The Na, K-ATPase in the failing human heart

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Abstract

The Na, K-ATPase consists of \(\alpha\)- and \(\beta\)-subunits and actively transports Na out and K into the myocyte. It is the receptor for cardiac glycosides exerting its positive inotropic effect by inhibiting enzyme activity, decreasing the driving force for the Na/Ca-exchange and increasing cellular content and release of Ca during depolarization. The specific binding capacity for cardiac glycosides is utilized as a tool for Na, K-ATPase quantification with high accuracy and precision. In treatment of patients with heart failure cardiac glycosides improve symptoms and reduce the need for hospitalization without affecting mortality. In endomyocardial biopsies from patients with compromised cardiac function total Na, K-ATPase concentration is decreased by \(\sim 40\%\) and a correlation between decrease in heart function and decrease in Na, K-ATPase concentration exists. At the subunit level, the \(\alpha_1\)-, \(\alpha_3\)- and \(\beta_1\)-proteins are reduced in human heart failure. During digitalization \(\sim 30\%\) of remaining Na, K-pumps are occupied by digoxin. Thus, a total of not less than half the Na, K-pumps may be out of function in the myocardium of digitalised heart failure patients. It is still a matter of debate whether a digitalis-like factor exists. There is a pressing need for the identification of its precise chemical structure, properties and quantitative relation to the Na, K-ATPase. It is recommended that cardiac glycosides are prescribed to heart failure patients who are still having heart failure symptoms after institution of mortality reducing therapy. Cardiac glycoside treatment is still the only safe inotropic drug for oral use that improves hemodynamics in patients with compromised cardiac function.

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1. Mechanism for the hemodynamic effects of digitalis glycosides

In 1997 Jens Christian Skou was awarded the Nobel Prize in chemistry for his discovery of the Na, K-adenosine triphosphatase (Na, K-ATPase or Na, K-pump) in peripheral nerves of the shore crab [1,2]. This membrane-bound Na, K-pump mediates the active transport of Na out and K into the cell and has been identified in virtually all animal tissues including the human myocardium. The active Na and K transport is specifically inhibited by cardiac glycosides [3]. While there is no doubt that cardiac glycosides are effective in heart failure, the underlying mechanisms are still debated. Two mechanisms are of major importance for their hemodynamic effects, that is their positive inotropic (Fig. 1) and their neurohumoral effect.

The inotropic effect is generally accepted to be due to high affinity binding of cardiac glycosides to the Na, K-ATPase. This leads to inhibition of the enzyme [4] and an increase in intracellular Na [5]. Hence, the Na/Ca-exchanger activity is affected so that less Ca is extruded from the cell. Consequently, more Ca is accumulated in the sarcoplasmic reticulum and is available during subsequent contractions leading to increased force of contraction [6].

Increased contractility can also be observed when intracellular Na is raised by other pharmacologic interven-
Fig. 1. Mode of action of cardiac glycosides. Three different pathways have been proposed to mediate the positive inotropic effect of cardiac glycosides. (A) Cardiac glycosides bind with high affinity to the Na, K-ATPase (NKA) and inhibit enzyme activity. Thereby, intracellular Na is increased and the driving force for the Na,Ca-exchanger (NCX) to extrude Ca from the cell is reduced. The remaining Ca is accumulated in the sarcoplasmic reticulum (SR) via the SR Ca-ATPase (SERCA) and is released during subsequent depolarizations leading to enhanced contractility. (B) The Ca-release from the SR has been found to be enhanced secondary to the interaction of cardiac glycosides with the Ca-release channel (ryanodine receptor, RyR). (C) Cardiac glycosides lead to a ‘slip-mode’ conduction of sodium channels (SOC) allowing Ca-ions to enter the cell via the channels. The interaction of cardiac glycosides with SOCs may be either direct or indirect (via NKA). See text for details and references.

