Hypokalemia in a mouse model of Gitelman’s syndrome

Ryan G. Morris, Ewout J. Hoorn, and Mark A. Knepper

Laboratory of Kidney and Electrolyte Metabolism, National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, Maryland

Submitted 23 October 2005; accepted in final form 11 January 2006

Hypokalemia in a mouse model of Gitelman’s syndrome. Am J Physiol Renal Physiol 290: F1416–F1420, 2006. First published January 24, 2006; doi:10.1152/ajprenal.00421.2005.—Hypokalemia is a cardinal feature of Gitelman’s syndrome, a genetic renal disorder also characterized by alkalosis, hypomagnesemia, hypocalciuria, and mild salt wasting (6). Loss-of-function mutations in the thiazide-sensitive sodium-chloride cotransporter (NCC) have been identified as underlying causes of Gitelman’s syndrome (23). Notably, administration of thiazide diuretics, agents widely used in the treatment of hypertension, generally results in the same physiological profile observed in Gitelman’s subjects, including potentially severe hypokalemia. Therefore, inhibition of NCC either through pharmacological means or as a result of genetic inactivation can result in clinically significant renal potassium loss.

A mouse model of Gitelman’s syndrome, NCC (−/−) mice, faithfully recapitulates many of the physiological findings observed in Gitelman’s patients, including hypomagnesemia, hypocalciuria (13, 22), and alkalosis (13). Surprisingly, hypokalemia has not been detected in this model. In the present study we sought to determine whether NCC (−/−) mice are more sensitive than wild-type mice to reductions in dietary potassium intake.

Hypokalemia is often induced in rodent models by severely restricting dietary potassium for a period of 2 wk. In addition to reducing plasma potassium, prolonged potassium deprivation also results in polyuria, polydipsia, renal hypertrophy, and impaired renal concentrating ability (18). Two weeks of potassium deprivation will induce the aforementioned changes in wild-type rodents with an intact NCC; therefore, we chose to shorten the duration and degree of potassium restriction in an effort to highlight any sensitivity of the NCC (−/−) mice relative to wild-type mice.

METHODS

Animals. A colony of NCC (−/−) mice on a C57/Bl6 background was established at the National Institutes of Health (NIH) from breeding pairs provided by Dr. Gary Shull (University of Cincinnati, Cincinnati, OH). Control mice, wild-type C57/Bl6 mice whose age and sex were matched to experimental NCC (−/−) mice, were purchased from Taconic and allowed to equilibrate for a minimum of 3 days in the NIH animal housing facility before experimentation. All studies were approved by the National Heart, Lung, and Blood Institute (NHLBI) Animal Care and Use Committee.

Serum chemistries. Mice were anesthetized with isoflurane, and blood was collected by retro-orbital puncture using a glass Pasteur pipette. Whole blood was transferred to a serum separator (StatSpin, Norwood, MA), and plasma was isolated by centrifugation. Electrolyte content of 150 μl of serum was analyzed at the Clinical Center Laboratory of Medicine.

Metabolic cage studies. Mice were housed in metabolic cages for durations ranging from 7 to 10 days. Mice were initially fed a standard rodent pellet food with ad libitum water. Mice were then fed gel food containing 4.5 g/25 g body wt of sodium- and potassium-free rodent meal supplemented with specific amounts of sodium (0.5 meq/day) and potassium chloride, as well as 3 ml of water, and agar. All mice, regardless of genotype, consumed essentially the entire daily portion of gel food. Our potassium-replete diet contained 0.48 meq of potassium chloride per day, whereas the reduced potassium diet contained 0.048 meq/day. In addition to the water present in the gel food, mice also had free access to water with the exception of the water restriction protocol. In the water restriction protocol, water intake was limited to 2.0 ml of water per 25 g body weight in the gel food. Water intake was measured daily, and mice were weighed at least every other day. Urine was collected under oil, and urine volume and osmolality were determined gravimetrically and with a vapor pressure osmometer, respectively. Electrolyte content of the urine was analyzed at the NHLBI Laboratory of Animal Medicine and Surgery.

