Brief Reviews

Epithelial Sodium Channel
Mendelian Versus Essential Hypertension

Bernard C. Rossier, Laurent Schild

Despite large changes in water and salt intake, the kidney is able to maintain the extracellular osmolarity and volume within narrow margins. Such fine control requires specific factors or hormones; among them, aldosterone and vasopressin play key roles. In aldosterone-responsive epithelial cells (kidney, colon), the epithelial sodium channel (ENaC) plays a critical role in the control of sodium balance, blood volume, and blood pressure. Tissue-specific expression of ENaC is observed in sodium transporting epithelia (lung, intestine, kidney, and exocrine and sweat glands). In lung, ENaC has a distinct role in controlling the ionic composition of the air liquid interface and thus the rate of mucusociliary transport. ENaC subunits are found in the aldosterone-sensitive distal nephron (ASDN; Figure), in the surface epithelia of the colon, and in the duct cells of exocrine glands. In human, imbalance of ENaC activity in aldosterone target cells leads to a large variety of pathologies: loss of glands. In human, imbalance of ENaC activity in aldosterone target cells leads to a large variety of pathologies: loss of function of ENaC leads to a severe renal salt loosing syndrome, with a hypotensive phenotype (pseudohypaldosteronism type 1), whereas ENaC gain of function leads to hypertension (Liddle’s syndrome).

The purpose of this brief review is to discuss recent developments pertaining to the role of ENaC in hypertension. We focus on the role of ENaC in Liddle’s syndrome, a mendelian form of hypertension, and essential hypertension.

ENaC and Liddle’s Syndrome

Human Disease

Liddle’s syndrome is an autosomal dominant form of salt-sensitive hypertension, characterized by early onset of severe hypertension, usually hypokalemia, metabolic alkalosis, repressed renin, and aldosterone secretion. In adolescents, salt restriction and amiloride are enough to control the blood pressure. Liddle’s syndrome results from mutations in the cytoplasmic C terminus of either the β- or γ-subunit of ENaC, which lead to constitutively increased channel activity. The mutation of the index case results in the elimination of 45 amino acids from the C terminus of the β-subunit. Missense mutations allowed to map a proline-rich domain as target for Liddle’s mutations in the β- and γ-ENaC subunits. Under physiological circumstances, the proline-rich domain motif binds to Nedd4-2, an ubiquitin E3 ligase protein, which promotes internalization of active ENaC at the cell surface and degradation (Figure). The lack of this repressor activity leads to a retention of active ENaC channels and an abnormally high Na+ reabsorption in the ASDN with its consequence on blood pressure. The molecular mechanism by which Nedd4-2 interacts with ENaC has also been reviewed recently in detail. Liddle’s syndrome is obviously a very rare condition. Fewer than 30 families (or isolated cases) have been so far described, worldwide. New families or sporadic cases have confirmed the critical importance of the C terminus of either β- or γ-subunit. A point mutation in the extracellular domain of the ENaC γ-subunit has been identified in 1 sporadic case in Finland. This Asn530Ser mutation is located in a critical region that controls channel gating.

