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EDITORIAL REVIEW

Regulation of the Na,K-pump in skeletal muscle

The regulatory problem

Over the last 30 years it has been firmly established that the active coupled transport of Na^+ and K^+ across the plasma membrane is mediated by the Na,K-ATPase, and that this enzyme plays a central role in the Na^+-K^+ homeostasis of virtually all animal cells [reviewed in 1 and 2].

More recently, it has become evident that both the activity and the concentration of this transport system are subject to regulation by a variety of hormonal and non-hormonal factors. This is of particular significance in the skeletal muscles, mainly because they contain the largest single pool of K^+ as well as a major part of the Na,K-pumps in the body.

The problem of K^+ homeostasis and the prominent role of skeletal muscle is perhaps best illustrated by the time course of changes in plasma K^+ during repeated bouts of maximum exercise. As shown in Figure 1, the K^+ concentration of arterial blood increases by more than 3 mmol/liter within less than one minute, followed by a complete reversal to the resting level within around four minutes. These dramatic changes demonstrate that during maximum activity the net loss of K^+ from the working muscles exceeds the capacity for reaccumulation via the Na,K-pump, whereby the heart is exposed to a K^+ concentration of at least 7 mmol/liter. Conversely, even a short resting period is sufficient to allow a complete clearing of the extra K^+ from plasma and the extracellular phase, illustrating the considerable functional capacity of the Na,K-pump [3]. Other studies have shown that during rest following the intense exercise of playing squash, plasma K^+ may reach values around 3.2 mmol/liter [4]. The fact that both extremes of plasma K^+ may cause cardiac arrest or interfere with contractile performance underscores the severity of the regulatory problem. It is obvious, therefore, that close regulation is essential for survival.

The long-term control of plasma K^+ ultimately depends on kidney function, but due to their large capacity for rapid Na^+-K^+ exchange the skeletal muscles play a dominant role in the acute, from minute-to-minute ongoing adjustment of plasma K^+ [5, 6]. The regulation of Na,K-transport in skeletal muscle has recently been reviewed in detail [7]. In the following presentation, more fundamental aspects and their clinical implications will be emphasized and the recent observations in this rapidly developing field will be described.

Quantification of Na,K-leaks and Na,K-pumps in muscle

A quantitative analysis of the K^+ leaking out of working muscles must be based on measurements of the K^+ efflux per contraction taking place in isolated muscles. Isotopic flux studies indicate that the unidirectional efflux of K^+ per contraction varies between 4 and 16 nmol/g wet wt depending on temperature and preparation [8–10]. In rat soleus at 30°C, a value of 9 nmol/g wet wt was found [9]. The same studies showed that there was a concomitant Na^+ influx of the same order of magnitude [8–10].

It is reasonable to assume that the unidirectional efflux of K^+ from working muscles will increase as a linear function of the frequency of contractions, and as shown in Figure 2, even moderate contractile activity leads to a considerable K^+ efflux. It should be noted that the frequency of contractions reached during a standard bicycle exercise experiment is around 25 per second [11]. In the leg muscles of the rat, this frequency would lead to a K^+ efflux of ($25 \times 60 \times 9$) or around 13,500 nmol/g per min at 30°C, and even more at body temperature.

In order to get an idea about the potential of the muscles to counterbalance this loss of K^+, the K^+ influx via the Na,K-pump should be quantified. As shown in Figure 2, this ouabain-suppressible K^+ influx amounts to only 250 nmol/g wet wt per min in the resting rat soleus muscle at 30°C.

Obviously, this is far from sufficient to restore the K^+ lost during work, and it is of considerable interest, therefore, to estimate the total capacity of K^+ accumulation via the Na,K-pump. Complete quantification of the concentration of Na,K-ATPase in muscle is difficult, mainly due to incomplete recovery and the formation of inside-out vesicles of the sarcolemma [12]. Measurements of ^{3}H-ouabain binding to intact muscle preparations or to muscle biopsies, however, have given highly reproducible values for the total concentration of the Na,K-ATPase [13].

Over a wide range of ^{3}H-ouabain binding site concentrations it could be shown that the ^{3}H-ouabain binding assay quantifies functional Na,K-pumps [14]. Furthermore, measurements of the total Na,K-ATPase concentration [15, 16] and 3-O-methylfluorescein phosphatase activity [17] in crude muscle homogenates have given values in good agreement with determinations of the total ^{3}H-ouabain capacity of intact muscles.

The maximum capacities for K^+ influx via the Na,K-pump in the isolated soleus muscle of 4-week-old rats at 30 and 37°C are indicated by dashed horizontal lines in Figure 2. It is evident that the K^+ loss during contractile activity may readily exceed even these maximum levels. This may account for the fact that during chronic electrical stimulation at 10 Hz in vivo, muscles undergo an appreciable net loss of K^+ and gain of Na^+, which is only recovered following a period of rest [18, 19]. This may be of importance for the pain and the development of degenerative changes associated with prolonged tonic muscle contractions.

In man, the concentration of ^{3}H-ouabain binding sites in skeletal muscle is around 280 pmol/g wet wt [20–22], corresponding to a theoretical maximum K^+ accumulation rate of 4,480 nmol/g wet wt/min at 37°C.

In conclusion, the concentration of Na,K-pumps in skeletal

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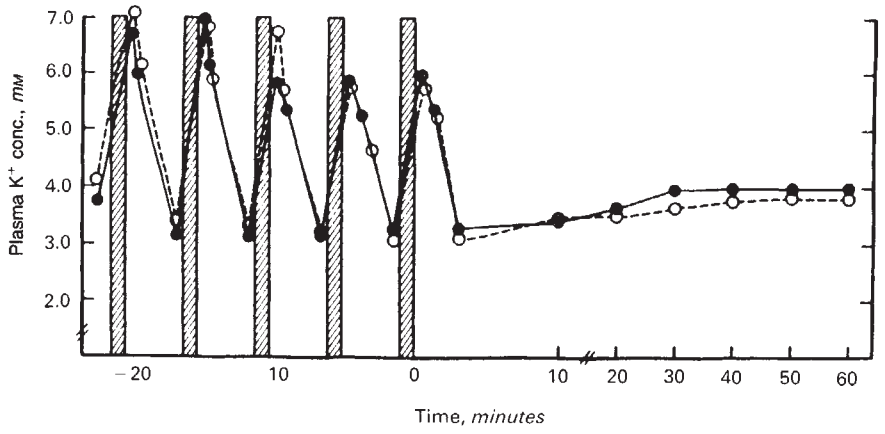

Fig. 1. *Changes in the K^+ concentration of arterial blood plasma in trained human subjects during repeated bouts of maximum exercise each lasting from 30 to 60 seconds (reproduced with permission from ref. 3).*

muscle can be quantified using biopsy specimens of around 5 to 20 mg and a relatively simple ^{3}H-ouabain binding assay [23, 24]. A comparison of the Na,K-pump concentration with the values for K^+ efflux per contraction shows that during contractile activity, the loss of K^+ and the gain of Na^+ via passive leaks often exceed the maximum capacity of the available Na,K-pumps for restoring the Na,K-gradients across the muscle cell membrane [9].

