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Na^+, K^+-ATPase: An Indispensable Ion Pumping-*Signaling* Mechanism Across Mammalian Cell Membranes

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Na^+, K^+-adenosine triphosphatase is a ubiquitous enzyme present in higher eukaryotes responsible for the maintenance of ionic gradients across the plasma membrane. It creates appropriate conditions for critical cellular processes such as secondary transport of solutes and water, for pH regulation, and also for creating an electrical potential that gives singular qualities to excitable cells. It also served as a platform for a higher level of cellular complexity because many important signaling networks appear to be downstream events of the pump's function. Renal physiology and pathology are affected significantly by its presence, and it seems that both molecular and pharmacologic manipulations of its action can create different venues to deal with diverse disease states.
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During the first decades of the 20th century, vigorous discussions were held among scientists regarding the fact that animal cells maintain both high K^+ and low Na^+ concentrations within the intracellular compartment, with the opposite relationship occurring in the extracellular milieu. It was well known that nondiffusible cytosolic proteins are negatively charged at physiologic conditions and that intracellular potassium neutralizes those charges with the resultant constitution of what is defined as the *Donnan effect of proteins*. This effect promotes entry of water into the cytosol and the consequent cellular swelling caused by the osmotic predominance of the compartment that, if not controlled, causes cells to burst. This fate seemed to be prevented by the compensatory high Na^+ concentration present outside the cell. However, the plasma membrane was at that time conceptualized according to the Danielli and Davson model, which did not consider the existence of proteins spanning the lipid bilayer such as transporting molecules, which could mediate a differential ionic distribution. It was the combination of the perceptiveness of Skou[1] with the insights of other physiologists that allowed the demonstration that the Na^+, K^+-adenosine triphosphatase (Na^+, K^+-ATPase), or *sodium pump*, could have these ions transferred across the plasma membrane and thus was responsible for the compensation of the Donnan effect.[1-3] Its discovery also helped to understand how the asymmetric concentration of cations maintains an energy source given by a chemical potential critical for transporting important substances across plasma membrane. Moreover, by pumping 3 cations out and only 2 into the cell, the sodium pump creates an electrical potential that is essential for all excitable cells.

Evolutionary Considerations of the Na^+, K^+-ATPase

The curious connection between the sessile nature of plants and the absence of a cell volume-regulating system such as the Na^+, K^+-ATPase was observed by Krogh more than 50 years ago. Contrary to plants, animal cells have this exquisite volume-regulating system but reciprocally lack a rigid cell wall that would limit their motility.[4] Plant cells regulate their