Mouse Models

To study the pathophysiology of salt sensitivity, a mouse model for Liddle’s syndrome with deletion of the C terminus of βENaC has been generated by Cre/loxP-mediated recombination. Under normal salt diet, mice heterozygous (L/+) and homozygous (L/L) for Liddle mutation (L) develop normally during the first 3 months of life. In these mice, blood pressure is similar to wild-type, despite evidence for increased sodium reabsorption in distal colon and hypervolemia (low plasma aldosterone). Under high salt intake, the Liddle mice develop high blood pressure, metabolic alkalosis, and hypokalemia, accompanied by cardiac and renal hypertrophy. This animal model reproduces to a large extent a human form of salt-sensitive hypertension and establishes a causal relationship between dietary salt, a gene expressed in kidney and hypertension. Investigations of the renal Na+(+) transport ex vivo (intact perfused tubules) and in vitro (primary cultured cortical collecting ducts) clearly established a major dysfunction of the ENaC expressed in the kidney of the Liddle mouse, bringing 3 independent lines of evidence for the constitutive hyperactivity of ENaC in cortical collecting ducts. In addition, measurements of ENaC-mediated Na+ currents by patch clamp in isolated cortical collecting ducts from mice homozygous for the Liddle mutation (L/L) and from wild-type showed that the L/L mice...
had much larger currents when the animals were fed a low-sodium diet or infused with aldosterone. Collectively, the data indicated that mineralocorticoid regulation of ENaC was maintained, even potentiated in the mouse model of Liddle’s syndrome. In the colon,22 aldosterone responsiveness of the ENaC in colon was also increased but may differ from that of the kidney in 2 aspects: ex vivo measurements of colonic tissues show a very significant increase in baseline currents, unlike the ex vivo cortical collecting duct patched tubules. Dysregulation of ENaC in colon may thus also have an impact on sodium balance. This may be pathophysiologically relevant in patients with Liddle’s syndrome, in particular on a high-salt diet, when suppression of plasma aldosterone is relevant in patients with Liddle’s syndrome, in particular on impact on sodium balance. This may be pathophysiologically Dysregulation of ENaC in colon may thus also have an impact on sodium balance. This may be pathophysiologically relevant in patients with Liddle’s syndrome, in particular on a high-salt diet, when suppression of plasma aldosterone is likely to be insufficient to reduce sodium absorption to an appropriate level both in kidney and colon.

ENaC and Essential Hypertension

Genetic diseases with mendelian transmission are often characterized by mutations with rare allelic frequency in human populations (<0.1%), often leading to very severe phenotypes. More frequent mutations (allelic polymorphisms), often single nucleotide polymorphisms (SNPs), have an allelic frequency of >1% or 2% in human populations. In addition to SNPs, other polymorphisms (eg, copy number variations or short tandem repeats) have to be considered. What can be the functional consequence of such polymorphisms? It is presently a key issue in understanding the genetic factors of multifactorial diseases. For the common forms of hypertension, genetic factors account for ≈30% of the variation of blood pressure in human populations.23 The development of hypertension depends therefore on the interaction between genetic factors and risk factors, such as excess weight, lack of physical training, smoking, drugs (ie, contraceptive pill), and salt intake.2 Many genes now have been associated with blood pressure control or essential hypertension: ENaC,25 11β-hydroxysteroid dehydrogenase type 2,26 angiotensinogen27 and genes on the renin angiotensin aldosterone axis,23,28–30 a subunit of adducin,31 glucagon receptor,32 Gαq,33 Gαs,34 endothelin 2,35 and α2 adrenergic receptor.36 Most of these genes are expressed in the kidney. They all may participate as candidate genes in the salt-sensitive phenotype. However, in most cases, the studies demonstrating positive associations correspond to an equal or larger number of studies showing lack of association with a specific polymorphism.23,30,37,38 We review the most recent and important studies in the field and discuss their potential interest and pitfalls.

Association Studies on ENaC and Associated Proteins

αENaC

A multicentric case-control study characterized the impact of αENaC polymorphisms (SNPs Trp493Arg and Ala663Thr) on the risk of ischemic cerebrovascular events.39 Carriers of 493Arg had a 1.78-fold increased risk for ischemic cerebrovascular events compared with Trp/Trp carriers. This study has not yet been duplicated and may experience the same difficulties as described above. One additional difficulty is to assess the functional effect of SNPs identified in the coding region of the protein. Recently, activity of wild-type (A334, C618, and A663) and polymorphic human αENaC expressed in Chinese hamster ovary cells was assessed with patch-clamp electrophysiology.40 The C618F and A663T polymorphisms have been reported to elevate ENaC activity, although this increase in channel activity remains small and less reproducible compared with ENaC gain of function in Liddle’s syndrome.