A few years ago Santana et al. found evidence that cardiac glycosides may induce a slip-mode conductance through Na-channels [18]. In this model Na-channels allow Ca-ions to pass, thereby increasing Ca-influx and contractility. However, recently this slip-mode phenomenon has been challenged [20]. Other investigators found that cardiac glycosides enhance the Ca-release from the sarcoplasmic reticulum (SR) by increasing single channel activity of ryanodine-receptors [21,22]. Thus, in addition to increasing SR load by Na, K-pump inhibition, amplification of SR Ca-release may play a role. However, no evidence was observed for enhancement of Ca-release from saponin skinned fibre preparations from human heart when exposed to ouabain (Schwinger and Müller-Ehmsen, unpublished observation).

Besides their direct positive inotropic effect, cardiac glycosides improve hemodynamics also by inhibition of extra-cardiac Na, K-ATPase. Hence, cardiac glycosides may restore baroreceptor activity in congestive heart failure [23]. In addition, ouabain treatment is known to stimulate vagal nerve activity decreasing heart rate [24]. This may be hemodynamically of special importance in patients with heart failure where contractility decreases at high heart rates due to the negative force-frequency-relationship [25]. The increased vagal activity may also be generally beneficial mimicking β-adrenoeceptor antagonist
treatment. Although this effect of digitalis glycosides may to some extent seem outdated in the era of the β-adrenoceptor antagonist for treatment of heart failure, it is of importance for patients not tolerating β-blockade. Thus, other effects than direct increase in contractility may play an important role for the benefits of cardiac glycosides in the treatment of patients with symptomatic heart failure.

2. Quantitative and kinetic aspects of myocardial Na, K-ATPase

Some decades ago cardiac glycoside binding was developed as a tool for Na, K-ATPase quantification [26]. This method allows quantification of muscular Na, K-ATPase with high accuracy and precision [27]. The Na, K-ATPase was found to be expressed in the human myocardium several years ago [28], and has been quantified in both normal and diseased myocardium. In normal human left ventricular myocardium a Na, K-ATPase concentration of ~700 pmol/g wet weight was found [29]. Hitherto, it has not been possible to quantify the absolute amounts of the various isoforms of the myocardial Na, K-ATPase. In human dilated cardiomyopathy endomyocardial biopsies showed a significant decrease of ~40% in total Na, K-ATPase concentration [30]. Later, using data from all available studies [30–33], it was concluded that there is a consistent and significant decrease of 26–32% in Na, K-ATPase protein in the failing human heart [34]. Concomitantly, the Na, K-ATPase activity is decreased in homogenates and membranes from failing human hearts [35].

Interestingly, a close correlation was observed between decreased left ventricular ejection fraction and decreased Na, K-ATPase concentration [30,36]. A very interesting and important question is, whether the reduction of Na, K-ATPase is part of the reason for cardiac dysfunction and/or hypertrophy, whether it serves compensatory purposes with effects comparable to digitalis treatment, or whether it just coincides with the disease progress. Data from hypertrophied dog heart indicate that reduced Na, K-ATPase expression occurs early in the development of heart failure [37]. Contradictory model-dependent results were found in hypertrophied rat hearts with either unchanged [38] or reduced levels of Na, K-ATPase [39]. Overall, it appears that a reduction of Na, K-ATPase expression occurs early in the development of heart failure, probably before heart failure symptoms are present.

