Particition of sodium-potassium adenosine triphosphatases in homeostasis regulation

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Abstract— A literature review presented an analysis of data regarding the mechanisms of the Na pump in nephron and hormonal regulators of enzyme activity, including enzymatic catalysts. Investigating the regulatory mechanisms of metabolic processes can facilitate the development of new strategies to repair various pathological conditions. Among these functional proteins, Na^+/K^+ATPase is responsible for the regulation of hydroionic homeostasis and signaling. Ion transport in different parts of the nephron is mediated via sodium transporters, which are characterized by a clear topographical expression. In the oligomeric Na^+/K^+ATPase molecule, the α-subunit comprises 10 transmembrane domains and performs a catalytic function. The signal function of Na^+/K^+ATPase and its interaction with the molecular environment in lipid microdomains involve rafts and caveolae. Analysis of the literature data demonstrated an important function of Na^+/K^+ATPase, along with its interaction with caveolin-1, in the regulation of intracellular cholesterol traffic. Moreover, reciprocal interactions of enzymes and cholesterol have been indicated. The status of Na^+/K^+ATPase activity is affected by hypoxia, reactive oxygen species, lipid peroxidation (LPO), increased cholesterol concentrations, and the viscosity of the cytoplasmic membrane. Ecological pollutants, including heavy metals, have significant effects on enzyme activity in nephron, hepatocytes and cardiomyocytes. Thus, available literature data indicate an important role of Na^+/K^+ATPase in the regulation of metabolic processes.

Keywords — caveolin, cholesterol, heavy metals, Na^+/K^+ATPase, Na-transporters

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1. Introduction

In recent years, modern biomedical science has been paying special attention to the study of the molecular mechanisms regulating homeostasis in the body's internal environment, a condition necessary for normal life. Notably, there is growing evidence supporting the role of functional proteins, considering their cellular localization and characteristics of the molecular environment. Understanding the cellular-molecular mechanisms of regulation can serve as the basis for the development of novelstrategies to rectifyvarious pathological conditions. One such important functional protein is Na^+/K^+ATPase, which is responsible for the regulation of hydroionic homeostasis and signaling. Reabsorption of ions, including sodium, in different parts of the nephron is mediated through sodium transporters [1,2]. Each transporter is characterized by a clear topographical expression: the $Na^+/K^+/2Cl^-$ cotransporter (NKCC2) is located in the thick section of the ascending limbof the loop of Henle; Na^+/Cl^-cotransporter (NCC), epithelial sodium channel (ENaC), and Na+/H+ exchanger (NHE- isoform 1) [3-10]. Interestingly, although the adenosine triphosphate (ATP)-dependent Na^+/K^+ATPase exchanger is present in all segments of the nephron, it is most common in the proximal tubule, the thick part of the ascending knee of the Henle loop, the distal tubules, and the medular collecting tubes. The oligomeric Na^+/K^+ATPase molecule consists of two subunits, α and β, in1:1ratio [11-13]. There are 4 isoforms of the α-subunit (α - α4), 3 isoforms of the β-subunit and seven proteins of the FXYD family. The isoform of the α1 subunit is expressed in the kidneys, as well as in red blood cells and the liver. The α2 isoform is mainly expressed in the myocardium, skeletal and smooth muscle [13,14]. The catalytic function is performed by the α-subunit, which includes 10 transmembrane domains (M1-M10). The ATP response and phosphorylation occurs along the long cytoplasmic loop M4-M5. The N-terminal domain is involved in the formation of the ion pathway M1-M4, which is involved in the binding of ouabain, an inhibitor of Na^+/K^+ATPase [15-17]. The extracellular loop of M7-M8 interacts with the β-subunit of the enzyme, contains one transmembrane domain, and acts as a chaperone. Furthermore, it allows the linkage the newly synthesized α-subunit, transport to the plasma membrane, and stabilization [18-21]. Distinctive

properties of the enzyme in the kidneys are provided by the combination of the α1 isoform with β-subunit and one of the FXYD proteins. For each hydrolyzable ATP, three Na^+ and two $K^+$are transported; this asymmetry leads to the generation of membrane potential, an indicator of cellularviability. During the process of Na^+/K^+ATPase functioning, there is a transition between two basic conformations of the protein (E1 and E2). E1 is characterized by a high affinity for ATP, Na^+, and low affinity for K^+; E2 is characterized by the inverse ratio of affinity for cations.