βENaC

Two commonly occurring βENaC variants (G589S and a novel intronic i12-17CT substitution) and a novel γENaC variant (V546I) were identified recently in Finland.41 At least 9% of Finnish patients with hypertension, admitted to a specialized center, carry genetic variants of βENaC and γENaC, a 3× higher prevalence than in the normotensive individuals or in random healthy controls. Patients with the variant alleles showed an increased urinary potassium excretion rate in relation to their renin levels.41 No increase in channel activity could be detected when these ENaC variants were expressed in Xenopus oocytes. Thus, the functional relevance of these variants remains uncertain.

A potentially novel polymorphic repetitive region (GT dinucleotide short tandem repeat) was identified recently in intron 8 of βENaC gene (SCNN1B).42 The authors characterized the prevalence and distribution of a polymorphic GT-short tandem repeat in SCNN1B in a Chilean population of hypertensive patients and analyzed the correlation between the different genotypes with 2 intermediate phenotypes: plasma renin activity and serum aldosterone. The main limitation is the size of the samples (133 hypertensive versus 69 normotensive).

Along the same line, association of preeclampsia with the R563Q mutation of the β-subunit of the ENaC was reported.43 The R563Q mutation was found in 7.8% of the women with
pre-eclampsia and in 2.6% of control. Again, the size of the population studied is small (230 test individuals and 198 matched controls).

**γENaC**
A relatively large number of independent studies have provided evidence for linkage between hypertension and quantitative trait loci on chromosome 16p12, a genomic region that encodes β- and γ-subunits.44–47 A recent study48 examined genotypes and haplotypes related to 26 SNPs across SCNN1G and its promoter in a large cohort (>2900 people from the Victorian Family Heart Study). By sampling unrelated subjects within the upper (mean 166 mm Hg) and lower (mean 98 mm Hg) 10% of the systolic pressure distribution, a significant genetic contrast was achieved, leading to the identification of relatively common polymorphisms in the SCNN1G gene that are associated with high systolic blood pressure in the general Australian white population.49,50 The observation that the γ-subunit comes out of many independent and large studies as candidate gene participating in determining systolic blood pressure is interesting. Indeed, the γ-subunit may have a specific role in ENaC activation by a novel mechanism (ie, proteolytic cleavage of its extracellular domain).49,50

**Associated Protein Nedd4-2**
The ubiquitylation–deubiquitylation signaling cascade has emerged a key mechanism for the membrane trafficking from, and to, the plasma membrane.5,6,51 Nedd4-2 is the E3 ubiquitylation enzyme that specifically interacts with the Liddle consensus motif proline-rich domain. As an ENaC-associated protein, it is thus a candidate gene for salt sensitivity or salt resistance and a determinant of blood pressure control. A recent transgenic mouse model harboring a Nedd4-2 gene deletion was shown to develop a severe form of salt-sensitive hypertension, suggesting that Nedd4-2 is a limiting factor in the aldosterone-dependent signaling cascade.52 Fava et al53 reported that 24-hour ambulatory blood pressure monitoring was linked to chromosome 18q21-22, and genetic variation of Nedd4-2 associates with cross-sectional and longitudinal blood pressure in Swedes. In a linkage study of 16 markers (including 2 SNP markers located within the Nedd4-2 gene) on chromosome 18 between 70 and 104 cM and ambulatory blood pressure, in 118 families, the strongest evidence of linkage was found for 24-hour and daytime systolic ambulatory blood pressure monitoring at the Nedd4-2 locus (82.25 cM). In a large population sample (>4000), the authors subsequently showed that a Nedd4-2 gene variant (rs4149601), which by alternative splicing leads to varying expression of a functionally crucial C2 domain, was associated with diastolic blood pressure. A genotype combination of the rs4149601 and an intronic Nedd4-2 marker (rs2288774) was associated with systolic and diastolic blood pressure. A quantitative transmission disequilibrium test in the family material of the rs4149601 supported this Nedd4-2 variant as being at least partially causative of the linkage result.53 Dahlberg et al54 reported that genetic Nedd4-2 variation appeared to affect salt sensitivity and plasma-renin in normotensive subjects, suggesting that genotyping of Nedd4-2 may be clinically useful to identify subjects who benefit from dietary salt restriction in the prevention of hypertension. Interestingly, Fouladkou et al55 provided in vitro evidence in heterologous expression system that the differential phosphorylation status between wild-type and a mutant Nedd4-2 (P355L) could account for their different potentials to inhibit ENaC activity. In a more recent similar study, Araki et al56 found that Nedd4-2 isoform I (generated by SNP rs4149601) may interact with other human isoforms in a dominant-negative fashion. Such interactions might abnormally increase sodium reabsorption, suggesting that the human Nedd4-2 gene, especially the evolutionarily new isoform I, is a candidate gene for hypertension.56

