Overexpression of the Sodium Chloride Cotransporter Is Not Sufficient to Cause Familial Hyperkalemic Hypertension

James A. McCormick, Joshua H. Nelson, Chao-Ling Yang, Joshua N. Curry, David H. Ellison

**Abstract**—The sodium chloride cotransporter (NCC) is the primary target of thiazides diuretics, drugs used commonly for long-term hypertension therapy. Thiazides also completely reverse the signs of familial hyperkalemic hypertension (FHHt), suggesting that the primary defect in FHHt is increased NCC activity. To test whether increased NCC abundance alone is sufficient to generate the FHHt phenotype, we generated NCC transgenic mice; surprisingly, these mice did not display an FHHt-like phenotype. Systolic blood pressures of NCC transgenic mice did not differ from those of wild-type mice, even after dietary salt loading. NCC transgenic mice also did not display hyperkalemia or hypercalciiuria, even when challenged with dietary electrolyte manipulation. Administration of fludrocortisone to NCC transgenic mice, to stimulate NCC, resulted in an increase in systolic blood pressure equivalent to that of wild-type mice (approximately 20 mm Hg). Although total NCC abundance was increased in the transgenic animals, phosphorylated (activated) NCC was not, suggesting that the defect in FHHt involves either activation of ion transport pathways other than NCC, or else direct activation of NCC, in addition to an increase in NCC abundance. *(Hypertension. 2011;58:888-894.)*  ● Online Data Supplement

**Key Words:** hypertension  ■ hyperkalemia  ■ sodium chloride symporters  ■ thiazides  ■ mice  ■ transgenic

Familial hyperkalemic hypertension (FHHt) is characterized by hyperkalemia, hypertension, and metabolic acidosis, with normal glomerular filtration rate. Plasma renin levels are low, but 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. Importantly, administration of thiazide diuretics, which inhibit the distal convoluted tubule (DCT)—specific sodium chloride cotransporter (NCC), is uniquely effective at ameliorating these abnormalities. FHHT is caused by mutations in 2 members of the with-no-lysine (K) (WNK) kinases, so named because of the unusual positioning of the lysine involved in coordinating ATP. Deletion of part of the first intron of WNK1 increases its expression in leukocytes and was proposed to be a gain-of-function mutation. Mice heterozygous for WNK1 display lower blood pressure than wild-type controls, supporting the hypothesis that WNK1 acts to increase blood pressure. Missense mutations within the WNK4 gene also lead to FHHT. WNK4 strongly inhibits NCC activity in *Xenopus* oocytes, whereas mutant WNK4 stimulates it; WNK1 increases NCC activity both through suppression of WNK4 and by activating STE20-and-SPS1-related proline/alanine-rich kinase (SPAK). Subsequent studies have shown that WNK1 itself is inhibited by a kidney-specific isoform lacking the kinase domain (KS-WNK1). Dysregulation of NCC activity has therefore been proposed to be the primary defect underlying FHHt. In vitro studies, however, have revealed that the WNK kinases regulate a wide variety of ion channels and transporters besides NCC (reviewed in), resulting in controversy regarding the central role of NCC in the etiology of FHHt.

Two mouse models that closely resemble FHHT have been reported. In the first, transgenic mice expressing 2 copies of *WNK4* with an FHHt-causing mutation, in addition to the 2 endogenous wild-type alleles, were generated. These mice displayed an FHHt-like phenotype, including elevated blood pressure, hyperkalemia, hypercalciiuria, and hyperplasia of the distal convoluted tubule, the nephron segment to which NCC is restricted. Interestingly, mice expressing an additional copy of wild-type *WNK4* displayed an opposite phenotype. Another model was generated in which an FHHt-causing *WNK4* mutation was knocked in, and similarly, an FHHT phenotype was observed. In both cases, the FHHt phenotype was completely reversed by administration of thiazides. Therefore, overexpression of NCC, achieved by other means, should be sufficient to cause an FHHt-like phenotype. The current experiments were designed to test this hypothesis.

