J Am Soc Nephrol 14: 538–540, 2003

The Thiazide-Sensitive Na-Cl Cotransporter and Human Disease: Reemergence of an Old Player

DAVID H. ELLISON
Division of Nephrology & Hypertension, Oregon Health & Science University, and VA Medical Center, Portland, Oregon.

The greatest breakthrough in the history of the drug treatment of hypertension came with the discovery of the orally effective diuretic, chlorothiazide (1).

Hypertension affects up to 25% of the adult population in industrialized countries. It contributes importantly to morbidity and mortality. While the pathogenesis of most hypertension remains enigmatic, molecular insights into renal ion transport pathways and genetic insights into Mendelian forms of hypertension have begun to open the door of understanding just a bit. Chlorothiazide is the prototype of the distal convoluted tubule diuretic. Frequently referred to as "thiazides," this group includes not only the true thiazides (hydrochlorothiazide and many others), but also the quinazolinones such as metolazone, the substituted benzophenone sulfonamides such as chlorthalidone (used in the ALLHAT trial), and the indolines such as indapamide (2). Although generated empirically without knowledge of renal ion transport pathways, the distal convoluted tubule diuretics are specific inhibitors of a protein that couples Na and Cl movement across the apical membrane of distal convoluted tubule cells. This protein, first cloned from flounder bladder by Gamba *et al.* (3), is the thiazide-sensitive Na-Cl cotransporter (NCC, TSC, or NCCT), a member of the cation-chloride cotransporter gene family (4).

The NCC, like many membrane transport proteins, is glycosylated on asparagine (N) moieties (3,5). N-linked glycosylation occurs first within the endoplasmic reticulum, generating a core-glycosylated protein that can be digested with endoglycosidase H. As processing continues, some of the sugar moieties are trimmed off the protein, after which it moves to the golgi apparatus, where it undergoes more extensive terminal glycosylation (6). Once fully glycosylated, the protein can be digested with peptide-*N*-glycosidase F, but not with endoglycosidase H. In this issue of *JASN*, Hoover *et al.* (7) demonstrate that glycosylation is essential for normal NCC function. They first confirmed that NCC is glycosylated in native renal tissue. They then expressed NCC constructs in which one or both consensus N-linked glycosylation sequences were mutated. Mutation of glycosylation sites increased both the chloride and metolazone affinity of the transporter, suggesting that the sugar moieties on the native transport protein sterically inhibit diuretic access to its binding site. Further, they support a long-held notion that chloride and distal convoluted tubule diuretics bind to the same site on the protein (8). The double mutant is not glycosylated and is not functional; its abundance at the membrane surface is significantly reduced. The effects of mutating both glycosylation sites on NCC trafficking resemble effects observed in humans suffering from Gitelman syndrome.

Gitelman syndrome is autosomal recessive disorder of hypokalemia, metabolic alkalosis, and "normal" blood pressure (9). Although Gitelman patients have been diagnosed frequently with Bartter syndrome, Gitelman patients typically manifest hypocalciuria and increased bone density, whereas Bartter patients frequently demonstrate hypercalciuria and nephrocalcinosis. Simon *et al.* (10) showed that mutations of the NCC (gene symbol: SLC12A3; gene locus:16q13) cause Gitelman syndrome, a finding confirmed by other laboratories (11–16). An NCC knockout mouse manifests several of the phenotypic features of Gitelman syndrome, suggesting that this disorder reflects loss of NCC function (17). Kunchaparty *et al.* (5) showed that Gitelman-causing mutations disrupt NCC function when expressed in a heterologous system. They demonstrated that many Gitelman mutations disrupt normal NCC folding, thereby activating the quality control mechanism of the endoplasmic reticulum. This system recognizes misfolded proteins and targets them for degradation rather than export to the golgi, where they are further processed and exported to the plasma membrane (18). Thus, misfolded NCC proteins remain inside of cells, where they are inactive, not because they are incapable of ion transport, but rather because they are not localized correctly. These results identify Gitelman syndrome as one of a growing number of diseases associated with protein processing defects (18).

A simple classification of ion transporter defects can be modified from that described for defective low-density lipoprotein receptors (19). According to this scheme, type 1 mutant transporters are normally synthesized and reach the cell surface but are inactive. Type 2 mutant transporters are normally synthesized, but they do not traffic appropriately to the cell membrane, primarily because the quality control mechanism has been activated. Type 3 mutant transporters are ineffectively

Correspondence to Dr. David H. Ellison, Division of Nephrology, Oregon Health & Science University, Suite 262, 3314 SW US Veterans Hospital Road, Portland, OR 97201. Phone: 503-494-8490; Fax: 503-494-5330; E-mail: ellisond@ohsu.edu

