Chapter 18
Regulation of Membrane Na^+-K^+ ATPase in Health and Disease

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Abstract Na^+-K^+ ATPase is primarily localized in the plasma membrane and occurs in the form of α-subunits (α-1, -2, and -3 isoforms) and β-subunits (β-1 and -2 isoforms) mainly. The inhibition of this enzyme by cardiac glycosides such as ouabain has been shown to raise the intracellular concentration of Na^+ and promote the increase in cardiac contractile force as a consequence of increased Na^+-Ca^{2+} exchange. Several studies have observed that Na^+-K^+ ATPase not only serves as a receptor for cardiac glycosides but also acts as a Na^+-pump for maintaining the electrolyte homeostasis as well as a signal transducer for the formation of reactive oxygen radicals and cellular growth. Different hormones and endogenous factors such as marinobufagenin are considered to regulate the Na^+-K^+ ATPase activity through protein kinase-induced phosphorylation of phospholemman, a membrane protein which is tightly associated with this enzyme. Stimulation of Na^+-K^+ ATPase by catecholamines and phosphorylation of phospholemman seems to be an adaptive mechanism for the prevention of Ca^{2+}-overload-induced arrhythmias whereas excessive increase in the plasma levels of marinobufagenin or high concentrations of cardiac glycosides are considered to induce cardiac dysfunction associated with depression of the Na^+-K^+ ATPase activity.

Keywords Cardiac Na^+-K^+ ATPase • Cardiac glycosides • Marinobufagenin • Phospholemman • Cardiac dysfunction • Signal transduction • Protein kinase-induced phosphorylation • Na^+-pump ATPase

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1 Introduction

Since the discovery of Na^+-K^+ ATPase in 1957 [1], extensive studies have examined the role of this enzyme in both health and disease conditions [2–10]. It is now well known that Na^+-K^+ ATPase or Na^+-pump ATPase is intimately involved in the transport of Na^+ and K^+, and thus maintains the electrochemical gradient across the cell membrane [2, 8, 11, 12]. Cardiac glycosides have been demonstrated to inhibit the Na^+-K^+ ATPase activity and increase the intracellular concentration of Na^+, which then increases the cytoplasmic concentration of Ca^{2+} through Na^+-Ca^{2+} exchanger and thus produces the positive inotropic action in the heart [3, 13, 14]. In addition to serving as receptor for cardiac glycosides for the treatment of heart failure [3], Na^+-K^+ ATPase is considered to act as a signal transducer for promoting cellular growth and thus may participate in the pathogenesis of cancer [15, 16]. While this enzyme has been suggested to be involved in cell survival, complete inhibition of Na^+-K^+ ATPase by ouabain was associated with cell death due to necrosis [17]. Likewise, ouabain at low doses has beneficial effects in the heart whereas at high concentrations, it exerts cardiotoxic actions [3]. Such actions of ouabain may be related to the high and low affinities of ouabain binding sites on Na^+-K^+ ATPase [18]. This chapter focuses on the structural and functional properties of Na^+-K^+ ATPase with particular emphasis on cardiac membranes. Some evidence has also outlined to indicate the involvement of Na^+-pump ATPase as a signal transducer. Furthermore, it is planned to describe some of the regulatory mechanisms for changes in the Na^+-K^+ ATPase activity.

2 Structural and Functional Properties

Although Na^+-K^+ ATPase is considered to be primarily localized in the cell membrane, the functional density of Na^+-K^+ pump current in the t-tubules was found to be 3–3.5-fold higher than that in the sarcolemma of myocardium [19]. Several studies have been carried out to examine the biochemical properties of partially purified Na^+-K^+ ATPase [20–23] from the tissue as well as that of the enzyme present in the sarcolemmal membrane. Treatment of the sarcolemmal membrane with phospholipase C and trypsin was observed to depress the Na^+-K^+ ATPase activity [22]. It was shown that Na^+-K^+ ATPase undergoes conformational changes during its activation by Na^+ and K^+, which subserve the possible mechanism underlying the active transport of cations [21]. The inhibition of the enzyme by ouabain, unlike that by Ca^{2+}, was allosteric in nature; Na^+-K^+ ATPase was also depressed by different inhibitors of sulfhydryl groups [23]. It should be mentioned that initial attempts for the solubilization and purification of Na^+-K^+ ATPase from the cardiac muscle by the use of both ionic and nonionic detergents yielded the enzyme with low activity in the range of 20–30 μmol P_i/mg protein/h [14, 24–26]. On the other hand, when the purification of Na^+-K^+ ATPase from cardiac tissue was carried out by the treatment with deoxycholate and NaI, the activity was found to be in the range of 140–160 μmol P_i/mg

