

Review

Na,K-ATPase in the Myocardium: Molecular Principles, Functional and Clinical Aspects

A. ZIEGELHÖFFER¹, K. KJELDSEN², H. BUNDGAARD², A. BREIER³, N. VRBJAR¹
AND A. DŽURBA¹

¹ *Institute for Heart Research, Slovak Academy of Sciences,
Dúbravská cesta 9, 842 33 Bratislava, Slovakia*

² *Department of Medicine B2142, The Heart Centre, Rigshospitalet,
National University Hospital, Blegdamsvej 9, DK-2100 Copenhagen, Denmark*

³ *Institute of Molecular Physiology and Genetics, Slovak Academy of Sciences,
Vlárska 3, 833 34 Bratislava, Slovakia*

Abstract. The role that Na,K-ATPase plays in Na⁺ and K⁺ antiport through the sarcolemma, in cation-homeostasis in cardiomyocytes as well as in excitation-contraction coupling and cell signalling in the myocardium is now widely recognized. It was its key importance for the cell membrane function that kept this enzyme intensively studied during the last three decades and finally brought to its discoverer the deserved Nobel Prize. Almost weekly are appearing new data concerning structure, function, regulation and role of the Na,K-ATPase in different physiological and pathological conditions. The special importance of the enzyme for heart function as well as the great amount of data that is concerned specifically with the heart Na,K-ATPase and accumulated since yet, started to call for setting them in order. The present paper updates basically important data on the cardiac Na,K-ATPase in relation to its specific properties, molecular mechanisms of function, mode of action, humoral and pharmacological modulation, adaptability, physiological role and clinical aspects.

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Correspondence to: Attila Ziegelhoffer, PhD., DSc., Institute for Heart Research, Slovak Academy of Sciences, 842 33 Bratislava, Dúbravská cesta 9, Slovakia.
E-mail: usrdzigy@savba.savba.sk

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Key words: Na,K-ATPase of heart sarcolemma — Secondary structure — ATP and cation-binding sites — Modulation and adaptability — Clinical relevance

1. The role of Na,K-ATPase in cardiac cells

Magnesium-dependent, sodium- and potassium-activated adenosine triphosphatase (Na,K-ATPase), colloquially referred to as the sodium pump, is the enzyme that proved to be responsible for transport of Na⁺ and K⁺ ions across the cell membrane (Skou 1965, 1975) and was shown to be involved in the regulation of many transport related processes in animal cells (Kjeldsen et al. 1987; Ziegelh  ffer et al. 1995, 1996a; Pekovi   et al. 1997; Kaloyianni et al. 1998; Breier et al. 1998; Sulov   et al. 1998). The enzyme is present in the heart sarcolemma of numerous species (Akera et al. 1969). In the heart, Na,K-ATPase is intimately involved in reestablishing the transmembrane ionic gradients, following influx of Na⁺ and Ca²⁺ as well as by efflux of K⁺ ions from cardiac cells during the processes of excitation and contraction (Dhalla et al. 1977). The enzyme fulfills the latter task by extruding Na⁺ from, and importing K⁺ ions into the myocytes. Via this action, Na,K-ATPase reestablishes the membrane potential and the cardiac muscle returns to the resting state (Dhalla et al. 1982).

The energy required for the countertransport of three molecules of Na^+ from intra- to extracellular space and two molecules of K^+ from extra- to intracellular space is provided by hydrolysis of one molecule of ATP (Matsui and Schwartz 1966; Skou and Esmann 1992) originating predominantly, if not exclusively, from glycolysis (Fedelešová et al. 1972). The 3Na:2K exchange ratio was confirmed for Na,K-ATPases from diverse sources (Chapman 1984) as well as for hearts of different species (Dhalla et al. 1977). Countercurrent exchange of non-equivalent amounts of Na^+ and K^+ ions between the intra- and extracellular spaces, performed by the enzyme, yields a net decrease of positive charged ions in intracellular space, in favor of the extracellular space. This electrogenic effect of the Na,K-ATPase (Chapman 1984; Eisner et al. 1984) leads *per se* to an increase in resting membrane potential amounting 5–10 mV i.e., to augmentation of hyperpolarization of cardiac cells (Breier and Monošíková 1990).

The electrochemical gradient across the sarcolemma, created by alkaline metals represents a form of transformed energy (Läuger 1984) that may be utilized for transport of substrates, such as for Na^+ -glucose, -aminoacids, -vitamins, Cl^- , H^+ cotransport, through the sarcolemma. The existence of Na^+ -glucose cotransport was indeed demonstrated in renal brush border membranes (Beck and Sacktor 1975; Aronson 1984). The finding of Fedelešová et al. (1972) that the function of heart sarcolemmal Na,K-ATPase relies on ATP produced by glycolysis, as well as the tight relationship that exists between glycolysis and the Na,K-ATPase (Balaban and Bader 1984) indicate that Na^+ -glucose cotransport might also be present in the heart (Breier and Monošíková 1990). Furthermore, the sarcolemmal Na gradient serves as energy storage for the Na-Ca exchanger that extrudes one Ca^{2+} ion for 3 Na^+ ions (Doohan and Rasmussen 1997). Thus, via modulation of intracellular Ca^{2+} concentration that plays a key role in contractility, the Na,K-ATPase may also affect heart performance. In cardiac cells, like in cells of other tissues, Na,K-ATPase is also actively involved in the maintenance of local homeostasis of K^+ , counterbalancing its loss during the excitation-contraction sequence. It should be noted that the permeability of the cardiac sarcolemma for K^+ ions exceeds the permeability for other ions. This implies that K^+ gradient is the major determinant of resting membrane potential of the cardiomyocytes.

By maintaining the homeostasis of cations in myocytes, cardiac Na,K-ATPase is also involved in numerous processes related to cell signalling and the reaction or adaptation of the myocardium to external and internal (also pathological) stimuli, and represents the target for action of cardiac glycosides. These aspects will be discussed in the following chapters.

2. Composition of Na,K-ATPase

Na,K-ATPase represents a complex with a relative molecular mass (M_r) of 379.400 ± 21.000 (maximal difference) consisting predominantly of two types of subunits: the catalytic α -subunit, $M_r = 112.000$ (difference 5–15%) and the β -subunit which, in its nature, is a glycoprotein, $M_r = 35.000$ (difference 3–7%). These data derive

from a comparison of results of a complete amino acid analysis of the Na,K-ATPase subunits from a number of different sources confirmed also by analytical centrifugation (Hastings and Reynolds 1979; Reynolds 1988; J  rgensen 1988). The mass relationship between the α and β subunits present in one active complex of the Na,K-ATPase (protomer) was approximately $\alpha:\beta = 65:35\%$. This might point to an $\alpha:\beta$ stoichiometry of $\alpha_2\beta_2$ (Esmann et al. 1979) or $\alpha_2\beta_4$ (Hastings and Reynolds 1979), depending on the type of non-ionic detergent used for solubilization of the purified enzyme. This indicates that the enzyme might form oligomers consisting of two or more protomers. Later on, however, it was found that the $\alpha_2\beta_4$ preparation contains 1 active site per 2 α -chains, a finding that implies that the functional unit itself might be a dimer formed by monomeric α and β subunits (one of each). The latter notion seems also to be supported by measurements obtained using the radiation inactivation technique (Jensen and Ottolenghi 1985). Nevertheless, results obtained by determination of n-terminal groups of the α and β subunits pointed to $\alpha\beta_2$ (Chetverin 1986), and electronmicroscopic investigations even to $\alpha_4\beta_4$ (Demin et al. 1988) as the possible stoichiometry of the active unit of the purified Na,K-ATPase. Very recent papers again report about a 1:1 ratio between α_1 and β_1 subunits in the renal Na,K pump (Pedersen et al. 1996a). These results indicate that the complacency on the subject of the oligomeric vs. protomeric state of the Na,K-ATPase as well as on the stoichiometry of its active state in different tissues, is still inappropriate and this may particularly concern the heart Na,K-ATPase.

According to classical concept, transient passages for ions can be formed at interfaces between peptide chains arranged in an oligomeric complex. However, when folded advantageously and exhibiting transmembrane loops, even one single peptide chain of a transport enzyme, such as of the monomeric α subunit of Na,K-ATPase, may provide intramembrane domains, suitable to form an alternative to oligomeric pump models (Andersen and Vilsen 1988). Arguments in favor of the α subunit as a monomeric system that might secure the transport of Na^+ and K^+ ions through the sarcolemma are as follows:

- i) In the native membrane, there is no unequivocal evidence for a minimum structural unit, with a size exceeding that of a monomer;
- ii) Solubilized monomers of transport ATPases are capable to occlude the ion to be transported in their E_1P state and to couple E_1P - E_2P transition (E_1P and E_2P represent two different phosphorylated conformational states of transport ATPases – for more details see chapter 3.2) with a concomitant increase in chemical potential of the occluded ion;
- iii) Stimulation of reaction rate of the Na,K- and other pumping ATPases by millimolar concentrations of ATP does not depend on subunit interaction;
- iv) Transition between E_1 and E_2 states of the Na,K-ATPase is accompanied by changes in the secondary and tertiary structure of the enzyme. These conformational changes are coupled with an increase in α -helicity of its molecule and may result in deeper immersion of the peptide into the lipid bilayer (Vrbjar et al. 1984, 1985; Ziegelh  ffer et al. 1986a; Andersen and Vilsen 1988).

After removal of the β subunit, the isolated α subunit of Na,K-ATPase exhibits

phosphohydrolase activity with well preserved sensitivity to stimulation by increasing amounts of Na^+ and K^+ , as well as to inhibition with ouabain (Freytag 1983). Recent studies on structure and localization revealed that the β subunit forms a single transmembrane segment. The majority of its hydrophilic residues with three N-linked glycosylation sites and three conserved disulfide bridges was found to be exposed to the extracellular surface (Capasso et al. 1992; Lingrel and Kuntzweiler 1994; Lutsenko and Kaplan 1995). A modification by 2-mercaptoethanol of the above mentioned disulfidic group(s) in the β subunit resulted in significant inhibition of ATP splitting on the α subunit (Kawamura and Nagana 1984). The inhibition could be prevented by binding of Na^+ and K^+ ions in their specific binding sites again on α subunit of the enzyme (Kawamura et al. 1985). The results indicate that, although the presence of the β subunit does not represent a "*conditio sine qua non*" for the expression of the ATP-hydrolase activity of the Na,K-ATPase, an interaction within the active complex between the catalytic α and regulatory β subunit (that may also play the role of a receptor), may be of essential importance for the functioning of the enzyme (Breier and Monošíková 1990).