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volume in such a singular way that they can even create an intracellular osmotic force that attracts water by accumulating Na^+ in vacuoles,[5] whereas animal cells dedicate much of their energy expenditure to remove Na^+ and water. In any case, sodium is excluded from the cytosolic compartment of most living cells because is causes osmotic stress and also has specific toxic consequences.[6] Indeed, it is accepted that the aqueous environment in which most cells are embedded creates a threat of death by flooding because of the Donnan effect, whose power of attraction is related to the number of intracellular molecules that is proportional to the structural complexity of the cytosol. To cope with this flooding challenge, animal cells pump sodium—and indirectly water, which follows Na^+ passively—toward the extracellular milieu through the Na^+, K^+-ATPase. In some tissues the cost of this process is up to 40% of the total ATP produced.[7] The Na^+, K^+-ATPase belongs to a superfamily of proteins collectively designated as *P-type ATPases*,[8] whose cloning and sequencing enabled tracking of their evolutionary links.[9] Two tentative phylogenetic trees suggest that the sodium pump appeared only once in evolution and developed from a proton pump constituting the primary cell volume regulator at the time life evolved in the oceans.[4] It has been suggested also that early plants developed in fresh water and for that reason did not have to cope with the sodium stress, clamping the cell volume through a semirigid wall, an event that resulted in a limited motion capacity despite being a multicellular organism. These conclusions are significant because they suggest that Na^+, K^+-ATPase–mediated transport is not a dispensable function, but instead is an essential necessity for evolution of animal cells. Many of their critical properties, if not their existence, may not have occurred without the appearance of Na^+, K^+-ATPase. Some of these qualities are indirectly yet fundamentally affected by the pump's activity. Lacking the straightjacket represented by a semirigid wall allows animal cells to integrate themselves in such a complex way that makes the stereotyped development pattern observed in superior species possible.[10] The latter also demands the presence of a myriad of proteins required for building a high level of intracellular complexity that in itself constitutes an osmotic challenge that parallels the evolutionary level of living organisms. Not surprisingly, genes related to both cell adhesion and signaling, and to DNA-binding proteins, which are critical for the development of multicellular eukaryotes, are significantly more abundant in the most evolved organisms such as the Homo sapiens.[10] Also, Na^+, K^+-ATPase constitutes a highly versatile membrane transport system because the same Na^+ gradient drives the transport of H^+, HCO_3^- and glucose, whereas plants have a specific ATP-dependent pump to extrude H^+.[11] Na^+-driven carrier proteins, such as Na^+-H^+ exchanger and Na^+-driven Cl^--HCO_3^- exchanger, are critical regulators of cytosolic pH, which directly affects the optimal work of cellular enzymes. The impact of intracellular pH on health and disease has been a matter of important debates that have challenged, for example, the usefulness of bicarbonate administration to correct extracellular pH in the face of severe acidosis.[12] Na^+, K^+-ATPase is designated as *electrogenic* because in contrast to other P-type ATPases it creates an electrical potential across the plasma membrane. No muscle contraction or communication between neurons would be possible without Na^+, K^+-ATPase. It has been suggested that a single residue holds this property and that its mutation can revert the electrogenic property. Conversely, the incorporation of this residue into the highly homologous, but nonelectrogenic, H^+, K^+ gastric pump can render it electrogenic,[13] which suggests an important level of evolutionary refinement in the development of this property. Interestingly, both Na^+, K^+-ATPase and H^+, K^+ gastric pump belong to the same branch of the P-type ATPases' phylogenetic tree.[14] Thus, this subtle change seems to have occurred in a short evolutionary period of time to enable the diversification of tissues.

Ionic Transporter and Signal Transducer

Apart from allowing cells to deal with the sodium stress, Na^+, K^+-ATPase gave rise to an even higher level of sophistication by guiding hierarchic cellular functions such as motility, polarity, and signaling. It has been shown convincingly that Na^+, K^+-ATPase is a signal transducer.[15] This quality appears to have arisen independently from the ion transport function during its evolution because the specific binding motifs that transfer signals are located outside the conserved regions common to the different P-type ATPases.[16] A key element in the nontransporting motifs is the domain for the binding of ouabain, a cardiac glycoside closely related to digoxin, which inhibits Na^+, K^+-ATPase and activates Src-family kinases in many cell types.[17] The only known plasma membrane receptor for cardiac glycosides is the sodium pump, although recently another binding site has been suggested.[18] By interacting with the sodium pump, ouabain regulates other signaling pathways such as the Ras-Raf–mitogen-activated protein kinase (MAPK) cascade through transactivation of epidermal growth factor receptors. In addition, ouabain stimulates the production of reactive oxygen species and the activation of protein kinase C (PKC) isoenzymes throughout the activation of Src. The physiologic implications of those signaling pathways are potentially countless. For example, a recently described mechanism termed $P \rightarrow A$[19] appears to produce a detachment of cell-cell and cell-substrate interactions after ouabain binding to Na^+, K^+-ATPase, and involves Rho/Rac and MAPK pathways. It has been suggested that it may be a protective mechanism by which injured epithelia could self-repair after cell detachment. Moreover, it could play a significant role in the development of metastasis.[20]

