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Endogenous modulators in the regulation of ion transporting enzymes: structure, function, interactions, recent advancements and future perspectives

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ABSTRACT

A prerequisite for life is the ability to uphold electrochemical imbalance across biomembranes. Ion transporting enzymes, known as specific pumps, are responsible for the transport of various ions across cell membranes to sustain the same. In all eukaryotes, the plasma membrane potential and secondary transport systems are maintained by the activity of P-type ion transporting enzymes, commonly known as ATPase membrane pumps. Malfunction of pumps leads to various cell disorders and subsequently diseases like cardiac problems, renal malfunctionings, diabetes, cataract, even cancer. Activities/functions of these pumps are regulated either by exogenous agents or by endogenous substances like proteins, peptides, hormones, etc., which are collectively known as modulators. Some of these endogenous modulators may be useful for developing drugs depending on the nature of regulation. For more than last two decades, researchers across the globe are exploring the mechanism of action of different endogenous modulators on these ion transporting enzymes with the aim of developing target-specific drugs. In this review, we have discussed recent advances in our understanding of ATPase pumps, e.g., Ca^{2+}-, Na^+, K^+-, Ca^{2+}, Mg^{2+}-, H^+, K^+-ATPases, with the emphasis on their functional regulation by a number of endogenous modulators, and the implications of development of some of these modulators as potential drugs.

Keywords: ATPases; Endogenous Protein/Peptides; FXYD; Phospholamban; Sarcolipin; Interleukin; Regulations

1. INTRODUCTION

Transport enzymes are those responsible for the transport of ions across the cell membranes. The transport takes place against ion gradient, energy required for the process is provided by ATP, which is hydrolyzed to ADP and Pi during these transport phenomena. These are a large group of evolutionary conserved ion pumps that are found in bacteria, archaea and higher eukaryotes and belong to P-type ATPases (includes Na^+, K^+, Ca^{2+}, H^+, K^+-ATPases) and are involved in performing different fundamental processes in biology and medicine, ranging from the generation of membrane potential to muscle contraction, the removal of toxic ions from cells, maintaining proper acidity inside cells etc. [1]. Mutation or dysfunction of these ATPases leads to several diseases. Malfunction of Ca^{2+}-ATPase may lead to defect in cardiac function, infertility, diabetes and even cancer [2]. Impairment of sodium pumps, on the other hand, cause diseases including osteoporosis, hypertension, familiar hemiplegic migraine. P-type ATPases, that transport monovalent and divalent cations such as Na^+, K^+, Ca^{2+}, Cu^+, Ag^+, Zn^{2+}, Cd^{2+}, Ni^{2+} etc., are divided into five subfamilies on the basis of the conserved sequences [3].

It is now well established that all these P-type ATPases are regulated by endogenous modualtors like, peptides, proteins and other small molecules.

1.1. Na^+, K^+-ATPase

The Na^+, K^+-ATPase commonly known as sodium pump, is responsible for coupled extrusion and uptake of Na^+ and K^+ ions across the plasma membranes of most eukaryotic organisms. Na^+, K^+-ATPase is a member of the P_{2c} family of P-type ATPases superfamily [4]. The pump drives three sodium ions out of the cell and two potassium ions into the cell against substantial concentration gradient. The activity of this enzyme is required for diverse functions like maintenance of cellular osmotic balance, generation of neuronal membrane potentials and renal as well as intestinal handling of solutes. This is a $(\alpha/\beta)_2$ dimeric integral membrane

protein and is composed of a 100-kDa α-subunit and a heavily glycosylated β-subunit of molecular weight about 55 kDa [5]. In addition, Na^+, K^+-ATPase is associated with a third subunit belonging to the FXYD protein family [6]. Isoforms exist for both the α (α_1, α_2, α_3) and β (β_1, β_2, β_3) subunits. These isoforms are expressed variedly in different mammalian tissues [7-10] with various $\alpha\beta$ combinations. The Na^+, K^+-ATPase can function as an $\alpha\beta$ protomer, but it is postulated that the Na^+, K^+-ATPase is composed of $(\alpha/\beta)_2$ dimer *in vivo*. α subunit contains ATP binding site, phosphorylation site, and amino acids essential for the binding of cations and cardiac glycosides which suggests that this subunit plays a major role in the catalytic function of the enzyme. The β subunit appears to be involved in maturation of the enzyme, localization of ATPase to the plasma membrane, and stabilization of a K^+-bound intermediate form of the protein [11]. The other low molecular mass transmembrane proteins were named after the invariant extracellular motif FXYD [6]. One central role of the FXYD proteins is to interact with the Na^+, K^+-ATPase and modulate its properties. There are seven FXYD proteins. Because each of these proteins has a different tissue distribution and functional effects, the current hypothesis is that FXYD proteins act as tissue-specific modulators of Na^+, K^+-ATPase that adjust or fine-tune its kinetic properties to the specific needs of a given tissue, cell type, or physiological state, without affecting it elsewhere [12,13].

1.2. Ca^{2+}-ATPase

Ca^{2+}-ATPase or the Ca^{2+}-pump on the other hand is responsible for the transport of Ca^{2+} across cell membranes and thus maintains intracellular calcium concentration. Because of its peculiar flexibility as a ligand, calcium regulates all important aspects of cellular activeity, beginning with the creation of new life at fertilization and ending with the dramatic event of apoptotic suicide at the end of the life cycle. Ca^{2+} may also function as a bonafide first messenger since it interacts with the exterior of cellular membrane as if it were a hormone or growth factor [14]. Ca^{2+} is distinctly ambivalent molecule that is essential for life. Cells have an absolute dependence on the messenger function of Ca^{2+}. In order to function properly, its homeostasis must be controlled with absolute precision, failing of which, there can be a sustained cellular Ca^{2+} overload leading to apoptotic and/or necrotic cell death [15]. The cytoplasmic free Ca^{2+} concentration in all cell types at rest is very low (50 nM - 150 nM) which is 10^3 - 10^4 times lower than the free Ca^{2+} concentration in the extracellular space (usually 1 mM) or in the lumen of sarco(endo)plasmic reticulum (SR/ER) (0.1 mM - 2.0 mM). Such large Ca^{2+} gradients across cellular boundaries are established and maintained by the powerful calcium pumps located in the plasma membranes and in sarco (endo) plasmic reticulum [16] with contributions from other cellular organelles.

The Ca^{2+} transporting systems can be classified into four basic transporting modes *i.e*. ATPases, exchangers, channels and uniporters. In general, whenever the situation demands the fine regulation of Ca^{2+} in submicromolar concentrations, the ATPase mode are chosen, since this appears to be the only transport system with the ability to interact with high Ca^{2+} affinity and is therefore used by plasma membrane and sarcoendoplasmic reticulum.

The ATP dependent Ca^{2+} pumps of sarco (endo) plasmic reticulm (SERCA) constitute a large family of proteins of 100 kDa - 138 kDa [17-21] and a proteolipid of molecular mass 6 kDa - 12 kDa [22], belonging to P_2 subfamily (subtype 2A) of P-type ATPases. They are structurally distinct from the Ca^{2+} pump of the plasma membrane, but share similarities in the mechanism of calcium translocation. The intracellular location of SERCA exclusively in SR/ER membranes is maintained by the presence of specific retention/retrieval motifs in their primary sequences. The Ca^{2+} transport is reversible and under favourable condition results in the formation of ATP molecule for two Ca^{2+} ions released from the lumen of SR [23]. Counter-transport of H^+ and fluxes of ions through the anion and cation channels of SR prevent large changes in membrane potential during Ca^{2+} transport. The SERCA pumps have high affinity for Ca^{2+} (K_m about 0.1 μM), and are capable of maintaining a resting cytoplasmic [Ca^{2+}] of 10 nM - 20 nM.

