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Biochimica et Biophysica Acta 1802 (2010) 1140–1149

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbadis

Review

Intracellular sodium sensing: SIK1 network, hormone action and high blood pressure ☆

Ariel Jaitovich [1], Alejandro M. Bertorello *

Membrane Signaling Networks, Atherosclerosis Research Unit, Department of Medicine, Karolinska Institutet, Karolinska University Hospital-Solna, 171 76 Stockholm, Sweden

ARTICLE INFO

Article history:
Received 30 November 2009
Received in revised form 12 March 2010
Accepted 20 March 2010
Available online 27 March 2010

Keywords:
Na^+
K^+-ATPase
High blood pressure
Dopamine
Angiotensin II
Aldosterone
Adducin
Salt-inducible kinase
Gene regulation

ABSTRACT

Sodium is the main determinant of body fluid distribution. Sodium accumulation causes water retention and, often, high blood pressure. At the cellular level, the concentration and active transport of sodium is handled by the enzyme Na^+,K^+-ATPase, whose appearance enabled evolving primitive cells to cope with osmotic stress and contributed to the complexity of mammalian organisms. Na^+,K^+-ATPase is a platform at the hub of many cellular signaling pathways related to sensing intracellular sodium and dealing with its detrimental excess. One of these pathways relies on an intracellular sodium-sensor network with the salt-inducible kinase 1 (SIK1) at its core. When intracellular sodium levels rise, and after the activation of calcium-related signals, this network activates the Na^+,K^+-ATPase and expel the excess of sodium from the cytosol. The SIK1 network also mediates sodium-independent signals that modulate the activity of the Na^+,K^+-ATPase, like dopamine and angiotensin, which are relevant *per se* in the development of high blood pressure. Animal models of high blood pressure, with identified mutations in components of multiple pathways, also have alterations in the SIK1 network. The introduction of some of these mutants into normal cells causes changes in SIK1 activity as well. Some cellular processes related to the metabolic syndrome, such as insulin effects on the kidney and other tissues, also appear to involve the SIK1. Therefore, it is likely that this protein, by modulating active sodium transport and numerous hormonal responses, represents a “crossroad” in the development and adaptation to high blood pressure and associated diseases.
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1. Sodium—a double-edged sword

Sodium, the most important determinant of extracellular osmolarity, plays a crucial role in the homeostasis and compartmental distribution of body fluids. In fact, water – the main intracorporeal solvent – is dragged across membranes by the osmolar differences between body compartments. No energy is spent *directly* to move water; instead, pumps and transporters move sodium ions across membranes, changing and balancing the osmolar composition of body compartments, thus *indirectly* affecting the distribution of water [1].

Frequently, the sodium concentration in blood, or *natremia*, reflects the water distribution status rather than the net sodium content. Most patients with hypernatremia are dehydrated, and similarly many of those with hyponatremia are over hydrated (dilutional hyponatremia). Indeed, patients with edema can show normal sodium concentrations in plasma but nonetheless have a total excess of sodium contained in the excess water in the form of edema [2]. Alterations of sodium homeostasis can cause water to accumulate abnormally in the intravascular compartment leading to blood pressure elevations, as occurs in patients with sodium-sensitive high blood pressure [3]. Most of these patients are instructed to limit their intake of sodium, but not of water, and are frequently given natriuretic drugs to control the blood intravascular volume and so the blood pressure [3]. In patients with heart failure, similarly, ingestion of free water scarcely affects their extracellular volume status, as opposed to sodium excess, which causes volume overload and eventually anasarca (disseminated edemas) and pulmonary edema [4]. Sodium restriction is the paramount measure to prevent abnormal accumulation of water in these patients [4]. Patients with liver cirrhosis and portal hypertension who consume too much sodium accumulate water in the peritoneal cavity and develop ascites; if this happens, they are requested to limit their sodium intake and take natriuretic drugs to correct the water excess [5]. Under ischemic conditions such as acute stroke, the amount of energy supplied to cells may be inadequate to maintain active sodium transport across the plasma membrane. Sodium then accumulates in the intracellular compartment [6]. This new status causes intracellular edema and cellular swelling which, because of the non-compliant nature of the skull, leads to intracranial hypertension and further exacerbates the injury. In fact, a common strategy to prevent intracranial injury is to

☆ This article is dedicated to the memory of Professor Adrian I. Katz (University of Chicago), a dear friend and a great mentor.

* Corresponding author.
E-mail addresses: ajaitovich@ccbhs.org (A. Jaitovich), alejandro.bertorello@ki.se (A.M. Bertorello).
[1] Present address: Department of Medicine, Cook County (Stroger) Hospital, 1901 W. Harrison St, Chicago, IL 60612, USA.

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doi:10.1016/j.bbadis.2010.03.009

administer hypertonic solutions to render the extracellular compartment relatively hyperosmolar and, as a consequence, to shrink cells back [7]. In critically ill patients, inflammatory injury to the lung renders the pulmonary epithelium incapable of handling water reabsorption, which causes accumulation of fluid in interstitial and alveolar space [8]. This situation is resolved when the alveolar epithelial cells recover their capacity to transport sodium back from the alveolar space to the blood [9]. Given that the resolution of pulmonary edema is associated with decrease of mortality of critically ill patients [10], there have been many attempts to manipulate sodium transport in order to hasten the reversion of lung edema. The use of diuretics [11], sodium restriction [12], delivery of sodium transporters to the air space by using aerosolized viral vectors [13] and other measures [14] have been proposed to hasten the resolution of pulmonary edema.

It has also been suggested that sodium can jeopardize the homeostasis not only through its effect on water but also on its own. Indeed, the "normal saline" solution commonly used in clinical settings (that contains 154 mmol of sodium per liter of water as opposed to the ~140 mmol/l in plasma), might be relatively harmful compared with other formulas such as Ringer's, which has a lower sodium content [15].

In summary, sodium is the primary determinant of water content in the body compartments and its misdistribution can cause intra- and extracellular edema, excess of intravascular water, and hypervolemia. In addition, independently of its osmotic properties, sodium excess can be deleterious by affecting the ionic composition of body fluids, pH, etc. Finally, as described in the next sections, intracellular sodium *per se* can influence the actions of hormones and also trigger activation of numerous signal transduction pathways, affecting multiples cellular processes.

2. Sodium sensing—a matter of cell survival

As primitive organisms originated in the salty oceanic medium, cells had to deal with the potential osmotic insult caused by entrance of sodium [22]. Indeed, given that sodium is potentially toxic for many biologically relevant components [16], cells had to develop mechanisms to cope with this challenge. The adaptations that have evolved over millions of years are both complex and extremely diverse. Some cells, such as those in plants, have a semi-rigid wall that prevents significant volume changes that might be detrimental to cellular functions. The disadvantage of that "solution" is that as multicellular organisms, plants with semi-rigid cell walls are sessile, confined to relatively fixed locations, which limits their ability to escape from adverse environmental conditions [17]. Although bacteria also have a semi-rigid cell wall, they can migrate long distances; yet their unicellular nature limits their complexity [17]. Mammalian cells, despite being "extremely modern" in evolutionary terms, cannot survive without proper control of intracellular sodium. Indeed, the aqueous environment in which most mammalian cells are embedded poses a threat of *death by flooding* due to the Donnan effect. The water-attracting power of this effect is related to the number of intracellular molecules, which in turn is proportional to the structural complexity of the cytosol [18]. Animal cells compensate this "sucking force from inside" by pumping sodium towards the extracellular milieu through the Na^+,K^+-ATPase located in the plasma membrane [19]: when Na^+ is actively ejected from the cell, water passively follows. In some tissues the cost of this process corresponds to as much as 40% of the total ATP produced. Without the Na^+,K^+-ATPase, evolutionarily advanced cells are defenseless against the sodium insult [20].

The Na^+,K^+-ATPase belongs to a superfamily of proteins known as P-type ATPases, whose evolutionary links have been traced through cloning and sequencing efforts [21]. It is accepted that the Na^+,K^+-ATPase appeared only once in evolution and developed from a proton ATPase that constituted the primary cell volume regulator at the time life evolved in the oceans [22]. Some have suggested that plants originated in fresh water and for that reason were not challenged with the sodium stress, an event that presumably resulted in the development of the semi-rigid wall [17]. These observations are significant because they suggest that Na^+,K^+-ATPase-mediated transport is not a dispensable function but an essential *requirement* for evolution of animal cells. Many of the critical properties of animal cells – not to mention the cells themselves – might never have developed without the appearance of the Na^+,K^+-ATPase. Some of these properties are indirectly yet fundamentally affected by the Na^+,K^+-ATPase activity. As indicated, the lack of the "straitjacket" represented by a semi-rigid wall allows animal cells to integrate themselves in a complex way that makes possible the stereotyped development pattern observed in advanced species. That complexity also demands a diverse array of proteins to guide the construction of sophisticated cellular networks. Indeed, that intracellular complexity in turn contributes to the cytosolic osmolarity that parallels the evolutionary level of living organisms. Not surprisingly, genes related to both cell adhesion and signaling, and genes encoding DNA-binding proteins, which are critical for the development of multicellular eukaryotes, are significantly more abundant in the highly evolved organisms such as *Homo sapiens* [23].

The Na^+,K^+-ATPase constitutes a highly versatile membrane transport system, as the same Na^+ gradient drives the transport of H^+, HCO_3^- and glucose. In other words, cells make use of their strategy for coping with sodium to perform other critical functions as well, such as nutrient uptake and pH regulation [24]. In some tissues, epithelia for example, the localization of the Na^+,K^+-ATPase to the basolateral domain of the plasma membrane is critical to vectorial transport (i.e. from one particular place to another) of water and solutes. In fact, loss of the pump's polarized localization is characteristic of the renal acute tubular necrosis and causes oliguria secondary to the inability to transport water and sodium across the tubular epithelium [25].

