

The Epithelial Na⁺ Channel as a Determinant of Blood Pressure

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Abstract: The epithelial Na⁺ channel (ENaC) forms the rate limiting step in transepithelial Na⁺ absorption across aldosterone-responsive tissues such as the distal nephron. After more than a decade of investigation it is clear that the mechanisms of ENaC regulation are complex with an array of ENaC-regulatory proteins having been identified. A variety of monogenic syndromes of low renin hypertension have been identified that serve, through different mechanisms, to up-regulate distal tubular ENaC activity. One of the most extensively studied, Liddle's syndrome, results predominantly from mutations in the C-termini of β and γ -ENaC subunits leading to a failure of Nedd4-2 (neuronal precursor cell-expressed and developmentally down-regulated protein 4-2) mediated channel endocytosis and an increased expression of ENaC at the cell surface.

The role of ENaC subunit polymorphisms as determinants of essential hypertension has been investigated in a number of studies. The T594M mutation in the β -subunit of ENaC, for example, has been screened in large study populations and there is conflicting evidence that it co-segregates with blood pressure. Reasons for such controversies are discussed. The authors conclude that ENaC is a pivotal convergence point in blood pressure regulation and that future studies must identify better ways to measure distal tubular ENaC activity and investigate the importance of combination polymorphisms of ENaC subunits and their regulatory proteins as genetic determinants of hypertension.

Key Words: Epithelial Na⁺ channel, hypertension.

EPITHELIAL NA⁺ CHANNELS

The epithelial Na⁺ channel (ENaC), first cloned by Canessa *et al.* in 1993 [1], constitutes the rate limiting step in transepithelial Na⁺ absorption in aldosterone-responsive tissues. It consists of three homologous subunits (α , β , and γ), each possessing two transmembrane domains, intracellular N and C-termini, and a glycosylated extracellular loop, that likely co-assemble as a heterotetramer with the stoichiometry $\alpha_2\beta\gamma$ [2]. In humans the genes encoding the α , β and γ -subunits of ENaC are located, respectively, in chromosome regions 12p13, 16p12 and 16p12 (GenBank accession numbers SCNN1A, SCNN1B and SCNN1G).

The mechanisms that regulate ENaC activity are complex and can involve changes in single channel conductance, channel open probability and the number of channels expressed at the plasma membrane. Amongst the factors known to affect ENaC activity are hormones (aldosterone, anti-diuretic hormone, insulin), intracellular second messengers (cAMP, the K-Ras family of small G-proteins), membrane-bound proteases, ion channels (cystic fibrosis transmembrane conductance regulator), ion concentrations (Na⁺, Ca²⁺, pH) and membrane stretch [3-5].

LIDDLE'S SYNDROME

Liddle's syndrome was originally described in two siblings presenting with features of primary aldosteronism (hypertension, hypokalaemia and alkalosis) but with negligible aldosterone secretion [6]. The clinical symptoms of the disorder were unresponsive to an aldosterone antagonist but

responded to a combination of dietary Na⁺ restriction and the K⁺-sparing diuretic triamterene. Although a large number of mutations responsible for Liddle's syndrome have now been identified, the vast majority localise to the C-terminal domains of either β or γ -ENaC subunits where they serve to either delete or modify a proline-rich PY motif [7, 8]. The PY motifs of β and γ -ENaC were shown by Staub *et al.* [9] to interact with a protein called Nedd4, originally identified in mouse brain as a protein expressed in neuronal precursor cells that is developmentally down-regulated. Subsequent experiments showed that the interaction between ENaC subunits and Nedd4 resulted in the ubiquitination of channels and their endocytosis (Fig. 1), a process prevented by Liddle's mutations [10-12]. It was proposed that the increased ENaC activity characteristic of Liddle's syndrome results from an increased channel number due to a failure of Nedd4 mediated down-regulation.

Over the last seven years the molecular details of ENaC dysfunction in Liddle's syndrome have received much attention. The description above may well prove to be an oversimplification. For example, Kamynina *et al.* [13] showed that mouse cortical collecting duct cells expressed two different Nedd4 isoforms (Nedd4-1 and Nedd4-2) and that only Nedd4-2 was able to regulate ENaC activity. The ability of Nedd4-2, but not Nedd4-1, to negatively regulate ENaC in two other epithelial cell lines was confirmed by Snyder *et al.* [14] using RNA interference techniques.