**Methods**

**Generation of NCC Transgenic Mice**

All procedures were performed in accordance with the National Institutes of Health *Guide for the Care and Use of Laboratory
Figure 1. Generation of sodium chloride cotransporter (NCC) transgenic mice. A, BAC clone RP24-263E2 was modified by recombinering in Escherichia coli to remove Herpud1 and the region of Nup43 extending to its 3′ untranslated region. The resulting NCC transgene contains 12.5 kb of sequence upstream of the NCC transcription start and the 3′ untranslated region of NCC (vector sequence is not shown). B, Semiquantitative polymerase chain reaction on genomic DNA extracted from the tails of potential founders identified lines 727 and 743 as containing >12 and 5 copies of the NCC transgene; amplification of the β-globin gene confirmed equal template input. M indicates DNA markers; WT, wild-type.

Dietary Manipulations
All diets used were obtained from Harlan Teklad: TD07309 (0.8% K+, control diet for K+ studies), TD07278 (5% K+), TD96208 (0.49% NaCl, control diet for NaCl studies), and TD92012 (8% NaCl). Mice were placed on each diet for 10 days.

Results

Generation of NCC Transgenic Mice
To generate mice overexpressing NCC, a BAC clone containing the entire mouse NCC gene, modified by recombinering to remove other genes, was used (Figure 1A). Twelve founders were identified, and semiquantitative polymerase chain reaction using primers targeting the NCC gene confirmed that the mice carried additional copies of the NCC gene (Figure 1B). A single transgene integration site was confirmed by fluorescence in situ hybridization analysis (Supplemental Figure II). Two lines were selected for further analysis, line 727, which carries >20 copies of the transgene, and line 743, which carries 5 copies (Figure 1B). Line 727 was not characterized extensively because of 80% perinatal mortality, which was most likely due to integration site of the transgene (see below and Supplemental Figure II), and hereafter “NCC transgenics” will refer to line 743, which has 5 copies of the transgene integrated. A single transgene integration site in line 743 was confirmed by fluorescence in situ hybridization analysis (Supplemental Figure II).

Western blotting revealed a 1.7-fold increase in total NCC expression in the kidneys of NCC transgenic mice (Figure 2A and 2B), similar to the increase observed in KS-WNK knockout mice.14 Expression levels of epithelial sodium channel (EnaC) subunits, the sodium-hydrogen antiporter 3 NHE3, total and phospho-NKCC2, and the NCC regulatory protein SPAK did not differ significantly between wild-type and transgenic mice (Supplemental Figure III).

Phosphorylation and Cellular Localization of NCC
Is Not Altered in NCC Transgenic Mice
WNK1 activates NCC indirectly, through phosphorylation and activation of SPAK kinase,15 which then phosphorylates NCC, a prerequisite for full transport activity.16,17 Western
Figure 2. Sodium chloride cotransporter (NCC) transgenic mice display increased total NCC protein expression. A, Western blot analysis of whole kidney extracts from wild-type (WT) and NCC transgenic (NCC-TG) mice was performed using antibodies against NCC and β-actin. B, Densitometric quantitation was performed normalizing to β-actin, and expression of total NCC in NCC-TG (filled bars) relative to WT (open bars) mice was calculated. WT, n=13; NCC-TG, n=16. Values are shown ±SEM, *p<0.005.

blotting using an antibody that recognizes p-T53 NCC revealed that the abundance of phosphorylated NCC did not differ between wild-type and NCC transgenics (Figure 3A and 3B), indicating that the ratio of phosphorylated to total NCC (Figure 2A and 2B) was reduced by 41% in the NCC transgenic animals. In addition to phosphorylation, for NCC to transport NaCl it must be localized to the apical membrane of the DCT. Overexpressed NCC could be mistrafficked and not reach the plasma membrane. Immunofluorescence, however, indicated that total and phospho-NCC cellular localization do not differ significantly between wild-type and transgenic mice (Figure 3C), but it could not clarify whether NCC expression in the luminal membrane was significantly different between genotypes.

