse cortical collecting duct (Nakhoul *et al.*, 1998), in human nasal airway cells in primary culture (Donaldson *et al.*, 2002), and in JME/CF15 airway cells derived from an individual with CF (Tong *et al.*, 2004), suggesting similar upregulation of ENaC by endogenous peptidases in mammalian epithelia. Further studies (Bridges *et al.*, 2001) suggested another layer of regulation by revealing inhibition of ENaC in cultured airway cells by a recombinant serine peptidase inhibitor (BAY 39–9437) derived from human placental bikunin, which may be an endogenous modulator of CAP activity. The exact mechanism of the effects of trypsin remains to be fully established. However, the overall effect on rat ENaC is to increase channel open probability of previously near-silent channels by lengthening the time spent open and diminishing time spent closed—without affecting open channel conductance to Na^+ (Caldwell *et al.*, 2004).

In other informative studies, aprotinin blocked ENaC-mediated Na^+ transport across primary rat alveolar type II cell monolayers by 70% without modifying α- and β-ENaC cell surface expression, whereas exogenous trypsin had little or no effect (Planes *et al.*, 2005). This suggested that aprotinin-sensitive epithelial serine peptidases activate ENaC in alveolar epithelium mostly by increasing channel open probability, and that this activation is maximal or near-maximal under usual culture conditions. Interestingly, pre-incubation of alveolar cells with aprotinin also completely abolished stimulation of Na^+ transport by β_2-adrenergic agonists. Inhibition of Na^+ transport by apically applied aprotinin also occurs in cultured mouse bronchioloalveolar epithelial cells, although to a lesser extent than in rat cells (Planes *et al.*, 2005). This variance may be due to interspecies differences in expression or aprotinin-sensitivity of endogenous peptidases, as shown between mouse and amphibian kidney cells (Vuagniaux *et al.*, 2002).

In vitro, cAMP agonists augment ENaC activity and transepithelial Na^+ transport in various cell types, including native alveolar epithelial cells. This occurs mostly by increasing turnover and promoting insertion of ENaC subunits at the cell surface (Planes *et al.*, 2002; Snyder, 2000). Serine peptidases and β_2-adrenergic agonists therefore could act synergistically, with peptidases activating Na^+ channels newly recruited to apical membrane under the influence of β_2-agonists. A similar synergism was reported between membrane-bound CAPs and serum- and glucocorticosteroid-regulated kinase in frog oocytes (Vuagniaux *et al.*, 2002).

B. Peptidases in ENaC Maturation and Disposal

ENaC is a heterotetrameric membrane protein built from products of homologous genes encoding α-, β-, and γ-subunits. Mature ENaC is thought to be composed of two α-, one β-, and one γ-subunits (Fig. 1). Each subunit has two transmembrane domains bridged by a large extracellular loop. After initial translation-coupled embedding in the endoplasmic reticulum membrane, subunits mature prior to insertion into the plasma membrane by addition and processing of N-linked sugars (Hughey *et al.*, 2004b). Some subunits also are processed by nicking of α at two sites and γ at one site by furin (Hughey *et al.*, 2003), a membrane-anchored subtilisin-type peptidase thought to reside principally in the lumen of the trans-Golgi network. Nicking of the α-subunit activates channel Na^+-transporting activity (Hughey *et al.*, 2004a), apparently by dissociating an inhibitory domain in the extracellular loop (Carattino *et al.*, 2006). Furin-mediated hydrolysis of ENaC α subunits also relieves "self-inhibition" by Na^+ (Sheng *et al.*, 2006), thereby increasing Na^+ transport. The importance of furin cleavage sites and furin itself has been suggested by loss of activity in channels with mutated furin cleavage sites and by reduced activity in furin-deficient cells (Hughey *et al.*, 2004a). At the cell surface, ENaC function appears to be further upregulated by one or more extracellular or plasma membrane-associated trypsin-like peptidases, as further reviewed below. Not all channels reaching the plasma membrane have been proteolytically processed (Hughey *et al.*, 2004b), suggesting that there is a population of channels available for proteolytic activation at the cell surface. Presently, there is no direct evidence that extracellular serine peptidases increase Na^+-transporting activity at the cell surface by cleaving ENaC at or near the unprocessed furin-sensitive sites. However, combinatorial peptide substrate profiling of one of the potential activators, prostasin, does suggest the potential to do so (Shipway *et al.*, 2004).

Although Na^+ transport across epithelia can be increased by "exocytotic" insertion of ENaC from an intracellular, subapical pool [some of which may be recycled (Butterworth *et al.*, 2005)], this does not seem to be the mechanism by which extracellular serine peptidases increase ENaC activity (Caldwell *et al.*, 2004). An alternative fate of ENaC endocytosed from the cell surface is ubiquitination by one or more ubiquitin ligases and subsequent translocation to the cytosol followed by destruction by the proteasome. This is an important means of regulating surface expression of ENaC and the magnitude of transepithelial transport of Na^+, as revealed by the hypertension and volume expansion in Liddle's syndrome, which is associated with genetic defects in parts of ENaC involved in binding to Nedd4 and related ubiquitin ligases (Shimkets *et al.*, 1994).

C. CAPs in Mammalian Epithelia

A number of peptidases are candidate endogenous activators of ENaC in mammalian epithelia. Some features of those peptidases for which there is biochemical or other functional support are listed in Table I. The mammalian peptidase that is the most likely orthologue of frog CAP1 is CAP1/prostasin. Like *Xenopus* CAP, mammalian prostasins are known or predicted to be type I tryptic serine peptidases with a C-terminal membrane anchor (Verghese *et al.*, 2004;Yu *et al.*, 1995). Furthermore, phylogenetic analysis suggests that prostasins are *Xenopus* CAP1's closest mammalian relatives (Caughey *et al.*, 2000;Verghese *et al.*, 2004;Vuagniaux *et al.*, 2002). Recombinant mouse CAP1/prostasin, when coexpressed with rat ENaC subunits in frog

oocytes, augments amiloride-sensitive transport of Na^+ (Vuagniaux *et al.*, 2000,2002). Patterns of expression of human and mouse prostasins are similar to those of ENaC itself, as one would expect if one protein were regulating the other (Donaldson *et al.*, 2002;Verghese *et al.*, 2004). Human prostasin is inhibited by aprotinin, which also inhibits ENaC-mediated epithelial Na^+ transport. Also, in rats, transgenic expression of human prostasin causes hypertension (Wang *et al.*, 2003), as one would predict of an enzyme augmenting Na^+ absorption. Direct evidence of a role for prostasin in modulating ENaC-mediated Na^+ currents was provided by results of siRNA-mediated knockdown of prostasin expression in CF epithelial cells (Tong *et al.*, 2004). Although the above collection of circumstantial and direct evidence builds a case favoring a prominent role for prostasin in regulating ENaC, such a role was not initially entertained for this enzyme, which was discovered as a soluble tryptic peptidase in secretions from the prostate gland (Yu *et al.*, 1994), where it is highly expressed. Although human prostasin exists free in solution in seminal fluid, it is synthesized initially with a C-terminal peptide anchor. The role of the peptide anchor is unclear, but in several cell types the peptide anchor is exchanged intracellularly for a lipid anchor, glycosylphosphatidyl inositol (GPI) (Chen *et al.*, 2001). In *Xenopus* CAP1, the GPI anchor consensus attachment motif and surface expression appear to be important for retention of ENaC stimulating activity (Vallet *et al.*, 2002;Verghese *et al.*, 2006). Intriguingly, mutation of *Xenopus* CAP1 to a catalytically incompetent form diminishes but does not abolish ENaC-stimulating activity, raising the possibility that nonenzymatic binding events upregulate Na^+ transport.

Other candidate endogenous regulators of human ENaC include TMPRSS4 (transmembrane protease, serine 4; also known as CAP2), matriptase (also known as membrane-type serine protease 1, ST14, and CAP3), and TMPRSS3. Although these peptidases, like *Xenopus* CAP1 and prostasin, feature trypsin-like catalytic domains, they differ in the mode of membrane anchoring (Fig. 1), being type II rather than type I transmembrane peptidases. For CAP3/matriptase, the actual mode of membrane association of the mature enzyme is not clear. Nonetheless, both CAP2/TMPRSS4 and CAP3/matriptase are coexpressed with ENaC in some epithelia and possess the potential to activate ENaC, as revealed by coexpression of recombinant versions of the peptidases and ENaC in frog oocytes (Vuagniaux *et al.*, 2002). Mutations of TMPRSS3 have an intriguing connection with congenital deafness (Guipponi *et al.*, 2002;Lee *et al.*, 2003). Although ENaC is expressed in the developing inner ear, a proposed link between defects in peptidase function and ENaC regulation presently is a matter of speculation.