**Perspectives and Conclusions**

**Association Studies Based on a Candidate Gene: A Biased Approach Based on a Physiological Hypothesis**
The candidate gene approach has major limitations in terms of reproducibility for reasons well summarized by Corvol23 and in a recent editorial comment by Tesson and Leenen.57 The latter authors suggested that a genomic unbiased approach should now be favored because by nature, it will reveal known and unknown novel, signaling cascades. As physiologists, we still believe that most of the significant signaling pathways have been established so far by the biased approach. Despite clear limitations to understand blood pressure regulation in hypertension, the identification of the most important limiting factors in a signaling cascade has come clearly from studying mendelian forms of hypertension.58 Loss-of-function mutations in the main salt transporters (NKCC [ie, furosemide receptor], NCC [ie, thiazide receptor], and ENaC [ie, amiloride receptor]) all lead to a severe salt loosing syndrome and hypertensive phenotype. Less severe loss-of-function mutations are expected to be protective against the development of hypertension (by a chronic “genetic” diuretic effect). This has been found to be true in the heterozygote carriers of a Gittelman mutation in a large Amish pedigree, with only one dose of the normal transport protein.59 More recently, rare independent mutations in renal salt handling (NCCT, NKCC2, and ROMK) were clearly shown to contribute to blood pressure variation in the general population (cohort from the Framingham Heart Study).60 As pointed out by the authors, these mutations, all heterozygous and rare, produce clinically significant blood pressure reduction and protect from development of hypertension. Their findings implicate many rare alleles that alter renal salt handling in blood pressure variation in the general population and identify alleles with health benefit that are nonetheless under purifying selection. These findings have implications for the genetic architecture of hypertension and other common complex traits. The candidate gene approach should still be pursued actively. Pseudohypoaldosteronism type 1 and Liddle’s syndrome have contributed greatly to the identifications of the main limiting factors of the aldosterone-dependent signaling cascade: mineralocorticoid receptor, Nedd4-2, and ENaC. The pathway is strongly repressed under standard or high-salt diet.61 Nedd4-2 is a physiological
negative regulator of salt reabsorption. Loss-of-function mutations of negative regulators could also be significant in blood pressure control. Interestingly, the NCC transporter is also under the control of strong negative regulators identified through the discovery of WNK4 and WNK1, genes causing disease of the Gordon syndrome.\textsuperscript{62} No doubt that a better understanding of the aldosterone and the WNKs signaling cascades will bring, in the near future, new candidate genes on the scene.

**Genomewide Association Studies: A Nonbiased Approach**

This approach is presently statistically powerful enough to detect multiple susceptibility variants for common and complex diseases.\textsuperscript{63} For instance, new variants were identified for type 2 diabetes in 2 independent studies.\textsuperscript{64,65} This constitutes proof of principle for the genomewide approach to the elucidation of complex genetic traits. Despite a very large population sampling (14 000 cases versus 3000 shared controls), no significant variants were identified for hypertension.\textsuperscript{63} The reasons for this failure are not yet clear, but 1 important factor is the clinical phenotyping. Type 2 diabetes is a relatively homogenous clinical entity, quantitatively well defined biochemically by measurement of blood glucose. Hypertension is likely to be a more heterogenous clinical entity. A number of confounding factors should be considered: age, gender, posture, activity, salt intake, circadian variation, etc. Large studies are required to stratify the population according to these variables, with special emphasis on salt intake, salt sensitivity, and circadian regulation of sodium balance. Twenty-four ambulatory blood pressure measurements are probably mandatory but difficult to implement on large populations. Day versus night sodium excretion has also been shown to be critical in interpreting circadian variation of blood pressure.\textsuperscript{65,66} The feasibility of such studies is methodologically good but limited by manpower and costs.