protein/h [23, 27]. The fact that detergents and chaotropic salts did not alter the key properties of Na^+-pump ATPase from those seen in native sarcolemmal enzyme indicates that Na^+-K^+ ATPase is an integral membrane protein.

The Na^+-K^+ ATPase is made up of α- and β-subunits and undergoes E_1–E_2 transition, a characteristic feature of the P-type ATPases [28–32]. The occurrence of three α-subunit isoforms (α_1, α_2, and α_3) and two β-subunit isoforms (β_1 and β_2) has been reported. It was suggested that these isoforms of the enzyme confer evolutionary advantage and regulation of gene expression during development in a tissue-specific manner [33]. The differential expression of Na^+-K^+ ATPase in different cells or tissues appears to be due to its structural heterogeneity [34]. By X-ray microdialysis of frozen cryosections, the Na^+-K^+ pump was identified to be present in the apical and basal cell membranes [35]. A monoclonal antibody, anti-BSP-3, directed against brain cell surface protein [36] recognized mouse Na^+-pump and immunoprecipitated Na^+-K^+ ATPase in microsomal fraction of the kidney; basolateral cell surface of polarized cells was stained by BSP-3 antibody. The α-subunit (MW 84–120 kDa) was considered to play a vital role in the catalytic activity of Na^+-pump ATPase. Sheep kidney Na^+-K^+ ATPase β-subunit (MW 55 kDa) cDNA was cloned and it was shown that p-subunit amino acid sequence was structurally similar to Kdp c subunit of Na^+-K^+ ATPase in *E. coli* [37]. The β-subunit of the Na^+-pump has been shown to play a critical role in cellular resistance to cardiac glycosides [38]. Decreasing the α-helix conformation of plasma membrane proteins was found to inhibit the Na^+-pump due to high concentrations of Ca^{2+} [39]. The amino acid sequence of the catalytic subunit of Na^+-K^+ ATPase (α-isoform) was derived from its cDNA [40]. Expression of the entire mouse cDNA coding for α-subunit conferred ouabain resistance in monkey CV-1 cells whereas deletion of the C-terminal of the α-subunit cDNA resulted in impairing this property [41]. Thus the behavior of Na^+-K^+ ATPase activity is considered to be determined by the composition of its isoforms.

Substitution of amino acids, Gln-Ala-Ala-Thr-Glu-Glu-Glu-Pro-Gln-Asn-Asp-Asn-ò Arg-Ser-Ala-Thr-Glu-Glu-Glu-Pro-Pro-Asn-Asp-Asp, in the N-terminal at extracellular domain of the α-subunit of the Na^+-K^+ ATPase in HeLa cells conferred ouabain resistance [42, 43]. The three α-subunit isoform gene structures have been characterized; all three isoforms have similar exon-intron structure [44]. Upregulation of β-subunit modulates the number of K^+-pump formation and translocation to the membrane surface in addition to regulating the α-β heterodimer formation [45]. Vascular smooth muscle Na^+-K^+ ATPase α-subunit isoform is regulated by alternative splicing of single transcript [46]. Since γ-thio ATP was found to be bound to ATP binding site in the Na^+-pump ATPase and mediate the inhibitory effect, addition of ATP was observed to remove this inhibition [47]. The activity of inactivated enzyme was also restored by incubating with a sulfhydryl reactive agent [48]. Substitution of amino acids α_1 C113 → Y and α_1 C113 → F of the Na^+-pump ATPase renders ouabain resistance compared to the wild type [49]. It has been shown that the Na^+-pump activity is regulated in part by a liver fatty acid binding protein by increasing the plasma membrane fluidity [50]. Substitution of amino acid Y317→ C in α_1-subunit ecto-domain H_3–H_4, caused an increased ouabain resistance in Madin-Darby canine