To estimate the functional impact of a reduced protein expression of Na, K-ATPase, it is important to know whether less protein necessarily means less activity. This is of course the case in $V_{max}$ measurements under experimental conditions in vitro [35,40], but it may not necessarily be the case in intact tissue, where factors like substrate availability and phosphorylation status influence Na, K-ATPase activity. In the absence of direct quantitative assessment of Na, K-ATPase current in intact human cardiomyocytes from failing hearts, we have to rely on indirect evidence from the parameters were measured. In experimental heart failure and in human heart failure increased intracellular Na-concentrations were observed [41,42]. The concentrations at 0.25 Hz in man were estimated to be 8–16 mM in non-failing ventricular myocytes and 12–22 mM in failing cells (~50% increase) [42]. The reduced Na, K-ATPase activity is certainly a major contributor to this finding in man, but other transporters like Na,H-exchanger [43], the sodium-bicarbonate co-transporter or Na-channels may play a role [42,44]. In rabbit, similar increases in Na were measured, but the Na, K-ATPase density was not reduced. Instead, an increased sodium influx was found to be responsible for increased Na. Whatever the reason, higher Na-concentration means more substrate for Na, K-ATPase and higher enzyme activity. The Na affinities that have been observed for heterologously expressed human Na, K-ATPase isoforms vary considerably with $K_{1/2}$ 8–25 mM in one study [45] and $K_{1/2}$ 1.5–2.8 mM in another study [46]. If the former results are representative for human cardiomyocytes, the activity of Na, K-ATPase enzymes will be considerably increased by the increase in intracellular Na, compensating at least in part for reduced pump abundance. In fact, in the above mentioned rabbit model of heart failure where no decrease in overall Na, K-ATPase capacity was observed, the sodium pump activity was even enhanced during heart failure due to increased intracellular Na concentrations [41]. However, if the lower $K_{1/2}$ reflects the actual situation in human cardiomyocytes, Na-dependent Na, K-ATPase activation is already saturated at Na-concentrations of ~10 mM. A further increase in intracellular Na-concentration will only insignificantly enhance overall Na, K-ATPase activity. In this case, a reduction in Na, K-ATPase protein will directly translate to reduced enzyme activity, thus mimicking cardiac glycoside treatment. In effect, contractility may be enhanced as compensation for the underlying disease.

3. Na, K-ATPase isoforms

The Na, K-ATPase is a heteromeric protein consisting of α and β subunits. While the α subunit contains the amino acids involved in catalytical function, ion transport and cardiac glycoside binding, the function of the β subunit is not completely understood although it is essential for the normal activity of the enzyme [47] and is involved in the transport of the functional Na, K-ATPase to the plasma membrane. Several isoforms of the Na, K-ATPase have been identified for both α (α1, α2, α3 and α4) and β subunits (β1, β2 and β3) [48] which are expressed in a tissue specific manner. In human heart α1, α2 and α3 are expressed together with β1 and at very low levels β2 in a region specific manner [35,49].
In the first report on Na, K-ATPase isoform expression in normal and failing human left ventricle, Allen et al. found no significant alteration in the mRNA expressions of the isoforms [50]. However, also the total Na, K-ATPase concentration was only non-significantly reduced by ~10% in that study. Later, Shamraj et al. [34] found that the mRNA expression pattern was different in samples from failing human hearts. On the protein level, α1 (~38%) and α3 (~30%) were lower in failing human hearts than in non-failing hearts. In parallel, abundance of β1 (~39%), maximal ouabain binding (~39%) and Na, K-ATPase activity (~42%) were lower, while the α2-isoform expression only showed a small tendency to a reduction [35]. In the right atrium from human failing hearts α1- and α2-isoforms, but not α3, were expressed at lower levels [51].

For interpretation of the specific regulation and expression of Na, K-ATPase isoforms it is important to know about their specific functional properties. It is generally accepted that in the human heart the affinities of the isoforms for cardiac glycosides are almost identical. However, two studies that investigated heterologously expressed human Na, K-ATPase isoforms found that ouabain had a two-fold lower affinity for α2β1 than for α1β1 or α3β1 [45,46]. The affinity for Na was similar for all cardiac heteromers in one study [46], while in the other α1β1 had the highest and α3β1 the lowest affinity for Na [45]. There were no differences in the ATP-affinity between the isoforms, but the K-affinity was lower in α2β1 than in α1β1 or α3β1. In consequence α2β1 requires higher extracellular K-concentrations (above 6 mM) to pump at maximal velocity. Interestingly, the turnover rates of the different isoforms were also different, with that for α1β1 being twice as high as that for α3β1. Thus, two α3β1 heteromers are needed to match the maximal velocity of one α1β1 enzyme [45,46]. These data indicate that downregulation of α1 may have a higher functional impact on overall cell Na, K-transport than downregulation of α2 or α3.

