Rapid Activation of the Na^+,K^+-pump - Mechanisms and Functional Significance

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Abstract

In a wide variety of tissues (skeletal muscle, heart, smooth muscle, nerve cells, pancreas and kidney), hormones induce a rapid increase in the rate of active Na^+,K^+-transport. In skeletal muscle, this was shown to lead to a decrease in intracellular Na^+, indicating that the affinity for intracellular Na^+ had increased. This activation of the Na^+,K^+-pump seems to be mediated by cAMP and protein kinases. During excitation, active Na^+,K^+-transport was found to increase up to 22-fold within 10 sec. This was also associated with a decrease in intracellular Na^+. Moreover, due to the electrogenicity of the Na^+,K^+-pump, acute activation leads to hyperpolarization. In muscles where contractility had been inhibited by exposure to high extracellular K^+, activation of the Na^+,K^+-pump with hormones or by repeated electrical stimulation restored contractile force within 5-10 min. This force recovery could be prevented by ouabain. In conclusion, the Na^+,K^+-pump is essential for the restoration and maintenance of excitability during continued exercise. This function of the Na^+,K^+-pump is considerably amplified by the potential to undergo rapid activation.

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Biol. Skr. Dan. Vid. Selsk. 1998, **49**:153–158

The transport of ions, amino acids and sugars across the plasma membrane is mediated by specific transport systems. This allows for selectivity and at the same time defines the transport capacity by the number of transport systems per unit of membrane area. It can be assumed that the theoretical maximum rate of transport in a tissue is a function of the number of transporters multiplied by the maximum turnover number of each transporter.

Transport capacity is likely to reflect the physiological need for performing a given type of transport. Thus the capacity for performing active Na^+,K^+-transport can be estimated by measuring the concentration of Na^+,K^+-ATPase. Among a wide variety of human tissues, from erythrocytes

to brain cortex, the concentration of Na^+,K^+-ATPase expressed as pmol/g tissue wet wt. has been found to vary over a 160,000-fold range (Clausen, 1997). This shows that the requirement for active Na^+,K^+-transport varies considerably and indicates that each tissue regulates the concentration of Na^+,K^+-ATPase, allowing its cells to meet the challenges arising from local changes in Na^+,K^+-distribution. Skeletal muscle is excited to contract by a rapid influx of Na^+. This together with the subsequent rapid efflux of K^+ leads to a net gain of Na^+, a net loss of K^+, depolarization and eventually a loss of excitability. Thus, the very process of excitation is the basis for a progressive loss of excitability (Juel, 1986; Balog and Fitts, 1996; Nielsen and Clausen, 1996; Nielsen and Overgaard, 1996). Moreover, the total pool of skeletal muscle cells in the body is so large that during intense exercise, the net loss of K^+ from the working muscles within 60 sec induces a doubling of plasma K^+ in arterial blood, sufficient to cause cardiac arrest if it took place under resting conditions (Medbø and Sejersted, 1990).

Thus, during intense exercise, there is an urgent need for rapid restoration of Na^+,K^+-gradients and membrane potential - not only in the working muscle cells, but also in several other cells of the body. To this end, muscle cells contain a high concentration of Na^+,K^+-ATPase and therefore have a large spare capacity for active Na^+,K^+-transport. For this to play an important role in the maintenance of the Na^+,K^+-gradients during work, the Na^+,K^+-ATPase must undergo prompt and substantial activation at the onset of contractile activity. Indeed, it was recently demonstrated that in isolated rat skeletal muscle, electrical stimulation induces a rapid increase in the rate of net Na^+ extrusion (Everts and Clausen, 1994). Following 10 sec of high frequency stimulation, net Na^+ extrusion increased up to 22-fold above the resting rate, reaching full activation of all available Na^+,K^+-pumps (Nielsen and Clausen, 1997). More importantly, a substantial part of this activation was shown to be independent of an increase in intracellular Na^+ ($[Na^+]_i$), and as shown below in muscles undergoing isometric contractions, electrical stimulation was found to lead to a substantial decrease in intracellular Na^+, which was maintained for up to 30 min after the cessation of stimulation. At constant $[Na^+]_i$, an increase in the rate of active Na^+,K^+-transport may in principle take place in the following ways:

