

Negative regulators of sodium transport in the kidney: Key factors in understanding salt-sensitive hypertension?

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J Clin Invest. 2003;111(7):947-950. <https://doi.org/10.1172/JCI18232>.

Commentary

Genetic evidence supporting Guyton's hypothesis: the kidney is a main player in the long-term control of blood pressure. Hypertension is the most common disease of the human population. Both genetic and non-genetic factors are involved and high salt intake has been proposed as a major risk factor. According to the hypothesis put forward by Guyton, over 20 years ago, control of blood pressure in the steady-state and on a long-term basis is critically dependent on renal mechanisms (1). During the last decade, a number of genes expressed in the distal part of the nephron have been shown to be directly involved in the control of blood pressure (2). Mutations in single genes that cause isolated Mendelian syndromes characterized by hypertension or hypotension have been identified and, remarkably, these genes have all turned out to be involved in the control of sodium chloride absorption by the distal nephron (Table 1). The distal nephron (Figure 1) is composed of the distal convoluted tubule, the connecting tubule and various segments of the collecting duct. The fact that these are the terminal segments of the nephron affords them the final word, so to speak, on how much salt is excreted. This underlies their critical importance in the maintenance of salt balance, which in turn controls the volume of the extracellular space and blood pressure. [...]

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Negative regulators of sodium transport in the kidney: Key factors in understanding salt-sensitive hypertension?

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J. Clin. Invest. **111**:947–950 (2003). doi:10.1172/JCI200318232.

Genetic evidence supporting Guyton's hypothesis: the kidney is a main player in the long-term control of blood pressure

Hypertension is the most common disease of the human population. Both genetic and non-genetic factors are involved and high salt intake has been proposed as a major risk factor. According to the hypothesis put forward by Guyton, over 20 years ago, control of blood pressure in the steady-state and on a long-term basis is critically dependent on renal mechanisms (1). During the last decade, a number of genes expressed in the distal part of the nephron have been shown to be directly involved in the control of blood pressure (2). Muta-

tions in single genes that cause isolated Mendelian syndromes characterized by hypertension or hypotension have been identified and, remarkably, these genes have all turned out to be involved in the control of sodium chloride absorption by the distal nephron (Table 1). The distal nephron (Figure 1) is composed of the distal convoluted tubule, the connecting tubule and various segments of the collecting duct. The fact that these are the terminal segments of the nephron affords them the final word, so to speak, on how much salt is excreted. This underlies their critical importance in the maintenance of salt balance, which in turn controls the volume of the extracellular space and blood pressure. The reabsorption of sodium in the distal tubule and the collecting tubules is closely regulated, chiefly by the action of the hormone aldosterone.

WNK kinases repress Na-Cl cotransporter activity

Pseudohypoaldosteronism type II (PHAII) or Gordon syndrome is an autosomal dominant disease, characterized by hypertension and hyper-

kalemia, despite a normal glomerular filtration rate. In addition, plasma renin levels are low, however plasma aldosterone is often in the normal range, but inappropriately low with respect to the observed high level of plasma potassium, a strong stimulus of aldosterone secretion (3, 4). Disease-associated physiological abnormalities can be corrected by administration of thiazide diuretics. The use of the term pseudohypoaldosteronism here is somewhat questionable since salt retention is observed in this syndrome. Hypertension, rather than salt wasting, is also observed. We prefer the term familial hyperkalemic hypertension, which more accurately describes the pathophysiology. The disease is genetically heterogeneous and three loci have been identified on chromosomes 1 (PHA2A), 17 (PHA2B), and 12 (PHA2C). Furthermore, genetic heterogeneity demonstrate the existence of at least a fourth locus (3). Mutations in two members of a novel serine/threonine kinase family named WNK (with no lysine K, i.e. lacking a lysine typical of the catalytic domain of this kinase family) – WNK1 and WNK4 – map to the PHA2A and PHA2B loci, respectively. The WNK1 mutations are large intronic deletions that were postulated to increase gene expression and increase enzymatic activity (4). In contrast, WNK 4 mutations (E562K, D564A, and Q565E) map to a small stretch of amino acids that lies outside the catalytic domain that is fully conserved between WNK1 and WNK4 (4). WNK1 is ubiquitously expressed in many tissues and organs and seems restricted to diverse chloride-transporting epithelia (5). In the kidney, WNK1 is restricted to the aldosterone-sensitive distal nephron

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Conflict of interest: The author has declared that no conflict of interest exists.