Overexpression of NCC Does Not Alter Basal Blood Chemistry Values or Body Weight

There were no significant differences in plasma chemistry values between wild-type and transgenics, including plasma [K⁺], which is typically elevated in FHHt (Table). Plasma renin concentration and aldosterone levels were determined and did not differ significantly, nor did body weights (Table). Significantly, line 727, which carries 20 copies of the transgene, did not display hyperkalemia or hypocalciuria (data not shown).

Table. Plasma Chemistry and Body Weights of Wild-Type and Sodium Chloride Cotransporter (NCC) Transgenic Mice

| Parameter            | Wild-Type, ±SD (n) | NCC Transgenic, ±SD (n) |
|----------------------|--------------------|-------------------------|
| Body weight (g), males | 31.6±3.6 (10)      | 28.9±2.4 (6)            |
| Body weight (g), females | 23.6±1.9 (9)       | 23.2±2.6 (17)           |
| Na⁺ (mmol/L)          | 147.5±1.86 (11)    | 147.9±3.08 (11)         |
| K⁺ (mmol/L)           | 3.85±0.29 (11)     | 3.85±0.31 (11)          |
| Mg²⁺ (mmol/L)         | 2.15±0.29 (11)     | 2.20±0.31 (11)          |
| Cl⁻ (mmol/L)          | 107.3±0.9 (11)     | 107.8±1.7 (11)          |
| iCa²⁺ (mmol/L)        | 1.22±0.03 (11)     | 1.25±0.05 (11)          |
| TCO₂ (mmol/L)         | 24.0±2.8 (11)      | 23.2±2.0 (11)           |
| Glucose (mg/dL)       | 241±37 (17)        | 226±45 (15)             |
| BUN (mg/dL)           | 21.8±2.7 (11)      | 22.6±2.9 (11)           |
| Creatinine (mg/dL)    | 0.24±0.07 (11)     | 0.23±0.05 (11)          |
| Hematocrit            | 0.42±0.01 (11)     | 0.42±0.03 (11)          |
| Plasma renin concentration (ng/mL per h) | 79±36 (10) | 108±39 (9) |
| Aldosterone (nmol/L)  | 0.79±0.12 (18)     | 0.90±0.29 (18)          |

For plasma values, 5 males and 6 females were used, except for renin activity (5 males and 5 females for wild-type; 5 males and 4 females for transgenic) and aldosterone (9 of each gender per group).

iCa²⁺ indicates ionized calcium; TCO₂, total CO₂; BUN, blood urea nitrogen.

Figure 3. Sodium chloride cotransporter (NCC) phosphorylation and cellular distribution are not altered in NCC-transgenic (NCC-TG) mice. A, Western blot analysis of whole kidney extracts from wild-type (WT) and NCC-TG mice was performed using antibodies against phospho (p)-NCC (T53) and β-actin. B, Densitometric quantitation was performed normalizing to β-actin, and expression of p-NCC in NCC-TG (filled bars) relative to WT (open bars) mice was calculated. n=12 for each group. Values are shown ±SEM. C, Immunofluorescence showed that in both WT and NCC-TG mice, total and p-NCC displayed expression at the apical membrane of the distal convoluted tubule.
blood pressure increased in both groups, but to a similar degree (19 mm Hg in wild-type and 22 mm Hg in transgenic) (Figure 4). The absence of functional differences likely reflected the fact that the abundance of total and phospho-NCC did not differ between wild-type and transgenic animals (Supplemental Figure IV). These data indicate that activation of NCC by fludrocortisone does not elicit a hypertensive phenotype in mice overexpressing NCC at the message level because NCC abundance at the protein level and NCC activity are not enhanced. Immunofluorescence showed that the degree of phospho-NCC expression at the apical membrane was similar in both groups (data not shown), suggesting that activation of NCC regulatory pathways, such as WNK/SPAK, may be required to fully stimulate the overexpressed NCC.