Due to its exchange stoechiometry of 3 Na^+ against 2 K^+, the sodium pump is, in contrast to the other P-type ATPases, *electrogenic* and thereby contributes by its pumping activity directly to the membrane potential. Many functions like ion channels' opening and closure in excitable and nonexcitable cells would not be possible without the activity of the Na^+,K^+-ATPase that establishes the Na^+ gradient across plasma membranes. It has been suggested that a single residue is responsible for the electrogenic property [26] and that mutation in this position can abolish such property. Conversely, the incorporation of this residue into the highly homologous, but non-electrogenic H^+,K^+ gastric ATPase can render it electrogenic, which suggests a high degree of evolutionary refinement in the development of this property [26]. Interestingly, both Na^+,K^+-ATPase and H^+,K^+-ATPase (gastric) belong to the same branch of the P-type ATPases' phylogenetic tree, so this subtle change seems to have occurred over a short evolutionary time span and enabled the diversification of tissues [27,28]. In summary, sodium challenge appears to have caused organisms to develop very sophisticated mechanisms that rendered them able to perform functions of which primitive and simpler cells were incapable.

As one delves deeper into the complexity of maintaining cell sodium and water homeostasis, it becomes clear that the presence of Na^+,K^+-ATPase in the plasma membrane provides a crucial platform that enables various specialized mechanisms to work in a concerted manner. Because so much depends on its function it is reasonable to think that cells might be better "armed" if they have in place a sodium-dependent signaling network controlling key mechanisms (active transport via the Na^+,K^+-ATPase) responsible for its cellular- and many other secondary-derived homeostatic processes. Because of the chaotic nature of the behavior of ions in solution, it is inferred,

hypothetically, the need for a considerable speed of diffusion of the sodium ions within the intracellular milieu (largely dependent on their effective radii) and also that this motion respect certain hierarchy (avoid deleterious cross-talk). A required signaling system can fulfill this criterion by amplifying, help to segregate in time and space and simultaneously propagate at certain speed the signal, and eventually efficiently shut down the process (Fig. 1). In the search for potential regulators, immunochemical methods were used to isolate proteins that were associated with the Na^+,K^+-ATPase subunits, and these proteins were then identified by mass spectrometry. The rationale for this strategy was that data obtained through the years showed that most, if not all, regulators of Na^+,K^+-ATPase activity, trafficking and signaling do so by physically interacting with the Na^+, K^+-ATPase subunits in what can be called a Na^+,K^+-ATPase-regulatory complex [29–35]. One of such molecule is the recently identified salt-inducible kinase 1 or SIK1 [36].

3. Sodium sensing—the SIK1 network

Salt-inducible kinase 1 (SIK1) was originally identified from adrenal gland of rats receiving a high salt diet [37]. It is also known as sucrose nonfermenting 1-like kinase (snf1-lk), an enzyme originally described in yeasts [38]. A myocardial snf1-lk was identified, but later studies indicated that it was snf1-lk, and the myocardial form was therefore renamed [39,40]. SIK1 belongs to the AMPK family of kinases [41] and has two related isoforms QIK (SIK2) and QSK (SIK3). The SIK1 gene is located in chromosome 21q.22.3 and has been well characterized. The QIK and QSK genes, however, are less well defined. These two genes are located close together on chromosome 11q23.1 and 11q23.3, respectively. All three isoforms have different molecular mass, a conserved kinase and ubiquitin domain [42] and large variability within regulatory regions. Among the isoforms, SIK1 has the better characterized regulatory region. A nuclear import/export domain spans between positions 567 and 612 of the amino acid sequence [43]. Several kinase regulatory sites have been identified, such as cAMP-dependent kinase (Ser-577) [44], calmodulin kinase (Thr-322) [36] and LKB (Thr-182) [45] as well as an autophosphorylation residue at Ser-186 in the A-loop, which is of importance for its kinase activity [46].

In renal epithelial cells, SIK1 associates with the Na^+,K^+-ATPase and regulates the maximal pumping capacity of the Na^+,K^+-ATPase measured by an activity assay (Fig. 2). It is not known whether SIK1 interacts directly with the enzyme's α- or β-subunit (or other subunits) or whether the interaction is structured via a linker protein. A modest rise in intracellular sodium results in increases in Na^+,K^+-ATPase activity, and this effect is blocked if SIK1 activity is suppressed either by reducing its cellular expression (by means of siRNA), or by transiently overexpressing a kinase-deficient SIK1 [36]. The fact that basal Na^+,K^+-ATPase activity (at normal intracellular sodium) remains unaffected under conditions when SIK1 activity is suppressed, indicates the possibility that only a selected pool of Na^+,K^+-ATPase subunits are subject to regulation via SIK1 upon changes in intracellular sodium. This pool is likely located at or in the vicinity of the plasma membrane. The results also suggest that SIK1 is probably not involved in the regulation of Na^+,K^+-ATPase molecules responsible for constitutive transport. Increases in sodium permeability are associated with transient increases in intracellular calcium [47], and it is not through sodium ions directly but via a calcium–calmodulin-dependent process that SIK1 becomes activated. CaMK1 phosphorylates a Thr-322 residue within SIK1, and removal of this residue in intact cells blocks the increases in Na^+,K^+-ATPase activity that normally occur when the intracellular concentration of sodium rises (Fig. 2). In transporting epithelia, phosphorylation of the Na^+,K^+-ATPase α-subunit has been associated with clathrin-dependent endocytosis of active α/β complex from the plasma membrane and with the reduction in active cell sodium transport [48,49]. It has been suggested that dephosphorylation of the Na^+,K^+-ATPase leads to the elevated catalytic activity in response to physiological or pharmacological stimuli [50]. In the presence of elevated intracellular sodium, the Na^+,K^+-ATPase α-subunit undergoes a calcium-dependent dephosphorylation [36]. This effect was abolished in cells transiently overexpressing an SIK1 mutant (lacking catalytic activity) but not in cells expressing wild type SIK1 [36]. In renal epithelial cells, increases in intracellular sodium activate protein phosphatase 2A (PP2A) in an SIK1-dependent manner, and the mechanism by which SIK1 controls PP2A activity appears to involve activation of a phosphomethylesterase-1 (PME-1) [36], possibly through its SIK1 consensus phosphorylation site ($\underline{V}^{69}Y\underline{K}SGSEGP\underline{V}$) (Fig. 2). Both PP2A (dimer PP2Ac/R65) and PME-1 (which is known to suppress PP2A activity by demethylation of its catalytic subunit [51]) associate with the Na^+,K^+-ATPase complex (Fig. 3) [36]; however, phosphorylation of PME-1 only favors its dissociation from the Na^+,K^+-ATPase/PP2A complex and permits the access of the PP2A regulatory subunit (Bs) to the PP2A catalytic subunit, which remains bound to the Na^+,K^+-ATPase (Fig. 3, *insert*). Thus, it is in heterotrimeric form (PP2Ac/R65/Bs) that PP2A develops its full activity [52]. We speculate that the association of a catalytically inactive PP2A with the Na^+,K^+-ATPase has a teleological justification because otherwise the phosphorylation state of Na^+,K^+-ATPase subunits would always be tilted toward a dephosphorylated/active state. However, experiments performed in intact cells revealed that a proportion of Na^+,K^+-ATPase α-subunits are in a phosphorylated state [48,49], suggesting the existence of various pools of Na^+,K^+-ATPase that might possibly be regulated by different signaling networks and under different conditions.

What are the structural arrangements in the cell that can hold the components of this network together and ensure that it can be activated in a hierarchical manner without any deleterious cross-talk? One possibility is the scaffolding system already known to be utilized by G protein-coupled receptors [53]. At the center of this scaffolding system is the multiple PDZ domain protein PATJ (Pals-associated tight junction protein). It is present in polar epithelial cells and cell lines derived from the renal or intestinal epithelia [54] and participates in the regulation of

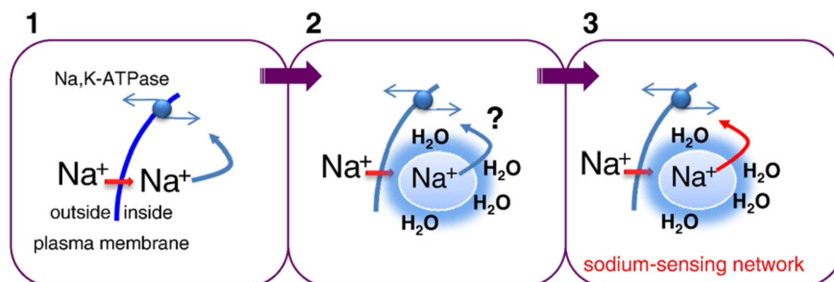

Fig. 1. Hypothetical explanation for why animal cells need an internal sodium-sensing network. Increases in sodium ions could increase Na^+,K^+-ATPase activity directly [1], however, considering that the radius of Na^+ ions in solution becomes larger due to the hydration cloud, and ionic movement is impeded [2], it is possible that a signaling network triggered by sodium ions mediates this effect [3]. A signaling network would provide accurate time and space control of active sodium transport.

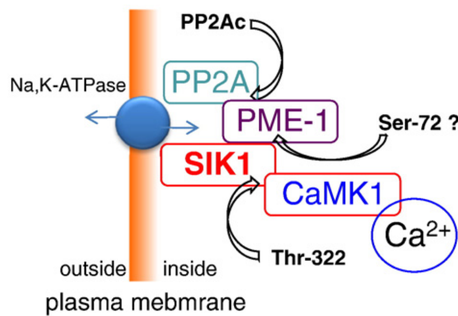

Fig. 2. Known partners comprising the SIK1 network that regulates active sodium transport. CaMK: calcium–calmodulin kinase; PME-1: phosphomethylesterase 1; PP2A: protein phosphatase 2A; NK: Na^+,K^+-ATPase; SIK1: salt-inducible kinase 1. Arrows denote potential interactive/phosphorylation domains.

Na^+,K^+-ATPase activity [55,56]. It appears that PATJ is at the crossroads of dopamine, angiotensin II and insulin signals during regulation of Na^+, K^+-ATPase activity in renal epithelial cells derived from proximal tubule segments. Different PDZ domains within PATJ are responsible for mediating stimulatory (angiotensin II and insulin) versus inhibitory (dopamine) actions on Na^+,K^+-ATPase activity. Whether SIK1 is functionally linked to this scaffolding network remains to be explored. Similarly, and also in renal epithelial cells, another PDZ domain-containing protein (sodium hydrogen exchanger regulatory factor-1) participates in the regulation of Na^+,K^+-ATPase activity in response to parathyroid hormone [57].