Although few years ago polarization was assumed to be a peculiar quality of epithelia and neurons, the general opinion has changed and nowadays it is considered that all cells from yeast to mammals display various degrees of polarized organization. Without polarization the monomer of a receptor protein sent at random to the plasma membrane would diffuse wandering on the plane of the bilayer until it met its counterpart by chance. In this sense, polarity may be seen as a principle of economy that speeds up the assembly of mem-

brane structures.[21] In the case of epithelia, polarity enables the transport of substances in a vectorial fashion. Na^+, K^+-ATPase appears to configure the cellular polarization of certain epithelia through the assembling and maintenance of tight junctions, which delimit accurately the basolateral and the apical domains of the plasma membrane. Formation of tight junctions requires the intervention of the adhesion molecule E-cadherin, but its role seems to be dispensable once the cell already has assembled this intercellular structure. In contrast, Na^+, K^+-ATPase appears to be critical for both the assembling and maintenance of tight junctions so that, at least in some cell types, polarization would be a downstream event of Na^+, K^+-ATPase function. In this regard, inhibition of Na^+, K^+-ATPase activity can increase tight junction permeability to both ionic and nonionic molecules without affecting the cell volume or other biomechanical properties that could nonspecifically alter the epithelial phenotype.[22]

Interestingly, apart from affecting cell polarity, Na^+, K^+-ATPase guides its own polarization through mechanisms that have been a matter of intense debate. Indeed, by creating a chimera through fusion of different domains of H^+, K^+-ATPase and Na^+, K^+-ATPase α-subunits it was possible to misguide the latter to the apical membrane.[23-25] The normal sodium pump's confinement to the basolateral domain appears to be determined by its inability to board the glycosphingolipid-rich rafts that carry proteins to the apical membrane.[26,27] Alternatively, it also has been proposed that because of the adhesiveness it confers, the Na^+, K^+-ATPase β-subunit may establish a linkage with similar subunits located in neighboring cells across the intercellular space and thus be responsible for the polarized expression of the pump in the lateral domain.[28] Notably, early in evolution an ancestor of the β-subunit was expressed independently of the catalytic α-subunit. In this regard, the β-subunit has the typical structure of an adhesion molecule and works as such, emphasizing its possible role as the lateralizing-polarization domain of the sodium pump that prevents its migration to the apical domain of the plasma membrane.[29,30]

Na^+, K^+-ATPase, particularly the β-subunit, affects the stabilization and organization of the cortical actin ring of epithelial cells, which is an important scaffold that platforms adhesion molecules and signaling mediators of cell-cell interaction and is altered significantly in Moloney sarcoma virus–transformed Madin-Darby canine kidney (MDCK) cells. Also, these cells that virtually lack both E-cadherin and Na^+, K^+-ATPase β-subunits, recover a normal cortical actin ring on repletion of the latter but not of E-cadherin. Likewise, stress fibers, which are dynamic actin filaments related to cell migration, seem to be affected by Na^+, K^+-ATPase by virtue of RhoA regulation, which is a small guanosine 5′-triphosphate (GTP) binding protein implicated in those fibers' configuration in fibroblasts and epithelial cells. In agreement with the previously mentioned P→A mechanism it has been speculated that the sodium pump could function as a motility suppressor in many normal epithelial cells, and could even give rise to an oncogenic behavior if disrupted or underexpressed.[7]

Na^+, K^+-ATPase Function: Impact in Health and Disease States

The mechanisms described previously suggest that Na^+, K^+-ATPase functions may be dissociated, one indirectly affecting cellular complexity through the transport of ions, which allows cells to cope with sodium stress and emerge as a consequence of the evolutionary pressure, and the other directly causing the cellular complexity by altering signaling pathways. It is worth emphasizing that some signaling functions not related to ion transport have been described clearly, although domain-dependent mutations that could preserve one function while deleting the other are lacking. Indeed, in physiologic terms both aspects constitute a continuum with significant interdependence because most, if not all, the functions of the enzyme involve the intervention of the ionic and nonionic facets. It has long been recognized that the kidney exerts a critical influence in the control (direct or indirect) of arterial blood pressure, blood volume and viscosity, vascular tone, and electrolyte composition of both intracellular and extracellular compartments. Na^+, K^+-ATPase plays a crucial role in all these variables, and its different operational levels have both physiologic and pathologic implications.