The plasma membrane Ca^{2+}-ATPase (PMCA) is the only high affinity Ca^{2+} transporting system present in the plasma membrane and belongs to the P_2 (subtype 2B) subfamily of P-type ATPases [24]. The molecular mass is 130 kDa - 140 kDa and are characterized by the formation of an aspartyl phosphate intermediate as part of their reaction cycle [25]. At variance with the closely allied SERCA, PMCA contains only one Ca^{2+} binding site, and indeed transports one Ca^{2+} as one ATP molecule is hydrolyzed.

In addition to Mg^{2+}-dependent Ca^{2+}-ATPase, another Ca^{2+}-ATPase which can be activated without any Mg^{2+} has also been reported from a number of tissues and sources with varying sensitivity to calcium and insensitivity to magnesium [26-34]. Both these ATPases are having similar properties [35-37]. They may either be the two forms of the same enzyme having separate catalytic sites or same catalytic site with different sensitivities to Mg^{2+} [36,38,39].

1.3. H^+, K^+-ATPase

The gastric H^+, K^+-ATPase is an alpha beta ($\alpha\beta$) heterodimeric member of the eukaryotic alkali-cation P2-type ion-motive ATPase family which undergoes a cycle of phosphorylation and dephosphorylation coupled to the outward and inward transport of hydrogen and potassium ions, respectively. The secretory canaliculus of the parietal cell present in the gastric glands of the stomach perform secretion of hydrochloric acid upon hormonal stimulation [40-43]. The ATPase sustains a 10-fold inward potassium gradient (150 mM K^+ in, 15 mM K^+ out) and a transmembrane outward hydrogen ion gradient of greater than 1 million fold to generate a luminal pH of 0.8. This is the largest ion gradient generated by a P2 type ATPase.

The molecular weight of α subunit is ~114 kDa and that of the glycosylated β subunit is ~65 kDa. The α subunit of hog, rat and sheep is predicted to span the membrane 10 times and a β subunit only once [40,41]. The primary structure of the α subunit of gastric H^+, K^+-ATPase (HK α_1) shows significant homology to the Na^+, K^+-ATPase (62%) and SR Ca^{2+}-ATPase (29%) while the β subunits of H^+, K^+-ATPase and Na^+, K^+-ATPase are 35% identical [41].

All these above mentioned ATPases have been reported to be regulated by endogenous proteins, peptides, hormones and/or other small molecules to different extent [44,45], collectively known as 'modulators'. For more than two decades scientists across the globe have been exploring different aspects of the regulation of these ion transporting enzymes by endogenous modultors.

In the present review, structure-functions of different ATPases, how they are regulated by endogenous modulators like peptides, proteins and other compounds, as well as the mechanism of their regulation and the implications/importance have been described.

2. STRUCTURE AND FUNCTION OF ATPASES

Jens Skou in 1957 first examined the effect of different cations, e.g. Na^+ and K^+ (later named as Na^+, K^+-ATPase) [46] in leg nerve homogenates of crabs. He was finally awarded the Nobel Prize in Chemistry in 2001, *i.e.*, 40 years after the discovery. It is specifically and characteristically identified in its inhibition by extra cellular binding of cardiac glycosides, the most widely used and well known one is ouabain [47].

Subsequent to his discovery in 1961, other ion pumps, like Ca^{2+}-pump [48] with comparable characteristics but different properties were identified from different tissues and organisms. Most important and well characterized of them are sarcoplasmic-reticulm (SR) Ca^{2+}-ATPase, which controls the contraction of skeletal muscl [17].

H^+, K^+-ATPase, another ion transporting enzyme mainly present in the gastric cells, is well known for its vigorous role during acid secretion process. It is homologous in sequence to the Na^+, K^+-ATPase and the Ca^{2+}-ATPase and has a similar pattern of transmembrane helices.

The biochemical properties that are common among these ATPases are: 1) formation of an acid-stable, phosphorylated aspartic acid residue during the pumping cycle (that is phosphorylated intermediate) and 2) inhibition by orthovanadate.

The mechanism of the Ca^{2+} ATPase is usually discussed in terms of the E_1-E_2 model developed from the Post-Albers scheme for Na^+, K^+-ATPase [49]. All these ATPases follow a similar catalytic cycle as described by Post-Albers [49] for Na^+, K^+-ATPase. The reaction cycle of the Ca^{2+} ATPase is shown in **Figure 1**.

Either ATP or Ca^{+2} can bind first to the E_1 conformation of the Ca^{2+}-ATPase. A series of conformational changes lead to the intermediate E_1"Ca_2.ATP, which undergoes phosphorylation to give E_2PCa_2. This leads to the release of Ca^{2+} into the lumen followed by dephosphorylation to form E_2, which then returns to E_1.

The above model proposes that Ca^{2+}-ATPase (SERCA) can exist in one of the two distinct forms, E_1 or E_2. In the E_1 conformation, the ATPase can bind two Ca^{2+} ions from the cytoplasmic site of the membrane with high affinity whereas in the E_2 conformation these two sites are closed. Following binding of ATP, the enzyme is phosphorylated on Asp-351 to give a phosphorylated intermediate E_2PCa_2, a state in which two Ca^{2+} binding sites are of low affinity and face inwards, the lumen of the SR. Following the loss of Ca^{2+} to the lumen of the SR, the ATPase dephosphorylates to E_2 and then recycles to E_1. The phosphorylation events on the Ca^{2+}-ATPase are reversible.

Putative structure of the plasma membrane Ca^{2+}-ATPase indicating the key functional sites are shown in **Figure 1**.

Putative structure of the plasma membrane Ca^{2+}-ATPase indicating the key functional sites [50] are shown in **Figure 2**.

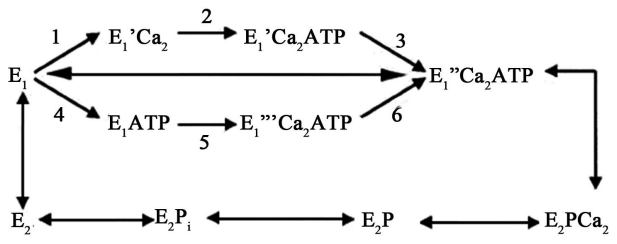

Figure 1. E_1/E_2 scheme for Ca^{2+}-ATPase.

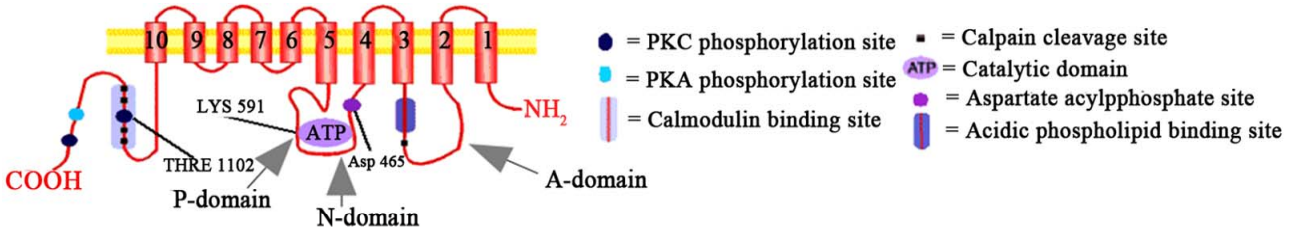

Figure 2. Putative structure of the plasma membrane Ca^{2+}-ATPase indicating the key functional sites [50].

