Na\(^{+}\)-K\(^{+}\) Pump Regulation and Skeletal Muscle Contractility

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Clausen, Torben. Na\(^{+}\)-K\(^{+}\) Pump Regulation and Skeletal Muscle Contractility. *Physiol Rev* 83: 1269–1324, 2003; 10.1152/physrev.00011.2003.—In skeletal muscle, excitation may cause loss of K\(^{+}\), increased extracellular K\(^{+}\) ([K\(^{+}\])\(_{o}\)), intracellular Na\(^{+}\) ([Na\(^{+}\])\(_{i}\)), and depolarization. Since these events interfere with excitability, the
processes of excitation can be self-limiting. During work, therefore, the impending loss of excitability has to be counterbalanced by prompt restoration of Na\(^+\)-K\(^+\) gradients. Since this is the major function of the Na\(^+\)-K\(^+\) pumps, it is crucial that their activity and capacity are adequate. This is achieved in two ways: 1) by acute activation of the Na\(^+\)-K\(^+\) pumps and 2) by long-term regulation of Na\(^+\)-K\(^+\) pump content or capacity. Depending on frequency of stimulation, excitation may activate up to all of the Na\(^+\)-K\(^+\) pumps available within 10 s, causing up to 22-fold increase in Na\(^+\) efflux. Activation of the Na\(^+\)-K\(^+\) pumps by hormones is slower and less pronounced. When muscles are inhibited by high [K\(^+\)]\(o\), or low [Na\(^+\)]\(o\), acute hormone- or excitation-induced activation of the Na\(^+\)-K\(^+\) pumps can restore excitability and contractile force in 10–20 min. Conversely, inhibition of the Na\(^+\)-K\(^+\) pumps by ouabain leads to progressive loss of contractility and endurance. 2) Na\(^+\)-K\(^+\) pump content is upregulated by training, thyroid hormones, insulin, glucocorticoids, and K\(^+\) overload. Down-regulation is seen during immobilization, K\(^+\) deficiency, hypoxia, heart failure, hypothyroidism, starvation, diabetes, alcoholism, myotonic dystrophy, and McArdle disease. Reduced Na\(^+\)-K\(^+\) pump content leads to loss of contractility and endurance, possibly contributing to the fatigue associated with several of these conditions. Increasing excitation-induced Na\(^+\) influx by augmenting the open-time or the content of Na\(^+\) channels reduces contractile endurance. Excitability and contractility depend on the ratio between passive Na\(^+\)-K\(^+\) leaks and Na\(^+\)-K\(^+\) pump activity, the passive leaks often playing a dominant role. The Na\(^+\)-K\(^+\) pump is a central target for regulation of Na\(^+\)-K\(^+\) distribution and excitability, essential for second-to-second ongoing maintenance of excitability during work.

I. INTRODUCTION

The Na\(^+\)-K\(^+\)-ATPase mediating the active coupled transport of Na\(^+\) and K\(^+\) across cell membranes was first identified in 1957 by Skou (392), who in 1997 was awarded the Nobel Prize in chemistry for this discovery. His review defining the role of this transport system, the Na\(^+\)-K\(^+\) pump, as a regulator of the transport and distribution of Na\(^+\) and K\(^+\) across cell membranes was published in this journal (393). This raised the question whether and how this transport regulator itself was regulated. Later reviews have described the regulation of the Na\(^+\)-K\(^+\) pumps in skeletal muscle (62), kidney (139), and heart (166). Many early or more general references may be found in these reviews. A related area, the K\(^+\) homeostasis in skeletal muscle and the heart during exercise, was recently reviewed (384). The detailed molecular mechanisms and specific signaling pathways involved in the regulation of the Na\(^+\)-K\(^+\) pumps in a wide variety of tissues have been reviewed (134, 408). The present review is written with the specific purpose of analyzing how regulation of the activity and the capacity of the Na\(^+\)-K\(^+\) pumps influences excitability and contractile performance in skeletal muscle. With the growing realization that the Na\(^+\)-K\(^+\) pumps undergo large regulatory changes both in their transport activity and capacity (tissue content of Na\(^+\)-K\(^+\)-ATPase), the functional implications of such changes are gaining interest; in particular for the understanding of the frequently occurring pathophysiological and pharmacological modifications of Na\(^+\)-K\(^+\) pump function. Therefore, the more general homeostatic role of Na\(^+\)-K\(^+\) pump regulation in skeletal muscle will also be illustrated by some clinical examples (for a more general review on the clinical significance of the Na\(^+\)-K\(^+\) pump in various tissues, see Ref. 65).

II. THE PLASMA MEMBRANE AND ITS Na\(^+\)-K\(^+\) TRANSPORT PATHWAYS

Skeletal muscle cells are surrounded by an outer plasma membrane (sarcolemma), which is covered by a glycocalyx layer. From the cell surface, invaginations of the sarcolemma extend into the cell interior, forming a grid of tubules running transversely across the cell. The lumina of these t tubules are open to the extracellular space, allowing ions and metabolites by simple diffusion to reach transport systems in the walls of t tubules in most parts of the muscle cell. This system not only offers a large membrane surface for the exchange of various compounds between the cytoplasm and the extracellular space, but also allows the propagation of action potentials from the sarcolemma to the terminal cisternae of the sarcoplasmatic reticulum (SR) in every part of the cell (89, 218). This ensures prompt and coordinated release of Ca\(^2+\) from the SR to the muscle fibrils in the entire cell. In skeletal muscle, Na\(^+\) and K\(^+\) are exchanged across the plasma membrane of sarcolemma and t tubules via a number of specific transport systems. As illustrated in Figure 1, the passive movements of Na\(^+\) and K\(^+\) are counterbalanced by one single active transport system, the Na\(^+\)-K\(^+\) pump. The major passive fluxes are mediated by the voltage-sensitive Na\(^+\) channels and at least four different categories of K\(^+\) channels (208). During excitation, the action potentials are elicited by a rapid and marked influx of Na\(^+\) via the Na\(^+\) channels, immediately followed by an almost equivalent efflux of K\(^+\). These fluxes may exceed the capacity of the Na\(^+\)-K\(^+\) pumps (basal or stimulated) for restoring Na\(^+\)-K\(^+\) distribution (see sect. viA). Studies on frog and snake skeletal muscle indicate that at variance with many other membrane proteins, the Na\(^+\) channels and most of the K\(^+\) channels are immobile, possibly due to an anchoring to the cell mem-
Bumetanide as well as furosemide clearly inhibited $^{22}\text{Na}$ uptake and induced progressive reduction in intracellular $\text{Na}^+$ content. This was not associated with any change in $^{42}\text{K}$ uptake or $\text{K}^+$ content. Neither was there any effect of bumetanide on $^{42}\text{K}$ efflux or net $\text{K}^+$ loss during excitation, nor on the reaccumulation of $\text{K}^+$ following stimulation. The conclusion of these studies is that in skeletal muscle, there is no functional NaK2Cl symport system, but apparently a NaCl symporter that can be activated under hyperosmolar conditions and suppressed by bumetanide or furosemide.

In addition, the transmembrane $\text{Na}^+$ gradient is driving the uptake of a variety of amino acids, inorganic phosphate (238), and the $\text{Na}^+$/Ca$^{2+}$ antiporter (17). Finally, a NaHCO$_3$ cotransport system has been identified in skeletal muscle (358). These processes and systems may all contribute to the uptake of $\text{Na}^+$, but little is known about their transport properties or capacity.

In resting rat soleus, the influx and efflux of $^{42}\text{K}$ are markedly inhibited by the above-mentioned membrane stabilizers (98, 78), and by Ba$^{2+}$ (83), which also inhibits $^{86}\text{Rb}$ efflux in frog semitendinosus muscle (53). This indicates that a large part of the resting passive $\text{K}^+$ fluxes are mediated by $\text{K}^+$ channels. The membrane stabilizers or Ba$^{2+}$ caused no change in the $\text{Na}^+$-K$^+$ pump-mediated $\text{Na}^+$-$\text{K}^+$ fluxes.

In frog semitendinosus muscle, metabolic poisoning with cyanide and iodoacetate induced a modest increase in $\text{K}^+$ efflux. Subsequent complete metabolic exhaustion elicited by electrical stimulation at 1 Hz for a few minutes induced a five- to sixfold increase in both $^{42}\text{K}$ and $^{86}\text{Rb}$ efflux. This increase was suppressed by Ba$^{2+}$, glibenclamide, tolbutamide, TEA, or local anesthetics, but not by inhibitors of the delayed rectifier or Ca$^{2+}$-activated K$^+$ channels (53). This indicates that during metabolic exhaustion, the ATP-sensitive K$^+$ channels mediate a large K$^+$ efflux.

There is substantial evidence that the $\text{Na}^+-\text{K}^+$ pump is electrogenic and therefore contributes to the maintenance of the membrane potential in skeletal muscle (2, 62, 158). In isolated rat soleus, blocking the $\text{Na}^+-\text{K}^+$ pump with ouabain (10$^{-3}$ M) caused 8-mV depolarization in 10 min. Conversely, stimulating the $\text{Na}^+-\text{K}^+$ pump with epinephrine caused 7.5-mV hyperpolarization in 10 min (72). It cannot be excluded that $\text{Na}^+-\text{K}^+$ pump stimulation, by reducing $[\text{K}^+]_i$, might induce hyperpolarization by increasing the $\text{K}^+$ equilibrium potential. Conversely, indirect depolarizing effects of elevated $[\text{K}^+]_o$ could be envisaged in tissues exposed to Na$^+$-$\text{K}^+$ pump inhibition. In frog extensor digitorum longus (EDL) muscles, rapid increase or decrease in temperature of 17°C caused, respectively, hyperpolarization and depolarization of ~10 mV within 2 s. About half of these changes were attributed to changes in the $\text{K}^+$ concentration in the t-tubular lumen,

**FIG. 1.** Na$^+$-K$^+$ transport pathways in skeletal muscle. The transmembrane Na$^+$-K$^+$ concentration gradients are generated by the continuous operation of the Na$^+$-K$^+$ pumps. The Na$^+$ gradient drives the cotransport of amino acids and chloride and the antiport of H$^+$ and Ca$^{2+}$. By far the major influx pathways for Na$^+$ are the voltage-sensitive Na$^+$ channels. Passive K$^+$ fluxes are mediated by the voltage-sensitive inward rectifier (inw.) and delayed rectifier (del.), the Ca$^{2+}$-sensitive and the ATP-sensitive K$^+$ channels. [Modified from Clausen (64).]

brane (367). This might limit lateral movements in the membrane or between t tubules and sarcotendinous.

In resting rat soleus muscle, $^{22}\text{Na}$ uptake is inhibited by a wide variety of membrane stabilizing agents, such as local anesthetics, barbiturates, phenothiazines, propranolol (78, 98), as well as the Na$^+$ channel blocker tetrodotoxin (TTX) (112), indicating that part of the resting uptake of Na$^+$ is mediated by Na$^+$ channels. This type of inhibition leads to a decrease in intracellular Na$^+$ ($[\text{Na}^+]_i$). In rat soleus, TTX reduced $^{22}\text{Na}$ uptake by 17%. This effect was additive to that of the NaK2Cl symport inhibitor bumetanide, which produced an inhibition of 23% and the Na$^+$/H$^+$ antiporter inhibitor amiloride, causing 19% inhibition. When exposed to all three inhibitors, $^{22}\text{Na}$ uptake was reduced by 54%, which is in good agreement with the sum of the observed effects of the three inhibitors (59%).
and the other half were directly related to the electrogenic action of the Na\(^+\)-K\(^+\) pump (158). On the other hand, in isolated rat skeletal myotubes, where diffusional limitations for K\(^+\) are negligible, ouabain or cooling from 37 to 13°C induced up to 20- to 25-mV depolarization (35). Studies with chick myotubes showed that ouabain induced a comparable depolarization within 2–5 s, which was not accompanied by any change in input resistance (40). When exposed to K\(^+\)-free buffer, which prevents the operation of the Na\(^+\)-K\(^+\) pump, the cells showed the same depolarization as in the presence of ouabain (10\(^{-3}\) M).

In conclusion, the Na\(^+\)-K\(^+\) pumps generate steep transmembrane gradients for Na\(^+\) and K\(^+\), allowing the maintenance of the membrane potential, excitability, and the operation of several secondary active transport processes. In addition, the Na\(^+\)-K\(^+\) pump has a direct and rapid (within seconds) electrogenic action, arising from its 3:2 exchange of Na\(^+\) against K\(^+\).

III. LOCALIZATION OF THE Na\(^+\)-K\(^+\) PUMPS

A. Sarcolemma

In skeletal muscle, the Na\(^+\)-K\(^+\) pumps are located in the sarcolemma and the t tubules. This has been documented using isolation of membrane preparations by differential centrifugation of muscle homogenates combined with electron microscopic identification of membrane vesicles (55) as well as by immunofluorescence labeling (136, 435). Although it has been possible to isolate and purify sarcolemma preparations from muscle homogenates, most procedures reported are complicated and inadequate from a quantitative point of view (see sect. mB). Measurements of \([3H]\)ouabain binding indicate that in sarcolemma, the density of Na\(^+\)-K\(^+\) pumps is \(\sim 3,350\) molecules/\(\mu m^2\) in the soleus of 4-wk-old rats (75), 2,500 molecules/\(\mu m^2\) in frog sartorius (422), and 1,000–1,800 molecules/\(\mu m^2\) in muscles from 8-wk-old pigs (196). However, these values are based on the assumption that all \([3H]\)ouabain binding sites are located in the sarcolemma. Because most of the \([3H]\)ouabain binding sites may be located in the t tubules, the density of Na\(^+\)-K\(^+\) pumps in sarcolemma is probably much lower (see sect. mB).

B. T Tubules

Already long ago, the t tubules were shown to constitute an extremely branched system. Because the visualization of the structure has not been surpassed since the publication of Peachey and Eisenberg in 1978 (348), their figure was selected to illustrate the extraordinary complexity (Fig. 2). Later studies using other techniques have demonstrated a network of similar complexity in muscles of the guinea pig (155) and the rat (435).

Single fibers prepared from frog semitendinosus muscles maintained contractile response even after the sarcolemma had been removed and the t tubules sealed (90), implying that the t-tubular membranes maintain concentration gradients for Na\(^+\) and K\(^+\) similar to those across the sarcolemma. This requires Na\(^+\)-K\(^+\) pumps, and the contractile response was abolished by inhibiting the Na\(^+\)-K\(^+\) pumps with strophanthidin, added before the removal of sarcolemma so as to allow access of this cardiac glycoside to the lumen of the t tubules. Thus, even when the connections between sarcolemma and the t tubules have been disrupted, the tubular membranes can carry excitatory action potentials, and this ability depends on functional Na\(^+\)-K\(^+\) pumps. Another important implication is that the propagation of the excitatory signals along...
the t-tubular network does not depend on diffusional Na⁺-K⁺ exchange between the t-tubular lumen and the interstitial water space. It is possible that most of the excitation-induced passive Na⁺-K⁺ fluxes are directly counterbalanced by the Na⁺-K⁺ pumps situated in the t-tubular walls and that this localized circuitry for Na⁺ and K⁺ is adequate for the maintenance of signal propagation into the interior of the cell.

T-tubular membranes isolated from rabbit sarcoplasmic skeletal muscle and identified by their morphological association with the terminal cisternae bind [³H]ouabain (55) and contain Na⁺-K⁺-ATPase (266). When [³H]ouabain was injected directly into the muscles 20 min before homogenization, or intravenously 2 h before homogenization, this tracer was trapped inside the lumen of resealed t-tubular vesicles and could be recovered in the microsomal fraction. Isolated t tubules prepared from rabbit sarcoplasmicus muscle were permeable to digitoxin, but not to [³H]ouabain (266). When pre-treated with deoxycholate, however, the t tubules took up [³H]ouabain and bound it with high affinity (37 pmol/mg protein, Kᵰ = 5.4 × 10⁻⁸ M). The tubules also accumulated Na⁺ by an ATP-dependent process, suppressible by digitoxin. Thus it is rather directly documented that the Na⁺-K⁺ pumps in the t tubules operate to maintain the transmembrane concentration gradient for Na⁺ required for action potential propagation.

In intact frog sartorius muscle, treatment with hyperosmolar glycerol disconnected 90% of the t tubules from the sarcolemma, as estimated from the accessibility of the t-tubular lumen to lanthanum as an extracellular marker. In this preparation, when incubated for 6–8 h, strophanthidin-suppressible ²²Na efflux was halved (421), indicating that the Na⁺-K⁺ pumps in the t tubules are major contributors to the active Na⁺-K⁺ transport in skeletal muscle.

An attempt was also made to quantify the amount of [³H]ouabain binding to the t tubules in the intact frog sartorius muscle by comparing untreated muscles with glycerol-pretreated muscles (422). The glycerol pretreatment only reduced [³H]ouabain binding by ~20%, indicating that the density of ouabain binding sites in the t-tubular membrane is only around 5% of that of the sarcolemma (125 vs. 2,500 sites/μm², respectively). This estimate for the density of [³H]ouabain binding sites in the t-tubular membrane is in good agreement with that which can be calculated from the above-mentioned data of Lau et al. (266) (180 sites/μm²). However, these values for t-tubular Na⁺-K⁺ pump density are likely to be much too low, because in a whole muscle, the glycerol pretreatment procedure may not give complete disruption of the t-tubular connections to sarcolemma. Even if only 10% of these connections are preserved, [³H]ouabain may gain access to the tubular lumen via the recently described longitudinal connections (356). Therefore, the measurements will not detect much of a decrease in [³H]ouabain binding after the glycerol pretreatment. More importantly, measurements of the content of [³H]ouabain binding sites in t-tubular and sarcolemmal membranes obtained from frog skeletal muscle showed similar values; 215 and 163 pmol/mg protein, respectively (222). The same study showed that the t tubules from rabbit skeletal muscle contained 169 pmol/mg protein. These rather high values would correspond to a Na⁺-K⁺-ATPase activity of ~80 μmol ATP split · h⁻¹ · mg protein⁻¹, which is not too far from the values of 39 and 50 μmol · h⁻¹ · mg protein⁻¹ reported for t tubules from rabbit muscle (200, 206). In the sarcolemma of rabbit skeletal muscle, Mitchell et al. (209) obtained a Na⁺-K⁺-ATPase activity of 57 μm · h⁻¹ · mg protein⁻¹, rather close to that of t-tubular membranes (39 μmol · h⁻¹ · mg protein⁻¹). In rat skeletal muscle, the Na⁺-K⁺-ATPase activity in a membrane fraction containing primarily t tubules was 58% of that in the fraction containing sarcolemma (20). Thus, as already pointed out by Hidalgo et al. (206), the contents of Na⁺-K⁺-ATPase in t tubules and sarcolemma are not as different as suggested by the [³H]ouabain binding studies by Venosa and Horowicz (422).

Following sarcolemmal leakage due to micropuncture or cuts, the t tubules in the adjacent segments of the muscle fiber within minutes underwent considerable swelling leading to vacuolation (52). Because this swelling could be prevented by ouabain, cooling, or inhibiting energy metabolism, it was attributed to the Na⁺ leaking into the cytoplasm being pumped into the t-tubular lumen and favoring the entry of water from the surrounding cytoplasm. These structural studies indicate that the Na⁺-K⁺ pumps in the t tubules possess a considerable transport capacity and are important for the exchange of Na⁺ and water.

Immunofluorescence labeling showed that in the human soleus muscle, the α₁-subunit isoform was mainly located in the sarcolemma, whereas the α₂-subunit isoform was observed both in the sarcolemma and diffusely distributed in the muscle fibers, possibly located in the t tubules (217). The plasma membrane seems to contain the α₂-subunit isoform. In the guinea pig heart, the t tubules and sarcolemma contain only the α₁-subunit isoform of the Na⁺-K⁺ pump, whereas in the rat heart, t tubules contained both α₁- and α₂-subunit isoforms (291). More recent immunofluorescence and immunoprecipitation studies of rat and mouse skeletal muscle showed that whereas the sarcolemma contains both the α₁- and the α₂-subunit, the t-tubular membranes contain only the α₂-subunit and no α₁-subunit (435). The same study showed that both the α₁- and α₂-subunit isoforms were associated with β-spectrin and ankyrin. This was proposed to concentrate both isoform subunits in costameres present at the sarcolemma over Z and M lines and in longitudinal strands. Thus, in mutant mice lacking β-spectrin, the Na⁺-K⁺-ATPase subunit isoforms were not concentrated in costameres, but seemed to be released from the anchor.
ing to become more uniformly distributed in the sarcolemma.

In conclusion, the preponderant evidence indicates that the content of Na\(^+\)-K\(^+\) pumps/\(\mu\)m\(^2\) of t-tubular wall is not far below that of sarcolemma. Because the area of the t-tubular membranes is severalfold larger than that of the sarcolemma, this implies that in the entire muscle cell, most of the Na\(^+\)-K\(^+\) pumps are situated in the t tubules, allowing rapid clearance of K\(^+\) from the t-tubular lumen during excitation. This transport mechanism may be more efficient than the diffusion of K\(^+\) out of the t-tubular network and therefore decisive for the maintenance of excitability.

C. Intracellular Pools

In cell cultures prepared from chick embryo leg muscles, immunolabelling showed that the Na\(^+\)-K\(^+\)-ATPase located in an intracellular pool corresponded to \(~60\)% of the total amount of the enzyme present in the cells (136, 436). Unfortunately, the precise localization of this pool was not identified, and it cannot be excluded that due to the use of saponin and formaldehyde during the procedures, the t tubules had been permeabilized so as to allow binding to the Na\(^+\)-K\(^+\) pumps situated in the t tubules. In this and some other studies, part of the pool of Na\(^+\)-K\(^+\) pumps termed “intracellular” may be residing in the t tubules (136). Several studies have described an intracellular pool, from where Na\(^+\)-K\(^+\) pumps may be recruited to the sarcolemma by hormones or excitation. This pool is not homogeneous and may include subsarcolemmal vesicles, triad junctions, and t tubules, and the relative distribution of Na\(^+\)-K\(^+\) pumps among these structures has not been quantified (216, 267, 268, 285). Another uncertainty in quantitative comparisons to the plasma membrane pool is that the protein recovery of intracellular membranes is eightfold larger than that of plasma membrane (216).

In conclusion, the intracellular pool, from where Na\(^+\)-K\(^+\) pumps are recruited to the sarcolemma, may in part be situated in the t tubules, but more precise identification and quantification is needed.

IV. STRUCTURE, IDENTIFICATION, AND QUANTIFICATION OF Na\(^+\)-K\(^+\) PUMPS

A. General Structure and Isoforms of Na\(^+\)-K\(^+\)-ATPase

As shown in Figure 3, the Na\(^+\)-K\(^+\)-ATPase is a heterodimer, composed of two protein subunits, a catalytic \(\alpha\)-subunit involved in the splitting of ATP (molecular mass \(~112\) kDa) and a \(\beta\)-subunit (\(~35\) kDa). The \(\alpha\)-subunit which actually pumps Na\(^+\) and K\(^+\) contains binding sites for Na\(^+\), K\(^+\), ATP, and digitalis glycosides. It has 10 transmembrane segments and depends on the \(\beta\)-subunit for transport activity. The \(\beta\)-subunit is a glycoprotein, necessary for the transfer of the entire enzyme molecule from its site of synthesis in the endoplasmic reticulum to its site of insertion in the plasma membrane. A \(\gamma\)-subunit (not shown) has been described in several tissues and seems to have regulatory function.

Like many other proteins, the subunits of the Na\(^+\)-K\(^+\)-ATPase are expressed in various isoforms, which can be detected using specific antibodies (403). Four isoforms of the \(\alpha\)-subunit (\(\alpha_1-\alpha_4\)) and three isoforms of the \(\beta\)-subunit (\(\beta_1-\beta_3\)) have been identified. In skeletal muscle, \(\alpha_1\), \(\alpha_2\), \(\beta_1\), and \(\beta_2\) are expressed, only minor amounts of \(\alpha_3\) have been found, and the \(\gamma\)-subunit has not been detected. Rat skeletal muscle in vivo expresses both the \(\alpha_1\)-, \(\alpha_2\)-, \(\beta_1\)-, and \(\beta_2\)-subunits (216, 267, 403). The ratio between \(\alpha_2\) and \(\alpha_1\) was reported to amount to 1.6 in the surface membranes and to approach 7 in the internal membranes (267), and \(\alpha_1\) seems to be mostly confined to sarcolemma (216). Primary cultures of rat skeletal muscle, however, express only the \(\alpha_1\)-, \(\beta_1\)-, and \(\beta_2\)-subunit isoforms, even though they contain mRNA for \(\alpha_2\) (387) and in L6 rat myogenic cells, only \(\alpha_1\)- and \(\beta_1\)-isoform protein and mRNA are expressed (388). In avian skeletal muscle cells, only the \(\alpha_1\)-isoform is expressed (405).

In a cell line obtained from mouse skeletal muscle (C2C12) the mRNA and protein levels of the \(\alpha_1\)-subunit isoform remained constant during differentiation. Concomitantly, the \(\alpha_2\)-subunit isoform underwent a marked increase, but this was found to contribute little to tonic homeostasis (207). In contrast, in the intact rat skeletal muscle advancing age from 6 to 30 mo was associated with marked increase in \(\alpha_1\)- and \(\beta_1\)-subunit and a decrease in \(\alpha_2\)- and \(\beta_2\)-subunit (396).

Cultured myotubes derived from chicken were found to bind 4.8 \(\times\) \(10^5\) molecules [\(^3\)H]ouabain/cell. Using a monoclonal \(^{35}\)S-labeled antibody, an almost identical number of antigenic sites was measured (135). A more general use of such comparisons would allow for a “translation” of specific antibody binding data to [\(^3\)H]ouabain binding data. This might solve some of the discrepancies arising from isoform studies.

Thus there seems to be little consensus with respect to the relative content of \(\alpha_1\)- and \(\alpha_2\)-subunit isoforms in skeletal muscle. With one exception (188), the content of isoform \(\alpha_1\)- or \(\alpha_2\)-subunits as expressed per gram tissue wet weight has unfortunately not been quantified. Using an \(\alpha_1\)-specific antibody to chicken kidney Na\(^+\)-K\(^+\)-ATPase, the content of \(\alpha_1\)-subunit isoform in soleus and EDL of 4-wk-old rats was determined. The content of \(\alpha\)-subunit varied from 15 to 30\% of the content of the \(\alpha_2\)-isoform quantified using [\(^3\)H]ouabain binding, thus representing
14–21% of the total content of Na⁺-K⁺-ATPase. Although these results seem more accurate than most other estimates of isoform abundancy, they leave some questions unanswered. Thus the antibody used was raised against the α₁-subunit from another species (chicken), and it cannot be excluded that it will interact differently with the α₁-subunits present in the muscles and the purified rat kidney α₁-subunit used as a reference. Moreover, the antibody may interact with newly synthesized α-peptide molecules, not necessarily representing functional Na⁺-K⁺ pumps situated in the membranes. Another recent study indicated that in the EDL muscle of mice the α₂-subunit isoform accounted for 87% of the total amount of α-subunit isoform of Na⁺-K⁺-ATPase, whereas the α₁ isoform accounted for only 13% (202).