Are there other sodium-regulatory processes directly involved in cell sodium homeostasis that could be mediated by activation/repression of SIK1 activity? Epithelial sodium channels (ENaC) constitute the major pathway of sodium entry into cells of the aldosterone-sensitive distal nephron. These channels are confined to the apical domain of epithelial cells and together with the Na^+,K^+-ATPase (which are confined to the basolateral domain), constitute the basis of vectorial sodium transport. Similar to the Na^+,K^+-ATPase, the plasma membrane ENaC activity is regulated upon changes of intracellular sodium [58]. This phenomenon has been attributed to sodium ions themselves operating through a feedback mechanism that regulate the availability of ENaC at the plasma membrane. It has recently been demonstrated that changes in intracellular sodium concentrations affect the proteolytic cleavage of ENaC [58]. It is not yet resolved whether this cleavage and/or a cleavage-independent regulation of ENaC are triggered directly by sodium ions or indirectly through a signaling mechanism, where SIK1 could possibly be involved. As mentioned before, the increases in intracellular sodium elicited by monensin are paralleled by transient increases in intracellular calcium; therefore, it is plausible that secondary increases in intracellular calcium could play a role via CaMK-SIK1 activation.

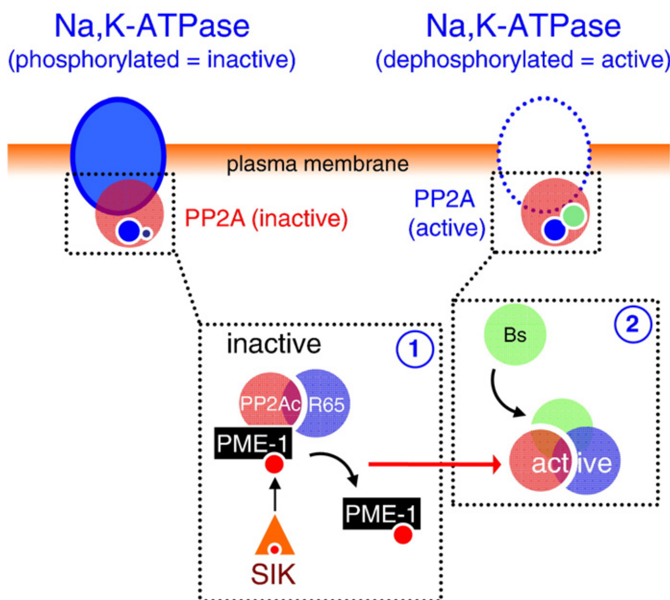

Fig. 3. Mechanism of PP2A activation by methylation/demethylation. An inactive PP2A complex associates with the Na^+,K^+-ATPase. *Inset 1:* PME-1 binds to the catalytic subunit PP2Ac (which exists as a dimer bound to the regulatory subunit, R65). SIK1-dependent phosphorylation of PME-1 favors its dissociation from the catalytic subunit, permitting the binding of the third PP2A regulatory (Bs) subunit, and thereby generating the active (heterotrimer) PP2A (*Inset 2*).

SIK1 is not present exclusively in animal cells but is also found in homologous form in plants [59] where a vacuolar proton pump is activated by SOS2 (a snf-kinase) [60,61] via SOS3 (a Ca^{2+}-dependent kinase) [62]. This network becomes critical in the adaption (survival) of plants to drought or to increases in the salinity of the soil. These observations further highlight the relevance of this evolutionary highly conserved network for the maintenance of cell sodium homeostasis.

Teleologically, the existence of a signaling system (with CaMK-SIK1 at its core) mediating the actions of sodium on its own transport has several potential explanations. First, in view of the random motion of molecules in solution, it could facilitate the "encounter" between sodium ions and the transporters. Because molecules and ions in solution move randomly and the chances of them interacting with a receptor depend on their concentration, the existence of an SIK1 network suggests that sodium actions in the cell may not only be the consequence of behaving *purely* as a randomly moving molecule in solution. In this hypothetical model, the "sodium signal" instead of "sodium ions" could traffic through the different levels of the SIK1 network using it as a molecular track that directs the signal towards the target (Na^+,K^+-ATPase), without the need of a bulk increase in intracellular sodium and in similar fashion to calcium signals through kinases, or to phosphate transfer over the different MAP kinases' signaling pathways in the process of phosphorelay. Second, it could set a hierarchical organization and coordinate the work of different mediators. Third, it could enable cells to amplify a sodium-triggered signaling process that causes sodium to be extruded from the cell. Without such amplification, the signal might require a transient, massive increase of intracellular osmolarity, which would jeopardize the cell by the potential "intracellular flooding" caused by the sodium entrance (Fig. 4). Instead, a subtle increase in sodium permeability would be sufficient to activate intracellular mechanisms that, without significantly affecting the osmotic load, would exert an effect that otherwise, with no intermediate network, could carry the risk of cell death by flooding (Fig. 4).

4. SIK networks—in addition to sodium sensing

Notwithstanding its crucial role in sodium homeostasis, the SIK network also appears to mediate the action of hormones that react to perturbations in that homeostasis as well as being involved in mechanisms where ionic imbalances have no obvious influence. The lack of ionic imbalance may be real, or it may be that the available data – though suggestive – still does not convincingly show the involvement of ion status. For example, SIK-dependent mechanisms associated with insulin action and glucose metabolism have recently been characterized. The SIK2 isoform phosphorylates (at Ser-794) the insulin receptor substrate-1 [63], and the activity/expression of SIK2 isoform is elevated in white adipose tissue from diabetic mice. Moreover, SIK2 can be activated in adipocytes by nutrient deprivation, whereas SIK2 activation suppresses the expression of lipogenic genes [64]. It is possible that secondary changes in ion gradients (amino acid-dependent transporters) may contribute

directly to increase SIK2 expression, or directly via the sodium-dependent SIK1 pathway. Of interest, in brown adipocytes, the SIK2-TORC2 signaling cascade mediates the effects of insulin on peroxisome proliferator-activated receptor-coactivator-1α and uncoupling of protein-1 gene expression [65].

The mediatory role of SIK in the actions of insulin extends beyond the adipose tissue. In hepatocytes, SIK influences TORC activity by direct phosphorylation [66]. SIK2-dependent phosphorylation of TORC and its ubiquitination is utilized by insulin during inhibition of gluconeogenesis [67]. Also in hepatocytes, SIK1 participates in the control of lipogenesis via phosphorylation of sterol regulatory element-binding protein 1c [68]. TORC is a key regulator of fasting glucose metabolism, and glucagon utilizes this mechanism to facilitate hepatic glucose production via the stimulation of gluconeogenic genes [69]. This effect requires the dephosphorylation of TORC by a coactivator of cyclic AMP responsive element (CRE)-binding protein (CREB), via calcineurin and possibly the suppression of cAMP-dependent phosphorylation of TORC via the SIK2 isoform [70].

LKB1 is a tumor suppressor kinase involved in many pathophysiological processes [71]. LKB1 regulates the cellular expression and activation of SIK1 [71], and the molecular mechanisms that mediate this process appear to involve the phosphorylation of threonine residues within the SIK A-loops. LKB1 and the downstream SIK pathway also play an important role in controlling CREB activity via the phosphorylation of TORC. Recent observations provided compelling evidence indicating that SIK1 mediates the link between LKB and p53, a critical regulator of apoptosis [72]. Loss of SIK1 function is associated with down-regulation of p53 and increases cell invasiveness in certain forms of cancer.

5. Sodium sensing—hormone actions in renal epithelia

Whereas renal sodium excretion can be affected by regulating the renal plasma flow and glomerular filtration rate, most hormones under physiological variations of salt intake influence sodium homeostasis by modulating its transport across the renal tubule segments. By affecting the activity of specific transporters (Na^+,K^+-ATPase, Na^+-antiporters, Na^+-channels, etc.), hormones can influence the vectorial sodium transport within specific segments of the nephron and thereby increase/decrease sodium reabsorption [73–75]. The multiple and concerted actions of diverse hormones along the nephron segments all serve the ultimate goal of adjusting the sodium excretion rate down or up depending whether the organism has sensed a status of sodium depletion or overload. Importantly, the final action of certain agents (such as aldosterone, atrial and brain natriuretic factors) will depend directly on their cellular levels and their capacity to bind their specific receptors. In other nephron segments, whenever natriuretic and antinatriuretic hormones operate simultaneously, such regulation becomes more complex and not fully understood.

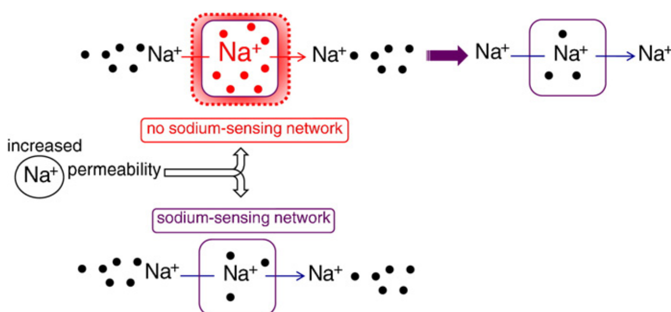

Fig. 4. Potential scenarios upon increase in sodium permeability in cells with/without a cell sodium-sensing network. Lack of a sodium-sensing network may require transient increases in intracellular sodium during vectorial transport.