Semiquantitative immunoblotting. Mice were euthanized by cervical dislocation, and right kidneys were processed as previously described (3). Equal loading was confirmed using staining gels as described previously (12). This gel was subsequently scanned with a

Address for reprint requests and other correspondence: R. G. Morris, National Institutes of Health, Bldg. 10, Rm. 6N260, 10 Center Dr. MSC 1603, Bethesda, MD 20892-1603 (e-mail: morrisr@nhlbi.nih.gov).

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linear fluorescence scanner (Odyssey; Li-Cor Biosciences) at an excitation wavelength of 700 nm. Affinity-purified primary antibodies against the sodium-potassium-chloride cotransporter 2 (NKCC2) and the water channel aquaporin-2 (AQP-2) have been characterized previously.

Aldosterone measurements. Trunk blood was collected after decapitation, and plasma was collected via a serum separator. Plasma aldosterone was measured by radioimmunoassay (Diagnostic Products, Los Angeles, CA) per the manufacturer’s instructions.

Statistics. ANOVAs were performed on multiple group comparisons, followed by Bonferroni post hoc tests. Significance was taken at $P < 0.05$. Student’s $t$-tests were utilized on comparisons between two groups.

RESULTS

**Serum potassium.** The plasma potassium concentration of NCC (-/-) mice on a low-potassium diet was significantly decreased ($P < 0.01$) by roughly 1 mM compared with all other groups (Fig. 1). As previously documented (22), NCC (-/-) mice display hypomagnesemia, and this was exacerbated by a low-potassium diet (Fig. 1). In contrast, plasma sodium and calcium concentrations were not significantly different among groups (data not shown).

![Fig. 1. Plasma potassium and magnesium concentrations of wild-type and NCC (-/-) mice after 7 days on normal and low-potassium diets. *$P < 0.05$, by ANOVA.](image)

**Urinary potassium excretion.** Rates of urinary potassium excretion were measured in NCC (-/-) and wild-type mice. After initiation of a low-potassium diet, NCC (-/-) mice had significantly higher rates of renal potassium excretion compared with wild-type mice (Fig. 2). Over time, the difference in urinary potassium excretion between NCC (-/-) and wild-type mice became indistinguishable, establishing a new steady state at a lower absolute rate of potassium excretion.

Time course of water intake, urine volume, and urine osmolality. Polydipsia and polyuria are well-known consequences of hypokalemia (18). Figure 3 presents a representative time course of water intake (A), urine output (B), and urine osmolality (C) of wild-type and NCC (-/-) mice. In the basal condition, days 1–3, there was no significant difference in water intake, urine output, or urine osmolality between wild-type or NCC (-/-) mice. After 2 days on a reduced potassium diet (day 5), there was a significant increase in the water intake of the NCC (-/-) compared with all other groups (Fig. 3A). This increase in water intake in the NCC (-/-) mice became increasingly pronounced over the duration of the experiment and was not observed in the other groups of animals. Significant increases in urine output (Fig. 3B) and significant reductions in urine osmolality (Fig. 3C) of the NCC (-/-) low-potassium group paralleled the increases in water intake. Among four metabolic cage studies performed, the onset of polyuria and polydipsia in the NCC (-/-) mice varied from 2 to 4 days after introduction of the low-potassium diet but always persisted through the duration of the experiment.

**Urinary concentrating ability.** A urinary concentrating test was performed on all four groups of mice to investigate whether the pronounced polyuria and polydipsia observed in the NCC (-/-) mice on a reduced potassium diet was associated with a reduced urine concentrating ability. Water intake, urine osmolality, and urine volumes before and after 24 h of water restriction for all four experimental groups are presented in Fig. 4. All groups of animals significantly increased urine osmolality and decreased urine volume in response to water restriction. Urine osmolality and urine volume of wild-type and NCC (-/-) groups on low-potassium diets were not significantly different after 24 h of water restriction.