The research on subunit isoforms has been motivated by the expectation that the different isoforms would be linked to specific tissues and cell structures or serve specific functions. In the rat, this expectation has been fulfilled by the observation of a preponderance of α₂-subunit isoform in the t tubules. An early suggestion was that the α₁-subunit isoform might be a “housekeeping version” of the Na⁺-K⁺ pump, involved in the maintenance of basic transport function, whereas the α₂ isoform would be the regulated version. This seems unlikely, however, since in the kidney, mainly the α₁ isoform is found, which would then preclude the well-documented regulation of renal Na⁺-K⁺-ATPase (139). Likewise, in avian muscle, only the α₁ isoform has been found (135, 405), with the same unfortunate implications for the regulation of Na⁺-K⁺ pumps in birds. In the rat heart, the α₁-subunit isoform is by far the most abundant. Still, the Na⁺-K⁺ pumps in the heart are definitely subject to regulation by hormones (166). In cultured human fibroblasts containing only the α₁-subunit isoform, insulin stimulates ⁸⁶Rb uptake (279). Moreover, in K⁺-deficient rats, where the ratio between α₂- and α₁-subunit isoform was reduced from 2.1 to 0.3, there was still a full stimulating effect of insulin and epinephrine on Na⁺-K⁺ pump mediated ⁸⁶Rb uptake (214). In rat skeletal muscle, exercise caused the same relative increase in plasma membrane content of the α₁- and α₂-subunit isoform (414).

Finally, the concept of regulation being exerted on a specific subunit isoform has not been causally linked to
any specific structural or functional properties of the Na\textsuperscript{+}-K\textsuperscript{+}-ATPase molecule. In brief, we have to know why the \(\alpha\textsubscript{2}\)-subunit isoform should be regulated and not the \(\alpha\textsubscript{1}\)-subunit isoform.

In conclusion, the detection and description of subunit isoforms has added much detail to the information about Na\textsuperscript{+}-K\textsuperscript{+} pumps, their localization, and regulation. However, the quantitative analysis is still inaccurate and the functional significance of the different isoforms is not yet precisely explained.

**B. Measurement of Na\textsuperscript{+}-K\textsuperscript{+}-ATPase Activity**

Crude homogenates of skeletal muscle contain very high concentrations of various ATPases, and only a minor fraction can be identified as specifically activated by Na\textsuperscript{+}, K\textsuperscript{+}, and Mg\textsuperscript{2+} or suppressed by cardiac glycosides. It is difficult, therefore, to obtain accurate values for the total content of Na\textsuperscript{+}-K\textsuperscript{+}-ATPase activity. Because most of the early studies on Na\textsuperscript{+}-K\textsuperscript{+}-ATPase activity were focused on the identification and molecular characterization of the enzyme, the strategy was to obtain highly purified membrane preparations containing high concentrations of Na\textsuperscript{+}-K\textsuperscript{+}-ATPase rather than to gain information about the total content of the enzyme in a given tissue. If these classical procedures developed for purification should be applied for quantification, detailed and precise information about the recovery of Na\textsuperscript{+}-K\textsuperscript{+}-ATPase activity is required. However, analysis of 17 papers with information on the yield of Na\textsuperscript{+}-K\textsuperscript{+}-ATPase activity showed that the methods published only allow between 0.02 and 8.9% recovery of the total content of Na\textsuperscript{+}-K\textsuperscript{+}-ATPase activity in skeletal muscle (189). Later estimates of the recovery have given values of 4% (336) and 1% (3) (see comment in Ref. 190). This is likely to give misleading information about the regulation of the total content of Na\textsuperscript{+}-K\textsuperscript{+}-ATPase activity in skeletal muscle, also because it is difficult to ascertain that such small samples of the total pool of Na\textsuperscript{+}-K\textsuperscript{+}-ATPase are representative for the plasma membrane. Thus the Na\textsuperscript{+}-K\textsuperscript{+}-ATPase obtained in the final steps of the purification procedures may have originated from contaminating nervous and vascular tissue, adipocytes, and fibrocytes, and not from the plasma membrane (190).

In crude homogenates of skeletal muscle, the content of Na\textsuperscript{+}-K\textsuperscript{+}-ATPase activity can be measured by a highly sensitive fluorometric assay using 3-O-methylfluorescein phosphate as substrate (215). This enzyme activity (3-O-MFPase) is stimulated by K\textsuperscript{+}, completely inhibited by ouabain, and reflects the amount of Na\textsuperscript{+}-K\textsuperscript{+}-ATPase (329, 330). On the basis of measurements of the molecular 3-O-MFPase activity (620 min\textsuperscript{-1}), the content of Na\textsuperscript{+}-K\textsuperscript{+}-ATPase activity in muscle homogenates could be quantified and was in satisfactory agreement with measurements of [\(^{3}\text{H}\)] ouabain binding capacity of the intact muscle (329). Modified versions of this procedure were used for quantification of 3-O-MFPase activity in rodent heart (330, 381) and human skeletal muscle (152, 154, 295). In biopsies of human vastus lateralis muscle, a significant correlation was found between [\(^{3}\text{H}\)] ouabain binding site content and 3-O-MFPase activity (153).

3-O-MFPase activity of muscle homogenates only reached its optimum value when deoxycholate was added. This was attributed to the opening of inside-out vesicles of sarcolemma, formed during the homogenization. Later studies showed that repeated freeze-thaw cycles produced a similar effect, possibly by opening vesicles or t-tubules (152). It is interesting that the binding of [\(^{3}\text{H}\)] ouabain to crude muscle homogenate was increased 10-fold by deoxycholate (266). It is possible that both phenomena reflect the opening of t-tubules and that most of the Na\textsuperscript{+}-K\textsuperscript{+}-ATPase in skeletal muscle resides in the lumen of these structures (see sect. \(\mu\)B).

In conclusion, the use of sophisticated purification procedures has yielded much valuable information about the localization, kinetics, molecular biology, and structure of the Na\textsuperscript{+}-K\textsuperscript{+}-ATPase in skeletal muscle. For the quantification of the enzyme, however, measurements of [\(^{3}\text{H}\)] ouabain binding or K\textsuperscript{+}-activated 3-O-MFPase activity are more accurate than estimates based on purified plasma membranes, corrected by the recovery.

**C. Measurement of [\(^{3}\text{H}\)] ouabain Binding**

Studies on Na\textsuperscript{+}-K\textsuperscript{+}-ATPase purified from various sources show that cardiac glycosides bind to the \(\alpha\)-subunit of the enzyme with a 1:1 molecular stoichiometry (186). This is the basis for the widespread use of [\(^{3}\text{H}\)] ouabain for the quantification of Na\textsuperscript{+}-K\textsuperscript{+} pumps in tissues, cells, and isolated membrane preparations.

Intact skeletal muscle preparations bind [\(^{3}\text{H}\)] ouabain to the sarcolemma and the luminal surface of the t-tubular membranes. In rat soleus muscles that had been equilibrated with [\(^{3}\text{H}\)] ouabain (10\textsuperscript{-6} M) for 120 min to reach complete saturation, the fractional loss of [\(^{3}\text{H}\)] ouabain during a subsequent washout in an ice-cold buffer was only marginally affected by cutting the fibers. This argues against any major intracellular accumulation of [\(^{3}\text{H}\)] ouabain (75).

Also in vivo, 15 min after intraperitoneal injection of a saturating dose of [\(^{3}\text{H}\)] ouabain to the rat, this ligand was bound to skeletal muscles and the content of [\(^{3}\text{H}\)] ouabain binding sites could be quantified after removing the unbound [\(^{3}\text{H}\)] ouabain by subsequent washout in ice-cold buffer (77). Over an eightfold range of values there was no significant difference between the content of [\(^{3}\text{H}\)] ouabain binding sites as determined by incubating intact muscles with the ligand in vitro or by injection of [\(^{3}\text{H}\)] ouabain into the intact animal. This indicates that the access and bind-
ing of [3H]ouabain in the isolated muscles in vitro is as complete as in the intact rat with preserved circulation and oxygenation. Furthermore, the in vivo studies showed that the method can be used for quantification of changes in [3H]ouabain binding site content under different conditions (K⁺ deficiency, ageing, denervation), yielding the same results as in vitro measurements. It should be recalled, though, that other animals than the rat might not tolerate being injected with ouabain at the required concentration. The reason why rats tolerate being injected with ouabain is that part of the Na⁺-K⁺ pumps in the heart, kidney, and brain contain the α₁-subunit isoform and therefore have a low affinity for ouabain. This allows the animals to avoid severe blockade of Na⁺-K⁺ pumps and functional impairment in these vital organs. This also implies that in the rat the [3H]ouabain binding assay cannot be used for these tissues.

In rat skeletal muscle, a minor fraction of the Na⁺-K⁺ pumps contain the α₁-subunit isoform, which may not be detected at the concentrations of [3H]ouabain used in the standard assay (10⁻⁶ to 5 × 10⁻⁶ M). Measurements of the maximum rate of ouabain-suppressible active Na⁺-K⁺ transport in intact rat muscles and 3-O-MFPase activity in crude homogenates have given values in reasonable agreement with those obtained in the standard [3H]ouabain binding assay (71, 329), indicating that this method quantifies the major part of the total content of Na⁺-K⁺ pumps in rat skeletal muscle.

The initial rate of [3H]ouabain binding can easily be quantified by exposing a muscle to [3H]ouabain for 15 min at 30°C, followed by four 30-min washouts in ice-cold buffer to remove [3H]ouabain not bound. Several in vitro studies have shown that when active Na⁺-K⁺ transport in intact muscles is stimulated by Na⁺ loading, insulin, insulin-like growth factor I (IGF-I), catecholamines, amylin, and excitation, the rate of [3H]ouabain binding increases (66, 76, 111, 127). Also in the intact organism, the rate of [3H]ouabain binding to rat soleus and EDL muscles is considerably accelerated (66–82%) by the β₂-agonist salbutamol (242). Conversely, pretreatment with tetracaine decreased Na⁺-K⁺ pump-mediated 42K uptake and reduced [3H]ouabain binding rate (76). A close correlation between ouabain-suppressible 42K uptake and the rate of [3H]ouabain was seen over a wide range of values (76). This correlation reflects that during increased rate of pumping, the Na⁺-K⁺-ATPase will more frequently exist in the conformation optimal for the binding of ouabain. An increase in the rate of [3H]ouabain binding can be taken as rather specific evidence that the pumping rate is accelerated.

As originally observed by Hansen et al. (191), vanadate (VO₄) can replace ATP in facilitating the binding of [3H]ouabain to the Na⁺-K⁺-ATPase. VO₄ is a PO₄ analog, and to exert this action, it must bind to the phosphorylation site of the Na⁺-K⁺ pumps on the intracellular surface of the plasma membrane. This high-affinity binding of VO₄ maintains the Na⁺-K⁺-ATPase in a configuration capable of binding [3H]ouabain to the extracellular surface of the molecule. This formed the basis for the development of a VO₄-facilitated binding assay for quantification of the content of [3H]ouabain binding sites in cut specimens of skeletal muscle. The assay is based on the concept that VO₄ via the cut ends of the muscle fibers gains access to the phosphorylation sites on the inner surface of the Na⁺-K⁺-ATPase. When incubated in a VO₄-containing buffer, cut muscle specimens bound the same amount of [3H]ouabain per gram wet weight as intact muscles obtained from the contralateral leg of the same rat and incubated in K⁺-free Krebs-Ringer bicarbonate buffer. Over a ninefold range of values, the two procedures gave the same results (331). Several methodological studies showed that to get accurate results, muscle specimens weighing ~5 mg should be equilibrated for 120 min at 37°C in a Tris buffer containing VO₄ and [3H]ouabain (10⁻⁶ M). Following a washout for 4 × 30 min in ice-cold buffer, to remove the [3H]ouabain not bound, the tissue specimens are blotted and taken for counting of [3H]ouabain. The values should be corrected for nonspecific uptake of [3H]ouabain, loss of specifically bound [3H]ouabain during washout, weight loss due to evaporation from the muscle specimens, impurity of the [3H]ouabain, and incomplete saturation of [3H]ouabain binding sites (241, 251). For measurements on rat skeletal muscle, these correction factors are usually lumped into one of around 1.3, by which the counted value for [3H]ouabain binding is multiplied. If the washout in the cold is omitted, and the results are corrected for the [3H]ouabain residing in the extracellular water space, the same values are obtained, albeit with a larger scatter (243). Measurements performed over a wide range of ouabain concentrations showed no evidence of heterogeneity of the [3H]ouabain binding sites in rat soleus muscle specimens, and the apparent Kᵦ was ~5 × 10⁻⁸ M (243). Neither in biopsies of human vastus lateralis muscle, nor in guinea pig skeletal muscle, Scatchard plots showed any evidence of heterogeneity (251, 328). The same procedure was developed and tested for guinea pig skeletal muscle and heart (251) as well as for the heart of dogs and pigs (381).

The VO₄-facilitated assay for [3H]ouabain binding has been widely used for measurement on biopsy specimens from the human vastus lateralis muscle and several other muscles. As shown in Table 1, the values obtained for control subjects are rather similar in 15 studies performed in 6 different laboratories.

Some of the values were higher because they were measured in rather well-trained control subjects (133, 298). It is important that the method can be used for frozen samples, allowing long-distance transport or long-term storage of the frozen samples for at least 11 wk (328), or, as found later, for up to 4 yr (Clausen, unpublished observations). Furthermore, the VO₄-facilitated [3H]ouabain binding assay has been adapted for measurements on specimens...
from the human heart (324, 325), porcine and canine heart (381), and uterine smooth muscle (132).

A comparison of the binding kinetics of \( ^{3} \text{H}\)ouabain in a variety of human tissues showed that the affinity of \( \alpha_{1} \), \( \alpha_{2} \), and \( \alpha_{3} \)-subunit isoforms for ouabain is similar (306, 426). This confirms repeated observations that in biopsies of human skeletal muscle and myocardium, only a single population of high-affinity sites can be detected (242, 328). This implies that in human skeletal muscle and myocardium, Na\(^{+}\)-K\(^{+}\) pumps can be quantified by measuring the content of \( ^{3} \text{H}\)ouabain binding sites.

The \( \text{VO}_{4}\)-facilitated binding assay also allows measurements to be performed on muscle samples taken post mortem (332). A comparison of the values obtained in fresh rat skeletal muscle with those determined after storage at 20°C for 12 h showed that the loss of the ability to bind \( ^{3} \text{H}\)ouabain was surprisingly slow, −1%/h. Studies on vastus lateralis muscle samples obtained from 10 human subjects 0.5–6 h after death showed that the content of \( ^{3} \text{H}\)ouabain binding sites declined by only 8% in 6 h (332). Another post mortem study on vastus lateralis in nine subjects (380) showed a value of 274 ± 26 pmol/g wet wt, which is well within the range reported for in vivo biopsies (Table 1).

Measurements performed on post mortem samples from four different human muscles with considerable variation in the ratio between type I (slow-twitch) and type II (fast-twitch) fibers showed rather modest differences in the content of \( ^{3} \text{H}\)ouabain binding sites. Again, the values were not significantly lower than those obtained in parallel measurements on in vivo needle biopsies obtained from vastus lateralis of six normal subjects (115). Although more observations are needed, these results indicate that with respect to \( ^{3} \text{H}\)ouabain binding, the vastus lateralis is representative for a substantial part of human skeletal muscles.

In conclusion, \( ^{3} \text{H}\)ouabain binds stoichiometrically to a specific receptor on the \( \alpha\)-subunit of the Na\(^{+}\)-K\(^{+}\)-ATPase, allowing accurate quantification of the content of Na\(^{+}\)-K\(^{+}\) pumps in intact skeletal muscle, biopsies, and membranes. The method can be applied to frozen or post mortem tissue samples, and the values obtained over a 20-yr period in several laboratories around the world are fairly consistent, allowing the detection of numerous regulatory changes.

### D. Measurement of Transport Capacity

The function of the Na\(^{+}\)-K\(^{+}\) pumps in intact skeletal muscle can be characterized by measurements of Na\(^{+}\)-K\(^{+}\) contents and isotope fluxes of Na\(^{+}\) and K\(^{+}\). The ouabain-suppressible components of the basal and hormone-stimulated uptake of \( ^{42}\text{K} \) and \( ^{86}\text{Rb} \) are rather similar, and both isotopes seem to be reliable tracers for the Na\(^{+}\)-K\(^{+}\) pump-mediated K\(^{+}\) transport (110). For other transport pathways for K\(^{+}\), however, \( ^{86}\text{Rb} \) is unreliable, yielding very different or even opposite results to those obtained with \( ^{42}\text{K} \) or in measurements of K\(^{+}\) content.

Due to diffusional limitations, the quantification of the maximum transport capacity of the Na\(^{+}\)-K\(^{+}\) pumps in intact skeletal muscle preparations is difficult. On the basis of a procedure developed by Sejersted et al. (385), an attempt was made to circumvent this problem by increasing the concentration of K\(^{+}\) in the incubation medium so as to compensate for the diffusional delay of the access of K\(^{+}\) to the Na\(^{+}\)-K\(^{+}\) pumps in intact isolated rat soleus (71). Following preloading with Na\(^{+}\) so as to increase [Na\(^{+}\)], to 126 mM, measurements of ouabain-suppressible \( ^{42}\text{K} \) and \( ^{86}\text{Rb} \) uptake, and the Na efflux, and the net changes in Na\(^{+}\)-K\(^{+}\) contents (by flame photometry) performed in a K\(^{+}\)-rich buffer gave values corresponding to 90% of the theoretical maximum predicted from measurements of the content of \( ^{3} \text{H}\)ouabain binding sites. In rat soleus, where the content of \( ^{3} \text{H}\)ouabain binding sites had undergone regulatory changes over a 4.5-fold range as a result of differentiation, K\(^{+}\) depletion or pretreatment with thyroid hormone, there was a close correlation between the content of \( ^{3} \text{H}\)ouabain binding sites and the maximum ouabain-suppressible \( ^{86}\text{Rb} \) uptake. These maximal transport rates were suppressed by cooling to 0°C or the metabolic inhibitor dinitrophenol and were not affected by K\(^{+}\) channel blockers (71). It seems reasonable to conclude, therefore, that the major part of the population of \( ^{3} \text{H}\)ouabain binding sites measured in rat soleus

### Table 1. \( ^{3} \text{H}\)ouabain binding site content in human skeletal muscle biopsies, as determined in six different laboratories

<table>
<thead>
<tr>
<th>Reference Nos.</th>
<th>Content, pmol/g wet wt</th>
</tr>
</thead>
<tbody>
<tr>
<td>328</td>
<td>278 ± 15</td>
</tr>
<tr>
<td>258</td>
<td>258 ± 16</td>
</tr>
<tr>
<td>276</td>
<td>276 ± 19</td>
</tr>
<tr>
<td>276</td>
<td>306 ± 27</td>
</tr>
<tr>
<td>271</td>
<td>317 ± 37</td>
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<tr>
<td>278</td>
<td>307 ± 43</td>
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<td>328</td>
<td>311 ± 41</td>
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<td>297</td>
<td>333 ± 19</td>
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<td>379</td>
<td>223 ± 13</td>
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<td>180</td>
<td>258 ± 13</td>
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<tr>
<td>169</td>
<td>339 ± 16</td>
</tr>
<tr>
<td>170</td>
<td>289 ± 22</td>
</tr>
<tr>
<td>171</td>
<td>268 ± 19 (Men)</td>
</tr>
<tr>
<td>317</td>
<td>243 ± 13 (Women)</td>
</tr>
<tr>
<td>173</td>
<td>345 ± 12</td>
</tr>
<tr>
<td>208</td>
<td>425 ± 11</td>
</tr>
<tr>
<td>133</td>
<td>343 ± 11 (Men)</td>
</tr>
<tr>
<td>297</td>
<td>281 ± 14 (Women)</td>
</tr>
<tr>
<td>270</td>
<td>306 ± 26</td>
</tr>
</tbody>
</table>
represents functional Na⁺-K⁺ pumps capable of operating close to their expected maximum theoretical transport rate. As described below, when stimulated at high frequency, the rates of active Na⁺-K⁺ transport of isolated muscles may reach this theoretical maximum (314).

In conclusion, there seems to be satisfactory agreement between the total content of [3H]ouabain binding sites in intact rat muscles and muscle segments and the maximum capacity for active Na⁺-K⁺ transport measured in the same muscles.

V. ACUTE REGULATION OF Na⁺-K⁺ PUMP ACTIVITY

This section and section VI describe the major factors involved in the acute and long-term regulation of the Na⁺-K⁺ pump in skeletal muscle, illustrated in the diagram shown in Figure 4. The largest single acute stimulus to the activity of the Na⁺-K⁺ pump seems to be excitation.

A. Excitation

1. Stimulatory effects

The action potentials causing excitation are elicited by an influx of Na⁺, followed by an efflux of K⁺. This leads to rapid increases in the concentrations of Na⁺ and K⁺ on the inner and the outer surfaces of sarcolemma, respectively, a strong stimulus for the activity of the Na⁺-K⁺ pumps. Indeed, electrical stimulation induced a marked increase in the rate of active Na⁺-K⁺ transport, both in young and adult animals (127, 200, 232, 296, 314). Ion-sensitive microelectrode recordings of the net recovery of intracellular K⁺ after electrical stimulation of isolated mouse soleus showed values in accordance with the theoretical maximum as estimated from the content of [3H]ouabain binding sites (232). Concomitant measurements of the net extrusion of Na⁺, however, showed values corresponding to only ~30% of the theoretical maximum. Since the activity coefficient for [Na⁺]i is relatively low, however, this value for Na⁺ efflux is likely to represent an underestimate. The postexcitatory net recovery of intracellular K⁺ and Na⁺ activity was suppressed by ouabain. Also in the perfused hindlimb muscles of adult dogs (30 kg), the reuptake of K⁺ taking place after electrical stimulation was suppressed by ouabain (200).

In isolated rat soleus mounted for unloaded contractions, continuous stimulation for 10 s at 60 Hz increased [Na⁺]i by 58%. In the following resting period, reextrusion of Na⁺ to the resting level was complete in 2 min and could be prevented by ouabain (10⁻⁴ M) or cooling to 0°C (127). The net reextrusion of Na⁺ as measured by flame photometry amounted to 4,430 nmol · g⁻¹ · min⁻¹, corresponding to an ~12-fold increase in Na⁺-K⁺ pump-mediated Na⁺ efflux or ~60% of the theoretical maximum rate. When the muscles were stimulated for 60 s at 10 Hz, the

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**FIG. 4.** Diagram of regulatory factors controlling the activity and contents of Na⁺-K⁺ pumps in skeletal muscle. **Left:** factors eliciting acute stimulation of the Na⁺-K⁺ pumps via second messengers. **Right:** factors influencing the content of Na⁺-K⁺ pumps by modifying their synthesis or degradation. IGF-I, insulin-like growth factor I. [Modified from Clausen (63).]
net Na$^+$ reextrusion as measured over the following minute amounted to 3,850 nmol · g$^{-1}$ · min$^{-1}$, indicating that also low frequencies may elicit large stimulation of the Na$^+$-K$^+$ pumps. Stimulation for 10 s at 120 Hz produced a 22-fold increase in net Na$^+$ extrusion as measured over the following 30 s, corresponding to the theoretical Na$^+$ maximum transport rate (314).

It should be noted that in these experiments with muscles allowed to shorten freely without any external load, excitation-induced Na$^+$ influx was fourfold larger than in muscles undergoing isometric contractions (see Table 3 and Ref. 314). In soleus muscles mounted for isometric contraction, stimulation at 60–120 Hz for 30 s increased net Na$^+$ extrusion rate to $\sim$50% of the theoretical maximum.

Electrical stimulation also increases $^{22}$Na influx and the uptake of $^{86}$Rb. All of these effects can be blocked by ouabain, indicating that they are the outcome of activation of the Na$^+$-K$^+$ pump (125–127). This interpretation is further supported by the observation that electrical stimulation also increases the rate of $[^3]$H]ouabain binding (127), a specific reflection of an increased rate of active Na$^+$-K$^+$ transport (see sect. vC and Ref. 76).

In rat soleus, direct electrical stimulation at 2 Hz increased the ouabain-suppressible $^{86}$Rb uptake 2.4-fold more than in EDL (126). In soleus, the large stimulation of $^{86}$Rb uptake was not associated with any detectable increase in [Na$^+$], whereas in EDL, the much more modest increase in $^{86}$Rb uptake was associated with an increase in [Na$^+$] of 63%. Also when [Na$^+$], was increased using monensin or veratridine, soleus showed a larger increase in $^{86}$Rb uptake than EDL. Thus, in muscles containing predominantly type I fibers, the Na$^+$-K$^+$ pump is considerably more sensitive to increases in [Na$^+$], than in muscles containing mainly type II fibers. Because the EDL muscles used contain 20% more $[^3]$H]ouabain binding sites than soleus, this phenomenon cannot be accounted for by differences in the content of Na$^+$-K$^+$ pumps.

In rat soleus, the early phase of electrical stimulation in vivo induced hyperpolarization and increased M-wave area, effects that were suppressed by ouabain (204, 262). This hyperpolarization is likely to reflect the electrogenic action of the Na$^+$-K$^+$ pump. M waves arise by summation of the action potentials generated in the muscle cells, and recordings of the amplitude or area of these waves provide information about the number of muscle cells undergoing excitation as well as the intensity and degree of synchronization of action potentials. It is widely used to assess changes in excitability. In human biceps brachii muscle, indirect stimulation via the nerve at 10 or 20 Hz induced a doubling of the M-wave area within 120 or 40 s, respectively (97). Also in human soleus and tibialis anterior muscles, repetitive stimulation caused an early enlargement of the M-wave area, which was ascribed to hyperpolarization due to increased activity of the Na$^+$-K$^+$ pumps (160, 289). This hypothesis was supported by the observation that cooling of the abductor pollicis brevis muscle to 20°C before and during contraction significantly reduced the M-wave amplitude (369). A lowering of the temperature from 30 to 20°C reduces the rate of active Na$^+$-K$^+$ transport in isolated rat soleus by almost 60% (79). In the human brachioradial muscle, the increase in muscle fiber conduction velocity during recovery from repeated ischemic isometric exercise was suppressed by intra-arterial injection of ouabain, indicating that it reflected activation of the Na$^+$-K$^+$ pump (368). Also in human subjects, excitation seems to stimulate active electrogenic Na$^+$-K$^+$ transport, leading to an initial increase in the intensity of the action potentials.

In conclusion, there is strong evidence that in intact skeletal muscle preparations, excitation induces a rapid stimulation of the Na$^+$-K$^+$ pumps which, dependent on frequency, mode of contraction, and fiber type, may activate anything up to all of the available Na$^+$-K$^+$ pumps so as to reach full utilization of the entire active transport capacity. There is evidence that excitation stimulates the Na$^+$-K$^+$ pump also in vivo and in human subjects, favoring excitability.

2. Mechanisms for excitation-induced stimulation of the Na$^+$-K$^+$ pump

The excitation-induced stimulation of the Na$^+$-K$^+$ pump has generally been seen as the result of an increase in [Na$^+$], which in turn activates the Na$^+$-K$^+$-ATPase. The following observations indicate that also other mechanisms are important for the activation of the Na$^+$-K$^+$ pump.

1) In rat soleus, electrical stimulation at 60 Hz for 1 s is sufficient to elicit highly significant stimulation (22%) of $^{22}$Na efflux (127) and $^{86}$Rb influx (40%), even though this caused no increase in [Na$^+$], (43).

2) Stimulation of rat soleus at 2 Hz more than doubles ouabain-suppressible $^{86}$Rb uptake without significant increase in [Na$^+$], (127, 130).

3) In soleus and EDL muscles mounted for isometric contractions, stimulation at frequencies from 30 to 90 Hz for 5–30 s elicits an initial short-lasting increase in [Na$^+$], followed by a 23–32% decrease below the resting control level, lasting up to 30 min (43, 314, 317) (Fig. 5).