Nonstandard abbreviations used: pseudohypoaldosteronism type II (PHAII); with no lysine K (WNK); Na-Cl cotransporter (NCC); epithelial sodium channel (ENaC).

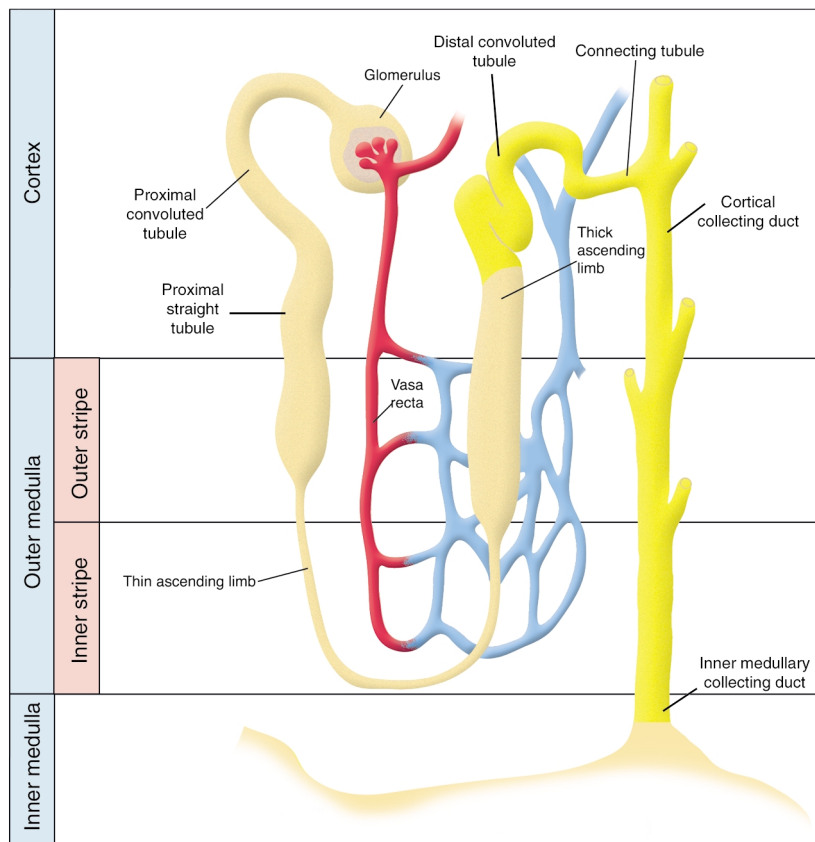


Figure 1
Diagram of the urinary system. The components comprising the aldosterone-sensitive distal nephron are shown in bright yellow.

and is cytoplasmic (Figure 2). WNK4 has a highly restricted expression profile. The protein is detected only in the distal convoluted tubule, connecting tubule and collecting duct (4). In the distal convoluted tubule WNK4 colocalizes with ZO-1, a specific tight junction protein, whereas in the cortical collecting duct WNK4 expression is mostly cytoplasmic, suggesting a specific function along the nephron axis (Figure 2).

Physiological and clinical studies on patients with PHAII have suggested at least two different cellular abnormali-

ties that may explain the hyperkalemia, low renin levels, and hypertension. Elegant physiological studies by Schambelan and colleagues (6) showed that that infusion of a cell-membrane impermeant sulfate anion led to correction of potassium secretion. They hypothesized that the primary mechanism underlying this result was increased chloride permeability in the distal convoluted tubule, which acts as an electric shunt. Decreasing the transepithelial voltage increases sodium reabsorption through the thiazide-sensitive Na-Cl cotransporter (NCC).