kidney cell line by affecting the ouabain binding to α-subunit [51]. Amino acid residues 496-HLLVMKGAPER-506 form the fluorescein isothiocyanate (FITC) binding domain in the catalytic α-subunit of the Na^+-pump ATPase. 494-PRHLL-498 is the most critical sequence for nucleoside binding [52]. Transfection of HeLa cells with double mutants generated by the amino acid substitutions at Asp111 and Arg122 in the H_1–H_2 extracellular domain of sheep Na^+-pump α-subunit rendered ouabain resistance to the cells. It was suggested that H_1–H_2 extracellular domain of Na^+-pump α-subunit possesses the functional unit of the Na^+-K^+ ATPase [43].

The yeast Na^+-K^+ ATPase is made up of α- (catalytic), β- (structural), and putative γ-subunit. Presence of α-β-subunit complex alone confers the ouabain-inhibitable Na^+-pump activity whereas the γ-subunit is redundant [47]. The Cys104 in H_1 transmembrane domain of α-subunit of Na^+-pump possesses the cardiac glycoside binding site [53]. The α-subunit hydrophobic domain H_1 and H_2 serves as signal-anchor type II whereas H_2–H_4 confers "halt" transfer signal. The membrane insertion property of Na^+-pump is conferred by amino-terminal possessing signal/anchor type II/halt transfer sequences [54]. The region corresponding to Gly554 → Pro785 in the cytoplasmic domain of α-subunit is essential for complex formation [55]. Substitution of amino acid at D369 → N porcine kidney Na^+-K^+ ATPase α-subunit caused an 18-fold increase in ATP binding by net reduction in negative charge in the phosphorylation site D369 → N [56]. Based on hydropathy plot, different numbers of transmembrane domains, varying from six [57], seven [58], and eight [40], have been proposed for the α-subunit. The H_1–H_2 and H_3–H_4 domains were orienting to the extracellular face and FITC [ATP] binding site, facing the cytoplasm [34]. Both N-terminus and C-terminus in the α-subunit are present in the cytoplasm; N-terminus is the most divergent whereas the H_1–H_2 and H_3–H_4 domains are shown to participate in ouabain binding. Amino acid residues Asp 369 and Lys 501 were labeled by FITC whereas Cys356, Asp 710, and Lys 719 were identified as ATP binding region of α-subunit [28, 40, 59]. It is pointed out that the α-subunit isoform distribution is well conserved across the species [28]. The β-subunit has been shown to mediate the membrane insertion and its transmembrane domain is also conserved across the species. The extracellular domain of different isoforms of β-subunit has different glycosylation sites. Six cysteine residues are critical for the disulfide bridge formation in all β-isoforms [60]. It has been conclusively demonstrated that the stoichiometry of Na^+-pump ATPase is 3 Na^+ (efflux):2 K^+ (influx) and is similar in epithelial and excitable cells [28].

3 Signal Transduction by Na^+-K^+ ATPase

It has become clear that the inhibition of Na^+-K^+ ATPase by cardiac glycosides affects cation fluxes and produces direct actions on cardiac contractility, electrical excitability, and conduction [61]. Synchronized modulation of Na^+-K^+ pump ATPase has been

shown to elicit membrane potential hyperpolarization in the cardiac cells [62]. It should also be noted that transmural gradient in the cardiac Na^+-K^+ pump has been observed to generate a transmural gradient on Na^+-Ca^{2+} exchange [63]. There is a functional communication between Na^+-K^+ ATPase and ATP-sensitive K^+ channels [64]; the activation of K^+-ATP channels by the inhibition of Na^+-K^+ ATPase has been shown to occur in the membrane [65]. It has been reported that the binding of ouabain to Na^+-K^+ ATPase converts this enzyme to a signal transducer and initiates different gene regulatory pathways through the activation of tyrosine kinase and Ras/Raf/MEK/MAPK pathway as well as the increased production of reactive oxygen species in the cardiomyocytes [66]. Not only did the inhibition of Na^+-K^+ ATPase activate tyrosine kinase-mediated signaling, ouabain was also found to increase mitochondrial production of reactive oxygen species and regulate the intracellular concentration of Ca^{2+} [67]. Furthermore, the inhibition of Na^+-K^+ ATPase resulted in an impairment of mitochondrial Ca^{2+}-retention, increased oxidative stress, and enhanced oxidative phosphorylation [68]. In fact, redox-activated protein kinases including PKA, PKC, and CaMKII have been shown to affect Na^+ and Ca^{2+} transporter as well as cationic channels [69]. It was interesting to observe that phosphorylation of sarcolemmal Na^+-K^+ ATPase (α-subunit) due to CaMKII caused a significant reduction in the activity of this enzyme [70]. These results clearly indicate that different protein kinases and redox-related system are intimately involved in the signal transduction mechanisms when Na^+-K^+ ATPase activity is inhibited by cardiac glycosides.