Acute regulation

The immediate, from minute to minute ongoing control of active Na^+ K^+ transport mainly seems to be exerted by activation of the Na,K-pump. Inhibitory factors have been detected in plasma, but at present their significance for acute regulation is not clarified [25]. The major physiological stimuli for the Na,K-pump are excitation, catecholamines and insulin.

Excitation

In single frog muscle fibers stimulated at a frequency of 1.5 Hz, only a 10% rise in intracellular Na^+ (Na_i) was observed [10]. Surprisingly this was associated with a concomitant stimulation of ^{24}Na efflux of 109%, indicating that the Na,K-pump may be activated over and above what can be predicted by the relative rise in Na_i. The observation is even more remarkable when taking into account that the entry of unlabelled Na^+ during excitation would be expected to cause dilution of the specific activity of the intracellular ^{24}Na with ensuing reduction of the fractional loss of ^{24}Na. The model for K^+ efflux and Na,K-pump activation during exercise developed by Hazeyama and Sparks [26] predicts that the Na,K-pump is activated by around 65% within the first three minutes of stimulation at a frequency of 4 Hz. According to their calculations, the rise in Na_i is not larger than 15% within this period. Recent experiments with isolated rat soleus muscle have shown that electrical stimulation at a frequency of 2 Hz is associated with a 74% increase in ouabain-suppressible ^{22}Na efflux [27] and a 110% increase in ouabain-suppressible ^{86}Rb uptake (Fig. 3). These values were obtained within a 10 minute period of stimulation and were accompanied by an increase in Na_i of 15% (as determined after the 10 min stimulation period).

Comparison of the effects of electrical stimulation at 2 Hz on the ouabain-suppressible ^{86}Rb uptake in rat soleus (slow-twitch) and extensor digitorum longus (EDL) (fast-twitch) muscle demonstrated that the stimulation of the Na,K-pump during excitation was more than three times larger in soleus than in EDL [27]. A similar difference between the two muscles has been observed when the Na,K-pump is stimulated with catecholamines [27–29].

It has been calculated that stimulation at a frequency of 15 Hz would result in a 60% increase in Na_i in skeletal muscle which would result in a similar relative increase in Na,K-pump rate [30]. Measurements with Na^+-sensitive electrodes have shown that the intracellular Na^+ activity in mouse soleus muscle may increase by around 80% within one minute of application of stimulus trains at a frequency of 40 Hz [31]. On the basis of the recovery of intracellular Na^+ activity after stimulation [31] it could be calculated that the Na,K-pump was stimulated to around 10 times the resting activity [7], corresponding to 40% of its theoretical maximum rate. The discrepancy between the relative rise in intracellular Na^+ activity and Na,K-pump activation was even larger when the activation of the Na,K-pump during recovery was calculated on the basis of the recovery of K_i, that is, for an 80% increase in intracellular Na^+ activity a 20-fold stimulation of K^+ influx could be calculated [31]. This is surprising in view of the observation that in resting skeletal muscle, the Na,K-pump mediated K^+ influx increases as a linear function of Na_i and that a rise in Na_i of 80% caused an increase of only 67% in Na,K-pump mediated K^+ influx [32].

Since the plasma catecholamine level rises considerably during intense exercise [33], partly due to norepinephrine release from sympathetic nerve endings in the muscles [34], the activation of the Na,K-pump induced by excitation might depend on a local action of norepinephrine. However, recent experiments with isolated rat soleus muscle have shown that the excitation-induced stimulation of ^{22}Na efflux could not be prevented by propranolol [27]. The same study showed that the effects of epinephrine and excitation were not additive in either soleus or EDL muscle, indicating that they may activate the Na,K-pump through the same mechanism.

At present it is only possible to speculate about the functional significance of an excitation-induced activation of the Na,K-pump. Recently, it has been proposed that development of fatigue might be partly explained by inexcitability due to accumulation of K^+ ions in the extracellular space [35, 36]. This suggests that resistance to fatigue might be partly ascribed to the capacity of the Na,K-pump to clear K^+ from the extracel-

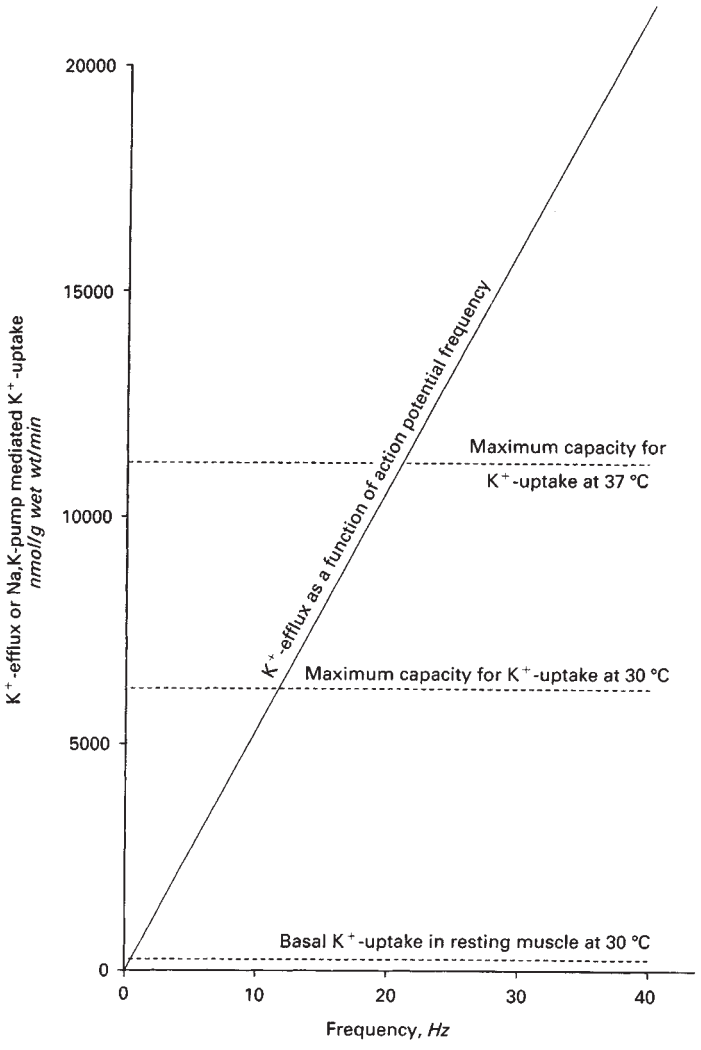

Fig. 2. *Comparison of K^+ loss induced by contractile activity and K^+ influx via the Na,K-pump.* In isotope flux studies at 30°C, a unidirectional K^+ efflux of 9 nmol/g wet wt per contraction was estimated for soleus muscle of 4 week-old rats [9]. On the basis of this value, the K^+ efflux was calculated at varying frequencies of contraction. For comparison, the ouabain-suppressible component of K^+ influx is indicated for resting [7] and for Na^+-loaded rat soleus muscle at 30°C (data from ref. 14). The theoretical maximum ouabain-suppressible K^+ influx at 37°C has been calculated on the basis of the ^{3}H-ouabain binding site concentration and the turnover number of the Na,K-pumps at this temperature (0.7 × 8,000 × 2 = 11,200 nmol K^+ per g wet wt per min).

