

WNK4, as thiazides, shuts off NaCl reabsorption to stimulate Na/K exchange*

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In a manuscript recently published in *Nature Genetics*, Lalioti *et al.* [1] show that Wnk4 switches distal nephron function from NaCl reabsorption to Na⁺/K⁺ exchange, mainly by dismantling the Na–Cl cotransporter expressing distal convoluted tubule segment DCT1. Interestingly, the structural integrity of this segment had already been shown long ago to depend on Na–Cl cotransporter function, as thiazides provoke similar DCT1 regression as Wnk4 overexpression [2].

The observation underlying the study by Lalioti *et al.* is that the rare autosomal dominant form of arterial hypertension Familial hyperkalemic hypertension (FHHt), also called Pseudohypoaldosteronism type II (PHAII) or Gordon's Syndrome, is caused by mutations in the gene encoding the protein kinase WNK1 or WNK4 [3]. The salient feature of FHHt is the unusual combination of Na⁺ and K⁺ retention. Additional symptoms are hyperchloraemia and metabolic acidosis. The central physiological question that is tackled by this monogenetic disease is how the differential response of the kidney to high potassium intake and/or to low extracellular volume (low NaCl) is coordinated and organized, in particular in view of the fact that both hypokalaemia and hypovolaemia are strong stimulators of aldosterone secretion.

First let us briefly mention how mutations in two different genes encoding related protein kinases lead to similar clinical features. In FHHt caused by mutations in the gene encoding WNK1, an intronic deletion leads to its increased expression, whereas in FHHt caused by WNK4, the mutations affect amino acids of a short specific region of this kinase [3]. As shown in Figure 1, WNK4 and WNK1 interact within a regulatory cascade that controls the thiazide-sensitive Na–Cl cotransporter NCC [4,5]. This explains how an increase in WNK1 expression can have the same effect as a mutation that prevents WNK4 from inhibiting NCC.

Transgenic mice expressing wild-type or FHHt mutant Wnk4

To address the question of the effect of Wnk4 and of its FHHt mutation *in vivo*, Lalioti *et al.* [1] generated transgenic mice harboring two additional genomic Wnk4 copies with and without an FHHt causative mutation. Importantly, the axial localization of these (over)expressed Wnk4 proteins along the nephron and their subcellular localization were found unchanged. The telemetrically measured mean systolic arterial blood pressure of Tg(Wnk4^{WT}) mice was significantly lower (109 mmHg) than that of control littermates (117 mmHg), whereas that of Tg(Wnk4^{FHHt}) mice was significantly higher (124 mmHg). In Tg(Wnk4^{WT}) mice, serum K⁺ and HCO₃⁻ were unchanged, Cl⁻ and Mg²⁺ slightly decreased and calciuria decreased compared to control littermates. In contrast, in Tg(Wnk4^{FHHt}) mice, serum K⁺, Cl⁻ and Mg²⁺ were significantly increased, HCO₃⁻ decreased and calciuria increased, recapitulating the situation known for Familial hyperkalemic hypertension. Upon a high K⁺ diet, mice harboring the FHHt transgene showed a clear defect in urinary K⁺ secretion which led to their death within several days, whereas the mice harboring the WT transgene maintained a lower K⁺ level than control littermates.

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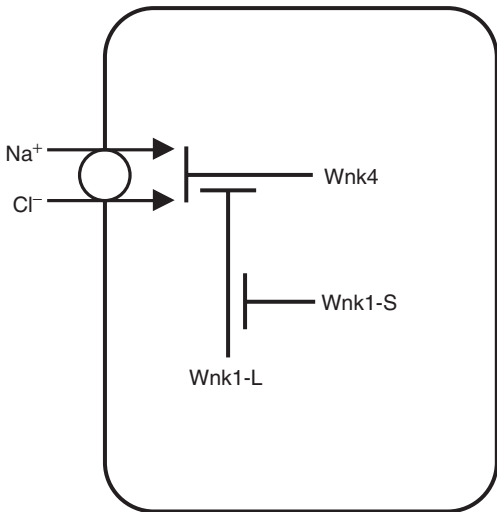


Fig. 1. Inhibition/down-regulation of Na-Cl cotransporter NCC by Wnk4. This simplified scheme shows that the Wnk4 mediated inhibition/downregulation of NCC can be relieved by full length Wnk1 (Wnk1-L=L-Wnk1). This effect of Wnk1-L indirectly activates NCC and can in turn be prevented by the kidney-specific short Wnk1 transcript Wnk1-S (KS-WNK1) that thereby indirectly inhibits NCC function/expression [5,19,20].