1046-6673/1402-0538
Journal of the American Society of Nephrology

translated or transcribed. Thus, individuals who inherit a type 3 mutation do not generate normal amounts of transporter protein. According to this scheme, the double mutant described by Hoover *et al.* is an example of a type 2 mutation. Berkman *et al.* (20) reported preliminary data that more than half of 25 tested Gitelman mutations are of this class, suggesting that, as for many such diseases, protein misfolding is a predominant mechanism. De Jong *et al.* (21), however, recently showed that NCC misfolding in Gitelman syndrome is not uniformly complete. Some mutant proteins associated with Gitelman syndrome do reach the plasma membrane to a limited extent and are thus partially active. These mutations represent a subtype of class 2 mutations; the single glycosylation site mutations described by Hoover *et al.* appear to represent mutations that are partially active for this reason. The observation that such mutations increase chloride and metolazone affinity is similar to one made in a preliminary report concerning effects of partially active Gitelman mutations (22). One Gitelman mutation increased chloride and metolazone affinity, although it is located in an area that is not adjacent to a glycosylation site. The mechanistic explanation for such affects awaits further exploration.

Gitelman syndrome, although less severe than some other salt-wasting phenotypes, is nearly always symptomatic (23). Thus, identifying potential therapeutic agents or approaches designed to alleviate symptoms is a worthy goal. Chemical chaperones, substances that assist protein folding, are being examined as potential therapeutic agents for other protein folding diseases such as cystic fibrosis and diabetes insipidus (24,25). In view of the fact that other transport proteins that are misfolded and retained within the cell have been shown to be active when delivered to the plasma membrane, screening for effects of chemical chaperones on mutant NCC may be worthwhile.

Perhaps even more significant, however, is the insight into blood pressure homeostasis derived from studies of NCC dysfunction. Cruz *et al.* (26) examined 199 members of an Amish kindred with an especially high incidence of Gitelman syndrome. By reducing background genetic variability, they were able to show the effects of mutant NCC alleles on blood pressure. When adjusted for age and gender, the diastolic blood pressure of individuals inheriting two mutant NCC alleles was 8.6 mmHg lower than in their wild type relatives. Although the blood pressure of heterozygous individuals was not different from wild-type controls, the 24-h urinary Na excretion was significantly elevated, suggesting that even one mutant NCC allele leads to a mild salt wasting phenotype, one that may be self-corrected by an increased dietary NaCl intake.

The data described above indicate that dysfunction of the NCC leads to a syndrome that includes not only hypokalemic alkalosis, but also relative hypotension. This raises the possibility that a syndrome of enhanced NCC activity would be associated with hypertension. Pseudohypoaldosteronism type II (PHAII) is a rare autosomal dominant disorder of hyperkalemia and hypertension. Lifton and colleagues showed that this syndrome is linked to mutations in two members of a novel protein kinase family known as WNK (*W*ith *N*o Lysine[*K*]) (28). These kinases are expressed along the distal nephron both within the cytoplasm, and in the case of WNK4, at the tight junction (27). Although the mechanisms by which WNK mutations cause hypertension is not known, Mayan *et al.* (29) recently reported data indicating that PHAII resembles the opposite of Gitelman syndrome. In a kindred that inherited mutant WNK4, affected members were shown to exhibit not only hypertension and hyperkalemia, but also hypercalciuria and low bone mineral density, the opposite of Gitelman patients. Furthermore, blood pressure of affected individuals was highly sensitive to thiazide diuretics; whereas thiazides given to essential hypertensives elicit a 13 and 10 mmHg drop in systolic and diastolic blood pressure, respectively, the PHAII patients exhibited a remarkable 45 and 25 mmHg decline. These authors suggest that marked sensitivity to thiazides in PHAII implies that the NCC is constitutively activated in these patients. Recent preliminary data from our laboratory, indicating that WNK4 inhibits NCC activity suggest a possible functional link between WNK kinases and the NCC (30)

The thiazide diuretics were developed nearly fifty years ago. They have proven to be remarkably safe and effective antihypertensive agents. Although they reduce mortality in hypertension (31), concern has been raised about their side effects, including hyperglycemia, hyperlipidemia, and hypokalemia (32). Although the clinical implications of these side effects has diminished with the popularity of low-dose approaches to thiazide use, it is interesting to note that hyperlipidemia and hyperglycemia are not common features of Gitelman syndrome, a syndrome in which complete functional NCC absence is observed (9). This suggests that these side effects may relate to nonspecific effects of DCT diuretics, unrelated to their ability to inhibit renal NCC action (33). If this is true, then it may be possible to develop more specific inhibitors, drugs with fewer side effects or drugs that can be used in higher doses to further enhance the efficacy of more recently developed agents. Thus, the distal convoluted tubule diuretics continue to be clinically useful and scientifically intriguing. Although their popularity as antihypertensive agents and as subjects for scientific investigation waned during the early 1990s, they have recently returned to the forefront in both regards; the recently reported ALLHAT results cement them as first-line treatment for hypertension in the twenty-first century (34).

