EPITHELIAL SODIUM CHANNELS AND HYPERTENSION

YAN RU SU AND ANIL G. MENON

Department of Molecular Genetics, Biochemistry and Microbiology, University of Cincinnati Medical Center, Cincinnati, Ohio

This paper is available online at http://dmd.aspetjournals.org

ABSTRACT:

Hypertension affects approximately 25% of the population in the United States and is a major risk factor for heart attack, stroke, and kidney failure. It is estimated to cause as many as 25% of all deaths in the United States, particularly for African Americans, in whom the disease is both more common and more severe. Essential hypertension is a multifactorial disorder influenced by both genetic and environmental factors. Physiological studies have shown that the kidneys play an important role in the maintenance of sodium balance, extracellular fluid volume, and long-term control of blood pressure. The sodium transporters in the kidney affect the amount of sodium and water reabsorption in the nephron and thus control extracellular fluid volume and blood pressure. Of the renal sodium transporters, the amiloride-sensitive epithelial sodium channels (ENaC), which are responsible for the rate-limiting step of sodium reabsorption in the distal nephron, are therefore important candidates in the development of hypertension. Moreover, mutations in this channel have been shown to cause a rare form of heritable hypertension (Liddle’s syndrome), and genetic linkage studies show that the β- and γ-subunits are linked to systolic blood pressure. Several polymorphisms have been identified in the β- and γ-subunits of this channel, of which the β-T594M variant is of particular interest. This variant is found in individuals of African American descent and not in Caucasians and may be associated with hypertension in some populations of African descent. Lymphocytes from individuals with this variant channel show an increased sodium conductance in response to cAMP in vitro. Studying the polymorphic variants in the various subunits of ENaC may further our understanding of the mechanisms that underlie sodium balance in mammals. These variants will provide an avenue to identify molecular targets for new diagnostic and therapeutic tools in the clinical treatment of hypertension.

The Kidney Plays a Key Role in the Long-Term Control of Blood Pressure

Among many factors affecting the blood pressure variation, the kidney plays an important role in the long-term regulation of sodium balance, blood volume, and blood pressure via pressure diuresis and pressure natriuresis (Guyton et al., 1972a; Liard et al., 1974). The kidney regulates extracellular fluid volume by tightly controlling sodium balance. The total amount of exchangeable sodium therefore determines both the extracellular fluid volume and blood pressure. Thus, although many systems can influence blood pressure in the short term, the long-term blood pressure setting ultimately depends on renal sodium handling (Guyton et al., 1972b). The sodium transporters in the kidney are responsible for sodium reabsorption and fluid balance and are therefore important candidates for involvement in the development of hypertension.

Sodium reabsorption is carried out by transporters that are present along the nephron. These transporters appear to be localized to specific segments of the nephron (Rosskopf et al., 1993) and mediate the entry of sodium across the apical membrane. These include the Na/H exchanger of the proximal tubule, the Na/K/2Cl cotransporter of the thick ascending limb of Henle, the NaCl cotransporter of the distal convoluted tubule, and the α-, β-, and γ-subunits of the epithelial sodium channel (ENaC$^\gamma$) of the distal tubule and the collecting duct (Fig. 1).

1 Abbreviations used are: ENaC, epithelial sodium channel; PHA-I, pseudohypoaldosteronism type I.
The detection of abundant levels of hormone receptors and other regulatory molecules that control sodium reabsorption in the distal nephron, combined with physiological evidence, strongly suggests that although the bulk reabsorption of sodium is carried out in the proximal tubule of the nephron, the fine control of sodium reabsorption is carried out in the distal nephron and collecting duct. The localization of the epithelial sodium channel makes it an important candidate gene for its involvement in blood pressure control.