1. by upregulation of the concentration of transporters in the plasma membrane
2. by translocation of transporters from an intracellular or membraneous pool to the plasma membrane
3. activation due to increased maximum turnover number of the transporters already located in the plasma membrane
4. activation due to increased affinity of the transporter for the transported agent

Fig. 1 exemplifies some major aspects of the acute and long-term regulation of active Na^+,K^+-transport in skeletal muscle. Long-term changes in the transport capacity are generally established by de novo synthesis of transporters and their subsequent insertion in the plasma membrane. These processes take place over hours and days and are obviously too slow to meet urgent needs for a rapid increase in the rate of transport. More expedient mechanisms are required to solve this problem. For a rapid increase in active Na^+,K^+-transport two mechanisms have been proposed - translocation and activation.

Insulin-induced translocation of the glucose transporter Glut 4 from a separate (possibly intracellular) pool to the plasma membrane is well-documented. There is some evidence that in skeletal muscle insulin may also induce translocation of Na^+,K^+-ATPase from an intracellular pool to the plasma membrane (for a review, see Ewart and Klip, 1995). The size of the intracellular pool is not defined, however, and due to the very low recovery of Na^+,K^+-ATPase from the tissue it cannot be ascertained whether the fractions isolated are representative. Moreover, studies on intact rat muscle, where the total pool of Na^+,K^+-ATPase located in the plasma membrane could be quantified gave no evidence for insulin-induced translocation of the enzyme to the plasma membrane (Clausen and Hansen, 1977).

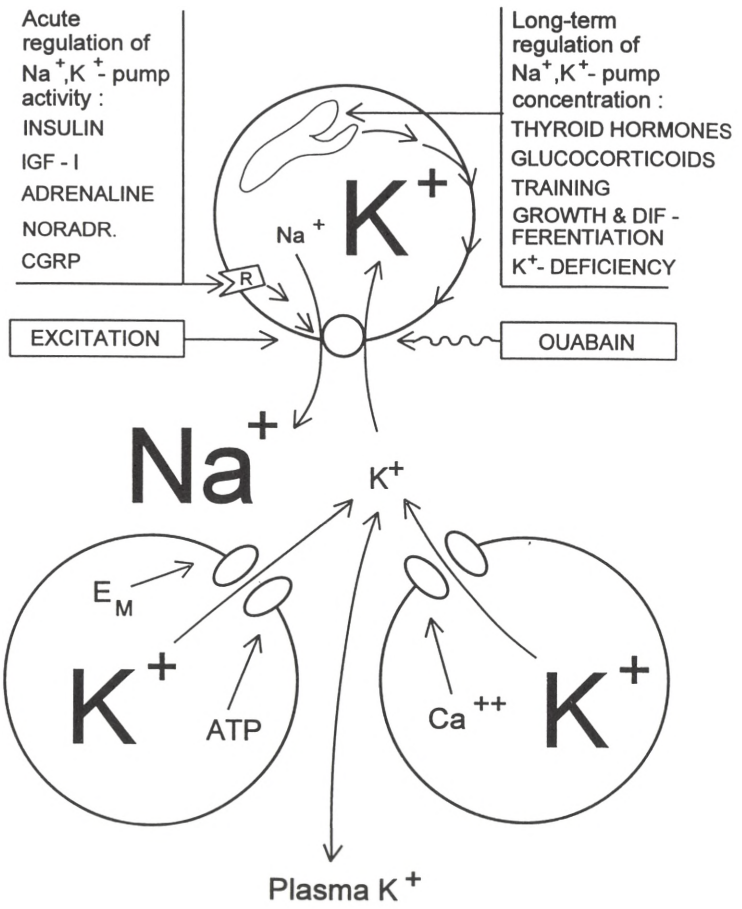

Figure 1. Some major aspects of the acute and long-term regulation of active Na^+,K^+-transport in skeletal muscle.