In the plasma membrane, the enzyme is organized with ten transmembrane domains, and the NH_2 and COOH termini are both located on the cytosolic side of the membrane. The bulk of the protein mass is facing the cytosol and consists of three major parts: the intracellular loop between transmembrane segments 2 and 3, the large unit between membrane-spanning domains 4 and 5, and the extended "tail" following the last transmembrane domain [51,52]. The first intracellular loop region between membrane-spanning domains 2 and 3 corresponds to the "transduction domain" is believed to play an important role in the long-range transmission of conformational changes occurring during the transport cycle. The large cytosolic region of ~400 residues between membrane spanning segments 4 and 5 contains the major catalytic domain including the ATP binding site and the invariate aspartate residue that forms the acyl phosphate intermediate during ATP hydrolysis. Finally, the extended COOH-terminal tail corresponds to the major regulatory domain (calmodulin-binding domain) of the PMCAs [53,54].

The PMCAs closely resemble that of the SERCA [sister pump of sarco (endo) plasmic reticulum] [55]. Indeed, the major global difference between the two types of calcium pumps is confined to the C-terminal tail, which is generally much smaller in the SERCAs (ranging from <20 to ~50 residues) than in the PMCAs (70 to 200 residues). Unlike SERCA pump, it only contains one Ca^{2+}-binding site, and indeed transports one Ca^{2+} as one ATP molecule is hydrolyzed.

All P-type ATPases have an architectural commonality, with cytoplasmic domains which are linked to a transmembrane module. The three cytoplasmic domains [known as the phosphorylation (P), nucleotide binding (N) and actuator (A) domains], as revealed from the first high resolution X-ray structure of the sarco (endo) plasmatic reticulum Ca^{2+}-ATPase (SERCA) which exchanges Ca^{2+} for protons) [17] are responsible for ATP hydrolysis. The P-type ATPases have six transmembrane helices (M1 - M6) that make up the core of the membrane transport domains. Both these subclasses have ten trans-membrane helices (M1 - M10): the core segment (M1 - M6) and an additional carboxyterminal transmembrane segment (M7 - M10). Many P-type ATPases also have regulatory (R) domains, which typically inhibit their function [24]. Crystallographic studies have provided detail information on the pumping mechanism of rabbit SERCA1a [17-19,56-58].

The functional cycle of P-type ATPases is typically denoted by E_1 and E_2 states which in case of SERCA relate to the binding and active transport of cytoplasmic Ca^{2+} and the countertransport of luminal H^+ to the cytoplasm, respectively. In Ca^{2+} translocation, ATP is involved as the key substrate in the formation of the Ca^{2+} occluded E_1-P state [56]. However, ATP at physio-logical concentrations also exhibits a general, stimulatory effect on the functional cycle of SERCA relating to a noncatalytic, modulatory mode of binding to the various ATPase intermediates. A key question as to the mechanism of SERCA is then to address the structural and functional properties of the modulatory ATP binding site in comparison to the catalytic site and to make possible extrapolations to other P-type ATPases [59].

There is evidence from mutational studies [60,61], and X-ray crystallography [19,56] which strongly suggests for a direct involvement of the conserved residues in the N-domain in nucleotide binding region of rabbit SERCA1a. Another puzzling observation relates to the structure of SERCA in the Ca_2E_1 state, which exhibits an open conformation with the N- and A-domains detached from the P-domain [17].

The complete structural shape of the Na^+, K^+-ATPase and identification of the individual domains based on a docking model derived from SERCA1a have been reported [62]. Recently, new information on the structure and function of the Na^+, K^+-ATPase and the H^+-ATPase has emerged from X-ray crystal structures of the auto-inhibited plasma membrane H^+-ATPase from Arabidopsis thaliana (AHA2) [63] and of the pig renal $\alpha_1\beta_1\gamma$ Na^+, K^+-ATPase complex (pNKA) [64,65]. A structure of shark Na^+, K^+-ATPase (sNKA) was determined at 2.4 A resolution [66] and was found to be similar to that of pNKA. This structure more accurately resolved the atomic interactions within the Na^+, K^+-ATPase, completed the β subunit ectodomain structure cholesterol [64,65]. The mechanism by which P-type ATPases function and how it is coupled to ATP hydrolysis, general and specific transport mechanisms have been proposed on

the basis of protein chemistry and mutagenesis experiments using various P-type ATPases [15,67-71] together with structural studies of the Ca^{2+}-ATPase from rabbit SERCA [19,69,72,73].

When the transported cations (three Na^+ for the Na^+, K^+-ATPase; one proton for H^+-ATPases) are bound at the membrane transport domain of the ATPase, a conformational change occurs and transmits to the P domain, predominantly by the M4 helix [17,18,58,59,74]. Conformational changes associated with the functional cycle can be examined from studying the Ca^{2+}-ATPase. The bound cations are occluded in the E_1P state. Occlusion occurs during phosphorylation owing to the formation of an ATP mediated cross link of the N and P domains, which moves the A domain to the side [19,73]. As the K^+ ions of the Na^+ pump bind (or when the H^+-ATPase has formed a proposed intramolecular salt bridge), the induced closure of the E_2P state stimulates dephosphorylation, and this is mediated by the TGE motif of the A domain which activates a water molecule at the phosphorylation site. Dephosphorylation leads to an occluded E_2 state, with two K^+ ions present in the occlusion cavity of Na^+, K^+-ATPase [19,64,75]. In the E_2 state, ATP is bound at the N domain and stimulates an E_2 to E_1 transition by interfering with residues involved in tertiary interactions between domains [59].

For the Na^+, K^+-TPase and SERCA, the M7 - M10 segments provide additional coordinating groups fo ions bound at the core region of the M domain and thus contribute to selectivity [17,64]. For Na^+, K^+-ATPase, the M7 - M10 helices were shown to have strong influence on the Na^+ affinity, as determined by the α subunit C terminus and its interaction network [64,65,76-79]. An overview of the structural aspects of plasma membrane Na^+, K^+-ATPase and H^+-ATPase ion pumps has been published recently [80].

3. DIVERSITY IN UNITY IN THE FUNCTION OF ATPASES

The mechanism of ion coordination and transport of P-type ATPase was first revealed after determination of the molecular structure of the sarcoplasmic reticulum Ca^{2+}-ATPase in an E_1 conformation at 2.6 A resolution [17]. Furthermore, the structure of the Ca^{2+}-ATPase in E_2 conformations bound with phosphate analogues has also been identified [19,57,73,81]. These structures show that the three cytoplasmic domains rearrange to move six out of ten transmembrane helices, thereby changing the affinity of the Ca^{2+}-binding sites and the gating of the ion pathway. These structural data have allowed construction of homology models that address the central questions of mechanism of active cation transport by all P-type cation pumps. However, for Na^+, K^+-ATPase and H^+, K^+-ATPase, which consist of both α and β-subunits, there may be some specific differences in regions of subunit interactions. Mutagenesis, proteolytic cleavage, and transition metal-catalyzed oxidative cleavages have provided evidence about residues involved in binding of Na^+, K^+, ATP, and Mg^{2+} ions and changes accompanying E_1-E_2 or E_1-P-E_2-P conformational transitions [67]. Recently Ogawa *et al.* [75] described the crystal structure of Na^+, K^+-ATPase with bound ouabain, a representative cardiac glycoside, at 2.8 A resolution in a state analogous to E_2. $2K^+$. Pi. Ouabain is deeply inserted into the transmembrane domain with the lactone ring very close to the bound K^+, in marked contrast to previous models. Due to antagonism between ouabain and K^+, the structure represents a low-affinity ouabain-bound state. Yet, most of the mutagenesis data obtained with the high-affinity state are readily explained by the present crystal structure, indicating that the binding site for ouabain is essentially the same [75]. The crystal structure also shows that the beta-subunit has a critical role in K^+ binding and explains, at least partially, why the homologous Ca^{2+}-ATPase counter-transports H^+ rather than K^+, despite the coordinating residues being almost identical [66]. Morth *et al.* [64] has shown that the beta- and gamma-subunits specific to the Na^+, K^+-ATPase are associated with transmembrane helices alpha-M7/alphaM10 and alphaM9, respectively. Electron microscopy has revealed the overall shape of proton pumps. The structure of a P-type proton pump determined by X-ray crystallography shows the ten transmembrane helices and three cytoplasmic domains define the functional unit of ATP-coupled proton transport across the plasma membrane, and the structure is locked in a functional state not previously observed in P-type ATPases [80].