lular space. This is, in particular, important in slow-twitch muscles like rat soleus, which have a rather high level of activity during the day [37]. In addition, the finding that the excitation-induced stimulation of the Na,K-pump was threefold larger in soleus than in EDL muscle, might be related to the fact that slow-twitch muscles have a much higher resistance to fatigue than fast-twitch muscles [38].

Catecholamines

A wide variety of experiments carried out over the last 50 years have shown that catecholamines stimulate the uptake of K^+ and increase the K^+ content in skeletal muscle. The injection of epinephrine was found to increase the arteriovenous difference for K^+ in the hindlimbs of dogs and frogs [39, 40] as well as in the human forearm [41–43]. In the perfused dog gracilis muscle, epinephrine induced an increased net K^+ uptake within three minutes [44], and in cats, an increase in the K^+ content of skeletal muscle [45].

In vitro studies have shown that both epinephrine and norepinephrine increase K^+ content as well as the intracellular concentration of K^+ in rat soleus [28, 46, 47] and EDL muscle [28, 48]. Isotopic flux measurements using ^{42}K or ^{86}Rb as tracers for K^+ have demonstrated that both epinephrine and norepinephrine stimulate the ouabain-suppressible component of K^+ influx quite markedly in rat and guinea pig soleus [28, 46, 47, 49–51]. As can be seen from Figure 3, this effect is more pronounced and rapid in onset than that induced by insulin, but almost as large as that elicited by electrical stimulation at a frequency of 2 Hz. Considerably smaller stimulation was obtained in rat EDL muscle, suggesting that in fast-twitch fibers the Na^+,K^+-pump responds less than in slow-twitch fibers [27, 28].

Epinephrine was shown to stimulate ^{22}Na efflux in frog sartorius [52, 53], mouse diaphragm [54] and rat soleus [46, 47, 51, 55]. Again, this effect was more pronounced in soleus than in EDL [28]. The fact that both the effects on K^+ influx and Na^+ efflux could be suppressed by ouabain indicate that they are the result of activation of the Na,K-pump. It should be emphasized that this could not be accounted for by increased Na_i or by a decrease in K^+ content. Indeed, several studies have shown that epinephrine (as well as other β_2-adrenoceptor agonists) induces a decrease in Na_i which in rat soleus may reach values as low as one third of the control level [46, 47, 52, 56]. As shown for ^{22}Na efflux this effect was larger in soleus than in EDL

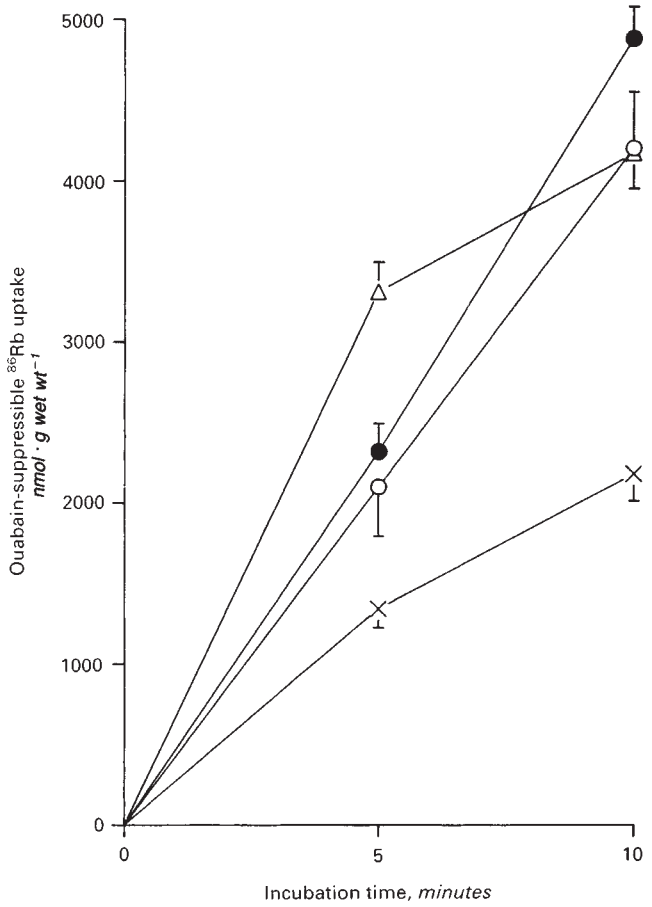

Fig. 3. *Comparison of the effects of insulin, epinephrine and electrical stimulation on ouabain-suppressible ^{86}Rb influx in isolated rat soleus muscle.* Intact muscles were prepared from 4 week-old rats and incubated in Krebs-Ringer bicarbonate buffer at 30°C. After preincubation for 15 min without or with ouabain (1 mM), the muscles were incubated for 5 to 10 min in buffer containing ^{86}Rb (0.1 μCi/ml) without or with ouabain (1 mM). Where indicated the radioactive buffer contained insulin (100 mU/ml) or epinephrine (10 μM). Part of the muscles was exposed to direct electrical stimulation of 2 Hz using 10 V pulses of 1 msec duration. On the basis of the specific activity of the incubation medium, the amount of ^{86}Rb taken up was calculated and expressed as nmol/g wet wt. The ouabain-suppressible ^{86}Rb uptake was taken as the difference of the values obtained in the absence and in the presence of ouabain. Symbols are: (x) rest, (△) + adrenaline (10 μM), (○) + insulin (100 mU/ml); and (●) electrical stimulation (2 Hz).

muscles [57]. Once this lower steady-state level for Na_i has been established, the rate of ^{22}Na efflux may be considerably smaller than that of the control muscles [55]. In the Purkinje fibers of the dog heart, both isoproterenol and norepinephrine induce a decrease in intracellular Na^+ activity which can be blocked by propranolol or strophanthidin [58, 59]. Recent studies with isolated rabbit cardiac myocytes indicate that this effect is not due to changes in extracellular K^+ [60]. Direct recordings with ion sensitive microelectrodes demonstrated that in human intercostal muscle fibers as well as in rat soleus muscle, epinephrine induces a decrease in the intracellular Na^+ activity, hyperpolarization and an increase in intracellular K^+ activity [61]. All these effects could be blocked by ouabain, indicating that they were secondary to stimulation of the Na,K-pump.