NCC Transgenic Mice Display Normal Responses to Dietary Electrolyte Modification

A distinctive characteristic of FHHt is the presence of hyperkalemia. On a control diet containing 0.8% potassium, NCC mice were normokalemic; increasing the dietary potassium level to 5% did not elicit hyperkalemia (Figure 5A). Similarly, NCC transgenic mice did not display a change in plasma sodium after 10 days on the high-potassium diet (Figure 5B). Western blotting revealed that expression of total NCC was not altered by potassium loading in either genotype, but phospho-NCC trended to lower levels in NCC mice (Supplemental Figure IV). These data indicate that activation of NCC by fludrocortisone does not elicit a hypertensive phenotype in mice overexpressing NCC at the message level because NCC abundance at the protein level and NCC activity are not enhanced. Immunofluorescence showed that the degree of phospho-NCC expression at the apical membrane was similar in both groups (data not shown), suggesting that activation of NCC regulatory pathways, such as WNK/SPAK, may be required to fully stimulate the overexpressed NCC.

**Figure 5.** Normal electrolyte homeostasis in sodium chloride cotransporter (NCC) transgenic mice. Plasma K⁺ (A) and plasma Na⁺ (B) did not differ between wild-type (WT) (open bars) and NCC transgenic (TG) (filled bars) mice on standard (0.8% K⁺) or high-potassium (5% K⁺) diets. Values are means±SEM, n=19 to 22. C, Urinary calcium:creatinine, measured from spot urine collections, did not differ between wild-type (open bars) and NCC transgenic mice (filled bars) on standard (0.49% NaCl) or high-NaCl (8%) diets. Values are means±SEM, n=18 to 22.
NaCl for 7 days did not induce hypercalciuria in either genotype (Figure 3C). Salt loading caused a trend toward lower expression of α-ENaC but significantly increased expression of γ-ENaC in both wild-type and NCC transgenic mice (Supplemental Table I); surprisingly, β-ENaC expression tended to increase in wild-type mice but decrease in NCC transgenic mice.

Responses of aldosterone secretion to both potassium and sodium loading did not differ between wild-type and NCC transgenic mice, significantly increasing in response to high K⁺ and significantly decreasing in response to high NaCl (Supplemental Figure V). Similarly, no differences in plasma renin activities were observed between wild-type and NCC transgenic mice following dietary manipulation (Supplemental Figure V).

Discussion
The current results add to an increasing body of evidence suggesting that increased NCC abundance alone is insufficient to cause hyperkalemia and hypertension. Initial studies in mice, coupled with clinical observations in humans, suggested that the predominant mechanism by which mutations in WNK kinases lead to FHHt is by increasing NCC abundance or trafficking to the apical membrane.9,10 However, studies from many laboratories have indicated that WNK kinases regulate the activity of multiple transport proteins, as well as modulating paracellular permeability (reviewed in11). We hypothesized that increased NCC abundance or trafficking to the apical membrane.9,10 However, studies from many laboratories have indicated that WNK kinases regulate the activity of multiple transport proteins, as well as modulating paracellular permeability (reviewed in11). We hypothesized that increased NCC abundance alone would be sufficient to cause the FHHt phenotype. Our data indicate that NCC transgenic mice, although they have increased total NCC abundance, are not hypertensive (Figure 3); furthermore, serum and urinary electrolyte levels are normal under standard dietary conditions (Table and Figure 3). In addition, none of the maneuvers initiated, including dietary electrolyte manipulation and fludrocortisone administration, resulted in any differences in blood pressure or electrolyte status compared with wild-type mice. This does not, however, indicate that an increased basal abundance of NCC is entirely without consequences. Following completion of this work and reported elsewhere, we found that the calcineurin inhibitor tacrolimus increased blood pressure more in transgenic than in wild-type mice.23