4. ATPASES AND ENDOGENOUS MODULATORS

4.1. Regulation of Na^+, K^+-ATPase

Na^+, K^+-ATPase is found to be regulated by the endogenous factors , e.g. peptides, proteins, hormones or other small molecules, known as modulators [45]. There are two types of factors: 1) peptidic and 2) nonpeptidic [82-84]. Physiologically, ATPase actions are controlled by endogenous regulator proteins. It has been reported that an endogenous inhibitor protein inhibits porcine spem motility and a competitive inhibitor of Na^+, K^+-ATPase and is identical to β-microseminoprotein [85]. The regulators of Na^+, K^+-ATPase can be divided into two groups: (a) direct modulators e.g., ouabain, some proteins and peptides and (b) indirect modulators which include many peptides, nonpeptideic hormones and neurotransmitters etc. [82,83]. The former group can bind directly with enzyme protein but the latter group of compounds affect via binding to membranes of specific re-

ceptors. Na^+, K^+-ATPase is also inhibited by some endogenous glycosides [86], insulin [87], aldosterone [88], endothelin-1 (ET-1) [89], thyroxin [90], bradykinin [91] and the N-terminal fragment of substance P (SPI-5) [92] most likely via receptor-mediated mechanism.

A group of proteins known as FXYD proteins, a family of seven homologous single transmem-brane segment proteins (FXYD1-7), has been observed to be expressed in a tissue-specific fashion and regulate Na^+, K^+-ATPase activity. Most of them are short chain single proteins (> 100 amino acids) except FXYD5 which is an atypically long N-terminal sequence [44]. It has been suggested that seven members of this family, FXYD1 (phospholemman) [93], FXYD2 (γ-subunit of Na^+, K^+-ATPase) [94,95], FXYD3 (Mat-8) [96], FXYD4 (CHIF) [97,98], FXYD5 (RIC, dysadherin) [99], FXYD6 (Phosphohippolin) (7) and FXYD7 [100], are auxiliary subunits of Na^+, K^+-ATPase. FXYD6 is unique in its expression in the inner ear, suggesting a role in endolymph compostion [101]. Another FXYD protein called FXYD10 with 74 amino acids has been reported to regulate the activity of shark Na^+, K^+-ATPase [102]. Some endogenous peptides of varying molecular weights regulate Na^+, K^+-ATPase [103,104]. Recent work of our laboratory has suggested that endogenous proteins of varying molecular masses isolated from different sources can either inhibit or stimulate ATPase activities [37,105-107]. The inhibittors were found to inhibit partly the H^+, K^+-ATPase also. The findings suggested that these proteins inhibit specifically monovalent ion transporting enzymes. Partial amino acid sequence of the 70 kDa inhibitor protein of Na^+, K^+-ATPase from goat spermatozoa cytosol [108] showed about 80% - 100% homology with mice [109], pig [110] and human [111] testicular aryl sulphatase. Another novel protein, MONaKA was reported to interact with plasma membrane Na^+, K^+-ATPase and modulates its activity [112].

Purification of non-peptidic endogenous Na^+, K^+-ATPase inhibitors to its high purity has been reported [113] and the structures of three peptidic inhibitors (SPAI-1, -2,-3) have been revealed [114]. A small peptide of molecular mass approximately 600 dalton isolated from human cerebrospinal fluid has been found to specifically inhibit Na^+, K^+-pump [115].

The involvement of cAMP-dependent protein kinase (PKA) in acute sodium pump regulation has been documented in 20 different mammalian tissues and in lower vertebrates [116]. The result can be either stimulation or inhibition of the pump, however, and in no case is the pathway completely understood. These structure of the Ca^{2+}-ATPase loop homologous to the Na^+, K^+-ATPase, it was suggested that PKA site should be inaccessible to the kinase [117]. Regulation occurs on several levels: biosynthesis and degradation; reversible recruitment to and internalization from the plasma membrane; alteration of affinity for Na^+; and either stimulation or inhibittion of activity.

4.2. Regulation of Ca^{2+}-ATPase

Fewer reports are available on endogenous protein stimulators/inhibitors of Ca^{2+}-ATPases. Calmodulin is the naturally occurring activator of plasma membrane Ca^{2+}-ATPase. A protein of molecular weight 63 kDa from human erythrocyte membrane [118], and another ~56 kDa - 60 kDa from dog and beef heart sarcolemma have been reported to stimulate Mg^{2+}, Ca^{2+}-ATPase. Regucalcin, a calcium binding protein plays a pivotal role in maintaining intracellular Ca^{2+} homeostasis due to activetion of Ca^{2+} pump enzymes in plasma membrane (basolateral membrane), microsomes (endoplasmic reticulum) and mitochondria of many cell types [119]. Other activators are acidic phospholipids and long chain polyunsaturated fatty acids [120]. Phosphorylation by protein kinase A and protein kinase A inhibitor (PKI) purified from bovine heart stimulates Ca^{2+}, Mg^{2+}-ATPase activity in human erythrocytes [121]. Recently, PDC-109, the major secretory protein from bull seminal vesicles has been described to stimulate bovine sperm membrane Ca^{2+}-ATPase. An analysis of the enzyme kinetics data suggested irreversible, cooperative interaction of PDC - 109 with the enzyme, the stimulation being organ specific, but not species specific [33]. A 12 kDa protein from rat brain cytosol reported from the author's laboratory has been found to inhibit Mg^{2+}-independent Ca^{2+}-ATPase while stimulating the Mg^{2+}-dependent form of the enzyme [37], another 14 kDa protein either from goat spermatozoa [122,123] or bovine brain [124,125] is found to stimulate Mg^{2+}-independent Ca^{2+}-ATPase without any significant effect on Mg^{2+}-dependent form of the ezyme. Narayanan *et al.* [126] reported a cytosolic protein fraction, termed CPF-I, obtained by $(NH_4)_2SO_4$ fractionation of rabbit heart cytosol which caused marked inhibition (up to 95%) of ATP-dependent Ca^{2+} uptake by cardiac sarcoplasm reticulumn. An endogenous inhibitor of SR Ca^{+2}-ATPase has been reported from human placenta, distributed in cytosol and microsomal fractions [127].