D. Candidate Physiological Inhibitors of ENaC-Activating Peptidases

Some possible endogenous inhibitors of human CAPs are listed in Table I. Furin can be inhibited by the largely intracellular serpin PI8 (Dahlen *et al.*, 1998). However, it is not known whether PI8 modulates furin processing of ENaC. Prostasin can be inactivated by the kunitz-type inhibitors placental bikunin/SPINT2 [a recombinant version of which depresses primate ENaC-mediated airway epithelial cell transport *in vitro* (Bridges *et al.*, 2001) and *in vivo* (Bowden *et al.*, 2006)] and by hepatocyte growth factor activator (HGFA) inhibitor-1B, also known as SPINT1 (Fan *et al.*, 2005). Prostasin also can be inhibited by a serpin-type inhibitor, protease nexin-1 (Chen *et al.*, 2004). Matriptase is likewise susceptible to SPINT1, but little is known about inhibitor susceptibilities of TMPRSS3 and TMPRSS4.

III. ENaC Regulation by Peptidases: *In Vivo* Evidence

A. Regulation of Alveolar Na^+ and Water Transport by Serine Peptidases

In mammals, the three ENaC subunits are expressed in Na^+-transporting epithelia in the collecting ducts of the kidney, the mucosal surface of distal colon, ducts of salivary and sweat glands, and airway and alveolar epithelium (Duc *et al.*, 1994). Surprisingly, ENaC subunits

also are expressed in non-transporting epithelia, such as epidermis (Brouard *et al.*, 1999). In distal nephron and colon, ENaC controls Na^+ balance, extracellular fluid volume and blood pressure, and is regulated principally by aldosterone. In airways and alveoli, ENaC helps to control epithelial lining fluid volume and is regulated mostly by glucocorticosteroids. Although activation of ENaC by various serine peptidases is seen in aldosterone- and glucocorticosteroid-responsive epithelial cells, the *in vivo* importance of this activation remains to be fully established. Nonetheless, recent investigations suggest that plasma membrane-associated or secreted ENaC-activating peptidases are likely to be physiologically and pathologically significant in several organs.

Active transcellular Na^+ transport by alveolar epithelial cells is a driving force for reabsorption of fluid from the alveolar space (accounting for the lung's remarkable ability to remove alveolar fluid at the time of birth) and is the main mechanism for resolution of alveolar edema (Basset *et al.*, 1987a, b; Matthay *et al.*, 1982, 2002). ENaC in the apical membrane of types I and II alveolar epithelial cells is rate-limiting for Na^+ absorption (Johnson *et al.*, 2002). Indeed, newborn mice with inactivated α-ENaC develop respiratory distress and die within 40 h of birth from failure to clear lungs of fluid (Hummler *et al.*, 1996). In fetal and adult alveolar epithelium, ENaC is regulated by glucocorticosteroids, which increase ENaC expression by transcriptional and posttranscriptional mechanisms. Alveolar ENaC also is regulated by β-adrenergic agonists, which increase the number of Na^+ channels at the cell surface and the probability of being open (Matthay *et al.*, 2002). Recent emergence of the concept of autocrine or paracrine regulation of ENaC by extracellular serine peptidases owes a great deal to recent studies of Na^+ and water transport across alveolar epithelium.

The potential role of serine peptidases in regulating ENaC was examined in rodent lung by measuring Na^+-driven alveolar fluid clearance (AFC) in the presence of broad-spectrum inhibitors or exogenous proteases, such as trypsin. Surprisingly, studies in a rat fluid-filled lung model (Swystun *et al.*, 2005) showed that addition of aprotinin to the instillate did not modify Na^+-driven AFC measured over 30 min. An *in situ* nonventilated mouse lung model (Planes *et al.*, 2005) also failed to find an acute inhibitory effect of intra-alveolar aprotinin on AFC measured over 15 min. Aprotinin's failure to change baseline AFC in these models, although it inhibits ENaC-mediated alveolar epithelial cell Na^+ transport *in vitro*, has several potential explanations, including the presence of endogenous bikunin, which is a native inhibitor of prostasin expressed in lung and detected in bronchial mucus. However, this explanation seems unlikely since baseline AFC was not increased by excess trypsin, which should circumvent potential AFC inhibition by endogenous inhibitors. Considering the long inhibitor incubation time (~75 min) needed to inhibit ENaC maximally *in vitro*, these shorter term measurements may be inappropriate for detecting delayed inhibition by aprotinin *in vivo*. In *in situ* mouse lung, aprotinin also had the interesting effect of abolishing terbutaline-stimulated AFC, consistent with *in vitro* findings in rat alveolar epithelial cells (Planes *et al.*, 2005). This finding suggested that serine peptidase-mediated activation of ENaC optimizes *in vivo* efficacy of β_2-adrenergic agonists, which markedly increase mammalian alveolar Na^+ transport and fluid clearance *in vivo* (Matthay *et al.*, 2002).

Among several serine peptidases with the potential to regulate Na^+ transport across murine alveolar epithelium, CAP1/prostasin is a prime candidate inasmuch as mRNA and protein corresponding to this peptidase is abundant in extracts of cultured or freshly isolated alveolar epithelial type II cells and in sections of alveolar epithelium (Planes *et al.*, 2005; Verghese *et al.*, 2004). Prostasin is expressed at the apical cell surface of cultured rat type II cells (Verghese *et al.*, 2004). In lung and kidney epithelial cells, prostasin is shed by endogenous GPI-specific phospholipase D1 (Verghese *et al.*, 2006). In mouse lung, mCAP1/prostasin is secreted into epithelial lining fluid. The presence of mCAP1/prostasin mRNA and protein in alveolar macrophages (Planes *et al.*, 2005) suggests that sources other than epithelial cells may

contribute to prostasin in epithelial lining fluid. Whatever the cells of origin, if secreted CAP1/prostasin is enzymatically active, as is true of human prostasin in seminal fluid (Chen *et al.*, 2001), then it may act not only as an autocrine but also a paracrine modulator of Na^+ absorption—able, for instance, to activate ENaC in type I cells, which cover most of the alveolar surface. In addition to CAP1/prostasin, CAP2 (mouse orthologue of human transmembrane serine protease TMPRSS4) and CAP3 (mouse orthologue of human matriptase MT-SP1) are expressed, at least at the mRNA level, in mouse alveolar epithelial cells but not in alveolar macrophages (Planes *et al.*, 2005). CAP2 and CAP3 potentially could regulate ENaC independently or via an activation cascade involving CAP1/prostasin (Rossier, 2004).

Other serine peptidases secreted into alveolar fluid by epithelial and inflammatory cells also could modulate ENaC activity and alveolar Na^+ and water transport in some conditions. In this connection, a soluble, trypsin-like protease (20–28 kDa on zymograms) in rat lung bronchoalveolar lavage fluid (Swystun *et al.*, 2005) may be important. This peptidase was proposed to facilitate lung liquid clearance because intra-alveolar addition of soybean trypsin inhibitor or α_1-antitrypsin decreased Na^+-driven AFC. Furthermore, addition of an excess of trypsin abolished the inhibition. The role of nonepithelial serine peptidases in alveolar Na^+ transport remains to be established, but the concept that pathologically activated alveolar inflammatory cells release paracrine regulators of ENaC deserves further investigation.

B. Regulation of Colonic Prostasin Expression by Aldosterone and Dietary Na^+

ENaC genes have been cloned from the distal colon of Na^+-deprived rats (Canessa *et al.*, 1993, 1994). Transcripts and protein corresponding to the three ENaC subunits are expressed by surface epithelial cells of the distal colon, but not by crypt cells (Duc *et al.*, 1994). Surface epithelium of the distal colon is typically responsive to aldosterone, which fine-tunes dietary absorption of Na^+. Hyperaldosteronism arising from depletion of dietary Na^+ or infusion of aldosterone increases amiloride-sensitive Na^+ transport and β- and γ-ENaC mRNA expression in distal colon (Greig *et al.*, 2002; Halevy *et al.*, 1986). Several serine peptidases shown to activate ENaC *in vitro* also are expressed in the gastrointestinal tract, with a tissue distribution broader than that of ENaC itself. CAP1/prostasin mRNA is present in stomach and colon in rats and in stomach, small intestine and distal colon in mice (Adachi *et al.*, 2001; Vuagniaux *et al.*, 2000). Mouse CAP2 and CAP3 mRNAs also are present in mouse stomach, small intestine, and colon (Vuagniaux *et al.*, 2002). Because CAPs are coexpressed with ENaC in distal colon, they may influence Na^+ reabsorption by modulating ENaC activity and may modulate physiological responses to aldosterone *in vivo*.