The stimulating effect of catecholamines on active Na,K-transport can be observed at physiological concentrations, and is mediated via β_2-adrenoceptors and activation of the adenylate cyclase [46, 49, 56]. This effect accounts for the hyperpolarizing action of catecholamines in muscle [46, 62–65].

The observation that catecholamines increase the rate of ^{3}H-ouabain binding is a specific indication that the Na,K-ATPase is activated [66], but there is no direct evidence that catecholamines can activate Na,K-ATPase prepared from skeletal muscle. Thus, the addition of cAMP caused no change in enzyme activity [67]. In kidney tubule cells, however, pretreatment with norepinephrine caused an increase in the activity of Na,K-ATPase subsequently prepared from the tissue [68].

In conclusion, catecholamines induce marked and rapid activation of the Na,K-pump in skeletal muscle, favoring net intracellular accumulation of K^+. This is important for the reduction of plasma K^+ during exercise. Furthermore, β_2-adrenoceptor agonists may be used to suppress hyperkalemic attacks [49, 69–71] as well as hyperkalemia in renal insufficiency [72]. The fatigue associated with treatment with β-blockers may be related to the hyperkalemia caused by suppression of the catecholamine activation of the Na,K-pump [73, 74]. It has been proposed that some cases of sudden death occurring after severe exercise are related to catecholamine-induced hypokalemia [4].

Insulin

The hypokalemic effect of insulin is the result of increased net uptake of K^+ in muscle cells which is separate from the effect on glucose transport [51, 75]. Thus, physiological concentrations of the hormone augment the arteriovenous difference for K^+ in the human forearm [76], and several in vitro studies have shown that insulin induces a net increase in the K^+ content in muscle [77–82]. The intracellular K^+ concentration is increased by around 10% [47, 78, 82, 83], and in cultured chicken heart cells, this effect is blocked by ouabain [84]. One study demonstrated an increase in the intracellular K^+ activity [85], whereas others were unable to detect any change in the intracellular concentration [81, 86] or activity of K^+ [87].

Insulin increases the influx of ^{42}K, ^{86}Rb or ^{134}Cs in isolated muscle preparations [47, 79, 82, 88–91], and in the rat soleus, insulin was found to increase the rate of ^{3}H-ouabain binding, a specific indication that the turnover of the Na,K-pump is augmented. The effects on isotope uptake were blocked by ouabain [47, 82, 88, 89, 91], indicating that the effect of insulin is mediated through the Na,K-pump [66].

As can be seen from Figure 3, the effect of a maximum stimulatory concentration of insulin on ouabain-suppressible ^{86}Rb-influx is somewhat smaller and slower in onset than that of epinephrine or electrical stimulation.

Insulin was shown to increase Na^+ efflux by between 25 and 70% [47, 55, 82, 83, 88, 92–94]. This effect could be blocked by cardiac glycosides or K^+-free buffer in frog muscle [88, 92], but not in rat soleus [82]. The effect of insulin on the Na,K-pump may account for the hyperpolarizing action of the hormone [47, 51].

Since insulin in intact muscle preparations induces a decrease in the intracellular concentration of Na^+ [78, 81, 83, 95] which

can be blocked by ouabain [47, 82, 92, 96], it is unlikely that the effects on Na^+ K^+ transport are the result of the Na,K-pump being activated by increased availability of Na^+ in the cytoplasm. In cultured myocytes, however, insulin was recently reported to stimulate Na,K-pump activity through a rise in intracellular Na^+ resulting from an increased passive Na^+ influx via the Na^+/H^+ exchange system [97].

Several attempts have been made to detect an effect of insulin on the activity of the Na,K-ATPase in muscle, but only one laboratory [67] has reported that the enzyme can be stimulated by the direct addition of insulin to a muscle membrane preparation [98].

The effects of insulin and epinephrine on K^+ accumulation and Na^+ efflux are additive, indicating separate mechanisms of action [47]. This is of particular importance since the insulin-induced hypoglycemia may induce stimulation of catecholamine secretion, and the simultaneous elevation of the plasma levels of both hormones was indeed shown to produce a larger decrease in plasma K^+ than either hormone alone [99]. The role of insulin in K^+ homeostasis has been documented by the demonstration of insulin secretion being stimulated by hyperkalemia [100, 101]. Whereas moderate hyperkalemia seems to be counterbalanced by insulin-induced stimulation of hepatic K^+ uptake, more pronounced elevations of plasma K^+ are associated with increased insulin levels in the peripheral blood and augmented K^+ uptake in skeletal muscle [6].

In conclusion, insulin induces activation of the Na,K-pump in skeletal muscle at physiological concentrations. This can account for the increase in intracellular K^+/Na^+ ratio, the hyperpolarization as well as a major part of the hypokalemic action. There is a negative feedback relationship between plasma K^+ and insulin secretion, whereby the hormone contributes to plasma K^+ homeostasis.

Other factors

Studies with metabolic inhibitors have shown that in rat soleus muscle, the Na,K-pump mediated Na^+ efflux and K^+ influx are suppressed [14, 82]. This is likely to reflect the requirement for ATP as an energy supply for the Na,K-pump. For the same reason, anoxia leads to inhibition of active Na^+K^+ transport with ensuing loss of intracellular K^+ and a rise in interstitial K^+. Since the ATP concentration required to produce half maximum saturation of the Na,K-ATPase is rather low (1 mM), cellular ATP has to undergo an appreciable reduction before severe interference with active Na^+K^+ transport is seen.

Vanadate (VO_4) is a potent inhibitor of Na,K-ATPase (as well as a number of other ATPases). Since muscle cells contain vanadate, this compound has been proposed as a potential regulator of the Na,K-pump, but as yet there is little support for this concept.

Long-term regulation

The long-term regulation of the Na,K-pump in skeletal muscle is achieved by variations in its concentration. These variations may occur as a physiological consequence of development and differentiation or training, or may be seen under pathological circumstances as hypo- and hyperthyroidism, uncontrolled diabetes, starvation, immobilization, K^+ deficiency and chronic renal failure. The mechanism of induction of the changes in Na,K-pump concentration is not necessarily identical under the various circumstances and will be discussed in the following paragraphs.

Thyroid hormones

It has been known for around 15 years that the Na,K-ATPase activity in skeletal muscle increases as function of the thyroid status [102–104]. This increased activity of the Na,K-pump was also suggested to account for the major part of the calorigenic action of thyroid hormones [102, 103]. However, measurements of the ouabain-suppressible heat production have shown that active Na,K-transport in intact skeletal muscle only accounts for a minor part (5%) of the total heat production [105], which fraction only increases modestly with the thyroid status [106].