High Blood Pressure

Arterial hypertension can result from an increase in total peripheral resistance (mostly dependent on vascular tone and blood viscosity), from an increase in heart rate, and from high stroke volume (directly related to volemia and strongly influenced by myocardial contractility and relaxation). An elegant set of experiments using knock-in and knock-out assays showed recently that the ouabain binding motive of Na^+, K^+-ATPase-α2 isoform mediates the development of high-vascular-tone arterial hypertension (nonhypervolemic hypertension). Indeed, replacement of 2 critical amino acids located in this domain prevented the development of adreno corticotropin hormone (ACTH)-induced hypertension such as in Cushing syndrome.[31,32] These experiments strongly suggest the existence of endogenous ligands for the ouabain-binding domain, a hypothesis that has created important controversies during the past 3 decades. Ouabain-like compounds have been found to be increased in patients suffering from essential hypertension, eclampsia, and heart failure, as well as in animal models of the same diseases.[33,34] Although the exact mechanisms by which ouabain regulates blood pressure remain to be elucidated, the two considered mechanisms are spatial changes in Na^+ and Ca^{2+} concentrations, and/or the triggering of a complex intracellular signaling cascade involving Src, PKC, or MAPK.[32] It is worth mentioning that different Na^+, K^+-ATPase isoforms with specific functions are located in particular areas of the plasma membrane.[35,36] For example, α2 isoform is located in microdomains juxtaposed to sarcoplasmic reticulum in close proximity to Na^+/Ca^{2+} exchanger, which is critically related to Ca^{2+} handling and muscle contraction produced by digoxin or ouabain. Low (therapeutic) doses of cardiac gly-

cosides do not evoke an increase in bulk cytosolic Na^+ concentration but only in the mentioned microdomains. Conversely, $\alpha 1$ isoform, which is the housekeeping variant, is less sensitive to cardiac glycosides but controls the general cellular ionic functions of the pump.[37] The existence of multiple types of the Na^+, K^+-ATPase α-subunit suggests that a primordial predecessor evolved to meet the requirements of increasingly complex multitissular organisms in which different isoforms may work singularly and even may be expressed variably according to a particular physiologic scenario.

Another form of arterial hypertension characterized by hypervolemia can be caused by a mutation in a cytoskeleton protein identified as adducin that is capable of increasing Na^+ reabsorption in renal tubules as a consequence of increased Na^+, K^+-ATPase activity.[38] Originally discovered in rats, known as *Milan hypertensive strain*, and carrying a mutated variant of adducin, it was observed that the mutation prevents the clathrin-coated vesicles–mediated sodium pump endocytosis in the proximal tubular epithelium, an event that is critical for the natriuretic response to different stimuli such as dopamine. The assembling of these vesicles requires the binding of the adaptor protein type 2 and its phosphorylation is a limiting step in the endocytosis-inhibition process. An anomaly in the phospho-dephosphorylation cycle of adaptor protein type 2-μ_2 was found in cells obtained from proximal tubules from Milan hypertensive rats and in renal tubule cells transfected with the hypertensive mutant form of α-adducin, strongly suggesting an association between this alteration, the increased Na^+, K^+-ATPase activity (by increasing its availability at the plasma membrane), and the abnormal renal sodium handling linked to the failure in the endocytotic process.[39] Also, transfected cells with the mutated α-adducin have an accelerated rate of polymerization and bundling of cortical actin cytoskeleton, which might create a structural obstacle for Na^+, K^+-ATPase endocytosis and by this means interfere with different natriuretic stimuli.[40] Interestingly, a retrospective study established that adducin-mutated carrier hypertensive patients had a lower incidence of myocardial infarction and stroke if treated with diuretic therapy instead of other antihypertensive drugs such as β-blockers,[41] whereas the benefit of both drugs proved to be similar in the general hypertensive population,[42] emphasizing the causal role of hypervolemia on hypertension and its sequels in the adducin-mutated cohort. An antihypertensive agent recently developed can selectively bypass the altered process of Na^+, K^+-ATPase endocytosis associated with the mutated adducin, probably by directly blocking the sodium pump's catalytic activity[43] and bypassing the endocytic process. Moreover, it appears that this compound also can prevent the prohypertrophic cardiac effects of chronic hypertension and thus potentially attenuate the deleterious effects of diastolic heart failure, which is a leading cause of congestive heart failure.[44] Significantly, this agent also attenuated the effects evoked by chronic treatment with ouabain, implying the mediation of Na^+, K^+-ATPase in this process.

