Clinical and therapeutic significance of the Na\textsuperscript{+},K\textsuperscript{+} pump*

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ABSTRACT

1. The Na\textsuperscript{+},K\textsuperscript{+}-ATPase or Na\textsuperscript{+},K\textsuperscript{+}-pump, mediating the active transport of Na\textsuperscript{+} and K\textsuperscript{+}, which was first identified 40 years ago, is a central target for acute and long-term regulation, as well as for therapeutic intervention. Acute stimulation of the Na\textsuperscript{+},K\textsuperscript{+}-pump in skeletal muscle by insulin, catecholamines, β\textsuperscript{2}-agonists or theophylline increases the intracellular uptake of K\textsuperscript{+} and accounts for the hypokalaemia elicited by these agents. Conversely, digitalis intoxication elicits hyperkalaemia via acute inhibition of the Na\textsuperscript{+},K\textsuperscript{+}-pump.

2. Simple and accurate methods have been developed for the quantification of the total concentration of Na\textsuperscript{+},K\textsuperscript{+}-pumps in small (0.5–5 mg) fresh or frozen biopsies of human skeletal muscle, myocardium or other tissues. This has allowed the identification of several long-term regulatory changes in the concentration of this transport system in human tissues. In skeletal muscle, upregulation is induced by training, thyroid hormones or glucocorticoids. Down-regulation is seen in hypothyroidism, cardiac insufficiency, myotonic dystrophy, McArdle disease, K\textsuperscript{+} deficiency and after muscle inactivity.

3. Since the skeletal muscles contain one of the major pools of Na\textsuperscript{+},K\textsuperscript{+}-pumps, these changes are important for the ability to counterregulate the hyperkalaemia elicited by exercise or the ingestion of K\textsuperscript{+}. Moreover, downregulation or inhibition of the Na\textsuperscript{+},K\textsuperscript{+}-pumps in skeletal muscle interferes with contractile performance. Since digitalis glycosides bind to the Na\textsuperscript{+},K\textsuperscript{+}-pump, the muscles constitute a large distribution volume for these agents and are therefore an important determinant for their plasma level.

4. In cardiac insufficiency, the decrease in the concentration of Na\textsuperscript{+},K\textsuperscript{+}-pumps in the myocardium is over a wide range correlated to the concomitant reduction in ejection fraction. The regulatory and pathophysiological changes in the activity and concentration of Na\textsuperscript{+},K\textsuperscript{+}-pumps are important for the contractile function of skeletal muscle and heart as well as for K\textsuperscript{+} homoeostasis and the response to digitalization.

INTRODUCTION

In 1997, Jens Christian Skou was awarded the Nobel Prize in Chemistry for the first identification of the membrane-bound Na\textsuperscript{+},K\textsuperscript{+}-ATPase – the Na\textsuperscript{+},K\textsuperscript{+}-pump – mediating the active transport of Na\textsuperscript{+} and K\textsuperscript{+} across the cell membrane [1]. Obviously, the significance of this discovery for the understanding of normal and abnormal cell function cannot be overestimated. It would be of interest, therefore – 40 years after the discovery – to assess its clinical and therapeutic impact. A search on Medline, however, shows that although there is an increasing number of original studies on the Na\textsuperscript{+},K\textsuperscript{+}-pump in human subjects, no comprehensive review of the clinical significance of the Na\textsuperscript{+},K\textsuperscript{+}-pump is available. This prompted the present general analysis of the importance

Key words: ATPase, catecholamines, digitalis, heart, insulin, muscle, potassium, sodium, thyroid hormones, training.

* This review was submitted before the award of the Nobel Prize in Chemistry to Jens Christian Skou was announced; the wording of the first sentence of the Introduction therefore was revised to take account of this event.

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of the Na\textsuperscript{+},K\textsuperscript{+}-pump for the understanding of pathophysiology and therapeutic intervention. The review was further motivated by three recent lines of development:

1. Increasing consensus that insulin, catecholamines and \(\beta\)-agonists induce acute activation of the Na\textsuperscript{+},K\textsuperscript{+}-pump in skeletal muscle, an effect which can account for the hypokalaemia induced by treatment with these agents [2-5].

2. Simple and accurate methods for the quantification of Na\textsuperscript{+},K\textsuperscript{+}-pumps in human tissues have allowed the detection of anomalies in the concentration of Na\textsuperscript{+},K\textsuperscript{+}-pumps in a wide range of diseases.

3. The demonstration that changes in endocrine and electrolyte status, physical activity and cardiac insufficiency are associated with long-term regulation of the concentration of Na\textsuperscript{+},K\textsuperscript{+}-pumps in human skeletal muscle and myocardium.

Accumulating evidence that the Na\textsuperscript{+},K\textsuperscript{+}-pump is subject to acute and long-term regulation [2,4,5] creates the basis for various types of intervention – either by means of acute stimulation or inhibition of the activity of this transport system or by the induction of changes in the concentration of Na\textsuperscript{+},K\textsuperscript{+}-pumps in various tissues.

This review will primarily focus on studies of human subjects or tissues, but evidence obtained in animal experiments is included where necessary. The possible roles of Na\textsuperscript{+},K\textsuperscript{+}, the Na\textsuperscript{+},K\textsuperscript{+}-pump and endogenous Na\textsuperscript{+},K\textsuperscript{+}-pump inhibitors in hypertension have recently been reviewed in detail [6] and will therefore not be discussed here.

**Na\textsuperscript{+},K\textsuperscript{+}-pump: Structure, Function and Regulation**

The Na\textsuperscript{+},K\textsuperscript{+}-pump is situated in the plasma membrane of virtually all animal cells. It is the major mechanism for maintenance of the low intracellular Na\textsuperscript{+} and high intracellular K\textsuperscript{+} concentrations required for a multitude of cellular functions. The concentration gradients for Na\textsuperscript{+} and K\textsuperscript{+} across the plasma membrane are generated by active coupled transport of Na\textsuperscript{+} and K\textsuperscript{+}, which takes place in cycles. In each cycle one molecule of ATP is split and 3 Na\textsuperscript{+} ions are extruded in exchange for 2 K\textsuperscript{+} ions entering the cell. This implies that the Na\textsuperscript{+},K\textsuperscript{+}-pump is electrogenic, i.e. generates and maintains the membrane potential. This electrogenic effect has been documented in measurements on preparations of the human atrium [7].

The Na\textsuperscript{+},K\textsuperscript{+}-pump is a protein composed of two subunits, a catalytic \(\alpha\) subunit involved in the splitting of ATP (molecular mass approx. 112,000 Da) and a \(\beta\) subunit (approx. 35,000 Da). Like many other proteins, the catalytic subunit of the Na\textsuperscript{+},K\textsuperscript{+}-pump is expressed in various isoforms (\(\alpha_1\), \(\alpha_2\) and \(\alpha_3\)), which can be detected using specific antibodies [8]. The functional significance of variations in the proportions of \(\alpha_1\) and \(\alpha_2\) isoforms among tissues is not yet clarified. A comparison of the affinities for Na\textsuperscript{+} and K\textsuperscript{+} showed no significant difference between \(\alpha_1\) and \(\alpha_2\), indicating that the transport functions of the Na\textsuperscript{+},K\textsuperscript{+}-pump are independent of the relative abundance of the \(\alpha_1\) and \(\alpha_2\) isoforms. In contrast, the \(\alpha_3\) isoform seems to show a lower affinity for intracellular Na\textsuperscript{+}, and in cells containing mainly this version of the Na\textsuperscript{+},K\textsuperscript{+}-pump the intracellular concentration of Na\textsuperscript{+} seems to be higher [9].