References

1. Freis ED: Origins and development of antihypertensive drug treatment. In: *Hypertension: Pathophysiology, Diagnosis, and Management*, edited by Laragh JH, Brenner BM, New York, Raven Press, 1990, pp 2093-2106
2. Ackerman DM, Hook JB: Historical background, chemistry, and classification. In: *The Physiological Basis of Diuretic Therapy in Clinical Medicine*, edited by Eknoyan G, Martinez-Maldonado M, Orlando, Grune & Stratton, Inc., 1986, pp 1-25
3. Gamba G, Saltzberg SN, Lombardi M, Miyanoshita A, Lytton J, Hediger MA, Brenner BM, Hebert SC: Primary structure and functional expression of a cDNA encoding the thiazide-sensitive, electroneutral sodium-chloride cotransporter. *Proc Natl Acad Sci USA* 90: 2749–2753, 1993
4. Mount DB, Delpire E, Gamba G, Hall AE, Poch E, Hoover RS, Hebert SC: The electroneutral cation-chloride cotransporters. *J Exp Biol* 201: 2091–2102, 1998

5. Kunchaparty S, Palcso M, Berkman J, Velázquez H, Bernstein P, Reilly RF, Ellison DH: Defective processing and expression of the thiazide-sensitive Na-Cl cotransporter as a cause Gitelman's Syndrome. *Am J Physiol Renal* 277: 1999
6. Helenius A, Aebi M: Intracellular functions of N-linked glycans. *Science* 291: 2364–2369, 2001
7. Hoover RS, Poch E, Monroy A, V_ázquez N, Nishio T, Gamba G, Hebert SC: N-glycosylation at two sites critically alters thiazide binding and activity of the rat thiazide-sensitive Na+:Cl- cotransporter. *J Am Soc Nephrol* 14: 271–282, 2003
8. Tran JM, Farrell MA, Fanestil DD: Effect of ions on binding of the thiazide-type diuretic metolazone to kidney membrane. *Am J Physiol* 258: F908–F915, 1990
9. Ellison DH: Salt-wasting disorders. In: *Acid-Base and Electrolyte Disorders*, edited by DuBose TD Jr, Hamm LL, Philadelphia, Saunders, 2002, pp 311-334
10. Simon DB, Nelson-Williams C, Bia MJ, Ellison D, Karet FE, Molina AM, Vaara I, Iwata F, Cushner HM, Koolen M, Gainza FJ, Gitelman HJ, Lifton RP: Gitelman's variant of Bartter's syndrome, inherited hypokalemic alkalosis, is caused by mutations in the thiazide-sensitive Na-Cl cotransporter. *Nat Genet* 12: 24–30, 1996
11. Lemmink HH, Lambert PW, van den Heuvel J, van Dijk HA, Merkx GF, Smilde TJ, Taschner PE, Monnens LA, Hebert SC, Knoers NV: Linkage of Gitelman syndrome to the thiazide-sensitive cotransporter gene with identification of mutations in three Dutch families [Abstract]. *Pediatr Nephrol* 10: 403–407, 1996
12. Pollak MR, Delaney VB, Graham RM, Hebert SC: Gitelman's syndrome (Bartter's variant) maps to the thiazide-sensitive cotransporter gene locus on chromosome 16q13 in a large kindred. *J Am Soc Nephrol* 7: 2244–2248, 1996
13. Lemmink HH, Knoers NV, Karolyi L, van Dijk H, Niaudet P, Antignac C, Guay-Woodford LM, Goodyer PR, Carel JC, Hermes A, Seyberth HW, Monnens LA, van den Heuvel LP: Novel mutations in the thiazide-sensitive NaCl cotransporter gene in patients with Gitelman syndrome with predominant localization to the C- terminal domain [In Process Citation]. *Kidney Int* 54:720–730, 1998
14. Mastrioianni N, De Fusco M, Bettinelli A, Ballabio A, Basilico E, Colussi G, Claris Appiani A, Casari G: Gitelman syndrome is caused by mutations in the human Na-Cl cotransporter gene: Molecular analysis in Italian families [Abstract]. *J Am Soc Nephrol* 7: 1617, 1996
15. Melander O, Orho-Melander M, Bengtsson K, Lindblad U, Rastam L, Groop L, Hulthen UL: Genetic variants of thiazide-sensitive NaCl-cotransporter in Gitelman's syndrome and primary hypertension. *Hypertension* 36: 389–394, 2000
16. Takeuchi K, Kure S, Kato T, Taniyama Y, Takahashi N, Ikeda Y, Abe T, Narisawa K, Muramatsu Y, Abe K: Association of a mutation in thiazide-sensitive Na-Cl cotransporter with familial Gitelman's syndrome. *J Clin Endocrinol Meta* 81: 4496–4499, 1996