Acute hypertension also appears to be affected by Na^+, K^+-ATPase in normal conditions. In mammalian nephrons the distal tubule is adjacent to its glomerulus of origin at the point at which about 80% of the filtrated Na^+ and water have been absorbed. The epithelial cells at this contact point—the macula densa—along with the smooth muscle cells of the afferent arteriole constitute the juxtaglomerular apparatus.[45] When the macula densa senses an increased Na^+ delivery, a signal that contracts afferent arteriole is triggered, preventing an increase in glomerular filtration rate and minimizing variations of salt concentrations in the fluid entering the distal portions of the nephron, which have a limited reabsorption capacity. It has been proposed that during acute hypertension a Na^+, K^+-ATPase blockage in the proximal tubule contributes the driving force for activating this tubuloglomerular feedback, an event that may explain how arterial pressure fluctuations occur without any measurable change in renal plasmatic flow. Internalization and inhibition of the apical sodium-hydrogen transporters parallels the sodium pump's blockage, leading to an increased delivery of sodium to the distal nephron.[46,47]

Adaptation to a High-Salt Diet

A thorough understanding of the sodium pump's aberrant endocytotic process in hypertensive rats and human beings carrying the adducin mutation resulted from a systematic approach started two decades ago that was aimed at studying both short- and long-term regulation of the enzyme's activity in the renal epithelia under physiologic conditions.[48] Three important facts regarding Na^+, K^+-ATPase short-term (ie, <5 minutes after stimulation) regulation were then appreciated: (1) both the dopamine-natriuretic and the angiotensin-antinatriuretic effects are governed by transient changes in intracellular sodium,[49] (2) changes in Na^+, K^+-ATPase activity in response to hormones are related directly to variations in the number of functional pumps on the plasma membrane,[50] and (3) specific signaling networks targeting Na^+, K^+-ATPase in response to G protein–coupled receptor stimuli take place at specific microdomains, located either at the plasma membrane in the case of endocytic signals, or at intracellular compartments if the process is exocytotic in nature.[50] Importantly, the Na^+, K^+-ATPase itself appears to constitute the scaffold anchoring the different signaling molecules that mediate such processes. A detailed review of similarities and differences between specific tissue networks was recently published.[50]

Long-term changes in Na^+, K^+-ATPase activity also have been shown, and it appears that they are dependent on the aforementioned short-term events, which become rate limiting. To note, ouabain evokes sodium-pump endocytosis in the range of 12 hours, with complete recovery within 12 to 24 hours after the drug's withdrawal.[51,52] The endocytic process also is mediated by clathrin-coated vesicles and requires the intervention of multiple mediators such as Src and phosphoinositide 3 kinase (PI3K). Interestingly, the endocytic-inhibitory process is paralleled by a downregulation in the pump's total mass,[53] whose recovery is dependent on de novo protein synthesis, implying the existence of a sustainable shutting-down process that prevents a permanent withdrawal of functional units as the sole Na^+, K^+-ATPase silencing mechanism.

