Nephron Remodeling Underlies Hyperkalemia in Familial Hyperkalemic Hypertension

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The molecular unraveling of Mendelian diseases often reveals previously unknown physiologic control systems. The molecular solution of Familial Hyperkalemic Hypertension (FHHt; also known as pseudohypoaldosteronism type 2 or Gordon syndrome) uncovered a complex signaling network in the mammalian distal nephron that permits the kidney to uncouple potassium and sodium excretion. Surprisingly, this network is most highly expressed along the distal convoluted tubule (DCT), a short nephron segment that transports very little, if any, potassium.

The central actors in this story, the thiazide-sensitive NaCl cotransporter (NCC), WNK and SPAK kinases, and the potassium channel Kir4.1, work together to adjust NaCl entry across apical membranes of DCT cells, modulating potassium secretion along downstream nephron segments secondarily. On the basis of work in animals and humans, a mechanistic model has been developed suggesting that NCC activation along the DCT is essential for the FHHt phenotype.1 Accord- ing to this model, NCC activation reduces Na+ delivery to the connecting tubule (CNT) and cortical collecting duct (CCD), thereby limiting potassium secretion in these segments.

However, components of this network are expressed not only by kidney cells, not only by DCTs, and not only in cytoplasmic compartments, suggesting that effects on proteins other than NCC may also contribute. Early hypotheses for FHHt focused on paracellular leakiness (the “chloride shunt hypothesis”)2, and WNKs have been reported to alter paracellular properties.3 WNK kinases also modulate transport along the thick ascending limb, CNT, and collecting duct. Finally, effects of WNK kinases outside the kidney have been suggested to be important.4 As reported in this issue of the Journal of the American Society of Nephrology, Grimm et al.5 generated an elegant mouse model to determine whether NCC activation along the DCT is sufficient to recapitulate FHHt. A knock-in approach was used to introduce cDNA encoding a constitutively active (CA) form of SPAK kinase, which phosphorylates and activates NCC,6 into the endogenous STK39 gene locus. The inclusion of loxP sites around the construct rendered the allele silent, unless coexpressed with Cre recombinase. By crossing these mice with transgenic mice expressing Cre recombinase only in the first segment of the DCT, DCT1, Grimm et al.5 were able to induce CA-SPAK expression specifically along this segment, while eliminating wild-type (WT) SPAK. The use of the Cre recombinase expressed only along DCT1 was crucial, because the thick ascending limb expresses the Na-K-2Cl cotransporter and DCT2 expresses epithelial sodium channels (ENaC) and apical potassium channels (ROMK) in addition to NCC; restricting CA-SPAK expression to the DCT1 allowed activation of NCC only, without potential confounding effects.

In WT mice, endogenous SPAK was expressed along TAL, DCT1, DCT2/CNT, and CCD as previously reported. As expected, in CA-SPAK mice, endogenous SPAK was completely absent, and CA-SPAK (identified by a tag) was exclusively expressed along DCT1. A third group of mice, which had the CA-SPAK gene but not Cre recombinase, exhibited no apparent SPAK expression (functional SPAK knockout). The last group of mice displayed a Gitelman syndrome–like phenotype, with metabolic alkalosis and lower plasma [K+] than WT mice, consistent with previous SPAK knockout mouse models. Expression of CA-SPAK not only reversed this phenotype but led to a full FHHt phenotype, with increased abundance of phosphorylated NCC, such as is commonly seen in mouse models of FHHt7,8; immunofluorescence revealed that these changes were restricted to DCT1. Analysis of plasma electrolytes revealed that CA-SPAK mice displayed increased plasma [K+] and decreased [HCO3−] compared with WT mice, consistent with an FHHt phenotype. Administration of hydrochlorothiazide (HCTZ) completely normalized these abnormalities, confirming a causative role for excessive NCC activity. Interestingly, the supplemental data also show that activation of NCC only along the DCT1 was sufficient to increase BP, an effect that was also reversed with HCTZ. Thus, CA-SPAK expressed only along the short DCT1 segment is sufficient to recapitulate all of the salient features of FHHt, and it does so by activating NCC. As the authors state, these data do not preclude effects of WNK kinases in segments other than the DCT1 or effects outside the kidney in the etiology of FHHt.