Most physiologic processes occur through reactions involving molecules in water solutions. Without these reactions, life as we know it would simply not be possible. As water distribution in the body is driven by osmolar strengths – of which sodium is a main determinant – the forces aimed at sodium conservation prevail in the human organism: they serve to maintain the amount of water needed as the “body solvent”. This means that in the absence of sodium intake, the renal epithelia will reabsorb it from urine in order to maintain water stores. In certain segments, such as proximal convoluted tubules (where the bulk of sodium is reabsorbed), sodium conservation is supported by prevailing antinatriuretic mechanisms usually under euvolemic conditions (i.e. angiotensin, at low concentrations, increases tubule Na^+,K^+-ATPase activity) [76]. Similarly, in intact cells, the Na^+,K^+-ATPase operates well below its maximal capacity [77], leaving considerable margin for increase in its activity if physiologically needed. Contrary to the constitutive sodium-retention mechanisms, in renal proximal tubules there is a facultative dopamine-mediated system that gets activated when there is an excessive sodium load [78,79]. This system is initially triggered by an increase in dopamine production within the proximal tubule [78] and an increase in the number of dopamine receptors at the plasma membrane of renal proximal tubules [80]. Cells in this segment do not store dopamine: rather, they produce it directly from its precursor, L-DOPA, the availability of which thus determines dopamine production. The cell transport (inward/outward) of L-DOPA is mediated in part (~25%) via a Na^+-dependent transporter and also via a Na^+-independent, pH-sensitive LAT2 transporter [an isoform of system L (leucine preferring) that transports large branched and aromatic neutral amino acids in a Na^+-independent manner] [81]. Both the molecular mechanisms behind the regulation of these two L-DOPA transport systems, and whether they operate simultaneously or independently of specific stimuli, remain to be explored. The mechanisms that link high salt intake/increased dopamine production and activation of L-DOPA transporters are not known. The actions of dopamine are associated with incorporation of dopamine receptors at the plasma membrane of tubule cells [80,82]. Is the sole presence of higher dopamine levels within renal proximal tubules enough to increase the availability of dopamine receptors at the plasma membrane? Stringent analysis of the available data indicates that this is probably not the case. Whatever the mechanism may be, increases in dopamine content within the tubule cell has little or no physiological relevance for dopamine action regarding down-regulation of Na^+,K^+-ATPase activity, because during inhibition of its activity, dopamine *per se* is ineffective. In general, dopamine-dependent inhibition of Na^+,K^+-ATPase activity in renal proximal tubules occurs when the enzyme is allowed to operate at V_{max} (highest activity). Under experimental *in vitro* conditions, this effect is created artificially by raising the concentration of sodium within the assay conditions. When cells in culture are incubated with dopamine, its inhibitory action on Na^+,K^+-ATPase-dependent ion transport is very poor (~10 %) and barely significant [83]. However, when the intracellular concentration of sodium rises above normal levels, there is a significant increase in Na^+,K^+-ATPase activity only in cells that were not exposed to dopamine. Based on this facts it is clear that sodium, is a prerequisite and the conditioning factor influencing the cell's response to dopamine during regulation of Na^+,K^+-ATPase activity and thereby sodium transport.

Most experiments aimed at studying the role of intracellular sodium *in vitro* have used permeabilized cells, a condition created by hypotonic stress. This harsh treatment causes a number of artificial conditions within cells that a priori prevent any meaningful interpretation of the results, mainly because it would be impossible to determine the magnitude and the duration of the intracellular sodium elevation, the behavior of other ions, and more importantly the stability of the cell architecture due to the rearrangements of the cell cytoskeleton [84]. Because of these impediments, the role of

intracellular sodium was further studied in intact cells in the presence of a sodium ionophore. Live cell imaging [47] provides a reliable quantitative analysis of the intracellular concentration of sodium and its dynamic changes over time. These studies revealed that monensin (one of the ionophores with highest selectivity for sodium) increases intracellular sodium over time and that, at certain concentrations, it elicits these effects without visible changes in the cell size. Under this optimized and controlled conditions, small elevations in intracellular sodium resulted in increased dopamine receptor immunoreactivity at the plasma membrane. This event coincides with the dopamine-dependent inhibition of Na^+,K^+-ATPase activity [47]. Based on these findings, it is perhaps more useful to consider the sodium factor *per se* when studying dopamine receptor-related phenomena (the activation of GRK, or its ability to activate intracellular messengers) in whole-cell systems rather than to focus independently on dopamine actions. Because of the multiple factors affecting sodium reabsorption in the proximal convoluted tubule, it is also relevant to ask whether it is only the redistribution of dopamine receptors at the plasma membrane that tilts the balance toward the dopamine-natriuretic effect. Along with the increase in dopamine receptors at the plasma membrane, there is a decrease in angiotensin II receptor immunoreactivity (Fig. 5) [80]. This strongly suggests that dopamine-dependent decrease in Na^+,K^+-ATPase activity not only requires more dopamine receptors at the plasma membrane but also a reduction in the opposing forces (provided by angiotensin II) that stimulate Na^+,K^+-ATPase and sodium reabsorption. Within renal tubule cells, a simple increase in sodium permeability by itself cannot generate such a complex cellular response resulting in the redistribution of various hormone receptors. Whether SIK1 provides the framework for these sodium-targeted actions remains to be explored.

Besides SIK1 being involved in the control of cell sodium homeostasis, it is possible that the network could also be used by hormones responsible for the increases of Na^+,K^+-ATPase activity in a sodium-independent manner. For example, one such hormone could be angiotensin II, and several experimental observations point at this direction (Fig. 6). In cells derived from renal proximal tubules, incubation with angiotensin II promotes an increase in SIK1 activity (Fig. 6A). The presence of angiotensin II in picomolar ($<$ 10 pM) concentrations also increases Na^+,K^+-ATPase activity in these cells [80]; however, if cells are transiently overexpressing SIK1 bearing a mutation (K56M) that renders the kinase inactive, angiotensin II fails to increase Na^+,K^+-ATPase activity (Fig. 6B). Angiotensin II-dependent stimulation of Na^+,K^+-ATPase requires the incorporation of new active subunits at the plasma membrane [35], and those units must also be in a dephosphorylated state. Because it is known that Na^+,K^+-ATPase α-subunit-dependent dephosphorylation at the plasma membrane is mediated by SIK1→PP2A activation under sodium stress conditions [36], it is possible that the role of SIK1 during angiotensin II-dependent stimulation of Na^+,K^+-ATPase is to dephosphorylate the α-subunits once they have reached the plasma membrane (Fig. 6C). Certainly, those Na^+,K^+-ATPase molecules trafficking to the plasma membrane in intracellular organelles do so in an inactive state. Alternatively, SIK1 could also mediate the traffic of Na^+,K^+-ATPase unit-containing vesicles to the plasma membrane (Fig. 6C, 1 and 1′). The strength of the latter hypothesis resides on the fact that certain cytoskeletal proteins such as kinesin heavy chain, kinesin light chain and dynein intermediate light chain contain SIK1-consensus phosphorylation sites, which are potential targets for regulation.

Among multiple and interdependent factors, variations in salt intake and the renin–angiotensin system are effective controllers of aldosterone production during salt deprivation [85,86]. Aldosterone synthase is at the center of the cellular mechanisms behind the regulation of aldosterone levels; CYP11B2 gene is a known target for angiotensin II and ACTH [87]. The potential cellular mechanism by which angiotensin II activates CYP11B2 involves a calcium-dependent

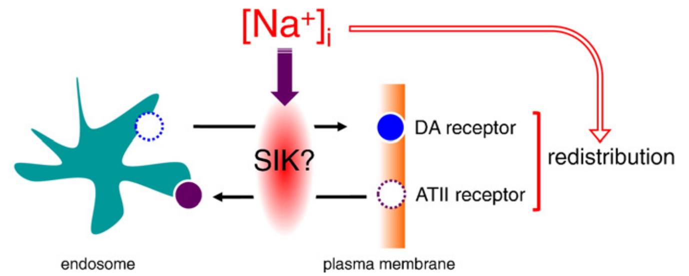

Fig. 5. Behavior of dopamine and angiotensin II receptor upon changes in intracellular sodium concentrations. The transition from basal to sodium-stimulated state promotes the redistribution of dopamine and angiotensin II receptor within the plasma membrane and intracellular organelles (endosomes).

activation of SIK1 via calmodulin kinase. It is likely that SIK1 regulates NURR1 (Nur-related factor 1, NR4A2), which is a known mediator of CYP11B2 activation in response to angiotensin II [88]. SIK1 can be regulated in a cAMP-dependent manner (during ACTH-dependent activation of CYP11b2) and in a calcium–calmodulin kinase-dependent manner [89]. Both cAMP-dependent activation of SIK1 and overexpression of SIK1 result in down-regulation of the CYP11B2 gene. During high salt intake, expression of the CYP11B2 gene in the adrenal gland decreases, which is also associated with elevated SIK1 activity. Although the available evidence is suggestive, very little attention has been directed to the SIK1-CYP11B2 axis, and it remains unclear whether there is a causal relationship between these two factors, and whether they might affect the production of aldosterone in the adrenal glands. At the cellular level, both long-term increases in sodium permeability and aldosterone exposure stimulate Na^+,K^+-ATPase activity at distal nephron segments [90]. Depending on the duration of the stimulus, this effect is mediated by some combination of increased catalytic activity of available pools of Na^+,K^+-ATPase units at the plasma membrane, recruitment of new molecules from intracellular organelles or increased synthesis of new subunits [90]. Could it be that the mediatory role of SIK1 on aldosterone actions is analogous to the effects of angiotensin II on SIK1-Na^+,K^+-ATPase? Since SIK1 controls the activity of different transcription factors, it is tempting to consider that it could also have a mediatory role in the regulation of specific aldosterone-targeted genes within the renal tubule segments.

6. Sodium sensing—elevations in arterial blood pressure

Despite the presence of multiple natriuretic systems (ANP, dopamine, ouabain-like factors, marinobufagenin, etc.) that provides a tight control of sodium homeostasis, a proportion of individuals develop hypertension when they increase their daily salt intake.

Over the years, animal models of hypertension have provided a useful tool for understanding the relevant mechanisms that fail at the cellular and/or organ level and their impact on the development of high blood pressure [91]. Moreover, the ability to manipulate specific genes (knock-out/knock-in) has provided a significant tool for studying diverse cellular factors and processes and their contribution to blood pressure regulation [91–95]. In particular, defining the role of Na^+/Ca^{2+} exchanger and Na^+,K^+-ATPase α-subunits and their sensitivity to ouabain provided important insights into the pathophysiology of hypertension [96]. Each one of these hypertensive models has its particular features, and faulty regulatory systems cannot be generalized. Also, in some models the different cell abnormalities do not coexist. However, despite this variability, the models have a common denominator in the response to the salt content of the diet.