Chromatographic separation with non-polar solvents revealed (in the kidney) the existence of a 12,000 dalton lipoprotein with an amino acid composition similar to that of the α subunit (Reeves et al 1980). However, removal of this chain, termed the γ subunit, remained without any effect on the Na,K-ATPase (Hardwicke and Freytag 1981). Moreover, electron microscopic studies could not confirm the existence of a γ subunit (Demin et al. 1988). Hence, much about the γ subunit and particularly about its possible presence in the heart still has to be elucidated.

It may be concluded that when fulfilling its physiological role, Na,K-ATPase has to respond actively to numerous modulatory and regulatory signals of diverse origin, and some may only be transmitted via the β subunit of the enzyme. This indicates that when localized "*in situ*", the enzyme may act as a heterodimer i.e., a molecule composed of two different subunits (Pedersen et al. 1996a). Results discussed in this chapter were predominantly obtained with Na,K-ATPase preparations from sources other than myocardium. However, it is highly probable that with little limitations they also apply to the cardiac enzyme.

3. Specificity and composition of cation binding sites and the reaction cycle of Na,K-ATPase

3.1. Specificity and composition of binding sites for cationic ligands

Na,K-ATPase is equipped by specific binding sites that can distinguish unequivocally between single cations and secure their biospecific binding to the enzyme. To fulfill this task, high resolution capabilities of the respective, specific binding sites are required. The ionic bonds of alkaline cations with S^{2-} , COO^- or PO_4^{3-} groups on the membrane do not meet the required criteria for specificity. On the other hand, bonds formed on the key – lock principle, called donor-acceptor or coordination bonds, of alkaline cations with -OH , -SH or -NH_2 groups as accep-

tors of free electron pairs provide sufficient specificity. In this type of bonds, the structure of the binding site always has to comply with the geometrical arrangement of hybrid orbitals of the cation to be bound (Hughes 1981; Ziegelh  ffer et al. 1986a, b; Breier et al. 1988). According to their usual coordination number (referring to the amount of electrons participating in binding) the alkaline cations and cations of alkaline earth metals may form bonds in four structural systems well known from crystallography: in binomic system with a coordination number 2 (Be^{2+}), tetraedric system with coordination number 4 (Li^+ , Be^{2+}), octaedric system with coordination number 6 (Li^+ , Na^+ , K^+ , Mg^{2+} , Ca^{2+} , Sr^{2+} , Ba^{2+}) and in the dodekaedric system with coordination number 8 (K^+ , Rb^+ , Mg^{2+} , Ca^{2+} , Sr^{2+} , Ba^{2+}). This principle termed the “coordination bond theorem” (CBT) may explain for instance, why Li^+ and Na^+ ions, both capable of forming coordination bonds in octaedric system may easily pass the Na-channel in the cell membrane, while the K-channel with specific cation-binding site in dodekaedric system is only very little permeable for these ions (Breier et al. 1988). CBT is also operational in cation-induced regulation of α -helicity of membrane proteins (Vrbjar et al. 1984, 1985, for more details see chapter 5) and may elucidate the mechanism of inhibition of the Mg^{2+} -dependent Na,K-ATPase by Ca^{2+} ions that is still often explained unsatisfactorily by simple competition of Mg^{2+} and Ca^{2+} ions for the same binding site on the enzyme molecule. In fact, Mg^{2+} and Ca^{2+} may bind to two different sites, one with octaedric the other one with dodekaedric structure, the former being associated with activation and the latter with inhibition of the enzyme (Vrbjar et al. 1986; Breier et al. 1998). The binding of Ca^{2+} to the inhibitory site may also exert an influence on the energy metabolism of the myocytes (Sulov   et al. 1998).

Nevertheless, although CBT is a principle of discrimination operational in all transport systems, it still admits numerous undesirable interchanges. Such cases require the use of another criterion in addition to CBT. Such a criterion may be also predominating, and it is given by the height of the energy barrier to be overcome i.e., the quantity of energy required to dehydrate the cation that is in solution surrounded with a cover of negatively oriented dipoles of water molecules. The latter always have to be removed prior to formation of a coordination bond (Hughes 1981). The process of dehydration requires energy that has to be donated in certain excess, by energy liberated either in interaction of a molecule of hydration water with an ionized carboxyl molecule, located within the binding site, or by energy liberated in coordination-interaction of the respective cation with its own specific binding site, or even by both of them. A schematic representation of energy requirements coupled with dehydration and coordination-interaction (solvation) of a cation within the transport system is presented in Fig. 1. The release of bound cation into the liquid environment represents a mirror picture of the process of its binding. It may only occur if the energy of its hydration exceeds that of its solvation.

The hydration energy (energy liberated in the process of hydration) of alkaline metals, their areal charge and the thickness of the hydration layer have been found to correlate linearly (Ziegelh  ffer et al. 1986b). This correlation was

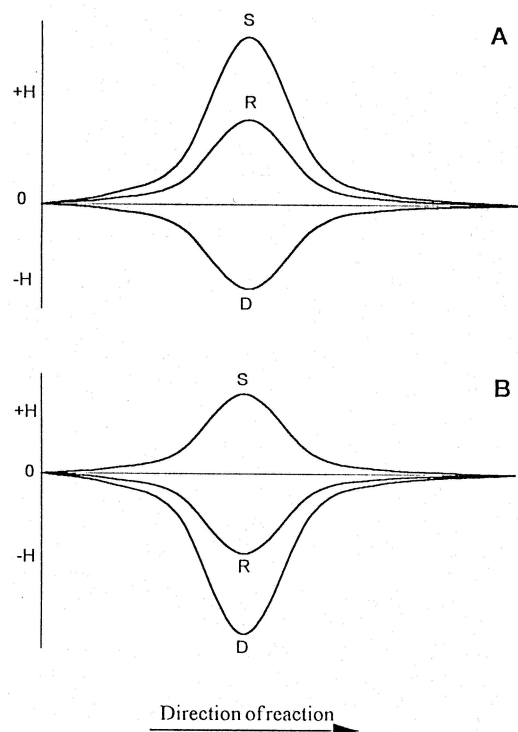


Figure 1. Schematic representation of energy requirements coupled with dehydration and subsequent coordination-interaction (solvation) of a cation in its specific binding site on the transport ATPase.

H^+ and/or H^- – energy expressed in terms of free enthalpy, D – energy needed for dehydration, S – energy liberated during solvation, R – final energy resulting from interference of the energies ($S - D$). Panel A: a case when energy liberated in the process of solvation of the cation exceeds the energy required for its dehydration i.e., coordination-interaction may be realized; Panel B: a case when energy required for dehydration of the cation exceeds the energy that may be liberated in the process of its solvation i.e., interaction of the cation cannot be realized.

based on the assumption that each ion has the shape of a ball with a radius identical with its own ionic radius; the smaller the ionic radius, the higher its hydration energy (Nagata and Aida 1984). Unfortunately, data that would confirm a proportional relationship between the hydration energy of cations and the energy of their solvation by bioorganic ligands are still lacking. Hence, the notion that the binding of ions with larger ionic radius into binding sites of ions with smaller ionic radius may be energetically advantageous, remains unplausible.

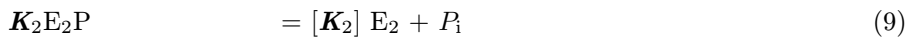
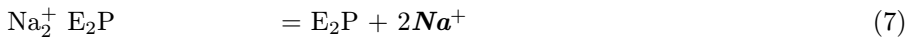
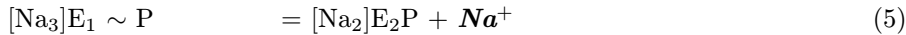
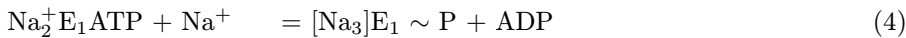
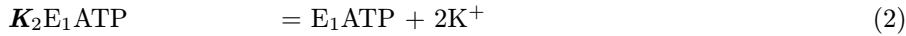
The above mentioned theoretical considerations were practically proved by testing the interactions of Li^+ , Na^+ , K^+ and Rb^+ with Na,K-ATPase in a par-

tially purified fraction of rat heart sarcolemma as well as in studies performed with diverse substances exerting inhibitory effect on the Na,K-ATPase. The latter studies revealed the existence of an essential and 2,4,6-trinitrobenzene sulfonic acid-sensitive amino group in the potassium-binding site of the rat heart sarcolemmal Na,K-ATPase and K⁺-dependent pNPPase (Breier et al. 1986, 1987). Experiments with the highly electrophilic substance, 1-(5-nitro-2-furyl)-2-phenylsulfonyl-2-furylcarbonyl ethylene showed that this compound inhibits the Na,K-ATPase by selective interaction with some essential nucleophilic (probably -SH and/or -NH₂) functional groups of an aminoacid residue located within or close to the K⁺-binding site of the enzyme (Breier et al. 1996b).

Because of the regulatory role of the Na,K-ATPase in processes associated with cardiac sarcolemma function a considerable part of investigatory work concerning elucidation of the structure, function and regulation of cation-binding sites has been done with the heart enzyme. Nevertheless, in spite of much effort the binding sites for cations represent the less understood part of the enzyme. Therefore, more interest in the future will be focused on elucidation of the structure and function of cation binding sites of the myocardial Na,K-ATPase.

