JCI The Journal of Clinical Investigation

Accessory factors and the regulation of epithelial sodium channel activity

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J Clin Invest. 1999;103(5):593-593. https://doi.org/10.1172/JCI6452.

Commentary

The contribution in this issue of the JCI by Abriel et al. (1) brings into sharp focus our expanding knowledge of the regulation of epithelial sodium channel (ENaC) activity by specific accessory proteins. It has long been recognized that interactions with cytoskeletal proteins, such as actin, can regulate ENaC in a number of model systems (2). The interactions with defined cytosolic regions of the ENaC subunits were demonstrated by earlier studies of the ubiquitin ligase Nedd4 by Staub et al. (3). The importance of this interaction was emphasized by the finding that the site of interaction was with specific proline-rich regions of the subunits and that these very sites were found to be mutated, or even missing, in patients with Liddle's syndrome (4). This autosomal dominant syndrome is a rare cause of human hypertension that has clearly been shown to result from the failure to properly regulate ENaC expression and activity, with volume-expanded low-renin hypertension as the direct consequence. It appears that Nedd4 negatively modulates ENaC activity; binding of its WW domains to the proline domains of ENaC is followed by ubiquitination of the channel subunits, with subsequent endocytosis and lysosomal degradation. Indeed, ENaC appears to turn over quite rapidly with critical NH2-terminal lysine residues identified as the sites of ubiquitination of the α and γ subunits (5). In the […]

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The contribution in this issue of the *JCI* by Abriel *et al.* (1) brings into sharp focus our expanding knowledge of the regulation of epithelial sodium channel (ENaC) activity by specific accessory proteins. It has long been recognized that interactions with cytoskeletal proteins, such as actin, can regulate ENaC in a number of model systems (2). The interactions with defined cytosolic regions of the ENaC subunits were demonstrated by earlier studies of the ubiquitin ligase Nedd4 by Staub *et al.* (3). The importance of this interaction was emphasized by the finding that the site of interaction was with specific proline-rich regions of the subunits and that these very sites were found to be mutated, or even missing, in patients with Liddle's syndrome (4). This autosomal dominant syndrome is a rare cause of human hypertension that has clearly been shown to result from the failure to properly regulate ENaC expression and activity, with volume-expanded low-renin hypertension as the direct consequence.

It appears that Nedd4 negatively modulates ENaC activity; binding of its WW domains to the proline domains of ENaC is followed by ubiquitination of the channel subunits, with subsequent endocytosis and lysosomal degradation. Indeed, ENaC appears to turn over quite rapidly with critical NH_2-terminal lysine residues identified as the sites of ubiquitination of the α and γ subunits (5). In the current studies, the *Xenopus* oocyte expression system was used to demonstrate that overexpression of Nedd4 with ENaC inhibited channel activity. This effect was critically dependent on the proline domains in the ENaC subunits and on intact ubiquitination activity of the Nedd4 protein (1). Of note, a catalytically inactive Nedd4 construct appeared to interact competitively with the wild-type Nedd4 protein and actually protected the expressed ENaC from ubiquitination. A similar dependence of the regulatory effect of Nedd4 on its COOH-terminal ubiquitin ligase domain was recently demonstrated by Goulet *et al.* (6). These changes in ENaC activity can be rationalized in terms of changes in the surface expression of the channel complex, consistent with the role of the Nedd4 protein in endocytosis and degradation of the assembled ENaC complex at the surface membrane.

While these effects are clearly explained by changes in the surface expression the ENaC complexes, there also appear to be direct kinetic effects of the various mutations described in Liddle's syndrome on the apparent open probability of the expressed ENaC (7). Certainly, endocytosis plays an important role in determining channel density or dwell time in the surface membrane, but other factors may also affect the rate of endocytosis (8), and even exocytosis, of the assembled channel complex to the surface membrane (9, 10). Other factors, including K-Ras2A, a small G protein that may in fact be one of the long-sought aldosterone-induced proteins (11), and an ENaC-associated serine protease termed channel activating protein-1 (12) can activate ENaC activity independently of changes in its surface expression. In fact, K-Ras2A markedly increases in ENaC activity despite a decrease in surface expression (11), while we find that syntaxin 1A increases ENaC surface expression but decreases functional ENaC activity (9).

This ensemble of studies has revealed important themes and details of the short-term regulation of ENaC activity. Although these studies have relied heavily on the *Xenopus* oocyte expression system, it can be expected that these results will provide novel approaches to the understanding, and even therapy, of human disorders of ENaC regulation. As the panoply of accessory proteins and factors unfolds, each provides a new candidate for the exploration of the functional regulation of ENaC activity, and even potential genetic linkage approaches to defined subsets of human low-renin hypertension.

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