The Na\textsuperscript{+},K\textsuperscript{+}-pump is markedly inhibited by digitalis glycosides which bind specifically to a defined segment of the \(\alpha\) subunit. In the majority of tissues and species, this binding takes place with high affinity in all the subunit isoforms. Because each Na\textsuperscript{+},K\textsuperscript{+}-pump molecule only binds one molecule of digitalis glycoside, \(^{3}H\)-labelled glycosides are frequently used for the quantification of Na\textsuperscript{+},K\textsuperscript{+}-pumps in homogenates, cells and tissues (see below).

In preparations of the human atrium, cardiac glycosides at concentrations down to the clinically relevant level (nanomolar) were shown to suppress the hyperpolarization induced by stimulation of the Na\textsuperscript{+},K\textsuperscript{+}-pump [7].

In a wide variety of tissues (e.g. skeletal and smooth muscle, heart, liver, kidney, intestine, exocrine glands and nervous tissue), the Na\textsuperscript{+},K\textsuperscript{+}-pump is subject to long- and short-term regulation. In Figure 1 this is exemplified for skeletal muscle, where the most detailed characterization has been carried out. A large number of studies have...
demonstrated that in this tissue, the activity and the capacity of the Na\(^+\),K\(^+\)-pump are controlled by several hormones, contractile activity, training, nutrition and electrolyte status [2,5]. As described in detail below, these regulatory factors have also been shown to exert similar actions in human subjects or tissues.

ACUTE ACTIVATION AND INHIBITION OF THE Na\(^+\),K\(^+\)-PUMP

Insulin and insulin-like growth factor I

Since the first preparations of insulin became available in the 1920s, it has been known that this hormone reduces plasma K\(^+\) levels and an overdose may result in dangerous hypokalaemia [10]. The rise in plasma insulin induced by a standard oral glucose load is sufficient to produce hypokalaemia [11]. Numerous studies on isolated preparations of skeletal muscle, heart and liver have documented that this effect can be accounted for by up to 50–100% stimulation of active Na\(^+\),K\(^+\)-transport, promoting intracellular accumulation of K\(^-\) and the clearance of extracellular K\(^+\) [2]. Furthermore, it was shown long ago that in the human forearm, physiological concentrations of insulin stimulate the uptake of K\(^+\) in skeletal muscle [12]. Conversely, acute inhibition of insulin secretion with somatostatin leads to hyperkalaemia. Hence, it is most likely that the hyperkalaemia associated with untreated diabetes can in part be related to insufficient action of insulin [13]. In hyperthyroid oriental subjects, hypokalemic attacks of paralysis may be induced by the ingestion of carbohydrates. This has been attributed to the resulting increase in plasma insulin [14]. Since hyperthyroidism leads to a marked increase in the concentration of Na\(^+\),K\(^+\)-pumps in skeletal muscle (see below), it can be assumed that insulin-induced stimulation of an enlarged pool of Na\(^+\),K\(^+\)-pumps will lead to a faster uptake of K\(^+\) into skeletal muscle and a more pronounced hypokalaemia.

It should be noted that the stimulating effect of insulin on active Na\(^+\),K\(^+\)-transport in isolated muscles is independent of changes in glucose transport. Furthermore, since the stimulation leads to a decrease in intracellular Na\(^+\), it cannot be attributed to increased Na\(^+\) influx [2,6].

Insulin-like growth factor I was recently shown to produce hypokalaemia in normal human subjects [15], an effect which may be due to the stimulating effect of insulin-like growth factor I on K\(^+\) uptake demonstrated in skeletal muscle [16].

In human subjects, insulin produces an anti-natriuretic effect [17], approximately doubling Na\(^+\) reabsorption [18]. This is also seen at the physiological concentrations reached during an oral glucose load [10]. These findings confirm the early observation of a direct inhibitory effect of insulin on net renal Na\(^+\) excretion in animals. Studies on isolated tubules and collecting ducts from animals demonstrate that this can be related to a stimulatory effect of insulin on active Na\(^+\),K\(^+\)-transport across the basolateral membrane of the tubular cells, favouring the tubular reabsorption of Na\(^+\) [19]. Since the Na\(^+\),K\(^+\)-ATPase in kidney is predominantly the \(\alpha_2\) isoform and in skeletal muscle is the \(\alpha_1\) isoform, these results strongly suggest that both isoforms are sensitive to insulin.

In skeletal muscle, insulin seems to induce an increase in the affinity of the Na\(^+\),K\(^+\)-pump for Na\(^+\), allowing the maintenance of a lower intracellular Na\(^+\). This could not be attributed to translocation of Na\(^+\),K\(^+\)-pumps from intracellular pools to the plasma membrane [2]. Studies on primary cultures of rat skeletal muscle indicate that the stimulating effect of insulin on the Na\(^+\),K\(^+\)-pump is mediated by activation of protein kinase C.

Catecholamines, synthetic β-agonists and theophylline

Early studies showed that the injection of adrenaline or noradrenaline induces a transient hyperkalaemia, followed by hypokalaemia. The hyperkalaemia was found to reflect increased release of K\(^+\) from the liver. The subsequent hypokalaemia was found to result from stimulation of β\(_2\) adrenoceptors [20].

Experiments with isolated rat muscles showed that catecholamines induce an even more marked stimulation of active Na\(^+\),K\(^+\)-transport than that elicited by insulin [2,20], indicating that the hypokalaemic action of adrenaline and noradrenaline is the result of Na\(^+\),K\(^+\)-pump activation. This effect was found to be mediated via β\(_2\)-adrenoceptors and adenylate cyclase activation [21], and the diagram shown in Figure 2 was developed to illustrate the mechanisms involved. The sequence of events and its implications for muscle function has been confirmed [5,22]. It is important to note that 3',5'-cAMP elicits a decrease in intracellular Na\(^+\), indicating that stimulation of the Na\(^+\),K\(^+\)-pump is not due to increased Na\(^+\) influx. Similar effects were observed in cultured cells from shark rectal glands [23] and isolated cardiomyocytes [24], indicating that it is a general phenomenon.