Since this voltage is the main driving force for potassium secretion through the apical potassium channel ROMK, this may lead to decreased potassium secretion and hyperkalemia (Figure 2). The second hypothesis is based on the observation that these patients are very sensitive to thiazide diuretic treatment. Since genetic evidence rules out the involvement of the Na-Cl cotransporter locus itself (SLC12A3), the primary underlying mechanism may be abnormal regulation of the NCC.

A study by Yang and colleagues (7) reported in this issue of the *JCI*, and another study by Wilson et al. (8) very recently published in the *Proceedings of the National Academy of Sciences*, both tested this second hypothesis and reached very similar conclusions. Using the *Xenopus* oocyte expression system, both studies clearly show that coexpression of thiazide receptor NCC with WNK4 leads to a significant (50% in ref. 8 and greater than 85% in ref. 7) decrease in thiazide-sensitive sodium uptake. Using two distinct methods to measure NCC cell surface expression (quantitative immunofluorescence of green fluorescent protein-tagged NCC, ref. 8, or immunodetection of cell surface biotinylated proteins, ref. 7), WNK4 was shown to consistently suppress cell surface expression of the transporter. The trafficking of NCC from the ER or Golgi was not affected by WNK4 (7) and the endocytic retrieval of NCC was apparently not disturbed (8). Altogether, the data suggest that WNK4 represses the activity of NCC by inhibiting its exocytic insertion from cytoplasmic vesicles into the plasma membrane. The effect of WNK4 appears to be specific and selective since the repression of NCC is abolished when a catalytic WNK4 dead mutant was coexpressed with NCC (8) or when an antisense construct was used as control (7). Yang et al. have made an excit-

Table 1
Monogenic diseases causing salt-sensitive hypertension or salt-losing syndromes

Mutation type	Monogenic disease or syndrome	Main location of affected transporters	Resultant phenotype
Loss-of-function	Bartter syndrome	Thick ascending limb	Salt-resistant, hypotensive
	Citelman syndrome	Distal convoluted tubule	
	Pseudohypoaldosteronism type I	Distal convoluted tubule, connecting tubule, and collecting duct	
Gain-of-function	Familial hyperkalemic hypertension syndrome	Distal convoluted tubule, connecting tubule, and collecting duct	Salt-sensitive, hypertensive
	Liddle syndrome	Distal convoluted tubule, connecting tubule, and collecting duct	

ing additional observation: WNK1, while inactive per se on NCC activity, was able to fully abolish the inhibitory effect of WNK4, suggesting that both kinases act on the same signaling pathway (7). The authors make the intriguing speculation that WNK1 may interact with WNK4 through an autoinhibitory domain, common to WNK1 and WNK4. Overexpression of WNK1 or its truncated isoform (expressing the inhibitory domain but not the catalytic domain) would nicely explain the dominant negative effect of the mutation. The use of a truncated WNK1 and/or a catalytically dead mutant could easily address this issue but these data are not presented in the Yang et al. study. It is more difficult to propose a molecular mechanism for the WNK4 mutations. Apparently they do not behave as dominant negative mutants in the *Xenopus* oocyte expression system (7). Even more curiously, Yang et al. report that only one of the mutations (Q565E) leads to the expected significant increase in NCC activity (7), but not the two closely related mutations (D564A or E562K), a result which is at variance with the findings of Wilson et al. (8). At present, it is difficult to understand the discrepancy.