3.2. The reaction cycle of Na,K-ATPase

The reaction mechanism of the Na,K-ATPase exhibits a sequence of well described partial reaction steps that involve binding, occlusion and translocation of cations as well as formation of phosphorylated, high-energy intermediate products (J  rgensen 1982, 1992). According to recent knowledge, a stepwise transition between a conformational state of the enzyme molecule called E₁, a different conformational state E₂ and again back to E₁ occurs during the reaction cycle of the Na,K-ATPase. E₁-E₂ transition involves a circular chain of molecular changes that are associated with countercurrent translocation of Na⁺ and K⁺ ions from one to another side of the membrane. Translocation of Na⁺ and K⁺ occurs in a sequence of events termed in enzymology as a ping-pong mechanism (Pedersen et al. 1996b) and may be described by the equations:



Notation: [] occluded form, $\mathbf{K}^+$ extracellular, K^+ intracellular, $\mathbf{Na}^+$ extracellular, $\mathbf{Na}^+$ intracellular

The phosphoforms ($\sim$ indicates a macroergic bond) can occlude $\mathbf{Na}^+$, and dephosphoforms can occlude K^+ or $\mathbf{Rb}^+$. Ions printed within brackets [$\mathbf{Na}^+$] or [K^+] are occluded and prevented from exchanging with ions in the medium. When printed both bold and Italic, $\mathbf{Na}^+$ or $\mathbf{K}^+$ are located on the extracellular, and when printed normally, $\mathbf{Na}^+$ or K^+ , are located on the intracellular surface of the cell membrane. Binding studies with ^{22}Na showed that [$\mathbf{Na}_3$] $\text{E}_1 \sim \text{P}$ occluded 3 $\mathbf{Na}^+$ ions, while the capacity [$\mathbf{Na}_2$] $\text{E}_2 \text{ P}$ corresponded to 2 $\mathbf{Na}^+$ ions per one α subunit (Pedersen et al. 1996b).

$\text{E}_1\text{-E}_2$ conformational changes of the α subunit are thought to mediate long-range interactions between the ATP binding site and the cation occluding sites in the membrane domain. The initial step represents the binding of ATP with low apparent affinity ($K_m \approx 0.2\text{--}0.4 \text{ mmol.l}^{-1}$) to the occluded [$\mathbf{K}_2$] E_2 conformation. This interaction accelerates the [$\mathbf{K}_2$] E_2 to $\mathbf{K}_2\text{E}_1$ -transition with deocclusion of K^+ at the cytoplasmic surface (reactions 1 and 2). ATP binds with increased affinity ($K_D \approx 0.1\text{--}0.2 \text{ mmol.l}^{-1}$) to the enzyme in E_1 conformation. This increase in binding energy of ATP is associated with the [$\mathbf{K}_2$] E_2 to $\mathbf{K}_2\text{E}_1$ -transition and represents the driving force for the transport of K^+ across the membrane (Läuger 1984). The following steps are: Na-dependent transfer of γ -phosphate from ATP to an acyl-bond at aspartate³⁶⁹ of the α subunit (reactions 3 and 4) and isomerization between the occluded [$\mathbf{Na}_3$] $\text{E}_1 \sim \text{P}$ and the [$\mathbf{Na}_2$] $\text{E}_2 \text{ P}$ phosphoforms (reaction 5). The latter step is coupled with reorientation of cation occluding sites coupled with giving up the occlusion of the $\mathbf{Na}^+$ ions (reaction 6) and their release at the extracellular surface (reaction 7). The reaction sequence is terminated by binding of extracellular K^+ ions and their occlusion using the energy liberated by dephosphorylation of the enzyme (reactions 8 and 9). This reaction cycle is also believed to apply to the cardiac sarcolemmal ATPase, and is represented schematically in Fig. 2.

4. Specificity and composition of the ATP binding site and the K^+ -pNPPase activity of Na,K-ATPase

4.1. Specificity and composition of the ATP binding site of Na,K-ATPase

From the aspect of substrate specificity, Na,K-ATPase may be considered as an enzyme with group substrate specificity for nucleotidetriphosphates (Fu et al. 1985; Ziegelhoffer et al. 1987a). Activation of cardiac sarcolemmal Na,K-ATPase as well as the velocity of hydrolysis of different nucleotidetriphosphates by the enzyme gradually decrease in the sequence: $\text{ATP} \gg \text{CTP} \gg \text{ITP} \gg \text{GTP} \gg \text{UTP} \gg \text{TTP}$, within the range from 100% for ATP to 0.5% for TTP. Concomitantly, the dissociation constant of the complex Na,K-ATPase-substrate is rising from $0.2 \mu\text{mol.l}^{-1}$ for

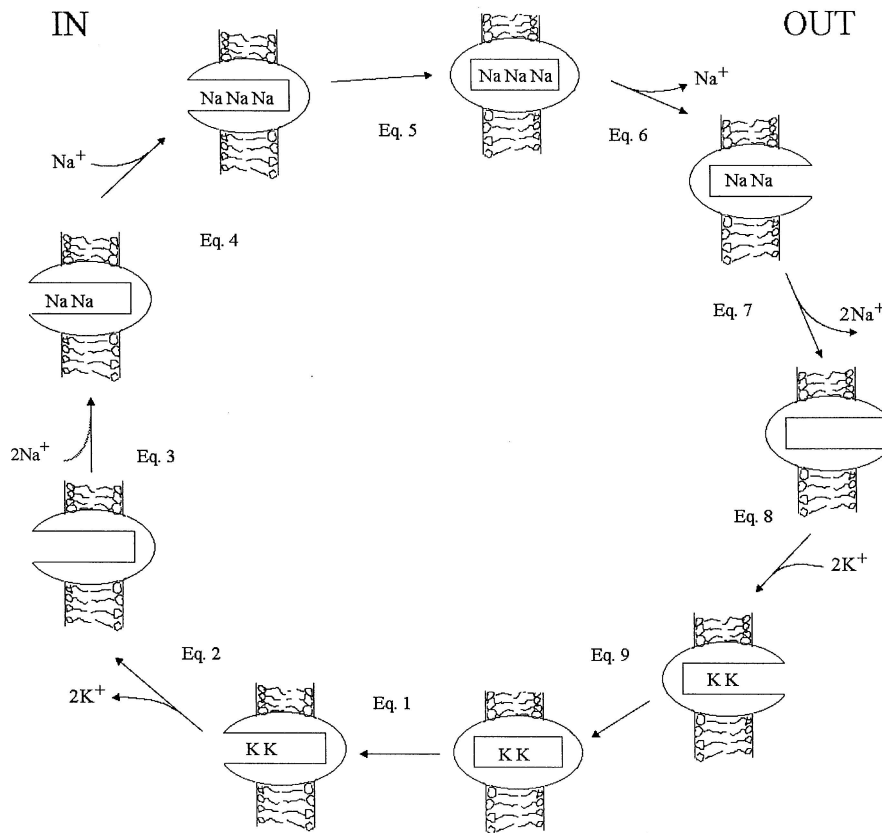


Figure 2. Schematic representation of the reaction cycle of cardiac sarcolemmal ATPase: relationship between the sequence of single reaction steps, E_1 to E_2 transition and side-orientation of interactions as well as occlusion of Na^+ and K^+ ions.

Eq. (1–9) – equation numbers of partial reactions according to the reaction scheme of the Na,K-ATPase presented in chapter 3.2; arrows point to the direction of the reaction and/or of the movement of single cations.

ATP to $2000 \mu\text{mol.l}^{-1}$ for TTP (Breier and Monořikov   1990). The Mg-dependent enzyme is also capable of hydrolyzing ADP, but with a considerably lower affinity and higher activation energy than in the case of ATP hydrolysis. It was speculated that this property may obtain special importance in energy deficient hearts (Pedersen et al. 1996b).

An essential step in exact, biospecific recognition of ATP from other possible

substrates of the Na,K-ATPase occurs in a specific ATP-binding site on the enzyme molecule where the substrate binds multiply, but minimally in three points.

Experiments with ATP analogues revealed that recognition of ATP from other nucleotides proceeds by creation of a hydrogen bond between the NH₂-group in position 6 on the adenine ring of ATP and an essential SH-group belonging to a cysteine residue, situated in the ATP-binding site of the enzyme (Patzelt-Wenczler and Schoner 1981; Schoner et al. 1982).

The terminal γ -phosphate group of ATP (distinguishing it from nucleotide di- and monophosphates) is recognized by specific interaction with a -COOH group on aspartate³⁶⁹, situated in the ATP-binding site of the Na,K-ATPase. The latter interaction is accompanied with considerable reduction of the negative charge on aspartate³⁶⁹ (site D369N) and opens the opportunity to examine several aspects of the transfer of energy from the ATP-binding domain to cation translocation (Pedersen et al. 1996a). The acylphosphate created after hydrolysis of ATP in the same site of the enzyme molecule, now already a phosphorylated intermediate of Na,K-ATPase, exhibits the character of a high energy compound (Jørgensen 1982; Pedersen et al. 1996a). In addition to its function as receptor of the γ -phosphate of ATP, aspartate³⁶⁹ has important short-range catalytic functions in modulating the affinity for ATP and long-range functions in governing the E₁-E₂ transitions that are coupled with reorientation of the cation-binding sites and with changes in affinity for ouabain. Experiments made with Woodward reagent K, a blocker of -COOH groups (Mårdh 1982) as well as with mutation to the phosphorylation site (D369N) and the presumptive K⁺-binding site (D807N), yielded inhibition of Na,K-ATPase and K⁺-pNPPase activities as well as of [³H]-ouabain binding (Pedersen et al. 1996a).

An interaction of the ribose part of the ATP molecule with the ATP-binding site of Na,K-ATPase is essential, since riboso-5-phosphate may completely prevent the binding of ATP to the enzyme (Monošíková et al. 1987a). An interesting role in this respect may be played by the hydroxyl group in position 2 on the ribose moiety of ATP, because deoxy-ATP (with this group absent) binds to the enzyme with an almost similar affinity, but it is split at a considerably lower rate than ATP (Monošíková et al. 1987b).

Essential tyrosine- (Cantley et al. 1978), arginine- (De Pont et al. 1977, Scheiner-Bobis and Schoner 1985) as well as NH₂-groups (Farley et al. 1984) were also found to be present in ATP-binding site of the Na,K-ATPase.