**Comparative Genomics**

A number of hypertensive rat strains (DAHL, SABRA, MILANO, spontaneously hypertensive rats, etc) have been characterized and selected for their ability to recapitulate many features of human essential hypertension. The genetic dissection of essential hypertension using a comparative genomic approach has been reviewed recently.\textsuperscript{68} The comparative mapping of blood pressure quantitative trait loci in human and rats may provide useful information to identify the best candidate genes involved in the hypertensive phenotype. The adducin gene nicely illustrates the power of such approach.\textsuperscript{69}

Members of the metabolizing cytochrome P450 family (CYP3A5 and CYP4A10) have been involved in salt-sensitive hypertension.\textsuperscript{70,71} One way to test the role of such a candidate gene is obviously to inactivate it in a transgenic mouse model. The disruption of the murine CYP4A10 gene causes a type of hypertension that is, like most human hypertensions, dietary salt sensitive. CYP4A10−/− mice fed low-salt diets were normotensive but became hypertensive when fed normal or high-salt diets. Hypertensive CYP4A10−/− mice had a dysfunctional kidney ENaC and became normotensive when amiloride was administered.\textsuperscript{70}

**Therapeutic Considerations: Diuretics Revisited**

Beginning in the early sixties, clinical trials documented the long-term benefit of lowering blood pressure. Diuretics, mainly thiazides, were the first antihypertensive drugs to be used on a large scale. Recently, the Antihypertensive and Lipid-Lowering treatment to prevent Heart Attack Trial (ALLHAT) study confirmed the efficacy of thiazide to prevent one of the major forms of cardiovascular diseases.\textsuperscript{72} In the United States, thiazide is preferred as first-step antihypertensive therapy, but this is not accepted by all. Diuretics are nevertheless an almost obligatory component of a biantihypertensive or triantihypertensive therapy, independent of salt sensitivity or salt resistance. Pharmacological and genetic evidence clearly demonstrates that the ASDN is playing a major role in blood pressure control. The interaction between the aldosterone signaling cascade mineralocorticoid receptor-SgK1−Nedd4-2-ENaC with the WNK signaling cascade in DCT2 (where ENaC and NCC are coexpressed) and in connecting tubule (where ENaC and ROMK are coexpressed) is probably the most important nephron segment to control sodium, potassium, and water balance, and ultimately blood pressure.

The prevalence of primary aldosteronism in the general population is debated,\textsuperscript{73,74} but 2 important factors may have been overlooked; one deals with the response of the adrenal gland to salt intake and the other with an increased responsiveness of the target cell. The first factor is that “normal” plasma aldosterone (with a normal target cell response) may contribute to essential hypertension with a greater prevalence, as discussed,\textsuperscript{75−77} the main reason being an inappropriately high aldosterone secretion in response to salt intake. The second factor is that “low” levels of plasma aldosterone (with an abnormal target cell) may inappropriately activate ENaC, as clearly seen in the Liddle’s mouse model in ASDN\textsuperscript{71} and in colon.\textsuperscript{22} Of course, a combination of these 2 factors will be synergistic and detrimental. The use of amiloride or spironolactone alone has been limited by its small natriuretic efficacy and by the fear of side effects (hyperkalemia). Until recently, no prospective studies addressed the question of the efficacy of amiloride (or spironolactone) in combination with thiazide. In a prospective, randomized, placebo-controlled, double-blind clinical trial, treatment with either amiloride or spironolactone provided an additional reduction in blood pressure in black patients already receiving conventional antihypertensive therapy (thiazide and calcium blocker).\textsuperscript{79} It would be highly desirable to extend this kind of study to larger and different populations around the world to definitively validate this protocol. The role of spironolactone in treating resistant hypertension has also been discussed recently in the journal, but it is not yet clear whether the mechanism involves volume- or nonvolume-related effects.\textsuperscript{79,80}

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None.

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