Since the Na\(^+\),K\(^+\)-pump extrudes 3 Na\(^+\) ions from the cell in exchange for 2 K\(^+\) ions, stimulation of the pump leads to hyperpolarization. This in turn could be expected to elicit hypokalaemia and recovery of excitability in muscles paralysed by elevation of extracellular K\(^+\). On the basis of these observations, the inhalation of salbutamol, a synthetic β\(_2\)-adrenoceptor agonist, was introduced for the treatment of hyperkalaemic periodic paralysis and shown to be efficient in the suppression of hyperkalaemia as well as the paralysis associated with the periodic attacks symptomatic of this disease [20,25,26]. Hyperkalaemic periodic paralysis arises from a defective voltage-activated Na\(^+\)-channel in skeletal muscle. Since this channel shows prolonged open-time, allowing ex-
leads to increased affinity for intracellular Na\(^+\) sarcolemma. Due to the electrogenic nature of the coupled active Na\(^+\) transport, the early increase in Na\(^+\) efflux and a subsequent maintainance of a steeper Na\(^+\) gradient across the sarcolemma, the increase in Na\(^+\) efflux leads to hyperpolarization, increased intracellular K\(^-\)/Na\(^+\) ratio, decreased extracellular K\(^-\) and hypokalaemia. These changes will influence excitability and contractile performance.

In haemodialysis patients, salbutamol was found to suppress the hyperkalaemia arising from renal failure [34]. Salbutamol and insulin plus glucose were found to be equally efficacious in lowering plasma K\(^+\). When combined, the hypokalaemic effects of the two agents were additive [3,35]. The advantage of \(\beta_2\)-agonists is that, unlike insulin, they do not elicit hypoglycaemia. Furthermore, they may be given by inhalation of nebulized solutions, allowing rapid treatment of hyperkalaemia ([36]; for review, see [3]). A low, in children with renal failure, inhalation of a nebulized \(\beta_2\)-agonist has been found to be an efficient treatment of hyperkalaemia [37].

The hypokalaemic effect of \(\beta_2\)-agonists has been noted as an undesirable side-effect of the treatment of bronchial asthma [38] and preterm labour [39]. A study of self-poisoning with salbutamol showed a close correlation between plasma salbutamol and hypokalaemia [40]. Hypokalaemia induced by salbutamol poisoning can be alleviated by propranolol [41].

Theophylline intoxication, which is a frequent complication in the treatment of asthma and chronic obstructive pulmonary diseases, has been shown repeatedly to be associated with hypokalaemia. This seems to be the result of theophylline-induced elevation of plasma catecholamines [42]. The hypokalaemic effect of the catecholamines, which is mediated by 3',5'-cAMP, may in turn be potentiated since theophylline inhibits the phosphodiesterase which degrades 3',5'-cAMP [27], indicating that this second messenger is also mediating the effect of the \(\beta_2\)-agonist on K\(^+\) uptake in vivo.

Studies on isolated rat skeletal muscle have demonstrated that stimulation of active Na\(^+\),K\(^-\)-transport with catecholamines, insulin or other hormones can alleviate the inhibitory effect of high extracellular K\(^+\) on excitability and contractile performance [28]. This effect can be attributed to a more efficient clearance of extracellular K\(^+\), and hyperpolarization of the sarcolemma. In human subjects catecholamines also induce hypokalaemia, an effect shown to be mediated via \(\beta_2\)-adrenoceptors [29,30]. In isolated human intercostal muscle fibres, adrenaline was shown to decrease intracellular Na\(^+\) and to produce hyperpolarization [31]. More recently, a \(\beta_2\)-agonist was shown to increase the net uptake of K\(^+\) in skeletal muscle in the human forearm [32]. During exercise, the plasma concentration of catecholamines increases in proportion to work intensity, reaching very high levels in some cases. This will stimulate the clearance of K\(^+\) from plasma and reduce the magnitude of exercise-induced hyperkalaemia. Since hyperkalaemia inhibits cardiac function and eventually may lead to cardiac arrest, the mobilization of catecholamines has survival value. Moreover, the catecholamines were shown to protect the heart against the inhibitory effects of high extracellular K\(^+\) [33].
seems likely, therefore, as noted by Morgan and Young [47], that the hypokalaemia seen after coronary occlusion could be elicited by the stimulating effect of the catecholamines on net uptake of K⁺ into skeletal muscle. It has been proposed repeatedly that hypokalaemia after coronary occlusion is important for the tendency to develop fibrillation [47,48]. Indeed, after acute myocardial infarction, hypokalaemia was shown to be associated with increased risk of cardiac arrhythmias and death [49,50]. Moreover, in decompensated heart failure plasma catecholamines increase and, as noted by Francis [51], the ensuing hypokalaemia may increase the risk of arrhythmias. The acute hypokalaemia associated with delirium tremens has also been related to the concomitant increase in plasma catecholamines and the ensuing stimulation of K⁺ uptake into skeletal muscle [52].

Several studies have demonstrated that treatment with non-selective β-blockers leads to hyperkalaemia, particularly during exercise [53-55]. This is the result of a blockade of the stimulating effect of endogenous catecholamines on the Na⁺,K⁺-pump-mediated uptake of K⁺ into skeletal muscle. The elevation of extracellular K⁺ may interfere with muscle excitability [5,28] and account, in part, for the fatigue associated with non-selective β-blocker treatment.

In canine heart, catecholamines and 3',5'-cAMP have been shown to stimulate active Na⁺,K⁺-transport [56]. Studies on pigs in vivo show that β₁-adrenergic stimulation increases Na⁺,K⁺-pump-mediated uptake of K⁺ in the myocardium by up to 2.5-fold [57]. This effect has been attributed to adenylate cyclase stimulation and could also be elicited by stimulation of cardiac sympathetic nerves.

**Digitalis glycosides**

A cute inhibition of the Na⁺,K⁺-pump decreases the net cellular uptake of K⁺ and leads to hyperkalaemia. Indeed, intoxication with cardiac glycosides has been found repeatedly to be associated with a marked increase in serum K⁺ (up to 13.5 mM) [58-60], which is positively correlated with mortality [59]. Conversely, treatment of digoxin poisoning with digoxin-specific Fab antibody fragments has been shown to normalize serum K⁺ by removing digoxin from plasma [60]. In patients with congestive heart failure, digitalization increases exercise-induced hyperkalaemia. This was shown to be associated with, and probably caused by, a pronounced rise (138%) in the net loss of K⁺ from working muscles [61]. In human subjects, standard digitalization with a dose of digoxin sufficient to give a plasma concentration of 1.2 nM was found to induce significant decreases in whole-body K⁺ and muscle K⁺ content of 8 and 6% respectively [62]. Intravenous infusion of ouabain in a therapeutic dose was found to induce a significant net loss of K⁺ from the human myocardium. This was seen within a few minutes and was associated with significant increases in systolic ejection rate index and left ventricular dp/dt [63]. More recently, it was demonstrated that in atrial tissue obtained from patients on standard digoxin treatment, the electrogenic effect of the Na⁺,K⁺-pump was significantly reduced [64].

For at least 200 years digitalis glycosides have been widely used for the treatment of cardiac insufficiency. In spite of several advances in the treatment of cardiovascular disorders, digoxin has remained one of the most commonly prescribed drugs in the U.S.A. and several other countries. There is considerable evidence that the inotropic effect of cardiac glycosides is primarily the result of inhibition of the Na⁺,K⁺-pump [65,66].

