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THE INDEPENDENCE OF MEMBRANE POTENTIAL AND POTASSIUM ACTIVATION OF THE SODIUM PUMP IN MUSCLE

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Summary

The membrane potential (E_m) of sartorius muscle fibers was made insensitive to [K^+] by equilibration in a 95 mM K^+, 120 mM Na^+ Ringer solution. Under these conditions a potassium-activated, ouabain-sensitive sodium efflux was observed which had characteristics similar to those seen in muscles with E_m sensitive to [K^+]. In addition, in the presence of 10 mM K^+, these muscles were able to produce a net sodium extrusion against an electrochemical gradient which was also inhibited by 10^{-4} M ouabain. This suggests that the membrane potential does not play a major role in the potassium activation of the sodium pump in muscles.

The ionic requirements of the Na^+-K^+ pump such as its activation by K_o^+, are the same as those for an ATPase isolated from membrane fractions [1], leading to the postulate that K^+ activation of the pump is similar in nature to an enzyme-substrate reaction. In fresh frog skeletal muscle both K^+ and azide, when applied externally, activate ouabain-sensitive Na^+ efflux and also depolarize membrane potential (E_m) [2, 3]. Since the relation between Na^+ efflux and transmembrane potential seemed to be the same in both instances, it was postulated that K^+ activation of the Na^+ pump is due to its effects on the membrane potential, not to a catalytic action on the pump mechanism. On the other hand, in high Na^+ sartorius muscles, the addition of K^+ does not immediately depolarize the membrane close to the K^+ equilibrium potential (E_K); rather, the potential is held at a higher value as a consequence of an electrogenic operation of the Na^+-K^+ pump [4, 5]. Under these conditions, a definite pump activation is observed with [K^+] as low as 1 mM [6]. Since other excitable tissues like squid axon [7] and *Anisodoris* giant neurone [8],

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as well as non-excitable human red blood cells [9], failed to show any membrane potential dependence of the Na^+-K^+ pump, it seemed worthwhile to re-examine the matter of the Na^+ pump activation in skeletal muscle. The idea was to work under conditions where the membrane potential and pump effects of K_o^+ could possibly be separated. This was done by taking advantage of the observation that the membrane potential of fibers which have been equilibrated in high K^+, normal Na^+ solutions is insensitive to reduction of K_o^+ while the membrane itself behaves as a Cl^- electrode [10, 11]. In this way it was possible to study Na^+ fluxes at constant E_m and variable K_o^+.

A basic experimental protocol and the constancy of membrane potential during the whole experiment are shown in Fig. 1. Fig. 2A summarizes all experiments performed to test the effect of $[K^+]_o$ on Na^+ efflux in muscles with membrane potentials clamped at about −23 mV. As can be seen, a $[K^+]_o$ as low as 1 mM was able to activate Na^+ efflux, while the shape of the curve indicates that the activation goes to saturation. As a comparison, a similar activation curve was studied in fresh muscles not subject to any treatment to clamp membrane potential, and this is shown in Fig. 2B. The characteristic of the activation curve in these conditions (e.g. membrane potential variable) was almost exactly the same as that observed when the membrane potential was

