COMMENTARY

WNK lies upstream of kinases involved in regulation of ion transporters

Gerardo GAMBA¹

Molecular Physiology Unit, Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán and Instituto de Investigaciones Biomédicas, Universidad Nacional Autónoma de México, Tlalpan 14000, Mexico City, Mexico

Two members of a recently discovered family of protein kinases {WNK1 and WNK4 [with no K (lysine) kinases-1 and -4]} are the cause of an inherited disease known as pseudohypoaldosteronism type II that features arterial hypertension. The family is known as WNK due to a lack of the invariant catalytic lysine in kinase subdomain II. The mechanisms by which WNKs regulate blood pressure are beginning to be understood at the physiological level from recent studies showing effects of WNK4 on several plasma membrane co-transporters and ion channels. However, little is known about the function of WNKs at the biochemical level. In this issue of the *Biochemical Journal*, Vitari et al. have

shown that WNK1 and WNK4 interact with other kinases, SPAK (STE20/SPS1-related proline/alanine-rich kinase) and OSR1 (oxidative stress response kinase-1), which are involved in the regulation of ion transporters. WNK1 and WNK4 phosphorylate SPAK and OSR1, which in turn phosphorylate the N-terminal domain of the basolateral $\text{Na}^+/\text{K}^+-2\text{Cl}^-$ co-transporter, NKCC1. The phosphorylation site involved in SPAK or OSR1 activation is identified as a threonine residue within the T-loop.

Key words: hypertension, ion transport regulation, kinase interaction, phosphorylation, pseudohypoaldosteronism type II.

Arterial hypertension is one of the most common and dangerous diseases of the industrialized world, which occurs in approx. 20–25 % of the adult population. Arterial hypertension is an asymptomatic disease that accelerates the process of atherosclerosis, increasing the risk of myocardial infarction and stroke. Although the origins of hypertension are unknown, it is a prototype of so-called polygenic diseases, in which single ‘normal’ changes throughout the genome (SNPs, or single nucleotide polymorphisms) predispose individuals for increased susceptibility to environmental factors, e.g. salt consumption, that will induce the increase in arterial pressure. In addition, it is believed that SNPs will help to explain the different responses to anti-hypertensive drugs observed within the population (pharmacogenomics). Thus the more we understand the genes involved in regulating blood pressure, the more we will be able to understand their participation in this complex disease. One approach that has been successfully used involves defining the genes causing monogenic diseases exhibiting high- or low-blood pressure levels; if altered function of a single gene is enough to produce an abnormal change in blood pressure, it is highly likely that the gene could be involved in the polygenic cause of essential hypertension. More than 15 genes have been identified by this strategy [1], leading the way to understanding their roles in blood pressure regulation and hypertension.

The gene family known as WNKs [with no lysine (K) kinases] was discovered by Xu et al. [2] in a cloning effort designed to identify novel members of the MEK [MAPK (mitogen-activated protein kinase)/ERK (extracellular-signal-regulated kinase) kinase] family. Lacking the invariant catalytic lysine in kinase subdomain II, the first identified member of the family was named WNK1. These serine/threonine kinases attracted widespread interest after Wilson et al. [3] informed us that two members of the family, WNK1 and WNK4, are the cause of an inherited disease known as Gordon’s syndrome or PHAII (pseudohypoaldosteronism type II), which features arterial hypertension and hyperkalaemia.

The WNK family is composed of four genes encoding the kinases WNK1, WNK2, WNK3 and WNK4, located on chromosomes 12, 9, X and 17 respectively [4]. Sequence analysis reveals that WNKs are composed of an N-terminal kinase domain and a C-terminal regulatory domain. Mutations in WNK1 are due to intronic deletions that result in overexpression of the kinase, whereas WNK4 contains missense mutations in an acidic domain of 10 residues highly conserved at the beginning of the regulatory domain in all four WNKs. Because both WNK1 and WNK4 are expressed in the kidney [3], and the clinical profile of PHAII suggested a renal origin of the hypertension (hyperkalaemia, metabolic acidosis and hypercalciuria), analysis of the effects of WNKs on ion transport pathways was a logical step in the study of WNK biology. It is now known that WNK4 regulates the activity of several plasma membrane co-transporters and channels in the kidney, including the thiazide-sensitive Na^+/Cl^- co-transporter, the basolateral isoform of the bumetanide-sensitive NKCC1 ($\text{Na}^+/\text{K}^+-2\text{Cl}^-$ co-transporter), CFEX (the $\text{Cl}^-/\text{HCO}_3^-$ exchanger), ROMK (the inwardly rectifying K^+ channel) and the tight-junction proteins, claudins (for a review, see [4]). It is also known that PHAII-like mutations in WNK4 alter the way in which these transport pathways are regulated by WNK4, providing an initial physiological explanation for the development of both hypertension and hyperkalaemia. The mechanism of disease in patients with WNK1 mutations is less clear, but evidence supporting WNK1 regulation of WNK4 activity provides a clue.

Although the mechanisms by which WNKs regulate blood pressure are beginning to be understood at the physiological level, there is a great deal of work to be done to understand WNKs at the biochemical level. The initial biochemical characterization of WNK1 revealed its activation by autophosphorylation, which in turn is regulated by the presence of an autoinhibitory domain [5]. The autoinhibitory domain of WNK1 inhibits not only its autophosphorylation, but also that of WNK2 and WNK4 [6], suggesting that autophosphorylation, and thus presumably the

¹ email gamba@biomedicas.unam.mx or gamba@quetzal.innsz.mx

activity of WNKs, can be regulated by itself or by the other WNKs. The pathways through which WNKs exert their functions are not yet known, but evidence suggests that WNK1 could be a MAP4K (MAPK kinase kinase), because WNK1 activates the ERK5 (MAPK) pathway, which contains MEK5 and MEKK2/3 (MAPK/ERK kinase kinases 2 and 3) as its upstream regulators, and WNK1 activation of ERK5 was observed to be due to a MEKK2/3-dependent mechanism [7].