4) In contrast, when [Na$^+$], was increased by inducing acute leakage of the sarcolemma by monensin or electroporation, ouabain-suppressible $^{86}$Rb uptake was clearly increased, but this was not followed by any “undershoot” in [Na$^+$], (43, 126).

5) Following electrical stimulation in vivo, hyperpolarization of the fibers has been observed in 10- to 12-wk old mice (146) and large rats (204, 262). This hyperpolarization was blocked by ouabain, indicating that it reflects
of the preserved axons to the muscle response to stimulation of the nerve (262). The connection to the other half denervated and with no contractile reaction of about half the axons supplying the muscle, leaving the soleus muscle of the rat was split so as to allow stimulation-induced changes in intracellular Na\(^+\) content during and after electrical stimulation in rat soleus. Isolated muscles were mounted for isometric contraction and either stimulated for 30 s at 60 Hz or allowed to rest. At each time point indicated, muscles were immediately transferred to ice-cold Na\(^+\)-free Tris-sucrose buffer, washed 4 × 15 min, blotted, and taken for determination of Na\(^+\) content (C, resting controls; ●, stimulated muscles). Each point represents a mean value ± SE of measurements on 6–27 muscles. [From Nielsen and Clausen (314).]

FIG. 5. Time course of changes in intracellular Na\(^+\) content during and after electrical stimulation in rat soleus. The stimulating effect of high [K\(^+\)]\(_o\) was suppressed by TTX, indicating that depolarization stimulates the Na\(^+\)-K\(^+\) pump, but that the effect depends on the opening of Na\(^+\) channels.

Taken together, these results indicate that during electrical stimulation and opening of the Na\(^+\) channels, the Na\(^+\)-K\(^+\) pump undergoes activation within seconds, leading to a conformational change allowing the transport system to maintain a lower intracellular Na\(^+\), probably reflecting an increased affinity for intracellular Na\(^+\) (43). This idea received further support from experiments with rat soleus that had been stimulated for 10 s at 60 Hz so as to achieve an undershoot in [Na\(^+\)]\(_i\) of 30%. When the muscles 10 min later were stimulated again for 15 s at 60 Hz, the increase in [Na\(^+\)]\(_i\) did not exceed the resting level. Despite this, during the following resting period, the net extrusion rate of Na\(^+\) was eightfold higher than in resting muscles (317).

The possible mechanism for the hyperpolarization induced by electrical stimulation in vivo was explored in an elegant experiment where the nerve supplying the soleus muscle of the rat was split so as to allow stimulation of about half the axons supplying the muscle, leaving the other half denervated and with no contractile response to stimulation of the nerve (262). The connection of the preserved axons to the muscle fibers was confirmed by the detection of action potentials elicited by stimulation of the nerve fibers. Surprisingly, electrical stimulation of these fibers elicited a hyperpolarization of 8–9 mV, not only in the contracting fibers, but also in the neighboring noncontracting fibers. This indicates that the hyperpolarization is not only due to the excitation-induced influx of Na\(^+\) and the ensuing rise in [Na\(^+\)]\(_i\), leading to stimulation of the electrogenic Na\(^+\)-K\(^+\) pump. It was suggested that some humoral factor is released in the contracting muscle and that this, in turn, stimulates the Na\(^+\)-K\(^+\) pump both in working and resting fibers. Because propranolol suppressed the hyperpolarization both in contracting and noncontracting fibers, it was proposed that the humoral factor could be norepinephrine released from the sympathetic nerve endings in the muscle. However, this is not in keeping with the earlier observation that the stimulating effect of 2-Hz stimulation on \(^{22}\text{Na}\) efflux from isolated rat soleus was not impaired by propranolol, or increased by theophylline (130). Moreover, the excitation-induced “undershoot” in [Na\(^+\)]\(_i\), of rat soleus (Fig. 5) could not be prevented by propranolol, indicating that it is not secondary to a norepinephrine-induced stimulation of the Na\(^+\)-K\(^+\) pump (314). Neither could it be attributed to calcitonin gene-related peptide (CGRP) released from nerve endings, to an increase in [K\(^+\)]\(_o\) or the formation of nitric oxide.

On the other hand, denervated muscles, where the nerve endings are depleted for CGRP, showed no stimulation-induced undershoot in [Na\(^+\)]\(_i\). In addition, pretreatment of normally innervated muscles with CGRP caused a decrease in [Na\(^+\)]\(_i\), and under these conditions, electrical stimulation produced no further undershoot in [Na\(^+\)]\(_i\). Likewise, in resting soleus, pretreatment with the \(\beta\)-agonist salbutamol caused a similar reduction in [Na\(^+\)]\(_i\), again preventing the excitation-induced undershoot in [Na\(^+\)]\(_i\) (314). The effects of epinephrine (10\(^{-5}\) M) and electrical stimulation at 2 Hz on \(^{22}\text{Na}\) efflux or \(^{86}\text{Rb}\) uptake were not additive either (130). CGRP, epinephrine, and salbutamol increase intracellular cAMP, and the lack of additivity between the effects of electrical stimulation, salbutamol, epinephrine, and CGRP suggests that cAMP is
important in mediating the effect of electrical stimulation on the Na\(^{+}\)-K\(^{+}\) pump.

In cultured rat skeletal myotubes, carbamylycholine elicited a rapid and almost complete depolarization, followed by a slower repolarization (36). The repolarization was associated with stimulation of \(^{86}\)Rb uptake and could be prevented by ouabain, indicating that it depended on stimulation of active electrogenic Na\(^{+}\)-K\(^{+}\) transport.

Muscle work has also been proposed to influence Na\(^{+}\)-K\(^{+}\) pump function by eliciting a translocation of the Na\(^{+}\)-K\(^{+}\) pumps from an intracellular pool to sarcolemma (414). Thus, after 1 h of treadmill running, the plasma membrane content of both \(\alpha_1\) and \(\alpha_2\)-subunit isoforms in both type I (red) and type II (white) fibers obtained from rat hindlimb showed a significant increase (43–94\%). More surprisingly, the running exercise also increased the content of mRNA for the \(\alpha_1\) and the \(\beta_1\)-subunits, but not for the \(\alpha_2\)-subunit isoform. It was concluded that increased presence of \(\alpha_1\)- and \(\alpha_2\)-subunits in the plasma membrane might be the mechanisms for exercise-induced stimulation of the Na\(^{+}\)-K\(^{+}\) pump in skeletal muscle.

The possibility of exercise-induced translocation was examined using giant sarcolemmal vesicles prepared from muscle tissue specimens obtained by needle biopsy. In the human vastus lateralis muscle, 4.6 min of exercise until fatigue increased the content of \(\alpha_2\)-isoform subunit by 70 and 26\%, respectively (234). In contrast to the above-mentioned study on rat muscle, however, no significant change could be detected in the content of \(\alpha_1\)-isoform. Recent studies on sarcolemmal vesicles prepared from rat skeletal muscle showed that following low-intensity treadmill running there was a 13–32\% increase in the \(\alpha_1\), \(\alpha_2\), and \(\beta_2\)-isoform subunits of the Na\(^{+}\)-K\(^{+}\)-ATPase and a 29\% increase in \(^{3}H\)-ouabain binding (223). This increase was almost completely reversible after a 30-min rest. When preparing sarcolemmal giant vesicles, however, only 0.3\% of the total amount of Na\(^{+}\)-K\(^{+}\) pumps present in the tissue is made available for quantification (233). Such a small sample may not be representative of the total pool of Na\(^{+}\)-K\(^{+}\) pumps or their regulatory changes.

Recently, the effects of a wide variety of frequencies and durations of electrical stimulation on the total capacity for the binding of \(^{3}H\)-ouabain to rat soleus and EDL muscles were characterized. No significant increase could be detected, neither in measurements on intact muscles nor on segments prepared and frozen immediately after acute or long-term stimulation, even under conditions where a large fraction (70\%) of all Na\(^{+}\)-K\(^{+}\) pumps were activated (296). Thus it seems that excitation is not mobilizing sufficient Na\(^{+}\)-K\(^{+}\) pumps to the sarcolemma to allow their detection as an increase in \(^{3}H\)-ouabain binding.

In conclusion, the activation of the Na\(^{+}\)-K\(^{+}\) pump elicited by excitation is most likely to reflect a rapid, but slowly reversible, increase in the affinity of the Na\(^{+}\)-K\(^{+}\) pump for [Na\(^{+}\)]\(_i\), possibly elicited by the depolarization during the action potentials. This would allow more efficient clearance of Na\(^{+}\) from the cytoplasm and K\(^{+}\) from the extracellular phase and reduce the risk of major rundown of the transmembrane Na\(^{+}\)-K\(^{+}\) gradients, depolarization, and loss of excitability during work. A translocation of Na\(^{+}\)-K\(^{+}\) pumps from intracellular pools or t tubules to the sarcolemma might contribute to these processes.

3. Excitation-induced inhibition of Na\(^{+}\)-K\(^{+}\)-ATPase activity

In contrast to these examples of excitation-induced stimulation of the Na\(^{+}\)-K\(^{+}\) pump, the activity of the Na\(^{+}\)-K\(^{+}\)-ATPase in skeletal muscle, measured as 3-O-MFPase activity, decreased shortly after exercise (295). Immediately after a quadriceps fatigue test (50 maximal isometric contractions), 3-O-MFPase activity in vastus lateralis biopsies was reduced by 14\% (153). Another study showed that immediately after 30 min of isometric single leg extension at 60\% of maximum voluntary contraction, 3-O-MFPase activity in biopsies from the vastus lateralis muscle of human subjects was decreased by 38\% (152). One or 4 h later, 3-O-MFPase activity returned toward normal levels. There was no significant change in the content of \(^{3}H\)-ouabain binding sites, but the early drop in 3-O-MFPase activity coincided with a decrease in force, M-wave area, and amplitude. There was a weak correlation between 3-O-MFPase activity and M-wave area. In rats, prolonged running exercise to fatigue caused a significant reduction in 3-O-MFPase activity of 12\% as measured in a series of different muscles (151).

These observations were taken to indicate that the activity of the Na\(^{+}\)-K\(^{+}\) pump could be reduced by a single period of exercise and that this could contribute to neuromuscular fatigue.

B. Catecholamines

The \(\beta\)-agonist isoproterenol was found to favor net Na\(^{+}\) extrusion and to induce hyperpolarization in isolated rat soleus (107). Isoproterenol stimulated the loss of Na\(^{+}\) from denervated rat diaphragm by 40\% (123) and \(^{22}\)Na efflux from frog sartorius by 45\% (199). In avian slow-twitch muscle, isoproterenol induced hyperpolarization, an effect that could be suppressed by ouabain (394). This was taken to indicate a stimulation of active electrogenic Na\(^{+}\)-K\(^{+}\) transport. In keeping with this, epinephrine and isoproterenol induced 3- to 4-mV hyperpolarization in rat diaphragm muscle (260). In guinea pig soleus and EDL, isoproterenol induced hyperpolarization, which was suppressed by ouabain (1.4 \(\times\) 10\(^{-6}\) M), K\(^{+}\) free buffer, or cooling (407). The hyperpolarization was more pro-
nounced in soleus than in EDL, indicating that the Na\(^+\)-K\(^+\) pump response is larger in type I than in type II fibers.

A more detailed analysis showed that both epinephrine and norepinephrine within minutes induced an acceleration of \(^{22}\)Na efflux and \(^{42}\)K influx. This was associated with hyperpolarization (5–8 mV), increased [K\(^+\)]\(_i\) (by a few percent), and decreased [Na\(^+\)]\(_i\) (by up to 67%) (72, 74). Also in vivo, epinephrine, salbutamol, and isoproterenol induced a hyperpolarization of up to 15 mV (73, 144, 219). All these effects were mediated by β\(_2\)-adrenoceptors (73, 427), blocked by ouabain, potentiated by theophylline, and mimicked by cAMP or dibutyl cAMP (72). The chain of events from the binding of catecholamines to β\(_2\)-adrenoceptors to the changes in Na\(^+\)-K\(^+\) distribution, membrane potential, and muscle contractility is illustrated in Figure 6. The effect of cAMP is likely to be mediated by activation of protein kinase A, which in turn activates the Na\(^+\)-K\(^+\) pump. This relation seems to be general and has also been described in isolated guinea pig ventricular myocytes, where forskolin increases the Na\(^+\)-K\(^+\) pump-mediated current, an effect that is abolished by protein kinase inhibitors (255). The central role of cAMP in mediating Na\(^+\)-K\(^+\) pump activation by other hormones is exemplified by the observation that peptide hormones (calcitonins, CGRP, and amylin) that cause an increase in the cAMP content in skeletal muscle also stimulate the Na\(^+\)-K\(^+\) pump (see sect. vC).

Epinephrine also increases the rate of \(^{3}\)Houabain binding, a specific indication that the Na\(^+\)-K\(^+\) pump is stimulated (76). The effects of catecholamines on the Na\(^+\)-K\(^+\) pump are more pronounced in muscle preparations containing predominantly slow-twitch fibers (130,

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**EPINEPHRINE NOREPIINEPHRINE β\(_2\)-AGONISTS**

![Figure 6. Sequence of events in the action of catecholamines and β\(_2\)-agonists on active Na\(^+\)-K\(^+\) transport, Na\(^+\)-K\(^+\) distribution, and contractile performance in skeletal muscle. Epinephrine, norepinephrine, or β\(_2\)-agonists bind to the β\(_2\)-adrenoceptors, which activate the adenylate cyclase to stimulate the conversion of ATP to cAMP. This second messenger in turn activates protein kinase A, which is assumed to induce a conformational change in the Na\(^+\)-K\(^+\)-ATPase. This leads to increased affinity for intracellular Na\(^+\), allowing for an acute increase in Na\(^+\) efflux and subsequent maintenance of a steeper Na\(^+\) gradient across the sarcolemma. Due to the electrogenic nature of the coupled active Na\(^+\)-K\(^+\) transport, the early increase in Na\(^+\) efflux leads to hyperpolarization, increased intracellular K\(^+\)/Na\(^+\) ratio, decreased [K\(^+\)]\(_o\), and hypokalemia. These changes will influence excitability and contractile performance. [Modified from Clausen (65).]
However, a recent study showed that both in isolated rat soleus and EDL, epinephrine produces a marked decrease in the intracellular Na\(^{+}\)/K\(^{+}\) ratio (225). Epinephrine stimulated \(^{86}\)Rb uptake in soleus muscles obtained from both normal and hypokalemic rats (214).

The effects of epinephrine on active Na\(^{+}\)-K\(^{+}\) transport and membrane potential in isolated rat soleus (72, 73) are seen at concentrations down to the physiological levels reached in plasma during heavy exercise in the human (2.4 \times 10^{-9} M; Ref. 159) and during running in the rat (6 \times 10^{-9} M; Ref. 366). Epinephrine (10^{-6} M) induces \sim 10\-mV hyperpolarization and a decrease in the activity of intracellular Na\(^{+}\) both in isolated rat soleus and human intercostal muscle fibers, effects that were blocked by ouabain (16).

Also in vivo, epinephrine stimulated the uptake of K\(^{+}\) in dog gracilis muscle via a \(\beta\)-adrenoceptor-mediated mechanism (357) and a \(\beta\)_\(2\)-agonist stimulated the net uptake of K\(^{+}\) in the skeletal muscles of the human forearm (148). Intravenous injection of epinephrine or the \(\beta\)_\(2\)-agonist salbutamol both induce hyperpolarization of rat soleus as determined in vivo (73, 144). In rats where the plasma epinephrine level had been increased by hemorrhage, the intracellular Na\(^{+}\)/K\(^{+}\) ratio of soleus and EDL was significantly decreased, and this in vivo effect was offset by adrenergic blockade (288).

In isolated rat soleus, the hyperpolarizing effect of epinephrine (10^{-5} M) was larger and earlier in onset than that of insulin (100 mU/ml) (74). Isoproterenol (10^{-6} M) produced 9-mV hyperpolarization in isolated rat soleus (272). This effect was mimicked by the adenylyl cyclase activator forskolin (10^{-5} M) and 8-bromo-cAMP (1 mM) and blocked by ouabain. Insulin (4 mU/ml) also induced hyperpolarization, and this was additive to the effect of isoproterenol, indicating that the two agents act via different mechanisms. As shown in Figure 7, the hyperpolarizing effect of insulin is seen at concentrations down to those found in plasma (10 \mu U/ml). The hyperpolarizing effect of isoproterenol reaches half-maximum values around 10^{-9} M.

Studies on rat skeletal myoballs showed that isoproterenol rapidly stimulates ouabain-suppressible transmembrane current by \sim 50\%. This effect was mimicked by 8-bromo-cAMP and attributed to a cAMP-mediated stimulation of the Na\(^{+}\)-K\(^{+}\) pump (273). In the same study, the contribution of Na\(^{+}\)-K\(^{+}\) pump-mediated current (\(I_{p}\)) to the resting membrane potential was calculated, and the value (5.7 mV) was identical to the depolarization induced by ouabain (1 mM) in untreated myoballs. These observations on single cells are important, because they provide strong evidence that the measured increases in pump current or membrane potential are not secondary to localized changes in [K\(^{+}\)]\(_{o}\).

Because the hyperpolarizing effect of isoproterenol in mouse lumbrical muscle fibers could not be blocked by ouabain (0.5 mM), but was blocked by 0.6 mM Ba\(^{2+}\) it has been proposed to be the result of K\(^{+}\) channel opening rather than a stimulation of Na\(^{+}\)-K\(^{+}\) pumps (418). This is not in keeping with the observation that Ba\(^{2+}\) does not prevent the hyperpolarizing effect of salbutamol on rat soleus (93). It cannot be excluded, however, that in certain types of skeletal muscle, K\(^{+}\) channels contribute to \(\beta\)_\(2\)-adrenoceptor agonist-induced hyperpolarization. The effects of salbutamol and CGRP on \(4^{2}\)K uptake and \([\text{Na}^{+}]_{i}\), in rat soleus were both independent of Ba\(^{2+}\)-sensitive and ATP-sensitive K\(^{+}\) channels (83).

Taken together, the stimulating effect of catecholamines on the Na\(^{+}\)/K\(^{+}\) pumps in skeletal muscle can adequately account for the hyperpolarization of muscle cells as well as the hypokalemic actions of these agents in the intact organism.

During intense exercise, the plasma catecholamines increase markedly (42, 159), reflecting rapid stimulation of secretion from the adrenal medulla and release of norepinephrine from the sympathetic nerve endings. These endogenous catecholamines are likely to counterbalance hyperkalemia by stimulating the uptake of K\(^{+}\) via the Na\(^{+}\)-K\(^{+}\) pump in resting or relatively inactive muscles (72, 130). Thus adrenalectomy reduces the tolerance to an intravenous infusion of K\(^{+}\) (26, 278). This intolerance to a K\(^{+}\) load was restored by epinephrine and could be mimicked by pretreatment with propranolol.

The significance of endogenous catecholamines in counterregulating exercise-induced hyperkalemia in human subjects is well-documented. Exercise-induced hyperkalemia was accentuated by propranolol (51, 434). During stepwise increasing bicycle work loads, propranolol pretreatment augmented the plasma K\(^{+}\) in femoral venous blood and prevented the postexercise hypokalemia (182). The more pronounced exercise-induced hyperkalemia induced by propranolol may be important for the fatigue often reported by propranolol-treated patients. Propranolol decreased the time to exhaustion from 86 to 39 min without any evidence of impaired energy metabolism (416).

On the other hand, after adrenalectomy, calves exercising by running at maximum speed showed the same degree of hyperkalemia as untreated controls (149), suggesting that in this species, the secretion of catecholamines from the adrenal medulla is of minor importance in the maintenance of K\(^{+}\) homeostasis during exercise.

The hypokalemic action of the \(\beta\)_\(2\)-agonists given as inhalation has found clinical application in the acute treatment of attacks of hyperkalemic periodic paralysis (HPP) (185, 427), as well as hyperkalemia arising from renal failure (301; for reviews, see Refs. 6 and 65). In premature infants, who often develop life-threatening hyperkalemia, treatment with salbutamol intravenously (175) or as inhalation (390) was efficient in reducing...
plasma K\(^+\). Also in normal human subjects the inhalation of salbutamol or fenoterol (another \(\beta_2\)-agonist) produced dose-dependent hypokalemia, which was a close mirror image of an up to sixfold concomitant increase in the plasma level of cAMP (377), indicating that this second messenger is mediating the effect of \(\beta_2\)-adrenoceptor stimulation on K\(^+\) homeostasis also in vivo. Acute theophylline intoxication leads to hypokalemia, partly because degradation of cAMP is inhibited, but more likely because in addition, the plasma catecholamines are increased up to four- to fivefold (386).

In conclusion, catecholamines stimulate the Na\(^+\)-K\(^+\) pumps in skeletal muscle within a few minutes, leading to hyperpolarization and a marked increase in the intracellular K\(^+\)/Na\(^+\) concentration ratio both in vitro and in vivo. These effects are mediated via \(\beta_2\)-adrenoceptors and adenylate cyclase activation and can be detected down to the plasma levels reached during intense exercise in animals and humans. This is important for the maintenance of K\(^+\) homeostasis during exercise and may thereby influence physical performance (see sect. VIII B). Moreover, catecholamines or \(\beta_2\)-agonists rapidly induce hypokalemia and can be used for acute treatment of hyperkalemia.

C. Peptide Hormones

A variety of peptide hormones stimulate active Na\(^+\)-K\(^+\) transport in skeletal muscle. For reviews, see References 62, 69, and 134.

1. Insulin

Insulin was shown to decrease [Na\(^+\)]\(_i\) in rat diaphragm (95). On the basis of similar results obtained in
frog sartorius (302) and rat soleus (79), it was proposed that the hormone acts by increasing the affinity of the Na⁺-K⁺ pump for intracellular Na⁺. This suggestion was documented by ²²Na flux studies on frog sartorius showing that insulin induced a clear-cut leftward shift of the relationship between Na⁺ efflux and the logarithm of [Na⁺]i concentration (240). In rat adipocytes, insulin stimulated the Na⁺-K⁺ pumps without any change in Vₘₐₓ but by augmenting the affinity for intracellular Na⁺ (282). In keeping with this, quantification of α₁- and α₂-subunit isoforms of Na⁺-K⁺-ATPase with immunogold labeling showed that insulin produced no increase in the Na⁺-K⁺-ATPase content of the plasma membrane, even under conditions where the hormone induced a sevenfold increase in the content of Glut4 (424). This is in keeping with the observation that in rat adipocytes, insulin produced no change in the total content of [³H]ouabain binding sites (76, 365). In cultured human fibroblasts that were shown to contain only the α₁-subunit isoform, insulin clearly increased ⁸⁶Rb uptake but caused no change in the content of [³H]ouabain binding sites (279). In rabbit cardiac myocytes, insulin stimulates Na⁺-K⁺ pump-mediated current by ~70% (192). This effect was abolished when [Na⁺]i was increased to 80 mM, leading to saturation of the Na⁺-K⁺ pumps. This indicates that in this cell type, the effect of insulin is not due to translocation of the Na⁺-K⁺ pumps to the plasma membrane but rather reflects an increase in the affinity of the Na⁺-K⁺ pump for intracellular Na⁺. Likewise, in isolated rat proximal convoluted kidney tubules, the stimulating effect of insulin on ouabain-suppressible ⁸⁶Rb uptake and Na⁺-K⁺-ATPase activity disappeared when [Na⁺]i was increased to saturating levels. Because insulin decreased the apparent dissociation constant for Na⁺ from 47 to 28 mM, it was concluded that the hormone acted by increasing the sensitivity of the Na⁺-K⁺-ATPase to Na⁺ and not by increasing Vₘₐₓ (138).

Insulin stimulates the uptake of ²²Na in rat soleus (74, 79). This effect was not reduced by amiloride or bumetanide, indicating that it is not the result of stimulation of the Na⁺/H⁺ antiporter or the bumetanide-sensitive NaCl cotransport system found in rat skeletal muscle (110). Also in human fibroblasts, insulin stimulated Na⁺ uptake by a bumetanide-resistant mechanism (279).

In rat soleus incubated in the presence of amiloride, insulin still produced a clear-cut stimulation of ⁸⁶Rb uptake as well as ²²Na efflux (74, 214). Likewise, in primary cultures of rat skeletal muscle, neither the stimulating effect of insulin on ⁸⁶Rb uptake nor its hyperpolarizing effect was reduced by amiloride (374).

The effect of insulin on the uptake of ⁴²K and ⁸⁶Rb was blocked by ouabain (74, 79). In the human forearm, the stimulating effect of an intra-arterial infusion of insulin on the net uptake of K⁺ was abolished by subsequent infusion of ouabain (140). In contrast, the effect of insulin on glucose uptake was preserved. This is in keeping with studies on isolated rat diaphragm (61) and soleus (74), showing that the transport of glucose is not coupled to active Na⁺-K⁺ transport or its response to insulin.

In rat soleus, insulin stimulated the uptake of ⁸⁶Rb and decreased [Na⁺]i effects that were both blocked by ouabain (430), but not by amiloride or the Na⁺ ionophore monensin. Insulin also increased the rate of [³H]ouabain binding, a rather specific indication that the rate of active Na⁺-K⁺ transport is accelerated.

In vivo recordings of the resting membrane potential of the rat soleus showed that insulin injected intravenously produced a hyperpolarization of 15 mV, which was additive to that of salbutamol (144). Also endogenous insulin released by injection of glucose produced a significant hyperpolarization (5.5 mV). A similar effect of intravenous insulin on the in vivo recorded membrane potential of soleus was observed (219). This effect was additive to that of isoproterenol and blocked by ouabain. In soleus of diabetic rats, insulin produced only ~70% of the hyperpolarization seen in normal muscles.

In primary cultures of rat skeletal muscle cells, insulin and phorbol esters induced stimulation of ⁸⁶Rb uptake and hyperpolarization (374). These effects were not additive, but suppressed by staurosporine or by prior downregulation of protein kinase C. This indicates that insulin stimulates the Na⁺-K⁺ pump activity via activation of protein kinase C. In keeping with this, phorbol ester induced a ouabain-suppressible hyperpolarization in isolated rat soleus (272).

In the rat skeletal muscle cell line L6, incubation for 5 min with insulin (100 nM) produced a significant increase in the activity of Na⁺-K⁺-ATPase measured in plasma membranes (359). Stimulating effects could be detected in the concentration range 0.1–100 nM and reached a maximum at ~100% after 15 min. The effect was associated with dephosphorylation of the α₁-subunit isoform, mediated by a serine/threonine protein phosphatase. In the rat, however, intravenous administration of insulin produced no change in the content of the α₁-subunit isoform in the plasma membrane and t-tubular fractions prepared from the hindlimb muscles, but an 80% increase in the content of Glut4 (108).

The physiological significance of the effects of insulin on the Na⁺-K⁺ pumps in skeletal muscle has been documented by the following.

1) Down to physiological concentration levels (10 μU/ml), insulin stimulates the net uptake of K⁺ in the human forearm (439).

2) During oral or intravenous glucose loads, the insulin release is sufficient to promote the clearance of K⁺ from plasma (310).

3) Acute inhibition of insulin secretion by the infusion of somatostatin causes an increase in plasma K⁺.
(102), indicating that basal insulin secretion/plasma levels are required for the maintenance of normal plasma K⁺.

4) Hyperkalemia has repeatedly been shown to increase plasma insulin (62, 87, 93).

For these reasons, it is likely that insulin contributes significantly to K⁺ homeostasis in skeletal muscle as well as in the entire organism.

