Cutting It Out: ENaC Processing in the Human Nephron

Evan C. Ray* and Thomas R. Kleyman**†
*Department of Medicine and **Department of Cell Biology, University of Pittsburgh, Pittsburgh, Pennsylvania


Studies over the past decade have shed light on how proteases activate the epithelial sodium channel (ENaC). Two ENaC subunits (α and γ) have short stretches of embedded amino acid residues that inhibit the channel. Following protease-dependent cleavage of these subunits at sites flanking these inhibitory tracks, fragments are released and channels are activated.

The serine protease furin is expressed in the trans-Golgi network and processes proteins transiting through the biosynthetic pathway. Furin cleaves the α subunit of ENaC twice at sites flanking an inhibitory track, transitioning channels from a low- to a moderate-activity state. Furin cleaves the γ subunit once, preceding its inhibitory track. Subsequent cleavage by a second protease at a site distal to the γ subunit inhibitory track releases another inhibitory track and transitions ENaC to a high activity state.1,2 A population of channels that have bypassed proteolytic processing has been observed at the cell surface, adding a layer of complexity regarding channel regulation by proteases (for reviews, see Kashlan and Kleyman3 and Kleyman et al.4).

A growing number of proteases have been shown to cleave the γ subunit at sites distal to the inhibitory track, including prostasin, transmembrane protease serine 4 (TMPRSS4), matriptase, kallikrein, plasmin, and elastase. While there is some evidence that kallikrein and prostasin may have a role in activating ENaC in the distal nephron or in other epithelia,5,6 the key proteases responsible for cleaving the γ subunit at a site distal to the inhibitory track under specific pathophysiologic conditions have not been clearly defined. To complicate matters, proteases function in cascades (e.g., clotting factors). Differentiating upstream proteases from those that directly cleave and activate the channel under different states is challenging. In addition, it is likely that we have not identified all of the proteases that can cleave and activate ENaC.

Multiple lines of evidence suggest that ENaC processing and activation is a physiologically relevant phenomenon. In the context of extracellular volume depletion or aldosterone administration in the absence volume depletion, increases in channel expression at the cell surface and proteolytic processing of the α and γ subunits have been described.7,8 Prostasin and/or kallikrein could have a role in cleaving the γ subunit in these settings. In the setting of proteinuric, filtration of plasminogen by damaged glomeruli and its subsequent conversion to plasmin by tubular urokinase may activate ENaC, contributing to the renal sodium retention seen in this setting.9,10

Antibodies directed against the amino- or carboxyl-termini of ENaC subunits can readily differentiate cleaved from non-cleaved ENaC subunits.11 The challenge is demonstrating that a subunit has been cleaved twice, shedding an inhibitory track.10,12 In this issue of JASN, Zachar et al. address important questions regarding the proteolytic processing of the γ subunit in human kidney.13 Specifically, they examined whether γ subunit processing in human kidney is affected by the administration of an angiotensin-converting enzyme inhibitor, angiotensin-receptor blocker, or a diuretic, and whether proteolytic processing is altered in the setting of nephrotic syndrome.

Two antibodies were used to answer these questions. One antibody, directed against the γ subunit inhibitory track, was previously described by Svenningsen et al.14 Zachar et al. generated a new antibody that is directed against residues immediately following the prostasin/kallikrein cleavage site. Surprisingly, this antibody appears to require cleavage at the prostasin/kallikrein site in order to recognize its epitope.

The authors asked whether individuals on diuretics exhibited altered γ subunit expression or proteolytic cleavage. Increases in full-length and presumably furin-cleaved γ subunits were noted in individuals on diuretics compared with controls. The ratio of furin-cleaved to full-length γ subunit was unchanged, suggesting that diuretics did not alter the extent of furin-dependent channel processing. Individuals receiving an angiotensin-converting enzyme inhibitor or angiotensin-receptor blocker had levels of expression of full-length and presumably furin-cleaved γ subunits that were similar to controls. Evidence of cleavage at the prostasin/kallikrein site, corresponding to a channel-activating event, was noted in some individuals receiving these medications, while absent in controls. The increased γ subunit expression in individuals receiving diuretics could reflect relative volume depletion. However, caveats in interpreting this work are that the reason these drugs were administered, and details
Individuals with proteinuria also had more full-length and presumably furin-cleaved γ subunit. A decreased ratio of furin-cleaved to full-length γ subunit was also noted, suggesting that a second processing event had occurred releasing the γ subunit inhibitory track. Evidence of cleavage at the prostasin/kallikrein site was noted in five of six individuals with proteinuria, providing additional support of a second processing event. It is interesting, but not surprising, that the two antibodies localized γ subunits to different sites within principal cells in the distal nephron of kidneys from individuals with proteinuria. While the antibody directed against the inhibitory track showed somewhat diffuse intracellular staining, the antibody recognizing a putatively prostasin/kallikrein-cleaved γ subunit showed punctate staining.

These results provide intriguing clues regarding γ subunit processing in humans under different states. First, there is furin-dependent processing of the γ subunit in the human kidney. Second, under certain conditions there appears to be cleavage at the prostasin/kallikrein site. This is particularly evident in individuals with proteinuria and provides an important clue regarding proteases that cleave the γ subunit and activate the channel in proteinuric states. If plasmin, generated by filtered plasminogen, is primarily responsible for cleaving the γ subunit in this setting, cleavage would occur following a lysine residue that is eight residues distal to the prostasin/kallikrein site.9 The resulting fragment would be unlikely to be recognized by the new antibody used by the authors because it would contain only two of the residues in the antigen used to raise the antibody. A simple test could confirm that the antibody would not recognize a plasmin-cleaved fragment. Given the previous work of Svenningsen et al. showing that plasmin cleaves and activates prostasin,14 the findings of Zachar et al. are consistent with prostasin-dependent cleavage of the γ subunit in proteinuric states.

There are several caveats regarding these findings. First, the prostasin/kallikrein cleavage site consists of a track of four basic residues, and it is likely that other proteases are present in the lumen of the distal nephron and are capable of cleaving the channel at this site. Second, the size of the fragment identified with the new antibody (45 kD) is not consistent with a γ subunit that is simply cleaved at the prostasin/kallikrein site.1 The size of the fragment should be about 5 kD less than the furin cleaved fragment, or about 70 kD. Possible explanations for this discrepancy include additional cleavage at a site preceding the second transmembrane domain, as has been observed by others,15 or that this 45-kD fragment is a cross-reacting polypeptide and not related to the γ subunit. Immunolocalization with this antibody is consistent with the antibody recognizing ENaC. Coimmunoprecipitation studies with this antibody and an antibody directed against the carboxyl terminus of the γ subunit could address the first possibility (the 45-kD fragment should not coimmunoprecipitate). Third, these findings do not exclude the possibility that a component of the γ subunit in proteinuric states is cleaved by plasmin at the lysine residue mentioned above. The antibodies used by the authors would likely not detect a plasmin-cleaved fragment. Fourth, on the basis of previous work it is difficult to explain how the 37-kDa fragment recognized by the antibody against the inhibitory track was generated. This is not consistent with a single cleavage at or in close proximity to the prostasin/kallikrein site.11 In summary, Zachar et al. have generated a novel antibody and presented new findings regarding human kidney γ subunit processing in the setting of proteinuria. Their work also raises new questions.

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DISCLOSURES

None.

REFERENCES