One early study showed that in patients receiving standard treatment with digoxin, the binding of digoxin to atrial myocardium was a linear function of serum digoxin and corresponded to around 70 pmol/g wet wt. [67]. More recently, it was found that in samples of the left ventricle obtained from digitalized subjects, the digitalis receptors occupied by digoxin corresponded to around 150 pmol/g wet wt., or 24% of the total binding capacity for H-ouabain [68]. This partial inhibition of the Na⁺,K⁺-pumps leads to a rise in intracellular Na⁺ which is sufficient to reduce Ca²⁺ efflux via the Na⁺/ Ca²⁺-exchange system. The resulting increase in the net uptake of calcium increases cytoplasmic Ca²⁺, favouring the activation of the contractile filaments during excitation. As pointed out by Levi et al. [66], an elevation of cytoplasmic Ca²⁺ is likely to increase the accumulation of calcium in the sarcoplasmic reticulum. This will give rise to a larger increase in the Ca²⁺ released from the sarcoplasmic reticulum during each heartbeat (Figure 3).

Several placebo-controlled studies have documented that in congestive heart failure, digoxin treatment induces symptomatic relief, haemodynamic improvement and gain in exercise performance (for review, see [69]). In patients with moderate heart failure, a low dose of digoxin (0.125 mg daily for 2 weeks) induced a significant increase in ventricular performance [70]. Conversely, in patients treated with digoxin for mild or moderate heart failure, the withdrawal of digoxin led to a highly significant reduction in left ventricular ejection fraction and exercise capacity [71].

Moreover, in a randomized double-blind clinical trial comprising 6800 patients with a left ventricular ejection fraction of 0.45 or less, digoxin treatment produced no change in overall mortality, but a highly significant decrease in the rate of hospitalization [72].

**Endogenous Na⁺,K⁺-pump inhibitors**

It has been suggested repeatedly that mammalian tissues contain substances capable of inhibiting the Na⁺,K⁺-pump via the digitalis binding site. Due to the high affinity of this site for digitalis it has been compared to a receptor with regulatory functions. This analogy is
The binding of glycoside inhibits the Na\(^{+}\),K\(^{+}\)-pump, reduces pumped Na\(^{+}\) extrusion from the cell and leads to a rise of intracellular Na\(^{+}\) ([Na\(^{+}\)_i], shown as the activity of Na\(^{+}\_i\) (aiNa\(^{+}\))]. This reduces Ca\(^{2+}\) extrusion from (or increases Ca\(^{2+}\) entry into) the cell via the Na\(^{+}\)/Ca\(^{2+}\) exchanger, and causes a rise of intracellular Ca\(^{2+}\) and cellular calcium content. By increasing sarcoplasmic reticulum (SR) Ca\(^{2+}\) release and generating a larger Ca\(^{2+}\) transient, this results in an increase of the force of contraction of cardiac muscle.

Reproduced from Levi et al. [66] with permission.

misleading, however, because it implies that the numerous other toxic substances with high binding affinity to, for example, ion channels might also be expected to play a role as regulatory endogenous factors. Furthermore, in spite of decades of efforts in numerous laboratories, no Na\(^{+}\),K\(^{+}\)-pump inhibitor has been isolated, purified and chemically identified. The evidence obtained to date is indirect and often based on studies with antibodies and incompletely purified preparations (for a detailed review, see [73]).

It was recently proposed that ouabain secreted from the adrenal glands is present in human plasma and potentially of importance for the development of hypertension. Later studies, however, have not been able to confirm these observations [74]. Besides, a quantitative analysis revealed that with the proposed rate of ouabain secretion from the adrenals, it would take 26 days to occupy only 1% of the ouabain binding sites available in skeletal muscle [75]. Such a slow inhibition of this minute fraction of the Na\(^{+}\),K\(^{+}\)-pumps cannot be expected to serve any regulatory role. Finally, the metabolic pathways required for the synthesis of ouabain are specific to certain plant cells, and have never been detected in mammalian cells [73]. In conclusion, endogenous Na\(^{+}\),K\(^{+}\)-pump inhibitors have not yet been adequately identified and their clinical significance is still elusive.

QUANTIFICATION OF Na\(^{+}\),K\(^{+}\)-ATPASE IN HUMAN TISSUES

As shown in Table 1, the concentration of Na\(^{+}\),K\(^{+}\)-pumps in human tissues varies over an enormous range with around a 160 000-fold difference between the lowest (in erythrocytes) and the highest concentration (in brain cortex). This marked variation reflects differences in functional requirements. Obviously, the activity of excitable tissues such as brain cortex or the heart is associated with much larger passive fluxes of Na\(^{+}\) and K\(^{+}\) than those taking place in erythrocytes and therefore gives rise to a need for a much larger Na\(^{+}\),K\(^{+}\)-pump capacity for restoring normal Na\(^{+}\),K\(^{+}\) contents. For the same reason, measurement of Na\(^{+}\),K\(^{+}\)-pumps in one cell type (erythrocytes or lymphocytes) is not likely to yield much information about the concentration of Na\(^{+}\),K\(^{+}\)-pumps and its regulation in other cell types like those of the heart, skeletal or vascular smooth muscle.

The first quantitative measurements of Na\(^{+}\),K\(^{+}\)-pumps in human cells were performed on erythrocytes. This was done by determining the total binding capacity for \(^{3}H\)-ouabain and values, from a wide range of studies, are around 0.07 pmol/ml cells (Table 1). Using similar procedures, it was found that lymphocytes, monocytes and leucocytes contain appreciably higher concentrations
of Na⁺,K⁺-pumps (2–4 pmol/ml cells). In such studies, the ³H-ouabain binding sites on the outer surface of the plasma membrane are readily accessible to the ³H-ouabain present in the incubation medium. Control measurements of Na⁺,K⁺-ATPase activity or maximum transport capacity enable confirmation of the values obtained by ⁴⁰Rb uptake and ³¹P efflux in rat soleus muscle showed that the Na⁺,K⁺-pumps measured with the ³H-ouabain binding assay are all functional, i.e. able to perform active Na⁺,K⁺-transport [94]. In other words, the values obtained for maximum transport capacity confirmed the theoretically predicted capacity for active transport of Na⁺ and K⁺.

In human skeletal muscle, ouabain-sensitive Na⁺,K⁺-ATPase has been detected in membranes isolated from homogenates by differential centrifugation [95]. More recently, studies on membranes obtained from human soleus muscle showed that α₁ and α₂ isoforms of the Na⁺,K⁺-ATPase are expressed [96].