Measurements of the ^{3}H-ouabain binding site concentration in intact mouse soleus muscles [106] or in strips and biopsies from rat soleus muscle [23, 107] indicate that in hyperthyroid animals, the entire population of Na,K-ATPase molecules in the muscles may be up to 10-fold larger than in hypothyroid animals (Fig. 4). Biopsies of human vastus lateralis muscles showed that the concentration of ^{3}H-ouabain binding sites varied over a sixfold range with the free T_4-index [20]. Furthermore, it was shown that these changes were fully reversible when the thyroid status was normalized by standard therapy [20].

Since skeletal muscles vary considerably with respect to their contractile, metabolic and fatigue properties [38, 108–110], the effects of thyroid hormones on Na,K-ATPase activity have been examined in muscles containing varying proportions of slow- and fast-twitch fibers. This demonstrated that the effect was present in soleus and diaphragm (mainly slow-twitch and red) as well as in EDL and gastrocnemius (mainly fast-twitch and white) muscle [107], but it was more pronounced in muscles with a large proportion of slow-twitch or red fibers in young and in adult rats (Fig. 4). The same study also showed that the thyroid hormone-induced changes in ^{3}H-ouabain binding site concentration could not be accounted for by differences in affinity or the rate of ^{3}H-ouabain binding [107]. The implication of these results is that the effect of thyroid status on the Na,K-pump concentration is present throughout the skeletal muscle pool and might be one of the major factors explaining the common clinical experience that digitalis glycoside tolerance increases with thyroid status [111, 112].

Recently, it has been demonstrated that the large increase in ^{3}H-ouabain binding site concentration in hyperthyroidism is associated with a proportional increase in the maximum rate of ouabain-suppressible ^{86}Rb uptake [14]. The latter was, however, measured in Na^+-loaded muscles exposed to high extracellular K^+, which is not a common physiological situation. With normal Na_i and extracellular K^+ an increase in basal ouabain-suppressible ^{42}K or ^{86}Rb uptake is found in hyperthyroid soleus and EDL muscles [29, 106] which was of the same order of magnitude as the respective increases in Na,K-pump concentration. Nevertheless, the total Na^+ and K^+ contents in skeletal muscles are unchanged with hypo- and hyperthyroidism [106, 107, 113]. This suggests that the increased influx of K^+ and efflux of Na^+ in hyperthyroidism is counter-balanced by an increased efflux of K^+ and influx of Na^+.

It has indeed been demonstrated that thyroid hormone increases the passive permeability of Na^+ and K^+ in skeletal

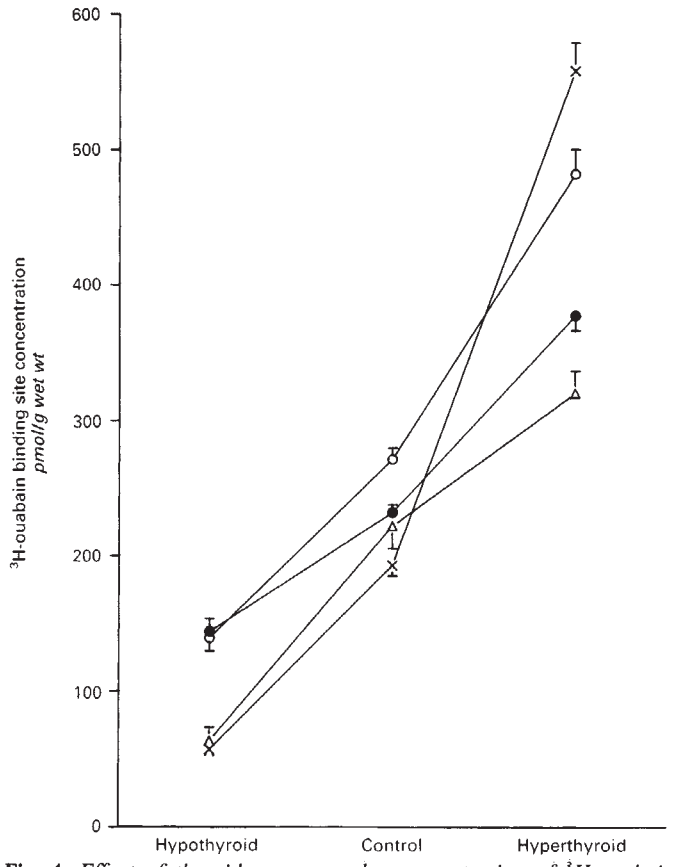

Fig. 4. *Effect of thyroid status on the concentration of ^{3}H-ouabain binding sites (Na,K-pumps) in various skeletal muscles of the rat.* Muscle specimens were prepared from 12 week old hypo-, eu- or hyperthyroid rats and the concentration of ^{3}H-ouabain binding sites was determined using the vanadate facilitated binding assay [23] (reproduced with permission from ref. 107). Symbols are: (x) soleus; (○) diaphragm; (●) EDL: (△) gastrocnemius.

muscle [114, 115]. More recently, it was shown for both rat liver, kidney tubules and skeletal muscle that the passive efflux of K^+ was increased after thyroid hormone treatment before any increase in Na,K-ATPase activity could be detected [116–118]. The time course of increase in Na^+ uptake was similar to that of the rise in K^+ efflux [118]. These observations lend further support to the idea that the increase in Na,K-ATPase activity after thyroid hormone treatment represents a specific response to the leak of K^+ from or influx of Na^+ into the muscles. The finding that the early phase of the thyroid hormone-induced increase in Na^+ uptake (12 to 24 hrs) could be suppressed by amiloride suggests that part of the increase in Na^+ uptake is due to stimulation of the Na^+/H^+ exchange, which in turn is stimulated by a rise in cytosolic Ca^{++} concentration [118].

The interesting point of course is that each action potential is associated with influx of Na^+ and efflux of K^+, which will account for the major part of the passive Na,K-leaks in skeletal muscle (Fig. 2). It remains to be established to which extent possible changes in Na,K-fluxes and Na,K-pump activity during work may account for the fatigue and weakness commonly observed in hypo- and hyperthyroid patients [119].

In conclusion, the concentration of Na,K-pumps in skeletal muscle is to a major extent controlled by thyroid hormones. The thyroid hormone-induced increase in Na,K-pump concentration seems to represent an adaptation to increased passive leaks of Na^+ and K^+. The rise in Na,K-ATPase concentration with thyroid status is observed throughout the skeletal muscle pool and is probably the major factor determining the increased digitalis glycoside tolerance seen with increasing thyroid hormone levels. Furthermore, it could be of importance for the hypokalemic attacks seen among certain hyperthyroid subjects [120].

The effect of thyroid hormone on the synthesis of Na,K-pumps is of considerable general significance in view of the fact that decreased serum T_3 is seen in a variety of diseases as well as during caloric restriction [121]. As exemplified below, this is associated with a reduction in the concentration of Na,K-pumps.