In isolated frog sartorius muscle, insulin was reported to increase the binding of [³H]ouabain, which was taken to indicate a mobilization of Na⁺-K⁺ pumps to the membrane surface (122). However, the measurements of the content of Na⁺-K⁺ pumps were performed before it could be ascertained that all Na⁺-K⁺ pumps were occupied. Later studies indicated that the observation was likely to be the result of insulin-induced stimulation of the rate of [³H]ouabain binding. Thus, when binding equilibrium was reached, insulin caused no significant change in [³H]ouabain binding (76, 111). More recently, it was found that in control subjects and type 2 diabetics, infusion of insulin did not allow accurate quantitation of the content of Na⁺-K⁺ pumps per square micron of plasma membrane or per gram of muscle. In the isolated split rat soleus preparation, insulin induced a 39% increase in the abundance of the α₂-subunit isoform in plasma membranes prepared by differential centrifugation (57).

In normal mice, injection of insulin caused no significant change in the content of the α₂-subunit isoform of the Na⁺-K⁺-ATPase detected in immunoblots of the plasma membranes prepared from hindlimb skeletal muscle. However, insulin induced a small but significant increase in the β₁-subunit of normal mice and a much larger increase in mice obtained from transgenic mice overexpressing the Glut4 glucose transporter (360).

By immunoelectron microscopy, the effect of insulin on the content of α₂-subunit subunits was assessed in rat skeletal muscle. Thirty minutes after an intravenous injection of insulin, the content of immunolabeled α₂-subunits per linear micron of plasma membranes had increased 1.5- to 3.7-fold (285). Unfortunately, these data do not allow accurate quantification of the content of Na⁺-K⁺ pumps per square micron of plasma membrane or per gram of muscle. In the isolated split rat soleus preparation, insulin induced a 39% increase in the abundance of the α₂-subunit isoform in plasma membranes prepared by differential centrifugation (57).

In normal mice, injection of insulin caused no significant change in the content of the α₂-subunit isoform of the Na⁺-K⁺-ATPase detected in immunoblots of the plasma membranes prepared from hindlimb skeletal muscle. However, insulin induced a small but significant increase in the β₁-subunit of normal mice and a much larger increase in muscles obtained from transgenic mice overexpressing the Glut4 glucose transporter (360).

In conclusion, the Na⁺-K⁺ pumps in skeletal muscle are stimulated within minutes by insulin over a range of concentrations down to the physiological level. This leads to hyperpolarization and redistribution of Na⁺ and K⁺ and is important for whole body K⁺ homeostasis and contractile performance (see sect. VIII). The stimulation of the Na⁺-K⁺ pumps depends on protein kinase C and seems to reflect an increase in the affinity of the enzyme for intracellular Na⁺. There is some evidence that insulin induces a recruitment of Na⁺-K⁺ pumps by translation from an undefined intracellular pool to the sarcolemma.

2. IGF-I

IGF-I is a polypeptide with structural homology to proinsulin. It interacts with the type I IGF receptor and to a minor extent also with the structurally similar insulin receptor. In lambs, the infusion of recombinant human IGF-I and insulin in doses resulting in the same relative drop in plasma glucose caused almost the same reductions (15–18%) in plasma K⁺ (118). Also in normal human subjects, IGF-I induced hypokalemia (163). The mechanism of the hypokalemic effect was explored in experi-
ments with isolated rat soleus (111). IGF-I stimulated 22Na eflux as well as 42K and 86Rb influx, leading to increased [K\(^{+}\)]_i and decreased [Na\(^{+}\)]_i. All these effects, except that on 22Na efflux, were blocked by ouabain (10^{-3} M). IGF-I decreased [Na\(^{+}\)]_i and increased intracellular K\(^{+}\) in EDL to the same extent as in soleus. IGF-I increased the rate of [\(^3\)H]ouabain binding but had no detectable effect on the total binding capacity for [\(^3\)H]ouabain. The effects of IGF-I and insulin on 86Rb uptake and [Na\(^{+}\)]_i, were not additive. In contrast, the effects of IGF-I and epinephrine on the same parameters were additive. This indicates that IGF-I and insulin stimulate the Na\(^{+}\)-K\(^{+}\) pumps via the same pathway, which is different from that activated by epinephrine. Taken together, these observations indicate that IGF-I may participate in the regulation of Na\(^{+}\)-K\(^{+}\) homeostasis of skeletal muscle, but more detailed in vivo studies are required to substantiate this possibility.

3. **CGRP and calcitonins**

CGRP is a 37-amino acid peptide present in a variety of central and peripheral neurons. In rat sciatic nerve, CGRP is transported in somatofugal direction in both motor and sensory nerves and accumulates in nerve terminals in the muscle (237). From these peripheral depots, CGRP may be released during electrical stimulation, exposure to high [K\(^{+}\)]_o, or specific agents such as capsaicin. CGRP binds to receptors on sarcolemma and stimulates adenylate cyclase activity leading to a rise in intracellular cAMP (406). Because this messenger mediates the stimulatory effects of catecholamines on the Na\(^{+}\)-K\(^{+}\) pumps (72), it was anticipated that CGRP would exert a similar action. In rat soleus, rat CGRP stimulated 22Na efflux and the influx of 42K and 86Rb, leading to increased [K\(^{+}\)]_i, decreased [Na\(^{+}\)]_i, and hyperpolarization. All these effects were blocked by ouabain or cooling, indicating that they reflect an acute stimulation of active electrogenic Na\(^{+}\)-K\(^{+}\) transport (10). Similar effects were exerted by human CGRP as well as rat and salmon calcitonin. Because the effects of rat CGRP and epinephrine on [Na\(^{+}\)]_i and 86Rb uptake were not additive, they may be exerted via the same messenger (cAMP). In contrast, the effects of rat CGRP and insulin were additive.

Capsaicin, which releases CGRP from the sensory nerve endings, induced a 51% decrease in the CGRP content of rat soleus (318). The CGRP released is available for action on the muscle cells, and capsaicin elicited effects similar to those of added CGRP on 22Na extrusion, [Na\(^{+}\)]_i, and 86Rb uptake (10). These effects were not impaired by propranolol, indicating that they were not the outcome of release of norepinephrine from the sympathetic nerve endings. Moreover, ruthenium red, which inhibits the effect of capsaicin on the release of CGRP, blocked the effects of capsaicin on 86Rb uptake and [Na\(^{+}\)]_i. The capsaicin-induced release of CGRP in rat soleus amounts to 4 pmol/ml extracellular fluid, corresponding to a local CGRP concentration of 4 \times 10^{-9} M, sufficient to elicit the above-mentioned stimulation of the Na\(^{+}\)-K\(^{+}\) pumps (10). This implies that the endogenous pools of CGRP in the nerve endings are adequate to influence Na\(^{+}\)-K\(^{+}\) homeostasis in skeletal muscle. Exogenously added CGRP (20–200 nM) or endogenously released CGRP also stimulated the short-circuit current in isolated frog skin, an effect that was partially suppressed by an adenylyl cyclase inhibitor and therefore proposed to be mediated by cAMP (54, 276).

The physiological significance of the stimulating effect of CGRP on the Na\(^{+}\)-K\(^{+}\) pumps is not known, but it seems most relevant that a localized rise in [K\(^{+}\)]_o induces a release of CGRP, which, in turn, might favor the local clearance of K\(^{+}\) (372, 375). CGRP is also a trophic factor generated in specific motoneurons. It is interesting that following unaccustomed downhill running, the number of CGRP-positive neurons supplying the hindlimb extensors at work increased threefold and the number of CGRP motor end plates increased even more (212). This would allow for more intense submaximal stimulation of the Na\(^{+}\)-K\(^{+}\) pumps during subsequent bouts of exercise and might thus constitute part of the training adaptation.

The excitation-induced release of CGRP from isolated muscles would suggest that during exercise, plasma CGRP increases. Although intense exercise produced no change (42), steady-state exercise gave \~50% increase in plasma CGRP (337).

In conclusion, there is good experimental basis for the idea that CGRP participates in the regulation of Na\(^{+}\)-K\(^{+}\) pump activity in skeletal muscle, possibly as a local mediator of nervous control of the Na\(^{+}\)-K\(^{+}\) pump during exercise and adaptation to localized increases in [K\(^{+}\)]_o or training. The similar stimulating effects of human and salmon calcitonins cannot yet be ascribed a defined regulatory significance.

4. **Amylin and other related peptides**

Amylin is a 37-amino acid peptide present in the pancreatic \(\beta\)-cells and is cosecreted with insulin into the bloodstream. Its amino acid sequence is 46% identical to that of CGRP, and it has been shown to mimic several of the actions of CGRP, among others an increase in cAMP in skeletal muscle. This would be expected to mediate stimulation of the Na\(^{+}\)-K\(^{+}\) pumps (see sect. 5B and Fig. 6) and as shown for CGRP, in rat soleus, amylin induced an increase in 22Na efflux and 86Rb influx, leading to a marked decrease in [Na\(^{+}\)]_i and a minor increase in [K\(^{+}\)]_i (63, 66). All these effects were blocked by ouabain, and similar actions were exerted on rat EDL (225). In contrast, neither the structurally similar peptides islet amyloid peptide and adrenomedullin produced any detectable change in [Na\(^{+}\)]_i or K\(^{+}\) uptake in rat soleus (66). Like insulin,
CGRP, and epinephrine, amylin also increased the rate of [3H]ouabain binding without causing any change in the total content of [3H]ouabain binding sites in rat soleus and EDL (66). The effects of supramaximal concentrations of insulin and amylin on 86Rb uptake in rat soleus are additive, indicating that they are exerted via two separate pathways. Thus there is good evidence that amylin stimulates the Na\(^+\)-K\(^+\) pumps both in type I and type II fibers. This may account for the significant hypokalemic action of the hormone (−16% in 1 h) when injected in large doses (423).

The physiological significance of the amylin-induced Na\(^+\)-K\(^+\) pump stimulation in skeletal muscle is not known, but it represents another example of a hormonal Na\(^+\)-K\(^+\) pump activation likely to be mediated by the second messenger cAMP.

5. Inhibitory agents

In view of the wide range of agents shown to stimulate the Na\(^+\)-K\(^+\) pumps, the existence of counterregulatory inhibitory agents would be expected. It has repeatedly been proposed that mammalian tissues contain substances exerting inhibitory effects on the Na\(^+\)-K\(^+\) ATPase, possibly via the digitalis receptor. In spite of decades of efforts in several laboratories, however, no specific Na\(^+\)-K\(^+\) pump inhibitor has been isolated, completely purified, and chemically identified. The evidence obtained has generally been indirect and based on studies with antibodies or incompletely purified preparations. It was proposed that ouabain secreted from the adrenal glands is present in human plasma (184). A later study, however, could not detect ouabain in human plasma (271). Moreover, with the proposed rate of ouabain secretion from the adrenals, it would take 26 days to occupy 1% of the ouabain binding sites in skeletal muscle (187). Such a slow inhibition of a minute fraction of the pools of Na\(^+\)-K\(^+\) pumps cannot be expected to play any regulatory role.

Leptin was reported to inhibit the uptake of 86Rb in cultured fibroblasts (404). This action was slow in onset and could be suppressed by the phosphatidylinositol 3-kinase inhibitor wortmannin. In the same preparation, insulin stimulated the uptake of 86Rb, and this action was also inhibited by wortmannin. In isolated rat soleus and EDL, however, leptin caused no significant change in 86Rb uptake or Na\(^+\)-K\(^+\) contents (unpublished observations).

In conclusion, no endogenous regulatory inhibitor of the Na\(^+\)-K\(^+\) pump in skeletal muscle has yet been identified and adequately documented.

D. Energy Depletion/Repletion

Active Na\(^+\)-K\(^+\) transport in resting muscle only consumes 5–10% of the total energy turnover, a percentage that is not likely to increase during work (for review, see Ref. 86). In rat soleus, 22Na efflux has a temperature coefficient of 2.3 (79). When isolated rat soleus was cooled to 4°C in K\(^-\)-free buffer, the resting membrane potential dropped to −47 mV. After rewarming to 37°C at a [K\(^+\)]\(_o\) of 4.5 mM, the fibers repolarized by 31 mV in 10 min. This effect was almost suppressed by the omission of K\(^+\) or the addition of ouabain, indicating that the electrogenic contribution of the Na\(^+\)-K\(^+\) pump is temperature dependent (272).

The Na\(^+\)-K\(^+\) pump requires ATP for its function. However, when exposed to anoxia or cyanide (2 mM), the rate of 22Na efflux from rat soleus showed no significant decrease (79). In contrast, the metabolic uncoupler 2,4-dinitrophenol (0.1 mM) induced a considerable inhibition of 22Na efflux to a level approaching that seen in the presence of ouabain (1 mM). These data indicate that in skeletal muscle, full inhibition of the Na\(^+\)-K\(^+\) pumps requires a substantial decline in cellular ATP content and that this can only be achieved by accelerating the degradation of ATP with an uncoupler of oxidative phosphorylation. It has repeatedly been discussed whether the ATP utilized by the Na\(^+\)-K\(^+\) pumps is preferentially supplied from glycolysis. Long ago, it was observed that inhibition of the Na\(^+\)-K\(^+\) pumps in cut rat diaphragm muscle by ouabain or K\(^-\)-free buffer leads to ~35% reduction in the rate of lactate production in the presence of O\(_2\) (aerobic glycolysis), but no change in glucose uptake. Glycogen synthesis was increased, and it could be shown that the glucose not degraded to lactate was incorporated into glycogen (60, 61). More recent studies on intact rat soleus and EDL showed that ouabain caused a similar reduction in resting aerobic lactate production (224). Moreover, when [Na\(^-\)]\(_i\) was increased by the Na\(^+\) ionophore monensin, aerobic lactate production was increased. This was suppressed by ouabain, indicating that it reflected stimulation of active Na\(^+\)-K\(^+\) transport. The same group showed that when the Na\(^+\)-K\(^+\) pump was stimulated by epinephrine or amylin, aerobic lactate production increased four- to fivefold, and these effects were markedly reduced by ouabain and K\(^-\)-free buffer (225, 288). Ouabain also reduced the degradation of glycogen elicited by epinephrine (225), and it was proposed that a substantial part of the stimulating effects of epinephrine and amylin on aerobic glycolysis and glycogenolysis in skeletal muscle is secondary to the stimulating effects of these hormones on active Na\(^+\)-K\(^+\) transport. Another group showed that in rat EDL, inhibition of glycolysis with iodoacetate caused up to fourfold increase in intracellular Na\(^+\) content, whereas inhibition of oxidative ATP production with hypoxia or carbonyl cyanide m-chlorophenylhydrazone (CCCP; 0.02–0.2 μM) caused no changes in [Na\(^-\)]\(_i\) (334).

In cardiac Purkinje cells, inhibition of glycolytic ATP synthesis by 2-deoxy-d-glucose or iodoacetic acid induced...
rapid decline in the Na⁺-K⁺ pump-mediated Na⁺ current (167). Also in perfused rat heart, there is good evidence that the Na⁺-K⁺ pump is fuelled primarily by glycolytic ATP (for review, see Ref. 105).

If the Na⁺-K⁺ pump depends on a supply of ATP from glycolysis, this would allow adequate and rapid supply for the prompt and sometimes marked activation of the Na⁺-K⁺ pump that occurs during high-frequency stimulation. Moreover, glycogen stores become essential for maintenance of Na⁺-K⁺ pump function. Hence, the fatigue associated with glycogen depletion may be related to reduction of Na⁺-K⁺ gradients and excitability. This is exemplified by McArdle disease, where a severe defect in glycolgen breakdown is associated with rapid loss of excitability and contractile force during continued electrical stimulation (119). This might be related to accelerated elevation of [K⁺]ᵢ, during exercise seen in these patients (183).

In conclusion, the Na⁺-K⁺ pump in skeletal muscle seems to maintain transport function even under anoxia, partly because it can utilize glycolytic ATP, and possibly also because activity can continue even at rather low cytoplasmic levels of ATP. A preferential utilization of glycolytic ATP will allow prompt availability of energy, and might explain the increased lactate production in muscles exposed to Na⁺-K⁺ pump stimulation.

E. General Mechanisms for Acute Regulation of the Na⁺-K⁺ Pump

The traditional concept for the regulation of Na⁺-K⁺ pump activity is that activation is elicited by an increase in [Na⁺]ᵢ, [K⁺]ᵢ, or both. This may arise during excitation, unspecific membrane leakage, or after exposure to agents increasing the permeability of the plasma membrane to Na⁺ or K⁺. Conversely, decreasing the passive leaks to Na⁺ and K⁺ by exposure to local anesthetics or other membrane stabilizers causes a marked decrease in [Na⁺]ᵢ (98) and a minor increase in [K⁺]ᵢ (78). Because this redistribution mimics the effects of Na⁺-K⁺ pump stimulation, measurements of passive Na⁺-K⁺ fluxes are required to ascertain whether a decrease in intracellular Na⁺/K⁺ ratio is brought about by the Na⁺-K⁺ pump.

Hormonal or pharmacological agents (insulin, IGF-I, epinephrine, norepinephrine, dibutylr cAMP, and amylin) as well as electrical stimulation that elicit activation of the Na⁺-K⁺ pumps in skeletal muscle all induce a highly significant decrease in [Na⁺]ᵢ (81). This indicates that acute stimulation of the Na⁺-K⁺ pumps might often be elicited by an increase in the affinity for intracellular Na⁺ without any rise in [Na⁺]ᵢ. This could reflect a reversible change into another conformation of the transport system, allowing it to function more efficiently and maintain a steeper Na⁺ gradient and membrane potential.

Another general mechanism for stimulation of active Na⁺-K⁺ transport is translocation of Na⁺-K⁺ pumps from a cellular pool to insertion in the plasma membrane. Although such effects have been documented in kidney (139), the observations on translocation in skeletal muscle are conflicting and too small to account for the large increases in Na⁺-K⁺ pump activity observed during excitation (see sect. V-A).

Stimulation of the Na⁺-K⁺ pumps may also reflect an increase in the turnover number of the transport system. In skeletal muscle, this is likely to account for the acceleration induced by azide (122) or elevated temperature (79). Recent studies indicate that insulin increases the turnover number of the Na⁺-K⁺ pumps in fibroblasts (279).

Whereas several studies have characterized the molecular mechanisms of Na⁺-K⁺ pump regulation in the kidney (139), little is known about skeletal muscle. Recently, a small protein, phospholemman, was identified in skeletal muscle and shown to associate with the Na⁺-K⁺-ATPase and to reduce its affinity for Na⁺ by ~50% (94). Phospholemman can be phosphorylated by several hormones and seems to be a target for protein kinases A and C. It is difficult, however, to understand how phospholemman can mediate protein kinase-dependent stimulation of the Na⁺,K⁺ pump in muscle cells.

In conclusion, acute regulation of Na⁺-K⁺ pump activity can be exerted via increased availability of Na⁺ and K⁺ at the membrane, by increasing the affinity for intracellular Na⁺, translocation of Na⁺-K⁺ pumps to the sarcolemma or increased turnover number of the Na⁺-K⁺ pumps.

VI. LONG-TERM REGULATION OF Na⁺-K⁺ PUMP CONTENT

This section describes a number of factors controlling the synthesis of Na⁺-K⁺ pumps, leading to upregulation or downregulation of the total content of Na⁺-K⁺ pumps in skeletal muscle. These factors are listed in the right side of the general regulatory diagram in Figure 4. To give an idea about the regulatory range as well as the clinical significance, Figure 8 illustrates the relative changes observed in measurements of [³H]ouabain binding sites in human muscle.

A. Training and Inactivity

1. Effects on the content of Na⁺-K⁺ pumps in skeletal muscle

Over the last 15 yr, numerous longitudinal and cross-sectional studies have demonstrated that training leads to an upregulation and inactivity to a downregulation of the content of Na⁺-K⁺ pumps in skeletal muscle. As shown in Table 2, activity-dependent regulation is seen in nine dif-
ferent species and in a wide variety of muscles, indicating that it is a general reaction to physical exercise. Since it is seen in the muscles participating in the activity and not in other muscles from the same animal, it cannot be due to endocrine mechanisms acting in the entire organism (252). Besides, swim training of rats caused no significant change in the thyroid hormone levels of plasma (252).

Training-induced upregulation of Na\(^+\)-K\(^+\) pumps was first detected in measurements of Na\(^+\)-K\(^+\)-ATPase activity in sarcolemma membranes prepared by differential centrifugation of homogenates of dog gracilis muscle (254). After 6 wk of treadmill training, a 165% increase was observed which is far above the upregulation observed in later reports. This discrepancy may be due to the very low recovery of the total content of Na\(^+\)-K\(^+\)-ATPase in the muscle (2–3%) and the difficulties in ascertaining representative samples (see sect. ivB). In another study, 18 wk of treadmill training of rats caused no change in the Na\(^+\)-K\(^+\)-ATPase activity of the sarcolemmal fraction prepared from five different skeletal muscles (220). Succinate dehydrogenase activity, however, was significantly increased by the training.

Swim training for 6 wk at a rather high intensity induced up to 46% increase in the total content of [\(^3\)H]ouabain binding sites in the hindlimb muscles of rats. Citrate synthase activity increased by 76% in the same muscles (252). The upregulation was reversible, returning to the control level after 3 wk of rest. Immobilization of hindlimbs caused a 20–22% downregulation of [\(^3\)H]ouabain binding sites. The activity-dependent increase, from the immobilized muscles to the swim-trained muscles, amounted to 83%. Immobilization caused a downregulation of up to 25% in the content of [\(^3\)H]ouabain binding sites in guinea pig hindlimb muscles (269) and in the soleus of young rats (428). When subsequently trained by running, the content of [\(^3\)H]ouabain binding sites in guinea pig muscles increased by 57%. The highest content of [\(^3\)H]ouabain binding sites in trained control muscles was 93% above the lowest level measured after 2 wk of immobilization. In sheep, 9 wk of immobilization of a hindlimb caused 22% decrease in the content of [\(^3\)H]ouabain binding sites in the vastus lateralis muscle. This loss was restored during a subsequent remobilization (226). In humans, immobilization of the deltoid muscle in
TABLE 2. Effects of training and inactivity on the content of Na\(^+\)-K\(^+\) pumps in skeletal muscle from nine different species.

<table>
<thead>
<tr>
<th>Species, Muscle, and Training</th>
<th>Relative Change, %</th>
<th>Reference Nos.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dog gracilis, running</td>
<td>+165</td>
<td>254</td>
</tr>
<tr>
<td>Rat soleus, swimming</td>
<td>+43</td>
<td>252</td>
</tr>
<tr>
<td>Rat EDL, swimming</td>
<td>+46</td>
<td>252</td>
</tr>
<tr>
<td>Rat diaphragm, swimming</td>
<td>+2</td>
<td>252</td>
</tr>
<tr>
<td>Pig longissimus dorsi, shivering</td>
<td>+58</td>
<td>99</td>
</tr>
<tr>
<td>Human vastus lateralis, swimming</td>
<td>+30</td>
<td>253</td>
</tr>
<tr>
<td>Human vastus lateralis, running</td>
<td>+32</td>
<td>253</td>
</tr>
<tr>
<td>Human vastus lateralis, weight-lift</td>
<td>+40</td>
<td>253</td>
</tr>
<tr>
<td>Guinea pig soleus, running</td>
<td>+25</td>
<td>269</td>
</tr>
<tr>
<td>Rabbit EDL, el. stim. in vivo</td>
<td>+86</td>
<td>168</td>
</tr>
<tr>
<td>Human vastus lateralis</td>
<td>+16</td>
<td>297</td>
</tr>
<tr>
<td>Human vastus lateralis</td>
<td>+14</td>
<td>169</td>
</tr>
<tr>
<td>Human vastus lateralis</td>
<td>+15</td>
<td>283</td>
</tr>
<tr>
<td>Pig longissimus dorsi, shivering</td>
<td>+84</td>
<td>196</td>
</tr>
<tr>
<td>Human vastus lateralis</td>
<td>+16</td>
<td>133</td>
</tr>
<tr>
<td>Human vastus lateralis</td>
<td>+22</td>
<td>170</td>
</tr>
<tr>
<td>Human vastus lateralis</td>
<td>+18</td>
<td>153</td>
</tr>
<tr>
<td>Horse glutue med.</td>
<td>+23</td>
<td>299</td>
</tr>
<tr>
<td>Horse glutue med.</td>
<td>+32</td>
<td>401</td>
</tr>
<tr>
<td>Angoni cattle</td>
<td>+30</td>
<td>419</td>
</tr>
<tr>
<td>Rat soleus, plaster immobilization</td>
<td>-20</td>
<td>252</td>
</tr>
<tr>
<td>Rat soleus, tenotomy</td>
<td>-22</td>
<td>252</td>
</tr>
<tr>
<td>Guinea pig plantaris</td>
<td>-23</td>
<td>269</td>
</tr>
<tr>
<td>Human deltoid muscle,</td>
<td>-27</td>
<td>270</td>
</tr>
<tr>
<td>immobiolized by impingement</td>
<td></td>
<td></td>
</tr>
<tr>
<td>shoulder syndrome</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sheep vastus lateralis,</td>
<td>-22</td>
<td>226</td>
</tr>
<tr>
<td>immobiolized by plaster</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

With one exception (254), where Na\(^+\)-K\(^+\) pump content was measured as Na\(^+\)-K\(^+\)-activated ATPase activity, all data were obtained using the VO\(_2\)-facilitated \[\text{H}\]ouabain binding assay. EDL, extensor digitorum longus.

patients with impingement syndrome of the shoulder was associated with a significant reduction (27%) in the content of \[\text{H}\]ouabain binding sites (270). Denervation also reduced the content of \[\text{H}\]ouabain binding sites in mouse and rat skeletal muscle (77, 85, 428).

Training caused the same increase in \[\text{H}\]ouabain binding site content in soleus and EDL, indicating that it does not depend on fiber type (252). Training caused no change in the affinity for \[\text{H}\]ouabain, neither in rat muscle (252) nor in horse muscle (401). There is no quantitative information on the effects of training on the distribution of subunit isoforms.

The time course of activity-induced upregulation of the content of \[\text{H}\]ouabain binding sites was studied by exposing rabbit fast-twitch muscles to chronic 10-Hz stimulation in vivo. A significant increase could be detected after 4 days, reaching a maximum of 86% after 10 days. This was followed by a somewhat later and even more pronounced increase (up to 3-fold) in mitochondrial citrate synthase activity (168). The same laboratory showed that stimulation-induced upregulation of Na\(^+\)-K\(^+\) pump content had a similar time course, reaching a value 114% above the control level after 20 days (205). From these studies based on rather different training paradigms it may be concluded that the activity-dependent regulatory range for the content of Na\(^+\)-K\(^+\) pumps in skeletal muscle is ∼100%.

Exercise training increased the content of \[\text{H}\]ouabain binding sites in horse glutus medius muscle by 23% (290) or 32% (398). Somewhat smaller increases were seen in the semitendinosus muscle (290), and in animals on a moderate training program, no significant increase was observed, possibly because this muscle contributes less to the exercise effort (401). In trained foals, where the hindlimb muscles showed a 20–30% upregulation of \[\text{H}\]ouabain binding sites, the masseter muscle showed no significant change (400, 401). This confirms that the upregulation of Na\(^+\)-K\(^+\) pumps in skeletal muscle is localized to the muscles directly participating in the training and cannot be attributed to a generalized stimulation of the synthesis of Na\(^+\)-K\(^+\) pumps. Recently, training was found to increase the content of \[\text{H}\]ouabain binding sites in the semitendinosus muscle of African Angoni draught cattle by 16–30% (419). Shivering induced by reducing the environmental temperature from 35°C (where there is no detectable shivering) to 10°C (shivering most of the time) for several weeks increased the content of \[\text{H}\]ouabain binding sites in the longissimus dorsi muscle of pigs by 58% at normal energy intake and by 127% at low energy intake (99). These observations were confirmed with the addition that more than 50% of the more pronounced upregulation seen at low energy intake could be attributed to reduced fiber size and hence a greater membrane area (196). Generally, there was a significant correlation between membrane area per unit volume and the content of \[\text{H}\]ouabain binding sites per gram wet weight. However, the increase in Na\(^+\)-K\(^+\) pump content in the cold-exposure experiments could not be explained by increased plasma levels of thyroid hormones.