With the observation that vanadate (VO₄³⁻) facilitates the binding of ³H-ouabain to biopsies of skeletal muscle, it became possible to measure the total binding capacity even in the small tissue specimens obtained using a biopsy needle [97]. Animal experiments show that this method gives the same values for Na⁺,K⁺-pumps in human cells and tissues determined from the biopsy needle [97]. Animal experiments show that this method gives the same values for Na⁺,K⁺-pumps in human cells and tissues determined from the biopsy needle [97]. Animal experiments show that this method gives the same values for Na⁺,K⁺-pumps in human cells and tissues determined from the biopsy needle [97]. Animal experiments show that this method gives the same values for Na⁺,K⁺-pumps in human cells and tissues determined from the biopsy needle [97].

Early studies were naturally designed to identify the Na⁺,K⁺-ATPase and to characterize its structure and function in various tissues. Therefore purification was considered more important than completeness of recovery. This led to the widespread use of purification procedures, where only a minor part of the Na⁺,K⁺-ATPase present in the tissue was recovered for characterization. This, however, is not true for all tissues. For example, in skeletal muscle, only 5% of the total Na⁺,K⁺-ATPase present in the tissue was recovered during the purification process. When it comes to studies of regulation, however, quantification of the total population of enzyme molecules is essential. Studies on isolated skeletal muscle preparations have shown that this can be achieved by determination of the binding capacity for Na⁺,K⁺-ATPase [93]. Measurements of Na⁺,K⁺-ATPase activity closely associated with the Na⁺,K⁺-ATPase, have provided quantitative confirmation of the results obtained in ³H-ouabain binding studies. Furthermore, measurements of the maximum rate of ⁴⁰Rb uptake and ⁴⁰N efflux in rat soleus muscle showed that the Na⁺,K⁺-pumps measured with the ³H-ouabain binding assay are all functional, i.e. able to perform active Na⁺,K⁺-transport [94]. In other words, the values obtained for maximum transport capacity confirmed the theoretically predicted capacity for active transport of Na⁺ and K⁺.

Clinical relevance of the Na⁺,K⁺-pump

Table 1 Concentration of Na⁺,K⁺-pumps in human cells and tissues determined from the ³H-ouabain binding capacity

<table>
<thead>
<tr>
<th>Tissue or cell type</th>
<th>Concentration of Na⁺,K⁺-pumps</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Erythrocytes</td>
<td>0.071 ± 0.0014 (pmol/ml)</td>
<td>[76]</td>
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<tr>
<td>Lymphocytes</td>
<td>3.5 ± 0.8 (pmol/ml)</td>
<td>[77]</td>
</tr>
<tr>
<td>Monocytes</td>
<td>4.1 (pmol/ml)</td>
<td>[77]</td>
</tr>
<tr>
<td>Leucocytes</td>
<td>1.9 ± 0.5 (pmol/ml)</td>
<td>[77]</td>
</tr>
<tr>
<td>Uterine smooth muscle</td>
<td>82 ± 9 (pmol/g)</td>
<td>[78]</td>
</tr>
<tr>
<td>Skeletal muscle, vastus lateralis</td>
<td>278 ± 15 (pmol/g)</td>
<td>[79]</td>
</tr>
<tr>
<td>Skeletal muscle, vastus lateralis</td>
<td>254 ± 8 (pmol/g)</td>
<td>[80]</td>
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<td>258 ± 16 (pmol/g)</td>
<td>[81]</td>
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<td>276 ± 19 (pmol/g)</td>
<td>[82]</td>
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<td>Skeletal muscle, vastus lateralis</td>
<td>360 ± 70 (pmol/g)</td>
<td>[83]</td>
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<td>333 ± 19 (pmol/g)</td>
<td>[84]</td>
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<td>339 ± 16 (pmol/g)</td>
<td>[85]</td>
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<td>Skeletal muscle, vastus lateralis</td>
<td>223 ± 13 (pmol/g)</td>
<td>[86]</td>
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<td>306 ± 27 (pmol/g)</td>
<td>[87]</td>
</tr>
<tr>
<td>Skeletal muscle, vastus lateralis</td>
<td>258 ± 13 (pmol/g)</td>
<td>[88]</td>
</tr>
<tr>
<td>Heart, endomyocardium</td>
<td>559 ± 62 (pmol/g)</td>
<td>[89]</td>
</tr>
<tr>
<td>Myocardium, left ventricle</td>
<td>505 ± 41 (pmol/g)</td>
<td>[90]</td>
</tr>
<tr>
<td>Myocardium, left ventricle</td>
<td>760 ± 58 (pmol/g)</td>
<td>[91]</td>
</tr>
<tr>
<td>Myocardium homogenate</td>
<td>507 ± 21 (pmol/g)</td>
<td>[92]</td>
</tr>
<tr>
<td>Brain cortex</td>
<td>11,400 ± 1000 (pmol/g)</td>
<td>[93]</td>
</tr>
</tbody>
</table>
ouabain. The total binding capacity can be obtained by counting the \( ^3\)H-activity of a trichloroacetic acid extract of the tissue. On the basis of the specific activity of the \( ^3\)H-ouabain in the incubation medium, the concentration of \( ^3\)H-ouabain binding sites can be expressed in pmoles per g of tissue (wet wt.). Since the binding is stoichiometric, this gives a value for the concentration of \( \mathrm{Na}^+\),\( \mathrm{K}^-\)-pumps in the tissue. It is important to note that contrary to what is observed in the rat, the \( \alpha_1\) isoform of human \( \mathrm{Na}^+\),\( \mathrm{K}^-\)-ATPase has a high affinity for ouabain, allowing the detection of both major isoforms (\( \alpha_1\) and \( \alpha_2\)) present in human skeletal muscle and heart [91]. The vanadate-facilitated \( ^3\)H-ouabain binding assay can readily be established in most standard laboratories. Consistent results have been obtained by about a dozen different research groups (Table 1).

One of the major advantages of the vanadate-facilitated binding assay is that it allows quantification of \( \mathrm{Na}^+\),\( \mathrm{K}^-\)-pumps to be performed on frozen biopsies [79]. Thus needle biopsies can be stored for at least 1 year in a deep-freezer or sent by air mail in dry-ice without any loss of \( ^3\)H-ouabain binding capacity. This greatly facilitates comparative or longitudinal studies.

In human skeletal muscle, a \( ^3\)H-ouabain binding capacity of around 275 pmol/g wet wt. is generally obtained [55,79–82,84–87,97–99]. In samples of human intercostal, rectus abdominis, rectus femoris, vastus lateralis and soleus muscle, values range from 215 to 310 pmol/g wet wt. [79,80], and seem independent of muscle-specific differences in fibre type [80]. These results could be compared with the uptake of digoxin in skeletal muscle under saturating conditions. In a case of digoxin intoxication [58], where it could be assumed that the binding of digoxin to skeletal muscle cells had reached saturation, muscle biopsies were found to contain 150 ng of digoxin/g wet wt., corresponding to around 200 pmol/g wet wt., which is not far below the values for total \( ^3\)H-ouabain binding capacity.