Besides the specific biochemical properties, a specific subcellular distribution of the Na, K-ATPase isoforms may influence their specific physiological and pathophysiological role. Thus, in rat cardiomyocytes α1 isoform is concentrated in the transverse tubules and α2 is uniformly distributed throughout the sarcolemma [52]. In rat arterial myocytes the α1 isoform was ubiquitously distributed over the surface of the cells, while α3 showed a reticular distribution similar to that of the Na/Ca-exchanger [53]. Such distribution may explain the findings that low dose ouabain can increase intracellular Ca-concentration without changes in bulk Na-concentration [10,12,13]. This hypothesis is further supported by findings of James et al. [54] in heterozygous knock-out mice in which cardiac expression of either α1 or α2 was reduced by ~50%. In this study, only the reduction of α2 led to hypercontractility of the heart, mimicking the effect of cardiac glycosides, although overall Na, K-ATPase activity was not significantly re-dced (because α1 accounts for the vast majority of pumps in the mouse heart). In contrast, the reduction of α1 expression did not mimic cardiac glycoside treatment, but was followed by hypocontractility. Thus, at least in mouse heart, the isoforms of the Na, K-ATPase have different impact on cardiac contractility which cannot be attributed to different affinities for cardiac glycosides or different biochemical properties, but which may be the result of distinct subcellular distribution. An important prerequisite for such conclusion, however, is the existence of subcellular Na gradients and a fuzzy space for Na, which have not been clearly demonstrated to date.

4. Myocardial Na, K-ATPase in digitalization

Occupancy of Na, K-ATPase, i.e. percentage of receptors occupied by cardiac glycosides, during digitalization was firstly evaluated by membrane potential measurements in human atrial biopsies revealing a 38% reduction in electrogenic effect [55]. Later occupancy in myocardial biopsies from digoxin treated patients was determined as the relative difference in digitalis glycoside binding before and after prolonged washing of the samples with buffer containing digoxin antibody. This revealed occupancies of 24–35% in the human heart [29,56]. These numbers of receptor occupancy appear relatively high when the therapeutic plasma concentrations of digoxin (~2.5 nM) or ouabain (~1 nM) are compared with the affinities of human isoforms for the drugs. In the absence of potassium, the $K_p$-values of ouabain binding to human α1 and α3 isoforms are 5–15 nM, and the $K_p$ of ouabain for the α2 isoform is even higher (25–30 nM) [45,46]. The presence of potassium in physiological concentrations further reduces this affinity with an approximate doubling of $K_p$-values [45] and, thus, reduces the fraction of Na, K-ATPase enzymes occupied by cardiac glycosides at therapeutic concentrations. The presumably low receptor occupancy in vivo may support the notion that other than positive inotropic effects (e.g. central nervous effects) may play an important role for the clinically beneficial effects of cardiac glycosides. On the other hand, the data observed in the Na, K-ATPase knock-out mice seem to indicate that the inhibition of the right isoform is more important than reducing overall Na, K-ATPase activity [54].

Some decades ago development of tolerance to chronic digoxin therapy was suspected based on the finding of Na, K-ATPase upregulation in various tissues such as erythrocytes. However, when Na, K-ATPase was later studied in the target organ for the inotropic action of digoxin, the human myocardium of patients with heart failure, no evidence for development of tolerance to long-term digitalization was found at the digoxin receptor level [29,56].

Recent studies using experimental animals indicate that myocardial Na, K-ATPase is also influenced by other drugs than digoxin used for treatment of heart failure.
Thus, potassium loss during diuretic therapy as well as hyperaldosteronism induced by heart failure has been found to reduce the myocardial Na, K-ATPase, whereas angiotensin converting enzyme (ACE) inhibitors may stimulate Na, K-pump activity [57–59].