Future directions: so many exciting questions

This is clearly just the tip of the iceberg. Unraveling a completely new signaling cascade is an exciting challenge for the physiologist. Many questions will now have to be addressed. What are the molecular mechanisms of the signaling cascade from WNK1 to WNK4 and then NCC? What are the associated proteins? Is NCC directly phosphorylated by WNK4? How does the interaction between WNK4 and NCC affect the exocytic pathway? What are the other substrates for WNK4 and WNK1? Localization of WNK4 at the tight junction (Figure 2), suggests that it could modulate anionic chloride permeability. The two hypotheses discussed here are not at all mutually exclusive. Other targets are possible: the epithelial sodium channel (ENaC); the epithelial calcium channel; ROMK; or other potassium channels. Nothing is known about the upstream physiological regulators. The aldosterone-signaling pathway is a good candidate to interact with the WNK pathway. Aldosterone is known

to induce or repress over 60 genes (9). Is WNK1 an aldosterone-induced transcript? Or, alternatively, is WNK4 an aldosterone-repressed transcript? Other candidate upstream regulators of WNK kinases may include insulin and vasopressin.

Negative regulators of the sodium transporter in the aldosterone-sensitive distal nephron are essential for achieving sodium balance and blood pressure control

Researchers like to think positively and we often favor positive regulators in our physiological working hypothesis. The switching on and off of an activator of sodium transport would at first seem sufficient to maintain control of our sodium balance. It may not be so simple. Liddle syndrome and PHAII teach us a very important lesson: repressors may be as important as activators. In Liddle syndrome, a specific mutation in the proline-rich domain of β or γ subunits of ENaC (PPXY motif) prevents the binding of the physiologic repressor Nedd4-2,

which normally promotes the endocytic retrieval of the channel. The Liddle mutations lead to channel retention at the cell surface. For PHAII, the mutations lead to the inactivation of a physiologic repressor that normally prevents the exocytic expression of the Na-Cl cotransporter at the cell surface. Are there other repressors of sodium transport present? Most probably, the answer is yes. Studying the vasopressin signaling cascade, we have recently demonstrated the existence of another repressor of ENaC, which acts not by changing ENaC cell surface expression but by drastically diminishing the probability of channel opening, at least in the oocyte expression system (10). Why has nature developed such a sophisticated repressor system? Of course dual control affords the biological response much greater flexibility in reaction to large variations in the external milieu. Human populations have only recently (during the last 10,000 years) been challenged by very large increases in salt intake in their diet. Without an efficient way of