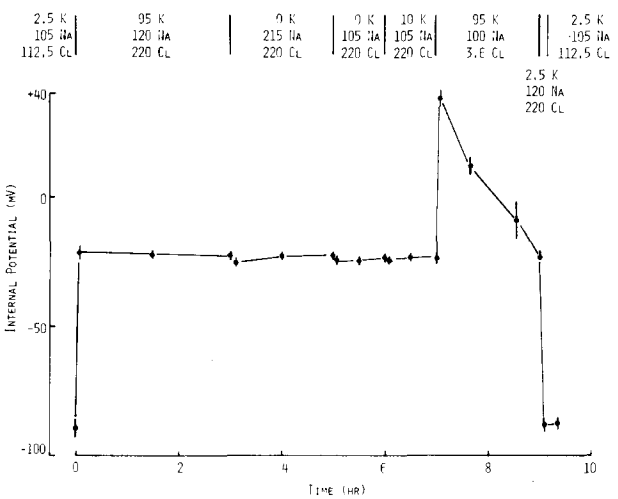

Fig. 1. Effect of different incubation solutions on membrane potential in frog sartorius. Once E_m was determined in normal 2.5 mM K^+-Ringer, the clamping period was begun by incubating the muscles for 3 h in a high K^+ solution. For Na^+ repletion with or without $^{22}Na^+$ loading (depending on the experiment), the muscles were incubated in K^+-free, high Na^+ solution for 2 h. The incubation in K^+-free, 105 mM Na^+ corresponds to the control Na^+ efflux in the absence of external K^+: external Na^+ was reduced to the usual concentration by partial replacement with Tris. In the next step, the muscles were in a solution with 10 mM K^+, $[Na^+]$ was 105 mM and Tris was reduced in accordance with the K^+ added. This gives the activation of Na^+ efflux by external K^+ at constant E_m. (In other experiments not shown here, E_m was also constant for different $[K^+]$.) Subsequent changes in solutions (sulfate for chloride and changing $[K^+]$) produced the expected changes in E_m, showing that the preparation was still viable. At the end of the experiment, the muscles remained excitable. In the net flux experiments, Na^+ content was determined after the K^+-free, 105 mM Na^+ period (before recovery) and after the 10 mM K^+ period (after recovery). In the labelled Na^+ efflux experiments, Na^+ content was followed for 1 h in K^+-free, 105 mM Na^+ and then for 0.5 h in K^+-containing media. In some cases ouabain was added (10^{-4} M) immediately thereafter and flux was followed for a further 0.5 h period. Each point is the mean ± S.E. of 16—24 punctures in four muscles. Temperature was 20 °C and pH 7.4. E_m was determined by a microelectrode technique.