Grimm et al.5 next turned to the mechanism by which hyperactivation of NCC along the DCT1 decreases potassium secretion along more distal segments. Evidence that NCC activation directly reduces distal potassium secretion was provided by normalization of plasma [K+] in CA-SPAK mice after HCTZ administration. This observation is consistent with the standard view that NCC modulates potassium secretion along the CNT/CCD by metering Na+ delivery to these...
In this scheme, increased NCC activity reduces Na\(^+\) delivery to ENaC, decreasing electrogenic Na\(^+\) reabsorption and thus, the drive for K\(^+\) secretion through ROMK. Although this is an often cited view, there is evidence that sodium delivery to distal segments does not have a dominant effect on potassium secretion in many conditions. Good and Wright\(^\text{10}\) showed that luminal [Na\(^+\)] along the distal tubule typically is not limiting to K\(^+\) secretion. Furthermore, a recent study from Bailey and coworkers\(^\text{1,1}\) reported that administration of HCTZ to mice induced natriuresis without kaliuresis initially, whereas after 3 days of dietary sodium restriction, the same treatment did increase potassium excretion. These data led to the proposal that molecular and structural changes in CNT/CDD are required for changes in NCC activity to alter distal K\(^+\) secretion. CA-SPAK mice, with NCC specifically activated in the DCT1, which lacks ENaC and ROMK, presented an ideal model in which to further test the sodium delivery model. Grimm et al.\(^\text{5}\) administered HCTZ to CA-SPAK mice daily and monitored changes in urinary Na\(^+\) and K\(^+\) excretion. They observed that, although HCTZ induced a potent and sustained natriuresis within 24 hours, increased urinary K\(^+\) secretion and a concomitant reduction in plasma [K\(^+\)] occurred slowly over a 3-day period. Because increased K\(^+\) secretion occurred long after inhibition of NCC had provoked Na\(^+\) excretion, these data also pointed to a mechanism beyond reduced distal sodium delivery. DCT atrophy and CNT/CCD hypertrophy have been well described in models of lower NCC activity,\(^\text{12}\) and expansion of DCT1 has been described in mouse models of NCC activation.\(^\text{8}\) Morphometric analyses after immunofluorescence with segment-specific markers revealed that DCT1 was expanded in CA-SPAK mice compared with WT mice. In contrast, CNT displayed decreased length and cross-sectional area. No changes in DCT2 or CCD were observed. Because the CNT is the segment along which increased NCC activity reduces Na\(^+\) delivery to ENaC, decreasing electrogenic Na\(^+\) reabsorption and thus, the drive for K\(^+\) secretion through ROMK, microperfusion of isolated tubules would verify that K\(^+\) secretion is indeed lower in CA-SPAK mice. Another minor limitation is that, although the evidence for CA-SPAK exerting a DCT1-specific effect is strong, the parvalbumin promoter is also active outside the kidney in central GABA-ergic interneurons.\(^\text{14}\) The WNK-SPAK/OSR1 pathway is well known to affect GABA-ergic pathways,\(^\text{1}\) which may influence activity of renal nerves. Thus, although Grimm et al.\(^\text{5}\) show that HCTZ administration completely reverses the effects of CA-SPAK, some of the upstream NCC-activating pathways may be extrarenal. One approach to circumvent this issue might be to introduce phosphomimetic mutations into NCC itself at SPAK/OSR1 phosphorylation sites using the same strategy that generated CA-SPAK mice.

A key unanswered question is how altered NCC activity induces remodeling of CNT. Does altered Na\(^+\) delivery itself induce remodeling, or is another mechanism involved? Although CA-SPAK displayed normal aldosterone levels, plasma renin activity is lower than in WT mice, raising the possibility that this or some other endocrine mechanism could be involved. It is well established that NCC is excreted in extracellular vesicles (e.g., exosomes). Recently, it has been reported that such vesicles can transmit microRNAs to cultured renal epithelial cells and alter expression of ion transporters.\(^\text{15}\) Similarly, extracellular vesicles derived from the DCT1 could transfer microRNAs or other signaling molecules to downstream segments, inducing remodeling. The cargo of these vesicles could vary depending on NCC activity or plasma [K\(^+\)].

