A potential second ion permeability barrier of the epithelial Na\(^+\) channel

Focus on “Point mutations in the post-M2 region of human α-ENaC regulate cation selectivity”

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Amiloride-sensitive Na\(^+\) channels constitute a new class of superfamily of ion channel proteins known as the degenerin/epithelial Na\(^+\) channel (Deg/ENaC) superfamily (1, 2). This superfamily is rapidly expanding with new members identified from vertebrates and invertebrates. These channels are ubiquitous in nature and have been shown to be expressed in a variety of epithelial and nonepithelial cell types (1, 2). These ion channels play a vital role in Na\(^+\) homeostasis and extracellular volume control. Derangement of these proteins has been implicated in the pathophysiology of a number of human genetic diseases, e.g., Liddle’s syndrome (salt-sensitive hypertension) and PHA-1 (pseudohypoaldosteronism type I) (1, 2). Interactions of ENaC with CFTR (cystic fibrosis transmembrane conductance regulator) have been implicated in yet another human genetic disease, cystic fibrosis (2).

Among various members of the Deg/ENaC superfamily, ENaC represents the most characterized prototype member (1, 2). Epithelial Na\(^+\) channels exhibit high selectivity for Na\(^+\) and Li\(^+\), inhibition by submicromolar concentrations of amiloride, small conductance, and voltage-independent slow kinetics (1, 2). ENaCs are expressed at the apical membrane of cells in many epithelial tissues, e.g., colon, lung, distal nephron, and secretory glands (1, 2). Because of the critical role of these ion channels in the physiology as well as pathophysiology of various human diseases, recent studies have intensely focused on elucidating the structural determinants of ion conductance-function relationship of these proteins. ENaCs have been shown to be composed of three nonidentical but homologous subunits: α, β, and γ. Each subunit has two membrane-spanning hydrophobic domains (M1 and M2), a large extracellular domain, and cytoplasmic NH\(_2\)- and COOH-terminal domains (1, 2). For the stoichiometry of ENaC or other members of the Deg/ENaC superfamily, two models have been proposed: 1) one composed of nine subunits (α\(_3\), β\(_3\), and γ\(_3\)) (3 or 2) one composed of four subunits (α\(_2\), β\(_1\), and γ\(_1\)) (9). Studies of the expression of the subunits of ENaCs, α, β, and γ, mainly in the Xenopus oocyte expression system, have shown that although heterologous expression of the α-subunit alone was sufficient for the ion conductance and amiloride inhibition characteristics of ENaCs, the expression of all three subunits was, however, required for the optimal targeting and functioning of these channels (2). On the basis of the above findings, it has been proposed that the α-subunit of the ENaC constitutes the main conductive moiety or pore region of the multimeric ENaC, whereas the β- and γ-subunits are auxiliary proteins that enhance the function of the ion channel (2). However, the detailed characteristics of the conductive pore of ENaCs have not been defined. Therefore, a number of recent studies have focused on elucidating the structural determinants of ion conductance, selectivity, and amiloride inhibition of the ENaC α-subunit (4, 7, 8, 12, 13). The current article in focus (Ref. 6; see page C64 in this issue) represents a significant contribution from a group of investigators who for many years have been focusing on molecular characterization of the epithelial Na\(^+\) channels. Their study provides strong evidence that a cation has to interact with at least two selectivity barriers in a series during its passage through the channel and greatly advances our knowledge with respect to characterization of the channel pore.

The critical function of ENaC in Na\(^+\) absorption depends on its ability to discriminate between Na\(^+\) and other cations (e.g., K\(^+\), Ca\(^{2+}\)) and its inability to conduct anions. In an attempt to characterize this selectivity filter and conductive pore, recent studies utilizing mutagenesis and subsequent functional analysis have indicated that amino acid residues of the hydro-
phobic region (H2) preceding the M2 region of the ENaC may form the selectivity filter of the channel (7, 8, 12, 13). It has been suggested that all three subunits (α, β, and γ) may participate in forming the selectivity filter and pore of the ion channel (7, 12, 13).

Studies with the α-subunit of rat ENaC (α-ENaC) suggested a P-loop model similar to K⁺ channels in which the pre-M2 (H2) region of the channel dips into the membrane, presumably forming the ion pore (11). Additionally, previous studies utilizing α-ENaC splice variants and chimeras indicated that the M2 region was important for channel function (14). Studies from the laboratory of the authors of the current article in focus (6) have recently demonstrated that the M2 region of α-hENaC was critical to channel function (10).

In that study, reversing the negative charges of the three amino acids of the M2 region (E568R, E571R, and D575R) significantly decreased the channel conductance without affecting ion selectivity. In contrast, similar mutation (E108R) of the M1 region of α-hENaC showed no functional consequences (10).

In the current article in focus (6), the authors have identified a novel arginine-rich sequence of conserved positively charged amino acids between residues 586 and 597 of α-hENaC that were identical in α-ENaCs from five different mammalian species but were absent in β- and γ-ENaCs. This arginine-rich region is localized just downstream to the M2 region in the cytoplasmic COOH-terminal domain of the α-hENaC. The current study was designed to test the hypothesis that this arginine-rich domain may comprise part of the inner mouth of the hENaC pore and may play a role in the conduction and/or ion selectivity of this channel. Two adjacent double point mutations of this region were generated, and the functional studies were performed in Xenopus oocytes at both the macroscopic and single-channel level by utilizing various concentrations of Na⁺, Li⁺, and K⁺. The functional analysis of the double point mutants showed significant differences with respect to steady-state kinetics and biophysical properties compared with the wild-type ENaC. The differences were noted in macroscopic current, open probability, apparent equilibrium dissociation constant (Kₘ) and maximal amiloride-sensitive current (Iₘₐₓ) for Na⁺, and ion selectivity. On the basis of these data, the authors have concluded that this region of the positive charge is a novel and important domain for ion permeation, serves as the potential second barrier for the ions moving through the channel, and may belong to the inner mouth of the conduction pore of the channel. The authors concluded that the ion selectivity resides at multiple sites, e.g., pre-H2, H2, proximal portion of the M2, and the arginine-rich post-M2 region. It is possible that the post-M2 regions of the β- and γ-subunits also may be similarly involved in ion selectivity.

In summary, the elegant studies of Ji et al. (6) provide strong evidence for a novel second ion selectivity barrier, presumably at the inner mouth of the channel pore, and raise interesting questions with respect to the role of this region in the other ENaC subunits (β and γ) and their possible contributions to the channel conductance and selectivity filter. Future studies focusing on further characterization of the structure/function of the human ENaC pore region should greatly increase our understanding of the physiology of the ENaC as well as the pathophysiological basis of the malfunctions of these channels in various human diseases.

REFERENCES