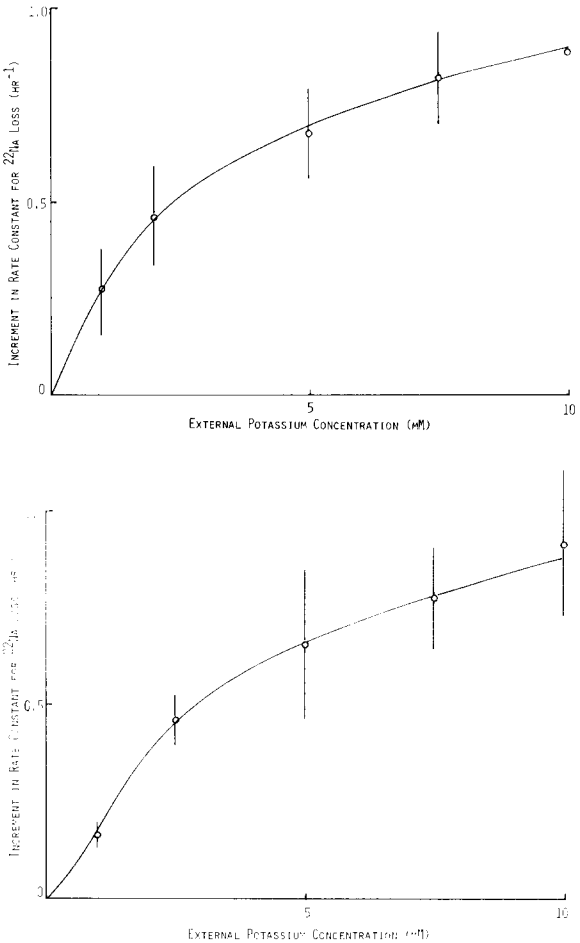

Fig. 2. Activation curve of Na^+ efflux as a function of $[K^+]_o$ in frog sartorius with membrane potential clamped at about −23 mV. Each point is the mean ± S.E. of three muscles and corresponds to an increment in the rate constant for $^{22}Na^+$ efflux over the value in K^+-free conditions. The average rate of Na^+ loss in the absence of K^+ was 1.06 ± 0.082 h^{-1} (n = 21). $^{22}Na^+$ efflux was determined as described elsewhere [12]. See legend of Fig. 1 for details. (B) Activation curve of Na^+ efflux by $[K^+]_o$ in fresh sartorius incubated in 105 mM Na^+. After loading with $^{22}Na^+$ by soaking the muscles for 2 h in 5 mM K^+, 114 mM Na^+ Ringer, the efflux of labelled Na^+ was followed for 1 h in K^+-free, 105 mM Na^+ and then for 0.5 h in solutions of varied $[K^+]$. The total osmolarity was maintained constant with Tris at 230 mosM. Points are the mean ± S.E. of three muscles, except that at 10 mM K^+ which includes two muscles. The rate of $^{22}Na^+$ loss in K^+-free conditions was 1.25 ± 0.09 h^{-1} (n = 18). Temperature was 20 °C.

clamped. On the other hand, and in opposition to the results of Horowicz and Gerber [2], $[K^+]_o$ as low as 1 mM was able to produce a detectable and consistent increase in ouabain sensitive Na^+ efflux above the values observed in K^+-free conditions. The main conclusion that can be drawn from Figs 2A and 2B is that the $[K^+]_o$ effect on Na^+ efflux in frog sartorius muscles is the same regardless of whether the membrane potential is variable or constant.

Since, by definition, the Na^+ pump is a mechanism which produces net Na^+ extrusion against an electrochemical gradient, as a further test, the effect of 10 mM $[K^+]_o$ on net Na^+ efflux against an electrochemical gradient in the

TABLE I

NET SODIUM EXTRUSION IN E_m CLAMPED MUSCLES

Net Na^+ extrusion against an electrochemical gradient in frog sartorius with membrane potential clamped at about −23 mV and incubated in 10 mM K^+, 105 mM Na^+ Ringer in the presence and absence of 10^{-4} M ouabain. Determinations of cell Na^+ were accomplished by the method proposed by Sjodin and Beaugé [12]. The number of muscles is given in parenthesis. See legend of Fig. 1 for details. Temperature was 20 °C.

Muscle Na^+ (μmol/g)					
Control			Ouabain (10^{-4} M)		
Before recovery	After recovery	ΔNa^+ (1 h)	Before recovery	After recovery	ΔNa^+ (1 h)
7.26* ±0.13 (9)	5.23* ±0.18 (9)	−2.04 ±0.15 (9)	8.23** ±0.50 (6)	9.95** ±0.36 (6)	1.88 ±0.26 (6)

*$P < 0.001$
**$P < 0.02$.

presence and absence of ouabain was investigated in muscles which had membrane potential clamped. A summary of these experiments is given in Table I. In the absence of an inhibitor of the Na^+ pump, 10 mM K_o^+ was able to produce a statistically significant net Na^+ extrusion against an electrochemical gradient. On the other hand, in the presence of 10^{-4} M ouabain the same $[K^+]_o$ was not only unable to produce any net Na^+ extrusion, but the muscle gained Na^+ after 1 h incubation; this Na^+ gain was also statistically significant. The Na^+ concentrations attained at the beginning of the experiment were very similar to those reported in the literature for fresh frog sartorius muscles [12].

The experiments reported in this paper seem to be clear evidence that K^+ is able to activate the Na^+ pump in frog skeletal muscle under conditions where their effects on the membrane potential are abolished. This strongly suggests that the mechanism by which this activation occurs in physiological conditions is not mediated through membrane potential changes, but is a direct consequence of the catalytic effect of K^+ on the enzymatic machinery which is responsible for the Na^+-K^+ pump. These results also agree with those found in squid axons [7], in red blood cells [9] and in molluscan neurons [8]. Although the characteristics of the curve of Na^+ efflux activation by K_o^+ found in this work are very similar in muscles whose potential is variable or clamped, this does not prove that all the characteristics of the pump remain constant. It might be possible that some other properties, such as the Na^+-K^+ coupling ratio, could be modified. Experiments to test these and other points are currently underway in this laboratory.

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