Diabetes and starvation

As insulin increases the rate of active Na,K-transport acutely, the question may arise whether the low plasma insulin level in diabetes will result in a reduction of the transport rate. Furthermore, if this is the case, the question will be whether this is due to a reduction of the molecular activity of the Na,K-ATPase or a downregulation of the enzyme concentration.

In streptozotocin-diabetic rats the soleus muscle showed a 48% increase in intracellular Na^+ content, without any change in intracellular K^+ content [122]. Recent measurements of ^{3}H-ouabain binding site concentration in soleus muscle biopsies have shown that streptozotocin-induced diabetes in rats is associated with a considerable (48%) decrease in Na,K-pump concentration without affecting the affinity of the Na,K-pump for ouabain [123]. In gastrocnemius muscle, a 38% decrease in ^{3}H-ouabain binding site concentration was found which was accompanied by a 33% increase in total Na^+ and no change in total K^+ content [123]. The same study also showed that the changes in Na,K-pump concentration and Na^+ content were completely prevented by insulin treatment.

Like the effect of thyroid hormones, the effect of diabetes on Na,K-ATPase concentration was seen in muscles containing various proportions of slow- and fast-twitch fibers, but it was most pronounced in the slow-twitch muscles [123]. In untreated insulin-dependent diabetic patients a 28% decrease in free T_3 has been demonstrated [124]. Since thyroid hormone is one of the major regulators of the Na,K-pump concentration in skeletal muscle, a reduction of the T_3 level might clearly play a role in the diabetes-induced decrease in Na,K-pump concentration. However, hypothyroidism in adult mice or rats is not associated with an increase in Na^+ content [106, 107], nor with a decrease in muscle weight. This implies that in uncontrolled diabetes one or more additional mechanisms are involved. It has been shown that protein synthesis in gastrocnemius muscle of streptozotocin-diabetic rats was only 30% of that in control rats [125], which might account for the decrease in muscle weight [123]. The observation that the Na^+ content was increased without a concomitant decrease in K^+ content might be related to dehydration.

Starvation is accompanied by a decrease in the plasma levels of thyroid hormones both in man [126] and the rat [127, 128].

The Na,K-ATPase activity in sarcolemma isolated from rat muscle has been shown to decrease by 50% after five days of complete fasting [129]. However, measurements of the total ^{3}H-ouabain binding capacity in intact muscle samples only showed a decrease of 25% [128]. Semi-starvation, induced by maintaining rats on one-third to one-half of their normal energy intake for three weeks, was associated with a 50% reduction of T_3 and T_4 levels, a 25% reduction in ^{3}H-ouabain binding site concentration in soleus muscle and only minor changes in total Na^+ and K^+ content [128]. Also in semi-starvation, the reduction of Na,K-pump concentration is seen throughout the skeletal muscle pool and is fully reversible after refeeding [128].

In conclusion, both uncontrolled diabetes and semi-starvation result in a down-regulation of the Na,K-ATPase concentration in skeletal muscle. The two disorders have in common that they are associated with a reduction of the T_3 level, which may, at least partly, account for the observed effects.

Age and development

Growth and differentiation of muscle cells is associated with a marked increase in the concentration of Na,K-pumps, both in the intact organism [130–132] and in cultures [133].

In rats and mice, the concentration of ^{3}H-ouabain binding sites increases three- to fivefold from birth to the fourth week of age. This rise is followed by a progressive decrease, *pari passu* with the increase in cell volume. In guinea pigs, which show earlier development, maximum concentration of ^{3}H-ouabain binding sites is seen at birth, followed by a drop [131, 132]. Studies in human subjects show no change in ^{3}H-ouabain binding site concentration in skeletal muscle from 18 to 80 years of age [21].

Little is known about what drives the synthesis of Na,K-pumps during development and differentiation, but at least three factors could be of importance: 1) the plasma levels of thyroid hormones increase over the first weeks of life [134]; 2) over the same interval of time, intracellular Na^+ undergoes a decrease from 30 to 8 mM [135]; and 3) muscle activity is increasing.

In conclusion, the early increase in Na,K-pump concentration seems to be part of the general development and differentiation. Since this rise is likely to favor the clearance of K^+ from the extracellular phase, it may contribute to the improvement of contractile performance observed in growing rats [136].

Training and inactivity

The exercise induced by hyperkalemia is reduced by training, both in man [137] and in the dog [138]. This could be due to an increased concentration of Na,K-pumps in skeletal muscle, and indeed, six weeks of training was found to augment the activity of Na,K-ATPase by 165% in the sarcolemma of dog gracilis muscle [138]. The ouabain-suppressible component of resting membrane potential in intercostal muscle fibers was also increased, indicating that the electrogenic effect of the Na,K-pump had become more pronounced. Measurements of the total concentration of ^{3}H-ouabain binding sites showed that following three to six weeks of swim training there was an increase of 43 to 46% in the soleus and the EDL (Fig. 5), and 22 to 25% in the spine muscles and the gastrocnemius. In contrast, the diaphragm showed no increase, all indicating that the effects were not exerted by a generally acting endocrine factor. This upregulation was completely reversible after three weeks of detraining [139]. In keeping with these observations, it was found that inactivity induced by denervation, tenotomy or plaster immobilization caused a reduction in the concentration of ^{3}H-ouabain binding sites [140–142]. The decrease in ^{3}H-ouabain binding site concentration with denervation was more pronounced in rat soleus than in EDL, and whereas plaster immobilization gave no change in EDL, the soleus showed a 33% decrease [143]. Conversely, denervation was reported to increase the concentration of Na,K-ATPase or ^{3}H-ouabain binding sites in membrane fractions by between 20 and 200% [144–146]. This discrepancy might be related to difficulties in ensuring representative recovery of Na,K-ATPase activity during the preparation of membrane fractions from skeletal muscle [discussed in 12].

In conclusion, muscle activity is associated with a reversible increase in the concentration of Na,K-pumps which could be part of the basis for the improved K^+ clearance during exercise in trained subjects. The observation that training increases the capacity to secrete epinephrine [147] suggests that increased stimulation of the Na,K-pump by circulating catecholamines contributes to the effect of training on K^+ clearance.

K^+-deficiency

Potassium deficiency is a frequently occurring complication to a large variety of diseases as well as to diuretic treatment. It is associated with a selective loss of K^+ from skeletal muscle [148–150], smooth muscle [151], and to a minor extent heart muscle [150].

Studies with cultured muscle cells as well as a variety of other cell types have shown that K^+ loss is associated with an increase in the intracellular Na^+ concentration and a progressive rise in the concentration of Na,K-ATPase in the plasma membrane [152–154]. This upregulation of Na,K-pumps is seen as a general response to a demand for increased transport capacity, allowing the clearance of Na^+ from the cytoplasm. Indeed, erythrocytes prepared from K^+ deficient animals or human subjects have been shown to contain an elevated level of Na,K-pumps and this is associated with virtually normal Na,K-content [155, 156].