Variations in salt intake determine blood pressure [97–99]. However, in some forms of hypertension such as α-adducin-dependent [95], elevation of arterial blood pressure may not only be associated with the elevated salt content of the diet but also caused by

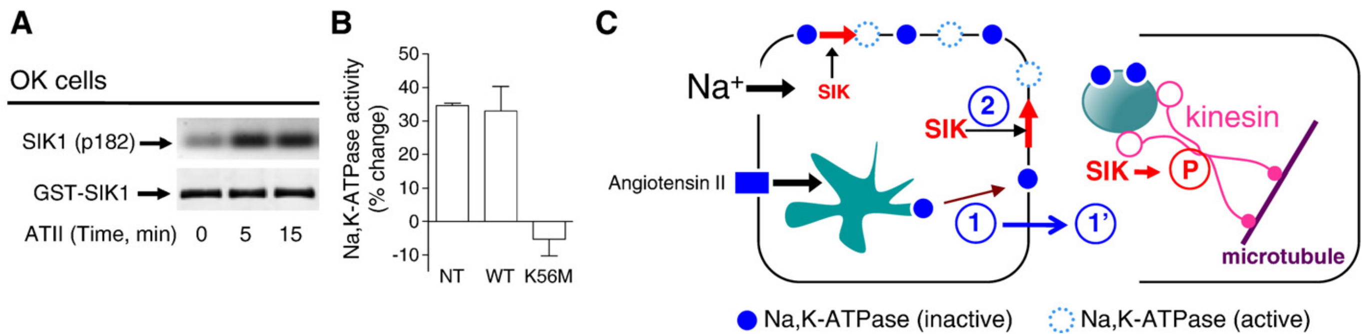

Fig. 6. Angiotensin II effects on sodium transport are mediated in part by the SIK1 network. (A) Transformed (GST-SIK1) OK cells were incubated with angiotensin II (1 pM at 23 °C) for 5 and 10 min. SIK1 activity was determined by Western blot as the degree of phosphorylation of its T182 residue (p182) using a specific phospho-antibody. (B) Na^+,K^+-ATPase activity was determined in OK cells transiently transfected with SIK1 wild type (WT), K56M mutant (which renders the kinase inactive) or nontransfected (NT) in the presence or absence of angiotensin II. Enzyme activity was examined as ouabain-dependent rubidium transport. Each bar represents the mean ± SEM of 5 experiments performed independently and in triplicate. (C) Cartoon depicting mechanistically the possible cellular mechanisms by which SIK1 may link the action of angiotensin II on Na^+,K^+-ATPase activity in renal epithelial cells (for detailed explanation see text).

a pre-existing partial impairment of cell sodium handling in renal tubule cells that gets exacerbated by increased salt intake [100]. For example, basal SIK1 activity is elevated in renal proximal tubules isolated from Milan hypertensive rats when compared to their normotensive counterparts [101]. Similarly, when a cell line derived from proximal tubules of the opossum kidney transiently express the hypertensive variant of human adducin, SIK activity increases in comparison to wild type cells. The molecular mechanisms by which a mutant variant of α-adducin associates with elevated SIK1 activity are yet to be fully explored. Hypothetically, it can be attributed either to a direct control of SIK by α-adducin, or to an indirect SIK1 activation by increased steady-state concentrations of intracellular sodium created by the presence of the hypertensive mutant variant of α-adducin.

In renal epithelial cells expressing the α-adducin variant carrying the hypertensive mutation, dopamine fails to promote the endocytosis of Na^+,K^+-ATPase subunits, and thereby the decrease in cell Na^+,K^+-ATPase activity [102,103]. The mechanism that is impaired appears to be a defective adaptor protein-2 phosphorylation/dephosphorylation process that leads to deficiencies in the formation of clathrin coated pits and vesicles [102]. Additionally, in Milan rats carrying the hypertensive variant of α-adducin it appears that ouabain has a stimulatory effect on renal Na^+,K^+-ATPase activity [104] with a consequent increase in sodium reabsorption. However, *in vitro* studies in cells transiently expressing the mutant α-adducin show that ouabain causes a decrease of Na^+,K^+-ATPase activity. Furthermore, long-term exposure to ouabain is also associated with the endocytosis of a proportion of active Na^+,K^+-ATPase pumps from the plasma membrane into endosomal compartments [105]. In summary, it appears that an array of malfunctioning systems could be responsible for the hypertensive phenotypes associated with mutations in the adducin molecule. A defect in the dopamine system associated with an elevated sodium transport activity could result in a net increase in sodium reabsorption. The defect in the dopamine-natriuretic system, although dormant, may further affect the response of these animals when subjected to a high salt intake. In Dahl salt-sensitive rats, dopamine also fails to down-regulate Na^+,K^+-ATPase activity. Another interesting feature of the Dahl rat model is that dopamine action is already impaired in the prehypertensive period [106], suggesting the presence of a pre-existing factor that is not triggered by salt intake *per se*. It is conceivable that there are multiple natriuretic systems that could act simultaneously in response to a single event such as an increase in the sodium content of the diet. This hypothesis requires further exploration. Apart from an inherited lack of dopamine receptor function, dopamine receptor dysfunction secondary to other factors such as the presence of ouabain-like compounds might occur. If elevated plasma ouabain-like factors suppressed the dopamine receptor effects [107], it could lead to increased sodium transport due to the lack of the inhibitory signals. This, alone or in combination with increased salt intake, may result in increased reabsorption and eventually lead to elevated blood pressure. Spontaneously hypertensive rats (SHR) are known to have dopamine receptor abnormalities (extensively described elsewhere in this issue); however, we still lack information concerning whether sodium and/or other preconditioning factors play a role in these abnormalities or if they are purely inherited. Could it be the case, in Milan as well as SHR rats – and in hypertensive individuals carrying the hypertensive adducin allele – that the actions of ATII are further potentiated by an inability of dopamine to operate efficiently beyond its direct actions on its receptors? The lack of an efficient dopamine-natriuretic system may lead to a prevailing antinatriuretic action of renal ATII on Na^+,K^+-ATPase activity: if basal SIK1 activity is high, lower concentrations of ATII might be sufficient to trigger larger increases in renal tubule reabsorption of sodium.

Hypertension is commonly observed in individuals with insulin resistance (also known as the metabolic syndrome). The mechanisms underlying the association of insulin resistance and diabetes with hypertension are poorly understood. Diabetes is the leading cause of renal dysfunction in adults. Both kidney function and insulin action are interdependently involved in blood pressure control, electrolyte metabolism and glucose metabolism. The renal actions of insulin could be divided into those affecting the glomerular function and those affecting the tubule transport. Insulin alone and elevated plasma glucose levels reduce renal sodium excretion, most likely by affecting proximal and/or distal tubule sodium transport and excretion [108,109]. At the tubular level, insulin has been found to stimulate Na^+,K^+-ATPase activity [110], increase sodium transport in proximal tubules, and potentiate the antinatriuretic effects of angiotensin II. Glucose-induced antinatriuresis is probably due to both enhanced glucose–sodium co-transport and enhanced sodium reabsorption in the proximal tubules during hyperglycemia. Compensatory hyperinsulinemia in insulin-resistant patients may alter the renal set point for sodium reabsorption by imposing a chronic antinatriuretic driving force on the kidney (enhanced by angiotensin II) and therefore play a critical role in the development and maintenance of high blood pressure. In unpublished experiments, we observed that the actions of insulin on renal proximal tubule cells Na^+,K^+-ATPase activity are abrogated in cells where SIK1 has been rendered inactive. These data provide another example of the involvement of the renal SIK1 network in the control of key cellular mechanisms responsible for the regulation of sodium transport and homeostasis. Furthermore, the data highlight the possibility that blocking SIK1 within the renal system could be of benefit in

preventing the actions of insulin and/or angiotensin II on sodium reabsorption.

7. Sodium sensing and hypertension—outlook

As new cellular systems, membrane transporters/receptors, and factors are discovered to be relevant for blood pressure control, evidence of different dysfunctional elements emerges. Many key regulatory proteins have been shown to have genetic variations that are associated with high blood pressure [111–113]; however, detailed functional studies in order to prove their causal relevance within mechanisms that control arterial blood pressure, need to be further explored.

Multiple natriuretic systems are controlled by salt, probably not directly but via numerous interdependent intermediate factors (environment based). Furthermore, the content of salt within the diet may not only be important for triggering a number of maladaptative responses leading to elevated blood pressure but also by influencing directly the action of other homeostatic systems operating under physiological conditions.

Acknowledgements

Dr A. Jaitovich is funded by the Cook County Bureau of Health Services, Illinois, and Dr A.M. Bertorello by a Senior Research Fellowship from the Swedish Heart and Lung Foundation. We thank all members of Membrane Signaling Networks and Professor H. Takemori for their input and contributions to this article. The Swedish Research Council and the Swedish Heart and Lung Foundation funded Dr Bertorello's work.