Sarcolipin (SLN) *in vitro* inhibits SERCA1 or SERCA2 pump activity. However the exact nature of regulation by sarcolipin is not fully understood [128]. Phopholamban (PLN), an oligomeric proteolipid has been reported to inhibit cardiac Ca^{2+}-ATPase [129]. It is composed of 52 amino acids and is organized in three domains: (a) cytosolic domain 1a (amino acids 1 - 20); cytosolic domain 1b (amino acids 21 - 30) and domain II

(amino acids 31 - 52) which tranverse the domains [130]. Various attempts have been made to therapeutically target of SERCA2a and PLN in animal and human heart failure models [131]. *In vitro* mutagenesis studies evaluating the interaction between PLN and SERCA2a have been directed toward the cytoplasmic domains (aa residue, 1 - 30), which include the phosphorylation sites in PLN. These studies demonstrate the importance of PLN interaction with SERCA2a and suggest that interferance with this interaction may provide a novel therapeutic approach for prevention of dilated cardiomyopathy. Convincing evidence suggested that the impaired function of the SR to cycle Ca^{2+} during diastole and systole is a critical defect in cardiomyocytes from failing hearts. Strategies to interfere with the PLN/SERCA2a interacttion have been proposed as therapy to improve Ca^{2+} cycling, contractility, and relaxation in failing and nonfailing animal and human heart models [132]. The recent mutation identified in a human genetic study has suggested the need for further refinement of the new therapeutic concept of PLN inhibition [133]. A class of inhibitors has emerged, named "caloxins" defined as substances that bind to the PEDs (putative extracellular domains) in PMCA to inhibit any conformational changes in them during the reaction cycle and hence modulate PMCA activity. The first caloxin (caloxin 2A1) was discovered as a peptide that would bind a synthetic peptide corresponding to the sequence of PED2 of PMCA [134]. Caloxin 2A1 does not affect the activities of Mg^{2+}-ATPase or Na^+, K^+ ATPase in the erythrocyte ghosts, SERCA1 Ca^{2+}, Mg^{2+}-ATPase in fast twitch skeletal muscle. Caloxin 3A1, based on PED3, inhibits the PMCA pump but not the sarcoplasmic reticulum Ca^{2+} pump [135].

Interleukin-2 (IL-2), one of the most important cytokines, generally produced by activated helper T-lymphocytes and stimulates proliferation and effector functions of various cells of immune system, has been shown to increase the activity of SR Ca^{2+}-ATPase in rat cardiomyocytes [136].

However, it also decreases the sensitivity of SR Ca^{2+}-ATPase to calcium. It has been demonstrated that 3,5,3'-tri-iodo-L-thyronine (T3), in rat thymocytes, increases plasma membrane Ca^{2+}-ATPase activity [137]. The effect of T3 is rapid, concentration related, evident at a physiological concentration as low as 1 pM. The amphiphilic peptide mastoparan, isolated from wasp venom, has been shown to be a potent inhibitor of the sarcoplasmic reticulum Ca^{2+}-ATPase. The peptide also decreases the affinity of the enzyme for Ca^{2+} and abolishes the cooperativity of Ca^{2+} binding [138]. Myotoxin a, a polypeptide of 43 amino acids from the prairie rattle snake Crotalus viridis viridis [139] and mellitin, a basic peptide isolated from bee venom inhibit the activity of the Ca^{2+}-ATPase of skeletal muscle sarcoplasmic reticulum [140]. Palytoxin, a coral toxin significantly reduces Ca^{2+} pumping of isolated SR vesicles [141]. A variety of polyamines, including spermine, spermidine and polyarginine inhibit the Ca^{2+}-ATPase of skeletal muscle [142].

The skeletal muscle SR Ca^{2+}-ATPase is stimulated by jasmine and jasmonate and increase the rate of dephosphorylation of the ATPase [143]. Reports show that ceramide stimulates the plasma membrane Ca^{2+}-ATPase activity in a dose dependent manner and an additive effect in activation was observed in presence of calmodulin and ethanol [144]. Ceramide affects the affinity for Ca^{2+} and V_{max} of the enzyme, and also stimulates Ca^{2+} transport in inside-out plasma membrane vesicles from erythrocytes. Sphingosine, on the other hand, inhibits the calmodulin stimulated enzyme [141]. Ivermectin, a macrocyclic lactone (IC_{50} = 7 μM), and cyclosporin A (a cyclic oligotide) have been shown to be potent inhibittors of SERCA1. Ivermectin inhibition has been shown to be competitive with respect to high concentrations of ATP, increase of K_m at the regulatory binding site [145].

5. MECHANISM OF REGULATION

It is evident from the above discussion that different endogenous regulators modulate various ion transporting enzymes. Questions come, what are the mechanisms of regulation?

Na^+, K^+-ATPase has been reported to be inhibited by a number of proteins of varying molecular weights. A 12 kDa - 13 kDa molecular mass protein from rat brain was found not to compete with ouabain (a specific inhibitor of Na^+, K^+-ATPase) in inhibiting the enzyme, whereas, an additive effect was observed in combination with ouabain, suggesting their different binding sites on ATPase. The inhibitor is responsible for controlling the phosphorylation of the ATPase and, thus, its activity and this is due to the binding to E_2 state of the enzyme. The inhibition was found to be due to conformational change of the ATPase on binding to the inhibitor [105].

Another group of high molecular mass (70 kDa - 75 kDa) proteins isolated from rat brain and goat spermatozoa cytosol was found to inhibit Na^+, K^+-ATPase without having any appreciable effect on Ca^{2+}-ATPase. In both the cases, inhibition was found due to blocking of the phosphorylation step of the overall reaction sequences [106,107]. The one isolated from rat brain (75kDa) inhibits Na^+, K^+-ATPase activity reversibly and binds to the E_2 state of the enzyme. CD spectra indicates a slight loss of α -helix and random coil in the enzyme-inhibitor complex [107]. While the inhibition of the Na^+, K^+-ATPase by 70 kDa protein isolated from goat spermatozoa was found to be competitive in nature with respect to its substrate, the binding is reversible and inhi-

bition was found to be due to the change in conformation of the enzyme [106]. The fact that both these inhibitors bind reversibly with the enzyme may act as control element of the transport enzyme and suggests their important role as endogenous regulators.

The SPAIs isolated from porcine duodenum, has been shown to inhibit Na^+, K^+-ATPase by the competitive mode against Na^+ and non-competitive to K^+ [146]. Another regulator, PE-60 has an activating influence on Na^+, K^+-ATPase from rat brain frontal cortex, the peptide stimulates the enzyme apparently due to Na^+-dependent steps of the Na^+, K^+-ATPase system. The activating effect was enhanced by preincubation at low concentrations of ATP that transforms the enzyme into the Na^+-form [103]. It is obvious that the interactions of PE-60, as well as SPIs, with Na^+, K^+-ATPase are relatively complex, both of them exerted their effect by binding to two different sites of the enzyme with different affinities. At low concentration, PEC-60 acts as an activator of the enzyme and at higher concentrations the activating effect is reversed by binding another molecule of PEC-60 [103]. Although SPIs have been reported to inhibit Na^+, K^+-ATPase, the effect was found to be biphasic, at submicro-molar concentration about 20% stimulation was observed from intestine whereas at higher peptide concentration inhibition was found [146]. The porcine sperm motility inhibitor (molecular mass, 15 kDa) is identical to β-microseminoprotein and is a competitive inhibitor of Na^+,K^+-ATPase [85]. With MONaKA, it has been revealed that it binds tightly to the β_1 and β_3 subunits of the Na^+, K^+-ATPase. The association between MONaKA and Na^+, K^+-ATPase β-subunit was confirmed by coimmunoprecipitation from transfected cells, mouse brain, and cultured mouse astrocytes [112]. Hormones like aldosterone has been reported to increase the Na^+, K^+-ATPase function in cultured AT2 cells. This was associated with an increase in the β_1-subunit mRNA levels and β_1-subunit protein abundance in AT2 cell plasma membranes. It has been demonstrated that the human Na^+, K^+-ATPase is transcriptionally regulated by aldosterone and may involve a direct interaction with potential hormone response elements present in the promoter region of these genes. Endothelin-1 (ET-1), a hormone, increases the Na^+, K^+-ATPase activity in epithelial cells by enhancing the mRNA and protein levels of the α_1 subunit suggesting ET-1 could play a homeostatic role in modulating aqueous humor formation by having differential effects on the activity and expression of Na^+, K^+-ATPase by the ciliary epithelium in the eye [89].