In addition, ouabain-mediated long-term stimulation of Na^+, K^+-ATPase in different cell lines, including renal ones, activates intracellular signaling complexes such as PKC,[54] MAPK,[55] and reactive oxygen species,[56] switching on transcription factors such as AP-1 and nuclear factor κB, and altering several growth-related genes.

The long-term effect in response to ouabain appears to be associated with the transference of functional Na^+, K^+-ATPase into the nucleus, suggesting either the existence of an ionic pumping mechanism throughout the nuclear membrane that could modulate chromatin structure and gene expression,[57] or a direct genomic effect of Na^+, K^+-ATPase. It has been proposed that the nuclear translocation could downregulate the expression of genes related to ionic transporters important for sodium handling in the proximal tubule.[58] More significantly, the existence of a chemical potential across the nuclear membrane may represent a novel mechanism for modulation of gene expression.

Acute Tubular Necrosis and Different Responses to Hypoxia

A close connection between renal ischemia and the loss in Na^+, K^+-ATPase polarity has been shown.[59] After 10 minutes of ischemia, Na^+, K^+-ATPase redistributes into the apical membrane with the consequent loss in the vectorial transport of solutes and water.[60] The transport of sodium into the lumen is a direct consequence of an aberrant distribution of Na^+, K^+-ATPase.[61] In fact, sodium transport by the proximal tubule epithelium is decreased on apical redistribution of Na^+, K^+-ATPase, independently of other variables known to influence sodium transport. Furthermore, correction of the high urinary excretion of sodium after renal ischemia proved to be dependent on the repolarization of Na^+, K^+-ATPase to the basolateral membrane.[60] In other nonischemic models of hypoxia, Na^+, K^+-ATPase behaves in a different manner. It is well known that hypoxic hypoxia evokes suppression of ATP-demanding cellular functions.[62] It also has been shown that after exposure of pulmonary alveolar epithelial cells to 1.5% O_2 for 60 minutes Na^+, K^+-ATPase endocytosis ensues, this process being mediated by mitochondria-derived reactive oxygen species.[63] Prolonged hypoxia promotes a downstream effect on Na^+, K^+-ATPase activity[64] through the ubiquitin/proteosome pathway-mediated degradation,[65] and probably also by shutting down gene transcription, affecting the resolution of pulmonary edema,[66] which is associated significantly with mortality in patients suffering from acute respiratory distress syndrome.[67] It appears that cytopathic hypoxia, which is an acquired mitochondrial dysfunction that leads to an abnormal ATP turnover, also is associated with a loss of epithelial-barrier permeability and the subsequent development of multiple-organ dysfunction syndrome in the context of systemic inflammation and sepsis.[68,69] In this model, both expression and activity of Na^+, K^+-ATPase are reported to be affected by interferon-γ and nitric oxide stimulation, which in turn alter the expression and/or targeting of tight junction proteins such as occludin, leading to a hyperpermeable epithelium.[70] It has been proposed that either nitration or lipid peroxidation may be the responsible mechanisms of Na^+, K^+-ATPase inactivation in this context.[71,72]

Finally, Na^+, K^+-ATPase inhibition can reduce metabolic demands in the face of anemic hypoxia,[73] so that cardiac glycosides may decrease cellular oxygen uptake in anemic lambs even in the presence of very high catecholamine levels.[74] It is worth emphasizing that Na^+, K^+-ATPase responses rest on complex intracellular signaling cascades that may be organ specific.[50] In this regard, most of the studies were focused on those organs for which a particular model of hypoxia proved to be physiologically more significant, namely, for example, ischemic or hypoxic hypoxia in the cases of the kidney and lung, respectively.