5. Endogenous digitalis-like factor

In 1982 a correlation was found between the concentration of a blood plasma component inhibiting Na, K-ATPase and blood pressure in humans [60]. A ouabain-like factor was later found in the adrenal cortex, pituitary and hypothalamus [61,62]. In bovine adrenocortical cells a secretion of ouabain was found, suggesting an endogenous synthesis of this hormone [63]. However, the precise chemical structure and its pathway of synthesis remain unknown [64,65]. Some studies have found a rapid increase in plasma levels of endogenous ouabain suggesting that it may play a role in acute circulatory regulation [66], others have found elevated plasma levels of endogenous ouabain in patients with essential hypertension [67]. However, not all investigators were able to identify an endogenous ouabain compound in human plasma [68]. In spontaneous hypertensive rats, no evidence of an endogenous digitalis-like factor was observed either when ouabain binding to muscular tissue in vivo and in vitro was compared, or when samples were exposed to prolonged washes before binding [69,70]. The role of an endogenous ouabain in blood pressure regulation was supported by the finding that prolonged infusion of ouabain induced sustained hypertension in rats [71]. On the other hand no hypertension was observed with digoxin or digitoxin [72], which may explain the lack of pro-hypertensive effects of digitalization in humans. Although it has been suggested that ouabain is secreted from the adrenal gland, subsequent quantitative evaluations showed that the production rate was so low that it would take ~1 month to produce enough ouabain to obtain only a 1% occupancy of the Na, K-ATPase in the entire muscular pool [73]. When myocardial samples from patients not treated with cardiac glycosides were exposed to prolonged washes with buffer containing digoxin antibody, no significant occupancy of the Na, K-ATPase was revealed in binding experiments [29,56]. In comparison, potassium-depletion has been found to be associated with significant reductions in ouabain binding site concentrations in resistance vessels of 33% [69] and skeletal muscles of 78% [74].

Thus, the results on the existence of endogenous ouabain and its putative significance are contradictory. It cannot be ruled out that some unspecific cross-reactions of the antibody used for the determination of the digitalis-like factor may play a role in some experiments. Moreover, the quantity of this factor seems minute as compared to the enormous quantity of Na, K-ATPase in the human body, and if existing, its physiological and pathophysiological role for blood pressure, heart rate and hemodynamics remains to be clarified.

6. Clinical aspects and perspectives

One major aspect of reduced myocardial Na, K-pump concentration in heart failure and an additional inhibition of a significant fraction of it during digitalization is its influence on Na and K homeostasis. Higher concentrations of intracellular Na have been observed in cardiomyocytes from heart failure patients and from failing rabbit hearts [41,42] which may be induced by decrease in Na, K-pump concentration [35], but also increased Na-influx [41]. Higher concentrations of Na may stimulate Na, K-ATPase activity [45,46], while at resting conditions only a few percent of the Na, K-pumps have been found to be usually active [75]. On the other hand, only a reduced capacity for Na handling is available when in demand. Thus, a Na rise during e.g. ischemia may secondarily limit Ca and H extrusion from myocytes inducing arrythmias and lead to further progression of heart failure due to cell necrosis.

Besides intracellular Na, extracellular K homeostasis is largely influenced by Na, K-ATPase activity. Here two important aspects should be considered, that is K handling of the myocytes per se, and handling of K arriving at the extracellular space of the heart via the blood stream. Both of these aspects of K homeostasis are affected by regulatory aspects, e.g. regulation of the Na, K-pump by physiological and pathophysiological conditions as well as medical treatment [57,76]. Thus, digitalization has been shown to affect both parameters [17,18]. Furthermore, in experimental animals K-loading as well as K-depletion are found to significantly affect K handling [77–79]. The effects of K-depletion are of special interest since this may occur often in heart failure patients due to treatment with diuretics [80]. Diuretics should clearly be used when fluid overload is present, but it may be more rational to reduce its use in chronic heart failure to the minimum and to add cardiac glycoside.

Finally, a major aspect of digitalization is the beneficial influence on hemodynamics [81]. In failing human heart, an increased sensitivity to interventions that enhance intracellular Na⁺ has been observed in vitro [34,82,83] and in vivo [84]. This is likely due to higher baseline cell sodium secondary to depressed sodium pump activity. The functional and pharmacological role of Na, K-ATPase isoforms needs further elucidation in additional studies. In the only large study on the use of cardiac glycosides for the treatment of heart failure, the Digitalis Investigation Group (DIG) trial, beneficial effects of digoxin on morbidity and need for hospitalizations without affecting mortality were found [85]. Therefore, the available data support the recommendations of the guidelines of the European Society of Cardiology [86] to use cardiac glycosides in the treatment of heart failure patients who...
remain symptomatic after institution of mortality reducing therapies. To date, digoxin is still the only safe positive inotropic drug for longer term use.

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