In contrast, in intact skeletal muscles in vivo, K^+ deficiency leads to a progressive and marked (up to 78%) loss of the Na,K-pumps [149, 150, 157]. This unexpected response is seen in a variety of species [reviewed in 158], as well as in human subjects suffering from K^+ deficiency induced by chronic diuretic treatment [22]. A possible explanation for the discrepancy between the responses to K^+ deficiency obtained in isolated cells or tissues and in vivo is that K^+ deficiency in the intact animal or man primarily involves the regulation of plasma K^+, whereas in isolated cells only the tissue K^+ content is subject to regulation. In the intact organism, therefore, other control mechanisms are overriding the strictly cellular regulation of Na,K-pump synthesis. This seems less surprising, perhaps, when considering that an upregulation of the Na,K-pump concentration is likely to favor the development of hypokalemia and ensuing paralysis.

The downregulation induced by K^+ deficiency was first detected by determination of the ^{3}H-ouabain binding site concentration [149], but has later been confirmed in measurements

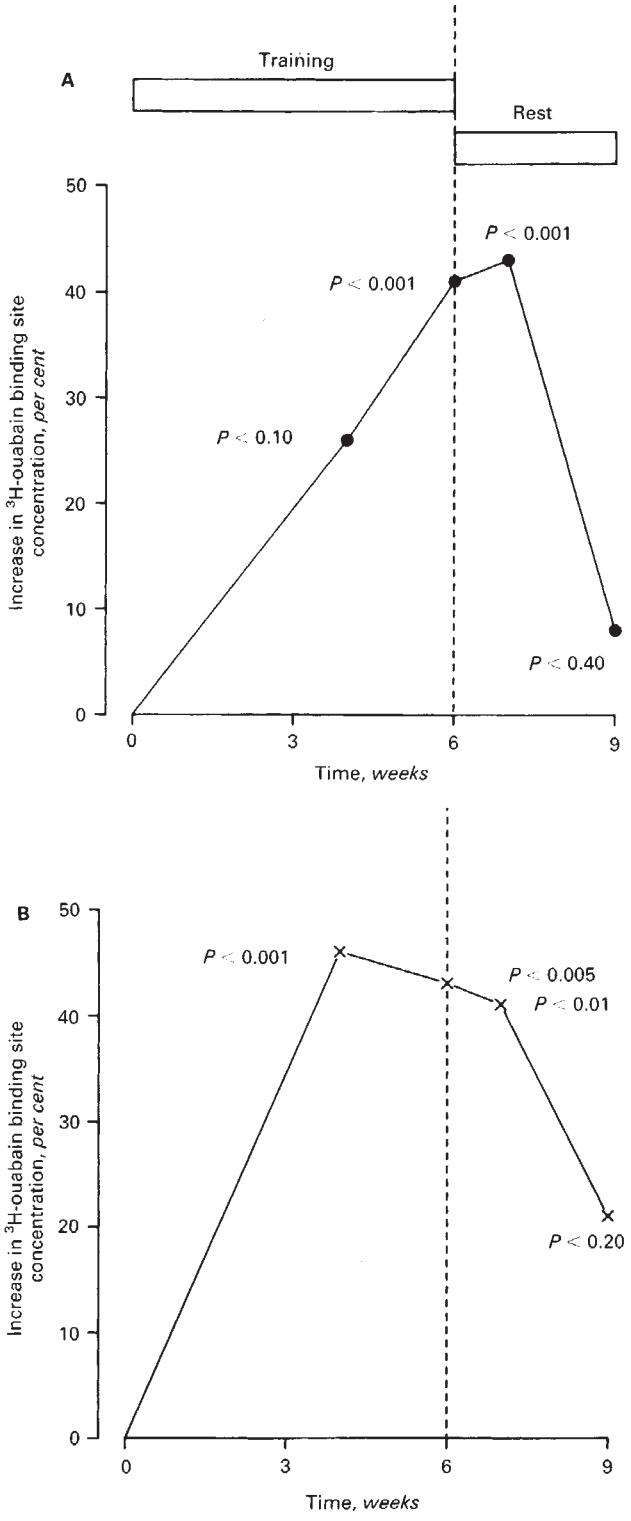

Fig. 5. *Effect of training and detraining on the concentration of ^{3}H-ouabain binding sites in rat soleus (A) and EDL (B) muscle (reproduced with permission from ref. 139).*

of 3-O-methylfluorescein phosphatase activity [17] as well as the ouabain-suppressible component of maximal K^+ influx [14].

A significant decrease in the concentration of Na,K-pumps in rat muscle can already be detected following three days of K^+ depletion, and full recovery is not achieved until after six days of K^+ repletion (Fig. 6), even though the K^+ content of the muscle cells was already normalized within a few hours [150]. There is a close correlation between the reduction in muscle K^+ content and the concentration of Na,K-pumps, and downregulation of the Na,K-pumps can be induced by K^+ deficient fodder as well as diuretics or mineralocorticoid treatment [150, 159]. The decrease in ^{3}H-ouabain binding site concentration induced by magnesium depletion seems to be accounted for by the concomitant reduction in K^+ content [160].

The downregulation is not initiated by a decrease in plasma thyroid hormone levels, nor is there any resistance to the action of thyroid hormone on the synthesis of Na,K-pumps [107]. Pronounced protein deficiency only gave modest reduction in the concentration of ^{3}H-ouabain binding sites in muscle, indicating that the effect of K^+ deficiency is not the result of a general reduction in protein synthesis [150]. The effect of K^+ deficiency is unimpaired by denervation, indicating that it is not mediated by the peripheral nerves [142]. On the other hand, immobilization by tenotomy, plaster or denervation induced normalization of the K^+ content of muscle within a few hours, indicating that contractile activity is important in eliciting the selective K^+ loss from the skeletal muscles [142].

After intravenous injection of ^{3}H-digoxin, the activity in plasma was higher in K^+-depleted rats than in controls [161]. More recently, it was shown that following the injection of a single dose of ^{3}H-ouabain, the acute rise in plasma ^{3}H-activity was 77% larger in K^+-deficient rats than in age-matched controls [141]. These observations indicate that consistent with the reduction in the skeletal muscle pool of digitalis receptors, less digitalis is cleared and more remains in plasma. In this connection it is interesting that in patients receiving digoxin treatment, the concentration of ^{3}H-ouabain binding sites in biopsies of the vastus lateralis muscle was not significantly different from that of comparable untreated controls [22].