References

[1] A.C. Gyuton, J.E. Hall, Textbook of Medical Physiology, Saunders Co, 2005.
[2] D.L. Kasper, E. Braunwald, S. Hauser, et al., Harrison's Principles of Internal Medicine, 17e, McGraw Hill Professional, 2008.
[3] M. Ernst, M. Moser, Use of diuretics in patients with hypertension, N. Engl. J. Med. 361 (2009) 2153–2164.
[4] M. Jessup, S. Brozena, Heart failure, N. Engl. J. Med. 348 (2003) 2007–2018.
[5] P. Gines, A. Cardenas, V. Arroyo, J. Rodes, Management of cirrhosis and ascites, N. Engl. J. Med. 350 (2004) 1646–1654.
[6] G.A. Donnan, M. Fisher, M. Macleod, S.M. Davis, Stroke, Lancet 371 (2008) 1612–1623.
[7] A.I. Qureshi, J.I. Suarez, Use of hypertonic saline solutions in treatment of cerebral edema and intracranial hypertension, Crit. Care Med. 28 (2000) 3301–3313.
[8] L.B. Ware, M.A. Matthay, The Acute, Respiratory distress syndrome, N. Engl. J. Med. 342 (2000) 1334–1349.
[9] J.I. Sznajder, P. Factor, D.H. Ingbar, Lung edema clearance: role of Na(+)-K(+)-ATPase, J. Appl. Physiol. 93 (2002) 1860–1866.
[10] J.I. Sznajder, Alveolar edema must be cleared for the acute respiratory distress syndrome patient to survive, Am. J. Respir. Crit. Care Med. 163 (2001) 1293–1294.
[11] H. Humphrey, J. Hall, I. Sznajder, M. Silverstein, L. Wood, Improved survival in ARDS patients associated with a reduction in pulmonary capillary wedge pressure, Chest 97 (1990) 1776–1780.
[12] J.I. Sznajder, L.D. Wood, Beneficial effects of reducing pulmonary edema in patients with acute hypoxemic respiratory failure, Chest 100 (1991) 890–892.
[13] Y. Adir, L.C. Welch, V. Dumasius, P. Factor, J.I. Sznajder, K.M. Ridge, Overexpression of the Na-K-ATPase alpha2-subunit improves lung liquid clearance during ventilation-induced lung injury, Am. J. Physiol. Lung Cell Mol. Physiol. 294 (2008) L1233–1237.
[14] A. Jaitovich, J.I. Sznajder, Improving survival by increasing lung edema clearance: is airspace delivery of dopamine a solution? Crit. Care 12 (2008) 135.
[15] S. Awad, S.P. Allison, D.N. Lobo, The history of 0.9% saline, Clin. Nutr. 27 (2008) 179–188.
[16] H.J. Bohnert, D.E. Nelson, R.G. Jensen, Adaptations to environmental stresses, Plant Cell. 7 (1995) 1099–1111.
[17] W.D. Stein, The sodium pump in the evolution of animal cells, Philos Trans R Soc Lond B Biol Sci. 349 (1995) 263–269.
[18] A. Jaitovich, A.M. Bertorello, Semin. Nephrol. 26 (2006) 386–392.
[19] A.G. Therien, R. Blostein, Mechanisms of sodium pump regulation, Am. J. Physiol. Cell Physiol. 279 (2000) C541–C566.
[20] J.H. Kaplan, A moving new role for the sodium pump in epithelial cells and carcinomas, Sci. STKE 289 (2005) pe31.
[21] W. Kuhlbrandt, Biology structure and mechanism of P-type ATPases, Nat. Rev. Mol. Cell Biol. 5 (2004) 282–295.
[22] W.D. Stein, Cell volume homeostasis: ionic and nonionic mechanisms. The sodium pump in the emergence of animal cells, Int. Rev. Cytol. 215 (2002) 231–258.
[23] B. Alberts, et al., Molecular biology of the cell, 4th ed, Garland Science, New York, 2002.
[24] W.F. Boron, Regulation of intracellular pH, Adv. Physiol. Educ. 28 (2004) 160–179.
[25] E.M. Fish, B.A. Molitoris, Alterations in epithelial polarity and the pathogenesis of disease states, N. Engl. J. Med. 330 (1994) 1580–1588.
[26] M.G. Burnay, G. Crambert, S. Kharoubi-Hess, K. Geering, J.D. Horisberger, Electrogenicity of Na,K- and H,K-ATPase activity and presence of a positively charged amino acid in the fifth transmembrane segment, J Biol Chem. 278 (2003) 19237–19244.
[27] K.B. Axelsen, M.G. Palmgren, Evolution of substrate specificities in the P-type ATPase superfamily, J. Mol. Evol. 46 (1998) 84–101.
[28] M.G. Palmgren, K.B. Axelsen, Evolution of P-type ATPases, Biochim. Biophys. Acta 1365 (1998) 37–45.
[29] G.A. Yudowski, R. Efendie, C.H. Pedemonte, A.I. Katz, P.O. Berggren, A.M. Bertorello, Phosphoinositide-3 kinase binds to a proline-rich motif in the Na^+, K^+-ATPase alpha subunit and regulates its trafficking, Proc. Natl. Acad. Sci. U. S. A. 97 (2000) 6556–6561.
[30] S.C. Doné, I.B. Leibiger, R. Efendiev, A.I. Katz, B. Leibiger, P.O. Berggren, C.H. Pedemonte, A.M. Bertorello, Tyrosine 537 within the Na+, K+-ATPase alpha-subunit is essential for AP-2 binding and clathrin-dependent endocytosis, J. Biol. Chem. 277 (2002) 17108–17111.
[31] R. Efendiev, G.A. Yudowski, J. Zwiller, B. Leibiger, A.I. Katz, P.O. Berggren, C.H. Pedemonte, I.B. Leibiger, A.M. Bertorello, Relevance of dopamine signals anchoring dynamin-2 to the plasma membrane during Na+, K+-ATPase endocytosis, J. Biol. Chem. 277 (2002) 44108–44114.
[32] A. Zatti, V. Chauvet, V. Rajendran, T. Kimura, P. Pagel, M.J. Caplan, The C-terminal tail of the polycystin-1 protein interacts with the Na, K-ATPase alpha-subunit, Mol. Biol. Cell. 16 (2005) 5087–5093.
[33] Z. Li, Z. Xie, The Na/K-ATPase/Src complex and cardiotonic steroid-activated protein kinase cascades, Pflugers Arch. 457 (2009) 635–644.
[34] T. Kimura, P.B. Allen, A.C. Nairn, M.J. Caplan, Arrestins and spinophilin competitively regulate Na+, K+-ATPase trafficking through association with a large cytoplasmic loop of the Na+, K+-ATPase, Mol. Biol. Cell. 18 (2007) 4508–4518.
[35] R. Efendiev, C.E. Budu, A.M. Bertorello, C.H. Pedemonte, G-protein-coupled receptor-mediated traffic of Na, K-ATPase to the plasma membrane requires the binding of adaptor protein 1 to a Tyr-255-based sequence in the alpha-subunit, J. Biol. Chem. 283 (2008) 17561–17567.
[36] M. Sjöström, K. Stenström, K. Eneling, J. Zwiller, A.I. Katz, H. Takemori, A.M. Bertorello, SIK1 is part of a cell sodium-sensing network that regulates active sodium transport through a calcium-dependent process, Proc. Natl. Acad. Sci. U. S. A. 104 (2007) 16922–16927.
[37] Z. Wang, H. Takemori, S.K. Halder, Y. Nonaka, M. Okamoto, Cloning of a novel kinase (SIK) of the SNF1/AMPK family from high salt diet-treated rat adrenal, FEBS Lett. 453 (1999) 135–139.
[38] M. Carlson, B.C. Osmond, D. Botstein, Mutants of yeast defective in sucrose utilization, Genetics 98 (1981) 25–40.
[39] J.C. Ruiz, F.L. Conlon, E.J. Robertson, Identification of novel protein kinases expressed in the myocardium of the developing mouse heart, Mech. Development 48 (1994) 153–164.
[40] A. Stephenson, G.Y. Huang, G.Y. Huang, N.T. Nguyen, S. Reuter, J.L. McBride, J.C. Ruiz, snf1lk encodes a protein kinase that may function in cell cycle regulation, Genomics 83 (2004) 1105–1115.
[41] D.G. Hardie, D. Carling, M. Carlson, The AMP-activated/SNF1 protein kinase subfamily: metabolic sensors of the eukaryotic cell? Annu. Rev. Biochem. 67 (1998) 821–855.
[42] H. Takemori, M. Okamoto, Regulation of CREB-mediated gene expression by salt inducible kinase, J. Ster. Biochem. Mol. Biol. 108 (2008) 287–291.
[43] Y. Katoh, H. Takemori, J. Doi, M. Okamoto, Identification of the nuclear localization domain of salt-inducible kinase, Endocr. Res. 28 (2002) 315–318.
[44] N. Horike, H. Takemori, Y. Katoh, J. Doi, M. Okamoto, Roles of several domains identified in the primary structure of salt-inducible kinase (SIK), Endocr. Res. 28 (2002) 291–294.
[45] M. Jaleel, A. McBride, J.M. Lizcano, M. Deak, R. Toth, N.A. Morrice, D.R. Alessi, Identification of the sucrose non-fermenting related kinase SNRK, as a novel LKB1 substrate, FEBS Lett. 579 (2005) 1417–1423.
[46] Y.K. Hashimoto, T. Satoh, M. Okamoto, H. Takemori, Importance of autophosphorylation at Ser186 in the A-loop of salt inducible kinase 1 for its sustained kinase activity, J. Cell Biochem. 104 (2008) 1724–1733.
[47] R. Efendiev, A.M. Bertorello, R. Zandomeni, A.R. Cinelli, C.H. Pedemonte, Agonist-dependent regulation of renal Na^+, K^+-ATPase activity is modulated by intracellular sodium concentration, J Biol Chem. 277 (2002) 11489–11496.
[48] A.V. Chibalin, C.H. Pedemonte, A.I. Katz, E. Féraille, P.O. Berggren, A.M. Bertorello, Phosphorylation of the catalypic alpha-subunit constitutes a triggering signal for Na^+, K^+-ATPase endocytosis, J. Biol. Chem. 273 (1998) 8814–8819.
[49] A.V. Chibalin, G. Ogimoto, C.H. Pedemonte, T.A. Pressley, A.I. Katz, E. Féraille, P. O. Berggren, A.M. Bertorello, Dopamine-induced endocytosis of Na^+, K^+-ATPase is initiated by phosphorylation of Ser-18 in the rat alpha subunit and Is responsible for the decreased activity in epithelial cells, J. Biol. Chem. 274 (1999) 1920–1927.
[50] E. Lecuona, L.A. Dada, H. Sun, M.L. Butti, G. Zhou, T.L. Chew, J.I. Sznajder, Na,K-ATPase alpha1-subunit dephosphorylation by protein phosphatase 2A is

necessary for its recruitment to the plasma membrane, FASEB J. 20 (2006) 2618–2620.

[51] S. Longin, J. Jordens, E. Martens, I. Stevens, V. Janssens, E. Rondelez, I. De Baere, R. Derua, E. Waelkens, J. Goris, C. Van Hoof, An inactive protein phosphatase 2A population is associated with methylesterase and can be re-activated by the phosphotyrosyl phosphatase activator, Biochem. J. 380 (2004) 111–119.

[52] E. Ogris, X. Du, K.C. Nelson, E.K. Mak, X.X. Yu, W.S. Lane, D.C. Pallas, A protein phosphatase methylesterase (PME-1) is one of several novel proteins stably associating with two inactive mutants of protein phosphatase 2A, J. Biol. Chem. 274 (1999) 14382–14391.

[53] T. Pawson, P. Nash, Assembly of cell regulatory systems through protein interaction domains, Science 300 (2003) 445–455.

[54] C. Lemmers, E. Medina, M.H. Delgrossi, D. Michel, J.P. Arsanto, A. Le Bivic, hINADl/PATJ, a homolog of discs lost, interacts with crumbs and localizes to tight junctions in human epithelial cells, J. Biol. Chem. 277 (2002) 25408–25415.