Fluorescein isothiocyanate (FITC) was for the first time used for labelling of the ATP-binding site of Na,K-ATPase by Carilli et al. (1981). Subsequently to labelling, the enzyme was treated with trypsin in a slightly alkaline milieu. The tryptic fragment carrying the FITC label was separated, purified, its amino acid sequence was determined and finally it was found to be attached to a NH₂ group of a lysine residue. The latter residue was then considered to belong to the main structure carrying the ATP-binding site of Na,K-ATPase (Farley et al. 1984; Kirley et al. 1984; Ohta et al. 1985). On the other hand, it was demonstrated that isothiocyanates (*p*-bromophenyl isothiocyanate, PBITC), isothiocyanatophenyl-(6-

thioureidoethyl)-carbamoylmethyl-ATP – the “*ITC-ATP*”, and also FITC, inhibit Na,K-ATPase by modifying an essential SH-group in the ATP-binding site of the enzyme (Ziegelh  ffer et al. 1983, 1987a; D  zurba et al. 1985; Breier et al. 1989, 1995, 1996a; Breier and Mono  ikov   1990). This controversy, i.e. cysteine vs. lysine (-SH or NH₂-group), may be explained finally in favour of the cysteine as follows: Isothiocyanates may react easily with NH₂-groups of amino acids providing N,N'-disubstituted derivatives of thiourea that are resistant to β -mercaptoethanol or dithiotreitol but are stable in alkaline milieu only. Nevertheless, isothiocyanates can also react with SH-groups. This reaction may occur in a broad range of pH values and far more readily than that with the NH₂-groups (Drobnica and Gemeiner 1976; Drobnica et al. 1977). Moreover, in neutral or slightly acidic conditions the reaction of isothiocyanates is limited to SH-groups of enzymes exclusively (Gemeiner and Drobnica 1979). However, the resulting S-esters of dithiocarbamic acid are less stable compounds and may dissociate already in slight alkaline milieu (Drobnica and Gemeiner 1976; Drobnica et al. 1977). Consequently, during tryptic hydrolysis in alkaline milieu the isothiocyanate label might easily dissociate from its original binding place (in neutral milieu most probably the SH group) and jump over to an advantageously situated NH₂-group of lysine. For the latter reasons, there is only little probability for the isothiocyanate-labeled peptidic fragment, obtained by tryptic cleavage of the Na,K-ATPase (Farley et al. 1984; Kirley et al. 1984; Ohta et al. 1985) to be identical with the structure carrying the ATP-binding site of Na,K-ATPase, but it is highly probable that it may be situated in close neighborhood of the ATP binding site.

Experiments with site-directed mutagenesis also indicated that if the FITC-sensitive lysine residue, believed to be involved essentially in binding of ATP (Farley et al. 1984; Kirley et al. 1984; Ohta et al. 1985), is replaced by either alanine, arginine or by glutamic acid, the expected loss of ATP binding due to the vacancy of lysine is missing. Nevertheless, the missing positive charge of lysine that was replaced by negative charged amino acid residues, such as that of glutamic acid, leads in a decrease in the affinity to ATP of the ATP binding site (Maruyama and MacLennan 1988; Tamura et al. 1989; Yamamoto et al. 1989; Wang and Farley 1992). The latter finding indicates that although the discussed lysine residue may not be essential for ATP binding, it should be situated close to the essential ATP-binding amino acid (Breier et al. 1996a). This assumption is strongly supported by studies of the sequence of amino acids constituting the α -subunit of the Na,K-ATPase (Shull et al. 1985). These studies revealed a triplet of amino acids consisting of: aspartic acid⁵⁰⁹, arginine⁵¹⁰ and cysteine⁵¹¹ that is situated close to the FITC-sensitive lysine residue referring to positions 496–506. Moreover, cysteine⁵¹¹ was found to be highly conserved in each mammalian P-type ATPase (with reaction sequence involving phosphorylation of the enzyme) being always localized in the same position (Breier et al. 1996a). The tripeptide: aspartic acid⁵⁰⁹ (may interact with the γ -phosphate of ATP), arginine⁵¹⁰ (may interact with the OH-group in position 2 on the ribose moiety of ATP) and cysteine⁵¹¹ (may react with the 6-NH₂-group of adenine by forming a hydrogen bond) has a big chance to fulfill the

criteria stated for the “true” ATP-binding site of the Na,K-ATPase. This sequence of amino acids also may be arranged in a three-dimensional structure that confines to geometry of the ATP molecule resulting from X-ray structural analysis and was resolved by means of computer-assisted molecular graphics (Breier et al. 1989). The probability of the proposed structure has also been supported by results of an independent NMR study (Stewart et al. 1988).

In the future, the number of studies utilizing genetic and molecular-biological techniques will further increase. Considerable efforts may be necessary particularly to elucidate the regulatory mechanisms of induction and repression as well as of modulation of the reactivity and specific properties of cardiac Na,K-ATPase in physiological and pathological conditions.

4.2. K^+ -pNPPase activity of Na,K-ATPase

Besides its ATP-hydrolyzing activity, Na,K-ATPase is well known to exhibit also K^+ -dependent phosphatase activity that is usually determined by using *p*-nitrophenyl phosphate (pNPP) as substrate – probably the reason why this enzyme activity is termed as K^+ -dependent *p*-nitrophenyl phosphatase or K^+ -dependent pNPPase activity (Hansen et al. 1979; Robinson et al. 1983). Askari et al. (1979) and Robinson (1985) postulated that K^+ -dependent pNPPase may manifest the phosphohydrolase reaction that is participating in the reaction cycle of the Na,K-ATPase, being responsible for potassium-dependent hydrolysis of E_2 phosphoenzyme-form of the Na,K-ATPase. The latter reaction step may be described by equation (10):



where K_2 represents bound and $[K_2]$ occluded potassium ions, both originating from intracellular space (for more details see chapter 3.2.). The above notion is supported by the following arguments:

- i) the dephosphorylation reaction of Na,K-ATPase and the reaction of K^+ -dependent pNPPase, both are K^+ -dependent processes;
- ii) K^+ -binding sites participating in the activation of the dephosphorylation reaction of the Na,K-ATPase and in the activation of pNPP hydrolysis by K^+ -dependent pNPPase exhibit similar properties of selectivity for potassium (Homareda and Matsui 1982).
- iii) results achieved with estimation of Na,K-ATPase activity by K^+ -dependent pNPPase activity in muscle tissues correspond well with the results of $[^3H]$ -ouabain binding and 3-*O*-MFPase activity measurements (Larsen and Kjeldsen 1995; Larsen et al. 1997).

However, there are also arguments particularly important for enzymological studies concerning the reaction kinetics and mechanism or the properties, structure and composition of the substrate binding site, that may call for caution in inter-exchanging promiscuously the K^+ -dependent pNPPase with the Na,K-ATPase. Such arguments are:

i) the structure of *p*-nitrophenyl phosphate resembles more that of a phospho-ester than that of the acylphosphate created and subsequently (K^+ -dependently) hydrolyzed in reaction mechanism of the Na,K-ATPase;

ii) some inhibitors such as oligomycin, 2,4,6-trinitrobenzenesulfonic acid and also ions of bivalent metals, exert differently strong inhibitory effects on the activities of Na,K-ATPase and K^+ -dependent pNPPase (Hansen et al. 1979; Robinson et al. 1984; Fleary et al. 1985; Breier et al. 1987);

iii) in contrast to vanadyl ions (IV) that are inhibitory to both Na,K-ATPase and K^+ -dependent pNPPase, vanadate ions (V) exhibit no interference with Na,K-ATPase and inhibit K^+ -dependent pNPPase only (Vysko  il et al. 1981).

At the present state of art it may be concluded that most differences between Na,K-ATPase and K^+ -dependent pNPPase concern the chemistry of their reaction mechanism, that may in part also follow from evident differences in the chemical nature of ATP and pNPP. On the contrary, there is little doubt that both enzymes may have identical binding sites for K^+ ions and that their binding sites for substrates are both localized on the α subunit of the Na,K-ATPase. The latter view is also confirmed by our finding that an approximately 25% downregulation of the dominating α_1 subunit of the rat heart Na,K-ATPase demonstrated by immunoblotting was accompanied by a very similar degree of decrease in cardiac K^+ -pNPPase activity, in chronically K^+ -supplemented rats (Bundgaard et al. 1998). For this reasons, in biological, pathophysiological and clinical studies, especially in tissues as well as in tissue homogenates with very low Na,K-ATPase activity and [3H]-ouabain binding, the data about enzyme expression obtained by estimating K^+ -dependent pNPPase rather than Na,K-ATPase are considered as reliable and are accepted with a high degree of validity.

5. Relationship between K^+ -, Na^+ -, Mg^{2+} - and Ca^{2+} -induced changes in secondary structure and the reaction kinetics of Na,K-ATPase

During the contraction-relaxation cycle of heart cells, membrane action potential plays a regulatory role in the modulation of the activity and the functional cooperation of ion-transporting systems in the sarcolemma (McNamara et al. 1974; Katz 1977; Dhalla et al. 1977). It is characteristic that in different phases of the time-course of membrane potential i.e., in different phases of the contraction-relaxation cycle, different cations are gradually binding to the sarcolemma. The cation-to-sarcolemma interactions, including those of Na^+ , K^+ and Mg^{2+} , exert specific influences on secondary and tertiary structure of the membrane proteins and are tightly coupled with the regulation of enzymatic and/or ion-transporting systems in the membrane (Vrbjar et al. 1986; Andersen and Vilsen 1988). Differences in secondary structure of membrane proteins, related to Na,K-ATPase activity, detected by changes in circular dichroism spectra that accompanied the ouabain-induced inhibition of the enzyme in a preparation of isolated cardiac sarcolemma, were demonstrated for the first time by L  llmann et al. (1975).