In human subjects, a cross-sectional study showed that the content of \[\text{H}\]ouabain binding sites in the vastus lateralis of 16 trained elderly men was 30–40% higher than in an age-matched group of untrained men (Fig. 8) (253). In young subjects, intense sprint training or endurance training increased the content of \[\text{H}\]ouabain binding sites in vastus lateralis muscle by 14–22% (133, 170, 283, 297, 298), an effect that was seen already after 1 wk (169), but showed no further increase over the next 10 wk (170). As shown in Table 1, in the vastus lateralis muscles of highly trained male cross-country skiers as well as alpine skiers, the content of \[\text{H}\]ouabain binding sites was in the highest reported range (∼350 pmol/g wt wt) (133) or 425 pmol/g wt wt (298). Before the training, female skiers showed 18% lower content of \[\text{H}\]ouabain binding sites than the males (133). The effects of long-lasting exercise in human subjects were explored by measuring the content of \[\text{H}\]ouabain binding sites in the vastus lateralis before and after a 100-km run, lasting on average...
10.7 h. This showed a significant increase of 13%, indicating that long-lasting continued exercise, already while it is taking place, promotes the synthesis of Na\textsuperscript{+}-K\textsuperscript{+} pumps (339).

In conclusion, there is massive evidence that the content of Na\textsuperscript{+}-K\textsuperscript{+} pumps in skeletal muscle depends on the contractile activity, with a regulatory range of \textasciitilde100\%. This is likely to reflect stimulation of the synthesis of Na\textsuperscript{+}-K\textsuperscript{+} pumps, restricted to the active muscles, possibly starting during early phases of activity.

2. Correlations to K\textsuperscript{+} clearance

Tibes et al. (412) observed that in trained human subjects, exercise-induced hyperkalemia was only half of that seen in untrained subjects. In dogs, 6 wk of training reduced the increase in plasma K\textsuperscript{+} induced by running from 1.6 to 0.4 mM (254). In Hereford calves, physical conditioning produced \textasciitilde50\% reduction in the hyperkalemia induced by performing the same strictly defined treadmill running. In contrast, if the animals were allowed to run at the higher maximum speed made possible by the training, hyperkalemia was 10\% higher (150).

In horses, 10 days of training reduced the exercise-induced peak values for plasma K\textsuperscript{+} by 13\% (299). The role of the Na\textsuperscript{+}-K\textsuperscript{+} pump in K\textsuperscript{+} homeostasis during exercise in domestic species (cats, dogs, horses, and cattle) was recently reviewed (124).

In humans, sprint training reduced exercise-induced hyperkalemia by 19\% and the ratio between exercise-induced rise in plasma K\textsuperscript{+} and work output by 27\% (297). Cycle training for 2 h/day for 6 days clearly improved the K\textsuperscript{+} clearance from plasma during exercise and increased the content of [\textsuperscript{3}H]ouabain binding sites in vastus lateralis by 14\% (169). During a 1-min sprint on a cycle ergometer, plasma K\textsuperscript{+} reached a significantly higher peak value in trained than in untrained subjects. Subsequently, plasma K\textsuperscript{+} underwent an exponential decay with time constants of 56 and 98 s in the trained and untrained subjects, respectively (284). This is in keeping with the above-mentioned study on calves, indicating that training increases the mass of muscles participating in the exercise, leading to a larger release of K\textsuperscript{+} into the circulating blood. Conversely, the ability to clear K\textsuperscript{+} during the subsequent resting phase seemed to be improved by training. Because the increase in plasma norepinephrine induced by a constant work load was reduced by \textasciitilde50\% after a 20-wk training program, the improved K\textsuperscript{+} clearance cannot be attributed to increased catecholamine levels (350). During incremental exercise, the increase in plasma K\textsuperscript{+} (delta K\textsuperscript{+}/work; in nmol L\textsuperscript{-1} J\textsuperscript{-1}) showed significant inverse correlation to 3-O-MFPase activity and [\textsuperscript{3}H]ouabain binding site content in biopsies of the human vastus lateralis muscle (153). On the other hand, the rate of decrease in plasma K\textsuperscript{+} after intense exercise was not correlated with the content of [\textsuperscript{3}H]ouabain binding sites in vastus lateralis (180).

In conclusion, there is good evidence that training improves the ability to clear the exercise-induced increase in plasma K\textsuperscript{+}, in part as a result of an upregulation of the content of Na\textsuperscript{+}-K\textsuperscript{+} pumps in skeletal muscle.

3. Correlation to energy turnover

There is evidence that the content of Na\textsuperscript{+}-K\textsuperscript{+} pumps in skeletal muscle is correlated to the capacity for energy turnover. Thus Na\textsuperscript{+}-K\textsuperscript{+}-ATPase activity was linearly correlated to the content of citrate synthase in different types of rat skeletal muscles (220). Comparison of four different skeletal muscles showed a similar correlation both in normal rats (58) and in rats with cardiac insufficiency (307). During a prolonged submaximal training program, a significant correlation ($r = 0.59$, $P < 0.05$) between the content of [\textsuperscript{3}H]ouabain binding sites in human vastus lateralis and peak aerobic power was found (170). Single leg cycle exercise increased both the content of [\textsuperscript{3}H]ouabain binding sites and citrate synthase (172). In contrast, cycle exercise of the contralateral leg during hypoxia produced a marked increase in citrate synthase, which was associated with a 14\% downregulation of [\textsuperscript{3}H]ouabain binding sites, indicating that it is possible to dissociate these two regulatory events. A similar dissociation was found in subjects exposed to high-altitude hypoxia (173).

In conclusion, exercise-induced upregulation of the content of Na\textsuperscript{+}-K\textsuperscript{+} pumps in skeletal muscle is associated with a concomitant upregulation of the oxidative potential. Even though these two changes are not necessarily causally coupled, the ability to maintain membrane excitability via upregulation of Na\textsuperscript{+}-K\textsuperscript{+} pump capacity is important for the ability to realize an increased $V_{O_{2}\max}$ (170).

4. Mechanisms for training-induced upregulation of Na\textsuperscript{+}-K\textsuperscript{+} pumps

The mechanisms eliciting training-induced upregulation of Na\textsuperscript{+}-K\textsuperscript{+} pump content in skeletal muscle was explored in primary cultures prepared from chick embryo leg muscle (436). With the use of monoclonal antibodies for the quantification of Na\textsuperscript{+}-K\textsuperscript{+} pumps on the cell surface, veratridine, which prolongs the open time of Na\textsuperscript{+} channels, produced a 60-100\% increase over a 24- to 36-h period (Fig. 9). This effect was reversible and blocked by TTX. Neither depolarization (with 50 mM K\textsuperscript{+}) nor elevation of cytoplasmic Ca\textsuperscript{2+} caused any change, indicating that the upregulation of Na\textsuperscript{+}-K\textsuperscript{+} pump content was elicited by the increased entry of Na\textsuperscript{+} via Na\textsuperscript{+} channels and not by depolarization or Ca\textsuperscript{2+} mobilization. The veratridine-induced increase in the content of Na\textsuperscript{+}-K\textsuperscript{+} pumps
was caused by a doubling of the rate of synthesis and a similar increase in their half-life (136).

The relative roles of $\alpha_1$- and $\alpha_2$-subunit isoforms in the regulation of the Na$^+$-K$^+$ pump induced by Na$^+$ influx have also been studied using cultured cells obtained from chicken and rat skeletal muscle. In chicken myogenic cultures, which only contain the $\alpha_1$-subunit isoform of the pump, 30 h of incubation with veratridine (10$^{-5}$ M) increased by 60% the amount of $\alpha_1$-subunit isoform associated with the membrane surface (measured using $^{125}$I-labeled monoclonal antibody) (436). There was no significant change in the intracellular fraction of Na$^+$-K$^+$-ATPase, which in these cells amounted to $\sim$60% of the total pool. The same study showed that when cells were incubated at low [K$^+$]o, the $\alpha_1$-isofrom increased by 20%. Veratridine induced an early and marked increase in the mRNA for the $\beta$-subunit isoform of the Na$^+$-K$^+$ pump followed by a somewhat slower and smaller increase in mRNA for the $\alpha_1$-subunit. Accordingly, there was an overexpression of the $\beta$-subunit protein, suggesting that the early upregulation of the $\beta$-subunit represented a drive for the assembly of the complete $\alpha\beta$-complex of the Na$^+$-K$^+$-ATPase (405).

In cultured rat myotubes, TTX, which lowered [Na$^+$]i, induced a progressive but reversible decrease in the content of [H]$^+$ouabain binding sites. Conversely, veratridine (10$^{-5}$ M) induced 50–75% increase in [H]$^+$ouabain binding and a 40–70% increase in ouabain-sensitive $^{86}$Rb uptake (41). Chronic pretreatment with ouabain induced 35% increase in the content of [H]$^+$ouabain binding sites. The upregulation elicited by veratridine or ouabain was both blocked by protein synthesis inhibitors. Elevation of intracellular Ca$^{2+}$ caused no change in the synthesis of ouabain binding sites. It was concluded that the [Na$^+$]i level plays a dominant role in the regulation of Na$^+$-K$^+$ pump synthesis. Veratridine elicited spontaneous oscillations in the membrane potential, mimicking the exposure to repeated action potentials during a training period (41). This is quite relevant for the understanding of training-induced upregulation of Na$^+$-K$^+$ pumps.

After 1–4 days of exposure to carbachol, myotubes showed hyperpolarization, caused by enhanced electrogenic Na$^+$-K$^+$ transport. This was associated with 45% upregulation of both $\alpha_1$- and $\alpha_2$-subunit isoforms of the Na$^+$-K$^+$ pump, as detected with monoclonal antibodies (203). In a myogenic cell line derived from mouse thigh muscle, 3-day exposure to carbachol elicited a 50% increase in the $\alpha_2$-subunit isoform, but no significant change in the $\alpha_1$-subunit isoform (258). In a muscle cell line obtained from mouse thigh muscle pretreatment with veratridine increased the $\alpha_2$-subunit isoform by 88%, but caused no change in the $\alpha_1$-isofrom (263). Conversely, TTX decreased the $\alpha_2$-isofrom by 58% without producing any change in the $\alpha_1$-isofrom. Taken together, these observations indicate that the effects of electrical stimulation or veratridine on the synthesis of Na$^+$-K$^+$ pumps is not restricted to a particular subunit isoform.

In conclusion, the activity-dependent regulation of Na$^+$-K$^+$ pump content in skeletal muscle can be mimicked by exposure of isolated myocytes to agents causing respectively opening or closing of Na$^+$ channels. Although increased [Na$^+$]i leads to stimulation of Na$^+$-K$^+$ pump synthesis in cell cultures, this may not be the only drive for activity-dependent upregulation in vivo.

B. Thyroid Hormones, Starvation, and Diabetes

1. Thyroid hormones

Thyroid hormones increase Na$^+$-K$^+$-ATPase activity in skeletal muscle (12). In soleus muscles of hyperthyroid rats, the content of [H]$^+$ouabain binding sites was $\sim$10-fold larger than in soleus from hypothyroid rats (245). In rat skeletal muscle, triiodothyronine (T$_3$) pretreatment for 12 days induced a fivefold increase in the relative abundance of the $\alpha_2$-subunit isoform, but no significant change in the $\alpha_1$-isofrom. The amount of mRNA showed similar relative changes (15). In human vastus lateralis muscle, the content of [H]$^+$ouabain binding sites increases with thyroid status from 100 to 600 pmol/g wet wt (Fig. 8). This increase was closely correlated to the free thyroxine (T$_4$) index, and after therapeutic correction of the hypothyroidism or hyperthyroidism, the content of [H]$^+$-ouabain binding sites returned to the level of euthyroid subjects (248).

In rat diaphragm muscle, hyperthyroidism increased the $\alpha_2$-subunit isoform threefold over that in the hypothyroid state, but caused no change in the $\alpha_1$-subunit isoform...
(181). Hypothyroidism decreased the \( \alpha_2 \)-isoform in rat skeletal muscle to one-sixth of that of the euthyroid controls, but there was a much smaller reduction in mRNA for the \( \alpha_2 \)-isoform (213). mRNA for the \( \alpha_1 \)-isoform showed no decrease in hypothyroidism, but a significant increase in hyperthyroidism.

In rats, the effect of thyroid hormones on the content of \(^3\)H]ouabain binding sites is more pronounced in muscles containing a high proportion of slow-twitch fibers (245). Moreover, the large increase in the content of \(^3\)H]ouabain binding sites is associated with a proportional increase in the maximum rate of ouabain-suppressible \(^86\)Rb uptake, indicating that the Na\(^+\)-K\(^+\) pump capacity undergoes the same relative upregulation (71). Also the basal rates of ouabain-suppressible uptake of \(^{42}\)K and \(^{86}\)Rb show the same relative increase as that of \(^3\)H]ouabain binding capacity (28, 300).

Studies on cultured rat skeletal myotubes indicate that the thyroid hormone-induced increase in the content of \(^3\)H]ouabain binding sites reflects a stimulation of the synthesis of Na\(^+\)-K\(^+\) pumps (37). Cells grown in the presence of \( T_3 \) or \( T_4 \) for 24 h showed an increase in \(^3\)H]ouabain binding, ouabain-suppressible \(^86\)Rb uptake, and resting membrane potential. These effects were abolished by blocking protein synthesis with cycloheximide or actinomycin D. When Na\(^+\) influx via Na\(^+\) channels or the Na\(^+\)/H\(^+\) antiporter were blocked using TTX or amiloride, the thyroid hormone-induced increase in Na\(^+\)-K\(^+\) pump synthesis was suppressed, indicating that it might be elicited by an early increase in Na\(^+\) influx. Indeed, thyroid hormones increased the content of Na\(^+\) channels, as quantified by saxitoxin binding, by 95% within 24 h (38).

Also in the rat, treatment with thyroid hormone induced early increases in the resting \(^{42}\)K efflux, \(^{86}\)Rb efflux, and \(^{22}\)Na influx of soleus muscles that were highly significant 12 h after the first injection with \( T_3 \), clearly preceding the increase in \(^3\)H]ouabain binding (125). Also in rat EDL, a significant stimulation of \(^{86}\)Rb efflux was seen 12 h after injection of \( T_3 \). Conversely, in muscles from hypothyroid rats, \(^{86}\)Rb efflux was decreased by 30%. The increase in the content of Na\(^+\)-K\(^+\) pumps was preceded by an upregulation of the content of Na\(^+\) channels (195). In rat soleus, the content of \(^3\)H]ouabain binding sites and \(^{42}\)K uptake were decreased by 61% in hypothyroidism and increased by 80% in hyperthyroidism (287). Total fasting decreased \(^3\)H]ouabain binding to the same level as in the hypothyroid rats. These changes were associated with similar relative changes in ouabain-suppressible \(^{42}\)K uptake. Refeeding or \( T_3 \) treatment of the fasting rats restored \(^3\)H]ouabain binding to the normal level.

Hypophysectomy decreased the content of \(^3\)H]ouabain binding sites in rat soleus by \(-80\%\). Subcutaneous injections of \( T_4 \) at doses restoring the serum concentrations of \( T_3 \) and \( T_4 \) to the physiological level increased the content of \(^3\)H]ouabain binding sites in the muscles of hypophysectomized rats to the control level in 11 days (128). Human growth hormone at doses causing normalization of serum IGF-I induced no increase in the binding sites. These observations indicate that the effect of thyroid hormones on the synthesis of Na\(^+\)-K\(^+\) pumps in skeletal muscle is exerted at physiological concentrations and does not depend on growth hormone or IGF-I.

Among Oriental males, attacks of hypokalemic periodic paralysis are a complication in 13% of the cases of hyperthyroidism (292). These attacks are seen following the ingestion of carbohydrates and have been related to an abnormally large hyperinsulinemic response to carbohydrates (389). Attacks can be provoked by a carbohydrate-rich meal combined with subcutaneous injection of insulin (292). Due to the increased content of Na\(^+\)-K\(^+\) pumps in the skeletal muscles of hyperthyroid subjects, the insulin may induce a more pronounced hypokalemia than in normal subjects. Because propranolol treatment prevents or abolishes the paralytic attacks induced by carbohydrate ingestion (88, 274), catecholamine-induced stimulation of the Na\(^+\)-K\(^+\) pumps may also be involved. Thus a glucose load increases the plasma concentration of catecholamines (13). In rats, the extrarenal clearance of an intravenous infusion of K\(^+\) is severely impaired by hypothyroidism and slightly improved by hyperthyroidism (261).

In conclusion, thyroid hormones in the physiological range are the major endocrine factors controlling the content of Na\(^+\)-K\(^+\) pumps in skeletal muscle. Their marked stimulating effect on the synthesis of Na\(^+\)-K\(^+\) pumps seems to be driven by an early increase in the passive leaks to Na\(^+\) and K\(^+\). This is important for whole body Na\(^+\)-K\(^+\) homeostasis and contractile performance (see sect. viiG). Moreover, the decrease in Na\(^+\)-K\(^+\) pump content in fasting or diabetes may be due to reduced thyroid status.

2. Starvation

Starvation leads to a decrease in the plasma levels of thyroid hormones in humans (415) and in rats (246, 383). Complete fasting for 5 days induced a 50% decrease in the content of Na\(^+\)-K\(^+\)-ATPase of sarcolemma prepared from rat skeletal muscle (402). Measurements on intact rat muscle samples showed that 5 days of starvation only caused a 25% decrease in the content of \(^3\)H]ouabain binding sites (246). Reducing the caloric intake to one-half the normal for 3 wk caused 45 and 53% decrease in plasma \( T_3 \) and \( T_4 \), respectively, and a 25% drop in the content of \(^3\)H]ouabain binding sites in soleus. A later study confirmed the downregulation induced by semistarvation (379), which was also seen in various other types of skeletal muscle. The downregulation of Na\(^+\)-K\(^+\) pumps was fully reversible after 3 days of refeeding (246). The downregulation could not be attributed to K\(^+\) deficiency.
and may be accounted for by the reduction in thyroid status. Because reduced caloric intake also decreases the mass of skeletal muscles, the total muscle pool of Na\(^+\)-K\(^+\) pumps is severely decreased (in rat by up to 58%), leading to impairment of K\(^+\) tolerance. In rats, fasting for 2 days induced a 33\% decrease in the Na\(^+\)-K\(^+\) ATPase activity in skeletal muscle (322). Four days of starvation decreased plasma T\(_3\) and the content of \(^{[3H]}\)ouabain binding sites in the soleus of 5-wk-old rats to the same value as in hypothyroid rats, effects that were completely restored to the control level by 5 days of refeeding or prevented by concomitant administration of T\(_3\) (287). Ouabain-suppressible \(^{42}\)K uptake showed similar relative changes as \(^{[3H]}\)ouabain binding. A more recent study on Shetland ponies showed that food restriction causing 30–50\% reduction in body weight gain produced significant decreases in serum T\(_3\) and free T\(_3\) (30 and 49\%, respectively), but only a minor (12–15\%) nonsignificant decrease in the content of \(^{[3H]}\)ouabain binding sites in the gluteus medius muscle (309). The downregulation of Na\(^+\)-K\(^+\) pumps seen during reduced caloric intake might in part reflect reduced physical activity, although it is more rapid than that seen during inactivity. However, it is unlikely to be due to reductions in fiber size, which would rather cause an increase leading to underestimation of the downregulation (196).

Globally, the downregulation of Na\(^+\)-K\(^+\) pumps in skeletal muscle elicited by reduced caloric intake may represent the most commonly occurring Na\(^+\)-K\(^+\) pump disorder, important for the ability to tolerate K\(^+\) ingestion as well as for muscle endurance. Still, there are no specific studies on this anomaly in humans. Moreover, it seems likely that reduced food intake in relation to cancer, infections, anorexia, and other diseases might also lead to downregulation of Na\(^+\)-K\(^+\) pumps in skeletal muscle.

3. Diabetes

In rats made diabetic by streptozotocin treatment, the content of \(^{[3H]}\)ouabain binding sites in skeletal muscle as measured after 4 wk was reduced by between 24\% (in EDL) and 48\% (in soleus). These changes were completely prevented by insulin treatment (244). Moreover, in the diabetic rats, 8 wk of treatment with insulin increased the content of \(^{[3H]}\)ouabain binding sites in skeletal muscle by 23\%. In rats made diabetic by streptozotocin or partial pancreatectomy, the content of \(^{[3H]}\)ouabain binding sites in soleus was reduced by 12–21\% (379). Again, insulin treatment of streptozotocin-diabetic rats increased the content of \(^{[3H]}\)ouabain binding sites to 18–26\% above controls. Moreover, in insulin-treated patients with non-insulin-dependent diabetes mellitus, where plasma insulin was increased, the content of \(^{[3H]}\)ouabain binding sites in vastus lateralis was increased by \(\sim 20\%\) compared with healthy controls (Fig. 8), and there was a significant correlation between plasma insulin and the content of \(^{[3H]}\)ouabain binding sites in vastus lateralis muscle (379). In skeletal muscle prepared from rats 2 and 14 days after the induction of diabetes with streptozotocin, the activity of Na\(^+\)-K\(^+\)-ATPase was decreased by 30–50\% (322). This may explain the observation that in diabetic rats, [Na\(^+\)], in skeletal muscle is increased (303). In contrast, crude membrane preparations obtained from skeletal muscle of streptozotocin showed a clear increase in the contents of both \(\alpha_1\) and \(\alpha_2\)-subunit isoforms, which could be partially reversed by insulin treatment (312). The same study showed that in the diabetic heart, the content of \(\alpha_2\)-isoform was significantly decreased, and this was partially restored by insulin treatment. These discrepancies remain unexplained.

In juvenile human diabetic subjects, the withdrawal of insulin treatment led to a significant drop in the nightly peaks of serum thyroid stimulating hormone (TSH) (40\%) and free serum T\(_3\) (28\%) (382), suggesting that insulin increases thyroid hormone levels via an effect on pituitary function. In rats, streptozotocin-induced diabetes was associated with a marked reduction in the plasma thyroid hormone level, which could be completely restored by insulin (397, 438).

Taken together, the observations indicate that insulin stimulates the synthesis of Na\(^+\)-K\(^+\) pumps in skeletal muscle, possibly indirectly by increasing the secretion of thyroid hormones. This might explain the downregulation of muscular Na\(^+\)-K\(^+\) pump content seen in untreated diabetes.

C. Steroid Hormones

It is well established that adrenal steroids influence the synthesis of Na\(^+\)-K\(^+\)-ATPase in a wide variety of tissues. However, almost no studies have explored the effects on skeletal muscle. In the rat, infusion of the glucocorticoid dexamethasone via osmotic minipumps was found to increase the content of \(^{[3H]}\)ouabain binding sites in soleus, EDL, gastrocnemius, and diaphragm muscles (113, 364). This increase was significant after 1 wk of treatment and after 2 wk, the upregulation was 34\% in soleus and 52\% in EDL, respectively. Effects could be detected down to a dose of 0.02 mg \cdot kg body wt\(^{-1}\) \cdot day\(^{-1}\) and seemed to reach its maximum at a dose of 0.05 mg \cdot kg\(^{-1}\) \cdot day\(^{-1}\). Thus the sensitivity is relatively low, and because the content of \(^{[3H]}\)ouabain binding sites in muscles was not reduced after adrenalectomy, the unstimulated level of endogenous glucocorticoids is of minor importance for the physiological regulation of the content of Na\(^+\)-K\(^+\) pumps in skeletal muscle. It is possible, however, that physiological stimulation of glucocorticoid secretion is sufficient to stimulate the synthesis of...
Na\(^{+}\)–K\(^{+}\) pumps in muscle. Furthermore, because large doses of glucocorticoids or ACTH are frequently used for treatment of a wide variety of disorders, upregulation is likely to occur in many patients. Indeed, intensive treatment of chronic obstructive lung disease with dexamethasone was shown to lead to an increase of between 31 and 61% in the content of \[^{3}H\]ouabain binding sites in the vastus lateralis muscle (364). In a follow-up on the above-mentioned studies on rats (410), both in soleus, EDL, gastrocnemius and diaphragm, dexamethasone treatment (0.1 mg · kg\(^{-1}\) · day\(^{-1}\) for 2 wk) was found to increase the abundance of the \(\alpha_2\)- and \(\beta_1\)-subunits ~1.5-fold. The \(\beta_1\)-subunit was increased by a 1.7-fold in the diaphragm, but in none of the other muscles. In the hindlimb muscles, dexamethasone treatment increased mRNA for \(\alpha_2\)- and \(\beta_1\)-subunits 6.4- and 1.5-fold, respectively.

The effects of dexamethasone on the synthesis of Na\(^{+}\)–K\(^{+}\) pumps could not be attributed to mineralocorticoid actions of the compound. Thus aldosterone induced a highly significant downregulation of the content of \[^{3}H\]ouabain binding sites in all four muscles examined. This could be related to the concomitant K\(^{+}\) deficiency elicited by aldosterone (113) and is in keeping with an earlier study showing that fluorohydrocortisone decreases the content of K\(^{+}\) and \[^{3}H\]ouabain binding sites in skeletal muscle (250).

In conclusion, high doses of glucocorticoid stimulate the synthesis of Na\(^{+}\)–K\(^{+}\) pumps in skeletal muscle. This might be of importance for K\(^{+}\) homeostasis and muscle contractility in patients treated with these agents.

D. Growth, Differentiation, and Fiber Type

As already reviewed in some detail (62), the early phases of growth and differentiation of muscle cells in rats and mice are associated with a marked increase in the content of \[^{3}H\]ouabain binding sites. In both species, the content reaches a peak value around 4 wk of age, followed by a decrease lasting several months, reflecting increasing fiber dimensions and ensuing reduction in surface-to-volume ratio. It was suggested that the early increase in \[^{3}H\]ouabain binding site content followed the development of the t-tubular system, which reaches full differentiation at the 4th week of life (249). A recent study on mice showed that postnatally, the \(\alpha_2\)-subunit isoform and the dihydropyridine receptor (a marker for t tubules) show parallel increases, reaching their full expression at 6 wk (92).

Measurements of 3-O-MFPase in crude homogenates of rat gastrocnemius muscles showed a time course similar to that of \[^{3}H\]ouabain binding with peak values reached at 4 wk. This argues against the possibility that the low \[^{3}H\]ouabain binding values found at birth are due to an undetected population of Na\(^{+}\)–K\(^{+}\) pumps with low affinity for ouabain.

Measurements of mRNA in rat skeletal muscle in the interval from fetal (14 days gestation) to 55 days of age showed unaltered abundance of \(\alpha_1\), but marked increases of \(\alpha_2\) and more modest increase in \(\alpha_3\) (338). This is in keeping with the above-mentioned increase in the content of Na\(^{+}\)–K\(^{+}\) pumps with high affinity for \[^{3}H\]ouabain, but due to uncertainties about the expression of the three mRNAs, precise comparisons are not yet possible.