[55] Z. Chen, I. Leibiger, A.I. Katz, A.M.A. Bertorello, Pals-associated tight junction protein functionally links dopamine and angiotensin II to the regulation of sodium transport in renal epithelial cells, Br. J. Pharmacol. 158 (2009) 486–493.

[56] M.D. Houslay, Disrupting specific PDZ domain-mediated interactions for therapeutic benefit, Br. J. Pharmacol. 158 (2009) 483–485.

[57] S.J. Khundmiri, E.J. Weinman, D. Steplock, J. Cole, A. Ahmad, P.D. Baumann, M. Barati, M.J. Rane, E. Lederer, Parathyroid hormone regulation of Na+, K+-ATPase requires the PDZ 1 domain of sodium hydrogen exchanger regulatory factor-1 in opossum kidney cells, J. Am. Soc. Nephrol. 16 (2005) 2598–2607.

[58] K.K. Knight, D.M. Wentzlaff, P.M. Snyder, Intracellular sodium regulates proteolytic activation of the epithelial sodium channel, J. Biol. Chem. 283 (2008) 27477–27482.

[59] A.M. Bertorello, J.K. Zhu, SIK1/SOS2 networks: decoding sodium signals via calcium-responsive protein kinase pathways, Pflugers Arch. 458 (2009) 613–619.

[60] J. Liu, M. Ishitani, U. Halfter, C.-S. Kim, J.-K. Zhu, The *Arabidopsis thaliana* SOS2 gene encodes a protein kinase that is required for salt tolerance, Proc. Natl. Acad. Sci. U. S. A. 97 (2000) 3730–3734.

[61] J. Liu, J.-K. Zhu, Zhu, A calcium sensor homolog required for plant salt tolerance, Science 280 (1998) 1943–1945.

[62] U. Halfter, M. Ishitani, J.-K. Zhu, The Arabidopsis SOS2 protein kinase physically interacts with and is activated by the calcium-binding protein SOS3, Proc. Natl. Acad. Sci. U. S. A. 97 (2000) 3735–3740.

[63] N. Horike, H. Takemori, Y. Katoh, J. Doi, L. Min, T. Asano, X.J. Sun, H. Yamamoto, S. Kasayama, M. Muraoka, Y. Nonaka, M. Okamoto, Adipose-specific expression, phosphorylation of Ser794 in insulin receptor substrate-1, and activation in diabetic animals of salt-inducible kinase-2, J. Biol. Chem. 278 (2003) 18440–18447.

[64] J. Du, Q. Chen, H. Takemori, H. Xu, SIK2 can be activated by deprivation of nutrition and it inhibits expression of lipogenic genes in adipocytes, Obesity 16 (2008) 531–538.

[65] M. Muraoka, A. Fukushima, S. Viengchareun, M. Lombès, F. Kishi, A.A. Miyauchi, M. Kanematsu, J. Doi, J. Kajimura, R. Nakai, T. Uebi, M. Okamoto, H. Takemori, Involvement of SIK2/TORC2 signaling cascade in the regulation of insulin-induced PGC-1alpha and UCP-1 gene expression in brown adipocytes, Am. J. Physiol. 296 (2009) E1430–E1439.

[66] Y. Katoh, H. Takemori, X.Z. Lin, M. Tamura, M. Muraoka, T. Satoh, Y. Tsuchiya, L. Min, J. Doi, A. Miyauchi, L.A. Witters, H. Nakamura, M. Okamoto, Silencing the constitutive active transcription factor CREB by the LKB1-SIK signaling cascade, FEBS J. 273 (2006) 2730–2734.

[67] R. Dentin, Y. Liu, S.H. Koo, S. Hedrick, T. Vargas, J. Heredia, J. Yates, M. Montminy, Insulin modulates gluconeogenesis by inhibition of the coactivator TORC2, Nature 449 (2007) 366–369.

[68] Y.S. Yoon, W.Y. Seo, M.W. Lee, S.T. Kim, S.H. Koo, Salt-inducible kinase regulates hepatic lipogenesis by controlling SREBP-1c phosphorylation, J. Biol. Chem. 284 (2009) 10446–10455.

[69] R.A. Screaton, M.D. Conkright, Y. Katoh, J.L. Best, G. Canettieri, S. Jeffries, E. Guzman, S. Niessen, J.R. Yates, H. Takemori, M. Okamoto, M. Montminy, The CREB coactivator TORC2 functions as a calcium- and cAMP-sensitive coincidence detector, Cell 119 (2004) 61–74.

[70] S.H. Koo, L. Flechner, L. Qi, X. Zhang, R.A. Screaton, S. Jeffries, S. Hedrick, W. Xu, F. Boussouar, P. Brindle, H. Takemori, M. Montminy, The CREB coactivator TORC2 is a key regulator of fasting glucose metabolism, Nature 437 (2005) 1109–1111.

[71] J.M. Lizcano, O. Göransson, R. Toth, M. Deak, N.A. Morrice, J. Boudeau, S.A. Hawley, L. Udd, T.P. Mäkelä, D.G. Hardie, D.R. Alessi, LKB1 is a master kinase that activates 13 kinases of the AMPK subfamily, including MARK/PAR-1, EMBO J. 23 (2004) 833–843.

[72] H. Cheng, P. Liu, Z.C. Wang, L. Zou, S. Santiago, V. Garbitt, O.V. Gjoerup, J.D. Iglehart, A. Miron, A.L. Richardson, W.C. Hahn, J.J. Zhao, SIK1 Couples LKB1 to p53-dependent anoikis and suppresses metastasis, Sci. Signal. 2 (2009) 35.

[73] A. Bertorello, T. Hökfelt, M. Goldstein, A. Aperia, Proximal tubule Na^+-K^+-ATPase activity is inhibited during high-salt diet: evidence for DA-mediated effect, Am. J. Physiol. 254 (1988) F795–F801.

[74] E. Féraille, A. Doucet, Sodium-potassium-adenosinetriphosphatase-dependent sodium transport in the kidney: hormonal control, Physiol. Rev. 81 (2001) 345–418.

[75] L.E. Yang, M.B. Sandberg, A.D. Can, K. Pihakaski-Maunsbach, A.A. McDonough, Effects of dietary salt on renal Na^+ transporter subcellular distribution, abundance, and phosphorylation status, Am. J. Physiol. 295 (2008) 1003–1016.

[76] A.R. Cinelli, R. Efendiev, C.H. Pedemonte, Trafficking of Na-K-ATPase and dopamine receptor molecules induced by changes in intracellular sodium concentration of renal epithelial cells, Am. J. Physiol. 295 (2008) F1117–F1125.

[77] J.C. Skou, The Na, K-pump, Methods Enzymol. 156 (1988) 1–25.

[78] R.W. Alexander, G.R. Gill, H. Yamabe, W. Lovenberg, H.R. Keiser, Effect of dietary sodium and acute saline infusion on the interrelationship between dopamine excretion and adrenergic activity in man, J. Clin. Invest. 54 (1974) 194–200.

[79] A.C. Aperia, Intrarenal dopamine: a key signal in the interactive regulation of sodium metabolism, Annu. Rev. Physiol. 62 (2000) 621–647.

[80] R. Efendiev, C.E. Budu, A.R. Cinelli, A.M. Bertorello, C.H. Pedemonte, Intracellular Na^+ regulates dopamine and angiotensin II receptors availability at the plasma membrane and their cellular responses in renal epithelia, J. Biol. Chem. 278 (2003) 28719–28722.

[81] P. Soares-Da-Silva, M.P. Serrão, M.J. Pinho, M.J. Bonifácio, Cloning and gene silencing of LAT2, the L-3, 4-dihydroxyphenylalanine (L-DOPA) transporter, in pig renal LLC-PK1 epithelial cells, FASEB J. 18 (2004) 1489–1498.

[82] H. Brismar, M. Asghar, R.M. Carey, P. Greengard, A. Aperia, Dopamine-induced recruitment of dopamine D1 receptors to the plasma membrane, Proc. Natl. Acad. Sci. U. S. A. 95 (1998) 5573–5578.

[83] F. Takemoto, H.T. Cohen, T. Satoh, A.I. Katz, Dopamine inhibits Na/K-ATPase in single tubules and cultured cells from distal nephron, Pflugers Arch. 421 (1992) 302–306.

[84] L. Mao, D. Hartl, T. Nolden, A. Koppelstätter, J. Klose, H. Himmelbauer, C. Zabel, Pronounced alterations of cellular metabolism and structure due to hyper- or hypo-osmosis, J. Proteome Res. 7 (2008) 3968–3983.

[85] H.S. Willenberg, S. Schinner, I. Ansurudeen, New mechanisms to control aldosterone synthesis, Horm. Metab. Res. 40 (2008) 435–441.

[86] K. Yanagibashi, M. Haniu, J.E. Shively, W.H. Shen, P. Hall, The synthesis of aldosterone by the adrenal cortex. Two zones (fasciculata and glomerulosa) possess one enzyme for 11 beta-, 18-hydroxylation, and aldehyde synthesis, J. Biol. Chem. 261 (1986) 3556–3562.

[87] M. Okamoto, Y. Nonaka, H. Takemori, J. Doi, Molecular identity and gene expression of aldosterone synthase cytochrome P450, Biochem. Biophys. Res. Com. 338 (2005) 325–330.

[88] M.H. Bassett, T. Suzuki, H. Sasano, P.C. White, W.E. Rainey, The orphan nuclear receptors Nurr1 and NGFIB regulate adrenal aldosterone production, Mol. Endocrinol. 18 (2004) 279–290.

[89] J. Doi, H. Takemori, X. Lin, N. Horike, Y. Katoh, M. Okamoto, Salt-inducible kinase represses cAMP-dependent protein kinase-mediated activation of human cholesterol side chain cleavage cytochrome P450 promoter through the CREB basic leucine zipper domain, J. Biol. Chem. 277 (2002) 15629–15637.

[90] V. Summa, D. Mordasini, F. Roger, M. Bens, P.Y. Martin, A. Vandewalle, F. Verrey, E. Féraille, Short term effect of aldosterone on Na, K-ATPase cell surface expression in kidney collecting duct cells, J. Biol. Chem. 276 (2001) 47087–47093.