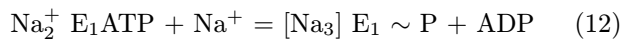
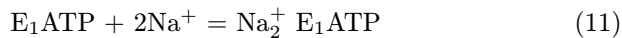
During the turnover cycle of Na,K-ATPase transitions occur from sodium-

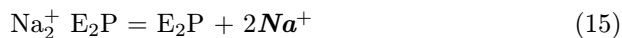
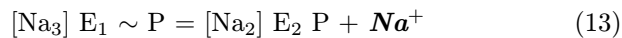
bound E_1 to potassium-bound E_2 and again back to E_1 conformation of the enzyme, and these transitions also involve changes in secondary and tertiary structure of the enzyme (Karlsh and Yates 1979; Jørgensen 1982; Pedersen et al. 1996b). Circular dichroism spectra of membrane-bound Na,K-ATPase revealed that approximately 80 amino acid residues may be involved in transitions between these two conformers of the enzyme (Gresalfi and Wallace 1984). However, it has been also shown that similar conformational changes in heart sarcolemmal proteins may be induced in the absence of ATP, by interaction of the sarcolemma with Na^+ , K^+ and Mg^{2+} ions only, when they are present in concentrations that may modulate the membrane bound Na,K-ATPase (Vrbjar et al. 1984). It was shown that approximately 80% of polypeptide chains in cardiac sarcolemma are arranged in an α -helical structure. Using optimal concentrations of Na^+ and Mg^{2+} ions, required for stabilization of the E_1 conformational state of Na,K-ATPase, only a slight increase (from 80 to 83%) in α -helical arrangement of the sarcolemmal proteins could be observed. On the other hand, simultaneous addition of K^+ and Mg^{2+} ions, in concentrations required to establish the E_2 conformational state of the enzyme, was followed by a significant 72% decrease in α -helical arrangement of the membrane proteins. When present at the same time, Na^+ , K^+ and Mg^{2+} ions had no influence on α -helicity of sarcolemmal proteins in comparison to the conformation established by Na^+ and Mg^{2+} ions only (Vrbjar et al. 1984). From these findings the following conclusions could be drawn:

i) In metal free state or in the presence of Na^+ and Mg^{2+} ions concentrations optimal for activation of the Na,K-ATPase, as well as in TRIS buffer, membrane proteins will predominantly exhibit a degree of α -helical arrangement, resembling that of the Na,K-ATPase in E_1 conformational state (Rempeters and Schoner 1983; Vrbjar et al. 1984).

ii) The E_1 -like conformational state of membrane polypeptides achieved after their interaction with Na^+ and Mg^{2+} ions can not be transferred to an E_2 -like state by simple addition of K^+ ions. A plausible explanation of this finding follows from events in the reaction cycle of Na,K-ATPase; i.e., K^+ ions can not interact with their specific (conformation changes-inducing) binding sites on the enzyme prior to the release of Na^+ ions from their – already occupied – specific binding sites. However, such a release of Na^+ ions might probably not occur in the absence of ATP (Pedersen et al. 1996b), since phosphorylation and dephosphorylation of the enzyme are required for its E_1 - E_2 transition (Eq. 11–16 – for more detailed description of the above reactions see chapter 3.2.).

iii) Conclusions sub i) and ii) indicate that changes in secondary structure of sarcolemmal proteins that are induced by metallic cofactors of the Na,K-ATPase and are expressed in changes of their α -helical arrangement, may predominantly reflect the changes in ATPase molecules (Vrbjar et al. 1984).





In contrast to changes in α -helicity when Mg^{2+} ions are also present, Na^+ ions alone are considerably depressing (instead of increasing), and K^+ ions alone considerably enhancing (instead of decreasing) the α -helical arrangement of membrane polypeptides. This confirms the regulatory role of Mg^{2+} ions in E_1 - E_2 transition of the Na,K-ATPase molecule (M  rdh 1982).

Nevertheless, although when the Na,K-ATPase is optimally activated by Na^+ , K^+ and Mg^{2+} ions, an addition of Ca^{2+} ions may inhibit the enzyme (Huang and Askari 1982; Yingst and Marcovitz 1983). The molecular mechanism of this inhibition involves a Ca^{2+} -induced depression in α -helical arrangement of sarcolemmal proteins, increase in the amount of enzyme molecules in E_2 form and slow-down their transition to E_1 form of the enzyme (Vrbjar et al. 1986).

Equations enabling quantitative determination of the relationship between secondary structure and activity of the membrane bound Na,K-ATPase, that are based on estimation of cation-induced changes in helical conformation of membrane proteins (Vrbjar et al. 1985, 1986) opened the way for further studies of regulatory relationships between the temperature-dependence of protein conformation, lipid motion and the kinetic behavior of the ATPases (Vrbjar et al. 1990, 1992).

6. Isoforms of the Na,K-ATPase α and β subunits: expression, composition, reactivity, pathophysiological and clinical implications

At least three different isoforms of the catalytic subunit α and two of the subunit β of the Na,K-ATPase have been identified definitely (Sweadner 1990). Isoforms of the α subunit are usually termed α_1 , α_2 and α_3 , but they have also been named as aI, aII and aIII, respectively. Moreover, the designation $\alpha(+)$ referring to a combination of isoforms α_1 and α_2 is also appearing in the literature. In addition, a putative fourth isoform indicated as α_4 has also been described (Shamraj et al. 1991; Munzer et al. 1994) however, it has not been demonstrated in the heart.

Studies of primary structure of different α subunits of the Na,K-ATPase revealed that their amino acid composition is similar to approximately 85% only (Lingrel et al. 1990; Takeyasu et al. 1990). Differences in the composition of the remaining (approximately 15%) amino acids are believed to be responsible for the enormous developmental, tissue and species diversity of α subunits of the Na,K-ATPase (Zahler et al. 1992, 1996a, b). This diversity becomes manifested in various properties of the Na,K-ATPase α isoforms, particularly in their affinity to intra- and extracellular concentrations of single cations such as $[\text{Na}^+]_i$, $[\text{Ca}^{2+}]_i$ and $[\text{K}^+]_e$, further in their reactivity with ouabain and probably also in their affinity to ATP

and p-NPP.

In various species, isoform α_1 appears to have the highest affinity to $[\text{Na}^+]_i$ reaching K_m values of 12 mmol.l^{-1} , reported for native human as well as rat isoforms. In comparison to α_1 , the α_2 and α_3 isoforms with K_m values of 22 and 33 mmol.l^{-1} respectively, exhibit considerably weaker affinities to $[\text{Na}^+]_i$ (Zahler et al. 1997). Isoforms of the α subunit not only show differences in their affinity to $[\text{K}^+]_e$, but severe changes in $[\text{K}^+]_e$ may exert even regulatory influences on their expression and activity. Generally, the affinity of the α_3 isoform to $[\text{K}^+]_e$ exceeds that of the α_1 and α_2 isoforms (Munzer et al. 1994). In the heart muscle and brain, hypokalemia characterized by low $[\text{K}^+]_e$ levels induced significant downregulation of the amount of the α_2 -isoform, but the α_1 isoform of Na,K-ATPase remained unaffected (Azuma et al. 1991). On the other hand, high $[\text{K}^+]_e$ has been shown to reduce the abundance of heart α_1 isoform (Bundgaard et al. 1998).

Differences in the regulation of α_1 and α_2 isoforms of the sodium pump by changes in the $[\text{Ca}^{2+}]_i$ level, that are still within the physiological range, may point to their different roles in maintaining membrane transport during the heart cycle. The α_2 isoform is very sensitive to calcium. It becomes half maximally inhibited already by a $[\text{Ca}^{2+}]_i$ concentration of 160 nmol.l^{-1} , present in muscle at relaxation. On the contrary, α_1 isoform that is less sensitive to $[\text{Ca}^{2+}]_i$ levels, becomes half maximally inhibited at 600 nmol.l^{-1} concentration of $[\text{Ca}^{2+}]_i$ only, i.e., at a calcium concentration exceeding by one order of magnitude that required for muscle contraction (McGeoch 1990). In the literature available, no data have been reported about natural differences that would concern the affinities of diverse α subunit isoforms to ATP or p-NPP.

As concerns their affinity to ouabain, isoforms of the Na,K-ATPase α subunit may be ordered in the following sequence $\alpha_3 < \alpha_2 < \alpha_1$, with K_D values amounting to $1.5 \times 10^{-9} \text{ mol.l}^{-1}$, $1.2 \times 10^{-7} \text{ mol.l}^{-1}$ and $5 \times 10^{-5} \text{ mol.l}^{-1}$, respectively (O'Brian et al. 1994). The latter data were obtained when investigating the rat isoforms. No direct information about K_D values of the human isoforms is available in the literature.

The α_1 , α_2 and α_3 isoforms of the sodium pump are encoded by three different genes (Lingrel et al. 1990). In studies of expression of specific α subunit isoforms these were usually detected as the quantities of isoform-specific mRNA, identified in northern blots by means of isoform-specific DNA probes. Another, immunological approach to this problem was based on the use of isoform-specific antibodies for quantitative estimation by western blotting of the amounts of isoform-specific proteins present in the tissue samples investigated. The results revealed that in the rat myocardium the α_1 isoform mRNA is distributed relatively uniformly in ventricles, including the conductive tissue; by its amount in the working myocardium that represents 75–90% of the total of all α isoforms, it is outnumbering the 10–25% amount of the α_2 isoform (Lucchesi and Sweadner 1991; Sweadner et al. 1994). Immunostaining demonstrated the presence of α_1 isoform also in rat atria, being expressed slightly more abundantly in the left atrium (Zahler et al. 1996a). The α_2 and α_3 isoforms were found to be present uniformly in the conductive system,

however, the expression of the α_3 isoform was found to be restricted to conductive tissue only (Zahler et al. 1992). In adult human myocardium all three α isoforms were found distributed evenly (Shamraj et al. 1991; Zahler et al. 1993). A survey of α subunit isoform composition in the myocardium of various species revealed that human, monkey, ferret and dog hearts are expressing all three α isoforms in mutually comparable amounts. On the contrary, in rat and guinea pig hearts the expression of the α_1 subunit isoform is predominating (Sweadner 1989). From the described isoforms of the β subunit of Na,K-ATPase, the expression of the β_1 isoform could only be detected in the heart (Zahler et al. 1996b).

The α_1 isoform remains at all time predominating in fetal, neonatal as well as in adult rat hearts. Nevertheless, the $\alpha_1:\alpha_2$ proportions gradually increase and that of $\alpha_1:\alpha_3$ gradually decrease during the development (Lucchesi and Sweadner 1991; Lingrel 1992).