In guinea pigs, the content of \[^{3}H\]ouabain binding sites seemed to reach its maximum at birth (600–900 pmol/g wet wt), followed by a decrease to ~350 pmol/g wet wt (249). Thus, at variance with the rat and mouse, the guinea pig is from birth equipped with a considerable amount of Na\(^{+}\)–K\(^{+}\) pumps, in keeping with its much earlier motor activity.

In pigs, the content of \[^{3}H\]ouabain binding sites in hindlimb skeletal muscle increased by ~100% during the last 30 days of gestation (101). The low content of Na\(^{+}\)–K\(^{+}\) pumps in fetal skeletal muscle reduces the ability to clear K\(^{+}\) from plasma and may explain the often marked hyperkalemia in premature children (175, 390). Measurements performed on four different pig muscles showed an increase from ~500 pmol/g wet wt at birth to ~700 pmol/g wet wt within the first week of life, followed by a decline to ~300 pmol/g wet wt at 8 wk (100). In horses, the content of \[^{3}H\]ouabain binding sites in gluteal muscle decreased by ~50% from 5 mo of age to maturity (353, 401).

In adult human subjects, the content of \[^{3}H\]ouabain binding sites in vastus lateralis muscle showed no significant change in the age range 25–80 yr (328). A post mortem study on the same muscle obtained from children in the age range 0–9 yr showed no age-dependent changes in the content of \[^{3}H\]ouabain binding sites (247).

Soleus and EDL muscles contain predominantly slow-twitch type I and fast-twitch type II fibers, respectively. Direct comparison showed that in the guinea pig in the age range 0–32 wk, EDL contains ~50% more \[^{3}H\]ouabain binding sites than soleus (249). In 4-wk-old rats, EDL contains ~20% more \[^{3}H\]ouabain binding sites, both when measured in isolated muscles in vitro (77, 126) and in vivo (77). In 8- to 10-wk old rats, in vitro measurements showed a difference of ~50% (44, 113). One study, however, showed that in the white portion of gastrocnemius, the content of \[^{3}H\]ouabain binding sites was 50% lower than in the red portion (307). Thus most observations indicate that type II fibers contain more Na\(^{+}\)–K\(^{+}\) pumps, possibly reflecting their documented higher rate of excitation-induced Na\(^{+}\)–K\(^{+}\) leaks (84).

In conclusion, the age-dependent changes in the content of Na\(^{+}\)–K\(^{+}\) pumps in skeletal muscle vary considerably among species, often reflecting differences in the motor activity or maturity, but sometimes also t-tubular development and the decrease in surface-to-volume ratio with muscle hypertrophy. Most data indicate that in rats
and guinea pigs type II fibers contain more Na\(^+\)-K\(^+\) pumps than type I fibers, possibly due to differences in passive Na\(^+\)-K\(^+\) fluxes.

E. K\(^+\) Deficiency and K\(^+\) Overload

1. K\(^+\) deficiency

K\(^+\) deficiency is one of the most common electrolyte disorders, arising from inadequate dietary supplies of K\(^+\) or Mg\(^{2+}\), diarrhea, treatment with diuretics, laxatives, antibiotics or the commonly used anticancer drug cisplatinum, or loss of K\(^+\) due to renal disease. The effects of K\(^+\) deficiency on Na\(^+\)-K\(^+\) homeostasis and the Na\(^+\)-K\(^+\) pump are therefore of considerable interest. When cells are cultured under conditions (with ouabain or in K\(^+\)-free buffer) leading to loss of cellular K\(^+\) and elevation of [Na\(^+\)]\(_{i}\), the synthesis of Na\(^+\)-K\(^+\) pumps is increased, leading to an up to severalfold rise in the Na\(^+\)-K\(^+\) pump content (355). A similar upregulation is also seen in cultured muscle cells and may in such cells also be obtained using veratridine to increase [Na\(^+\)]\(_{i}\) (39, 436). It was surprising, therefore, that in animals maintained on K\(^+\)-deficient fodder, the Na\(^+\)-K\(^+\) pump content of skeletal muscle underwent a marked decrease of up to 80% (327). This downregulation was further documented in measurements of 3-O-MFPase activity and 42K uptake in muscles prepared from the K\(^+\)-deficient animals (for details, see Ref. 62). Measurements on isolated soleus muscles prepared from K\(^+\)-deficient rats showed a close correspondence between the reduction in the content of [\(^3\)H]ouabain binding sites and the reduction in maximum capacity for Na\(^+\)-K\(^+\) pumping (71).

In vivo measurements of [\(^3\)H]ouabain binding capacity in skeletal muscle showed that K\(^+\) deficiency caused the same downregulation as observed when [\(^3\)H]ouabain binding took place in vitro (77). The K-deficient rat also showed impaired ability to clear injected [\(^3\)H]ouabain from the plasma. [\(^3\)H] activity measured in plasma 15 min after an intraperitoneal injection of [\(^3\)H]ouabain was 77% higher in K\(^+\)-deficient rats than in controls receiving the same dose of [\(^3\)H]ouabain per kilogram body weight (77).

K\(^+\)-deficient rats showed a reduced capacity to clear K\(^+\) from plasma following oral administration of KCl (371). This is important because treatment of K\(^+\) deficiency with intravenous or oral K\(^+\) supply carries an extra risk of inducing life-threatening levels of hyperkalemia. Recently, however, a more detailed study showed that in K\(^+\)-deficient intact rats, the clearance of intravenously infused K\(^+\) was more efficient than in the controls (44). This could be attributed to a more efficient renal clearance and a higher rate of K\(^+\) uptake in skeletal muscles, probably due to the increased [Na\(^+\)]\(_{i}\), causing stimulation of the Na\(^+\)-K\(^+\) pump.

Following the onset of K\(^+\) deprivation, the relative decrease in [\(^3\)H]ouabain binding site content in skeletal muscle was somewhat larger than the relative decrease in K\(^+\) content (250, 327). This supports the idea that downregulation of Na\(^+\)-K\(^+\) pump capacity in skeletal muscle is an early regulatory adaptation to K\(^+\) deficiency, favoring a net release of K\(^+\) from the large muscular K\(^+\) pool.

K\(^+\) deficiency is associated with inhibition of growth and protein synthesis (109). However, experiments with protein-deficient rats showed that the downregulation of [\(^3\)H]ouabain binding sites could not be attributed to reduced protein synthesis (250). In rats given K\(^+\)-deficient fodder, serum IGF-I decreased by ~50% in 7 days. When serum IGF-I was restored by infusion of the hormone, however, the downregulation of [\(^3\)H]ouabain binding sites was not counteracted (114). Neither was there any stimulating effect of IGF-I on [\(^3\)H]ouabain binding sites in control rats. Another study showed that the downregulation of [\(^3\)H]ouabain binding sites seen in K\(^+\) deficiency was not due to reduced sensitivity to thyroid hormone (245).

Studies on the relative abundancy of the \(\alpha\)-subunit isoforms of Na\(^+\)-K\(^+\)-ATPase showed that following 1–4 wk on low-K\(^+\) diet, the \(\alpha_2\)-isoform in hindlimb muscles showed a progressive decrease and disappeared after 3 wk (214). The \(\alpha_1\)-isoform showed a somewhat smaller decrease. Measurements of Na\(^+\)-K\(^+\)-ATPase activity showed that the \(\alpha_2\)-isoform with high affinity to ouabain disappeared after 3 wk on low-K\(^+\) diet, whereas the \(\alpha_1\)-isoform decreased by 40%. Surprisingly, in the muscles from the K\(^+\)-deficient rats, the mRNA for the \(\alpha_2\)-isoform underwent a marked increase to a level above that of the untreated control rats. It was proposed that when K\(^+\) deficiency is developed under in vivo conditions, the expected increase in the production of Na\(^+\)-K\(^+\)-ATPase is prevented, possibly due to suppression of the translation of the mRNA (214).

Another study showed that following 2 wk on K\(^+\)-deficient diet, the \(\alpha_2\)-isoform of Na\(^+\)-K\(^+\)-ATPase in homogenate of rat hindlimb muscles had decreased by 82%, whereas the \(\alpha_1\)-isoform showed no change (14). Total K\(^+\)-pNPPase activity, which is closely associated with Na\(^+\)-K\(^+\)-ATPase activity, was reduced by 42%. The mRNA for, \(\alpha_2\), was only reduced by 35%, again suggestive of suppressed translation. Already after 2 days on K\(^+\)-deficient fodder, the \(\alpha_2\)-isoform in skeletal muscle had decreased by 36%, whereas the relative drop in muscle K\(^+\) content was only ~10%. This is in keeping with the above-mentioned data for relative changes in the content of [\(^3\)H]ouabain binding sites and K\(^+\). During K\(^+\) restriction in the rat, the downregulation of the \(\alpha_2\)-isoform of Na\(^+\)-K\(^+\)-ATPase was more pronounced in muscles containing predominantly type II fibers than in those containing mainly type I fibers (411). Although this conclusion received support from comparison of red and white gastrocnemius
muscles, comparison of soleus and EDL showed no difference in downregulation in spite of the marked differences in the relative abundance of type I and type II fibers in these two muscles (409). In keeping with this, measurements on soleus and EDL had shown that after 1 wk of K+-deprivation in rats, mice, and guinea pigs showed that during K+-deficiency elicited by dietary K+-restriction, fluorohydrocortisone, or diuretics, the K+-content was closely correlated to the content of [3H]ouabain binding sites over a wide range.

In hindlimb muscles from ferrets maintained for 20 days on K+-deficient diet, K+-pNPPase activity was decreased by 27%, the α2-subunit isoform by 51%, whereas the α1-subunit isoform showed no change (311).

Taken together, the data demonstrate that during K+-deficiency, skeletal muscle cells in several species undergo a downregulation of Na+-K+-pump content. This, in turn, is perhaps the cause of the rather selective loss of K+-efficiency elicited by long-term treatment with diuretics or mineralocorticoids caused a loss of K+-efficiency from skeletal muscle cells. This type of K+-deficiency was also associated with a downregulation of the content of [3H]ouabain binding sites (113, 250). Also in humans, K+-deficiency elicited by long-term treatment with diuretics was associated with a significant reduction in the content of [3H]ouabain binding sites in biopsies of the vastus lateralis muscle (115–117). The content of [3H]ouabain binding sites showed a significant correlation to the K+/Na+ ratio in the muscle biopsies. In patients treated with diuretics, the renal loss of Mg2+-favors the loss of K+. Oral Mg2+-supplements, therefore, limit the loss of K+-efficiency from the muscles. In a group of 76 patients, who had received diuretics for 1–17 yr, oral administration of Mg2+ for 26 wk restored the contents of K+, Mg2+, and [3H]ouabain binding sites in the vastus lateralis toward the normal levels (117). Mg2+- and K+-deficiency developing in alcoholics is associated with a minor, but significant, downregulation of [3H]ouabain binding sites (8–14%) in vastus lateralis (1).

Taken together, these observations indicate that K+-deficiency arising from a variety of causes leads to early adaptations, involving reduced insulin sensitivity of the Na+-K+-pump, downregulation of the content of Na+-K+-pumps in the muscles, and ensuing transfer of K+ from muscles to plasma and vital organs. This response delays the onset of severe hypokalemia with ensuing paralysis and may therefore have survival value. K+-deficiency also leads to a loss of Na+-K+-pumps from skeletal muscle in human subjects (for reviews, see Refs. 65 and 109), which may contribute to the fatigue known to be associated with K+-deficiency (see sect. viiD). Moreover, a reduction in the total pool of Na+-K+-pumps in skeletal muscle may increase the risk of inducing severe hyperkalemia if the condition is treated with K+-infusion.

2. K+-overload

Animals fed a diet enriched in K+ show an adaptation allowing more efficient clearance of an intravenous K+-load from the extracellular phase. This is in part due to increased uptake of K+ in extrarenal tissues (4, 29, 45). Thus, after an intravenous injection of 86Rb, the uptake of the tracer into skeletal muscle of K+-supplemented rats was 87% larger than in the controls (29). Liver, heart, and spleen showed no change in 86Rb uptake, indicating that the increased clearance of K+ primarily took place by accumulation in skeletal muscle.

In rats maintained on K+-supplemented fodder for 10 days, the Na+-K+-ATPase activity and [3H]ouabain binding in microsomal fractions prepared from skeletal muscle were increased by 64 and 100%, respectively (29). A more recent study showed that whereas 1–2 wk of K+-supplementation (200 mmol/100 g Chow) caused only a minor increase (20%) in the content of [3H]ouabain binding sites in soleus, gastrocnemius and EDL muscles showed increases of 42–68%, indicating that upregulation was more pronounced in type II fibers (45). The K+ supplementation induced similar relative increases in the activity of K+-pNPPase, in crude muscle homogenates. The upregulation of Na+-K+ pump content of skeletal muscles seems to explain that during an intravenous KCl infusion the K+ supplemented rats showed a 62% lower increase in plasma K+ (29). The improved K+-clearance was confirmed in measurements on arterial blood from K+-supplemented rats where the increase in plasma K+ induced by a K+ load was reduced by 42% (45).

In conclusion, dietary K+-overload induces the opposite change in the content of Na+-K+-pumps in skeletal muscle to that seen in K+-deficiency. This upregulation might facilitate the clearance of excessive loads to K+ in connection with the repeated ingestion of a large amounts of K+-rich fodder (meat, fruit, vegetables, in particular in dry form).
F. Heart Failure and Hypoxia

1. Heart Failure

In patients with congestive heart failure (mean ejection fraction 32%), the content of [3H]ouabain binding sites in the vastus lateralis muscle showed a significant reduction of 25% as compared with an age-matched group with paroxysmal tachycardia but an ejection fraction of 60% (Fig. 8). The [3H]ouabain binding site values were correlated to the ejection fraction (325). In another study, the content of [3H]ouabain binding sites in the vastus lateralis of 11 heart failure patients was reduced by 37% (380). It cannot be excluded, however, that this downregulation is the result of decreased physical activity. In patients suffering from mitral stenosis, the content of [3H]ouabain binding sites in biopsies of vastus lateralis was low (228 pmol/g wet wt). Immediately after operation, there was no change, but 4 mo later a significant increase to 265 pmol/g wet wt (+16%) was found (21). In the entire group of patients there was a significant negative correlation between the content of [3H]ouabain binding sites in muscle and the exercise-induced hyperkalemia, suggesting that Na\(^+-\)K\(^+\) pumps assisted in the clearance of K\(^+\) during exercise.

In patients with heart failure, exercise-induced hyperkalemia was more pronounced, reaching the same level as in control subjects already at \(~40\%\) lower work loads (22). The same study showed that physical training reduced the exercise-induced hyperkalemia and that this could not be attributed to differences in plasma catecholamine levels reached during work.

Surprisingly, a recent study showed that in 36 patients with moderate chronic heart failure (ejection fraction 25%), the content of [3H]ouabain binding sites in vastus lateralis was not significantly different from that of sedentary controls (171). Moreover, even though training produced a significant (19%) increase in peak oxygen uptake of these patients, no significant increase in the content of [3H]ouabain binding sites was found. However, there was a weak positive correlation (\(r = 0.4\) and \(P < 0.05\)) between the content of [3H]ouabain binding sites and peak oxygen uptake. The discrepancy between the absence of downregulation in this study, in spite of the larger reduction in ejection fraction, is unexplained.

In rats where chronic heart failure had been induced by ligation of the left coronary artery, measurement of the content of [3H]ouabain binding sites in soleus and plantaris muscles 26 wk later showed a significant decrease of \(~20\%\) (352). Another study showed that 8--10 wk after ligation of the left coronary artery, rats had moderate or severe cardiac dysfunction (307). Among those with severe dysfunction, the content of [3H]ouabain binding sites was significantly reduced (21--28%) in three hindlimb muscles. The downregulation was not the result of deconditioning and was not associated with or accounted for by any change in the apparent affinity for [3H]ouabain (307, 352). The content of [3H]ouabain binding sites was correlated with VO\(_{2}\text{max}\) and the content of citrate synthase. At variance with this, another study showed that chronic heart failure in rats, induced by a similar procedure, induced no significant decrease in the content of \(\alpha_1-\) and \(\alpha_2\)-subunit isoforms as assessed using immunoblot analysis of sarcosomal vesicles from soleus and EDL (281). In these rats, the muscles showed little or no functional impairment.

Taken together, these results are not conclusive, and the discrepancies have no simple explanation. There is good evidence that heart failure may be associated with a reduction in the content of Na\(^+-\)K\(^+\) pumps in skeletal muscle. However, this can in part be attributed to reduced physical activity, and it has not yet been settled whether it reflects a downregulation more directly related to the heart failure or to the concomitant hypoxia (see below).

2. Hypoxia

Whereas it has not been possible to find reports on the effects of hypoxia on Na\(^+-\)K\(^+\) pump contents in resting muscle, one study showed that 8 wk of bicycle training under hypoxic conditions (13.5% \(O_2\)) induced a 14% decrease in the content of [3H]ouabain binding sites in human vastus lateralis muscle. In the same subjects, the other leg was training under normoxic conditions, and this induced a 14% increase in the content of [3H]ouabain binding sites (170). During a 21-day mountain expedition climbing to a maximum altitude of 6,200 m, the content of [3H]ouabain binding sites in the vastus lateralis decreased by 14% (173). These observations indicate that hypoxia in general may lead to a downregulation of the Na\(^+-\)K\(^+\) pumps in skeletal muscle. The elegant experiments with bicycle training strongly suggest that the downregulation is due to local effects of the hypoxia rather than general endocrine mechanisms. However, because hypoxia is likely to cause an increase in \([Na^+]_o\), an upregulation would be expected, and more studies are required to solve this paradox.

In conclusion, a hypoxia-induced downregulation of Na\(^+-\)K\(^+\) pumps is of considerable general importance because hypoxia caused by respiratory, cardiac, or local circulatory failure or living at high altitudes is a very common disorder.

G. Muscular Dystrophy and McArdle Disease

1. Muscular dystrophy

In homogenates of skeletal muscle samples from patients with myotonic muscular dystrophy, the content of [3H]ouabain binding sites was three- to sixfold lower than in control subjects (104). The reduced Na\(^+-\)K\(^+\) pump content may explain the observation that in patients with
muscular dystrophy, the muscles show increased $[\text{Na}^+]_i$ and depolarization (120, 178). The reduced $\text{Na}^+\text{-K}^+$ pump content in the muscles of these patients may in part be due to their limited physical activity. However, muscle cells cultured from patients with myotonic dystrophy contained 30–40% fewer $[^3\text{H}]\text{ouabain}$ binding sites than those obtained from age-matched controls (24), data that were confirmed by measurements of 3-O-MPFase activity (Fig. 8). Obviously, this downregulation cannot be attributed to reduced physical activity. A reduced $\text{Na}^+\text{-K}^+$ pump capacity may contribute to the abnormally high exercise-induced hyperkalemia observed in patients with muscular dystrophy (2.2 vs. 0.8 mM increase in plasma K$^+$) (433).

Microelectrode measurements of intracellular $\text{Na}^+$ activity in EDL, soleus, and gastrocnemius muscles of dystrophic mice showed a marked increase, and intracellular K$^+$ activity was decreased (146, 429). However, the rate of $\text{Na}^+$ clearance measured following an excitation-induced $\text{Na}^+$ loading showed no difference between dystrophic and normal mice, indicating that the ability to extrude $\text{Na}^+$ was not affected (146).

In conclusion, muscular dystrophy is associated with downregulation of the content of $\text{Na}^+\text{-K}^+$ pumps in skeletal muscle. The ensuing impairment of $\text{Na}^+\text{-K}^+$ homeostasis may contribute to the physical disability of these patients.

2. McArdle disease

In patients with muscle phosphorylase deficiency, severely restricting the energy supply from glycolgenolysis (McArdle disease), exercise-induced hyperkalemia is more pronounced (183, 346). During stimulation of the ulnar nerve at 20 Hz, the amplitude of the compound action potential in the muscles undergoes ~50% decline in 50 s, which is markedly faster than in control subjects. This suggests that membrane excitability is reduced by the elevated $[\text{K}^+]_i$ (34, 119, 176). Measurements of $[^3\text{H}]\text{ouabain}$ binding to muscle biopsies showed a significant (27%) reduction (Fig. 8), which could contribute to the doubling of exercise-induced hyperkalemia observed in the same patients (183). It cannot be settled, however, whether the downregulation of $\text{Na}^+\text{-K}^+$ pumps reflects a primary reduction in the synthesis of $\text{Na}^+\text{-K}^+$ pumps or the reduced physical activity of these patients. An alternative explanation of the exercise-induced hyperkalemia and accelerated loss of excitability might be inadequate ATP supply from glycolysis to the $\text{Na}^+\text{-K}^+$ pump (see sect. vD).

H. Digitalis and Amiodarone

1. Digitalis

Digitalis glycosides are widely used in the treatment of cardiac insufficiency, and the usual therapeutic plasma level corresponds to ~$10^{-9}$ M digoxin. This level was found sufficient to induce a significant decrease in whole body K$^+$ and muscle K$^+$ content of 8 and 6%, respectively, but no change in plasma K$^+$ (121). In patients with congestive heart failure, exercise-induced hyperkalemia was characterized before and after standard digitalization. After digitalization the total loss of K$^+$ from the working leg had increased by 138%, reflecting a marked increase in the venoarterial difference in plasma K$^+$ due to inhibition of the $\text{Na}^+\text{-K}^+$ pump by the digoxin (378).

Measurements of the occupancy of digitalis receptors in human skeletal muscle during a standard digitalization have given values of 13% (380) and 9% (378), and there was no evidence of compensatory upregulation of the content of $[^3\text{H}]\text{ouabain}$ binding sites in the digitalized subjects. A more recent study confirmed this observation (171). It is important that such a modest reduction in the total pool of functional $\text{Na}^+\text{-K}^+$ pumps in skeletal muscle seems sufficient to cause the above-mentioned marked rise in exercise-induced hyperkalemia.

In conclusion, even at therapeutic plasma levels of digitalis, the $\text{Na}^+\text{-K}^+$ pumps in skeletal muscle undergo sufficient inhibition to interfere with K$^+$ homeostasis during work. This provides independent evidence that the comparable and modest downregulation of the $\text{Na}^+\text{-K}^+$ pump content often seen after inactivity and in a variety of diseases is important for the clearance of plasma K$^+$ during exercise.

2. Amiodarone

Patients on long-term treatment with the antiarrhythmic amiodarone showed a significant (36%) decrease in the content of $[^3\text{H}]\text{ouabain}$ binding sites in vastus lateralis muscle (326). This downregulation was not associated with any significant change in the plasma levels of $T_3$ or $T_4$, but may reflect amiodarone-induced interference with the binding and action of thyroid hormones (264). Thus long-term treatment with amiodarone is often associated with hypothyroidism.

I. Implications of Acute Plus Long-Term Regulation for $\text{Na}^+\text{-K}^+$ Pump Capacity

The potential implications of $\text{Na}^+\text{-K}^+$ pump regulation in skeletal muscle are perhaps best illustrated by calculating the complete regulatory range for the combined effects of acute and long-term upregulation. In rat soleus, thyroid hormones induce an up to 10-fold increase in the content of $[^3\text{H}]\text{ouabain}$ binding sites (245). This together with the up to ~20-fold acute increase in the activity of the $\text{Na}^+\text{-K}^+$ pumps elicited by electrical stimulation in the same muscle (314) implies that the maximum transport rate of the fully activated $\text{Na}^+\text{-K}^+$ pumps in hyperthyroid soleus is ~200-fold larger than the basal
transport rate in a resting hypothyroid soleus. This enormous regulatory range emphasizes the functional significance of the Na\textsuperscript+-K\textsuperscript+ pump in controlling Na\textsuperscript+-K\textsuperscript+ homeostasis and excitability in skeletal muscle.

VII. EXCITATION, PASSIVE Na\textsuperscript+-K\textsuperscript+ FLUXES, AND CONTRACTILITY

A. Excitation-Induced Na\textsuperscript+-K\textsuperscript+ Leaks May Exceed Na\textsuperscript+-K\textsuperscript+ Pump Activity/Capacity

Action potentials (AP) are elicited by Na\textsuperscript+ influx followed by an efflux of K\textsuperscript+. These passive fluxes have repeatedly been measured, but it has generally been overlooked that the values obtained in skeletal muscle are rather large. In single fibers prepared from the semitendinosus muscle prepared from Rana temporaria, excitation-induced Na\textsuperscript+ influx, when expressed per unit sarcolemma area, amounts to 19.4 pmol \cdot cm\textsuperscript{-2} \cdot AP\textsuperscript{-1}, or 11 pmol/g wet wt (209). In whole sartorius muscles from Rana pipiens, a value of 27 pmol \cdot cm\textsuperscript{-2} \cdot AP\textsuperscript{-1} was measured, or 15 nmol \cdot g wet wt\textsuperscript{-1} \cdot AP\textsuperscript{-1} (420). A recent isotope flux study on rat EDL reported an excitation-induced Na\textsuperscript+ influx as high as 20 nmol \cdot g wet wt\textsuperscript{-1} \cdot AP\textsuperscript{-1} (257).

As indicated in Table 3, Na\textsuperscript+ influx and K\textsuperscript+ efflux per AP measured in different muscle preparations show appreciable variation. The differences can be related to fiber type, species, and conditions of stimulation. In mouse soleus undergoing isometric contraction, the Na\textsuperscript+ influx as monitored using Na\textsuperscript+-sensitive electrodes amounted to 5.5 nmol \cdot g wet wt\textsuperscript{-1} \cdot AP\textsuperscript{-1} (232). In frog semitendinosus muscles, ion-sensitive electrode recordings showed a Na\textsuperscript+ influx and K\textsuperscript+ efflux of 4 and 6 nmol \cdot g wet wt\textsuperscript{-1} \cdot AP\textsuperscript{-1}, respectively (18).

Thus, even after half a century of attempts to quantify the Na\textsuperscript+-K\textsuperscript+ fluxes per AP, the results are still not satisfactory. The use of high frequencies or long-lasting stimulation may lead to loss of excitability, overestimation of the number of effective action potentials causing contractions, and ensuing underestimation of the Na\textsuperscript+-K\textsuperscript+ fluxes per AP. Thus some of the published values are likely to be too low.

Excitation-induced Na\textsuperscript+ influx is around fourfold larger in muscles allowed to shorten freely during contraction than in muscles undergoing isometric contractions (314, 320, and Table 3). This marked difference is unexplained but very important for the evaluation of Na\textsuperscript+-K\textsuperscript+ homeostasis and endurance during contractile activity. The much higher value measured in muscles allowed to shorten freely may represent a physiological response to the more complete shortening taking place during contractions at low loads. This suggests that the Na\textsuperscript+ influx causing action potentials is influenced by a feedback from the processes of contraction, changes in muscle shape, and tension, events that may be important for the maintenance of excitability. The rate of fatigue was faster in unloaded than in isometric contractions, possibly because the excitation-induced Na\textsuperscript+-K\textsuperscript+ fluxes were larger, leading to a faster loss of excitability (321).