Although these results were surprising initially, 2 other studies obtained results that are highly consistent with ours. As noted above, KS-WNK1 is believed to be a dominant-negative regulator of WNK1, thereby suppressing the effects of WNK1 to stimulate NCC.8 KS-WNK1-deficient mice did not display an FHHt-like phenotype, despite a 1.8-fold increase in renal NCC expression levels and despite hypertrophy of the DCT.14 Similar to our findings, these mice did not display overt hypertension, although there was a small increase in diastolic blood pressure. Furthermore, no hyperkalemia was observed in KS-WNK1 knockout mice, even when they were placed on a high-potassium diet. In the second study, KS-WNK1 knockout mice were shown to be neither hypertensive nor hyperkalemic when consuming a normal diet, although mild hypertension and hyperkalemia developed when the mice were challenged with high salt and potassium intakes. In these mice, the abundance of NCC was increased,24 as in the present results. Hadchouel et al observed a reduction in expression of both α- and γ-ENaC, which they proposed to compensate for increased NCC expression.4 In our studies, there were no differences in expression of ENaC isoforms on a standard diet, but following sodium loading, NCC transgenic mice displayed a reduction in β-ENaC expression, which could be a compensatory mechanism in these mice. Our findings in wild-type mice are the same as those obtained in salt-loaded rats by Song et al.25 As noted, we observed a discrepant effect of salt loading on β-ENaC expression in NCC transgenic mice. Noncoordinate regulation of ENaC subunit expression in vivo (ie, manipulations leading to changes in expression of only some subunits, or changes in expression of subunits in opposite directions) is frequently observed and has been well described.26

In contrast to the mild phenotypes observed in these studies, other mouse models, in which FHHt mutants of WNK4 were overexpressed9 or knocked in,10 developed clear hyperkalemic hypertension in association with increased NCC expression; the abundance of NCC increased by 2-fold in 1 model.10 One possible reason for the observed differences between the models results from differential effects on other transporters. Expression of ENaC is downregulated in KS-WNK1 knockout mice mice,14 whereas it is upregulated in mice overexpressing an FHHt mutant of WNK4.9 Taken together with our observations in NCC transgenic mice, these data suggest that WNKs must alter the activity of several transport proteins to induce the FHHt phenotype.

Another possibility is that WNKs generate hyperkalemic hypertension by activating NCC via SPAK, in addition to increasing its abundance. In NCC transgenic mice, although total NCC expression was increased, there was no difference in the phospho-NCC level between wild-type and transgenic mice (Figure 3). We reasoned that by increasing NCC abundance and phosphorylation, fludrocortisone might uncover a difference between wild-type and transgenic mice, but fludrocortisone increased blood pressure similarly in both groups. Notably, the phenotype of KS-WNK1 knockout mice, in which both total and phospho-NCC levels are elevated, is intermediate between the phenotype observed in transgenic NCC mice and the FHHt-mutant WNK4 mice, with a mild increase in diastolic pressure under basal conditions.14 Thus, it seems unlikely that an increase in phospho-NCC alone would generate the complete FHHt phenotype.

There are several possible explanations for the ability of thiazides to completely correct FHHt that are not directly related to their inhibition of NCC. First, it is well established that thiazide diuretics inhibit activity of carbonic anyhydrase in the proximal tubule.28 More recently, it has been shown that NCC knockout mice treated with thiazides still display a significant increase in urinary sodium output,29 showing that thiazides act on other sodium transport mechanisms. Finally, thiazide treatment leads to changes in kidney structure, including apoptosis and dedifferentiation in the DCT1,30 where NCC is the predominant sodium entry pathway. These changes may result from reduced intracellular sodium concentration or increased intracellular calcium levels. Atrophy of the DCT is also observed in NCC knockout mice31 and in transgenic mice overexpressing wild-type WNK4, in which
NCC activity is presumably lower. Therefore, it is possible that some of the effects of thiazides in patients with FHHt are secondary to structural changes in the distal nephron.