Kamynina & Staub [15] have proposed that Nedd4-2 is a phosphorylation target for Sgk-1 (serum and glucocorticoid-inducible kinase type 1), a protein kinase originally identified in a differential screen for glucocorticoid-inducible transcripts in a rat mammary tumour cell line. Sgk-1 is induced by aldosterone and stimulates ENaC activity. It is proposed

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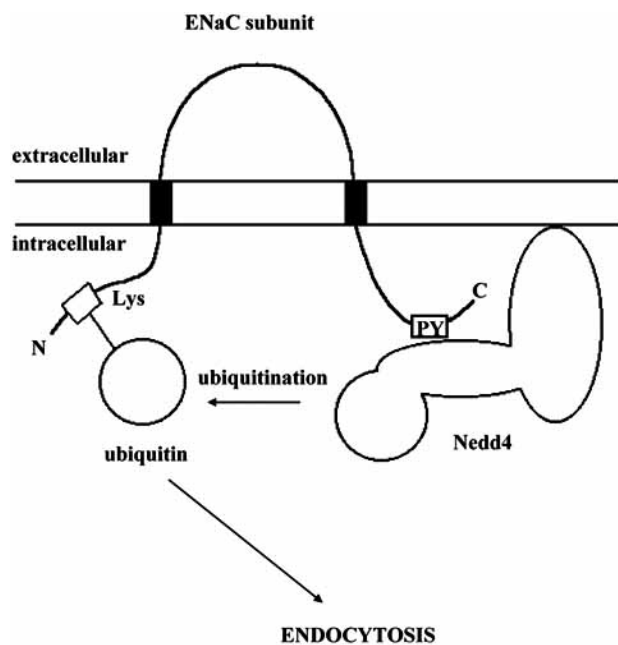


Fig. (1). Proposed mechanism for the regulation of ENaC activity by Nedd4. The Nedd4 protein has distinct functional domains: a Ca^{2+} sensitive lipid binding domain that interacts with the plasma membrane, a series of WW domains that mediate interactions with the PY motifs of ENaC subunits and a ubiquitin protein ligase domain. Binding of Nedd4 to ENaC results in N-terminal ubiquitination of Lys residues in the α and γ subunits of ENaC. For simplification, the diagram suggests that Nedd4 binding results in ubiquitination of the same subunit although there is no evidence for this at present. Ubiquitination then acts as a signal for endocytosis.

that Sgk-1 mediated phosphorylation of Nedd4-2 weakens the interaction between ENaC and Nedd4-2 reducing ENaC ubiquitination and resulting in the accumulation of ENaC at the cell surface.

Recently a novel type of Liddle's mutation has been identified. Hiltunen *et al.* [16] identified a 25 year old male with the full Liddle's phenotype possessing a substitution mutation, serine for asparagine, at codon 530 of the γ -ENaC subunit. This mutation is unusual in that it resides in the extracellular domain of the channel subunit rather than the C-terminus. When the mutant channel was expressed in oocytes a two-fold increase in ENaC activity was observed, compared with the wild type, without a change in cell surface expression. It was suggested that the abnormally high Na^+ reabsorption in this individual resulted from increased channel open probability. Other authors have suggested that Liddle's mutations in the C-termini of β and γ -ENaC increase channel activity at least in part due to an increase in channel open probability. Ismailov *et al.* [17] showed that C-terminal truncation mutants of β and γ -ENaC, which resemble the Liddle's channel phenotype, have a higher open probability when expressed in lipid bilayers. They proposed that the C-terminus acts as an intrinsic inhibitor of channel opening.

As new Liddle's mutations are identified the complexities of ENaC regulation have become increasingly apparent.

OTHER MONOGENIC FORMS OF LOW RENIN HYPERTENSION

Although the monogenic forms of low renin (salt-sensitive) hypertension are rare, they are of interest because they have assisted in elucidating the complex mechanisms of distal tubular salt handling and ENaC regulation. Monogenic syndromes leading to low renin hypertension include, in addition to Liddle's syndrome, glucocorticoid remediable aldosteronism (GRA), familial hyperaldosteronism type II, apparent mineralocorticoid excess (AME), the syndrome of hypertension exacerbated in pregnancy, and deficiencies of steroid 11β -hydroxylase and steroid 17α -hydroxylase [18].

GRA (or familial hyperaldosteronism type I) results from a chimeric gene duplication, between the 11β -hydroxylase and aldosterone synthase genes, leading to ectopic aldosterone synthesis in the cortisol-secreting zona fasciculata of the adrenal gland under the control of adrenocorticotrophic hormone (ACTH). Affected individuals typically develop hypertension in youth which is refractory to standard anti-hypertensive treatment but responds to ACTH suppression with dexamethasone [19]. Familial hyperaldosteronism type II is another autosomal dominant form of hyperaldosteronism but differs in that aldosterone hypersecretion is not suppressible by dexamethasone. The genetic cause is presently unknown, but a genome-wide search has revealed that the disorder is linked with a locus in chromosome region 7p22 [20].