FXYD proteins modulate the function of Na^+, K^+-ATPase by affecting its kinetic properties in specific way. Although effects of FXYD proteins on parameters such as $K^+_{1/2}$, $Na^+_{1/2}$, K_mATP and V_{max} are modest, usually two folds, however these effects may have important long-term consequences for homeostasis of cation balance. Functional modulators are likely to affect the ATPase activity possibly by altering rate limiting steps, particularly the E_1P-E_2P and $E_2(K)$-E_1 conformational transitions, or binding of the transported cations, particularly cytoplasmic Na^+ ions (or competing K^+ ions), which may limit the rate of active Na^+ pumping *in vivo*. Although some conclusions on the effects of FXYD on cation binding and conformational transitions have been drawn, direct observations with purified α/β/FXYD complexes would be more conclusive [44].

Evidence suggests multiple sites of interactions between FXYD and α/β subunits. Data supporting this came from the fact that the anti-γC terminus neutralizes the effect of γ on the apparent ATP affinity in renal Na^+, K^+-ATPase or HeLa cells transfected with γ, but not with K^+: Na^+ antagonism [16,44]. Expression in HeLa cells of γ with either 4 or 10 C-terminal residues or 7-deleted N-terminal residues removes the effect of γ on ATP affinity but does not affect the K^+: Na^+ antagonism. Replacement of the deleted 7 N-terminal residues with 7 alanines restores the effect on ATP affinity [93].

Mutational analysis combined with expression in Xenopus oocytes reveals that Phe^{956}, Glu^{960}, Leu^{964}, and Phe^{967} in TM9 of the Na^+, K^+-ATPase α subunit represent one face interacting with the three FXYD proteins *i.e.* FXYD2, FXYD4, and FXYD7. Leu^{964} and Phe^{967} contribute to the efficient association of FXYD proteins with the Na^+, K^+-ATPase α subunit, whereas Phe^{956} and Glu^{960} are essential for the transmission of the functional effect of FXYD proteins on the apparent K^+ affinity of Na^+, K^+-ATPase. The relative contribution of Phe^{956} and Glu^{960} to the K^+ effect differs for different FXYD proteins, due to the intrinsic differences of FXYD proteins on the apparent K^+ affinity of Na^+, K^+-ATPase. In contrast to the effect on the apparent K^+ affinity, Phe^{956} and Glu^{960} are not involved in the effect of FXYD2 and FXYD4 on the apparent Na^+ affinity of Na^+, K^+-ATPase. The mutational analysis followed by a docking model of the Na^+,K^+-ATPase/FXYD7 complex, predicted the importance of Phe^{956}, Glu^{960}, Leu^{964}, and Phe^{967} in subunit interaction [147]. It has been suggested that FXYD proteins modulate Na^+, K^+-ATPase activity in close cooperation with post-translational modifications such as phosphorylation [148].

Recent study shows FXYD1 associated with the Na^+, K^+-ATPase α and β subunits, and that the effects of phosphorylation by PKA on the Na^+, K^+-ATPase regulatory activity of FXYD1 could be due primarily to changes in electrostatic potential near the membrane surface and near the Na^+/K^+ ion binding site of the Na^+, K^+-ATPase α subunit [149]. Furthermore, protein kinase phos-

phorylation also seems to involve direct modification of Na^+, K^+-ATPase catalytic activity both in vitro and *in vivo*, at least in some systems [149]. Interestingly, FXYD proteins are found to be involved in the regulation of some diseases. Recently Okudela *et al.* [150] has reported that FXYD3 levels were also lower in a considerable proportion in primary lung cancers than in non-tumoral airway epithelia; its expression levels decreased in parallel with the dedifferentiation process. Therefore, it was suggested that inactivation of FXYD3 through a gene mutation or unknown mechanism could be one of the causes of the atypical shapes of cancer cells and play a potential role in the progression of lung cancer.. The latest addition of FXYD11 gene (zFXYD11) regulating the transport ability of NaK-MRCs (mitochondrion-rich cells) by modulating Na^+, K^+-ATPase activity may be involved in the controlling of body fluid and electrolyte homeostasis [151].

Anionic phospholipids increase the intermolecular cross-linking between the FXYD10 C-terminus (Cys74) and the Cys254 in the Na^+, K^+-ATPase (in shark) A-domain. However it has been suggested that phosphorylation involves only modest structural rearrangements between the cytoplasmic domain of FXYD10 and the Na^+, K^+-ATPase A-domain [147]. The salinity-dependent expression of pFXYD (pufferfish FXYD gene) protein and Na^+, K^+, as well as the evidence for their co-localization and interaction in pufferfish gills suggested that pFXYD regulates Na^+, K^+-ATPase activity in gills of euryhaline teleosts upon salinity challenge [152].

The ATPase described here gets phophorylated by different protein kinases for their function. The protein kinase A/protein kinase C (PKA/PKC) phosphorylation profile of H^+, K^+-ATPase is very similar to the one found in the Na^+, K^+-ATPase. PKC phosphorylation is taking place in the N-terminal part of the α-subunit with a stoichiometry of 0.6 mol Pi/mole α-subunit. PKA phosphorylation is in the C-terminal part and requires detergent, as is also found for the Na^+, K^+-ATPase. The stoichiometry of PKA-induced phosphorylation was 0.7 mol Pi/mole α-subunit. The Na^+, K^+-ATPase is also known to be regulated by membrane trafficking controlled by N-terminal PKC phosphorylation [63] through a mechanism that involves binding of phosphoinositide 3-kinase to a polyproline motif in the N-terminus [153]. Controlled proteolysis of the N-terminus abolished PK C phosphorylation of native H^+, K^+-ATPase [102].

A number of low molecular mass proteins reported from the author's laboratory has been found to affect Mg^{2+}, Ca^{2+}-ATPase and Ca^{2+}-ATPase differentially. One, molecular mass of 12 kDa from rat brain has been reported to stimulate Ca^{2+}, Mg^{2+}-ATPase and inhibit Ca^{2+}-ATPase (SERCA family), the binding between enzymes and the protein was found to be reversible and non-competitive in nature. The modulator was found to be negatively charged protein and could be a good tool in distinguishing the regulation of these two ATPases [37]. On the other hand, a protein with molecular mass 13,961 from goat testes cytosol [122] has been found to stimulate Mg^{2+}-independent Ca^{2+}-ATPase without having any appreciable effect on Mg^{2+}-dependent one. The stimultorbinds to a single site of the enzyme. The effect was due to enhancement of the dephosphorylation rate of the overall reaction steps and acceleration of the calcium uptake [123]. Another 14 kDa mass stimulator of Mg^{2+}-independent Ca^{2+}-ATPase (belongs to SERCA family) has been reported by Ghoshal *et al.* [124,125] from bovine brain and was found to enhance the Ca^{2+}-uptake. It also stimulates sperm motility suggesting its role in fertility. Proteomic analysis suggests its similarity with thyroid hormone-responsive protein [154]. The effects in both the above cases were found to be non-species specific against SERCA.

Myotoxin a, a polypeptide from the prairie rattle snake was found to inhibit Ca^{2+}-ATPase by decreasing the rate of dephosphorylation, with no effect on the Ca^{2+}-transport step]139].

Based on the findings of the author's laboratory, following schemes (**Figures 3(a**) and **(b)**) have been proposed for the regulation of Mg^{2+}-independent and Mg^{2+}-dependent Ca^{2+}-ATPases by various endogenous modulator proteins.