Consequences of altering plasma levels of aldosterone include reported changes in prostasin and ENaC mRNA expression in colonic epithelium (Fukushima *et al.*, 2004). In these studies, adult rats were given normal or Na^+-depleted diets, and continuous infusion of vehicle or aldosterone for 2 or 4 weeks. Prostasin and ENaC subunit mRNAs in extracts of colonic epithelial cells were quantitated by PCR or mRNA blot analysis. Dietary Na^+ depletion and aldosterone infusion both dramatically increased plasma levels of aldosterone, accompanied by a marked increase in arginine vasopressin. Expression of α-ENaC mRNA was detected in epithelial cells from proximal and distal colon under basal conditions, but was induced at 1, 2, and 4 weeks in both models only in the distal colon. β-ENaC mRNA was absent or barely detected under basal conditions, but was also clearly enhanced in distal colon in the context of prolonged hyperaldosteronism (2 and 4 weeks). γ-ENaC mRNA, quantified by real time RT-PCR because of low basal expression, also increased at 2 weeks in distal (but not proximal) colonic epithelium from Na^+-depleted and aldosterone-infused rats. Prostasin mRNA increased ~3-fold at 2 and 4 weeks in distal colonic epithelial cells from Na^+-depleted and aldosterone-infused rats. Prostasin mRNA tended to increase in proximal colonic epithelial cells from aldosterone-infused rats, but not significantly. These data show that a rise in plasma

levels of aldosterone enhances prostasin and ENaC subunit mRNA expression in distal colonic epithelium *in vivo*. It remains to be established whether prostasin and ENaC are coexpressed in the same colonic cells and whether prostasin induction in the aldosterone response is physiologically important.

An interesting question raised by the above study was the potential role of arginine-vasopressin on prostasin expression in the distal colon, since the stimuli used in both models dramatically increased plasma levels of arginine-vasopressin, together with the expected increase in aldosterone. The authors (Fukushima *et al.*, 2004) evaluated the effect of arginine-vasopressin on prostasin expression *in vitro* by exposing mineralocorticoid receptor-expressing T84 colon cancer cells to aldosterone or arginine-vasopressin alone, or to the combination of aldosterone and arginine-vasopressin. Surprisingly, although neither aldosterone nor arginine-vasopressin alone had much effect, together they markedly stimulated expression of prostasin mRNA, suggesting synergism. Additional studies are needed to evaluate whether arginine-vasopressin exerts effects on ENaC or prostasin complementary to those of aldosterone, as reported for ENaC in the kidney (Ecelbarger *et al.*, 2000).

C. Regulation of Prostasin by Aldosterone in the Kidney

Although ENaC-activating serine peptidases such as CAP1/prostasin, CAP2, or CAP3/MT-SP1 matriptase were initially cloned from kidney cell lines (Vallet *et al.*, 1997; Vuagniaux *et al.*, 2000, 2002), direct evidence that these peptidases regulate renal Na^+ transport *in vivo* is lacking. However, reports that prostasin expression in rat and human kidney is regulated by aldosterone suggest that CAPs could participate in stimulation of Na^+ transport by aldosterone (Narikiyo *et al.*, 2002; Olivieri *et al.*, 2005). Fine regulation of Na^+ excretion in the distal nephron occurs principally in the collecting duct and is mediated chiefly by aldosterone, via effects on ENaC. In kidney, the ENaC subunits are specifically expressed in epithelial cells from the aldosterone-sensitive distal nephron (ASDN) (Duc *et al.*, 1994; Masilamani *et al.*, 1999), where ENaC expression and activity at the cell surface is generally rate-limiting. The mechanism whereby aldosterone stimulates ENaC activity and Na^+ transport in distal nephron is complex, tissue-specific, and not completely understood. Aldosterone effects are mainly mediated via mineralocorticoid receptor-induced changes in expression of various gene products, such as serum glucocorticoid-induced kinase 1, which are elements of a network that controls function of the Na^+ transport machinery and expression of ENaC itself (Loffing *et al.*, 2001). In rats, elevation of aldosterone levels in plasma rapidly and selectively increased the abundance of ENaC mRNA and protein along the entire ASDN, and induced apical translocation of ENaC subunits in the initial portion of the ASDN (Masilamani *et al.*, 1999). Interestingly, aldosterone also induced a shift in size of γ-ENaC from 85 to 70 kDa. The authors proposed that this shift, the functional consequences of which are not known, could result from cleavage of the extracellular loop by peptidases.

Several studies using kidney epithelial cell lines showed that exposure to apical aprotinin induced a substantial decrease in ENaC-mediated transepithelial Na^+ transport. CAP1/prostasin (and CAP2 and CAP3), coexpressed with ENaC in these cells, was proposed to be targets of aprotinin and physiological activators of ENaC (Liu *et al.*, 2002; Vallet *et al.*, 1997; Vuagniaux *et al.*, 2000, 2002). A few studies subsequently evaluated aldosterone's effects on prostasin expression *in vitro*, with conflicting results. One set of studies (Liu *et al.*, 2002) failed to detect an effect of aldosterone treatment on the magnitude either of aprotinin-induced decrease in Na^+ current or of prostasin/CAP1 mRNA expression in M-1 cortical collecting duct cells, even though cells were exposed for 48 h to a high concentration (1 μM) of aldosterone. In contrast, exposing the same cell line to the same hormone concentration for 6–48 h, other investigators (Narikiyo *et al.*, 2002) found that aldosterone progressively increased CAP1/prostasin mRNA and protein expression. Surprisingly, prostasin was detected neither

in the cytosolic nor in the membrane fraction; rather, it was found in cell culture medium in a soluble, secreted form, which increased following cell exposure to aldosterone. Prostasin induction was associated with an increase in the magnitude of aprotinin inhibition of amiloride-sensitive ^{22}Na uptake, suggesting that prostasin in part mediates stimulation of Na^+ transport by aldosterone. To assess the *in vivo* relevance of the findings, these investigators exposed rats for 7 days to continuous aldosterone (100 μg/100 g body weight/day) or vehicle infusion, before evaluating renal expression and urinary excretion of prostasin (Narikiyo *et al.*, 2002). Rats treated with aldosterone developed systemic hypertension and metabolic alkalosis. Prostasin protein could not be detected in cytosolic or membrane fractions of renal cortex or medulla with or without aldosterone. However, soluble prostasin was detected in rat urine under basal conditions, suggesting massive secretion of the protein by kidney tubular cells. Indeed, urinary excretion of prostasin increased fourfold in aldosterone-treated rats. This study also examined urinary prostasin excretion in patients with primary aldosteronism before and 7 days after adrenalectomy, and in patients undergoing surgery for another disease. Interestingly, urinary prostasin excretion was high in subjects with primary aldosteronism as compared with controls, and adrenalectomy normalized plasma aldosterone concentration and decreased urinary prostasin excretion. Of note, urinary prostasin excretion before and after adrenalectomy correlated well with the urinary Na^+/K^+ ratio, reflecting the effect of aldosterone on kidney cells.

These data suggesting a relationship between urinary prostasin and aldosterone production were confirmed recently in normotensive and hypertensive subjects with inappropriate aldosterone secretion. This study first evaluated the changes in urinary prostasin in healthy normotensive individuals after spironolactone-induced blockade of mineralocorticoid receptor or acute volume expansion (Olivieri *et al.*, 2005). The investigators detected prostasin in urine from all normotensive subjects tested, regardless of gender and dietary Na^+ intake. Treatment with spironolactone induced an expected 30% increase in urinary Na^+/K^+ ratio and at the same time decreased urinary prostasin in normotensive subjects in whom the renin/aldosterone axis was activated by a low Na^+ intake, but was ineffective in normotensive subjects with high Na^+ intake. Saline infusion decreased both plasma aldosterone levels and urinary prostasin excretion in all normotensive subjects investigated. In contrast, urinary prostasin paradoxically increased after saline infusion in hypertensive patients with primary aldosteronism. Two-dimensional immunostaining revealed a complex pattern of expression with up to seven distinct bands with different glycosylation patterns. Some of these isoforms were paradoxically overexpressed following volume expansion in patients with primary aldosteronism. The authors concluded that some prostasin is excreted independently of Na^+ balance and plasma levels of aldosterone, and that additional prostasin is excreted under the influence of aldosterone.