More than 70% of sodium, potassium, chloride, bicarbonate, phosphate, water, almost all glucose, and amino acids are reabsorbed in the proximal tubules [22]. About 15% of sodium is reabsorbed in the thick section of the ascending knee of the Henle loop; potassium, bicarbonate, calcium, magnesium, and urea are also reabsorbed here. The urine formed here is iso- or hypotonic, and the interstitium of the medulla is hypertonic. In the nephron, aldosterone can play the role of a stimulator of intracellular ion reabsorption involving Na^+/K^+ATPase [23-24].

In the main cells of the collecting tubes, sodium reabsorption is associated with potassium secretion through a two-step mechanism: the transport of sodium from cells and that of potassium into them using the Na,K-ATP-basal membrane [23,25]. This serves as a driving force for the entry of Na^+ and the exit of K^+ through the apical membrane. Na^+ entry into the cells is carried out mainly through the epithelial sodium channel (ENaC), constructed from homologous α-, β-, and γ-subunits in a 2:1:1 ratio [26,27]. K^+ secretion, through the apical membrane, occurs through the potassium channels of the external medulla of the kidneys (ROMK). Water reabsorption involves the participation of the vasopressin-induced aquaporin 2 (AQP-2) apical membrane and constitutive aquaporins 3 and 4 present on the basolateral membrane. The driving force for water transport is the sodium reabsorption in the thick section of the ascending part of the loop of Henle. The Na^+/H^+ exchanger of the NHE-1 basolateral membrane is involved in the regulation of intracellular pH [28,29].

In this section of the nephron, aldosterone plays the role of a positive regulator of sodium reabsorption. Aldosterone increases sodium reabsorption and stimulates the excretion of potassium and protons. Additionally, it facilitates the action of vasopressin on water reabsorption. The effect of aldosterone includes delayed transcription induction of mainly α-subunits of Na^+/K^+ATPase and faster activation of a previously synthesized enzyme playing the role of an allosteric regulator of Na^+/K^+ATPase. The second but functionally most significant effect of aldosterone is the effect on the epithelial sodium channel (ENaC) on the apical surface of cells, which is believed to be a limiting element for the sodium reabsorption system [30]. In relation to this effect, a double effect of the hormone was observed: stimulation of transcription of subunits (mainly α-subunits) and activation of a previously synthesized protein. Aldosterone, acting at the transcription level, rapidly (after 30 min) increases the expression of glucocorticoid-regulated kinase 1 (Sgk1), which phosphorylates the ubiquitin-ligase E3 enzyme and blocks the ubiquitin-protein ligase interaction with the epithelial sodium channel [31]. As a result, the rate of ENaC degradation decreases, and sodium transport through the apical membrane of the distal convoluted tubule cells and collecting tubes increases.

Additionally, vasopressin also increases ENaC activity by inducing phosphorylation, but the cAMP-dependent kinase, PC-A, is the effector kinase in this case [32].

In addition to creating a driving force for potassium excretion (activation of Na^+/K^+ATPase and the sodium channel), aldosterone stimulates the expression of the potassium channel of the outer medullary layer (ROMK) and its incorporation into the apical membrane. In this mechanism, aldosterone induces the expression of a glucocorticoid-regulated protein kinase (Sgk1), a phosphorylating potassium channel of the outer medullary layer (renal outer medullary potassium channel of ROMK) [33,34].