Each of the modulator reported from the author's laboratory [37,105-107,122,124] has been found to bind to the respective ATPase in a reversible manner. Thus it has been hypothesized that they can act as endogenous regulators of the ATPases. Moreover, an "on" and "off" type of mechanism has been proposed, whereby the modulator remains inactive under normal condition (off). It gets activated (on) when a malfunction of the ATPase/pump occurs, binds to the enzyme, brings it to the normal condition then dissociates. A cartoon as shown below in **Figure 4** has been proposed.

The most important among the regulators of Ca^{2+}-ATPase is calmodulin. The PMCA pump has high affinity for Ca^{2+} (K_m < 0.5 μM) when complexed with calmodulin, the physiological activator of the pump [155]. Calmodulin stimulates the V_{max} of the enzyme, but especially decreases the K_m (Ca^{2+}) by one order of magnitude *i.e.* from about 10 μM - 20 μM to about 0.5 μM. The pump interacts with calmodulin in a Ca^{2+}-dependent manner and has high affinity for it: a K_d of about 1 nM has been observed [53]. The regulation of the pump by calmodulin binding in its C-terminal tail provides a striking example of the autoregulation of the Ca^{2+}. Ca^{2+}-calmodulin binds to a region in the COOH-terminal portion of the PMCAs, located ~40 residues downstream of the last transmembrane domain[156]. In the absence of

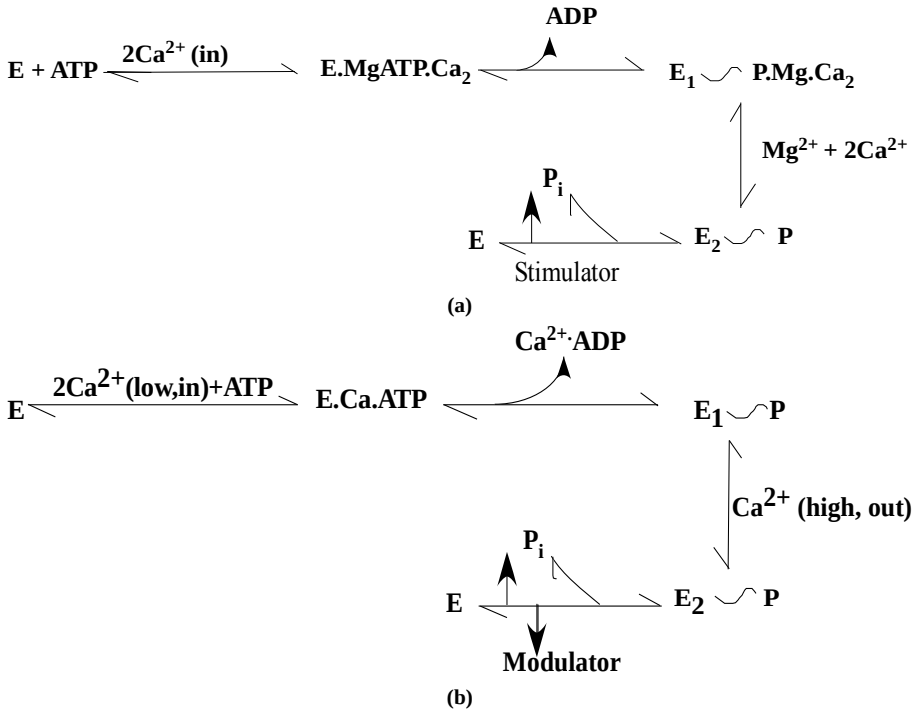

Figure 3. (a) Mg^{2+}-dependent Ca^{2+}-ATPase. Phosphorylation is followed by a complex formed in presence of Mg^{2+} and ATP followed by dephosphorylation. Endogenous protein was found to stimulate (↑) the enzyme activity. (b) Mg^{2+}-independent Ca^{2+}-ATPase. Phosphorylation and dephosphorylation are controlled by low (high affinity) and high (low affinity) concentration of Ca^{2+}. The modulator proteins can either stimulate (↑) or inhibit (↓) the enzyme activities.

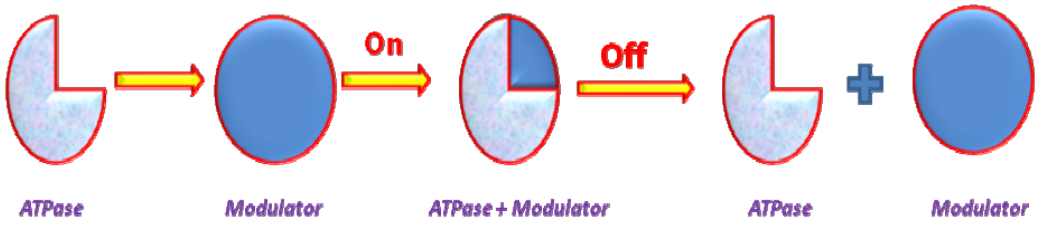

Figure 4. A schematic model showing binding of the modulator with the ATPase and its dissociation.

Ca^{2+}-calmodulin, this sequence acts as an "autoinhibitory domain"; cross-linking studies using labeled peptides demonstrated that the calmodulin binding domain interacts intramolecularly with two separate regions of the pump, one located in the first cytosolic loop and the other in the major catalytic unit between the phosphorylation and the ATP binding site [155]. This intramolecular interaction probably hinders the access of Ca^{2+} and/or ATP to the active site, preventing catalytic turnover, keeping the pump in an inhibited state. An elevation in the cytoplasmic Ca^{2+} results in an increase in Ca^{2+}-calmodulin, which then binds with high affinity to the autoinhibitory domain of the PMCA, thereby releasing the inhibition and stimulating pump activity to near-maximal potential.

The peptide, caloxin inhibits Ca^{2+}, Mg^{2+}-ATPase activity in leaky erythrocyte ghosts with a K_i value of 0.4 mmol/L - 0.8 mmol/L. The inhibition is noncompetitive with respect to the substrates and calmodulin [157]. Caloxin1A1 inhibits PMCA by binding to the first extracellular domain [135] while caloxin1B1 is isoform selective with a higher affinity for PMCA4 than PMCA1 [158]. The effect of spermine, particularly, is highly specific; inhibition resulting from the decrease in the rate of dissociation of Ca^{2+} from the phosphorylated ATPase ($Ca_2E_1P \rightarrow E_2P$) [142]. It has been suggested that regucalcin (maintains intracellular Ca^{2+}-homeostasis) may act on the SH groups of Ca^{2+}-ATPase by binding to microsomal membranes [119]. Effects of different endogenous modulators on affecting activities and/or affinities of the enzymes to the substrate and/or co-factors are shown in **Tables 1** and **2**.

Table 1. Effects of different modulators on the activities and kinetic pameters of Na^+, K^+-ATPase. ↑ stimulation and ↓ inhibition of the enzyme activities.