Taken together, the above studies strongly support the concept that prostasin expression and urinary excretion are regulated by aldosterone. Although urinary prostasin is a possible marker of ENaC activation in the kidney, several issues relating to its roles and importance *in vivo* remain to be addressed, starting with its cellular origin. In this regard, it should be noted that expression of prostasin, unlike that of ENaC, is not restricted to the distal nephron, for prostasin/CAP1 mRNA is highly expressed in mouse proximal tubule, a segment of the nephron that is aldosterone-unresponsive (Vuagniaux *et al.*, 2000). This “proximal” source of urinary prostasin may contribute to aldosterone-independent basal secretion. It also remains to be established if any of the several soluble forms of urinary prostasin are enzymatically active. If this is so, then urinary prostasin may exert a paracrine effect on renal tubular cells and activate apically expressed ENaC. The functional importance of aldosterone-induced prostasin expression to renal Na^+ reabsorption also remains to be answered. Tissue-specific inactivation of prostasin/CAP1 gene should help to answer these questions.

D. Potential Role of Prostasin in Regulating Aldosterone Production and Hypertension

The physiological links between prostasin, aldosterone, and ENaC function might be more complex than prostasin being an aldosterone-induced protein that activates ENaC. Indeed, a study suggested that prostasin could influence the processing of aldosterone itself (Wang *et al.*, 2003) based on effects of adenovirus-mediated human prostasin gene delivery on blood pressure and electrolyte homeostasis in rats. Animals receiving a single injection of adenovirus achieved high-level expression of human prostasin mRNA in adrenal gland and liver and low-level expression in kidney, lung, heart, and aorta. Immunoreactive prostasin was detected in blood and urine for almost 3 weeks. Prostasin gene transfer induced a marked and prolonged (3–4 week) increase in blood pressure. Interestingly, this increase was preceded by an increase in plasma aldosterone and a moderate decrease in plasma renin. Because human prostasin mRNA was expressed in adrenal gland (the principal site of aldosterone generation), as well as in vessel walls [where aldosterone also may be produced (Takeda *et al.*, 1995)], the authors suggested that prostasin could be involved in aldosterone maturation, processing, or secretion. In this regard, it is uncertain whether endogenous prostasin normally is expressed in rat adrenal gland and vessel walls. Previous studies failed to identify prostasin mRNA and protein expression in rat and human aorta, vein, and artery (Yu *et al.*, 1994, 1995). It is possible that prostasin-induced aldosterone production in this study is a non-physiological consequence of unregulated, ectopic production of human prostasin. The mechanism whereby prostasin gene transfer-induced aldosterone production elevated systemic blood pressure also remains to be fully explained. Although plasma aldosterone levels increased in rats receiving prostasin-adenoviral construct, urinary Na^+ and K^+ excretion did not change at Day 3, 7, or 21 postinjection, and body weight curves did not differ from those of control rats. Paradoxically, increased urinary Na^+ excretion with concomitantly decreased K^+ excretion was seen at Day 14 in treated animals. The authors proposed that this could be explained by escape from the aldosterone effect, perhaps mediated by upregulation of the renal kallikrein–kinin system. Possibly, the change in Na^+ balance is due to effects of elevated aldosterone on vascular resistance. Whatever the mechanism, these intriguing data emphasize the complexity of interactions between prostasin and aldosterone.

E. Role of CAPs in Maintaining the Epidermal Permeability Barrier

In humans and rodents, the skin is thought not to be involved in ion or fluid absorption, but surprisingly, ENaC is expressed in the epidermis, the outermost layer of the skin (Brouard *et al.*, 1999; Roudier-Pujol *et al.*, 1996). The epidermis is a self-renewing epithelium, consisting mostly of keratinocytes. Basal keratinocytes in the inner layer of epidermis proliferate and migrate upward while terminally differentiating, leading to the generation of flattened, anucleated corneocytes, which are interconnected by desmosomes and embedded in a lipid matrix. This structure constitutes the watertight stratum corneum, which prevents excessive loss of body fluids, and protects against mechanical, chemical, or biological insults. Human keratinocytes express the three ENaC subunits (Brouard *et al.*, 1999). However, ENaC's role in the skin is unclear. Previous *in vitro* studies found that expression of ENaC, especially β-subunit, increased dramatically during cell growth and differentiation of human cultured keratinocytes, suggesting that ENaC may play a role in terminal differentiation (Brouard *et al.*, 1999; Oda *et al.*, 1999). Patch-clamp experiments in keratinocytes showed a channel similar to ENaC in a small proportion or recorded cells, but no transepithelial Na^+ transport was identified. Interestingly, there is an epidermal Ca^{2+} gradient, with more Ca^{2+} in upper than lower epidermis. This gradient appears to regulate keratinocyte growth and differentiation (Dotto, 1999). The fact that ENaC subunit expression exhibits a similar vertical gradient suggests a possible interrelationship between Na^+ and Ca^{2+} flux in these cells (Guitard *et al.*, 2004). Whatever the mechanism, ENaC's importance in skin differentiation and development was highlighted by findings in α-ENaC-deficient mice, in which newborns exhibited epidermal

thickening, disordered differentiation, premature lipid secretion in the upper epidermis, and abnormal keratohyalin granules (Mauro *et al.*, 2002).

Numerous peptidases are expressed in the epidermis. Some of them such as profilaggrin endopeptidase 1 (Resing *et al.*, 1995), calpain-1, cathepsin L (Benavides *et al.*, 2002), or stratum corneum chymotryptic enzyme (Ekholm and Egelrud, 1998) are implicated in processing and maturation of profilaggrin to filaggrin filaments, and in epidermal differentiation and desquamation. Among the peptidases expressed in mouse skin are ENaC-activating CAP1/prostasin (Vuagniaux *et al.*, 2000) and CAP3/MT-SP1 matriptase (Vuagniaux *et al.*, 2002). CAP1/prostasin was detected in the top layers of the stratum granulosum and at the transition between stratum granulosum and stratum corneum (Leyvraz *et al.*, 2005). *In vitro*, CAP1/prostasin and ENaC are coexpressed in keratinocytes with similar patterns of expression, that is, with mRNA levels increasing gradually as cells differentiate. Interestingly, two recent studies revealed that inactivation of either CAP3/MT-SP1 matriptase or CAP1/prostasin in mice induced a lethal phenotype with deficient epidermal barrier function. In the first study, introduction of a null mutation into the CAP3/MT-SP1 matriptase gene (List *et al.*, 2002) results in newborns with dry, red, shiny, and wrinkled skin. Epidermal barrier function was compromised, as demonstrated by increased toluidine blue penetration and transepidermal water loss, which led to rapid and fatal dehydration. In the second study, mice lacking epidermal CAP1/prostasin (Leyvraz *et al.*, 2005) were generated by crossing mice with a conditional "floxed" CAP1/prostasin allele with mice expressing Cre recombinase controlled by the keratin 14 promoter, which is active in basal keratinocytes. Newborn pups lacking epidermal CAP1/prostasin had low body weight and died within 60 h of birth. Their stratum corneum was severely malformed with abnormal lipid composition, corneocyte morphogenesis, and profilaggrin processing. As in CAP3/MT-SP1 matriptase-deficient pups, early death was due to impaired skin barrier function and dehydration. Among the most interesting findings was the complete absence of occludin in tight junctions of CAP1/prostasin-deficient epidermis. This was associated with increased permeability of tight junctions, as evidenced by failure to prevent diffusion of the subcutaneously injected biotin (600 kDa) to the skin surface. This implied involvement of CAP1/prostasin in maintaining tight junction integrity is consistent with prior studies in which exposure of renal and alveolar epithelial cell monolayers to apical aprotinin (an inhibitor of CAP1/prostasin) reduced transepithelial resistance (Liu *et al.*, 2002; Planes *et al.*, 2005), whereas treatment with exogenous trypsin increased it (Swystun *et al.*, 2005).

Collectively, these data strongly suggest that CAP1/prostasin can modulate tight junctions and paracellular permeability in various epithelia, possibly through regulation of occludin expression or function. It is possible that occludin is cleaved by prostasin, though it lacks preferred polybasic cleavage sites (Leyvraz *et al.*, 2005). Another issue to be resolved is the relationship between ENaC and CAP3/MT-SP1 matriptase or CAP1/prostasin in the skin. Presently, there is no evidence that ENaC dysfunction plays a role in skin abnormalities observed in CAP1/prostasin- or CAP3/MT-SP1 matriptase-deficient mice. Nonetheless, these studies suggest a potential role of CAPs in regulating paracellular permeability beyond an effect on function of ENaC.