It has been assumed that muscle cells only undergo net loss of K\textsuperscript+ and gain of Na\textsuperscript+ at high stimulation frequencies. Already in 1963, however, Sreter (395) observed that when stimulated indirectly for 4 min at a frequency of 5 Hz in vivo, rat gastrocnemius muscles showed a 14–20% decrease in [K\textsuperscript+], and a 81% increase in [Na\textsuperscript+]. Thirty minutes of stimulation at 2 Hz induced ~100% increase in [Na\textsuperscript+], (395). In the rat, direct as well as indirect stimulation in vivo induced a loss of K\textsuperscript+ and a gain of Na\textsuperscript+ which was considerably more pronounced in EDL than in soleus. In EDL, significant changes could be demonstrated down to 1–5 Hz and further documents that even at very low frequencies, the passive Na\textsuperscript+-K\textsuperscript+ leaks may exceed the Na\textsuperscript+-K\textsuperscript+ pump-mediated restorative fluxes. These effects were attributed to differences in Na\textsuperscript+-K\textsuperscript+ fluxes per stimulation and were proposed to be related to the differences in the fatigue resistance between the two muscles (309). In vitro stimulation for 60–180 min at 0.5 Hz induced a progressive and up to threefold increase in intracellular Na\textsuperscript+ content in rat soleus (164). These results are in keeping with the repeated observation that voluntary biking exercise, which is normally assumed to be associated with an average stimulation frequency of ~6 Hz, leads to a net loss of K\textsuperscript+ from human muscle (182, 391).

Thus there is good evidence that also at moderate excitation frequencies, the transmembrane concentration gradients for Na\textsuperscript+ and K\textsuperscript+ may undergo significant reduction, in particular in fast-twitch muscles.

It is generally agreed upon that during excitatory
activity, the Na\textsuperscript{+}-K\textsuperscript{+} pumps restore and maintain the transmembrane Na\textsuperscript{+}-K\textsuperscript{+} gradients and the membrane potential. The question is how rapidly this can take place. During the action potential, the opening of K\textsuperscript{+} channels allows efficient restoration of the membrane potential within milliseconds. The cost of this expediency is that the extracellular concentration of K\textsuperscript{+} undergoes a rapid rise. This, in turn, may cause depolarization of the sarcolemma and t-tubular membranes with loss of excitability and impairment of contractility in the contracting fibers as well as the surrounding fibers. Moreover, the muscle cells may lose K\textsuperscript{+}, leading to a reduction in the equilibrium potential for K\textsuperscript{+} \((E_K)\) and the resting membrane potential \((E_m)\).

In conclusion, there is abundant evidence that during work, skeletal muscles may undergo a net loss of K\textsuperscript{+} and gain of Na\textsuperscript{+} both in vitro and in vivo (384). This is a strong indication that the excitation-induced leaks to Na\textsuperscript{+} and K\textsuperscript{+} often exceed the activity/capacity of the Na\textsuperscript{+}-K\textsuperscript{+} pump for restoring the loss of K\textsuperscript{+} and the gain of Na\textsuperscript{+}. The question is when the accumulation of K\textsuperscript{+} in the t-tubular lumen or the interstitial water space becomes sufficient to interfere with excitability.

B. Excitation-Induced Na\textsuperscript{+}-K\textsuperscript{+} Leaks and Endurance Depend on Fiber Type

It is well-established that in muscles containing predominantly type II fibers, the excitation-induced influx of Na\textsuperscript{+} and efflux of K\textsuperscript{+} are appreciably larger than in muscles with predominantly type I fibers (126, 309, 395). This is in part due to the higher content of Na\textsuperscript{+} channels in type II fibers (165). Intermittent stimulation via the sciatic nerve in vivo for 1–24 h induced a marked increase in Na\textsuperscript{+} content and decrease in K\textsuperscript{+} content in rat EDL and tibialis anterior muscles (predominantly containing type II fibers), but no significant changes in soleus (129).

Contractile endurance also depends on the fiber type, being considerably lower in muscles containing mainly type II fibers (84, 160, 286, 313, 321, 343–345). Thus, in isolated mouse soleus exposed to repetitive direct stimulation at 30 Hz for 0.5 s every 2.5 s, the time to 50% loss of force was 4.4-fold longer than in EDL from the same animals (344). During ischemia, stimulation at 50 Hz caused much faster force decline in the white gastrocnemius than in the soleus muscle of the cat, in keeping with the higher content of type II fibers in gastrocnemius (305). The much lower endurance in muscles containing predominantly type II fibers could be related to a faster reduction in the amplitude of compound action potentials (343).

Comparisons of soleus and EDL prepared from young rats (~4 wk) showed that the endurance was considerably larger in soleus (84, 313, 321). During chronic stimulation at 60 Hz, the rate of force decline in EDL was 5.5-fold larger than in soleus. Comparison of excitation-induced Na\textsuperscript{+}-K\textsuperscript{+} fluxes showed that in EDL, Na\textsuperscript{+} influx and K\textsuperscript{+} efflux were respectively 5.5- and 6.5-fold larger than in soleus (84, 321), indicating that the rate of force decline was related to the passive leaks to Na\textsuperscript{+} and K\textsuperscript{+}. As shown in Table 3, the K\textsuperscript{+} efflux per action potential values in isolated rat soleus and EDL are 1.6 and 10 nmol/g wet wt, respectively. From these values and the measured extracellular water space of around 20%, it could be calculated that when stimulated at a frequency of 60 Hz, \([K^+]_o\) will increase by 0.46 and 2.9 mM/s in soleus and EDL, respectively. In EDL this would be sufficient to cause severe interference with excitability and contractility within 5 s, provided K\textsuperscript{+} is not removed by diffusion. In soleus, excitability may be maintained for an around six-fold longer period. Parallel measurements of M-wave area and tetanic force showed close correlations both in EDL and soleus, indicating that the force decline during the continued stimulation is due to progressive loss of excitability (84, 321). This, in turn, seems to be the result of the larger content of Na\textsuperscript{+} channels in EDL and the higher rate of Na\textsuperscript{+} influx per AP (see Table 3).

On the basis of measurements of the total content of Na\textsuperscript{+}-K\textsuperscript{+} pumps in EDL, it can be calculated that even if the theoretical maximum rate of K\textsuperscript{+} reuptake is reached, this would only allow the clearance of 23% of the net K\textsuperscript{+} release induced by 60-Hz stimulation. In contrast, even though the soleus contains slightly fewer Na\textsuperscript{+}-K\textsuperscript{+} pumps than EDL, they would be able to keep pace with the excitation-induced K\textsuperscript{+} loss, provided they are fully active. In addition, because the stimulating effect of excitation on active Na\textsuperscript{+}-K\textsuperscript{+} transport in EDL is smaller than in soleus (130), the restoration of Na\textsuperscript{+}-K\textsuperscript{+} gradients may be less efficient in EDL.

In conclusion, the excitation-induced passive fluxes of Na\textsuperscript{+} and K\textsuperscript{+} are markedly larger in muscles containing predominantly type II fibers than in those composed of mainly type I fibers. Therefore, the rundown of Na\textsuperscript{+}-K\textsuperscript{+} gradients and the accumulation of extracellular K\textsuperscript{+} are much faster in fast-twitch muscles than in slow-twitch muscles. This difference cannot be compensated by the Na\textsuperscript{+}-K\textsuperscript{+} pumps and may have a large extent account for the much lower endurance in muscles containing predominantly type II fibers.

C. Effects of Na\textsuperscript{+} Channel Modulation on Contractile Endurance

Veratridine and aconitine prolong the open time of voltage-sensitive Na\textsuperscript{+} channels (56) and can therefore be used to assess the importance of the leak-to-pump ratio for Na\textsuperscript{+} during excitation in acute experiments. In rat soleus, veratridine and aconitine increased the excitation-induced influx of \(^{22}\text{Na}\) by 118 and 91%, respectively. Dur-
ing continuous stimulation at 90 Hz, veratridine and ac-

conitine increased the initial rate of force decline by 69 and

19%, respectively, and reduced the rate of poststimulation

force recovery by 86 and 59%, respectively (198). Thus, by

increasing the leak-to-pump ratio for Na\(^{+}\), defined as the

ratio between excitation-induced Na\(^{+}\) influx and Na\(^{+}\)-K\(^{+}\)
pump-mediated Na\(^{+}\) efflux, both contractile endurance

and force recovery show significant impairment. As dis-
cussed in section VIII, treatment with thyroid hormone
leads to upregulation of the content of Na\(^{+}\) channels,

increased excitation-induced Na\(^{+}\) influx, and reduced en-
durance.

The role of permanent Na\(^{+}\) channel anomalies in
determining contractility is strikingly illustrated in HPP, a

genetic disorder caused by a defect in the normal voltage-
dependent inactivation of the Na\(^{+}\) channels, brought

about by a few amino acids in the Na\(^{+}\) channels being

replaced by others (147). This implies that the open time of

the channels is somewhat longer, leading to depolar-
ization of the skeletal muscle cells, loss of excitability,
and paralysis (50). The attacks of paralysis are elicited by
the hyperkalemia induced by the ingestion of K\(^{+}\)-rich
meals, or by rest after exercise, and the disorder is seen in
human subjects and in certain breeds of horses. The

membrane of resting muscle cells from patients and
horses with HPP are depolarized by 16–19 mV (96, 354).
This reflects an increase in the \(P_{Na}/P_{K}\) ratio, and in
intercostal muscle fibers from HPP horses, TTX essentially
abolished the depolarization.

Attacks of paralysis seem to be initiated by increased
Na\(^{+}\) influx in the muscle fibers. In keeping with this, the

early phase of the attacks is accompanied by a rather
marked drop (3–7 mM) in plasma Na\(^{+}\), reflecting the
redistribution of Na\(^{+}\) into the intracellular phase of the
muscles (87). During induced or spontaneous attacks, the

resting membrane potential depolarized by 22 and 17 mV,
respectively (96). Measurements on intercostal muscle
fibers obtained from HPP patients showed that exposure
to slightly elevated K\(^{+}\) (7 mM) induced a rise in intracel-

lular Na\(^{+}\) activity accompanied by an excessive depolar-
ization (16). It could be envisaged, therefore, that the

repeated occurrence of attacks and the increased influx of

Na\(^{+}\) would lead to an upregulation of Na\(^{+}\)-K\(^{+}\) pumps in
skeletal muscle. However, measurements of [\(^{3}H\)]ouabain
binding in the gluteal muscles of foals or adult horses with
attacks of HPP showed no significant difference from
clinically normal animals (353). Epinephrine induces hy-

perpolarization in muscle fibers prepared from patients or
horses with HPP, and epinephrine, \(\beta_{2}\)-agonists, and insu-
lin may alleviate paralysis (354, 427). Thus hormonal stim-
ulation of the Na\(^{+}\)-K\(^{+}\) pumps available in the muscles

seems sufficient to counterbalance the increased Na\(^{+}\)
influx, the hyperkalemia, and the paralytic attacks.

In conclusion, acutely induced or longer lasting in-
creases in the excitation-induced Na\(^{+}\) channel mediated

influx of Na\(^{+}\) lead to considerable impairment of contrac-
tile endurance and the force recovery after long-lasting
contractions. These defects may be counterbalanced by
stimulation of the Na\(^{+}\)-K\(^{+}\) pumps.

D. Effects of K\(^{+}\) Channel Modulation
on Contractile Endurance

The K\(^{+}\) channel blocking aminopyridines prolong the
action potential duration, causing increased force of con-
traction. In rat diaphragm muscle, 3,4-diaminopyridine
(DAP) markedly increased contractile force elicited by
direct stimulation of curarized muscles in the frequency
range 1–50 Hz (417). The delayed repolarization and re-
duced accumulation of K\(^{+}\) in the t tubules and the extra-
cellular phase may also contribute to the improved con-
tractility. DAP has been used in the treatment of myas-
thetic syndrome and has been shown to improve muscle
force by up to 81%.

The K\(_{\text{ATP}}\) channel opener pinacidil and cromakalim
increased the rate of tetanic force decline taking place
during repetitive stimulation of rat EDL, but only under
anoxic conditions (431). This effect was abolished by the
K\(_{\text{ATP}}\) channel blocker glyburide. In mouse soleus and
EDL, pinacidil produced a similar increase in the rate of
force decline during repetitive stimulation (286). Parallel
experiments showed that pinacidil increased the rate of
K\(^{+}\) efflux as assessed from measurements of \([^{86}\text{Rb}]\) wash-
out. The authors concluded that the major mechanism by
which K\(_{\text{ATP}}\) channels suppress force involved an amplified
increase in \([K^{+}]_{o}\). The ensuing inhibition of excitation was
seen as a protective mechanism, preserving muscle en-
ergy and function.

The role of K\(^{+}\) channels in the maintenance of excit-
ability and their importance for the effects of hormones
stimulating the Na\(^{-}\)-K\(^{+}\) pump were explored using the K\(^{+}\)
channel blockers Ba\(^{2+}\) and glibenclamide. In rat soleus,
Ba\(^{2+}\) produced marked inhibition of the influx and the
efflux of \([^{43}\text{K}]\), a concomitant depolarization, and loss of
contractility (83). This was associated with a pronounced
decrease in contractile force that was closely correlated
to a reduction in M-wave area. In the presence of Ba\(^{2+}\),
both salbutamol and CGRP induced significant stimula-
tion of the Na\(^{-}\)-K\(^{+}\) pump, repolarization, and recovery of
contractility and M-wave area. When the Ba\(^{2+}\)-inhibited
muscles were stimulated at 1-min intervals with tetanic
pulse trains (1.5 s at 30 Hz), both membrane potential,
tetanic force, and M-wave area showed a significant re-
covery. In the presence of Ba\(^{2+}\) (0.1 mM), blocking the
ATP-sensitive K\(^{+}\) channels with glibenclamide (10\(^{-4}\) M)
produced no change in force development or the force
recovery induced by Na\(^{-}\)-K\(^{+}\) pump stimulation. These
results give evidence that the stimulating effect of \(\beta_{2}\)-
adrenoceptor agonists, CGRP, and repeated excitation on
excitability and contractility does not depend on the function of $K^+$ channels. More importantly, perhaps, the many experimental data collected, when compared, showed close and highly significant correlations between tetanic force and M-wave area, tetanic force and membrane potential, as well as between M-wave area and membrane potential (93). These three pairs of correlations allowed a combined presentation of all the data in the three-dimensional diagram shown in Figure 10.

In conclusion, acute modification of the activity of $K^+$ channels has a marked influence on contractility, probably due to changes in $[K^+]_o$ and the membrane potential. $Ba^{2+}$-induced blockade of $K^+$ channels does not prevent activation of the $Na^+-K^+$ pump by excitation or hormones and makes it possible to identify the correlations between membrane potential, M wave, and tetanic force.

E. Are the Processes of Excitation in Skeletal Muscle Self-limiting?

As discussed in section VII A, continued muscle activity may lead to a rundown of $Na^+-K^+$ gradients, depolarization, and progressive loss of excitability. These events were repeatedly proposed to contribute to the development of fatigue. Thus, already Fenn (137) proposed that the $K^+$ lost from working muscles was “one of the factors which causes the intensity of contraction to decrease.” More detailed and complete models for the role of $K^+$ in the loss of excitability and force have repeatedly been proposed and further developed (27, 48, 62, 64, 69, 80, 82, 227, 315, 384, 391).

During a few minutes of direct electrical stimulation of a curarized rat diaphragm muscle fiber at 32–40 Hz, the excitation threshold of the fiber membrane increases three- to sixfold in comparison with that recorded at 20 Hz (259). Continued stimulation of skeletal muscles was repeatedly shown to reduce the amplitude and conduction velocity of action potentials in muscle fibers (193, 275, 305, 308). This may be related to the observation that in the anesthetized rat 10–30 s of tetanic stimulation of the gastrocnemius muscle elicited a depolarization of $\sim 20$ mV (277). Also in rat EDL, 30 s of stimulation at 10 Hz caused a highly significant depolarization (193). More recently, repetitive stimulation of frog semitendinosus muscle for 300 s was shown to cause $\sim 10$-mV depolarization, $\sim 12$-mV decrease in AP overshoot, and marked reduction in the rate of depolarization during the AP (19). These changes were accompanied by a 92% loss of tetanic force, however developing with a rather different time course. It was concluded, therefore, that the fatigue was not caused by a loss of the excitability of the sarcolemma, but by impairment of impulse propagation in the t-tubular system.

Several studies have shown that increasing the frequency of stimulation exacerbates fatigue (46, 229, 308, 370). As shown in Figure 11, the decline in force developing during continuous stimulation at 50 Hz is increased when the frequency is doubled and that the loss of force may be partially reversed by lowering the frequency of stimulation, both in mouse and human muscle. Other experiments showed that loss of force could also be reversed by increasing the voltage or the duration of the

**FIG. 10.** Relationships between membrane potential, M-wave area, and tetanic force. Corresponding data points are shown for simultaneous recordings of membrane potential, M-wave area, and tetanic force in 11 individual rat soleus muscles during exposure to $Na^+-K^+$ pump stimulation with calcitonin gene-related peptide (CGRP), salbutamol, or electrical stimulation in the presence or absence of $Ba^{2+}$ (0.1 mM). A regression plane was fitted to the data points in the cube. [From Clausen and Overgaard (83).]
stimulating pulses (228, 229, 313). Furthermore, brief (1–4 s) cessation of chronic stimulation caused a considerable force recovery (46). This is taken to indicate that there is a loss of excitability related to the rundown of Na\(^{+}\)-K\(^{+}\) gradients elicited by the action potentials. By reducing the frequency of stimulation, this process is slowed down, allowing for a more efficient recovery of Na\(^{+}\)-K\(^{+}\) gradients and ensuing recovery of excitability and contractile force. Moreover, continued contractile activity has repeatedly been shown to be associated with a decline in the M-wave area and amplitude in vivo (27, 156, 160, 162). However, direct comparison of the simultaneous changes in plasma K\(^{+}\), M waves, and force during sustained contraction in human quadriceps muscles showed no evidence for loss of muscle membrane excitability during the hyperkalemic phase (432). On the other hand, there was a strong correlation between twitch torque and venous plasma K\(^{+}\), indicating an inhibitory effect of K\(^{+}\) distal to the surface membrane, probably in the t-tubules.

Conditioning fatiguing stimulation of part of the medial cat gastrocnemius muscle caused a strong decline of both tension and electromyogram in the unstimulated part of the muscle, indicating lateral diffusion of inhibitory factors like K\(^{+}\) (256). Due to structural and mechanical hindrances, however, the precise relations between contractility and M waves in vivo are often difficult to analyze. Studies with isolated rat soleus have circumvented some of these problems, and recently it was shown that during continuous indirect stimulation for 120 s at 30 Hz and during the subsequent recovery period, there is a very close correlation between M-wave area and tetanic force (342). Also in muscles where M-wave area and tetanic force were recorded every 5 min and the Na\(^{+}\) channels were progressively blocked by graded exposure to TTX, M-wave area and tetanic force showed a rather similar and close correlation. This supports the idea that the decline in force and M-wave area elicited by continuous stimulation reflects progressive loss of Na\(^{+}\) channel function. During continuous stimulation, the rate of force decline was around fivefold larger in EDL than in soleus muscle. This was associated with a corresponding difference in excitation-induced influx of Na\(^{+}\) and efflux of K\(^{+}\). Again, there was a close correlation between force and M-wave area (84, 321).

Taken together, these observations indicate that due to the excitation-induced passive leaks to Na\(^{+}\) and K\(^{+}\), the processes of excitation in skeletal muscle can perhaps be described as self-limiting, being impaired by increasing stimulation frequency, the activity or content of Na\(^{+}\) channels. As described in section VIII, the excitation-induced rundown of excitability may be counterbalanced by activation of the Na\(^{+}\)-K\(^{+}\) pump. Indeed, in rabbit tibialis anterior muscle, the content of Na\(^{+}\)-K\(^{+}\) pumps was significantly correlated to the M-wave amplitude (205).

**F. Role of t Tubules**

The area of t-tubular walls has been estimated and varies with fiber type, age, and species. In frog sartorius, the ratio between t-tubular and sarcolemmal area is 7:1 (347). In mouse EDL and soleus, the volume and surface area of the...
t tubules increases 5- to 12-fold from birth to adulthood (280). In adult mice, the surface area of the t-tubular membranes corresponds to 3.1 and 1.8 cm²/cm² fiber surface area in EDL and soleus, respectively. In adult rats (250–300 g body wt), the ratio between the area of t-tubular membranes and sarcolemma varied from 3 to 5 (11).

Analysis of the K⁺ efflux during the action potential and the appearance of the afterdepolarization indicated that ~50% of the voltage-dependent ("delayed rectifier") K⁺ channels are situated in the t tubules (239). On the basis of electrophysiological measurements it was estimated that 70–90% of the inward rectifier K⁺ channels reside in the t tubules, that the K⁺ diffusion in the t-tubular lumen was not very different from that in free solution, and that following a perturbation of intraluminal K⁺, recovery should be half-complete in ~0.4 s (7,8). At variance with this, the diffusion constant for K⁺ in the t tubules was around sixfold larger than in free solution (239).

The t tubules in frog sartorius muscle contain a high density of Na⁺ channels quantified as TTX binding sites (40–50 μm⁻²), albeit lower than that of the sarcolemmal membrane (175 μm⁻²) (223). Measurements of [³H]ethylendiamine TTX binding showed that t-tubular and sarcolemmal membranes of frog skeletal muscle contained 4.8 and 9.0 pmol/mg protein, respectively, and in rabbit muscle the t tubules contained 3.5 pmol/mg protein (222). Voltage-clamp studies indicate that the density of voltage-dependent Na⁺ channels is similar for surface and tubular conductances (304). Recently, elegant studies demonstrated that in rat EDL, action potentials spread both transversely and longitudinally in the t-tubule system and that this propagation is suppressed by TTX (356).

The t-tubular system is unique by its extremely large surface-to-volume ratio. Thus "every cubic cm of tubule lumen is surrounded by 50–100 m² of tubular membrane" (8). This implies that during excitation, the ionic composition of the tiny volume of the t-tubular lumen is likely to be dominated by the fluxes via the many Na⁺ and K⁺ channels present in the walls. It has been calculated that for each action potential, the K⁺ concentration in the t-tubular lumen increases by 0.37 mM (239). This implies that during stimulation at 40 Hz, the K⁺ concentration in the tubular lumen will increase by ~15 mM in 1 s, sufficient to block further impulse propagation. Others have reported similar estimates (8).

Based on the structural data reported by Luff and Atwood (280), which seem to represent an average of reported values for the ratio between t tubule and sarcolemmal area for the fully developed mouse EDL, it can be calculated that the t-tubular membrane area corresponds to ~4,200 cm²/ml muscle and 1,400,000 cm²/ml t-tubular volume. This is 200 times larger than the surface-to-volume ratio for the same muscle. As shown in the diagrammatic presentation in Figure 12, the excitation-induced rise in K⁺ concentration is likely to be much more pronounced in the t-tubular lumen than in the interstitial or vascular water spaces. The source of the K⁺ causing repolarization of t tubules is the K⁺ channels in the extensive t-tubular walls, and unless this K⁺ is returned to the cytoplasm by the Na⁺-K⁺ pumps in the t tubules it has to be cleared into the surroundings of the cell. Because this has to take place via the narrow lumen of the relatively long t tubules, however, the clearance of K⁺ from the t-tubular lumen is likely to be considerably delayed (see Ref. 239) and to a large extent will depend on reaccumulation of K⁺ via the Na⁺-K⁺ pumps into the cytoplasm.

Due to the small volume of t-tubular lumen and the vast number of surrounding transporters, the Na⁺-K⁺ turnover in this narrow space must be exceedingly fast during excitation. Therefore, the maintenance of stable...
concentrations of Na\(^+\) and K\(^+\) and excitability of the t-tubular membranes depend on a very close match between the rates of Na\(^+-\)K\(^+\) transport via the Na\(^+\) channels, the K\(^+\) channels, and the Na\(^+-\)K\(^+\) pumps.

The role of K\(^+\) concentration changes for the action potentials and membrane currents were analyzed by computer simulation. It was concluded that only in the outer tubular compartment of the cell, the diffusion of K\(^+\) into the surroundings was of major importance, whereas in the inner compartment, reuptake into the cytoplasm via the Na\(^+-\)K\(^+\) pumps or the inward rectifier was the major process (425). However, the analysis might well have underestimated the role of the Na\(^+-\)K\(^+\) pump capacity in the t-tubules, which was assumed to be one-tenth of that of the sarcolemma.

The rise in t-tubular K\(^+\) will be accompanied by a similar decrease in the luminal concentration of Na\(^+\). Therefore, Bouclin et al. (32) mimicked the expected changes in the Na\(^+-\)K\(^+\) concentrations in the t-tubular lumen by exploring the combined effects of increased [K\(^+\)]\(_o\) and decreased [Na\(^+\)]\(_o\) in the incubation medium on contractile performance in frog sartorius. The major new result was that the inhibitory effects of increased [K\(^+\)]\(_o\) and decreased [Na\(^+\)]\(_o\) were synergistic. As shown in Figure 13, the inhibitory effect of elevated [K\(^+\)]\(_o\) became much more pronounced when [Na\(^+\)]\(_o\) was lowered from 120 to 100 or 60 mM.

The effects of elevated [K\(^+\)]\(_o\) reduced [Na\(^+\)]\(_o\) and their combination were also explored in the isolated rat soleus. Solely increasing [K\(^+\)]\(_o\) from 4 to 8–9 mM or reducing [Na\(^+\)]\(_o\) from 147 to 85 mM produced no significant change in tetanic force (1.5-s stimulation at 30 Hz) or M-wave area. When these changes in Na\(^+-\)K\(^+\) concentra-

![Image](image_url)

**Fig. 13.** Effects of K\(^+\) on tetanic force of frog sartorius at different Na\(^+\) concentrations. Na\(^+\) was replaced by an equimolar concentration of N-methyl-D-glucamine, whereas K\(^+\) concentration was increased by adding appropriate amounts of KCl. Tetanic force was measured during stimulation at 60 Hz in buffers containing the indicated concentrations of Na\(^+\). Each point represents the mean of observations on 5 muscles with vertical bars denoting SE. *Mean tetanic force was significantly different from mean measured at 3 mM K\(^+\) and the same Na\(^+\) concentration. [modified from Bouclin et al. (32).]

**VIII. THE Na\(^+-\)K\(^+\) PUMP AND CONTRACTILE PERFORMANCE**

**A. Effects of Excitation-Induced Na\(^+-\)K\(^+\) Pump Stimulation**

In isolated rat soleus, the inhibitory effect of high [K\(^+\)]\(_o\) on tetanic or twitch force could be counteracted by reducing the intervals between short tetanic stimuli from once per 10 min to once per minute (82, 318, 340). This excitation-induced force recovery was elicited by both direct and indirect stimulation (318). It was associated...
with a decrease in [Na\(^+\)], and completely blocked by ouabain (10\(^{-3}\) M), indicating that it was the result of Na\(^+\)-K\(^+\) pump stimulation. When contractile force had been inhibited by raising [K\(^+\)]\(_o\) to only 10 mM, excitation-induced force recovery reached 97% of the force developed in the standard buffer containing 5.9 mM K\(^+\). At higher [K\(^+\)]\(_o\), however, force recovery decreased in proportion to the level of [K\(^+\)]\(_o\). It should be noted also that tetanic stimulation of muscles every minute completely prevented the inhibitory effect of increasing [K\(^+\)]\(_o\) to 10 mM (340).

The possible role of CGRP in eliciting excitation-induced force recovery was explored in muscles in which the endogenous CGRP pools had been reduced by prior (7 days) denervation, by in vitro pretreatment with capsaicin, or repeated electrical stimulation. In all three instances, excitation-induced force recovery was suppressed, indicating that it depended on activation of the Na\(^+\)-K\(^+\) pumps by CGRP released from endogenous stores (318). Moreover, capsaicin alone induced partial recovery of tetanic and twitch force in rat soleus exposed to a [K\(^+\)]\(_o\) of 12.5 mM (10, 318).

