WNK kinases, distal tubular ion handling and hypertension

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Familial hyperkalaemic hypertension (FHH) was originally described by Paver and Pauline in 1964 [1]. Also referred to as Gordon syndrome [2] or pseudohypoaldosteronism type 2 [3], the FHH syndromes are rare autosomal dominant diseases characterized by the unusual association of low renin hypertension with hyperkalaemia and metabolic acidosis despite normal glomerular filtration rate. These clinical and biological features are the mirror image of the Gitelman syndrome. Together with the peculiar sensitivity of FHH patients to thiazide diuretics [4,5], it suggests an increased sodium reabsorption through the thiazide-sensitive Na–Cl co-transporter (NCCT) as the main mechanism of the disease.

Genetic analyses, however, have excluded NCCT as being directly involved in all the families studied so far, but, instead, found evidence for three loci on chromosomes 1, 17 [6] and 12 [7], and for further genetic heterogeneity [8]. More recently, the two responsible genes corresponding to the chromosome 12 and 1 loci have been identified [9]. These genes do not correspond to ionic transporters but to unexpected proteins, WNK1 and WNK4, which are two closely related members of a novel serine–threonine kinase family. Their identification paved the way for experimental and clinical studies that recently have provided important new insights into the regulatory pathways that control ion transport in the distal nephron.

WNK4 is expressed predominantly in the kidney where it is restricted to the distal convoluted tubule (DCT), connecting tubule and collecting duct. Four discrete WNK4 missense mutations responsible for FHH have been identified in four different families. All are located within highly conserved short sequences respectively 20–25 amino acids downstream of the first and second coiled-coil domains of the enzyme [9] (Figure 1). These similarities and the absence of other classical loss-of-function mutations strongly suggested that their deleterious mechanism could be indirect, through modifications of the interplay between WNK4 and unknown partners [9]. Two groups have examined the hypothesis that WNK4 could regulate NCCT activity in vitro [10,11]. The co-expression of NCCT and WNK4 in Xenopus oocytes showed that WNK4 decreases thiazide-sensitive sodium transport. The marked decrease induced by WNK4 was the consequence of a nearly complete inhibition of NCCT insertion from the cytoplasm into the plasma membrane. One of the FHH-causing mutations, (Q562E)-WNK4, reduced NCCT activity significantly less than the wild-type WNK4. Although performed in non-renal cells, these studies strongly suggest that WNK4 is a negative regulator of the thiazide-sensitive sodium transport. The concomitant decrease induced by WNK4 was the consequence of a nearly complete inhibition of NCCT insertion from the cytoplasm into the plasma membrane. One of the FHH-causing mutations, (Q562E)-WNK4, reduced NCCT activity significantly less than the wild-type WNK4. Since calcium reabsorption is inversely related to sodium reabsorption in the distal tubule, the decreased NCCT activity in patients with the Gitelman syndrome or in patients receiving thiazide therapy is accompanied by hypocalciuria. Hypercalciuria would thus be expected in FHH if the disorder is mainly the consequence of an excessive NCCT activity (Figure 2).

Since calcium reabsorption is inversely related to sodium reabsorption in the distal tubule, the decreased NCCT activity in patients with the Gitelman syndrome or in patients receiving thiazide therapy is accompanied by hypocalciuria. Hypercalciuria would thus be expected in FHH if the disorder is mainly the consequence of an excessive NCCT activity (Figure 2).

Indeed a detailed analysis of an Israeli family bearing...
the (Q562E)-WNK4 mutation [12] showed that mean levels of urinary calcium excretion in affected subjects with FHH were more than twice as high as those of the unaffected relatives, thus providing strong support for an increased activity of NCCT as the underlying mechanism of the disease caused by this specific mutation. Nonetheless, a definitive conclusion must await the demonstration that WNK4 actually controls NCCT membrane expression in renal epithelial cells. An important issue will then be to determine if WNK4 is a specific negative regulator of NCCT or if this regulatory function extends to other apical and/or basolateral transport systems.