Potassium deficiency is also associated with a reduced capacity to clear an oral load of K^+ [162], possibly in part because the pool of functional Na,K-pumps in skeletal muscle is reduced. However, the role of renal adaptation to low K^+ diet was not evaluated and might have contributed to the impaired K^+ clearance [162]. Conversely, K^+ loading by administration of K^+-enriched fodder for 10 days was found to cause a considerable improvement in the capacity to clear an intravenous load of K^+ in the rat [163]. This was associated with increased microsomal Na,K-ATPase concentration and in vivo ^{86}Rb uptake in hind limb skeletal muscle, indicating that K^+ loading leads to upregulation of Na,K-pump concentration in muscle. Others, however, failed to detect any effect of K^+ loading on ^{3}H-ouabain binding site concentration of soleus muscle [150].

In conclusion, the concentration of Na,K-pumps in the skeletal muscles of the intact organism is to a major extent controlled by the availability of K^+. In particular, K^+ deficiency leads to a pronounced downregulation of the Na,K-pump concentration which has implications for the capacity to clear intravenous loads of digitalis glycosides or K^+. The functional significance of this phenomenon remains to be explored, but it can be expected to reduce the tendency to develop early severe hypokalemia and paralysis, thereby prolonging

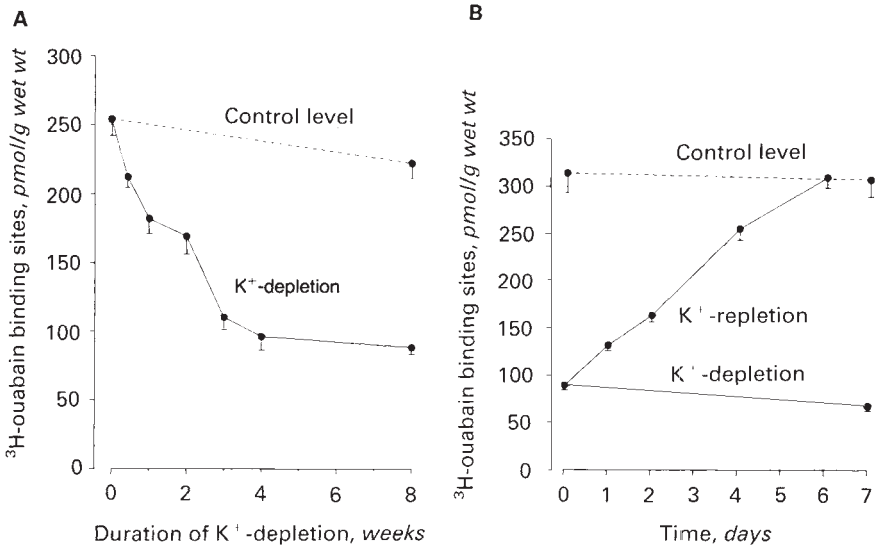

Fig. 6. *Time-course of changes in the concentration of ^{3}H-ouabain binding sites in rat soleus during K^+ depletion* (**A**) *and K^+ repletion* (**B**) *(reproduced with permission from ref. 150).*

survival and increasing the chance to gain access to K^+ repleting nutrients.

Chronic renal failure

As discussed in the beginning of this review, the long-term regulation of K^+ homeostasis ultimately depends on kidney function. Although patients with chronic renal failure can generally maintain a fairly satisfactory water and electrolyte status, due to the compensatory action of surviving nephrons, the end stage of the disease is accompanied by water and Na^+ retention as well as hyperkalemia [164]. These disturbances might have consequences for the Na^+ and K^+ content and Na,K-pump function in the extrarenal tissues.

Na,K-pump function in chronic renal failure has recently been reviewed by Deepak and Kahn [165], but as these authors pointed out there is little information on the Na,K-pump mediated ion transport in tissues other than the uremic erythrocyte. However, we can try to make some speculations on the basis of the available data on Na^+K^+ contents and membrane potential in uremic muscle cells, combined with the changes in K^+ homeostasis and endocrine status observed in patients with chronic renal failure.

A typical feature of the uremic muscle cell is a significant increase in intracellular water [164] indicating inadequate volume regulation of the muscle cells. The same study also reported a minor decrease in the intracellular K^+ concentration of the muscles. In addition, measurements of membrane potential and intracellular Na^+ have shown that the membrane potential is decreased in uremic muscles, which was associated with a proportional increase in intracellular Na^+ [166].

The observations in muscles also suggests that Na,K-pump activity is reduced in uremia, which might be due to a reduced concentration of Na,K-pumps or an intrinsic inhibition of the Na,K-pump by the uremic plasma. In sarcolemma fractions isolated from the heart, a decrease in Na,K-ATPase activity has indeed been reported [167, 168]. Although some studies have indicated the presence of inhibitory factors in the uremic plasma, this question has not been settled yet. The observations that muscle membrane potential and intracellular Na^+ and K^+ are not normalized until after 6 to 12 weeks of dialysis [166] would rather suggest that the abnormalities in the uremic muscle are of more sustained character.

At least two of the factors responsible for the long-term regulation of the Na,K-pump concentration in muscle tissue may be of importance for the changes occurring during chronic renal failure. These include K^+ depletion and thyroid status. Similar to the effects of K^+ deficiency in intact animals or man, the K^+ depletion in uremia might in itself cause a reduction of the Na,K-pump concentration of the muscle tissue. Furthermore, it has been shown that plasma T_3 is decreased in uremia [169] resulting in "tissue hypothyroidism" [170] which also would result in a reduced concentration of Na,K-pumps.

It is concluded that, although direct measurements of Na,K-pump concentration in muscles of uremic patients are lacking, the available data indicate that the Na,K-pump concentration might be reduced. This could be secondary to a low T_3 level and perhaps in part to K^+ depletion, but remains to be established.

Conclusions

The largest acute physiological rise in plasma K^+ occurs during exercise and is caused by the net release of K^+ from the working muscles. This K^+ loss is restored by reaccumulation into the muscle cells via Na,K-pump dependent transport. Therefore, the pool of Na,K-pumps in skeletal muscle plays a central role in K^+ homeostasis and is subject to both acute and long-term regulation.

Acute activation of the Na,K-pump can be induced within minutes by excitation, catecholamines (a β-adrenoceptor effect) or insulin. This counterregulates the hyperkalemia induced by exercise or the ingestion of K^+-rich nutrients.

Long-term regulation is exerted by control of the concentration of Na,K-pumps. The major endocrine factor stimulating the synthesis of Na,K-pumps is thyroid hormone, and in man and animals the Na,K-pump concentration varies with thyroid status over a 6- to 10-fold range. In diabetes or during starvation, where plasma T_3 is reduced, the concentration of Na,K-pumps is decreased. The Na,K-pump concentration increases markedly during early growth and differentiation of muscle cells

and is upregulated in proportion to muscle activity. In contrast to what is seen in cell cultures, K^+ deficiency in the intact organism leads to a progressive downregulation of Na,K-pump concentration in muscle cells. The regulatory and clinical implications of these extrarenal control mechanisms are presented and discussed.

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