The Epithelial Sodium Channel Is an Important Candidate Gene for Blood Pressure Control

The epithelial sodium channel is responsible for the rate-limiting step of sodium reabsorption and thus plays an important role in the maintenance of sodium balance, extracellular fluid volume, and blood pressure. The channel is composed of at least three subunits: \( \alpha \), \( \beta \), and \( \gamma \) (Canessa et al., 1994) (Fig. 2). Each subunit is proposed to have two transmembrane domains, a large extracellular loop and amino and carboxyl termini within the cytoplasm (Rossier et al., 1994). Human and rat genes encoding all three subunits have been identified and cloned (McDonald et al., 1994, 1995; Voilley et al., 1994, 1995). This channel is expressed in the distal nephron, distal colon, salivary glands, sweat glands, and the epithelia of the lung. In the kidney, all three subunits are colocalized in the apical membrane of the cortical collecting ducts and outer medullary collecting ducts (Duc et al., 1994). The \( \alpha \)-subunit appears to be the conducting unit, since expression of the subunit by itself in Xenopus laevis oocytes results in low levels of amiloride-sensitive Na\(^+\) current. The role of the other two subunits is less certain. Neither the \( \beta \)-nor \( \gamma \)-subunit, when expressed alone or together, produced any measurable Na\(^+\) current. However, coexpression with the \( \alpha \)-subunit greatly enhanced the amplitudes of the Na\(^+\) current (Canessa et al., 1994; Schild et al., 1995). These results indicate that the \( \beta \)- and \( \gamma \)-subunits probably have a structural and/or regulatory role in the stabilization and function of the channel. The activity of amiloride-sensitive sodium channels is regulated by aldosterone and vasopressin, hormones that modulate sodium balance and thus control blood volume and blood pressure (Kemendy et al., 1992; Schafer and Hawk, 1992; Pacha et al., 1993).

Genetic Linkage of the Epithelial Sodium Channel with Liddle’s Syndrome, a Rare Autosomal Dominant Form of Hypertension

One of the major breakthroughs in understanding the genetics of hypertension was the demonstration of linkage between the ENaC and a rare form of heritable human hypertension (Liddle’s syndrome) (Shimkets et al., 1994). Deletions and mutations in the C terminus of the \( \beta \)- and \( \gamma \)-subunit of the ENaC have been identified in patients with Liddle’s syndrome. Homozygosity mapping has shown that the genes encoding subunits of ENaC are the disease loci for pseudohyppaldosterone type I (PHA-I), a rare salt-wasting disorder characterized by dehydration, hyperkalemia, metabolic acidosis, and hypotension (Chang et al., 1996). Mutations in all three subunits of ENaC have been found in patients with PHA-I (Schild et al., 1995; Strautnieks et al., 1996). These mutant channels, when expressed in a X. laevis oocyte system, show significant increases (Liddle’s syndrome) or decreases (PHA-I) in amiloride-sensitive sodium current using whole cell measurements (Schild et al., 1995; Firsov et al., 1996; Strautnieks et al., 1996). Liddle’s syndrome and PHA-I therefore serve as an important “proof of principle” showing that altered function of the epithelial sodium channel can directly affect blood pressure (Fig. 3).

The C Terminus of the \( \beta \)- and \( \gamma \)-Subunit of ENaC Are Critical for Regulation of the Channel Activity

In Liddle’s kindred, deletion or truncation of the C terminus of the \( \beta \)- or \( \gamma \)-subunit results in an increased channel activity. Recent studies have shown that the increased channel activity is due to an increase in the number of Na\(^+\) channels expressed on the apical membrane and an increase in open state probability. A proline-rich motif in the C termini of the \( \beta \)- and \( \gamma \)-subunits of ENaC has been identified and shown to be critical for regulation of the channel activity (Hansson, 1995; Schild et al., 1996; Staub et al., 1996). Deletions of the PY motifs in the \( \beta \)- and \( \gamma \)-subunit in Liddle’s patients result in constitutively increased channel activity. In addition, Oh et al. (1995) have studied a series of Liddle’s truncations by expression in X. laevis oocytes and identified another functionally important domain in the C
terminus of the β-subunit at amino acid residues 591 to 596, which includes a putative protein kinase C target site.