In this issue of the *Biochemical Journal*, Vitari et al. [8] shed new light on the biochemical mechanisms of action of WNK1 and WNK4 by showing that they are able to interact with other kinases known to be involved in regulation of ion transport proteins. The authors first used polyclonal antibodies against WNK1 to identify tissues with high expression of this kinase for subsequent immunoprecipitation analysis and observed the highest level of expression in the testes. Four protein bands were detected in the WNK1 immunoprecipitate that were not seen in the control: two corresponded to WNK1 and WNK3, which was not a surprise since it is known that WNKs interact with each other [6]; a third band was the SDB84 antigen, but direct interaction between WNK1 and SDB84 was not confirmed; and the fourth band was a member of the STE20 family of kinases known as SPAK (STE20/SPS1-related proline/alanine-rich kinases). This last observation was pursued since it has been shown that SPAK, and the related OSR1 (oxidative stress response kinase-1), are involved in regulation of the basolateral isoform of NKCC1, and that WNK4 and SPAK can interact in a yeast two-hybrid system [9,10]. Of the four members of the STE20 family, the authors observed that WNK1 and WNK4 are able to physically interact with SPAK and OSR1, but not with the pseudokinases STRAD α (STE20 related adaptor α) and STRAD β . Most importantly, using the kinase domain of WNK1 and WNK4, they went on to show that WNK1 and WNK4 phosphorylate SPAK and OSR1. Phosphorylation was more intense in OSR1 than in SPAK, and required the kinase activity of WNKs, because concurrent experiments using kinase-dead WNK1 or WNK4 resulted in no phosphorylation of SPAK or OSR1. To investigate the effect of SPAK and OSR1 phosphorylation by WNKs, the authors analysed the effect of WNKs and SPAK or OSR1 on phosphorylation of the N-terminal domain of NKCC1. This was a very important experiment, because it is well known that the N-terminal domain of this co-transporter contains two threonine residues that are phosphorylated during its activation [11], and that two SPAK-binding motifs are located upstream of these threonine residues [10], in addition to experimental data suggesting that SPAK regulates NKCC1 activity [10,12]. The authors observed that SPAK or OSR1 induced phosphorylation of the NKCC1 N-terminal domain, but only when co-incubated with WNK1 or WNK4. Any one of these kinases alone, i.e. WNK1, WNK4, SPAK or OSR1, had no effect on NKCC1 phosphorylation. In addition, the kinase activity of both STE20 and WNK was required. Thus co-incubation of wild-type SPAK or OSR1 with kinase-dead WNK1 or WNK4, or co-incubation of kinase-inactive SPAK or OSR1 with wild-type WNK1 or WNK4, resulted in no phosphorylation of NKCC1.

Thr-233 and Ser-373 (Thr-185 and Ser-325 in OSR1) were identified as the phosphoacceptor sites in SPAK. Finally, phosphorylation of OSR1 at Thr-185, but not at Ser-325, was shown to be required for activation. In summary, Vitari et al. [8] demonstrated that WNK1 and WNK4 interact with SPAK and OSR1 and phosphorylate these kinases at two sites. Phosphorylation within the T-loop activates SPAK and OSR1, which phosphorylate the N-terminal domain of NKCC1.

The results of Vitari et al. [8] are important, because they show that WNKs can interact, physically and functionally, with

key kinases already known to be important in the regulation of ion transport pathways. Another recent study [13] shows that WNK1 activates the serum- and glucocorticoid-induced protein kinase (SGK1), which is also known to be involved in regulation of ion transport pathways in the distal nephron of the kidney. The study of Vitari et al. [8] is purely biochemical; however, a concomitant study provides the physiological counterpart of their findings. Gagnon et al. [14], following their previous observations that SPAK interacts with NKCC1 and that SPAK and WNK4 can interact in a yeast two-hybrid system [9,10], studied the effects of SPAK and WNK4 together on the activity of NKCC1. Gagnon et al. [14] observed that co-injection of *Xenopus laevis* oocytes with NKCC1, SPAK and WNK4 cRNA resulted in a significant increase in NKCC1 activity and insensitivity to external osmolarity or cell volume. In this regard, it is known that NKCC1 is activated by external hypertonicity (induces cell shrinkage) and inhibited by hypotonicity (induces cell swelling), accompanied by phosphorylation or dephosphorylation respectively, of the N-terminal domain threonine residues [11]. Thus the fact that SPAK and WNK4 together activate NKCC1 and render this co-transporter insensitive to hypotonicity strongly suggests that SPAK and WNK4 together phosphorylate the N-terminal domain threonine residues of NKCC1. This hypothesis was confirmed by Vitari et al. [8]. Because a previous study showed that WNK4 alone resulted in significant inhibition of NKCC1 activity [4], and Vitari et al. [8] show that WNK4 alone or WNK4 kinase-inactive mutant cannot phosphorylate NKCC1, it is tempting to speculate that WNKs probably possess both activities. That is, they can act as activators and inhibitors of the same transport pathway, depending on their ability to interact with other kinases involved. Some of these effects will depend on the kinase activity of the WNKs, as with SPAK and OSR1 [8], whereas others will depend more on WNKs' protein-protein interactions, and not on their kinase activity, as with SGK1 [13]. Thus the observations of Vitari et al. [8] provide significant clues to understanding WNKs at the biochemical level. This is a very important issue, since WNKs are potential targets for new anti-hypertensive drugs, and because they probably hold important biochemical explanations for the development of arterial hypertension.

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