As WNK4 is expressed all along the distal nephron, it is not known if the proposed increase in NCCT activity occurs in the DCT, to which NCCT expression is restricted under physiological conditions, or if the mutation is responsible for a distal extension of the NCCT expression pattern to the connecting tubule (Figure 2). In other words, whether WNK4’s physiological role is to control the density of functional NCCT in the DCT, or to control the length of the transitional segment (DCT2) where NCCT and ENaC are co-expressed, remains to be determined.

WNK4 and paracellular permeability

One of the intriguing results obtained by Lifton’s group is the co-localization of WNK4 with the tight junction protein ZO-1 in the DCT, whereas downstream its expression seems cytoplasmic [9]. These results support the hypothesis that the kinase may subserve different, albeit not necessarily exclusive, physiological functions in the early and late distal nephron [9]. The marked preferential localization of WNK4 at the tight junction in the DCT suggests that, in this segment, WNK4 could participate in the regulation of paracellular permeability (Figure 3). Evidence that this is indeed the case, and that specific WNK4 mutations alter this function, would substantiate the chloride shunt hypothesis proposed by Schambelan et al. [3] >20 years ago. In this model, the consequence of enhanced paracellular leak of chloride from the lumen is the dissipation of the electrical transepithelial gradient that drives potassium secretion, resulting in volume-expanded hypertension and hyperkalaemia. It is possible that both increased NCCT activity and abnormal chloride shunting underlie the pathophysiology of WNK4-related FHH. The possibility remains open even that distinct muta-
tions alternatively cause the disease through one or the other mechanism since, unlike (Q562E)-WNK4, co-expression of two other disease-causing mutations, (D561A)-WNK4 and (E559K)-WNK4, had the same inhibitory effect on NCCT processing to the oocyte membrane as the wild-type WNK4 [11].

FHH and WNK1

Two main WNK1 isoforms have been identified: a ubiquitously expressed 12 kb transcript, and a more specifically and highly expressed 10 kb transcript in the kidney, where its expression seems restricted to the cytoplasm of distal epithelial cells. Disease-causing mutations are large deletions within the first intron, and have been suggested to lead to an increased expression of the short kidney-specific transcript (without alteration of the translated protein structure; Figure 1). The WNK1 enzyme could phosphorylate an as yet unknown substrate and/or interact with membrane proteins.

When co-expressed with NCCT in Xenopus oocytes, the long WNK1 transcript (L-WNK1) did not alter the resulting thiazide-sensitive sodium transport [11]. However, when NCCT was expressed with both WNK4 and L-WNK1, the inhibitory effect of WNK4 on NCCT surface expression was no longer observed [11]. These findings are consistent with the possibility that the physiological role of L-WNK1 is to counter-regulate WNK4’s negative control of NCCT surface localization (Figure 3). Whether the short kinase-defective isoform, that predominates in the distal tubule (and whose abnormally high expression is thought to cause the disease), also interacts with WNK4 directly or through L-WNK1 remains to be investigated (Figure 3).

Certainly, the unifying hypothesis is compelling that loss-of-function WNK4 mutations and gain-of-function WNK1 mutations similarly cause increased NCCT functional expression leading to excessive sodium reabsorption in the distal convoluted tubule. In that case, one would expect the two mutations to have comparable clinical consequences. However, phenotypic analyses of a large French family bearing the WNK1 deletion highlighted notable differences from the clinical feature resulting from the (Q562E)-WNK4 mutation [12,13]. In the French pedigree, urinary calcium excretion was normal and similar in both affected and unaffected subjects. This observation is not readily explained by the hypothesis that increased NCCT activity accounts for WNK1-induced FHH. Furthermore, hypertension, when present, was much less severe than in the Israeli family, and also did not develop until the fourth decade. Among the 17 affected patients, the eight youngest were normotensive, but presented metabolic abnormalities of similar severity to the hypertensive affected