Surprisingly, salt loading did not increase urinary calcium excretion in wild-type mice. In humans, urinary calcium excretion is directly related to sodium intake. Furthermore, urinary calcium excretion was ~5 times as high in salt-loaded rats compared with controls drinking deionized water, irrespective of dietary calcium content. In their mouse model of FHHt, Lalioti et al. saw little change in urinary calcium excretion by wild-type mice on an 8% NaCl diet, the same level of NaCl provided in our studies. These data suggest that renal calcium handling in mice may respond differently to salt loading. Another explanation is that in the mouse studies, animals were provided with 8% NaCl for only 10 days, whereas the rat studies involved 8 weeks of salt loading.

Perspectives

These unanticipated data suggest that NCC overexpression alone is insufficient to induce hyperkalemic hypertension accompanied by hypercalciuria and that dysregulation of NCC activity or of other transporters/channels plays a significant role in the etiology of FHHt. In vitro, the WNK kinases have been shown to regulate the activities of a broad range of sodium and potassium transport mechanisms in the kidney (reviewed in ). Therefore, it is likely that the WNK kinases play a broad role in ion homeostasis in normal physiology. Alternatively, the mouse may not precisely model human physiology and pathophysiology with regard to NCC function, because NCC knockout mice do not precisely mimic the catalytic lysine in subdomain II of NCC. Therefore, it is likely that the WNK kinases play a broad role in ion homeostasis in normal physiology.

Acknowledgments

We thank Nicole Desmarais for technical assistance with the animal studies and Tom Roeschel for assistance with immunofluorescence.

Sources of Funding

This work was supported by grants from the National Institutes of Health (Career development award K01 DK076617 to J.A.M., 5T2DOK067864-05 to J.H.N., and DK51496 to D.H.E.). D.H.E. is also supported by a Merit Review from the Department of Veterans Affairs and a Grant-In-Aid from the American Heart Association.

Disclosures

None.

References

1. Farfel Z, Iaina A, Rosenthal T, Waks U, Shibolet S, Gafni J. Familial hyperpotassaemia and hypertension accompanied by normal plasma aldosterone levels: possible hereditary cell membrane defect. *Arch Intern Med*. 1978;138:1828–1832.

2. Wilson FH, Disse-Nicodeme S, Choaite KA, Ishikawa K, Nelson-Williams C, Desitter I, Gunel M, Milford DV, Lipkin GW, Achard JM, Feely MP, Dussol B, Berland Y, Unwin RJ, Mayhan H, Simon DB, Farfel Z, Jeunemaitre X, Lifton RP. Human hypertension caused by mutations in wnk kinases. *Science*. 2001;293:1107–1112.

3. Achard JM, Disse-Nicodeme S, Fiquet-Kempf B, Jeunemaitre X. Phenotypic and genetic heterogeneity of familial hyperkalaemic hypertension (Gordon syndrome). *Clin Exp Pharmacol Physiol*. 2001;28:1048–1052.

4. Mayhan H, Vezed I, Mouallem M, Tzadok-Wilkon M, Pauzner R, Farfel Z. Pseudohypoaldosteronism type II: marked sensitivity to thiazides, hypercalciuria, normomagnesemia, and low bone mineral density. *J Clin Endocrinol Metab*. 2002;87:3248–3254.

5. Xu B, English JM, Wilsbacher JL, Stippec S, Goldsmith EJ, Cobb MH. Wnk1, a novel mammalian serine/threonine protein kinase lacking the catalytic ly sine in subdomain II. *J Biol Chem*. 2000;275:16795–16801.

6. Zambrowicz BP, Abuin A, Ramirez-Solis R, Richter L, Piggott J, Beltran-Rodrigo H, Buxton EC, Edwards J, Finch RA, Friddle CJ, Gupta A, Hansen G, Hu Y, Huang W, Jaing C, Key BW Jr, Kipp P, Kohlhauff B, Ma SQ, Markesich D, Payne R, Potter DG, Qian N, Shaw J, Schrick J, Shi ZZ, Sparks MJ, Van Sligtenhorst I, Vogel P, Walke W, Xu N, Zhu Q, Person C, Sands AT. Wnk1 kinase deficiency lowers blood pressure in mice: a gene-trap screen to identify potential targets for therapeutic intervention. *Proc Natl Acad Sci U S A*. 2003;100:14109–14114.