Modulators	Enzymes	Kinetic parameters change	Activity (↑↓)	Refs.
	Na^+, K^+-ATPase	$K_{1/2}$ Na^+	-	[93]
FXYD1	(rat kidney, bovine and rat cardiac sarcolema);	$K_{1/2}$ K^+		
	rat cardiac Na^+, K^+-ATPase	-	↑	[159]
	Na^+, K^+-ATPase (renal)	K_mATP	-	[94]
FXYD2	knockout mouse	$K_{1/2}$ Na^+	-	[94]
	(kidney membrane)	K_mATP		
FXYD3	Na^+, K^+-ATPase	$K_{1/2}$ Na^+		
	(X.oocytes)	$K_{1/2}$ K^+		
FXYD4	Na^+, K^+-ATPase (mammalian)	$K_{1/2}$ Na^+	-	[160]
FXYD7	Na^+, K^+-ATPase (brain)	$K_{1/2}$ K^+	-	[100]
SPAI-1	Na^+, K^+-ATPase	$K_{1/2}$ Na^+	↓	[114]
	(mammalian kidney	$K_{1/2}$ K^+		
12 kDa - 13 kDa	Na^+, K^+-ATPase	$K_{1/2}$ Na^+	↓	[105]
Protein (rat brain)	(rat brain)	$K_{1/2}$ K^+		
75 kDa protein	Na^+, K^+-ATPase	$K_{1/2}$ Na^+	↓	[107]
(rat brain)	(rat brain)	$K_{1/2}$ K^+		
70 kDa protein	Na^+, K^+-ATPase	$K_{1/2}$ Na^+	↓	[106]
(goat testis)	(rat brain)	$K_{1/2}$ K^+		

Table 2. Effects of different modulators on the activities and kinetic pameters of Mg^{2+}-independent and Mg^{2+}-dependent Ca^{2+}-ATPases. ↑ stimulation and ↓ inhibition of the enzyme activities.

Modulators	Enzymes	Kinetic parameters change	Activity (↑↓)	Refs.
PDC-109	Mg^{2+}-independent	K_mATP	↑	[33]
(bovine seminal and dependent vesicles)	Ca^{2+}-ATPase			
	(bovine spermatozoa)			
12 kDa protein	Mg^{2+}-independent	$K_{1/2}$ Ca^2	↓	[37]
(rat brain)	Ca^{2+}-ATPase	K_mATP		
	Mg^{2+}-dependent			
	Ca^{2+}-ATPase	$K_{1/2}$ Ca^{2+}	↑	[37]
	(goat spermatozoa)	K_mATP		
4 kDa protein	Mg^{2+}-independent	$K_{1/2}$ Ca^{2+}	↑	[124]
(bovine brain)	Ca^{2+}-ATPase			
4 kDa protein	Mg^{2+}-independent	$K_{1/2}$ Ca^{2+}	↑	[122]
(goat testis)	Ca^{2+}-ATPase			

Two other low molecular weight proteins which can modulate SERCA pump activities are phospholamban and sarcolipin [161,162]. *In vitro*, phospholamban inhbits SERCA1 and SERCA2, but not SERCA3. The phosphorylation of phospholamban by the catalytic subunit of the cAMP-dependent protrein kinase reverses the inhibitory effect of Ca^{2+}-pump. This suggests that phosphorylation of an inhibitor of the Ca^{2+}-ATPase in cardiac SR and dephopshorylation relieves its inhibition. The direct interaction between hydrophilic portion of PLN may be one of the mechanisms of the regulation. It is clear that SERCA2a and PLN have a critical role in SR Ca^{2+} cycling and contractility. Thus, elucidating more specific roles that these proteins play in the development of cardiomyopathy may aid in development and improvement of drugs for the treatment of cardiac disease, and ultimately lead to the generation of novel genetic therapy for human heart failure [129]. PLN binding inhibits the Ca^{2+} pumps by lowering their Ca^{2+} affinity [161]. It can exist as a monomer or as a pentamer and it is the latter which appears to be responsible for the inhibition [163]. The transmembrane interaction sites (a residue, 31 - 52) were also shown to mediate the regulatory effects of PLN on SERCA2a affinity, with some mutations yielding increased inhibition while others abolished the PLN inhibitory effects on SERCA2a in vitro [164]. The total expression levels of one phospholamban per Ca^{2+}-ATPase result in full inhibition of the enzyme activity. The excess PLN expressed in the heart over that required for inhibition suggests a capability for graded responses of the Ca^{2+}-ATPase activity to endogenous kinases and phosphatases that modulate the level of phosphorylation necessary to relieve inhibition of the Ca^{2+}-ATPase by PLN [165]. Mutagenesis studies show the SERCA sequence Lys^{397}-Asp Acid-Asp Acid-Lys-Pro-Val^{402} are essential for regulation by phospholamban [133]. This sequence interacts with the cytoplasmic domain of phospholamban. Since this sequence is missing in SERCA3, it is not inhibited by PLN. It interacts with the SERCA pump both at the cytosolic nucleotide binding domain (Lys^{400} in the N domain) [166] and with the transmembrane sector, maintaining the pump in an inhibited state. The inhibition is removed by phosphorylation of residues Ser^{16}, Thr^{17} and Ser^{10} in the hydrophilic portion by protein kinase A, a Ca^{2+}/calmodulin-dependent kinase-II and protein kinase C respectively. The phosphorylation is assumed to induce a conformational change of PLN that forces its detachment from the pump both at the cytosolic and the transmembrane interacting sites. PLN can be again dephosphorylated by protein phosphatases.

Sarcolipin is a 31 amino acid peptide expressed predominantly in the fast twitch skeletal muscle [167], although in vitro it inhibits SERCA1 or SERCA2 pump activity. Amino acid sequence comparison and modeling studies have shown that the transmembrane helices of SLN and PLN share considerable homology, suggesting both proteins interact in a similar way with SERCA [128, 168-170]. A recent study suggests that the lumenal domain could be involved in the retention of SLN in the ER, although it might have a different function too [171]. The flexible nature of the C-terminus also leaves Tyr-29 and Tyr-31 residues available for interactions with various aromatic residues in the transmembrane helices of SERCA and suggests that lumenal domain could be involved in the regulation of SERCA-SLN interaction [128,171]. Recently Morita *et al* has proposed the interactions among PLN, NF-SLN and SERCA1a and found that mutation of amino acids Ile^{40}, Ile^{47}, and Ile^{48} in PLN and mutation of Val^{19}, Ile^{22} and Trp^{23} in NF-SLN diminished either the super-inhibition imposed on SERCA1a function by the PLN-NF-SLN binary complex or the physical interactions between PLN and NF-SLN or both [172].

6. CONCLUSIONS

The foregoing discussion along with the supporting information suggest that endogenous modulators of varying molecular masses regulate ATPase activities and are believed to act as physiological regulators of different ATPases. The question may be raised whether the modulator(s) particularly when they are proteins would reach the target site in their native form when use from outside since they may be degraded by proteolysis. The other possibility is to develope antibody against these modulators due to prolong use of them.

However, inspite of these short comings, protein regulators may be useful tool for develping drugs with better acceptability since they are endogenous in nature. We have found that one of the modulators of Ca^{2+}-ATPase [37] can act as a female contraceptive agent when tested on rat and rabbit. An Indian patent has been awarded on this work. Simuilarly, it is clear that SERCA-2a and PLN have a critical role in SR Ca^{2+} cycling and contractility. Thus, elucidating more specific roles that these proteins play in inducing cardiomyopathy may aid in development and improvement of drugs for the treatment of cardiac disease, and ultimately lead to the generation of novel genetic therapy for human heart failure [131]. SERCA is responsible for the reuptake of cytoplasmic Ca^{2+} in muscle, where it ensures the efficient relaxation at the end of a contraction event. Recently it has been reported that FXYD3 levels were decreased in a considerable proportion of primary lung cancers than in nontumoral airway epithelia. Its expression levels decreased in parallel with the dedifferentiation process.

Therefore, it has been suggested that inactivation of FXYD3 through a gene mutation or unknown mechanism could be one of the reasons of the atypical shapes of cancer cells and play a potential role in the progresssion of lung cancer [150]. Therefore activation of FXYD3 by any out side agent may provide a lead of developing an anticancer drug.

Hence, it may be suggested that some of these modulators may be utilized as drug against a specific disease which is considered to be quite important and interesting from physiological, biochemical and medicinal point of view.

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