An acute increase in the activity of Na^+,K^+-ATPase already located in the plasma membrane may be achieved by increasing the maximum turnover number or the affinity of the transporter for intracellular Na^+. Several studies have shown that in muscle cells insulin produces a decrease in intracellular Na^+ (for a review, see Clausen, 1986), indicating that the stimulating effect of insulin on active Na^+,K^+-transport is the result of increased affinity for intracellular Na^+. More recently, insulin was found to reduce $[Na^+]_i$ also in renal tubular cells (Feraille et al., 1994).

Several other hormones have been found to stimulate the Na^+,K^+-pump in skeletal muscle (Clausen, 1996), in isolated cardiac myocytes (Desilets and Baumgarten, 1986) and other tissues. It is important that in such experiments, intracellular Na^+ decreases to values below the control level, indicating that the stimulation of the Na^+,K^+-pump cannot be related to increased influx of Na^+. Adrenaline-induced stimulation of the Na^+,K^+-pump was also shown to lead to a decrease in the activity of intracellular Na^+ in isolated rat and human muscle (Ballanyi and Grafe, 1988). As shown in Fig. 2, insulin, insulin-like growth factor I (IGF-I), adrenaline, noradrenaline and calcitonin gene related peptide (CGRP) all induce a marked decrease in intracellular Na^+ in isolated rat soleus muscle. Dibutyryl 3',5' cAMP has the same effect, and there is further evidence that 3',5'cAMP mediates the stimulating effect of the