F. ENaC Dysregulation in the Pathophysiology of Cystic Fibrosis: CAPs as Potential Drug Targets

The mucosa of proximal and distal airways is covered by airway surface liquid (ASL), which is a first line of defense against inhaled pathogens. The ASL is composed of the watery periciliary liquid layer (PCL) surrounding the cilia on the apical face of ciliated cells, and of the mucin-rich mucous layer, which resides atop of the PCL. Maintenance of proper ASL volume/depth and ionic composition is critical to optimize ciliary beating and mucus clearance

(Matsui *et al.*, 1998). Fine regulation of ASL volume is mainly achieved by active transepithelial transport of salt, leading to liquid absorption or secretion. Airway epithelial cells have the capacity to actively reabsorb Na^+ and to secrete Cl^- (Boucher, 2003; Tarran *et al.*, 2001). In the basal state, the dominant ion transport across the airway epithelium is amiloride-sensitive Na^+ absorption through ENaC, which induces passive Cl^- absorption. ENaC subunits are expressed throughout the respiratory tract (with a large predominance of α- and γ- over β-subunits in proximal airways), and are upregulated by glucocorticoids (Farman *et al.*, 1997; Pitkanen *et al.*, 2001; Renard *et al.*, 1995). Active Cl^- secretion also can occur following stimulation by agents such as cAMP agonists and ATP. Cl^- secretion mostly occurs through the apical Cystic Fibrosis Transmembrane Conductance Regulator (CFTR), and possibly through other channels such as the Ca^{2+}-activated Cl^- channel. CFTR is a protein kinase A-activated Cl^- channel in the apical membrane of secretory epithelia, but also regulates other ion transport systems. In particular, CFTR influences ENaC activity. In various epithelial cells and overexpressing recombinant cells, activation of CFTR inhibits ENaC currents, most likely by increasing intracellular concentration of Cl^- (Konig *et al.*, 2001; Mall *et al.*, 1998).

Attention has focused on airway epithelial ion transport processes because CF is caused by mutations in the CFTR gene (Rowe *et al.*, 2005). CF is characterized by a generalized exocrine dysfunction, but lung disease is the leading cause of death. Respiratory manifestations of CF include airway obstruction by hyperviscous secretions leading to iterative bacterial infections, chronic airway inflammation, and bronchiectasis. Although it is clear that CF lung disease reflects a defect in the innate defense of airway surfaces against bacterial infection, the basis of this defect and its relationship with CFTR dysfunction are not well understood. One of the prevailing explanations, called the “low volume hypothesis,” emphasizes the role of the CFTR defect in both Cl^- secretion and ENaC regulation. According to this hypothesis, absence of CFTR leads to lack of Cl^- (and water) secretion, but also to enhanced Na^+ (and water) absorption because tonic ENaC inhibition by CFTR is lost. This Na^+ hyperabsorption decreases ASL volume, impairs clearance of mucus, and promotes accumulation of hyperviscous mucus, thereby favoring bacterial infection (Knowles *et al.*, 1986; Matsui *et al.*, 1998, 2000).

Recent animal and human studies underscore the potential importance of ENaC in the pathogenesis of CF. Increased airway epithelial Na^+ absorption caused by airway-specific overexpression of β-ENaC induced a severe CF-like lung disease in mice, including mucus obstruction, goblet cell metaplasia, neutrophilic inflammation, and decreased bacterial clearance (Mall *et al.*, 2004). ENaC hyperactivity in the airways was associated with ASL volume depletion, increased mucus concentration, delayed mucus transport, and mucus adhesion to cell surfaces. Furthermore, an association study searching for genetic modifiers identified *SCNN1B* and *SCNN1G* (genes encoding β- and γ-ENaC, respectively) as potential modulators of CF severity (Stanke *et al.*, 2006).

Pharmacological inhibitors of ENaC are being actively investigated as treatment of CF lung disease (Hirsh *et al.*, 2004). Physiological activators of ENaC in the airways—including CAPs—also can be regarded as candidates for therapeutic inhibition. Prostasin is abundantly expressed in normal and CF nasal epithelial cells (Bridges *et al.*, 2001; Donaldson *et al.*, 2002; Tong *et al.*, 2004), and silencing prostasin expression in CF cells using siRNA decreases ENaC-mediated Na^+ transport by 75% (Tong *et al.*, 2004). As noted, the kunitz-type inhibitor aprotinin markedly decreased ENaC-mediated Na^+ transport across primary human nasal and bronchial epithelial cells from individuals with and without CF (Bridges *et al.*, 2001; Donaldson *et al.*, 2002). Interestingly, Bridges and colleagues reported that BAY 39-9437, a recombinant inhibitor derived from the human kunitz-type inhibitor bikunin, also inhibited ENaC in normal and CF bronchial epithelial cells. In contrast, two broad-spectrum non-kunitz inhibitors (soybean trypsin inhibitor and α_1-antitrypsin) were ineffective (Bridges *et al.*, 2001). It is therefore tempting to conclude that kunitz-type inhibitors targeting prostasin-like

peptidases possess therapeutic potential for reversing Na^+ hyperabsorption in CF. In this regard, the report that recombinant-truncated bikunin/SPINT2 produces long-lasting changes in nasal potential difference in monkeys *in vivo* is encouraging (Bowden *et al.*, 2006). The effects of chronic inflammation on expression and activity of prostasin-like peptidases in the airways are unknown. Of note, exposure of mouse cortical collecting duct M-1 cells to transforming growth factor (TGF)-β1 markedly downregulated expression of CAP1/prostasin (Tuyen do *et al.*, 2005). This raised the possibility that proinflammatory cytokines released in CF airways influence expression and activity of prostasin or other epithelial CAPs. Also, there are most likely some physiological endogenous inhibitors of prostasin-like proteases (such as bikunin and HGFA inhibitor-1B) in ASL. Their regulation and potential role under inflammatory conditions are currently unknown. Finally, epithelial CAPs may not be the sole activators of ENaC in the airways; other peptidases released in bronchial mucus by inflammatory cells could also influence ENaC activity under pathological conditions. For instance, human neutrophil elastase (hNE), a serine protease, activates ENaC and transepithelial Na^+ transport in a human bronchial cell line (Caldwell *et al.*, 2005). Under normal conditions, extracellular airway hNE is inactive and bound to an endogenous inhibitor (e.g., α_1-antitrypsin or secretory leukoprotease inhibitor), but can be present and active in CF airways because the large number of disintegrating neutrophils overwhelms the antiprotease shield. Thus, hNE could participate in ENaC activation in CF airways. Accordingly, development of pharmacological inhibitors of prostasin-like proteases and hNE may be useful for treatment of CF.

References

Adachi M, Kitamura K, Miyoshi T, Narikiyo T, Iwashita K, Shiraishi N, Nonoguchi H, Tomita K. Activation of epithelial sodium channels by prostasin in *Xenopus* oocytes. J Am Soc Nephrol 2001;12:1114–1121. [PubMed: 11373334]

Basset G, Crone C, Saumon G. Fluid absorption by rat lung *in situ*: Pathways for sodium entry in the luminal membrane of alveolar epithelium. J Physiol 1987a;384:325–345. [PubMed: 3116209]

Basset G, Crone C, Saumon G. Significance of active ion transport in transalveolar water absorption: A study on isolated rat lung. J Physiol 1987b;384:311–324. [PubMed: 3656149]

Benavides F, Starost MF, Flores M, Gimenez-Conti IB, Guenet JL, Conti CJ. Impaired hair follicle morphogenesis and cycling with abnormal epidermal differentiation in nackt mice, a cathepsin L-deficient mutation. Am J Pathol 2002;161:693–703. [PubMed: 12163394]

Boucher RC. Regulation of airway surface liquid volume by human airway epithelia. Pflugers Arch 2003;445:495–498. [PubMed: 12548395]

Bowden A, Stevens L, Morton M, Tomkinson A, Tepper J. Effect of the serine protease inhibitor, AER 002, on epithelial sodium channel activity: A novel treatment for CF and COPD. Am J Respir Crit Care Med 2006;3:A727.