Differences have been revealed also in cellular localization of different α subunit isoforms of the myocardial Na,K-ATPase. Cytochemical and immunocytochemical studies demonstrated that the myocardial α_1 subunit was present predominantly in sarcolemmal membranes and it is the only α isoform present in the T-tubules (Zahler et al. 1996a, b; Slez  k et al. 1996, 1997). Hence, its localization as well as its weak sensitivity to inhibition by $[\text{Ca}^{2+}]_i$, that occurs at 10^{-4} mol.l $^{-1}$ concentrations of calcium only, predestinate the α_1 subunit to be the isoform most suitable for effective functional interaction with the $\text{Na}^+/\text{Ca}^{2+}$ exchange system in cardiac cell membranes. The α_2 isoform (being the minor component in the rat heart), was predominantly found to be localized in external sarcolemma, but a slight signal was also dispersed throughout the whole myocyte (Slez  k et al. 1996, 1997). This localization as well as the ouabain sensitivity exceeding that of the α_1 isoform indicate that the specific role of the α_2 isoform in the heart still needs further elucidation. Small amounts of the α_3 isoform were found to be present diffusely and uniformly, but only in the ventricles of neonatal rat hearts. However, the functional interpretation of this finding still awaits elucidation. In adult rat hearts the presence of the α_3 isoform is limited exclusively to the conduction system, to the junctional complex between the cardiomyocytes and to presynaptic neuromuscular junction (Zahler et al. 1993; Sweadner et al. 1994; Slez  k et al. 1996). This may indicate that the α_3 isoform could mainly be involved in neuromuscular and myocardial signal transduction (Zahler et al. 1994, 1996c).

The α subunit isoform composition of Na,K-ATPase has been shown to also be perturbed by pathological processes. Upregulated expression of the α_1 isoform in the myocardium was reported in cardiac hypertrophy induced by aortic banding in dogs (Zahler et al. 1996b). A similar effect was also observed in the rat, but in this species the synthesis of new isoenzyme subunits was also accompanied with an alteration of their kinetic properties towards those estimated in neonatal hearts (Charlemagne et al. 1986). Downregulation in α_1 isoform expression was also reported in rat and guinea pig hearts subjected to chronic K^+ supplementation (Bundgaard et al. 1998). On the other hand, no changes in ouabain binding properties or activity of the α_1 isoform were found in dog hearts after coronary oc-

clusion (Maixent and Lelievre 1987) and in genetically hypertensive rats (Herrera et al. 1988).

Expression of the α_2 isoform was found to be downregulated in adult rat hearts with hypertrophy induced by aortic banding (Charlemagne 1990), but not in rat hearts with elevated $[K^+]_i$ due to chronic potassium supplementation (Bundgaard et al. 1998). Significant depression in ouabain binding to high affinity binding sites as well as in activity of the α_3 isoform was demonstrated in dog hearts upon coronary occlusion (Maixent and Lelievre 1987).

A shift in the mutual ratios of the single α subunit isoforms may also be anticipated in most cases that are characterized by down- or upregulation of the total Na,K-ATPase content in the myocardium, such as in diabetes (Kjeldsen et al. 1987) or in cardiac hypertrophy (Charlemagne et al. 1986; Zahler et al. 1996b) etc.

Although much knowledge has accumulated up to now about the isoforms of the diverse subunits of the cardiac Na,K-ATPase, it becomes more and more evident that considerable effort is still needed to understand their specific role and mechanism of regulation in health and disease to a satisfactory and useful extent.

7. Adaptability and induction of Na,K-ATPase

7.1. Changes in function, properties and kinetics associated with adaptation of Na,K-ATPase

7.1.1. Adaptation to hypoxia

It is well-known that acclimatization to high altitudes, with considerably lower values of pO_2 , involves a complex of adaptation changes to hypoxia, able to protect the myocardium by increasing its tolerance against the effects of acute hypoxia (Reimer and Jennings 1992; Ošťádal et al. 1995) or ischemia induced by acute coronary occlusion. In the heart, the protective effect of acclimatization to high altitude hypoxia was manifested in the preserved function of membrane systems, particularly of the cardiac sarcolemma, thus confirming the regulatory role of membranes in the heart function (Dhalla et al. 1977). Sarcolemmal preparations obtained from right ventricles of acclimatized hearts exhibited a decrease in Na,K-ATPase activity accompanied with a decrease in its V_{max} value, but also a significant decrease in the K_m value of the enzyme. The latter finding signalized an increase in affinity of the active site of Na,K-ATPase to ATP – a property that may be of great advantage when the enzyme has to cope with decreased availability of ATP, such as in hypoxia or ischemia. Based on increased anoxia-tolerance and well preserved potential for post-anoxic functional recovery of superfused right ventricles from acclimatized hearts, it was concluded that when equipped with Na,K-ATPase operating with kinetics more advantageous in conditions with decreased availability of ATP, the cardiac sarcolemma may preserve its role in excitation contraction coupling as well as in maintaining ion homeostasis at decreased energy requirements. This phenomenon was first described by ourselves, and it was found to also apply

to other sarcolemmal ATPases as well as to different regimens of adaptation to high altitude hypoxia, and it was termed “adaptation on enzyme level” (Ziegelh  ffer et al. 1987b, 1993). The molecular mechanisms involved in adaptation of the Na,K-ATPase to hypoxia have not yet been elucidated. Among others, it may also be speculated that changes in α subunit isoform constitution of the cardiac Na,K-ATPase, that need not be linked with changes in the amount, but also may be in concert with up- or downregulation of the enzyme in myocardial tissue, may play an important role in this process.

7.1.2. Adaptation to ischemia

Adaptation of heart sarcolemmal ATPases similar to that found in hypoxia was also confirmed to occur in global ischemia of the myocardium at 37  C, first in the case of Na,K-ATPase (Vrbjar et al. 1991), then also in the case of Ca^{2+} -ATPase and again the Na,K-ATPase (Vrbjar et al. 1993, 1995). The process of adaptation exhibits a time-dependence that seems to be little influenced by the severity of ischemia. The values of V_{max} for all ATPases decrease abruptly within the first 15 min after the onset of ischemia. During the next time interval (between 15–60 min from the beginning of ischemia) the values of V_{max} decrease at a very slow rate only. In comparison to changes in V_{max} the decrease in K_m values occurs with a delay, after 30 min for Ca^{2+} -ATPase and as many as after 45 min for the Na,K-ATPase, but the values of K_m remain stabilized if ischemia persists. An especially advantageous feature of Na,K-ATPase adapted to ischemia is that after a transient increase during the first 30 min of ischemia, the activation energy of the enzyme also decreases considerably. Quantification of myocardial Na,K-ATPase content in dog and guinea pig hearts, subjected to 20 min acute coronary occlusion and subsequent reperfusion of the occluded area revealed no ischemia-induced changes in the content of the enzyme in the myocardium (Schmidt et al. 1990). This finding strongly supports the view that the above mentioned ischemia-induced changes in the properties of cardiac sarcolemmal Na,K-ATPase may reflect its adaptation to unfavourable working conditions rather than damage to the enzyme.

Adaptation of membrane bound ATPases to ischemia has been shown to play an important role also in ischemic preconditioning of the myocardium (Ziegelh  ffer et al. 1992a, b, 1995). The adapted Na,K-ATPase is believed to be responsible for the stabilization of sarcolemmal functions in the acute-, as well as in the late “second window” preconditioning of the heart (Ziegelh  ffer et al. 1995; Szekeres 1996).

Little is known yet about the molecular mechanisms that may participate in the adaptation of ATPases. Originally, it was speculated that they may involve changes in the vicinity of the ATP binding sites (Vrbjar et al. 1995). These changes may either concern the α -helical arrangement of the enzyme molecule or spatial reorganization of local charges within, or close to the ATP-binding site. Nevertheless, there are also other even more plausible options e.g., a shift in the composition of the α subunit isoforms in the myocardium resulting either from increased expression of an isoform with a higher affinity to ATP or from a depression of α subunit

isoforms with lower affinity to ATP, etc.

7.1.3. *Adaptation to diabetes mellitus*

Another type of adaptation of the Na,K-ATPase was demonstrated in chronically diabetic rats with fully developed diabetic cardiomyopathy (Ziegelh  ffer et al. 1996b). Isolated perfused hearts of these animals exhibit a high resistance against calcium overload, even if induced in its most severe form i.e., by calcium paradox (Tribulov   et al. 1996). It turned out that in spite of serious abnormalities in sarcolemmal functions, Ca-resistant diabetic hearts dispose with a considerably well preserved activity of the Na,K-ATPase and that the enzyme exhibits highly preserved sensitivity to stimulation with increasing concentrations of Na⁺- and partially also with K⁺ ions. It is known that, due to impaired sequestration and extrusion, the diabetic myocardium is permanently exposed to elevated intracellular Ca²⁺ levels (Ziegelh  ffer et al. 1997). In these circumstances a conservation of activity and properties of the Na,K-ATPase may be referring to an adaptation mechanism that protects the heart against a further rise in intracellular free Ca²⁺ by preventing its entry into the myocytes. The particular role of Na,K-ATPase in this adaptation mechanism is to prevent any increase in Na⁺ concentration in the fuzzy subsarcolemmal space (Ziegelh  ffer et al. 1996a) thus preventing any enhancement of the Na-Ca exchange system.

The conservation of Na,K-ATPase in diabetic hearts seems to belong to a more broad circle of adaptation changes on molecular level since it was also accompanied by significant increases in Mg²⁺- and Ca²⁺-ATPase activities, and its functional importance was so strong that the calcium paradox remained without any significant effect on the activities and properties of these enzymes. Consequently, 83% of diabetic hearts survived a calcium paradox that was lethal to all control hearts (Ziegelh  ffer et al. 1996b). Experiments with the application of resorcyllidene aminoguanidine revealed that remodelling of sarcolemma induced by non-enzymatic glycation of proteins and coupled with free radical formation was responsible for the functional conservation of Na,K-ATPase in diabetic hearts (Ziegelh  ffer et al. 1997). The described adaptation of Na,K-ATPase to diabetes was considered as a rare example for a beneficial effect of non-enzymatic glycation and the related free radical production (Ziegelh  ffer et al. 1999).

A decrease in activity (Pierce et al. 1991) and particularly in the amount of Na,K-ATPase have also been observed in the diabetic myocardium (Kjeldsen et al. 1987). It can not be excluded that these changes concerning the bulk of the enzyme present in the myocardium may differ in regard to the content and activities of the single isoforms of the cardiac sodium pump. Hence, it may be expected that future experiments may underline the role of changes in quantity and subunit composition of the Na,K-ATPase in adaptation changes of the enzyme.