The relationship between NCC activity and plasma [K\(^+\)] has been appreciated for many years as a result of studying Gitelman syndrome and FHH. Although this reflects an effect of dysregulated NCC activity on potassium homeostasis, it has become evident more recently that changing plasma [K\(^+\)] in nondisease states (e.g., when dietary potassium intake is altered) can itself affect NCC activity. A model is emerging in which changes in plasma [K\(^+\)] are sensed by DCT cells and transmitted to NCC via changes in intracellular Cl\(^-\), which directly modulate WNK kinase activity. Under conditions of low K\(^+\), NCC is activated, and as K\(^+\) increases, it is inhibited in a process that may also include effects on phosphatases.\(^\text{16}\) The dogma has been that changes in NCC activity homeostatically control plasma [K\(^+\)] by metering Na\(^+\) delivery to K\(^+\)-secreting segments. Grimm et al.\(^\text{5}\) have provided strong evidence that this view may be incorrect and that changes in NCC activity alter potassium secretion by inducing structural remodeling of the CNT, the major K\(^+\)-secreting segment.

**DISCLOSURE**

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MicroRNA as Novel Exercise Mimetic for Muscle Wasting in CKD

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Cachexia with muscle wasting is prevalent and closely associated with mortality and morbidity in patients with CKD. The pathophysiology of muscle wasting in CKD is complex. Inadequate nutritional intake, physical inactivity from muscle weakness, systemic inflammation and aberrant signaling of neuropeptides have been implicated. To date, there is no effective therapy. Exercise training has well documented health benefits, including maintenance of muscle mass as well as increased physical performance resulting from changes in muscle fiber phenotype leading to increased mitochondrial biogenesis. The molecular signaling mechanism underlying adaptations to increased physical activity in CKD-associated muscle wasting is not well understood.

Wang et al.3 investigated microRNA-23a (miR-23a) and miR-27a, located in an NFAT-regulated gene cluster, as potential candidates for exercise mimetics in mice with CKD cachexia. miR-23a expression is decreased in mice with CKD. Resistance overload exercise increased muscle miR-23a and miR-27a expression in CKD. In vivo transfection of miR-23a/miR-27a cluster precursor in CKD mice attenuated muscle wasting, improved grip strength, and corrected the aberrant signaling of ubiquitin ligases, muscle ring finger 1, and atrogin 1, which are the signature of muscle wasting in CKD. In silico analysis identified PTEN and caspase-7 as targets for miR-23a and Fox-O1 as target of miR-27a in muscle. The authors concluded that the miR-23a/miR-27a cluster might play an important role in the molecular signaling of exercise-induced adaptations in CKD muscle and suggested that this pathway may provide the basis of pharmacological exercise mimetics for CKD cachexia and wasting.

miRs have long been associated with myogenesis. miR-1 knockout flies show premature death from failure of skeletal muscle development. Knockout of miRs in mice, however, produces little in terms of pathologic phenotype, such as would be predicted from in vitro studies. Overlap and redundancy of various miRs could be the reason. Double knockout of miR-133a-1 and miR-133a-2 resulted in skeletal muscle myopathy, which was not shown in the single knockouts. miRs regulate satellite cell proliferation. In vitro miR-27a is known to promote myoblast proliferation by inhibiting myostatin. However, miR-23a affects myoblast differentiation via regulation of myosin heavy-chain gene transcription. Exercise has known effects on miR expression. Endurance exercise increases miR-1 and miR-133 in the short term but decreases the resting levels of miR-1 and miR-133 in the long term.

In this study, Wang et al.3 first determined the effect of resistance exercise–prevented muscle wasting in both transverse abdominal (TA) and soleus muscles. Muscle overloading specifically improved miR-23a/miR-27a expression, whereas miR-24 and miR-29a expressions did not change in CKD mice. Then, they showed that overexpression of miR-23a/27a/24–2 by transfection of vectors into the TA muscle improved TA muscle mass as well as grip strength in CKD mice. The TA muscle provides thoracic and pelvic stability, which may improve efficiency of muscle recruitment in the extremities. In this study, only grip strength, which is mostly a test of upper limb muscle function, was tested. Rotarod activity and treadmill running tests, which

See related article, “Constitutively Active SPACK Causes Hyperkalemia by Activating NCC and Remodeling Distal Tubules,” on pages 2597–2606.