Cerebral Salt-Wasting Syndrome

Ouabain-like compounds (OLC) have been implicated in the development of severe hyponatremia and hypovolemia associated with cerebral salt-wasting syndrome, a catastrophic condition associated with subarachnoid hemorrhage and other intracerebral events.[75] In one trial, plasma OLC immunoreactivity was identified in 18 of 25 patients with aneurysmal subarachnoid hemorrhage, and the level of OLC correlated with the amount of bleeding.[76] Despite this, ouabain administration does not cause significant diuresis and the potential function of Na^+, K^+-ATPase in this process is unknown, which suggests that OLC may play a role in the cerebral salt-wasting syndrome, albeit being an unlikely cause of it.[77] However, it recently was shown that digoxin antibodies, commonly used to treat digoxin toxicity, decrease diuresis and natriuresis in the presence of cerebral bleeding[78] so that an endogenous unidentified OLC could be an important mediator of this pathologic condition.

Potassium Homeostasis

Potassium regulation also is affected significantly by P-type ATPases. Na^+, K^+-ATPase provides the driving force for trafficking solutes of different natures, and classic works on potassium handling by Na^+, K^+-ATPase explored variations of its activity during periods of hyperkalemia and hypokalemia.[79] The kidney adapts to K^+ restriction by upregulating H^+, K^+-ATPase number and activity with a consequent increase in K^+ reabsorption, along with decreased abundance of renal outer-medullary K channel (ROMK) secretory K^+ channels.[80]

Skeletal muscle Na^+, K^+-ATPase plays a central role in adjusting K^+ variations and, again, different isoforms of the pump have specific functions in this regard. Skeletal muscle has an altruistic specialization to lose K^+ in the face of hypokalemia, this response being the converse of what occurs in cultured cells. In the latter, a decrease in extracellular K^+ levels increases its gradient and limits Na^+, K^+-ATPase activity, which eventually increases extracellular K^+ and intracellular Na^+ levels. These events stimulate sodium-pump synthesis and delivery to the plasma membrane, which oppose the K^+ loss from the cell, thus resulting in a net protective effect on the intracellular K^+. Skeletal muscle responds in the opposite way in vivo by diminishing the Na^+, K^+-ATPase activity and thus buffering the decrease in extracellular K^+,

protecting the extracellular K^+ concentration, which has a fundamental impact on cell excitability and thus on the organism's viability.[81] This process is attributed to the $\alpha 2$ isoform without a significant change of $\alpha 1$ activity.[82] By contrast, the observed process in cultured cells rests on the $\alpha 1$-subunit, which reinforces the aforementioned idea that diversification of isoforms was aimed at providing an evolutionary advantage in the face of a more complex scenario, namely in this case fluctuations in the extracellular K^+ availability. The muscular response to hyperkalemia also depends on the $\alpha 2$ isoform function. There is evidence that in the postprandial period K^+ is absorbed by virtue of insulin-mediated uptake through Na^+, K^+-ATPase-$\alpha 2$, which may redistribute from internal endosomal vesicles. The same mechanism helps to buffer K^+ excess of uremic patients during interdialysis periods.[82]

Conclusions

The appearance of Na^+, K^+-ATPase is a significant event in evolution. Its intrinsic properties make the simultaneous occurrence of advanced cellular processes with their inherent fragilities possible. Also, it allowed the existence of new phenomena such as its signaling properties, which are of paramount importance for diverse cellular processes. Discovering that Na^+, K^+-ATPase activity could be affected in a short-term fashion in intact cells in response to physiologic agonists was critical for understanding that variations in its activity depends on changes in the availability of active pumps on the plasma membrane. The latter is affected by complex networks of intracellular messengers in which Na^+, K^+-ATPase acts as a scaffold protein that organizes the anchoring of different mediators in time and space, working either on the plasma membrane or on intracellular organelles.

Expanding this knowledge undoubtedly will be useful in the design of new agents that can modulate Na^+, K^+-ATPase function in an isoform-specific manner and at specific locations within the cell.

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