It seems probable that the various types of adaptation described in chapters 7.1.1–3 will not represent the only ones that the heart Na,K-ATPase is able to undergo.

7.2. Regulation of Na,K-ATPase by induction: Mode of induction and properties of the induced enzyme

An increase in cardiac and diaphragm Na,K-ATPase exceeding several times its original activity, was observed to occur in a time interval 2–76 hours after *in vivo* administration of stable derivatives of prostacyclin such as of 7-oxo-prostacyclin (PGI₂) (D  zurba et al. 1991; Ziegelh  ffer et al. 1993; Stankovi  ov   et al. 1995) or of Iloprost^{  } (Szekeres 1996) in the rat. This increase in Na,K-ATPase activity proved to be sensitive to cycloheximide and was attributed to enhanced synthesis of the enzyme (D  zurba et al. 1991). However, the effect of PGI₂ may not be specific for ATPases only, since PGI₂ also promotes the synthesis of phosphodiesterase isoforms I and IV in the myocardium (Borchert et al. 1994). A special feature of Na,K-ATPase induced by PGI₂ is that it exhibits a decreased K_m value, similar to that in Na,K-ATPase adapted to hypoxia or ischemia. The kinetic predisposition of the PGI₂-induced enzyme to cope with ATP-deficiency in the hypoxic or ischemic myocardium proved to be similar to, and additive with that acquired in the process of enzyme adaptation *in vivo* (Ziegelh  ffer et al. 1993). This indicates that both, the induction- and the adaptation-induced increase in affinity of Na,K-ATPase to ATP may, at least in part, reflect a similar mechanism, with roots somewhere in the enzyme synthesis. Hence, they might have more general importance, and these phenomena should probably be taken into consideration also in experiments with mutants of the Na,K-ATPase.

8. Modulation of the heart Na,K-ATPase in health and disease: Effects of hormones, regulatory peptides and some intermediates

Induction of the Na,K-ATPase by 7-oxo-prostacyclin (PGI₂) was utilized successfully as a useful tool for investigation of the role and function of the Na,K-ATPase in different experimental conditions. For instance, PGI₂-pretreated hearts with elevated content and activity of Na,K-ATPase were shown to be able to resist to membrane deterioration induced by perfusion with calcium free solution that causes an almost complete diminution of the Na,K-ATPase activity. In PGI₂-pretreated hearts, calcium depletion of the same duration was not able to depress the activity of the enzyme below the control level, and these hearts did not develop calcium paradox (Ravingerov   et al. 1991; Ziegelh  ffer et al. 1996a). This result supported the hypothesis about the essential role of an influx of Na⁺ ions, prior to influx of Ca²⁺ ions, in triggering the mechanism of the calcium paradox (Chapman et al. 1991; Ziegelh  ffer et al. 1991).

Pretreatment with PGI₂ yielding induction of Na,K-ATPase with higher affinity to ATP proved to be favorable also in protection of the heart against global ischemia (Ravingerov   et al. 1991) and regional ischemia due to acute coronary occlusion, as well as against reperfusion arrhythmias (Ravingerov   et al. 1993). The described manipulation of the Na,K-ATPase led to stabilization of sarcolemmal functions manifested in decreased incidence of both, ventricular tachycardia and

ventricular fibrillation from 100% and 92% to 33% each, as well as to a spontaneous recovery to sinus rhythm within the first 2 min after the onset of reperfusion. Based on these findings it was hypothesized that upregulation of the sarcolemmal Na,K-ATPase, preservation of its activity or at least its adaptation to ischemia, may play an essential role in ischemic- as well as in the second window preconditioning of the heart against ischemia and also against ischemia-reperfusion injury (Ziegelh  ffer et al. 1995). In order to prove this hypothesis and also to investigate the possible involvement of catecholamines (the late effect) in the regulation of the sarcolemmal Na,K-ATPase activity, rats were pretreated with noradrenaline *in vivo* 24 h prior to an experimental ischemic insult. The hypothesis became supported by the evidence that the hearts of pretreated animals showed significantly better preserved Na,K-ATPase activities, and this was accompanied with considerable suppression of ischemia-induced severe ventricular arrhythmia (Ravingerov   et al. 1996, 1997).

In a majority of cases, free radicals attack Na,K-ATPase causing an irreversible damage to the essential -SH group in its ATP-binding site and, in addition, also increasing the affinity of the enzyme to Na⁺ ions that may slow down or inhibit the transition of E₁ to E₂ conformer of the Na,K-ATPase (Shao et al. 1995). The same mechanism of action of oxyradicals on cardiac sarcolemmal Na,K-ATPase is also believed to participate in reperfusion arrhythmia (Ravingerov   et al. 1994).

As concerns the affinity of its Na⁺-binding sites, cardiac Na,K-ATPase was found targeted in diverse pathological conditions. Experiments in rats with NO-deficient hypertension revealed a decrease in affinity of Na⁺-binding sites of the enzyme to Na⁺ ions. This alteration was attributed to failure of the sodium pump to cope with persistently elevated intracellular sodium concentrations [Na]_i in hearts of hypertensive rats. The effect of NO deficiency on heart Na,K-ATPase proved to be reversible since after termination of experimental hypertension, when [Na]_i also became normal, the enzyme regained its original affinity to Na⁺ ions (Vrbjar et al. 1996). A stimulation of Na⁺-binding manifested in a 67% increase in V_{max} and 33% decrease in apparent K_a value for Na⁺ of the Na,K-ATPase, leading to augmented extrusion of Na⁺ ions by the enzyme, was demonstrated to participate in the molecular mechanism of the calcium antagonistic and antiarrhythmic action of nitrendipine (D  zurba et al. 1993).

Sarcolemmal Na,K-ATPase also serves as a target for estradiol action on the heart. The molecular mechanism of protection of the myocardium by estradiol involves an increased activation of the Na,K-ATPase by increasing concentrations of K⁺ ions (Ziegelh  ffer et al. 1990). This occurs via an increase in affinity of the enzyme to potassium, but remains without any influence on the K_D value of ouabain binding to the heart (D  zurba et al. 1997). A similar principle was found to be operating when estradiol was applied to protect the myocardium against acute ischemia (Barta et al. 1989).

K⁺-Mg²⁺-(D,L)-aspartate, was also shown to act on the heart via the Na,K-ATPase. When applied in form of a racemic salt, and particularly in conjunction with cardiac glycosides, the substance was shown to protect the heart in cardiac insufficiency caused by ischemia, anoxia, cardiomyopathies or coronary insufficiency,

and reduced the toxicity of ouabain and digoxin by 37% and 75% respectively (Greef and Knippers 1964). This protection involves a specific interaction of aspartate moiety of the compound with the heart sarcolemmal Na,K-ATPase. This interaction occurs in the K^+ -binding site of the enzyme (Fedele  sov   et al. 1973). At the same time, aspartate is also competing with ouabain for the same binding site (Fedele  sov   et al. 1975a). In addition, K^+ - Mg^{2+} -(D,L)-aspartate may protect the sodium pump against the deleterious effect of toxic doses of isoproterenol and prevent the isoproterenol-induced calcium overload of the heart (Fedele  sov   et al. 1975b).

Results described in chapters 6–8 indicate that the data concerning disease-induced alterations in activity and function of the cardiac sodium pump as well as those concerning the mechanisms of interaction of various substances and physiological modulators with the cardiac Na,K-ATPase, may be utilized in elucidation and treatment of diverse cardiac disorders.

9. Quantitative aspects of myocardial Na,K-ATPase

Na,K-ATPase content in the myocardium of different species exhibits considerable diversity; however, within certain limitations, it seems to be in inverse relationship to the body weight. Quantification of the Na,K-ATPase content by measuring either 3H -ouabain binding or K^+ -dependent 3-*O*-methylfluorescein phosphatase (3-*O*-MFPase) activity in samples from the left ventricular myocardium of mature animals yielded the following values (in $pmol \times g^{-1}$ w.wt.): Syrian hamster, 3,500 (N  rgaard et al. 1987); rat, 2,100 (N  rgaard et al. 1985); guinea pig, 1,079 (Kjeldsen et al. 1985); pig, 1,037 (Schmidt et al. 1990); and dog, 1,410 (Schmidt et al. 1990). These data are in good agreement with those obtained by measuring K^+ -dependent pNPPase activity in the rat heart that provided an assumed molecular activity of $1,345 \times min^{-1}$ and a Na,K-ATPase content of $2,200 pmol \times g^{-1}$ w.wt. (Larsen and Kjeldsen 1995).

9.1. Human myocardial Na,K-ATPase quantification

The content of Na,K-ATPase in the myocardium has also been assessed (for review see Bundgaard and Kjeldsen 1996). The values reported for adult human individuals (means $\pm$ S.E.M., in $pmol \times g^{-1}$ w.wt.) varied between 305 ± 15 ($n = 8$, Schmidt and Kjeldsen 1991; Schmidt et al. 1991) and 728 ± 58 ($n = 5$, Schmidt et al. 1993), being higher in infants (Kjeldsen and Gr  n 1990). The observed differences may be attributed, at least in part, to differences in conditions of sample taking and in the methods applied for quantification of the myocardial Na,K-ATPase. Postmortal Na,K-ATPase degradation, the cause of death, previous medication and particularly treatment with cardiac glycosides may have influenced the results (N  rgaard et al. 1986). Even studies using samples harvested *in vivo* from patients with suspected heart disease, but having normal ejection fraction, used as criteria for considering the tissue to be normal, can not guarantee definitely that these patients were true healthy controls. Moreover, the methodology

used for quantification of the myocardial Na,K-ATPase could itself be a source of differences. For example, a lower ^3H -ouabain binding observed in homogenates (Ellingsen et al. 1994) in comparison to intact samples (Schmidt et al. 1993) could be also considered as being responsible for reduced recovery of the enzyme in the homogenates (Hansen and Clausen 1988). Considering all the above problems it may be assumed that the most reliable value for human myocardial Na,K-ATPase content for a healthy adult person would amount to $700 \text{ pmol} \times \text{g}^{-1} \text{ w.wt.}$ This value is close to that obtained by vanadate facilitated ^3H -ouabain binding in intact vital samples originating from healthy human donors in heart transplantation program.