7. Yang CL, Angell J, Mitchell R, Ellison DH. Wnk kinases regulate thiazide-sensitive Na-C1 cotransport. *J Clin Invest*. 2003;111:1039–1045.

8. Subramanya AR, Yang CL, Zhu X, Ellison DH. Dominant-negative regulation of WNK1 by its kidney-specific kinase-defective isofom. *Am J Physiol Renal Physiol*. 2006;290:F619–F624.

9. Lalioti MD, Zhang J, Volkman HM, Kahle KT, Hoffmann KE, Toka HR, Nelson-Williams C, Ellison DH, Flavell R, Booth CJ, Lu Y, Geller DS, Lifton RP. Wnk4 controls blood pressure and potassium homeostasis via regulation of mass and activity of the distal convoluted tubule. *Nat Genet*. 2006;38:1124–1132.

10. Yang SS, Morimoto T, Rai T, Chiga M, Sohara E, Ohno M, Uchida K, Lin SH, Moriguchi T, Shibuya H, Kondo Y, Sasaki S, Uchida S. Molecular pathogenesis of pseudohypoaldosteronism type II: generation and analysis of a Wnk4(D561E/+ ) knockin mouse model. *Cell Metab*. 2007;5:331–344.

11. McCormick JA, Ellison DH. The WNKs: atypical protein kinases with pleiotropic actions. *Physiol Rev*. 2011;91:177–219.

12. Lazarak A, Liu Z, Huang CL. Antagonistic regulation of ROMK by long and short splice variants of the mouse Na-K-2Cl co-transporter. *Biochem Biophys Res Commun*. 2008;366:181–186.

13. Feng M, Diperri K. Non-invasive blood pressure measurement in mice. *Methods Mol Biol*. 2009;573:45–55.

14. Hachouch J, Soukaesem C, Bussi C, Zhou XO, Baudrie V, Zarier T, Cambillau M, Elghozi J, Lifton RP, Loffing J, Jeunemaitre X. Decreased EnaC expression compensates the increased NCC activity following inactivation of the kidney-specific isoform of WNK1 and prevents hypertension. *Proc Natl Acad Sci U S A*. 2010;107:18109–18114.

15. Moriguchi T, Uruishiyama S, Isamato N, Iemura SI, Uchida S, Natsume T, Matsumoto K, Shibuya H. WNK1 regulates phosphorylation of cation-chloride-coupled cotransporters via the STE20-related kinases, SPAK and OSR1. *Proc Natl Acad Sci U S A*. 2005;280:42685–42693.

16. Richardson C, Rafaji FH, Karlsson HK, Moleleki N, Vandewalle A, Campbell DG, Morrice NA, Alessi DR. Activation of the thiazide-sensitive Na(+)-Cl(-) cotransporter by the WNK-regulated kinases SPAK and OSR1. *J Cell Sci*. 2008;121:675–684.

17. San-Cristobal P, Pacheco-Alvarez D, Richardson C, Ring AM, Vazquez N, Rafaji FH, Chari D, Kahle KT, Leng Q, Bobadilla NA, Hebert SC, Alessi DR, Lifton RP, Gamba G. Angiotensin II signaling increases activity of the renal Na-C1 cotransporter through a WNK4-SPAK-dependent pathway. *Proc Natl Acad Sci U S A*. 2009;106:4384–4389.

18. Ni XP, Pearce D, Butler AA, Cone RD, Humphreys MH. Genetic disruption of gamma-melanocyte-stimulating hormone leads to salt-sensitive hypertension in the mouse. *J Clin Invest*. 2003;111:1251–1258.

19. Kim GH, Maslamani S, Turner R, Mitchell C, Wade JB, Knepper MA. The thiazide-sensitive Na–Cl cotransporter is an aldosterone-induced protein. *Proc Natl Acad Sci U S A*. 1998;95:14552–14557.