[91] J.P. Rapp, Genetic analysis of inherited hypertension in the rat, Physiol. Rev. 80 (2000) 135–172.

[92] S.D. Crowley, S.B. Gurley, M.J. Herrera, P. Ruiz, R. Griffiths, A.P. Kumar, H.S. Kim, O. Smithies, T.H. Lee, T.M. Coffman, Angiotensin II causes hypertension and cardiac hypertrophy through its receptors in the kidney, Proc. Natl. Acad. Sci. U. S. A. 103 (2006) 17985–17990.

[93] I. Dostanic-Larson, J.W. Van Huysse, J.N. Lorenz, J.B. Lingrel, The highly conserved cardiac glycoside binding site of Na, K-ATPase plays a role in blood pressure regulation, Proc. Natl. Acad. Sci. U. S. A. 102 (2005) 15845–15850.

[94] N. Makhanova, M.L. Sequeira-Lopez, R.A. Gomez, H.S. Kim, O. Smithies, Disturbed homeostasis in sodium-restricted mice heterozygous and homozygous for aldosterone synthase gene disruption, Hypertension 48 (2006) 1151–1159.

[95] D. Cusi, C. Barlassina, T. Azzani, G. Casari, L. Citterio, M. Devoto, N. Glorioso, C. Lanzani, P. Manunta, M. Righetti, R. Rivera, P. Stella, C. Troffa, L. Zagato, G. Bianchi, Polymorphisms of alpha-adducin and salt sensitivity in patients with essential hypertension, Lancet 349 (1997) 1353–1357.

[96] T. Iwamoto, S. Kita, J. Zhang, M.P. Blaustein, Y. Arai, S. Yoshida, K. Wakimoto, I. Komuro, T. Katsuragi, Salt-sensitive hypertension is triggered by Ca^{2+} entry via Na^+/Ca^{2+} exchanger type-1 in vascular smooth muscle, Nat. Med. 10 (2004) 1193–1199.

[97] P. Meneton, X. Jeune, H.E. de Wardener, G.A. MacGregor, Links between dietary salt intake, renal salt handling, blood presure, and cardiovascular diseases, Physiol. Rev. 85 (2005) 679–715.

[98] O. Melander, F. von Wowern, E. Frandsen, P. Burri, G. Willsteen, M. Aurell, U.L. Hulthén, Moderate salt restriction effectively lowers blood pressure and degree of salt sensitivity is related to baseline concentration of renin and N-terminal atrial natriuretic peptide in plasma, J. Hypertens. 25 (2007) 619–627.

[99] A. Jaitovich, A.M. Bertorello, Salt, Na^+, K^+-ATPase and hypertension, Life Sci. Life Sci. 86 (2010) 73–78.

[100] G. Tripodi, F. Valtorta, L. Torielli, E. Chieregatti, S. Salardi, L. Trusolino, A. Menegon, P. Ferrari, P.C. Marchisio, G. Bianchi, Hypertension-associated point mutations in the adducin alpha and beta subunits affect actin cytoskeleton and ion transport, J. Clin. Invest. 97 (1996) 2815–2822.

[101] K. Stenström, H. Takemori, G. Bianchi, A.I. Katz, A.M. Bertorello, Blocking the salt-inducible kinase 1 network prevents the increases in cell sodium transport caused by a hypertension-linked mutation in human alpha-adducin, J. Hypertens 27 (2009) 2452–2457.

[102] R. Efendiev, R.T. Krmar, G. Ogimoto, J. Zwiller, G. Tripodi, A.I. Katz, G. Bianchi, C.H. Pedemonte, A.M. Bertorello, Hypertension-linked mutation in the adducin alpha-subunit leads to higher AP2-mu2 phosphorylation and impaired Na^+, K^+-ATPase trafficking in response to GPCR signals and intracellular sodium, Circ. Res. 95 (2004) 1100–1108.

[103] L. Torielli, S. Tivodar, R.C. Montella, R. Iacone, G. Padoani, P. Tarsini, O. Russo, D. Sarnataro, P. Strazzullo, P. Ferrari, G. Bianchi, C. Zurzolo, alpha-Adducin mutations

increase Na/K pump activity in renal cells by affecting constitutive endocytosis: implications for tubular Na reabsorption, Am. J. Physiol. 295 (2008) 478–487.
[104] P. Ferrari, M. Ferrandi, G. Tripodi, L. Torielli, G. Padoani, E. Minotti, P. Melloni, G. Bianchi, PST 2238: a new antihypertensive compound that modulates Na, K-ATPase in genetic hypertension, J. Pharm. Eexp. Ther. 288 (1999) 1074–1083.
[105] J. Liu, R. Kesiry, S.M. Periyasamy, D. Malhotra, Z. Xie, J.I. Shapiro, Ouabain induces endocytosis of plasmalemmal Na/K-ATPase in LLC-PK1 cells by a clathrin-dependent mechanism, Kidney Intern. 66 (2004) 227–241.
[106] A. Nishi, A.C. Eklöf, A.M. Bertorello, A. Aperia, Dopamine regulation of renal Na^+, K^+-ATPase activity is lacking in Dahl salt-sensitive rats, Hypertension 21 (1993) 767–771.
[107] Y. Zhang, Z. Yuan, H. Ge, Y. Ren, Effects of long-term ouabain treatment on blood pressure, sodium excretion, and renal dopamine D(1) receptor levels in rats, J. Comp. Physiol. 180 (2009) 117–124.
[108] E. Muscelli, A. Natali, S. Bianchi, R. Bigazzi, A.Q. Galvan, A.M. Sironi, S. Frascerra, D. Ciociaro, E. Ferrannini, Effect of insulin on renal sodium and uric acid handling in essential hypertension, Am. J. Hypertens. 9 (1996) 746–752.
[109] M.W. Brands, T.D. Bell, N.A. Rodriquez, P. Polavarapu, D. Panteleyev, Chronic glucose infusion causes sustained increases in tubular sodium reabsorption and renal blood flow in dogs, Am. J. Physiol. 296 (2009) R265–R271.
[110] E. Feraille, S. Marsy, L. Cheval, C. Barlet-Bas, C. Khadouri, H. Favre, A. Doucet, Sites of antinatriuretic action of insulin along rat nephron, Am. J. Physiol. 263 (1992) 175–179.
[111] W. Ji, J.N. Foo, B.J. O'Roak, H. Zhao, M.G. Larson, D.B. Simon, C. Newton-Cheh, M. W. State, D. Levy, R.P. Lifton, Rare independent mutations in renal salt handling genes contribute to blood pressure variation, Nat. Genet. 40 (2008) 592–599.
[112] C. Newton-Cheh, T. Johnson, V. Gateva, M.D. Tobin, M. Bochud, L. Coin, S.S. Najjar, J.H. Zhao, S.C. Heath, S. Eyheramendy, K. Papadakis, B.F. Voight, L.J. Scott, F. Zhang, M. Farrall, T. Tanaka, C. Wallace, J.C. Chambers, K.T. Khaw, P. Nilsson, P. van der Harst, S. Polidoro, D.E. Grobbee, N.C. Onland-Moret, M.L. Bots, L.V. Wain, K.S. Elliott, A. Teumer, J. Luan, G. Lucas, J. Kuusisto, P.R. Burton, D. Hadley, W.L. McArdle, Wellcome Trust Case Control Consortium, Brown M., Dominiczak A., Newhouse SJ., Samani N.J., Webster J., Zeggini E., Beckmann J. S., Bergmann S., Lim N., Song K., Vollenweider P., Waeber G., Waterworth D. M., Yuan X., Groop L., Orho-Melander M., Allione A., Di Gregorio A., Guarrera S., Panico S., Ricceri F., Romanazzi V., Sacerdote C., Vineis P., Barroso I., Sandhu M.S., Luben R.N., Crawford G.J., Jousilahti P., Perola M., Boehnke M., Bonnycastle L.L., Collins FS., Jackson A.U., Mohlke K.L., Stringham H.M., Valle T.T., Willer C.J., Bergman R.N., Morken M.A., Döring A., Gieger C., Illig T., Meitinger T., Org E., Pfeufer A., Wichmann H.E., Kathiresan S., Marrugat J., O'Donnell C.J., Schwartz S.M., Siscovick D.S., Subirana I., Freimer N.B., Hartikainen A.L., McCarthy M.I., O'Reilly P.F., Peltonen L., Pouta A., de Jong P.E., Snieder H., van Gilst W.H., Clarke R., Goel A., Hamsten A., Peden J.F., Seedorf U., Syvänen A.C., Tognoni G., Lakatta E.G., Sanna S., Scheet P., Schlessinger D., Scuteri A., Dörr M., Ernst F., Felix S.B., Homuth G., Lorbeer R., Reffelmann T., Rettig R., Völker U., Galan P., Gut I.G., Hercberg S., Lathrop G.M., Zelenika D., Deloukas P., Soranzo N., Williams F.M., Zhai G., Salomaa V., Laakso M., Elosua R., Forouhi N.G., Völzke H., Uiterwaal C.S., van der Schouw Y.T., Numans M.E., Matullo G., Navis G., Berglund G., Bingham S.A., Kooner J.S., Connell J.M., Bandinelli S., Ferrucci L., Watkins H., Spector T.D., Tuomilehto J., Altshuler D., Strachan D.P., Laan M., Meneton P., Wareham N.J., Uda M., Jarvelin M.R., Mooser V., Melander O., Loos R.J., Elliott P., Abecasis G.R., Caulfield M., Munroe P.B. Genome-wide association study identifies eight loci associated with blood pressure. *Nat. Genet.* PMID: 19430483.
[113] Y. Wang, J.R. O'Connell, P.F. McArdle, J.B. Wade, S.E. Dorff, S.J. Shah, X. Shi, L. Pan, E. Rampersaud, H. Shen, J.D. Kim, A.R. Subramanya, N.I. Steinle, A. Parsa, C.C. Ober, P.A. Welling, A. Chakravarti, A.B. Weder, R.S. Cooper, B.D. Mitchell, A.R. Shuldiner, Y.-P.C. Chang, Whole-genome association study identifies STK39 as a hypertension susceptibility gene, Proc. Natl. Acad. Sci. U. S. A. 106 (2009) 226–231.