17. Schultheis PJ, Lorenz JN, Meneton P, Nieman ML, Riddle TM, Flagella M, Duffy JJ, Doetschman T, Miller ML, Shull GE: Phenotype resembling Gitelman's syndrome in mice lacking the apical Na+- Cl- cotransporter of the distal convoluted tubule [In Process Citation]. *J Biol Chem* 273: 29150–29155, 1998
18. Kuznetsov G, Nigam SK: Mechanisms of disease: Folding of secretory and membrane proteins. *N Engl J Med* 339: 1688–1695, 1998
19. Morello JP, Bichet DG: Nephrogenic diabetes insipidus. *Annu Rev Physiol* 63: 607–630, 2001
20. Berkman J, Reilly RF, Ellison DH: Mechanisms of thiazide-sensitive Na-Cl cotransporter dysfunction in Gitelman's syndrome [Abstract]. *J Am Soc Nephrol* 10: 1291, 1999
21. De Jong JC, Van Der Vliet WA, Van Den Heuvel LP, Willems PH, Knoers NV, Bindels RJ: Functional Expression of Mutations in the Human NaCl Cotransporter: Evidence for Impaired Routing Mechanisms in Gitelman's Syndrome. *J Am Soc Nephrol* 13: 1442–1448, 2002
22. Sabath E, Meade P, Vazquez N, Berkman J, Ellison DH, Gamba G: Characterization of functional mutations in Gitelman's disease. *J Am Soc Nephrol* 13: 75A, 2002
23. Cruz DN, Shaer AJ, Bia MJ, Lifton RP, Simon DB: Gitelman's syndrome revisited: An evaluation of symptoms and health-related quality of life. *Kidney Int* 59: 710–717, 2001
24. Howard M, Welch WJ: Manipulating the folding pathway of delta F508 CFTR using chemical chaperones. *Methods Mol Med* 70: 267–275, 2002
25. deCarvalho AV, Ndi CP, Tsopmo A, Tane P, Ayafor J, Connolly JD, Teem JL: A novel natural product compound enhances cAMP-regulated chloride conductance of cells expressing CFTRΔF508. *Mol Med* 8: 75–87, 2002
26. Cruz DN, Simon DB, Nelson-Williams C, Farhi A, Finberg K, Burleson L, Gill JR, Lifton RP: Mutations in the Na-Cl cotransporter reduce blood pressure in humans. *Hypertension* 37: 1458–1464, 2001
27. Wilson FH, Disse-Nicodeme S, Choate KA, Ishikawa K, Nelson-Williams C, Desitter I, Gunel M, Milford DV, Lipkin GW, Achard JM, Feely MP, Dussol B, Berland Y, Unwin RJ, Mayan H, Simon DB, Farfel Z, Jeunemaitre X, Lifton RP: Human hypertension caused by mutations in WNK kinases. *Science* 293: 1107–1112, 2001
28. Xu B, English JM, Wilsbacher JL, Stippec S, Goldsmith EJ, Cobb MH: WNK1, a novel mammalian serine/threonine protein kinase lacking the catalytic lysine in subdomain II. *J Biol Chem* 275: 16795–16801, 2000
29. Mayan H, Vered I, Mouallem M, Tzadok-Witkon M, Pauzner R, Farfel Z: Pseudohypoaldosteronism type II: marked sensitivity to thiazides, hypercalciuria, normomagnesemia, and low bone mineral density. *J Clin Endocrinol Metab* 87: 3248–3254, 2002
30. Yang C-L, Angell J, Mitchell R, Ellison DH: WNK4 downregulates thiazide-sensitive NaCl transport [Abstract]. *J Am Soc Nephrol* 13: 75A, 2002
31. The sixth report of the Joint National Committee on prevention, detection, evaluation, and treatment of high blood pressure *Arch Intern Med* 157: 2413–2446, 1997
32. Pollare T, Lithell H, Berne C: A comparison of the effects of hydrochlorothiazide and captopril on glucose and lipid metabolism in patients with hypertension. *N Engl J Med* 321: 868–873, 1989
33. Calder JA, Schachter M, Sever PS: Potassium channel opening properties of thiazide diuretics in isolated guinea pig resistance arteries. *J Cardiovasc Pharmacol* 24: 158–164, 1994
34. Appel LJ: The verdict from ALLHAT—Thiazide diuretics are the preferred initial therapy for hypertension. *JAMA* 288:3039–3042, 2002

See related article, "N-Glycosylation at two sites Critically Alters Thiazide Binding and Activity of the Rat Thiazide-Sensitive Na^+:Cl^- Cotransporter," on pages 271–282.