In addition to the hormonal regulation of the functional activity of Na^+/K^+ATPase, the glucocorticoid-regulated kinase (Sgk1) of the proximal tubule and the thick part of the ascending knee loop of Henle should be noted. It was first discovered in rat adrenal glands on a diet high in Na^+ and K^+ and belongs to 5-AMP-activated protein kinases (AMPK). With an increase in the concentration of cellular Na^+, there is an increase in the influx of Ca^{2+} through the Na^+ / Ca^{2+} exchanger (NCE1) and the Ca^{2+} / calmodulin-dependent protein kinase 1 (CAMK1) is activated, which activates the Na^+/K^+ATPase. Protein phosphatase 2A (PP2A) is also involved in the activation of Na^+/K^+ATPase, which is part of a multi-protein complex [35]. Along with several pathological processes, including the influence of ecopathogenic factors on renal, cardiovascular diseases, hypoxia plays a role in the regulation of Na^+/K^+ATPase activity. It is inevitably present in conditions such as metabolic syndrome, diabetes mellitus, pulmonary pathologies, toxic situations and causes tissue damage.

One of the main causes of cell damage and death during hypoxia is the disruption of the ion transporting systems and the ion balance necessary for normal cellular activity [36].

Furthermore, the lipid microenvironment plays a role in regulating the properties and functions of Na^+/K^+ATPase. It is known that the lipid environment affects membrane proteins, particularly, Na^+/K^+ATPase. Reportedly, lipids (including cholesterol) play an important role in the stabilization of Na^+/K^+ATPases in the plasma membrane.

To realize the signaling function of Na^+/K^+ATPase, rafts and caveolae play key roles inits interaction with the molecular environment in lipid microdomains. In lipid rafts and caveolae, protein–protein and lipid–protein interactions occur, realizing the most diverse signal functions. The regulators of protein functions in caveolae and raft are lipids themselves, including cholesterol. Presumably, Na^+/K^+ATPase interacts with the N-terminus of caveolin-1 [37,38] and caveolin, which directly interacts with cholesterol, is involved in the regulation of intracellular cholesterol trafficking [37,39].

Thus, another important function of Na^+/K^+ATPase was revealed – its interaction with caveolin-1 in the distribution of cholesterol between intracellular membranes and the plasma membrane. It was observed that disruptions in the interaction

of the α1-isoform of Na^+/K^+ATPase and the formation of caveolae,as wellas the synthesis of cholesterol and its transport, are bilateral [11,40].

Structural changesin the enzyme affect the formation of caveolin; indeed, cholesterol itself is involved in the regulation of Na^+/K^+ATPase. A decreased cholesterol content in the cell membrane causes endocytosis and impairs activity of the α1-isoform of Na^+/K^+ATPase through tyrosine kinase and ubiquitin-dependent regulatory pathways [41]. Presumably, there is reciprocal regulation between the α1-isoform of Na^+/K^+ATPase and cholesterol through the participation of caveolin-1. Thus, lipids themselves, including cholesterol, are regulators of functional proteins in caveolae and rafts.

Our data allow us to consider the possibility of reciprocal regulation between the α-1 isoform of Na^+/K^+ATPase and cholesterol. An increased concentration of cholesterol, which contributes to a change in the viscosity of lipids in the cytoplasmic membrane, affects the molecular structure of Na^+/K^+ATPase, causing a decrease in its functional activity. In our studies, we found that many pathological conditions, including diabetes, coronary heart disease, and toxic conditions, are caused by heavy metal salts; an inverse correlation was recorded between these indicators. In experimental studies of diabetes mellitus, an increase in the microviscosity of the cytoplasmic membranes of red blood cells and cells of internal organs was observed. These changes in the cell membrane were accompanied by a decrease in the activity of Na^+/K^+ATPase and receptor apparatus.

Thus, two forms of Na^+/K^+ATPase are present in the body: one functions as an ionic pump, while the other participates in the membrane and intracellular signal transduction [42- 44]. Furthermore, Na^+/K^+ATPase can react with tyrosine kinase (Src kinase) in specialized structural domains [45,46].