Genetic Linkage of the ENaC to Human Essential Hypertension

Affected sibling pair analysis is commonly used to study candidate marker loci for evidence of linkage with hypertension. This approach does not require that specific assumptions be made about the number of loci involved in the expression of the trait and the underlying mode of inheritance. If a genetic locus is linked to a particular phenotype (in this case hypertension), the number of observed alleles shared by the affected siblings would be higher than expected, suggesting a linkage between the candidate locus tested and the trait (hypertension). Genetic linkage of angiotensinogen to essential hypertension has been demonstrated using this method (Jeunemaitre et al., 1992). Several studies (see Table 1) have shown that the β- and γ-subunits of the ENaC are linked to systolic blood pressure (Munroe et al., 1998; Niu et al., 1999; Wong et al., 1999). However, a study with more than 300 affected sibling pairs collected in China failed to show linkage between the β- and γ-subunits of ENaC and hypertension, indicating possible genetic heterogeneity in different ethnic groups for the involvement of ENaC in essential hypertension. In addition, both our preliminary data and that of others do not support the linkage between α-ENaC and hypertension. Note that the power to detect linkage is affected by sample size; a large number of samples is required to either confirm or refute the linkage.

Polymorphisms in the Epithelial Sodium Channel and Essential Hypertension

The demonstration of genetic linkage between a monogenic form of human hypertension, Liddle’s syndrome, and ENaC suggests the physiological involvement of ENaC in blood pressure regulation. In addition to the Liddle’s and PHA-I mutations, several polymorphisms have been identified in the β- and γ-subunits of ENaC. Figure 4 shows a summary of the polymorphisms found in the β- and γ-subunits of ENaC (Su et al., 1996; Persu et al., 1998, 1999).

Among the polymorphisms identified in the β- and γ-subunits of ENaC, β-T594M and β-G442V are only seen in individuals of African origin (Su et al., 1996; Persu et al., 1998). Lymphocytes from patients carrying the β-T594M polymorphism show an increased sodium conductance in response to cAMP. Moreover, the protein kinase C inhibition in the epithelial sodium channel with β-T594M polymorphisms is lost (Cui et al., 1997). Most recently, association of β-T594M polymorphisms with hypertension among blacks in London has been reported (Baker et al., 1998). No association has been found between β-G442V and hypertension.

African Americans have the highest incidence of hypertension in the world, and they often develop end-stage renal disease in the early course of the disease. In addition, 75% of the patients are salt-sensitive, i.e., show a blood pressure increase with dietary salt intake. The physiological significance of the T594M polymorphism could partly explain the high incidence of salt-sensitive hypertension in African Americans.

In summary, the epithelial sodium channel plays a key role in controlling sodium balance and blood pressure. Many mutations identified in this channel have been shown to be involved in blood pressure regulation. The “gain of function mutations” (Liddle’s syndrome mutations) increase channel activity, resulting in excess reabsorption of sodium leading to hypertension, whereas the “loss of function mutations” (PHA-I mutations) decrease channel activity causing salt wasting, dehydration, and hypotension. The evidence of

<table>
<thead>
<tr>
<th>Genes</th>
<th>Markers Used in Study</th>
<th>Number of Sibling Pairs Analyzed</th>
<th>Trait</th>
<th>P Value</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>αENaC</td>
<td>D12S889</td>
<td>61</td>
<td>HTN</td>
<td>0.10</td>
<td>Munroe et al., 1998</td>
</tr>
<tr>
<td>β-γENaC</td>
<td>D16S403</td>
<td>206</td>
<td>SBP</td>
<td>0.0003</td>
<td>Wong et al., 1999</td>
</tr>
<tr>
<td>β-γENaC</td>
<td>D16S420</td>
<td>172</td>
<td>SBP</td>
<td>0.005</td>
<td>Wong, et al., 1999</td>
</tr>
<tr>
<td>β-γENaC</td>
<td>D16S420</td>
<td>63</td>
<td>HTN</td>
<td>0.14</td>
<td>Munroe et al., 1998</td>
</tr>
<tr>
<td>β-γENaC</td>
<td>D16S403</td>
<td>304</td>
<td>HTN</td>
<td>N.S.</td>
<td>Niu et al., 1999</td>
</tr>
<tr>
<td>β-γENaC</td>
<td>D16S403</td>
<td>301</td>
<td>HTN</td>
<td>N.S.</td>
<td>Niu et al., 1999</td>
</tr>
</tbody>
</table>

HTN, hypertension; SBP, systolic blood pressure.
genetic linkage of the β- and γ-subunits to systolic blood pressure strongly suggests the involvement of this channel in the development of essential hypertension in humans. The T594M variant in the African American population is associated with increased sodium content in beta subunit of epithelial sodium channels in black people resident in London. Proc Natl Acad Sci USA 94:9962–9966.