9.2. Human myocardial Na,K-ATPase in heart disease

Myocardial Na,K-ATPase is quantitatively regulated in response to physiological as well as pathophysiological changes. A major physiological determinant for myocardial Na,K-ATPase content represents the age: the contents of the enzyme during differentiation and growth is exceeding those in mature hearts. In addition, animal studies indicate that changes in plasma levels of thyroid hormones, insulin, angiotensin or aldosterone may have a considerable influence on the regulation of the Na,K-ATPase contents in the myocardium (Doohan and Rasmussen 1997). In animals, changes in myocardial Na,K-ATPase content were reported in relation to K^+ -depletion (Nørgaard et al. 1985) or adaptation to high K^+ levels (Blachley et al. 1986, Bundgaard et al. 1998), as well as in relation to diseases that may induce structural changes in the myocardium, like those which may occur in hypertensive heart disease, different cardiomyopathies and in other forms of secondary hypertrophy caused by pressure or volume overload of the heart.

Heart failure is generally associated with decreased Na,K-ATPase contents in the human myocardium. In patients with hearts failing due to ischemic heart disease, dilated cardiomyopathy or hypertrophic heart disease, reduced myocardial Na,K-ATPase contents have been observed.

Short-term regional ischemia (20 min) and subsequent reperfusion of the ischemic area remained without any effect on the content of Na,K-ATPase in the canine myocardium (Schmidt et al. 1990). It is not surprising that data concerning the content of Na,K-ATPase in human hearts, related to the first minutes of acute coronary occlusion, are not available. On the other hand, chronic ischemic heart disease in man was found to be associated with significant downregulation of the myocardial Na,K-ATPase of up to 47% (Shamraj et al. 1993). It should be stressed that in the latter, but in other studies as well (Schmidt et al. 1991, 1993; Ellingsen et al. 1994) great attention was focused to keeping the rule that samples used for measurement of ^3H -ouabain binding should not be taken from the infarcted, scarred areas, but from the well perfused, if possible not remodeled parts of the myocardium. Myocardial infarction is usually followed by remodeling of the left ventricle associated with a compensatory hypertrophic response. If the expression of Na,K-ATPase in hypertrophic myocardium is regulated by similar mechanisms as it is regulated in skeletal muscle during the development of physical

training-induced muscle hypertrophy (Green et al. 1993; McKenna et al. 1993), an upregulation of the myocardial Na,K-ATPase content might be anticipated. This type of upregulation would be expected to occur particularly in the initial remodeling phase after myocardial infarction, i.e. in the compensated stage of the disease. Nevertheless, in ischemized human heart, no such upregulation of the myocardial Na,K-ATPase has been observed. This may either indicate that different regulatory mechanisms may be involved or that the investigated patients were either in more progressed stages of the disease such as the stage when the process of hypertrophy has already reached its stabilized phase or even in an early decompensation stage of ischemic heart disease.

In patients with dilated heart disease a reduction in myocardial Na,K-ATPase content amounting up to 89% (N  rgaard and Kjeldsen 1989) has been reported. Moreover, a significant, positive, linear correlation between the content of myocardial Na,K-ATPase and the left ventricular ejection fraction was discovered (N  rgaard and Kjeldsen 1989). This means that with the progressing disease, the content of Na,K-ATPase in the myocardium becomes progressively reduced. But unfortunately, the above correlation between the Na,K-ATPase content and the value of ejection fraction fails to provide any information about their cause-response relationship i.e., whether the reduced Na,K-ATPase content is compromising a weakened left ventricular function or *vice versa*.

Similarly as it was discussed in the case of ischemic heart disease, the dilating heart may also transiently exhibit hypertrophy in some areas of the ventricular wall, at least in the initial phases of the disease.

In hypertrophic heart disease significant myocardial Na,K-ATPase reductions of up to 42 and 60% have been reported in spontaneously hypertensive rats and in humans with an aortic valve disease, respectively (Larsen et al. 1997). In early phases of the development of myocardial hypertrophy in the spontaneously hypertensive rats a significant 11% upregulation of myocardial Na,K-ATPase was observed. However, in later phases of hypertrophy, the initial upregulation of the enzyme gradually declined. These findings may be consistent with the increase in myocardial Na,K-ATPase content observed in patients with hypertrophic cardiomyopathy (N  rgaard and Kjeldsen 1989). However, the latter report only included two patients with hypertrophic cardiomyopathy. Hence, the observed upregulation of myocardial Na,K-ATPase may also be attributed to a mere play of chance. Nevertheless, these observations may indicate that the presently established knowledge concerning the regulation of myocardial Na,K-ATPase content in patients with ischemic, dilated and hypertrophic heart disease, may be mainly related to changes occurring in advanced stages of diseases. Little if any reliable data are available about changes in the amount and particularly in specific properties of cardiac Na,K-ATPase in early phases of different heart diseases, that would be related to human material. A rare exception in this respect may represent the study performed by Shamraj et al. (1993) who, however, were not able to detect any changes in properties of the Na,K-ATPase or its isoforms in failing human hearts. In contrast to clinical studies, data obtained from animal experiments do confirm numerous, early,

qualitative as well as quantitative changes in expression, function and properties of the heart sarcolemmal Na,K-ATPase in diverse pathological conditions. It may be of particular importance that many of these changes clearly exhibit the character of adaptation (for more details see the chapters 6–8). This seemingly controversial gap between experimental data and those obtained from clinical material makes further thorough studies of the sarcolemmal Na,K-ATPase from healthy and diseased human hearts even more appreciable.

It is known that, irrespective of the etiology of the heart disease, numerous hormonal and/or metabolic disturbances occurring coincidentally may have an additional effect on the content and function of Na,K-ATPase in the myocardium. For example, heart failure is often associated with increased plasma catecholamine levels, reduced myocardial α -adrenergic receptor density and changes in the renin-angiotensin-aldosterone axis. In such cases, the complexity of combined effects on the heart Na,K-ATPase may cause interpretation difficulties that can particularly increase in the clinical setting where also the effect of pharmaca used in the treatment of these diseases has to be considered.

9.3. Human myocardial Na,K-ATPase and digoxin treatment

Na,K-ATPase serves as a specific receptor for cardiac glycosides that bind reversibly in a 1:1 ratio to extracellular domains of the sodium pump (for review, see Lingrel et al. 1990). Binding of cardiac glycosides inactivates the Na,K-ATPase apparently by induction of a conformational change in the enzyme molecule. The inhibited active Na^+ and K^+ transport results in increased intracellular Na^+ concentrations, which improves myocardial force of contraction due to secondarily increased intracellular Ca^{2+} concentrations. Different species exhibit considerable variability in affinities of the myocardial Na,K-ATPase to ouabain. In rats the α_1 -subtype with low affinity to ouabain constitutes around 90% (Hensley et al. 1992) of the total Na,K-ATPase, which indicates that the rat is rather resistant to cardiac glycosides. It is of interest that the rat α_1 isoform exhibits an approximately 1,000-fold weaker affinity to ouabain than reported for the human myocardial Na,K-ATPase. For the transfer of experimental results to clinical practice it is important to realize that the major determinant of Na,K-ATPase affinity to cardiac glycosides is the rate of their dissociation from the enzyme rather than the rate of their association (Akera and Brody 1977). The differences in ouabain-affinity between single α isoforms of the Na,K-ATPase have been utilized in chromosome-mediated gene transfer and site-directed mutagenesis studies of the ouabain binding to the Na,K-ATPase (for review see Lingrel et al. 1990).

Binding of cardiac glycosides to myocardial Na,K-ATPase depends on the amount of the α -subunit isoform that is prevailing. In the human heart all three α isoforms have been demonstrated at mRNA (Shamraj et al. 1993) as well as at protein levels (Wang et al. 1996). Ouabain-sensitivity of the single isoforms present in the human myocardium has not been determined, but it is generally accepted that all three isoforms have high affinities to this glycoside (Wang et al. 1996). Measurements based on vanadate-facilitated ^3H -ouabain binding in myocardial samples

taken from digitalized patients with heart failure, that were performed before and after removal of bound digoxin by washing of samples with an excess of digoxin antibody, revealed a receptor occupancy of 24–34% (Schmidt et al. 1991, 1993). The latter degree of receptor occupancy is in concert with a 33% reduction in maximum hyperpolarisation of the resting membrane potential observed in atrial tissue from digitalized humans (Rasmussen et al. 1990). An upregulation of myocardial Na,K-ATPase induced by persisting digitalization, as suggested by studies performed in cardiac cell cultures, has not been confirmed in long-term digitalized patients with heart failure (Schmidt et al. 1991, 1993).

The reduced transport capacity of the myocardial Na,K-ATPase that occurs in heart failure may be further compromised by treatment with cardiac glycosides. The resulting aggravated reduction in the capability of Na,K-ATPase to maintain the transport of Na⁺ and K⁺ ions may act particularly negatively in situations when maximum pump function may be called for, such as during exercise-induced hyperkalemia or during post-ischemic reperfusion, when a locally increased extracellular K⁺ contents has to be cleared. Moreover, an increase in intracellular Ca²⁺ content, secondary to increased Na⁺ content resulting from the hypofunction of the myocardial Na,K-ATPase, may potentially cause an overload of the heart by calcium, and eventually lead to damage or death of myocytes. On this basis, chronic treatment of patients with heart failure with higher doses of cardiac glycosides, as it has been recommended previously, may lead to problems originating from lowered functional capacity of the myocardial Na,K-ATPase. Recent studies prefer administration of reduced doses of cardiac glycosides that may be without any disadvantage with respect to cardiac inotropy during chronic treatment of patients with heart failure (Gheorhiade et al. 1995).

Emerging knowledge about heart Na,K-ATPase constitution, molecular mechanisms of regulation, induction and adaptation, affinities to ligands as well as about expression of isoforms of the enzyme and their specific functional role in cell signalling and function in the heart, is far exceeding the interest of biologists, biochemists and physiologists. New findings about the Na,K-ATPase have a permanently increasing importance for proper understanding of the role of the enzyme in the diseased heart. Such informations seem to be necessary in order to establish a modern and progressive clinical rationale for the management of various heart diseases that would focus on the Na,K-ATPase beyond the administration of cardiac glycosides, used already for two centuries.

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