From these measurements it can be calculated that in a human subject weighing 70 kg, the pool of skeletal muscles (40% of body weight) contains around 8000 nmol of \( ^3\)H-ouabain binding sites or \( \mathrm{Na}^+\),\( \mathrm{K}^-\)-ATPase. After the administration of 1000 nmol of \( ^3\)H-digoxin, 480 nmol was found to be distributed to the skeletal muscles and only 6 and 24 nmol to the heart and extracellular volume respectively [100]. This indicates that modest changes in the large amount of digoxin bound to muscles may elicit marked variations in plasma digoxin.

The vanadate-facilitated binding assay for \( ^3\)H-ouabain binding has also been adapted for biopsies of the human myocardium. A series of studies have given values from the left myocardium of 500–760 pmol/g wet wt. (Table 1). These values are in good agreement with that reached (507 ± 21 pmol/g wet wt.) by measuring the \( ^3\)H-ouabain binding to total membrane sediments obtained by ultra-

centrifugation of homogenized human myocardium [91]. In tissue homogenates prepared from the atria, ventricles and septa of hearts of human organ donors, immunoblot assays established the expression of \( \alpha_1\), \( \alpha_2\) and \( \alpha_3\) isoforms. In keeping with earlier studies on animals, the concentration of \( \mathrm{Na}^+\),\( \mathrm{K}^-\)-ATPase was found to be significantly lower in the atria than in the septum and the ventricles [101].

In specimens of non-pregnant human myometrium, obtained during hysterectomy, a\( ^3\)H-ouabain binding site concentration of 83 pmol/g wet wt. was measured [79]. In samples of the pregnant myometrium obtained during caesarian section almost the same value (72 pmol/g wet wt.) was determined.

In tissue specimens obtained from the human brain cortex, measurements of the total binding capacity for \( ^3\)H-ouabain have shown values of around 11 000 pmol/g wet wt. [92]. This value is in good agreement with that obtained by measurement of total activity of \( \mathrm{Na}^+\),\( \mathrm{K}^-\)-ATPase in crude homogenates of bovine brain cortex.

## Physiological and Pathophysiological Changes in Na\(^+\),K\(^-\)ATPase Concentration in Human Tissues

It is well established that in several tissues, the concentration of \( \mathrm{Na}^+\),\( \mathrm{K}^-\)-ATPase is subject to regulation by a wide variety of factors [2,4,5]. This phenomenon has been termed long-term regulation and is often the result of changes in the rate of synthesis of \( \mathrm{Na}^+\),\( \mathrm{K}^-\)-ATPase. The most detailed characterization of the normal and pathological changes in the concentration of \( \mathrm{Na}^+\),\( \mathrm{K}^-\)-pumps has been performed in skeletal muscle, and Figure 4 illustrates the relative changes in the values obtained. The possible functional significance of these changes is discussed below.

### Thyroid hormones

Several animal studies have shown that the major single endocrine factor controlling the synthesis of \( \mathrm{Na}^+\),\( \mathrm{K}^-\)-ATPase is thyroid hormone. Thyroid hormones stimulate the synthesis of \( \mathrm{Na}^+\),\( \mathrm{K}^-\)-pumps, and this effect seems to be secondary to an early increase in the passive leaks to \( \mathrm{Na}^+\) and \( \mathrm{K}^-\). In human subjects, the concentration of \( ^3\)H-ouabain binding sites in vastus lateralis muscle increases with thyroid status from 100 to 600 pmol/g wet wt., and this increase is closely correlated to the free \( T_4\) index. After standard treatment of the thyroid disorders, the concentration of \( \mathrm{Na}^+\),\( \mathrm{K}^-\)-pumps returns to the control range [97].

A minal studies show that the thyroid-hormone-induced increase in \( \mathrm{Na}^+\),\( \mathrm{K}^-\)-pumps is seen throughout
Clinical relevance of the Na$^+\cdot$K$^+$-pump

The Na$^+\cdot$K$^+$-pump is involved in maintaining the electrochemical gradient across the plasma membrane of skeletal muscle cells. This gradient is essential for muscle contraction. The Na$^+\cdot$K$^+$-pump also plays a role in regulating intracellular potassium concentration, which is critical for normal muscle function. Conditions such as hyperthyroidism, hypothyroidism, and glucocorticoid treatment can affect the expression and activity of the Na$^+\cdot$K$^+$-pump, leading to changes in muscle function and overall health.

**Figure 4** Relative changes in the concentration of Na$^+\cdot$K$^+$-pumps in human skeletal muscle associated with some physiological and pathophysiological conditions

All values are based on the determination of the total concentration of $^3$H-ouabain binding sites in needle biopsies of the vastus lateralis muscle. Each condition and the reference number are given on the left side of the diagram, and the percentage increase or decrease is expressed in relation to values obtained in parallel measurements on control subjects.

The pool of skeletal muscles [2]. It would be expected, therefore, that thyroid-hormone-induced upregulation of the concentration of Na$^+\cdot$K$^+$-pumps in skeletal muscle improves the clearance of K$^+$ from plasma. Indeed, after infusion of K$^+$ to nephrectomized hyperthyroid rats, the increase in plasma K$^+$ is smaller than in the euthyroid controls [105]. Conversely, hypothyroid rats show more pronounced hyperkalaemia when exposed to the same intravenous load.

Since skeletal muscles contain a large fraction of the digitalis binding sites, it can be expected that upregulation favours the clearance of digitalis from plasma, reducing the rise in the plasma concentration after digitalization. Indeed, after the administration of the same dose of $^3$H-digoxin, serum levels were highest in hypothyroid, intermediate in euthyroid and lowest in hyperthyroid subjects [106]. In keeping with this, hyperthyroid patients show a better tolerance to digitalis, and conversely, hypothyroid patients are more likely to develop digitalis toxicity [107].

Because active Na$^+\cdot$K$^+$-transport is an energy-requiring process, it has been proposed that the upregulation of Na$^+\cdot$K$^+$-pumps induced by thyroid hormones could account for their thermogenic action. However, detailed analysis of Na$^+\cdot$K$^+$-transport rates, Na$^+\cdot$K$^+$-pump-related heat production and oxygen consumption in rat skeletal muscle has shown that the Na$^+\cdot$K$^+$-pump-related energy utilization can at most account for 15% of the thyroid-hormone-induced thermogenesis (for details, see [108]).

In hyperthyroid subjects it has been shown that the concentration of $^3$H-ouabain binding sites is reduced by about 25% in lymphocytes, whereas no significant change could be detected in hyperthyroid subjects [109]. In leucocytes from hyperthyroid subjects, the concentration of $^3$H-ouabain binding sites, Na$^+\cdot$K$^+$-ATPase activity and active Na$^+\cdot$K$^+$-transport showed modest (15–33%), albeit significant increases [110]. In platelets from thyrotoxic subjects, the concentration of Na$^+\cdot$K$^+$-ATPase was increased by 89%, and in patients suffering from thyrotoxic hypokalaemic periodic paralysis a significant further increase (179%) was observed [111]. If this extra upregulation of Na$^+\cdot$K$^+$-pumps also takes place in skeletal muscle, it would increase the capacity for clearance of K$^+$ from the plasma considerably and might explain the development of attacks of hypokalaemic paralysis seen in these patients.