Bridges RJ, Newton BB, Pilewski JM, Devor DC, Poll CT, Hall RL. Na^+ transport in normal and CF human bronchial epithelial cells is inhibited by BAY 39–9437. Am J Physiol Lung Cell Mol Physiol 2001;281:L16–L23. [PubMed: 11404240]

Brouard M, Casado M, Djelidi S, Barrandon Y, Farman N. Epithelial sodium channel in human epidermal keratinocytes: Expression of its subunits and relation to sodium transport and differentiation. J Cell Sci 1999;112(Pt 19):3343–3352. [PubMed: 10504339]

Butterworth MB, Edinger RS, Johnson JP, Frizzell RA. Acute ENaC stimulation by cAMP in a kidney cell line is mediated by exocytic insertion from a recycling channel pool. J Gen Physiol 2005;125:81–101. [PubMed: 15623897]

Caldwell RA, Boucher RC, Stutts MJ. Serine protease activation of near-silent epithelial Na^+ channels. Am J Physiol Cell Physiol 2004;286:C190–C194. [PubMed: 12967915]

Caldwell RA, Boucher RC, Stutts MJ. Neutrophil elastase activates near-silent epithelial Na^+ channels and increases airway epithelial Na^+ transport. Am J Physiol Lung Cell Mol Physiol 2005;288:L813–L819. [PubMed: 15640288]

Canessa CM, Horisberger JD, Rossier BC. Epithelial sodium channel related to proteins involved in neurodegeneration. Nature 1993;361:467–470. [PubMed: 8381523]

Canessa CM, Schild L, Buell G, Thorens B, Gautschi I, Horisberger JD, Rossier BC. Amiloride-sensitive epithelial Na^+ channel is made of three homologous subunits. Nature 1994;367:463–467. [PubMed: 8107805]

Carattino MD, Sheng S, Bruns JB, Pilewski JM, Hughey RP, Kleyman TR. The epithelial Na^+ channel is inhibited by a peptide derived from proteolytic processing of its alpha subunit. J Biol Chem 2006;281:18901–18907. [PubMed: 16690613]

Caughey GH, Raymond WW, Blount JL, Hau LWT, Pallaoro M, Wolters PJ, Verghese GM. Characterization of human γ-tryptases, novel members of the chromosome 16p mast cell tryptase and prostasin gene families. J Immunol 2000;164:6566–6575. [PubMed: 10843716]

Chang SS, Grunder S, Hanukoglu A, Rosler A, Mathew PM, Hanukoglu I, Schild L, Lu Y, Shimkets RA, Nelson-Williams C, Rossier BC, Lifton RP. Mutations in subunits of the epithelial sodium channel cause salt wasting with hyperkalaemic acidosis, pseudohypoaldosteronism type 1. Nat Genet 1996;12:248–253. [PubMed: 8589714]

Chen LM, Skinner ML, Kauffman SW, Chao J, Chao L, Thaler CD, Chai KX. Prostasin is a glycosylphosphatidylinositol-anchored active serine protease. J Biol Chem 2001;276:21434–21442. [PubMed: 11274175]

Chen LM, Zhang X, Chai KX. Regulation of prostasin expression and function in the prostate. Prostate 2004;59:1–12. [PubMed: 14991861]

Dahlen JR, Jean F, Thomas G, Foster DC, Kisiel W. Inhibition of soluble recombinant furin by human proteinase inhibitor 8. J Biol Chem 1998;273:1851–1854. [PubMed: 9442015]

Donaldson SH, Hirsh A, Li DC, Holloway G, Chao J, Boucher RC, Gabriel SE. Regulation of the epithelial sodium channel by serine proteases in human airways. J Biol Chem 2002;277:8338–8345. [PubMed: 11756432]

Dotto GP. Signal transduction pathways controlling the switch between keratinocyte growth and differentiation. Crit Rev Oral Biol Med 1999;10:442–457. [PubMed: 10634582]

Duc C, Farman N, Canessa CM, Bonvalet JP, Rossier BC. Cell-specific expression of epithelial sodium channel alpha, beta, and gamma subunits in aldosterone-responsive epithelia from the rat: Localization by *in situ* hybridization and immunocytochemistry. J Cell Biol 1994;127:1907–1921. [PubMed: 7806569]

Ecelbarger CA, Kim GH, Terris J, Masilamani S, Mitchell C, Reyes I, Verbalis JG, Knepper MA. Vasopressin-mediated regulation of epithelial sodium channel abundance in rat kidney. Am J Physiol Renal Physiol 2000;279:F46–F53. [PubMed: 10894786]

Ekholm E, Egelrud T. The expression of stratum corneum chymotryptic enzyme in human anagen hair follicles: Further evidence for its involvement in desquamation-like processes. Br J Dermatol 1998;139:585–590. [PubMed: 9892900]

Fan B, Wu TD, Li W, Kirchhofer D. Identification of hepatocyte growth factor activator inhibitor-1B as a potential physiological inhibitor of prostasin. J Biol Chem 2005;280:34513–34520. [PubMed: 16103126]

Farman N, Talbot CR, Boucher R, Fay M, Canessa C, Rossier B, Bonvalet JP. Noncoordinated expression of alpha-, beta-, and gamma-subunit mRNAs of epithelial Na^+ channel along rat respiratory tract. Am J Physiol 1997;272:C131–C141. [PubMed: 9038819]

Fukushima K, Naito H, Funayama Y, Yonezawa H, Haneda S, Shibata C, Sasaki I. *In vivo* induction of prostasin mRNA in colonic epithelial cells by dietary sodium depletion and aldosterone infusion in rats. J Gastroenterol 2004;39:940–947. [PubMed: 15549446]

Greig ER, Baker EH, Mathialahan T, Boot-Handford RP, Sandle GI. Segmental variability of ENaC subunit expression in rat colon during dietary sodium depletion. Pflugers Arch 2002;444:476–483. [PubMed: 12136266]

Guipponi M, Vuagniaux G, Wattenhofer M, Shibuya K, Vazquez M, Dougherty L, Scamuffa N, Guida E, Okui M, Rossier C, Hancock M, Buchet K, et al. The transmembrane serine protease (TMPRSS3) mutated in deafness DFNB8/10 activates the epithelial sodium channel (ENaC) *in vitro*. Hum Mol Genet 2002;11:2829–2836. [PubMed: 12393794]

Guitard M, Leyvraz C, Hummler E. A nonconventional look at ionic fluxes in the skin: Lessons from genetically modified mice. News Physiol Sci 2004;19:75–79. [PubMed: 15016907]

Halevy J, Budinger ME, Hayslett JP, Binder HJ. Role of aldosterone in the regulation of sodium and chloride transport in the distal colon of sodium-depleted rats. Gastroenterology 1986;91:1227–1233. [PubMed: 3758615]

Hirsh AJ, Sabater JR, Zamurs A, Smith RT, Paradiso AM, Hopkins S, Abraham WM, Boucher RC. Evaluation of second generation amiloride analogs as therapy for cystic fibrosis lung disease. J Pharmacol Exp Ther 2004;311:929–938. [PubMed: 15273255]

Huang P, Gilmore E, Kultgen P, Barnes P, Milgram S, Stutts MJ. Local regulation of cystic fibrosis transmembrane regulator and epithelial sodium channel in airway epithelium. Proc Am Thorac Soc 2004;1:33–37. [PubMed: 16113409]

Hughey RP, Mueller GM, Bruns JB, Kinlough CL, Poland PA, Harkleroad KL, Carattino MD, Kleyman TR. Maturation of the epithelial Na^+ channel involves proteolytic processing of the alpha- and gamma-subunits. J Biol Chem 2003;278:37073–37082. [PubMed: 12871941]

Hughey RP, Bruns JB, Kinlough CL, Harkleroad KL, Tong Q, Carattino MD, Johnson JP, Stockand JD, Kleyman TR. Epithelial sodium channels are activated by furin-dependent proteolysis. J Biol Chem 2004a;279:18111–18114. [PubMed: 15007080]

Hughey RP, Bruns JB, Kinlough CL, Kleyman TR. Distinct pools of epithelial sodium channels are expressed at the plasma membrane. J Biol Chem 2004b;279:48491–48494. [PubMed: 15466477]

Hummler E, Barker P, Gatzy J, Beermann F, Verdumo C, Schmidt A, Boucher R, Rossier BC. Early death due to defective neonatal lung liquid clearance in alpha- ENaC-deficient mice. Nat Genet 1996;12:325–328. [PubMed: 8589728]

Johnson MD, Widdicombe JH, Allen L, Barbry P, Dobbs LG. Alveolar epithelial type I cells contain transport proteins and transport sodium, supporting an active role for type I cells in regulation of lung liquid homeostasis. Proc Natl Acad Sci USA 2002;99:1966–1971. [PubMed: 11842214]

Knowles MR, Stutts MJ, Yankaskas JR, Gatzy JT, Boucher RC Jr. Abnormal respiratory epithelial ion transport in cystic fibrosis. Clin Chest Med 1986;7:285–297. [PubMed: 3522073]