In order to examine the role of Na^+-K^+ ATPase inhibition due to cardiac glycosides in producing the increase in intracellular Ca^{2+} in cardiomyocytes, a pharmacologic approach was employed to understand the involvement of different signal transduction mechanisms [71–73]. The results were found to support the view that in addition to sarcolemmal Na^+-Ca^{2+} exchanger, sarcolemmal L-type Ca^{2+}-channels and store-operated Ca^{2+}-channels may be involved in raising the intracellular concentration of Ca^{2+} upon the inhibition of Na^+-K^+ ATPase. Furthermore, both sarcolemmal Na^+-H^+ exchanger and Na^+-channels may play a critical role in increasing the ouabain-induced increase in the concentration of Ca^{2+} in cardiomyocytes [71, 72]. These results are interpreted to suggest that the inhibition of sarcolemmal Na^+-K^+ ATPase markedly affects the function of other membrane proteins. The involvement of sarcoplasmic reticulum in raising the intracellular concentration of Ca^{2+} due to the Na^+-K^+ ATPase inhibition was also tested by incubating cardiomyocytes with agents that affect the sarcoplasmic reticulum Ca^{2+} stores. Treatments with caffeine, ryanodine, and cyclopiazonic acid attenuated the ouabain-induced increase in the levels of intracellular Ca^{2+}. Inhibitors of CaMKII, PKA, and inositol-3-phosphate receptors were also observed to depress the ouabain-induced increase in the intracellular Ca^{2+} in cardiomyocytes [73]. These results show that the participation of sarcoplasmic reticulum in raising the intracellular concentrations due to the inhibition of Na^+-K^+ ATPase by ouabain is of indirect nature and may involve multiple signal transduction mechanisms in cardiomyocytes.

4 Regulation of Na^+-K^+ ATPase

Various hormones, prostaglandins, and neuropeptides have been shown to exert regulatory effect on Na^+-K^+ ATPase in a tissue-specific manner. For example, catecholamines were found to stimulate the Na^+-K^+ ATPase activity in the heart through the activation of β-adrenoceptors and PKA mechanisms [74, 75]. Angiotensin II showed biphasic effects on renal Na^+-K^+ ATPase as the enzyme was stimulated at its low concentrations and inhibited by its high concentrations [76]. The stimulatory effect of angiotensin II was associated with increased activities of MAP kinase, tyrosine kinase, and NADPH oxidase. Estradiol-induced expression of Na^+-K^+ ATPase catalytic isoforms in vascular tissue was mediated through the formation of nitric oxide [77]. A neuropeptide, myomodulin, was observed to depress the Na^+-K^+ ATPase activity in heart interneurons [78]. Different purine nucleosides, inosine, guanosine, and adenosine, were found to stimulate Na^+-K^+ ATPase activity in a dose-dependent manner in erythrocytes [79]. Marinobufagenin, an endogenous ligand of α-1 subunit of Na^+-K^+ ATPase, has been reported to depress the myocardial enzyme activity [80]. Likewise, thromboxane B2, a product of thromboxane A2 during the synthesis of prostaglandin, was shown to inhibit the Na^+-K^+ ATPase activity in the heart [81]. Thus the activity of Na^+-K^+ ATPase is regulated by a wide variety of hormones and endogenous factors and seems to play an important role in the adaptation and maladaptation of different organ function in the body.