**Glucocorticoids**

In patients receiving intensive treatment with dexamethasone, the concentration of $^3$H-ouabain binding sites in vastus lateralis muscle biopsies was found to be increased by 61% [102]. Parallel studies in rats showed that dexamethasone infused via osmotic minipumps induces a similar upregulation of the $^3$H-ouabain binding site concentration in skeletal muscle [112].

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Training and inactivity
Several animal studies have shown that training leads to upregulation of the concentration of $\text{Na}^+,\text{K}^+$-pumps in skeletal muscle [2]. This might be elicited by the increased passive fluxes of $\text{Na}^+$ and $\text{K}^+$ associated with the action potentials acting as a drive on the synthesis of $\text{Na}^+,\text{K}^+$-pumps – analogous to the upregulation elicited by thyroid hormones. In human subjects who had been physically very active for several years, the concentration of $\text{Na}^+,\text{K}^+$-pumps ($^3\text{H}$-ouabain binding sites) was found to be 30–40% higher than in age-matched controls [82].

Longitudinal studies show that within weeks various types of training produce an increase of 13–16% in the concentration of $^3\text{H}$-ouabain binding sites in human skeletal muscle [84,85,99]. After 5 months intense training, elite cross-country skiers show a 16% increase in the concentration of $^3\text{H}$-ouabain binding sites in biopsies of vastus lateralis was found to be significant correlation ($P < 0.003$) or during skiing ($P < 0.01$) [113]. Conversely, localized muscle inactivity is associated with a downregulation of 27% [87].

Several studies have shown that after training, exercise-induced hyperkalaemia is blunted [84,114], possibly as a result of an increased capacity for active $\text{Na}^+,\text{K}^+$-transport and ensuing improvement of the intracellular accumulation of $\text{K}^+$ in skeletal muscle. Since the exercise-induced rise in extracellular $\text{K}^+$ may impair excitability of the muscle cells [5], a more efficient clearance of $\text{K}^+$ could be expected to favour contractile performance. In one study, the concentration of $\text{Na}^+,\text{K}^+$-pumps in vastus lateralis muscle showed significant correlation ($P < 0.01$) with maximal isometric strength [82]. In two other studies, no correlation could be detected between $\text{Na}^+,\text{K}^+$-pump concentration and physical performance [84,99]. More recently, however, $^3\text{H}$-ouabain binding capacity in biopsies of vastus lateralis was found to be significantly correlated to performance during a treadmill test ($P < 0.003$) or during skiing ($P < 0.001$) [113].

In patients with heart failure, the concentration of $^3\text{H}$-ouabain binding sites in skeletal muscle is reduced by about 25%, possibly as a result of limited physical activity [115]. In such patients, exercise-induced hyperkalaemia is more pronounced than in subjects with normal cardiac function, probably due to the decreased capacity for $\text{Na}^+,\text{K}^+$-pump-mediated accumulation of $\text{K}^+$ in skeletal muscle [114]. In rats with chronic heart failure, the $^3\text{H}$-ouabain binding site concentration in skeletal muscle was recently found to be reduced by 18–22% [116]. It remains to be explored whether this downregulation of $\text{Na}^+,\text{K}^+$-pump capacity contributes to the reduced physical performance and fatigue often associated with cardiac insufficiency.

$\text{K}^+$ deficiency
Studies on rats, mice and guinea pigs have shown that $\text{K}^+$ deficiency leads to a downregulation of the concentration of $\text{Na}^+,\text{K}^+$-pumps in skeletal muscle ([117]; for reviews, see [118] and [119]). A comparison of skeletal muscles from several species with varying degrees of $\text{K}^+$ deficiency shows that the concentration of $^3\text{H}$-ouabain binding sites is correlated with the tissue concentration of $\text{K}^+$ [118]. In human subjects, $\text{K}^+$ deficiency induced by treatment with diuretics was shown to be associated with a decrease in the concentration of $^3\text{H}$-ouabain binding sites in skeletal muscle [84,98]. This loss of $\text{Na}^+,\text{K}^+$-pumps could be corrected by restoring normal $\text{K}^+$ content in skeletal muscles [98].

In rat soleus muscle, where the concentration of $\text{Na}^+,\text{K}^+$-pumps had been reduced by 60% by prior $\text{K}^+$ depletion of the animals, the rate of force decline seen during high-frequency stimulation was increased by 111% [120]. A comparable increase (88%) in the rate of force decline was observed after the induction of a similar decrease (53%) in the concentration of functional $\text{Na}^+,\text{K}^+$-pumps by preincubation with ouabain. These observations offer an explanation for the fatigue experienced after digitals intoxication as well as during $\text{K}^+$ deficiency caused by chronic treatment with diuretics.

Another implication of a reduction in the concentration of $\text{Na}^+,\text{K}^+$-pumps in skeletal muscle is that the muscular pool of digitals receptors is decreased. Thus, the administration of digitalis glycosides, a larger fraction of the dose given will be available for distribution in the extracellular volume, leading to a higher plasma concentration. In keeping with this it is a well-known clinical experience that $\text{K}^+$-deficient patients are more sensitive to digitization [107]. This relation has been explored in experiments with $\text{K}^+$-deficient rats, where the concentration of $\text{Na}^+,\text{K}^+$-pumps in skeletal muscle was found to be reduced by 63%. In these animals injection of $^3\text{H}$-ouabain was found to produce a 77% greater rise in plasma $^3\text{H}$-ouabain than in controls given the same dose of $^3\text{H}$-ouabain per kg of body weight [121]. This illustrates the major influence of the large muscular pool of digitals glycoside receptors on the availability of digitals glycosides in plasma and offers an explanation for the increased sensitivity to digitals in patients suffering from $\text{K}^+$ deficiency. It should be added that since $\text{K}^+$ interferes with the binding of digitals to the $\text{Na}^+,\text{K}^+$-pump, the hypokalaemia associated with $\text{K}^+$ deficiency favours the binding of digitals to all cells and increases the risk of intoxication. Conversely, hyperkalaemia reduces the risk of digitalis intoxication.

Diabetes
In rats, streptozotocin-induced diabetes induces a 24–48% decrease in the concentration of $^3\text{H}$-ouabain binding sites in skeletal muscle [122]. This anomaly is completely restored by 4 weeks of insulin treatment. After prolonged (8 weeks) treatment of diabetic rats with insulin, the concentration of $^3\text{H}$-ouabain binding sites

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increased to 23% above the control level [86]. Thus, insulin seems to stimulate the synthesis of Na\(^{+},K\)\(^{-}\)-pumps in skeletal muscle. This offers an explanation for the observation that in insulin-treated diabetic subjects, where plasma insulin is elevated, the concentration of \(^{3}H\)-ouabain binding sites in vastus lateralis muscle is increased by 17–22% and shows a positive linear correlation to the concentration of insulin in plasma [86]. In keeping with this, insulin-treated diabetic patients show an improved capacity for extrarenal clearance of an acute K\(^{+}\) load [123].