When incubated at a [K\(^+\)]\(_o\) of 10 mM, soleus muscles were depolarized by ~15 mV, and after 20 tetanic stimulations (1.5 s, 30 Hz) applied via the nerve at 1-min intervals, there was a significant repolarization of 7 mV (340). Concomitantly, the M-wave area and amplitude recorded extrajunctionally underwent a marked decrease, followed by recovery. During the repeated tetanic stimulations, tetanic force showed an 80% recovery with almost the same time course as the 90% restoration of M-wave area (Fig. 14).

The force recovery was not prevented by propranolol (10\(^{-6}\) M), indicating that it is not elicited by norepinephrine released from local pools in the muscle. These experiments provided some evidence, however, that the force recovery was induced by a compound released from nerve endings, possibly brought about by an effect of the muscle contractions per se. Thus, in isolated frog sartorius muscle, stretching stimulates the efflux of isotopic Na\(^+\) (194) and reduces [Na\(^+\)] (363).

The observation that repeated tetanic stimulation prevents the inhibitory effect of exposing rat muscles to a [K\(^+\)]\(_o\) of 10 mM may explain the well-documented experience that in patients suffering from attacks of HPP, where plasma K\(^+\) is often above 7 mM and the muscle cells are severely depolarized, the diaphragm which maintains its activity will not get paralyzed. Likewise, paralysis of individual muscle groups can be prevented by maintaining contractile activity in these muscle groups, even at a stage where the others get paralyzed. As described already 100 yr ago and several times later (see Ref. 87; and for review, see Ref. 161), these patients know from their daily life that paralytic attacks can be prevented by maintaining general physical activity, for instance by walking.

In conclusion, both in vitro and in vivo, the inhibitory effect of high [K\(^+\)]\(_o\) on contractility can be counteracted by excitation-induced activation of the Na\(^+\)-K\(^+\) pump. Due to its electrogenic action, the Na\(^+\)-K\(^+\) pump thereby contributes to the maintenance of excitability during work or acute increases in [K\(^+\)]\(_o\).

### B. Effects of Acute Hormonal Na\(^+\)-K\(^+\) Pump Stimulation

Although there is consensus that in skeletal muscle the Na\(^+\)-K\(^+\) pumps are subject to acute hormonal stimulation, relatively few studies have explored the implications of such effects for contractile performance. In standard incubation media, hormones stimulating the Na\(^+\)-K\(^+\) pump produce little or no change in contractility. When muscles are exposed to high [K\(^+\)]\(_o\), however, leading to depolarization, rather marked effects are seen. Thus Bowman and Raper (33) showed that epinephrine, norepinephrine, and isoproterenol all reduced the inhibitory

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**FIG. 14.** Excitation-induced recovery of tetanic force and M-wave area in rat soleus. Tetanic force and M-wave area recorded from the extrajunctional region were measured in standard Krebs-Ringer solution containing 4 mM K\(^+\) and after incubation at 10 mM K\(^+\). Values are expressed as percentages of the average value in standard buffer. After 60 min of exposure to 10 mM K\(^+\), tetanic stimulations were applied at 1-min intervals for 20 min. Symbols represent mean values from 6 muscles with error bars representing SE. At the arrows, the following resting membrane potentials were recorded: a, –78 mV; b, –62 mV; c, –69 mV. [From Overgaard and Nielsen (340).]
effect of high \([K^+]_o\) on single twitches in rat diaphragm. Tomita (413) showed that in isolated guinea pig soleus exposed to a \([K^+]_o\) of 11 mM, the loss of twitch force could be restored by isoproterenol \((10^{-6} \, \text{g/ml})\). Because this force recovery was suppressed by ouabain \((5 \times 10^{-6} \, \text{M})\), it was proposed to reflect stimulation of the \(\text{Na}^+-\text{K}^+\) pumps. At higher levels of \([K^+]_o\) \((30 \, \text{and} \, 67 \, \text{mM})\), isoproterenol no longer induced force recovery. The \(\beta_2\)-agonist terbutaline as well as insulin also counteracted the inhibitory effect of high \([K^+]_o\) on contractile force in guinea pig soleus \((210, 211)\).

The physiological significance of the inhibitory effects of high \([K^+]_o\) on contractility depends on the \(K^+\) levels reached in the interstitial water space during exercise. Studies with microdialysis probes indicate that in the human gastrocnemius muscle \(K^+\) values up to 11 mM may be reached after 5 min of plantar flexion at a power output of 6 W \((174)\). This is slightly higher than the values reported by Juel et al. \((235)\), but lower than those recorded using \(K^+\)-sensitive microelectrodes. The limitation of microdialysis probes is the time lag of the recordings and the somewhat indirect nature of the measurements. Moreover, like the measurements performed using \(K^+\)-sensitive microelectrodes, local muscle cell damage might cause artefactual increases in interstitial \(K^+\).

The role of the \(\text{Na}^+-\text{K}^+\) pumps in compensating the inhibition of contractile performance induced by an acute increase in \([K^+]_o\) was explored by exposing isolated rat soleus to graded stimulation with hormones or inhibition with ouabain \((70)\). When exposed to a \([K^+]_o\) of 12.5 mM, the resting membrane potential as measured in central fibers decreased by 11 mV with a maximum time lag of 18 min, corresponding to the time required to reach full inhibition of subtetanic force elicited by repeated pulse trains of 12 Hz. Both insulin, epinephrine, and salbutamol clearly delayed the inhibitory effect of a \([K^+]_o\) of 12.5 mM on twitch and subtetanic contractions. Conversely, ouabain \((10^{-6} \, \text{to} \, 10^{-5} \, \text{M})\) increased the rate of force decline. There was a close correlation between the \(\text{Na}^+-\text{K}^+\) pump-mediated rate of \(K^+\) uptake (measured using \(^{86}\text{Rb}\)) and the time until full inhibition of subtetanic force development. The epinephrine-induced delay of the force decline was significant down to the physiological level \((10^{-8} \, \text{M})\). This strongly supports the idea that the clearance of \(K^+\) from the interstitial water space depends on the \(\text{Na}^+-\text{K}^+\) pumps, their capacity, and the hormonal regulation of their activity.

When exposed to 10 mM \(K^+\), the isometric twitch and tetanic force of rat soleus undergo a decline of 40–70\%, depending on the time of exposure. The subsequent addition of epinephrine, salbutamol, or CGRP produced almost complete force recovery in 5–10 min. Insulin \((100 \, \text{mU/ml})\) induced 62\% force recovery in 15 min. All these recovery effects were abolished by prior addition of ouabain \((10^{-5} \, \text{to} \, 10^{-3} \, \text{M})\). Nine different hormones or compounds that acutely stimulate \(\text{Na}^+-\text{K}^+\) pump activity (insulin, epinephrine, norepinephrine, salbutamol, terbutaline, CGRP, salmon calcitonin, amylin, cAMP) all produced a ouabain-suppressible force recovery in rat soleus inhibited by high \([K^+]_o\) \((10–12.5 \, \text{mM})\) \((10, 66, 68)\). At a \([K^+]_o\) of 15 mM, however, salbutamol or insulin produced no significant force recovery. Also dibutyryl cAMP and theophylline induced force recovery in rat soleus inhibited by high \([K^+]_o\), and these agents potentiate each other (unpublished data).

In rat soleus exposed to 12.5 mM \(K^+\), a large concentration range of different agents stimulating the \(\text{Na}^+-\text{K}^+\) pumps (insulin, salbutamol, alone or in combination, and epinephrine) caused force recovery \((68)\). There was a close correlation \((r = 0.82; P < 0.001)\) between the decline in \([Na^+]_i\) and the concomitant subtetanic force recovery. Force recovery was also closely correlated with the ouabain-suppressible stimulation of \(^{86}\text{Rb}\) uptake. In the \(K^+\)-inhibited muscles, all these agents induced a similar recovery of twitch force, and this was also correlated with the decline in \([Na^+]_i\) and the stimulation of \(^{86}\text{Rb}\) uptake. These correlations obtained using rather different agents stimulating the \(\text{Na}^+-\text{K}^+\) pumps support the idea that the common mechanism underlying the force recovery is an acute stimulation of \(\text{Na}^+-\text{K}^+\) pumps.

Hormonal or nonhormonal stimulation of the \(\text{Na}^+-\text{K}^+\) pumps leads to a decrease in \([Na^+]_i\) \((81)\), favoring the clearance of extracellular \(K^+\) and increased intracellular \(K^+\). Due to the electrogenic action of the \(\text{Na}^+-\text{K}^+\) pump and the increased steepness of the transmembrane \(K^+\) gradient, sarcotendinous undergoes hyperpolarization. The increased steepness of the transmembrane \(K^+\) gradient is likely to increase the amplitude of the action potential. All of these actions contribute to the restoration of contractility seen in \(K^+\)-enriched buffer, but it is difficult to quantify their relative importance. In one study, rat soleus was exposed to a \([K^+]_o\) of 11 mM, causing a 50% loss of force. Salbutamol, insulin, and CGRP were all shown to produce partial restoration of tetanic force \((47)\). All of these agents produced a highly significant decrease in \([Na^+]_i\) but only salbutamol caused hyperpolarization. Thus force recovery may be elicited by an increase in the transmembrane \(Na^+\) gradient, but hyperpolarization is not essential.

Salbutamol also restores contractile force during attacks of HPP, probably because stimulation of the electrogenic \(\text{Na}^+-\text{K}^+\) pump counterbalances the depolarization causing these attacks \((427)\). Measurement of the compound action potential in the abductor digiti minimi showed that during an attack there was a 85% decrease in amplitude \((185)\). After treatment with salbutamol, an attack caused only 18% decrease in amplitude, and paralysis was prevented, indicating that excitability could be maintained, possibly by \(\text{Na}^+-\text{K}^+\) pump stimulation.

The effects of acute stimulation of the \(\text{Na}^+-\text{K}^+\) pumps...
pumps on contractility were also examined in experiments where the electrochemical gradient for Na⁺ was reduced by replacing NaCl with choline chloride (341). This caused a modest depolarization and marked reduction in contractile force. Insulin and salbutamol completely repolarized the muscles and caused a substantial drop in [Na⁺]o. This was associated with a force recovery of 80–88%. Force recovery was closely correlated to the drop in [Na⁺]o, and could be abolished by ouabain. Repolarization induced by reducing [K⁺]o from 4 to 2 mM caused 96% force recovery that could almost entirely be attributed to the repolarization. The observations indicate that force recovery may be restored by hyperpolarization, a decrease in [Na⁺]o, or a combination of both. The more general implication is that elevated [K⁺]o, and/or [Na⁺]o as well as reduced [Na⁺]o, act by lowering the driving force for Na⁺ influx, with ensuing impairment of action potential generation and propagation. In addition, elevated [K⁺]o interferes with Na⁺ channel function. During repeated excitation, all these factors act in concert to reduce excitability but are counterbalanced by Na⁺-K⁺ pump stimulation, allowing rapid restoration of contractile performance.

In conclusion, skeletal muscles exposed to high [K⁺]o or low [Na⁺]o undergo a loss of excitability and contractility. This can be restored by hormonal stimulation of the Na⁺-K⁺ pumps, a combined result of hyperpolarization, decreased [Na⁺]o, and improved clearance of extracellular K⁺.

C. Effects of Temperature, Extracellular Ca²⁺, and pH

Recent studies showed that in muscles where force generation and M-wave area had been reduced by exposure to high [K⁺]o at 20°C, elevation of the temperature to 30°C induced a marked recovery of both parameters (349). The temperature elevation increased the resting membrane potential and ouabain-suppressible ⁸⁶Rb uptake by 93%, and the force recovery was suppressed by ouabain. Thus, due to the high Q₁₀ of the Na⁺-K⁺ pump, increasing muscle temperature may contribute to the clearance of extracellular K⁺ and the restoration of excitability.

The inhibitory effects of elevated [K⁺]o alone or in combination with lowered [Na⁺]o on tetanic force is also counteracted by elevating [Ca²⁺]o from 1.3 to 10 mM (49). This rather marked effect was attributed to the observed repolarization of 5 mV. Interestingly, the progressive loss of force elicited by repeated tetanic stimulation was delayed at high [Ca²⁺]o and increased by the omission of Ca²⁺ from the buffer. These observations indicate that the maintenance of the membrane potential is important for the protection of excitability.

It should be noted that muscle fatigue not only arises from impairment of the excitability of sarcolemma and t-tubular membranes, but also from reduced release of Ca²⁺ from the SR (5, 30) as well as several other changes in the cytoplasm of the working muscle cell. The role of these factors is beyond the scope of this review, but has been described in detail elsewhere (141).

In soleus muscles inhibited by high [K⁺]o, acidification with lactic acid, CO₂, or propionic acid elicited a force recovery that was closely correlated with a concomitant restoration of M-wave area. This effect was not accompanied by hyperpolarization, but perhaps arises by improved function of the Na⁺ channels (316). The force recovery induced by acidification was additive to that elicited by epinephrine, a further indication of the difference between the mechanisms (103). The combination of lactic acid and epinephrine allowed force recovery even in muscles exposed to 15 mM K⁺, a concentration where β₂-adrenoceptor stimulation alone failed to induce any force recovery (68). These observations are important for the understanding of muscle fatigue during intense exercise, which is associated with acidosis and a concomitant elevation of [K⁺]o and plasma catecholamines.

In conclusion, the inhibitory effect of high [K⁺]o on contractility is counteracted by Na⁺-K⁺ pump stimulation due to increased temperature, hyperpolarization induced by high [Ca²⁺]o, and acidosis, possibly acting on Na⁺ channel function.

D. Effects of Na⁺-K⁺ Pump Inhibition or Downregulation

As described in section vii, B and C, increasing excitation-induced Na⁺ influx augments the leak-to-pump ratio, leading to loss of force and endurance. Leak-to-pump ratio may as well be increased by inhibiting the Na⁺-K⁺ pump. Several studies have shown that inhibition of the Na⁺-K⁺ pumps with high concentrations of cardiac glycosides leads to marked reduction in twitch force in isolated skeletal muscles (9, 197, 437). On the other hand, low (μM) concentrations of ouabain only produced modest changes in twitch force, probably because only a minor fraction of the Na⁺-K⁺ pumps are blocked and the remaining Na⁺-K⁺ pumps are further activated by elevation of [Na⁺]o. When Na⁺ permeability in mouse diaphragm muscle was increased by veratridine, aconitine, or monensin however, twitch force was potentiated two-to fourfold, and under these conditions ouabain (5 × 10⁻⁶ M) suppressed the twitch potentiation (323). Thus inhibitory effects of low concentrations of ouabain on twitch contraction may only be detectable when the passive Na⁺ leak is high enough to require the participation of a relatively large fraction of the Na⁺-K⁺ pump capacity.

During continuous electrical stimulation leading to a
larger acceleration of the passive Na\(^+\) leak and a more rapid rundown of transmembrane Na\(^+\)-K\(^+\) gradients, the effects of cardiac glycosides or other mechanisms causing reduced Na\(^+\)-K\(^+\) pump capacity are more likely to be seen. Indeed, preincubation of soleus with ouabain at 10\(^{-5}\) and 10\(^{-3}\) M increased the rate of force decline recorded during continuous stimulation by 200 and 360\%, respectively (313). As shown in Figure 15, also pretreatment with lower concentrations of ouabain so as to achieve partial inhibition of the Na\(^+\)-K\(^+\) pumps induced a considerable acceleration of force decline. A similar decrease in endurance was seen in muscles where the Na\(^+\)-K\(^+\) pump content had been decreased by prior K\(^+\) depletion of the rats. The graded reduction of the functional Na\(^+\)-K\(^+\) pumps over a wide range (from 756 to 110 pmol/g wet wt) produced a proportionate decrease in the contractile endurance (313). These data explain that among patients with digitalis intoxication, fatigue or weakness is the predominant symptom.

In rat soleus, the increase in the rate of force decline induced by preincubation with ouabain (10\(^{-5}\) M) was highly significant already within the first seconds of continuous tetanic stimulation at 90 Hz. At 2–5 s after the onset of stimulation, the rate of force decline showed a threefold increase (198), and in muscles from K\(^+\)-deficient rats, force decline as measured after 2 s of stimulation showed a similar increase. Thus already during the first seconds of high-frequency stimulation, the capacity of the Na\(^+\)-K\(^+\) pumps becomes inadequate for the maintenance of contractility. This is in keeping with the observation that a major part of the Na\(^+\)-K\(^+\) pumps are activated within a few seconds (see sect. VI A), indeed at the moment where maintenance of excitability is most needed.

The increased rate of decline in twitch force of the isolated rat soleus elicited by ouabain (10\(^{-5}\) M) was significantly correlated with the concomitant decreases in M-wave area and amplitude recorded using a glass micro-electrode positioned in the center of the muscle (197). This indicates that the inhibitory effect of ouabain on contractility is the result of interference with excitability. All the effects were reversible upon washout of ouabain.

After continuous stimulation at 90 Hz until an 80% loss of force, soleus muscles pretreated with 10\(^{-5}\) or 10\(^{-3}\) M ouabain showed, respectively, 81 and 100% reduction in the rate of force recovery. In soleus obtained from K\(^+\)-deficient rats where the content of Na\(^+\)-K\(^+\) pumps was downregulated by 69\%, the rate of force recovery was reduced by 38\% (198).

It should be noted that in the muscles obtained from K\(^+\)-deficient rats, the initial tetanic force development was not reduced. This observation differs from earlier studies (201) demonstrating that in K\(^+\)-deficient dogs, both the initial tetanic and twitch force as measured in the anterior calf muscles in vivo were considerably reduced (by 90 and 71\%, respectively). Taken together, however, the data indicate that K\(^+\) deficiency may lead to severe impairment of contractile performance.

Also the age-dependent decrease in Na\(^+\)-K\(^+\) pump content in rat soleus was associated with a considerable impairment of endurance and force recovery (198). Thus, in muscles obtained from 10-wk-old rats (containing 350 pmol Na\(^+\)-K\(^+\) pumps/g wet wt), the rate of force decline during 90-Hz stimulation was 97\% larger than in muscles from 4-wk-old rats (containing 817 pmol/g wet wt), and the rate of subsequent force recovery was reduced by 34\%.

Although there are few measurements of the muscle performance of the K\(^+\)-deficient organism, there is objective evidence of impaired endurance. Thus, in rats maintained on K\(^+\)-deficient fodder (plasma K\(^+\) 1.9 mM), swimming led to exhaustion in only 44 min, whereas the paired controls continued swimming for 175 min (31). K\(^+\) deficiency is associated with fatigue, which is a returning complaint of patients losing K\(^+\) due to, e.g., the commonly occurring chronic treatment with diuretics (145). Moreover, decreased K\(^+\) intake is associated with a decline in muscle grip strength in elderly subjects (231).
(91, 177). More recent studies showed that the endurance of diaphragm, soleus, and EDL prepared from streptozotocin diabetic rats was reduced (293, 294). These effects might be due to the downregulation of Na\(^+-\)K\(^+\) pumps associated with diabetes (see sect. viB) and relate to the clinical experience that the fatigue experienced by untreated type 1 diabetics is rapidly alleviated following standard insulin treatment. In soleus and EDL prepared from diabetic rats, a significant depolarization was observed and insulin caused repolarization (294).

In patients suffering from idiopathic dilated cardiomyopathy, the left ventricular ejection fraction was significantly correlated to the content of [\(^{3}H\)]ouabain binding sites of the endomyocardium (324) or the myocardium of the right ventricle (221), indicating that also in the heart, contractile performance depends on the content of Na\(^+-\)K\(^+\) pumps.

In conclusion, these studies provide strong evidence that the maintenance of contractility and excitability in working skeletal muscle requires a fairly large population of functional Na\(^+-\)K\(^+\) pumps. Reductions in the activity or content of Na\(^+-\)K\(^+\) pumps induced by cardiac glycosides, K\(^+\) deficiency, increasing age, and diabetes clearly interfere with contractility and endurance and cause fatigue as well as impairment of force recovery after continuous stimulation. As briefly reviewed elsewhere, there is accumulating evidence that the sodium pump keeps us going (67).

E. Energy Depletion, Loss of Excitability, and Contractility

Hypoxia is known to interfere with contractility and endurance in skeletal muscle, and this is usually attributed to changes in the intracellular concentrations of ATP, ADP, P\(_i\), and H\(^+\). Anoxia accelerates the decrease in twitch force elicited by repeated stimulation (431). Several studies indicate that mechanisms proximal to the contractile apparatus contribute to the potentiating effect of hypoxia on fatigue. In cat geniohyoid muscles exposed to severe hypoxia in situ, contractile endurance was significantly reduced (373). In the muscles of the rat tongue, hypoxia in vivo caused considerable loss of contractile force (157), which was associated with and weakly correlated to the concomitant changes in M-wave area. This was proposed to reflect fatigue-related changes in transmembrane electrolyte gradients. In human soleus and tibialis anterior muscles undergoing fatiguing isometric contractions, ischemia induced by arterial cuff occlusion caused a considerable acceleration of the decline in M-wave amplitude and area, and within 2–3 min after cuff release, M waves showed full recovery (160). These changes were taken to reflect accumulation of K\(^+\) induced by the arrested circulation, followed by washout after its reestablishment. These observations do not exclude, however, that in addition, the effects of hypoxia on contractile performance reflect impairment of SR function.

In conclusion, although there is good evidence that hypoxia interferes with excitability and contractile performance, more studies with isolated muscles are required to identify the role of Na\(^+-\)K\(^+\) distribution and membrane potential.

F. Training, Na\(^+-\)K\(^+\) Pump Upregulation, and Contractile Performance

Several studies have shown that treadmill running leads to increased running endurance in the rat (for references to training effects, see Refs. 23 and 143). In intact perfused soleus muscles of rats trained by running, the rate of fatigue caused by repeated tetanic stimulation was fourfold slower than in soleus from untrained rats (143). This increase in fatigue resistance was attributed to improved respiratory capacity, leading to a smaller decrease in high-energy phosphate concentration, reduced lactic acid accumulation, and acidification with ensuing improvement of the Ca\(^2+\) binding to troponin. One contributing factor may be training-induced upregulation of Na\(^+-\)K\(^+\) pump capacity allowing improvement of Na\(^+-\)K\(^+\) homeostasis during exercise and ensuing better maintenance of muscle cell excitability.

The content of Na\(^+-\)K\(^+\) pumps in vastus lateralis showed a weak, but significant, correlation to maximum isometric strength (253). Sprint training leads to increased content of [\(^{3}H\)]ouabain binding sites in vastus lateralis. Although this was associated with improved K\(^+\) clearance, there was no correlation to isometric muscle function (297). In 39 endurance-trained men, intensified running training increased the content of [\(^{3}H\)]ouabain binding sites in vastus lateralis muscle by 15%, but there was no correlation to indices of endurance performance (283). There was only a weak correlation between [\(^{3}H\)]ouabain binding site content and VO\(_{2}\)max measured before the training. However, more detailed studies showed significant correlation between the content of [\(^{3}H\)]ouabain binding sites in vastus lateralis and VO\(_{2}\)max, performance during a 20-min treadmill test, and performance during cross-country skiing (133). Strenuous strength training of top alpine skiers increased the content of [\(^{3}H\)]ouabain binding sites by 15%. Although the individual improvement in an endurance test correlated with the individual changes in Na\(^+-\)K\(^+\) pump content, there was no significant correlation between pump content and muscle strength or endurance (298). In another study (153), peak O\(_{2}\) consumption was significantly correlated to both [\(^{3}H\)]ouabain binding site content and 3-O-MFPase activity in biopsies of human vastus lateralis.
muscle. [3H]ouabain binding site content showed a significant inverse correlation to fatigue index.

In rats maintained on a diet of hard pellets, the fatigue of masseter muscles induced by repeated stimulation at 80 Hz increased by ~50% with age. This decrease in endurance was associated with a 22% reduction in the content of [3H]ouabain binding sites (333).

A recent analysis of the human Na+-K+-ATPase α2 gene suggested that DNA sequence variation at the locus of this gene or a locus in close proximity is associated with the responsiveness of VO₂ max and maximum work to a 20-wk endurance training program (362).

In conclusion, although it is well-documented that various types of training increase physical performance as well as the content of Na+-K+ pumps in skeletal muscle, these two parameters are not always well-correlated, perhaps because the relative increases in pump content are sometimes modest.

G. Effects of Thyroid Hormones

Hyperthyroid patients complain of muscle weakness and fatigue, symptoms that disappear when the euthyroid status is reestablished (361). Intercostal muscle fibers prepared from thyrotoxic patients were markedly depolarized (by 19 mV), and the muscle weakness was attributed to reduced muscle excitability (179). Studies on isolated rat muscles showed that, whereas pretreatment with thyroid hormone for 6 wk produced minor changes in the contractile properties of the EDL, the force-frequency curve of the soleus was shifted to the right, resembling the curve of a euthyroid EDL (142). This was attributed to the shortened twitch duration and the ensuing higher fusion frequency. During repeated direct stimulation for 15 min, the force decline in soleus muscles prepared from hyperthyroid rats was approximately doubled in comparison with those from euthyroid controls, measured from a euthyroid rat, the initial force was unaltered (131). After 8 days of T3 pretreatment, the initial rate of force decline in rat soleus exposed to continuous stimulation was increased by 53%. Significant acceleration of force decline was already seen 24 and 48 h after T3 injection (195). This decrease in endurance was attributed to the concomitant increase in the content of Na+ channels (154%) and the resulting rise in excitation-induced Na+ influx. This would accelerate the rundown of the transmembrane Na+ gradients and the rate of depolarization. The thyroid hormone-induced up-regulation of the content of Na+ channels confirms the demonstration that in cultured skeletal myotubes, thyroid hormone stimulates the synthesis of Na+ channels (38).

In hypothyroid dogs, where the content of [3H]ouabain binding sites in the sternothyroid muscle was reduced by 14%, resting plasma K+ as well as exercise-induced hyperkalemia were significantly higher than in euthyroid dogs (376).

In conclusion, thyroid hormones upregulate the content of Na+ channels and increase excitation-induced Na+ influx in skeletal muscle. This may accelerate the rundown of Na+ gradients, causing depolarization and loss of excitability. This could contribute to the fatigue in hyperthyroidism.

IX. MAJOR CONCLUSIONS

AND GENERAL PERSPECTIVES

The physiological significance of the Na+–K+ pump and its regulation for contractile performance is perhaps best illustrated by the following findings.

1) The Na+–K+ pumps are located in sarcolemma and a large fraction in the t-tubular membranes, in strategically close vicinity to Na+ and K+ channels.
2) The content of Na+–K+ pumps has repeatedly been quantified and their total transport capacity found to agree with the theoretical maximum predicted by the content of [3H]ouabain binding sites.
3) During excitation, the activity of the Na+–K+ pump is increased within seconds, rapidly enough to induce a subsequent undershoot in [Na+]i.
4) During high-frequency excitation, active Na+–K+ transport may reach its theoretical maximum rate, which is comparable in size to the passive leaks of Na+ and K+ eliciting the action potentials.
5) In muscles inhibited by high [K+]o, low [Na+]o, or depolarization, Na+–K+ pump stimulation induced by excitation, hormones, or elevated temperature elicits rapid restoration of excitability.
6) Training upregulates and inactivity downregulates the content of Na+–K+ pumps in skeletal muscle.
7) Inhibition or downregulation of the Na+–K+ pump increases the leak-to-pump ratio and leads to reduction in contractility and endurance of isolated muscles. Likewise, increasing leak-to-pump ratio by augmenting the activity or content of Na+ channels also reduces endurance.
8) Several diseases are associated with a downregulation of the content of Na+–K+ pumps in skeletal muscle, which may contribute to the impairment of physical performance seen in these disorders.

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