**Kidney weight.** Kidney weights and kidney weights normalized for body weight were analyzed for evidence of renal hypertrophy. Two of four individual experiments demonstrated
a significant increase in kidney weight in NCC (−/−) mice maintained on a low-potassium diet compared with knockout mice on a normal potassium diet (0.116 ± 0.004 vs. 0.149 ± 0.008 g, respectively; n = 10, P < 0.002). However, cumulative results from the four experiments (30–40 mice from each experimental group) did not demonstrate significant differences in kidney weight or kidney weight normalized to body weight despite the consistent polydipsia and polyuria (data not shown).

Western blotting analysis. Alterations in the expression pattern of transport proteins involved in renal concentrating ability were analyzed by Western blotting of whole kidney homogenates prepared from mice with ad libitum access to water. The protein abundance of the water channel AQP-2 was significantly reduced in both wild-type and NCC (−/−) mice on low-potassium diets. In contrast, the abundance of NKCC2 was not significantly different among the groups (Fig. 5).

Plasma aldosterone. Plasma aldosterone measurements were performed on the four experimental groups (Fig. 6). Plasma aldosterone was significantly higher (P < 0.001) in NCC (−/−) mice compared with wild-type mice regardless of diet. As expected, low-potassium diets suppressed plasma aldosterone levels, although statistical significance was achieved only for the NCC (−/−) mice (P < 0.01). Even on a low-potassium diet, aldosterone levels in NCC (−/−) mice were markedly elevated at 2.2 ± 0.5 nM (compared with the K_d of the mineralocorticoid receptor for aldosterone, 1.3 nM).

DISCUSSION

In this study, we examined the effects of manipulating dietary potassium intake in a mouse model of Gitelman’s syndrome. In response to a diminished potassium intake, NCC (−/−) mice demonstrated a pronounced polydipsia and polyuria concurrent with elevated renal potassium excretion and significantly reduced serum potassium and magnesium concentrations; findings consistent with the development of potassium depletion. These findings were not observed in wild-type mice on a reduced potassium diet or in wild-type and NCC (−/−) mice on a diet replete with potassium. Thus the NCC (−/−) mice are more sensitive than wild-type mice to dietary potassium restriction.

In contrast to the 7-day potassium depletion studies we have presented, many experimental protocols for potassium deple-
tion are 2 wk in duration (1, 2, 9, 15) and encompass both central and renal responses. For example, Berl et al. (1) demonstrated that polydipsia precedes, and is independent of, the ultimate appearance of urinary concentrating defects in potassium depletion. Our results demonstrated the early onset of polydipsia and polyuria in the NCC (−/−) mice coincidently with significant renal potassium loss. In NCC (−/−) mice, the polydipsia and polyuria persisted throughout the duration of the experiment despite wild-type and NCC (−/−) mice ultimately achieving similar rates of renal potassium excretion. The potassium loss accrued in the NCC (−/−) mice before the new steady-state level of potassium excretion was established resulted in significantly lower plasma potassium in the NCC (−/−) mice that persisted throughout the duration of the experiments. The persistent polydipsia and polyuria are thought to be driven, at least in part, by the effects of hypokalemia on neural regulation of the thirst drive. However, many compensatory changes with various time courses are undoubtedly occurring in response to potassium depletion, including possible changes in the vasopressin axis and angiotensin II levels that may directly or indirectly contribute to the polydipsia. Nevertheless, the absence of a frank renal concentrating defect at the specific time point examined in this model is consistent with an early-onset primary polydipsia. Such a process undoubtedly contributes to the polyuria seen in Gitelman patients.

Renal hypertrophy and a urinary concentrating defect are normally considered hallmarks of potassium depletion induced by restricting potassium intake. However, despite the obvious polydipsia, polyuria, and significantly reduced plasma potassium levels, at this time point there was no evidence of a concentrating defect or consistent findings of renal hypertrophy in the NCC (−/−) mice maintained on a low-potassium diet. We propose that the lack of a urinary concentrating defect and inconsistent findings of hypertrophy are a result of the relatively short duration of low-potassium diet, which is further aggravated by the variable onset of polydipsia and polyuria.