Inhibition of NCC by Wnk4 triggers dismantling of the DCT1 nephron segment

Morphological analysis of the kidneys by immunostaining showed that the main difference between the Wnk4 genotypes was the size of the first part of the distal convoluted tubule also called DCT1 (Figure 2) [1]. This is the segment that expresses as sole apical Na⁺ transport protein the thiazide-sensitive Na-Cl cotransporter NCC. Whereas in Tg(Wnk4^{FHHt}) mice this nephron segment was hypertrophied compared to littermates, its size (length and size of cells) was strongly reduced in Tg(Wnk4^{WT}) mice as previously observed after thiazide treatment of rats and in NCC^{-/-} mice [2,6]. Correspondingly, the amount of NCC mRNA was also strongly changed, whereas the mRNAs of ROMK1 and ENaC, the main luminal channels of the following tubular segments, were not much affected. To confirm the central role played by the Na-Cl cotransporter for this regulation, NCC^{-/-} alleles were bred into the Tg(Wnk4^{FHHt}) background. Importantly, the knock out of NCC reverted the phenotype of the Wnk4 FHHt transgenic

Wnk4 transgenic mouse model	Tg(Wnk4 ^{WT})	control	Tg(Wnk4 ^{FHHt})
DCT1 Na-Cl cotransport (NCC) segment			
CNT Electrogenic Na ⁺ transport (ENaC) segment			
Wnk4 mediated NCC-inhibition	+++	+	-
Resembling human monogenetic disease	Gitelman's Syndrome (NCC defect)		Familial hyperkalemic hypertension (Wnk4 ^{FHHt})
Diet inducing similar state	high K⁺ diet low renin-angiotensin and high aldosterone	normal diet low RAAS activity	low NaCl diet Unlike in FHHt, RAAS is activated and ENaC segments are stimulated

Fig. 2. Wnk4 switches the distal nephron from Cl⁻ reabsorption to K⁺ secretion. The impact of transgenic Wnk4 WT and mutant alleles is shown in a simplified two-compartment model [15]. As shown in the left panels, wild-type Wnk4 overexpression inhibits NCC and this leads to regression of the distal convoluted tubule segment DCT1, similar to that observed with pharmacological NCC inhibition by thiazides or with genetic NCC disruption (Gitelman's syndrome). The consequent lack of NaCl reabsorption in DCT1 increases the Na⁺ load to the following DCT2 and CNT segments that express ENaC such that increased electrogenic Na⁺ reabsorption drives K⁺ secretion through ROMK and maxi K channels. A similar situation is observed under high K⁺ diet. A mirror image situation corresponding to Familial Hyperkalemic Hypertension is observed when the dominant Wnk4 FHHt allele is expressed (right panels). This leads to a lack of NCC inhibition, such that very little Na⁺ reaches the following ENaC expressing segments, leading to a defect in K⁺ secretion. A similar activation of NCC is also observed when the renin-angiotensin-aldosterone system is induced by low NaCl diet. Unlike in FHHt, there is high aldosterone in this condition that stimulates ENaC expressing segments.

mice at the morphological and at the functional levels, to a phenotype very similar to that of the Wnk4 WT transgenic mice. However, the intriguing question of how Na–Cl cotransport activity via NCC controls the epithelial cell number and size of the DCT1 segment remains open.

Do the other Wnk4 functions described *in vitro* play a physiological role?

The reversal of the FHHt phenotype by knocking out NCC raises the question as to whether any action of Wnk4, other than NCC inhibition, is physiologically relevant. Indeed, many coexpression studies of wild-type WNK4 with proteins potentially participating in the FHHt phenotype suggested additional effects of this kinase, in particular the inhibition of ROMK1, TRPV4, NKCC1 and K⁺–Cl[–] cotransporters and the up-regulation of TRPV5 and of the paracellular Cl[–] permeability [7–13]. In this respect, one can conclude from the experiments presented in the paper of Lalioti *et al.* that in the physiological conditions tested, potential additional effects of Wnk4 are not required to explain the phenotypes [14]. This means probably that the functional effects mediated via NCC are clearly dominant in the tested overexpression conditions (Figure 2). However, this does not mean that some of the other effects described *in vitro* do not participate in the graded physiological effects that lie on the continuum between the tested extremes. In this context, it is important to note that the experiments described by Lalioti *et al.* clearly demonstrate that Wnk4 must have a tonic activity in control conditions, since the regulation of the NCC compartment by WNK4 can act both ways, towards building up or dismantling DCT1. The very powerful but quite energy-consuming K⁺ secretion system that is driven by Na⁺ reabsorption via ENaC can indeed function only when sufficient luminal Na⁺ is available downstream of the NCC compartment, that is when NaCl reabsorption via NCC is not maximal [15]. Interestingly, the ENaC and K channel-mediated Na⁺/K⁺ exchange system situated downstream of the NCC compartment appears to be activated even more efficiently by the need for increased K⁺ secretion than by low Na⁺ conditions [16,17].

Conclusions

Taken together, the study by Lalioti *et al.* stresses the central role of thiazide-sensitive Na–Cl cotransporter (NCC) regulation for extracellular volume control. Wnk4 is elegantly shown *in vivo* to be a strong negative regulator of NCC expression, whereas its mutant form which causes dominant FHHt has the opposite effect. By tuning NaCl reabsorption at the level of the DCT1, the regulatory network of WNK's switches the action of aldosterone from supporting

NaCl reabsorption, in the case of hypovolaemia, to that of stimulating the secretion of K⁺ in the case of hyperkalaemia. One clear difference between the stimulation of aldosterone in the context of hypovolaemia compared to its stimulation by hyperkalaemia is the activation via the renin–angiotensin system. As suggested by the authors of the Wnk4 transgene study, it could be that angiotensin II itself plays a regulatory role here, at the level of the kidney tubule [1].

However, how potassium intake controls Wnk4 activity and thus the activity of Na–Cl cotransport and the integrity of the distal convoluted tubule remain open questions. Finally, it appears likely that genetic variants of WNK's (expression) and thus of distal convoluted tubule integrity and function impact on individual arterial blood pressure regulation [1,18].

Conflict of interest statement. None declared.

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