Konig J, Schreiber R, Voelcker T, Mall M, Kunzelmann K. The cystic fibrosis transmembrane conductance regulator (CFTR) inhibits ENaC through an increase in the intracellular Cl- concentration. EMBO Rep 2001;2:1047–1051. [PubMed: 11606421]

Lee YJ, Park D, Kim SY, Park WJ. Pathogenic mutations but not polymorphisms in congenital and childhood onset autosomal recessive deafness disrupt the proteolytic activity of TMPRSS3. J Med Genet 2003;40:629–631. [PubMed: 12920079]

Leyvraz C, Charles RP, Rubera I, Guitard M, Rotman S, Breiden B, Sandhoff K, Hummler E. The epidermal barrier function is dependent on the serine protease CAP1/Prss8. J Cell Biol 2005;170:487–496. [PubMed: 16061697]

List K, Haudenschild CC, Szabo R, Chen W, Wahl SM, Swaim W, Engelholm LH, Behrendt N, Bugge TH. Matriptase/MT-SP1 is required for postnatal survival, epidermal barrier function, hair follicle development, and thymic homeostasis. Oncogene 2002;21:3765–3779. [PubMed: 12032844]

Liu L, Hering-Smith KS, Schiro FR, Hamm LL. Serine protease activity in M-1 cortical collecting duct cells. Hypertension 2002;39:860–864. [PubMed: 11967240]

Loffing J, Summa V, Zecevic M, Verrey F. Mediators of aldosterone action in the renal tubule. Curr Opin Nephrol Hypertens 2001;10:667–675. [PubMed: 11496063]

Mall M, Bleich M, Greger R, Schreiber R, Kunzelmann K. The amiloride-inhibitable Na^+ conductance is reduced by the cystic fibrosis transmembrane conductance regulator in normal but not in cystic fibrosis airways. J Clin Invest 1998;102:15–21. [PubMed: 9649552]

Mall M, Grubb BR, Harkema JR, O'Neal WK, Boucher RC. Increased airway epithelial Na^+ absorption produces cystic fibrosis-like lung disease in mice. Nat Med 2004;10:487–493. [PubMed: 15077107]

Masilamani S, Kim GH, Mitchell C, Wade JB, Knepper MA. Aldosterone-mediated regulation of ENaC alpha, beta, and gamma subunit proteins in rat kidney. J Clin Invest 1999;104:R19–R23. [PubMed: 10510339]

Matsui H, Randell SH, Peretti SW, Davis CW, Boucher RC. Coordinated clearance of periciliary liquid and mucus from airway surfaces. J Clin Invest 1998;102:1125–1131. [PubMed: 9739046]

Matsui H, Davis CW, Tarran R, Boucher RC. Osmotic water permeabilities of cultured, well-differentiated normal and cystic fibrosis airway epithelia. J Clin Invest 2000;105:1419–1427. [PubMed: 10811849]

Matthay MA, Landolt CC, Staub NC. Differential liquid and protein clearance from the alveoli of anesthetized sheep. J Appl Physiol 1982;53:96–104. [PubMed: 7118651]

Matthay MA, Folkesson HG, Clerici C. Lung epithelial fluid transport and the resolution of pulmonary edema. Physiol Rev 2002;82:569–600. [PubMed: 12087129]

Mauro T, Guitard M, Behne M, Oda Y, Crumrine D, Komuves L, Rassner U, Elias PM, Hummler E. The ENaC channel is required for normal epidermal differentiation. J Invest Dermatol 2002;118:589–594. [PubMed: 11918703]

Nakhoul NL, Hering-Smith KS, Gambala CT, Hamm LL. Regulation of sodium transport in M-1 cells. Am J Physiol 1998;275:F998–F1007. [PubMed: 9843918]

Narikiyo T, Kitamura K, Adachi M, Miyoshi T, Iwashita K, Shiraishi N, Nonoguchi H, Chen LM, Chai KX, Chao J, Tomita K. Regulation of prostasin by aldosterone in the kidney. J Clin Invest 2002;109:401–408. [PubMed: 11828000]

Oda Y, Imanzahrai A, Kwong A, Komuves L, Elias PM, Largman C, Mauro T. Epithelial sodium channels are upregulated during epidermal differentiation. J Invest Dermatol 1999;113:796–801. [PubMed: 10571736]

Olivieri O, Castagna A, Guarini P, Chiecchi L, Sabaini G, Pizzolo F, Corrocher R, Righetti PG. Urinary prostasin: A candidate marker of epithelial sodium channel activation in humans. Hypertension 2005;46:683–688. [PubMed: 16172430]

Pitkanen OM, Smith D, O'Brodovich H, Otulakowski G. Expression of alpha-, beta-, and gamma-hENaC mRNA in the human nasal, bronchial, and distal lung epithelium. Am J Respir Crit Care Med 2001;163:273–276. [PubMed: 11208656]

Planes C, Blot-Chabaud M, Matthay MA, Couette S, Uchida T, Clerici C. Hypoxia and beta 2-agonists regulate cell surface expression of the epithelial sodium channel in native alveolar epithelial cells. J Biol Chem 2002;277:47318–47324. [PubMed: 12372821]

Planes C, Leyvraz C, Uchida T, Angelova MA, Vuagniaux G, Hummler E, Matthay M, Clerici C, Rossier B. *In vitro* and *in vivo* regulation of transepithelial lung alveolar sodium transport by serine proteases. Am J Physiol Lung Cell Mol Physiol 2005;288:L1099–L1109. [PubMed: 15681398]

Renard S, Voilley N, Bassilana F, Lazdunski M, Barbry P. Localization and regulation by steroids of the alpha, beta and gamma subunits of the amiloride-sensitive Na^+ channel in colon, lung and kidney. Pflugers Arch 1995;430:299–307. [PubMed: 7491252]

Resing KA, Thulin C, Whiting K, al-Alawi N, Mostad S. Characterization of profilaggrin endoproteinase 1. A regulated cytoplasmic endoproteinase of epidermis. J Biol Chem 1995;270:28193–28198. [PubMed: 7499312]

Rossier BC. The epithelial sodium channel: Activation by membrane-bound serine proteases. Proc Am Thorac Soc 2004;1:4–9. [PubMed: 16113404]

Roudier-Pujol C, Rochat A, Escoubet B, Eugene E, Barrandon Y, Bonvalet JP, Farman N. Differential expression of epithelial sodium channel subunit mRNAs in rat skin. J Cell Sci 1996;109(Pt 2):379–385. [PubMed: 8838661]

Rowe SM, Miller S, Sorscher EJ. Cystic fibrosis. N Engl J Med 2005;352:1992–2001. [PubMed: 15888700]

Sheng S, Carattino MD, Bruns JB, Hughey RP, Kleyman TR. Furin cleavage activates the epithelial Na $^+$ channel by relieving Na^+ self-inhibition. Am J Physiol Renal Physiol 2006;290:F1488–F1496. [PubMed: 16449353]

Shimkets RA, Warnock DG, Bositis CM, Nelson-Williams C, Hansson JH, Schambelan M, Gill JR Jr, Ulick S, Milora RV, Findling JW, Canessa CM, Rossier BC, et al. Liddle's syndrome: Heritable human hypertension caused by mutations in the beta subunit of the epithelial sodium channel. Cell 1994;79:407–414. [PubMed: 7954808]

Shipway A, Danahay H, Williams JA, Tully DC, Backes BJ, Harris JL. Biochemical characterization of prostasin, a channel activating protease. Biochem Biophys Res Commun 2004;324:953–963. [PubMed: 15474520]

Snyder PM. Liddle's syndrome mutations disrupt cAMP-mediated translocation of the epithelial Na(+) channel to the cell surface. J Clin Invest 2000;105:45–53. [PubMed: 10619860]

Snyder PM. Minireview: Regulation of epithelial Na^+ channel trafficking. Endocrinology 2005;146:5079–5085. [PubMed: 16150899]

Stanke F, Becker T, Cuppens H, Kumar V, Cassiman JJ, Jansen S, Radojkovic D, Siebert B, Yarden J, Ussery DW, Wienker TF, Tummler B. The TNFalpha receptor TNFRSF1A and genes encoding the amiloride-sensitive sodium channel ENaC as modulators in cystic fibrosis. Hum Genet 2006;119:331–343. [PubMed: 16463024]

Stutts MJ, Canessa CM, Olsen JC, Hamrick M, Cohn JA, Rossier BC, Boucher RC. CFTR as a cAMP-dependent regulator of sodium channels. Science 1995;269:847–850. [PubMed: 7543698]