Under hypoxic conditions, against the background of eco pathogenic factors, the activity state of Na+/K+ATPase is influenced by ROS, inducing the LPO process. LPO substrates are unsaturated fatty acids, which are the main components of phospholipids of cytoplasmic and intracellular membranes [47]. A change in the molecular structure of phospholipids of cell membranes disrupts the activity of Na^+/K^+ATPase (Table 1).

Table 1. LPO process indicators, Na+/K+ATPase activity and cholesterol content against the background of ecopathogenic factor (cobalt chloride) in the experiment

Indicators	Control	$CoCl_2$
MDA (red blood cells), nmol / ml	4,41±0,06	5,65±0,045[1111])
SOD (red blood cells), conventional units	87,18±0,98	62,76±0,99[1111])
MDA (cortical substance), nmol / mg protein	2,162±0,042	2,87±0,027[1111])
MDA (medulla), nmol / mg protein	2,69±0,056	5,41±0,019[1111])
Na^+/K^+ATPase (cortical substance), μmol Rn pH / mg protein / hour	2,16±0,042	2,87±0,027[1111])
Na^+/K^+ATPase (brain substance), μmol Rn pH / mg protein / hour	2,69±0,056	5,41±0,019[1111])
Cholesterol, mmol / L	1,79±0,042	3,62±0,043[1111])
HDL cholesterol, mmol / l	0,68±0,021	0,52±0,022[1111])
LDL cholesterol, mmol / l	1,12±0,029	3,09±0,012[1111])
Note: [1111]) - p <0,001; [111]) - p <0,01; [11]) - p <0,02; [1]) - p <0,05 reliability relative to control.		

In renal tissue, the functional ability of ATPase activated by Na^+ and K^+ decreases (p <0,001). Data on the Na-pump indicate its insufficiency in the toxic syndrome, its combination with L-nitroarginine with methyl ether (L-NAME), in contrast to the effects of L-arginine. The regulatory role of Na^+/K^+-dependent ATPase is mediated through a change in the molecular structure of cell membrane phospholipids of the renal tubules, occurring as a result of lipoperoxidation. Unidirectional changes in the enzyme also occurred in hepatocytes and cardiomyocytes due to a lack of energy and inefficiency of the respiratory chain. In rats with toxic syndrome, intersystem analysis between malondialdehyde and the state of the Na-pump in the renal, cardiac, and liver tissues revealed an close inverse correlation, as well as in combination of intoxication with an inhibitor of eNOS expression (r=-0,72; r=-0,67; r=-0,59). Notably, intramolecular changes in the phospholipids of cytoplasmic membranes occurs under conditions of systemic intoxication and activation of free-radical oxidation, manifested as an increase in viscosity and inhibition of the activity of functional proteins including Na^+/K^+ATPase.

2. Conclusion

The literature data confirm the existence of two populations of Na^+/K^+ATPase as an ion pump and a regulator of the signal function of cells. The participation of Na^+/K^+ATPases as a component of ion transporters in the distal nephron and cardiomyocytes was shown. An important role of the enzyme in the thick part of the ascending knee of the Henle loop, as well as in the distal tubules and medullary collecting tubes, is noted. The transport mechanisms in the nephron are regulated by aldosterone. This hormone is responsible for the expression of Na^+/K^+ATPase and ion channels. An important role of Na^+/K^+ATPase in the formation of the hyperosmoticity of the medullary layer of the renal tissue is noted. On the other hand, in the distal tubules of the kidneys, the enzyme contributes to the retention of sodium, fluid, increased blood pressure - hypertension and overload of the heart muscle. Its interactions with the lipid microenvironment, in particular, cholesterol, are described. The signal function of Na^+/K^+ATPases is shown for hypoxic conditions that activate lipid peroxidation. Violation of the structure of phospholipids of the cytoplasmic and intracellular membranes is accompanied by inhibition of enzyme activity.

The prospect of work is the development of new methods for the correction of hypoxic conditions and the restoration of the functional ability of Na^+/K^+ATPase.

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Conflict of Interest

The authors declare no conflict of interest.

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