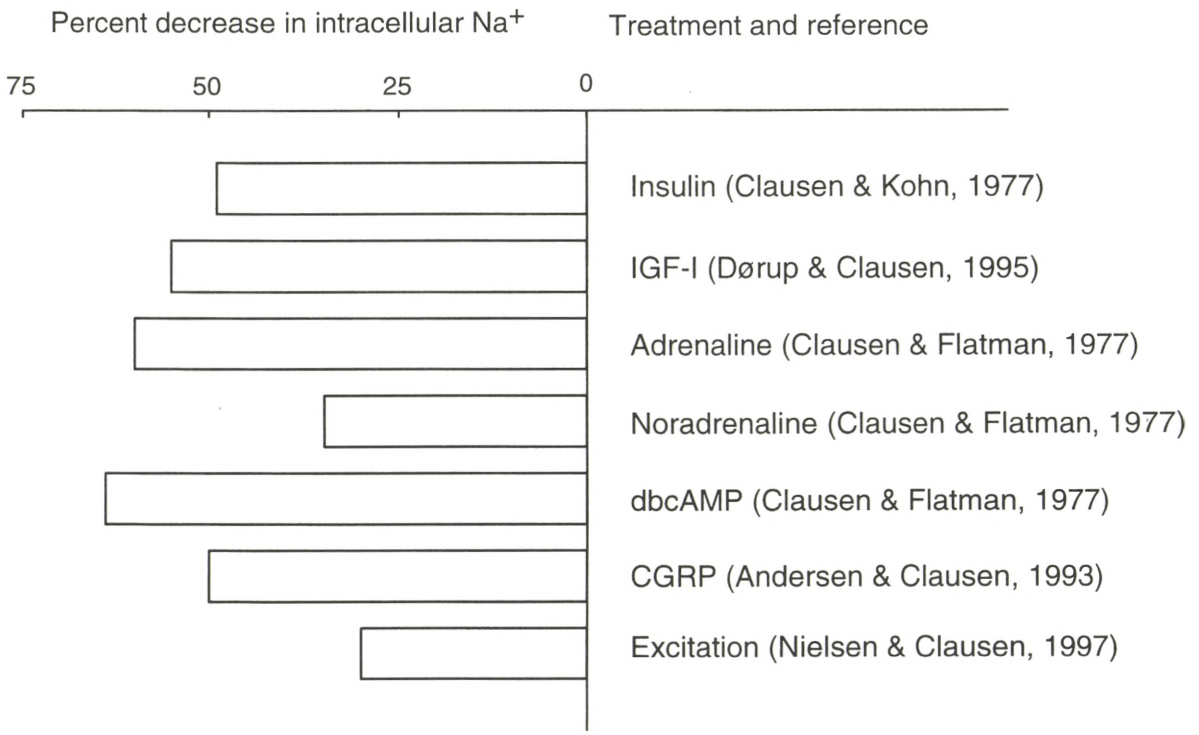

Figure 2. Effects of insulin, insulin-like growth factor I (IGF-I), adrenaline, noradrenaline, dibutyryl cyclic AMP (dbcAMP) and electrical stimulation (60 Hz for 30 sec, followed by 10 min rest) on $[Na^+]_i$ in isolated rat soleus muscle. Data were obtained from the publications indicated by references.

catecholamines and CGRP (Clausen and Flatman, 1977; Andersen and Clausen, 1993). In cells cultured from shark rectal glands, 3',5'cAMP induces stimulation of the Na^+,K^+-pump. This leads to a decrease in intracellular Na^+ (Lear et al., 1992), indicating that this is a general mechanism.

Taken together, the observations presented in Fig. 2 indicate that there is a general mechanism for rapid activation of the Na^+,K^+-pump, leading to a decrease in intracellular Na^+. The hormonal activation takes place within minutes and seems to be mediated by second messengers and protein kinases. The excitation-induced stimulation of the Na^+,K^+-pump, however, may reach maximum within 10 sec. We assume that these rapid changes reflect conformational modifications of the Na^+,K^+-pump, allowing it to function with a higher affinity for intracellular Na^+. This is analogous to the observation that the Ca^{++}-ATPase located in the plasma membrane exists in two interconvertible functional states, one with a considerably higher affinity for intracellular Ca^{++} than the other (Scharff and Foder, 1993).

The molecular mechanisms underlying the rapid activation of the Na^+,K^+-pump are poorly understood. We need more information about how the Na^+,K^+-pump might be influenced by 3',5'cAMP, protein kinases, rapid changes in membrane potential or the opening of Na^+-channels. A model for the plasma membrane must include mechanisms for a rapid increase in the rate of active Na^+,K^+-transport that is independent of a rise in $[Na^+]_i$.

Implications of Activation of the Na^+,K^+-pump:

Due to the electrogenicity of the Na^+,K^+-pump, an acute activation leads to hyperpolarization. This would allow restoration of excitability in muscles exposed to high extracellular K^+ ($[K^+]_o$). Indeed, in isolated rat soleus muscles which had been inhibited by exposure to a $[K^+]_o$ of 10-12.5 mM, the addition of hormones inducing stimulation of the Na^+,K^+-pump was found to produce hyperpolarization and a marked force recovery. This force recovery was completely suppressed by ouabain and closely correlated to the concomitant stimulation of ^{86}Rb uptake and the decrease in intracellular Na^+ (Clausen et al., 1993). These experiments strongly suggest that during exercise, activation of the Na^+,K^+-pump by the elevated plasma level of catecholamines is important for the maintenance of excitability and contractile performance in skeletal muscle. The much more rapid activation of the Na^+,K^+-pump associated with excitation allows the muscle cells to restore excitability at the moment where this is most needed. Recently, it was shown that in the isolated rat soleus muscle, intensified electrical stimulation could prevent the inhibitory effect of high $[K^+]_o$ on force development (Clausen et al., 1996).

Contrary to earlier assumptions, the Na^+,K^+-pump is more than a slow mechanism for gradual compensation of excitation-induced rundown of Na^+,K^+-gradients. The new concept is that the Na^+,K^+-pump restores and maintains excitability by undergoing prompt and sometimes large-scale activation.

References

Andersen, S.L.V., and T. Clausen. 1993. Calcitonin gene-related peptide stimulates active Na^+-K^+ transport in rat soleus muscle, *Am. J. Physiol.* 264:C419-C429.

Ballanyi, K., and P. Grafe. 1988. Changes in intracellular ion activities induced by adrenaline in human and rat skeletal muscle, *Pflügers Arch.* 411:283-288.