Swystun V, Chen L, Factor P, Siroky B, Bell PD, Matalon S. Apical trypsin increases ion transport and resistance by a phospholipase C-dependent rise of $Ca2^+$ Am J Physiol Lung Cell Mol Physiol 2005;288:L820–L830. [PubMed: 15626748]

Takeda Y, Miyamori I, Yoneda T, Iki K, Hatakeyama H, Blair IA, Hsieh FY, Takeda R. Production of aldosterone in isolated rat blood vessels. Hypertension 1995;25:170–173. [PubMed: 7843766]

Tarran R, Grubb BR, Gatzy JT, Davis CW, Boucher RC. The relative roles of passive surface forces and active ion transport in the modulation of airway surface liquid volume and composition. J Gen Physiol 2001;118:223–236. [PubMed: 11479349]

Thomas CP, Itani OA. New insights into epithelial sodium channel function in the kidney: Site of action, regulation by ubiquitin ligases, serum- and glucocorticoid-inducible kinase and proteolysis. Curr Opin Nephrol Hypertens 2004;13:541–548. [PubMed: 15300161]

Tong Z, Illek B, Bhagwandin VJ, Verghese GM, Caughey GH. Prostasin, a membrane-anchored serine peptidase, regulates sodium currents in JME/CF15 cells, a cystic fibrosis airway epithelial cell line. Am J Physiol Lung Cell Mol Physiol 2004;287:L928–L935. [PubMed: 15246975]

Tuyen do G, Kitamura K, Adachi M, Miyoshi T, Wakida N, Nagano J, Nonoguchi H, Tomita K. Inhibition of prostasin expression by TGF-beta1 in renal epithelial cells. Kidney Int 2005;67:193–200. [PubMed: 15610243]

Vallet V, Chraibi A, Gaeggeler HP, Horisberger JD, Rossier BC. An epithelial serine protease activates the amiloride-sensitive sodium channel. Nature 1997;389:607–610. [PubMed: 9335501]

Vallet V, Pfister C, Loffing J, Rossier BC. Cell-surface expression of the channel activating protease xCAP-1 is required for activation of ENaC in the *Xenopus* oocyte. J Am Soc Nephrol 2002;13:588–594. [PubMed: 11856761]

Verghese GM, Tong ZY, Bhagwandin V, Caughey GH. Mouse prostasin gene structure, promoter analysis, and restricted expression in lung and kidney. Am J Respir Cell Mol Biol 2004;30:519–529. [PubMed: 12959947]

Verghese GM, Gutknecht MF, Caughey GH. Prostasin regulates epithelial monolayer function: Cell-specific Gpld1-mediated secretion and functional role for the GP1 anchor. Am J Physiol Cell Physiol. 2006in press

Voilley N, Galibert A, Bassilana F, Renard S, Lingueglia E, Coscoy S, Champigny G, Hofman P, Lazdunski M, Barbry P. The amiloride-sensitive Na^+ channel: From primary structure to function. Comp Biochem Physiol A Physiol 1997;118:193–200. [PubMed: 9366043]

Vuagniaux G, Vallet V, Jaeger NF, Pfister C, Bens M, Farman N, Courtois-Coutry N, Vandewalle A, Rossier BC, Hummler E. Activation of the amiloride-sensitive epithelial sodium channel by the serine protease mCAP1 expressed in a mouse cortical collecting duct cell line. J Am Soc Nephrol 2000;11:828–834. [PubMed: 10770960]

Vuagniaux G, Vallet V, Jaeger NF, Hummler E, Rossier BC. Synergistic activation of ENaC by three membrane-bound channel- activating serine proteases (mCAP1, mCAP2, and mCAP3) and serum- and glucocorticoid-regulated kinase (Sgk1) in *Xenopus* oocytes. J Gen Physiol 2002;120:191–201. [PubMed: 12149280]

Wang C, Chao J, Chao L. Adenovirus-mediated human prostasin gene delivery is linked to increased aldosterone production and hypertension in rats. Am J Physiol Regul Integr Comp Physiol 2003;284:R1031–R1036. [PubMed: 12626364]

Warnock DG. Accessory factors and the regulation of epithelial sodium channel activity. J Clin Invest 1999;103:593. [PubMed: 10074474]

Yu JX, Chao L, Chao J. Prostasin is a novel human serine proteinase from seminal fluid. Purification, tissue distribution, and localization in prostate gland. J Biol Chem 1994;269:18843–18848. [PubMed: 8034638]

Yu JX, Chao L, Chao J. Molecular cloning, tissue-specific expression, and cellular localization of human prostasin mRNA. J Biol Chem 1995;270:13483–13489. [PubMed: 7768952]

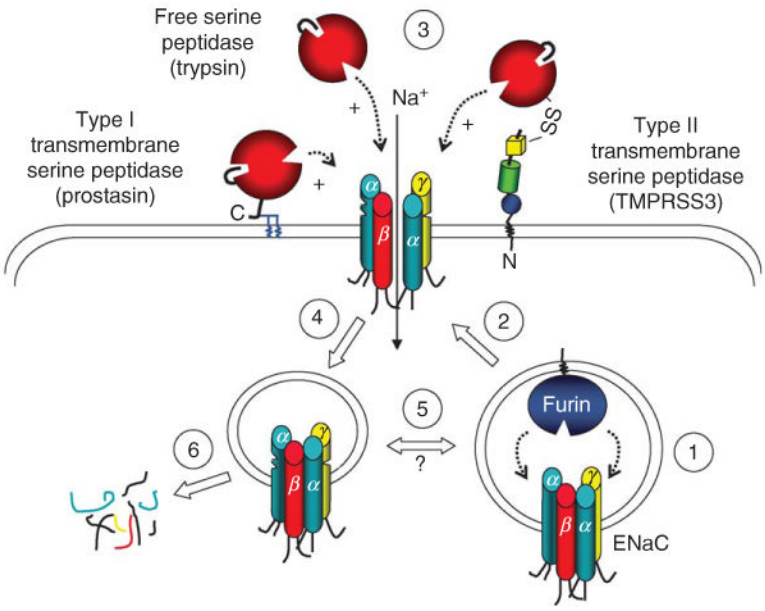

Figure 1.

Proteolytic regulation of ENaC. The ENaC $\alpha\alpha\beta\gamma$ heterotetramer is thought to be assembled initially in the endoplasmic reticulum. The N-terminal and C-terminal ends of each subunit reside in the cytosol. (*Step 1*) Portions of the extracellular domains of α- and γ-subunits are nicked by the transmembrane peptidase furin, probably in the lumen of trans-Golgi network. These intracellular processing events may increase ion transport activity when ENaC is inserted into the plasma membrane (*Step 2*). As shown in *Step 3*, trypsin-family "CAPs" upregulate Na^+ transport via ENaC. These interactions probably are extracellular, as depicted. Known ENaC-activating peptidases include the type I transmembrane peptidase prostasin, shown here attached to the plasma membrane via a GPI lipid anchor. Certain type II transmembrane peptidases, including TMPRSS3 shown here, also activate ENaC. In addition, some free trypsin-like serine peptidases, notably trypsin itself, can increase ENaC-mediated Na^+ transport. The target of these peptidases is not known; it may be ENaC or a protein that regulates ENaC. The amount of functional ENaC on the cell surface can be decreased by endocytic uptake (*Step 4*). A portion of endocytosed ENaC may be subject to further processing and recycled to the membrane (*Step 5*). Otherwise, ENaC in endosomes is likely to be ubiquinated, thereby tagging it for denaturation and total destruction by the peptidases and other proteins associated with the cytosolic proteasome (*Step 6*).

Table I

Candidate Endogenous Serine Peptidase Activators of ENaC

Peptidase (Human)	Aliases	Gene	Location	Membrane Anchor	Inhibitors
Prostasin	CAP1 (frog, mouse)	*PRSS8*	16p11.2	Type I; peptide or lipid/GPI	SPINT2/bikunin SPINT1/HAI-1B PN1
Transmembrane protease, serine 4	CAP2 (mouse)	*TMPRSS4*	11q23.3	Type II; peptide	?
Matriptase	MT-SP1 ST14 Epithin/CAP3 (mouse)	*ST14*	11q24	Type II; peptide	SPINT1/HAI-1B
Transmembrane protease, serine 3		*TMPRSS3*	21q22.3	Type II; peptide	
Furin	PACE	*FUR*	15q25.6	Type I; peptide	Serpin PI8

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