20. Friedman PA. Codependence of renal calcium and sodium transport. *Am J Physiol Renal Physiol*. 2006;38:1124–1132.

21. Mayhan H, Munter G, Shaharabany M, Mouallem M, Pauzner R, Holtzman EJ, Farfel Z. Hypercalciuria in familial hyperkalemia and hypertension accompanies hyperkalemia and precedes hypertension: Description of a large family with the Q565E WNK4 mutation. *J Clin Endocrinol Metab*. 2004;89:4025–4030.
22. Achard JM, Warnock DG, Disse-Nicodeme S, Fiquet-Kempf B, Corvol P, Fournier A, Jeunemaitre X. Familial hyperkalemic hypertension: pheno-
typic analysis in a large family with the WNK1 deletion mutation. *Am J Med*. 2003;114:495–498.
23. Hoorn EJ, Walsh SB, McCormick JA, Furstenberg A, Yang C-L, Roeschel T, Paliege A, Howie AJ, Conley J, Bachmann S, Urowin RI, Ellision DH. Calcineurin inhibitors activate the renal sodium chloride cotransporter to cause hypertension. *Nat Med*. In press.
24. Liu Z, Xie J, Wu T, Truong T, Auchus RJ, Huang CL. Downregulation of NCC and NKCC2 cotransporters by kidney-specific WNK1 revealed by gene disruption and transgenic mouse models. *Hum Mol Genet*. 2011; 20:855–866.
25. Song J, Hu X, Shi M, Knepper MA, Ecelbarger CA. Effects of dietary fat, NaCl, and fructose on renal sodium and water transporter abundances and systemic blood pressure. *Am J Physiol Renal Physiol*. 2004;287:F1204–F1212.
26. Weisz OA, Johnson JP. Noncoordinate regulation of ENaC: paradigm lost? *Am J Physiol Renal Physiol*. 2003;285:F833–F842.
27. Gordon RD, Hodsman GP. The syndrome of hypertension and hyperka-
laemia without renal failure: long term correction by thiazide diuretic. *Scott Med J*. 1986;31:43–44.
28. Velazquez H. Thiazide diuretics. *Ren Physiol*. 1987;10:184–197.
29. Leviel F, Hubner CA, Houllier P, Morla L, El Moghrabi S, Brideau G, Hatim H, Parker MD, Kurth I, Kougountzes A, Sinning A, Pech V, Riemondy KA, Miller RL, Hummler E, Shull GE, Aronson PS, Doucet A, Wall SM, Chambrey R, Eladari D. The Na+-dependent chloride-
bicarbonate exchanger SLC4A8 mediates an electroneutral Na+ reab-
sorption process in the renal cortical collecting ducts of mice. *J Clin Invest*. 2010;120:1627–1635.
30. Loffing J, Loffing-Cueni D, Hegyi I, Kaplan MR, Hebert SC, Le Hir M, Kaim B. Thiazide treatment of rats provokes apoptosis in distal tubule cells. *Kidney Int*. 1996;50:1180–1190.
31. Loffing J, Vallon V, Loffing-Cueni D, Aregger F, Richter K, Pietri L, Bloch-Faure M, Hoenderop JG, Shull GE, Meneton P, Kaim B. Altered renal distal tubule structure and renal Na(+) and Ca(2+) handling in a mouse model for Gitelman’s syndrome. *J Am Soc Nephrol*. 2004;15:2276–2288.
32. Saric M, Piasek M, Blanusa M, Kostial K, Ilich JZ. Sodium and calcium intakes and bone mass in rats revisited. *Nutrition*. 2005;21:609–614.
33. Morris RG, Hoorn EJ, Knepper MA. Hypokalemia in a mouse model of Gitelman’s syndrome. *Am J Physiol Renal Physiol*. 2006;290:F1416–F1420.
34. 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. *J Biol Chem*. 1998;273:29150–29155.
35. Ernst ME, Moser M. Use of diuretics in patients with hypertension. *N Engl J Med*. 2009;361:2153–2164.