Balog, E.M., and R.H. Fitts. 1996. Effects of fatiguing stimulation on intracellular Na^+ and K^+ in frog skeletal muscle, *J. Appl. Physiol.* 81:679-685.

Clausen, T. 1986. Regulation of active Na^+-K^+ transport in skeletal muscle, *Physiological Reviews* 66:542-580.

Clausen, T. 1996. Long- and short-term regulation of the Na^+-K^+ pump in skeletal muscle, *News in Physiol. Sci.* 11:24-30.

Clausen, T. 1997. The clinical and therapeutic significance of the Na^+,K^+-pump. Submitted for publication.

Clausen, T., S.L.V. Andersen, and J.A. Flatman. 1993. Na^+-K^+ pump stimulation elicits recovery of contractility in K^+-paralysed rat muscle, *J. Physiol.* 472:521-536.

Clausen, T., and J.A. Flatman. 1977. The effect of catecholamines on Na-K transport and membrane potential in rat soleus muscle, *J. Physiol.* 270:383-414.

Clausen, T., and O. Hansen. 1977. Active Na,K-transport and the rate of ouabain binding. The effect of insulin and other stimuli on skeletal muscle and adipocytes, *J. Physiol.* 270:415-430.

Clausen, T., and P.G. Kohn. 1977. The effect of insulin on the transport of sodium and potassium in rat soleus muscle, *J. Physiol.* 265:19-42.

Clausen, T., O.B. Nielsen, and L. Hilsted. 1996. Excitation-induced force recovery in K^+-inhibited muscle, *The Physiologist* 39:A58 (abstr.).

Desilets, M., and C.M. Baumgarten. 1986. Isoproterenol directly stimulates the Na^+-K^+ pump in isolated cardiac myocytes, *Am. J. Physiol.* 251:H218-H225.

Dørup, I., and T. Clausen. 1995. Insulin-like growth factor I stimulates active Na^+-K^+ transport in rat soleus muscle, *Am. J. Physiol.* 268:E849-E857.

Everts, M.E., and T. Clausen. 1994. Excitation-induced activation of the Na^+-K^+ pump in rat skeletal muscle, *Am. J. Physiol.* 266:C925-C934.

Ewart, H.S., and A. Klip. 1995. Hormonal regulation of the Na^+,K^+-ATPase: mechanisms underlying rapid and sustained changes in pump activity, *Am. J. Physiol.* 269:C295-C311.

Feraille, E., M.L. Carranza, M. Rousselot, and H. Favre. 1994. Insulin enhances sodium sensitivity of Na-K-ATPase in isolated rat proximal convoluted tubule, *Am. J. Physiol.* 267:F55-F62.

Juel, C. 1986. Potassium and sodium shifts during in vitro isometric muscle contraction, and the time course of the ion-gradient recovery, *Pflügers Arch.* 406:458-463.

Lear, S., B.J. Cohen, P. Silva, C. Lechene, and F.H. Epstein. 1992. cAMP activates the sodium pump in cultured cells of the elasmobranch rectal gland, *J. Am. Soc. Nephrol.* 2:1523-28.

Medbø, J.I., and O.M. Sejersted. 1990. Plasma potassium changes with high intensity exercise, *J. Physiol.* 421:105-122.

Nielsen, O.B., and T. Clausen. 1996. The significance of active Na^+,K^+ transport in the maintenance of contractility in rat skeletal muscle, *Acta Physiol. Scand.* 157:199-209.

Nielsen, O.B., and K. Overgaard. 1996. Ion gradients and contractility in skeletal muscle: the role of active Na^+,K^+ transport, *Acta Physiol. Scand.* 156:247-256.

Nielsen, O.B., and T. Clausen. 1997. Regulation of Na^+,K^+-pump activity in contracting rat muscle, *J. Physiol.* 503:571-581.

Scharff, O., and B. Foder. 1993. Regulation of cytosolic calcium in blood cells, *Physiol. Rev.* 73:547-582.