The regulation of Na^+-K^+ ATPase is considered to be affected mainly by phosphorylation and dephosphorylation of phospholemman, a small transmembrane protein which is associated with the enzyme [82–85]. Phospholemman is a member of the FXYD family of proteins and is a major substrate for different protein kinases [86, 87]. In its dephosphorylated form, phospholemman exerts an inhibitory action on Na^+-K^+ ATPase whereas it is stimulatory in the phosphorylated form. Phospholemman phosphorylation and modulation of Na^+-K^+ ATPase due to catecholamines and ischemia in the heart has been suggested to function in a manner similar to the interaction of phospholamban and Ca^{2+}-pump ATPase in the sarcoplasmic reticulum [88, 89]. It should be mentioned that both PKA and PKC have been shown to phosphorylate phospholemman at conserved serine residue (Ser 68) in its cytoplasmic domain and induce major changes in the protein conformation and thus alter the Na^+-K^+ ATPase activity [86]. Furthermore, phospholemman phosphorylation and Na^+-K^+ ATPase stimulation has been suggested to be an integral part of sympathetic fight-or-flight response for limiting Ca^{2+}-overload-induced arrhythmias as a consequence of attenuated rise in the intracellular concentration of Na^+ [83].

Na^+-K^+ ATPase is regulated by different hormones not only in physiological conditions, but this enzyme is also a target of regulation by various factors under diseased situations. Extensive work has indicated marked changes in the Na^+-K^+ ATPase activity in diverse cardiovascular diseases [4–7, 9]. In view of the ability of Na^+-K^+ ATPase to maintain cell volume [90], it is possible that changes in the enzyme activity may alter cellular function by inducing changes in cell volume. During cardiac hypertrophy and transition to heart failure due to hypertension, a decrease in α-1 isoform and an increase in α-3 isoform in cardiac Na^+-K^+ ATPase are associated with

an increase in the plasma levels of endogenous ligand, marinobufagenin [91]. In fact, depressed cardiac Na^+-K^+ ATPase activity and associated reduction in Na^+-K^+ ATPase α-1 isoform in heart failure were seen upon the infusion of marinobufagenin [92]. Different hypertrophic stimuli have been reported to alter the enzyme activity by affecting the gene expression of Na^+-K^+ ATPase β-1 and α-3 isoforms [93]. The inhibition of Na^+-K^+ ATPase and the development of cardiac dysfunction in endotoxemia were associated with myocardial TNF-α protein expression via calcium/mTOR signaling [94]. A modest diet-induced increase in serum cholesterol was found to increase the sensitivity of sarcolemmal Na^+-K^+-pump to intracellular Na^+, whereas a large increase in cholesterol levels decreased the sensitivity to Na^+ [95]. Some investigators have also observed the arrhythmogenic role of Na^+-K^+ ATPase in human heart failure and have suggested its involvement as an important modulator of excitability and refractoriness in human atria [96, 97]. Such results provide evidence that Na^+-K^+ ATPase is regulated by diverse mechanisms during the development of heart disease. Different diseases including diabetes [98] have been shown to alter the properties of this enzyme and thus can be seen to regulate the Na^+-K^+ ATPase.

5 Conclusions

From the foregoing discussion, it is evident that the membrane Na^+-K^+ ATPase not only serves as a Na^+ pump for maintaining the homeostasis of electrolytes in the cell but also forms an essential component of the signal transduction mechanisms. Various hormones and endogenous factors have been shown to regulate the Na^+-K^+ ATPase activity through phosphorylation and dephosphorylation of phospholemman, a membrane protein which is associated with this enzyme. Although α-isoforms (α-1, -2, and -3 subunits) and β-isoforms (β-1 and -2 subunits) of the Na^+-K^+ ATPase are tissue specific, their role in the regulation of enzyme activities has been suggested in the development of disease processes. A shift in the Na^+-K^+ ATPase isoform composition seems to occur during the transition of cardiac hypertrophy to heart failure. Particularly, the plasma level of the endogenous ligand, marinobufagenin, has been shown to increase in heart failure due to hypertension which may phosphorylate phospholemman, change the composition of Na^+-K^+ ATPase isoforms, and depress the enzyme activity.

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