AME is a syndrome that results from defective 11β -hydroxysteroid dehydrogenase type 2 (11β -HSD2). This enzyme is co-expressed with the mineralocorticoid receptor in the kidney and converts cortisol to its inactive metabolite cortisone. Its deficiency allows unmetabolised cortisol to bind to the mineralocorticoid receptor inducing sodium retention, hypokalaemia, and hypertension. Mutations in the gene encoding 11β -HSD2 account for the inherited form of the disease, but a similar clinical picture occurs following the ingestion of competitive inhibitors of 11β -HSD2 such as bioflavonoids, liquorice and carbenoxolone [21].

The syndrome of hypertension exacerbated in pregnancy is a rare autosomal dominant condition characterised by severe hypertension with suppressed renin and aldosterone levels. The striking feature is a severe exacerbation of hypertension and hypokalaemia during pregnancy. The disease is caused by an activating mutation in the gene encoding the mineralocorticoid receptor, which allows progesterone and other mineralocorticoid antagonists to function as agonists [18].

11β -hydroxylase deficiency is the second most common form of congenital adrenal hyperplasia and is responsible for nearly 5 % of cases. The enzyme deficiency results in impaired conversion of 11 -deoxycortisol to cortisol. Due to the accumulation of mineralocorticoids, approximately 50% of patients develop hypertension. In addition cortisol precursors are diverted through the $17,20$ -lyase pathway resulting in androgen excess and varying degrees of genital ambiguity in females [22]. The much rarer genetic disorder of 17α -hydroxylase deficiency also results in hypertension through mineralocorticoid accumulation. However, the lack of $17,20$ -

lyase activity prevents androgen synthesis resulting in undervirilisation in males and failure of spontaneous pubertal development in females [23].

EPITHELIAL NA⁺ CHANNEL SUBUNIT POLYMORPHISMS IN ESSENTIAL HYPERTENSION

Although the full Liddle's phenotype is rare, the condition has aroused speculation that genetic polymorphisms in ENaC subunit genes contribute to the aetiology of essential hypertension.

T594M

The T594M mutation in the β -subunit of ENaC and its relationship with essential hypertension has been extensively studied. The mutation was first described by Jackson *et al.* [24] in a kindred with at least four affected members suffering from Liddle's syndrome. DNA sequencing revealed insertion of an additional cytosine between codons 593 and 595 resulting in a sequence frame shift predicted to produce a protein truncated by 34 amino acids. In a study by Persu *et al.* [25] analysing β -ENaC polymorphisms in essential hypertension in 525 subjects (475 whites, 50 Afro-Caribbeans) seven amino acid changes were detected including T594M. The frequency of genetic variants in β -ENaC was approximately 1 % in the white population but reached 44 % in patients of African origin. The major part of this high prevalence in the African group was due to 2 frequent polymorphisms: T594M (6 %) and G442V (36 %).

In a case control study of 348 black people resident in London the T594M variant was present in 8.3 % of hypertensive participants compared with 2.1 % of normotensive participants [26]. A high proportion of participants with the T594M variant were women. Plasma renin activity was significantly lower in hypertensive participants with the T594M variant compared to untreated hypertensive individuals without the variant. In a further study of the black African population of London β -subunit polymorphisms were analysed in 459 first generation immigrants [27]. The frequencies of the T594M and G442V variants (heterozygotes and homozygotes) were 4.6 % and 27 %, respectively. Whilst the frequency of the T594M variant increased with increasing blood pressure category, being more common in hypertensives than normotensives, the G442V variant did not vary across blood pressure categories. Dong *et al.* [28] have shown in 186 individuals from Ghana that the frequency of the T594M mutation is higher in the indigenous population than in a London-based migrant population, thus implicating this mutation as a significant cause of hypertension in Ghana.

The efficacy of amiloride as an anti-hypertensive in individuals bearing the T594M polymorphism has been investigated in a number of studies. Baker *et al.* [29] showed in 14 such black hypertensive individuals that amiloride alone (at 10 mg BD) controlled blood pressure to the same level as previous combination therapy. Other studies have been less supportive of a role of the T594M polymorphism in essential hypertension. In a large study by Hollier *et al.* [30] including 3137 Dallas County subjects and 1666 Jamaican blacks, the T594M allele was not predictive of systolic blood pressure and amiloride treatment did not lower the blood pressure in

T594M heterozygotes more than in control subjects. They concluded that the T594M allele does not contribute significantly to blood pressure in blacks. Nkeh *et al.* [31] reached a similar conclusion in a study of 1033 South African individuals of African ancestry in which the frequency of the T594M allele was 4.2 % in hypertensive participants and 4.5 % in normotensive participants with no differences in any blood pressure parameter between the two groups.