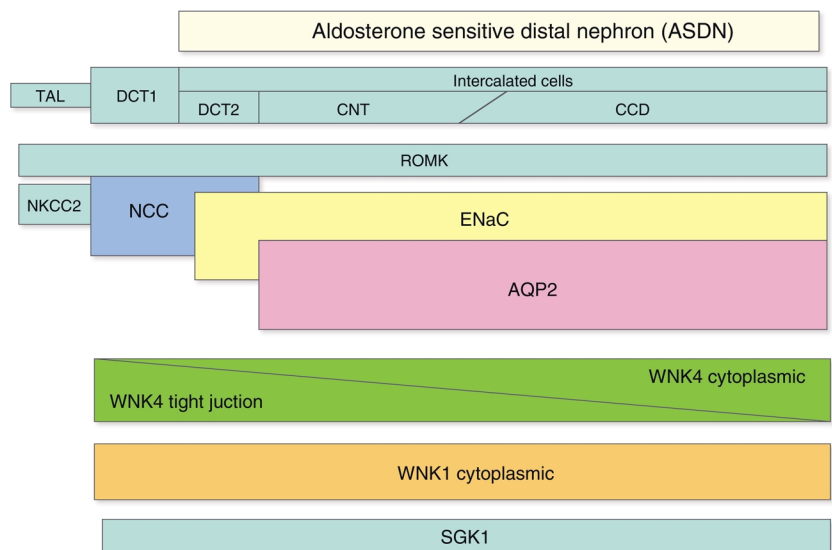


Figure 2

Location of Na-Cl transporters and their regulatory proteins along the aldosterone-sensitive distal nephron. Sodium balance is ultimately achieved in this part of the renal tubule by fine aldosterone-mediated regulation of apically located Na-Cl cotransporters in the distal convoluted tubule (DCT); the epithelial sodium channel (ENaC) in the collecting duct (CD), connecting tubule (CNT), and overlapping expression in the distal convoluted tubule 2 (DCT2); and the aquaporin water transporter 2 (AQP2), which are all limiting factors in transepithelial fluid reabsorption (see review in ref. 11). The aldosterone-sensitive distal nephron expresses genes needed for the specificity of the aldosterone signaling cascade, i.e. the mineralocorticoid receptor (MR) and 11- β HSD2, an enzyme that protects MR from illicit occupation by cortisol. Mutations in these genes also cause severe salt-sensitive hypertensive phenotypes (see recent reviews in refs. 2 and 12). TAL, thin ascending limb; CCD, cortical collecting duct; NKCC2, Na-K-2Cl cotransporter; SGK1, serum glucocorticoid-regulated kinase. Figure adapted with permission from Kriz and Kaissling (13), and Loffing and Kaissling (11).

repressing our sodium transporters in our kidney, the frequency of salt-sensitive hypertension would have been even higher and hypertension even much more severe.

Acknowledgments

We thank Jean-Daniel Horisberger, Dmitri Firsov, and Laurent Schild for critically reading the manuscript and Nicole Skarda for secretarial work. This work was supported by a grant from the Swiss National Foundation (no. 31-061966.00 to B.C. Rossier).

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Peanut allergy: a growing phenomenon

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J. Clin. Invest. **111**:950-952 (2003). doi:10.1172/JCI200318233.

Peanut allergy is one of the most serious of the immediate hypersensitivity reactions to foods in terms of persistence and severity and appears to be a growing problem (1). The prevalence of food hypersensitivity in adults is reportedly less common, but a recent survey in the US found that 1.3% of adults are allergic to peanuts or tree nuts (2). Recently, in a cohort of American children referred for the evaluation of atopic dermatitis, the prevalence of allergic reactivity to peanuts was nearly twice as high as that in a similar group evaluated a decade earlier (3). In spite of increased recognition and understanding of food allergies, food is the single most common cause

of anaphylaxis seen in hospital emergency departments (4), accounting for about one-third of anaphylaxis cases seen. It is estimated that about 30,000 food-induced anaphylactic events are seen in American emergency departments each year, 200 of which are fatal (5). Either peanuts or tree nuts cause more than 80% of these reactions.

Due to the persistence of the reaction and the lack of effective treatment, peanut-specific immunotherapy is currently being examined as a treatment option. An understanding of the molecular mechanisms is vital to ensure the eventual, effective treatment of peanut-allergic patients.

Th2 polarization of cytokines

The study by Turcanu et al. (6) in this issue of the *JCI* uses a novel approach, that of CFSE staining to separate peanut-specific lymphocytes by flow cytometry and subsequent cloning. Due to technical difficulties arising from the low frequency of allergen-specific cells in the blood, previous

studies have been complicated and not easily interpreted. Human T cells, when repeatedly stimulated in vitro, will often develop a Th2 phenotype regardless of their origin (7, 8).

In this study, the authors compare peanut-allergic individuals, individuals who have outgrown peanut allergy, and those who had always tolerated peanuts (6). Peripheral blood lymphocytes in the peanut-allergic individuals demonstrated a Th2 polarization of cytokine production by peanut-specific cells with low levels of IFN- γ and TNF- α and high levels of IL-4, IL-5, and IL-13. In the individuals who had outgrown their peanut allergy and the nonallergic controls, the cytokines showed a Th1 bias with high levels of IFN- γ and TNF- α and low levels of IL-4, IL-5, and IL-13. An interesting part of the study investigated individuals who were allergic to eggs, using similar laboratory and cloning techniques. As in the peanut-specific cells, the egg-allergic individuals' peripheral blood lymphocytes had a Th2 skewing of their ovalbumin-specific T cells (Figure 1).

This evidence that peanut antigens do not in themselves induce a Th2 cytokine response is not surprising, since all food allergy patients share similar clinical symptoms in the skin, gastrointestinal tract, and respiratory tract. The results of the present study (6) will help in the evaluation of future immunomodulatory treatments for food allergy and in studies