**Cardiac insufficiency**

In patients with dilated cardiomyopathy, the concentration of \(^{3}H\)-ouabain binding sites was measured in endomyocardial biopsies obtained from the left myocardium and withdrawn by catheter via the femoral artery. The values obtained were considerably reduced and distributed over a wide range, depending on the degree of functional deficit [88,89,115]. In comparison to subjects with normal left ventricular function, an average decrease of 41% was found. A close correlation with left ventricular ejection fraction was observed, indicating that contractile performance of the myocardium decreases in proportion to the loss of Na\(^{+},K\)\(^{-}\)-pumps. Later studies have shown that in myocardial tissue from patients with heart failure, the total binding capacity for \(^{3}H\)-ouabain is reduced by 19% [90], 36% [68] or 42% [91]. It is important to note that the relative decrease is almost the same whether expressed per g wet wt. (42%), per g dry wt. (44%), per g DNA (37%), per g protein (36%) or per g myosin (35%) [91]. Moreover, the Na\(^{+}\),K\(-\)ATPase activity in the failing myocardium shows a similar relative reduction (40-47%). In one study, heart failure was not associated with any significant change in the relative abundance of the messenger RNA for the \(\alpha_{1}\), \(\alpha_{2}\), and \(\alpha_{3}\) isoforms of Na\(^{+}\),K\(-\)ATPase [90]. A further study showed some change, but the variation was too large to allow any definitive conclusion [91]. Post-mortem measurements of \(^{3}H\)-ouabain binding capacity in samples of the left ventricle of hypertrophied hearts showed a 25% lower (P < 0.02) value than in controls [124]. A recent study on tissues removed during aortic valve replacement showed that in the myocardium of patients with aortic stenosis, regurgitation and a combination thereof, the concentration of \(^{3}H\)-ouabain binding sites was reduced by 56, 46 and 60% respectively [125]. In all instances, this downregulation was associated with myocardial hypertrophy.

Although the downregulation of Na\(^{+}\),K\(-\)ATPase activity in failing hearts is well documented and seems related to decreased contractile performance, it remains to be determined whether the loss of Na\(^{+}\),K\(-\)-pumps is the primary cause of cardiac insufficiency. It is paradoxical and unexplained that whereas a reduction in Na\(^{+}\),K\(-\)-pump concentration is associated with decreased contractile performance, further inhibition of the Na\(^{+}\),K\(-\)-pump induced by digitalization results in improved performance. Thus a recent study pointed out that in patients with heart failure, where the concentration of Na\(^{+}\),K\(-\)-pumps was reduced by about 25%, digoxin treatment produced a further decrease of 15%, leading to a total reduction in the concentration of functional Na\(^{+}\),K\(-\)-pumps of 40% [126].

The observation that digitalization was associated with an upregulation of the \(^{3}H\)-digoxin binding site concentration in human erythrocytes gave rise to speculation that the treatment elicits a compensatory upregulation of the concentration of Na\(^{+}\),K\(-\)-pumps [127]. Measurements of the \(^{3}H\)-ouabain binding site concentration in the left cardiac ventricle, however, showed no evidence of upregulation in digitalized subjects [68]. Furthermore, it was shown that in atria prepared from patients on digoxin treatment, the electrogenic effect of active Na\(^{+}\),K\(-\)transport stimulation was inhibited [64].

**Myotonic dystrophy**

In homogenates of muscle biopsies obtained from patients with myotonic muscular dystrophy, the concentration of \(^{3}H\)-ouabain binding sites was found to be 3-6-fold lower than in normal muscle [95]. Muscle cells cultured from patients with myotonic dystrophy have been shown to contain 30-40% fewer \(^{3}H\)-ouabain binding sites than those obtained from age-matched control subjects [83,104]. Measurements of the Na\(^{+}\),K\(-\)-ATPase related enzyme activity, 3-O-methylfluorescein phosphatase, confirmed this observation. The reduced concentration of Na\(^{+}\),K\(-\)-pumps in the muscle cells may explain the earlier observation of increased intracellular Na\(^{+}\) and depolarization in muscles from patients with myotonic dystrophy [128,129]. Furthermore, the reduced Na\(^{+}\),K\(-\)-pump capacity may explain the abnormally high exercise-induced rise in plasma K\(^{+}\) observed in these patients [130], and contribute to the reduced physical performance.

**McArdle disease**

In patients suffering from McArdle disease (an inherited muscle phosphorylase deficiency) the concentration of Na\(^{+}\),K\(-\)-pumps in skeletal muscle was found to be reduced by 30%. This downregulation was associated with abnormally high exercise-induced hyperkalaemia, attributed to the reduced capacity for clearing extracellular K\(^{+}\), an effect that was proposed to contribute to the marked fatigue experienced by these patients [103]. It cannot be excluded, however, that the more pronounced exercise-induced hyperkalaemia seen in patients suffering from myotonic dystrophy or McArdle disease in part reflects increased K\(^{+}\) efflux via K\(^{+}\) channels.
Central nervous system disorders
In the brain cortex of human subjects, dementia is associated with a 62% reduction in the total concentration of H-ouabain binding sites measured in tissue specimens [92]. Measurements of H-ouabain binding to microsomes obtained from brain cortex of patients with Alzheimer’s disease show a decrease of 40% [131], and in microsomes from the basal ganglia of patients with Huntington’s chorea a decrease of 50% has been reported [132]. Such large reductions in the Na⁺-K⁺-pump capacity are most likely to be associated with functional impairment of the tissue, but it cannot be discerned whether they are primary or secondary to the overall pathological process.

Cystic fibrosis
In cystic fibrosis, the underlying genetic defect in epithelial chloride secretion is accompanied by an increased Na⁺ reabsorption. Studies in human nasal epithelium have shown that this is associated with a 60% increase in the concentration of Na⁺-[K⁺-pumps (measured as ouabain binding sites) in the basolateral membranes of the cells, where the active transport of Na⁺ takes place [133]. Recent measurements of Na⁺-[K⁺-ATPase activity in human bronchial epithelium obtained during lung transplantation showed that in preparations from cystic fibrosis patients, the level of ouabain-suppressible component is 2-fold higher than in those obtained from controls [134]. These observations suggest that in the affected airway epithelia in patients with cystic fibrosis the increased Na⁺ entry leads to a considerable compensatory upregulation of Na⁺-[K⁺-pumps. It is interesting that in the tracheal epithelium of normal subjects, only one population of high-affinity H-ouabain binding sites could be detected [134].

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REFERENCES
T. Clausen


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Clinical relevance of the Na\(^+\), K\(^+\)-pump


123 Smoller, S., Rashid, K., Perez, G. O., Oster, J. R. and Carver, J. G. (1979) The acute changes seen in cardiac glycoside receptor sites, \(^86^\)Rb uptake and intracellular sodium concentrations in the erythrocytes of patients during the early phases of digoxin therapy are not found during chronic therapy: pharmacological and therapeutic implications for chronic digoxin therapy. Br. J. Clin. Pharmacol. 8, 135–142