The absence of a renal concentrating defect and inconsistent signs of renal hypertrophy would at first appear to preclude a renal phenotype at this specific time point in the model. However, there were significant decreases in renal AQP-2 transporter abundance in both wild-type and NCC (−/−) mice in response to a low-potassium diet. The diminished expression of AQP-2 would be expected to contribute to the subsequent development of a renal concentrating defect known to be present after 2 wk of potassium depletion (1, 7, 17). Furthermore, the decreases in AQP-2 abundance seen in wild-type and NCC (−/−) mice on low-potassium diets are independent of water intake, given that water intake is significantly elevated only in the NCC (−/−) low-potassium group, whereas AQP-2 levels are decreased in both. Again, these findings are consistent with the observations that the ultimate development of a concentrating defect in potassium depletion is independent of water intake (1) and imply that the kidneys are responding to dietary potassium through an unknown mechanism.

Given the exacerbated hypomagnesemia observed in these studies, the potential role of magnesium depletion in this model should be considered. Magnesium depletion is known to cause tissue potassium depletion (24) but does not result in a urinary concentrating defect (16). However, in models of primary magnesium depletion, there is no evidence of polyuria or polycystin (16). Furthermore, the hypomagnesemia described in this study is enhanced only in response to reductions in dietary potassium intake. Therefore, the exacerbated hypomagnesemia would appear to be secondary to alterations in potassium metabolism and not directly involved the polydipsia and polyuria.

There are many potential renal mechanisms contributing to the potassium deficiency in NCC (−/−) mice on a low-potassium diet. First, even on a low-potassium diet, circulating aldosterone in the NCC (−/−) mice is markedly elevated.
(presumably because of the salt wasting), and this would serve as a potent stimulus for potassium secretion by the aldosterone-sensitive segments of the renal tubule (4, 20). The hypocalciuria previously reported in the NCC (−/−) mice also may play an indirect role in promoting potassium secretion. Urinary calcium has been shown to inhibit active potassium secretion (19); therefore, hypocalciuria could relieve this inhibition and promote potassium secretion. Furthermore, calcium also inhibits the activity of epithelial sodium channels (5, 8). Again, a reduction of this inhibitory influence would allow enhanced electrogenic sodium reabsorption, resulting in a depolarization of the apical membrane of the collecting duct principal cells, thereby increasing the driving force for potassium secretion (21). Finally, increased luminal flow, as might be encountered in this model, has been shown to stimulate potassium secretion in in vivo-perfused cortical collecting ducts (14). Further investigation is required to identify the precise molecular mechanisms in this model.

The pathogenesis of hypokalemia in Gitelman’s syndrome remains incompletely understood (10). Most explanations favor one or more of the aforementioned mechanisms. We have established that in a mouse model of Gitelman’s syndrome, a modest reduction in dietary potassium intake can induce the hypokalemic phenotype, allowing more detailed investigation of mechanisms contributing to the development of hypokalemia. Previous studies have shown that there are significant fluctuations in dietary potassium intake in humans and that these are associated with blood pressure changes and cardiovascular risk (11). Conceivably, hypokalemia in Gitelman’s syndrome may be associated with a low dietary potassium intake and may explain why some patients develop hypokalemia and polyuric spells, whereas others patients remain normokalemic.

In conclusion, NCC (−/−) mice are sensitive to reductions in dietary potassium and begin to demonstrate signs of potassium depletion before wild-type mice do. Our findings are consistent with previous studies demonstrating polyuria and polydipsia occurring early in the development of potassium depletion, with alterations in renal concentrating ability and morphology occurring at later time points. Thus the absence of hypokalemia in NCC (−/−) mice on a normal diet appears to be due to compensation by sufficient amounts of dietary potassium.

ACKNOWLEDGMENTS

We thank Dr. Gary Shull for the NCC (−/−) mice, David Caden at the NHLBI Laboratory of Animal Medicine and Surgery, and members of LKEM for helpful discussions.

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AJP-Renal Physiol • VOL 290 • JUNE 2006 • www.ajprenal.org

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