Fig. 2. Location of water and solute transport systems and WNK kinases in the distal nephron.
members of the pedigree. In this family, the delayed development of hypertension as a manifestation of an inherited defect, contrasting with the early expression of hyperkalaemia, is difficult to reconcile with a model according to which hyperkalaemia results from primarily increased sodium reabsorption. Alternatively, a primary alteration of the normal regulation of aldosterone-dependent K\(^+\) secretion could underlie the disorder. Then, if hypertension is not, or not solely, the result of volume expansion, progressive alterations of peripheral vascular resistance with ageing are likely to be involved in its pathogenesis. The moderate but chronically sustained elevation of aldosterone in response to hyperkalaemia might perturb the normal long-term regulation of vascular remodelling, ultimately resulting in the development of high blood pressure. An alternative hypothesis is that WNK1, which unlike WNK4 is ubiquitous and is heavily expressed in different tissues such as heart and skeletal muscle [9,14], could be directly involved in the regulation of the vascular remodelling process through its expression in vascular smooth muscle cells.

Perspectives

The understanding of the respective physiological roles of WNK4 and WNK1 is in its infancy, and the various factors involved in the different pathophysiological forms of FHH remain to be more clearly defined. Unravelling the threads of these novel regulatory pathways will lead to a deepened understanding of the integrated physiology of ion transport in the distal nephron. The most exciting perspective offered by the discovery of the WNK kinases is the promise of unexpected insights into new mechanisms potentially involved in essential hypertension.

During the last 10 years, a significant number of genes causing relatively uncommon Mendelian hypertensive diseases have been identified. Interestingly, all of them are responsible for increased renal sodium reabsorption in the distal nephron with subsequent severe, low renin hypertension of early onset [15]. As recently outlined by Rossier [16], this puts on a firm basis Guyton’s hypothesis that long-term control of blood pressure in the steady state is critically dependent on the renal ability to excrete sodium. Although the molecular genetic characterization of these genes has largely confirmed a long-established role in sodium homeostasis of the corresponding encoded proteins (ion channels, transporters, enzymes or receptors), it is uncertain whether any of them plays a significant role in essential hypertension [17]. The unique feature of WNK1 among all these genes, which when mutated causes the blood pressure to increase with a time course mimicking essential hypertension, deserves particular attention. The opportunity to decipher mechanisms responsible for a progressive and moderate alteration of blood pressure control with ageing resulting in hypertension in middle adulthood opens up particularly relevant novel perspectives in hypertension research. Even if physicians have often been taught by physiologists that high blood pressure is just a matter of balance between salt intake and its renal excretion, they generally appreciate that salt sensitivity and suppressed plasma renin activity are not the most common features among essential hypertensives. If as discussed above, WNK1 mutations turn out to be responsible for a form of hypertension that is not exclusively salt dependent, the identification of the WNK kinases may well represent a major breakthrough in hypertension research.

Conflict of interest statement. None declared.

References

Calcium-sensing receptor and renal cation handling

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Renal tubular calcium reabsorption is a critical determinant of extracellular fluid (ECF) calcium concentration; for the need of constancy of ECF calcium concentration, the renal tubular handling of calcium is tightly controlled in order to match renal calcium excretion to the net amount of calcium entering the ECF. Both parathyroid hormone (PTH) and vitamin D metabolites are involved in the control of renal tubular calcium reabsorption and ECF calcium concentration [1]. Besides this hormonal control, it has been recognized recently that ECF calcium is able to regulate its own reabsorption by the mammalian tubule. Indeed, a large body of evidence supports the view that ECF calcium exerts this action by activating the calcium/polyvalent cation-sensing receptor (CaSR) located in the plasma membrane of many tubular cell types. First, increasing ECF calcium concentration elicits a marked increase in urinary calcium (and magnesium) excretion [2,3] and this occurs independently of any change in the calcium-regulating hormones [2,3]. Second, the inhibitory effect of ECF calcium on its own reabsorption is shared by other CaSR agonists, e.g. magnesium [4]. Third, the relationship between ECF calcium and urinary calcium excretion is altered in patients bearing mutations of the CASR gene: renal tubular calcium reabsorption is enhanced in patients with inactivating mutations [5,6] and decreased in patients with activating mutations. Therefore, there is abundant evidence that renal tubular CaSR plays a role in the control of divalent cations reabsorption under both normal and pathological conditions.

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