Other β -ENaC Polymorphisms

The significance of allelic variation in β -ENaC as a cause of essential hypertension in different populations has attracted interest and caused controversy. In a South African study by Rayner *et al.* [32] an R563Q polymorphism in β -ENaC was found in 10 of 139 black hypertensives but was not present in any of 103 black normotensives. The frequency of the mutation was highest in the low renin subgroup. Chang & Fujita [33] sequenced the C-terminal portion of β -ENaC in an unselected cohort of Japanese patients with essential hypertension and were unable to identify significant mutations. In a further Japanese study (the Ohasama study), 803 randomly selected subjects were screened for polymorphisms in exons 8 and 12 of β -ENaC [34]. Although this study revealed a number of novel polymorphisms, none were shown to be associated with hypertension.

γ -ENaC and α -ENaC Polymorphisms

In a study of 215 essential hypertensive patients and 137 normotensive controls Poch *et al.* [35] failed to show a relationship between polymorphisms in codon 649 of γ -ENaC and either blood pressure or salt sensitivity. Persu *et al.* [36] extended their screen to the entire coding sequence of γ -ENaC in a subset of 65 patients with a low renin profile but were unable to implicate a role for γ -ENaC polymorphisms in essential hypertension. In other studies the screening of hypertensive patients with the lowest plasma renin levels has proved fruitful. In such a study Hannila-Handelberg *et al.* [37] identified three ENaC polymorphisms (including γ -ENaC V546I and β -ENaC G589S) in a group of 27 Finnish patients with low renin hypertension. When these variants were screened for in 347 Finnish subjects with treatment-resistant hypertension their prevalence was found to be 9 %, or 3-fold greater than in random normotensive controls.

The relationship between α -ENaC polymorphisms and hypertension has received less attention, presumably owing to the fact that mutations responsible for Liddle's syndrome reside in the β -ENaC and γ -ENaC genes. However, in a study by Iwai *et al.* [38] in a cohort of 3898 Japanese subjects 8 polymorphisms in α -ENaC were identified and investigated for linkage to blood pressure. Interestingly, one particular polymorphism, A(2139)G in the promoter region co-segregated with hypertension and proteinuria. It is of note that previous studies in the Japanese population failed to identify linkage between β -ENaC polymorphisms and hypertension.

WIDER PERSPECTIVES

The above studies demonstrate varying degrees, if any, of co-segregation between ENaC subunit polymorphisms and hypertension. These differences may reflect the power of

individual study designs or true population differences relating to ethnicity, age or plasma renin level. It is unlikely however that studies of single subunit polymorphisms can elucidate the importance of distal tubular ENaC activity as a determinant of blood pressure. The reason lies in the complexity of ENaC regulation which has been eluded too. It is likely that variations in ENaC activity between individuals derive from the additive effect of polymorphisms not only in the ENaC subunits themselves but in the array of ENaC regulatory proteins. This is the likely explanation for the genotype-phenotype mismatch seen with some ENaC subunit mutations. For example heterozygosity for the T594M mutation in β -ENaC was identified by Jackson *et al.* [24] as the cause of Liddle's syndrome in a kindred of four affected members. In other studies this same mutation has failed to co-segregate not only with the full Liddle's phenotype, but with hypertension alone [30].

A few studies have investigated the relationship between blood pressure and polymorphisms in ENaC regulatory proteins. Russo *et al.* [39] typed multiple single nucleotide polymorphisms in the Nedd4L gene on human chromosome 18q21 in a collection of US whites, Greek whites, and African-Americans with essential hypertension. A significant association between several polymorphisms and hypertension was observed in all 3 populations. Von Wöern *et al.* [40] have studied the association between genetic variance in Sgk-1 and blood pressure. A promoter C/T, an intron 6 C/T and an exon 8 C/T polymorphism in the Sgk-1 gene were genotyped in 4830 subjects. Significant differences were observed in diastolic and systolic blood pressure between groups thus identifying Sgk-1 genotypes that confer a risk for hypertension.

The main difficulty in assessing the importance of distal tubular ENaC activity as a determinant of blood pressure is our inability to measure channel function directly *in vivo*. Resultantly a number of studies have addressed the issue indirectly. Baker *et al.* [41] measured the transnasal potential difference in three brothers with genetically proven Liddle's syndrome, their unaffected sister, and 40 normotensive controls. Compared to controls, Liddle's patients had a greater lumen-negative transnasal potential difference and a greater voltage response to topical amiloride. Similar studies in white patients with essential hypertension unexpectedly showed a reduction in the transnasal potential difference response to amiloride compared to controls [42]. Interpretation of such results is difficult as it is an unsafe assumption that the pathways regulating ENaC activity are comparable in the kidney and respiratory epithelia.

Other surrogate markers of ENaC activity include plasma renin suppression, blood pressure response to amiloride and urinary prostaticin [43]. Due to confounding factors their use is limited.

In summary, the evidence is strong that ENaC is a pivotal convergence point in blood pressure regulation. Further studies must now identify better ways to measure distal tubular ENaC activity and investigate the importance of combination polymorphisms of ENaC subunits and their regulatory proteins as genetic determinants of hypertension.

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