Resolution of the Potassium Ion Pump in Muscle Fibers Using Barium Ions

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ABSTRACT When frog sartorius muscles recover from Na enrichment in the presence of external K, net K entry into the fibers occurs by both passive movement and active inward transport via a K pump. Under normal conditions, it has not been possible to experimentally distinguish these processes. Fractionation into the flux components must be accomplished from inferences concerning the K conductance or permeability during a period of rapid Na extrusion. The best estimates indicate that 60-80% of the K entry occurs via the K pump. In the presence of Ba ions, the membrane permeability to K is very much reduced. Under these conditions, Na-enriched muscles underwent a normal recovery in the presence of external K, and the amount of inward K movement due to the K pump rose to over 90% of the total K entry. The characteristics of the K pump studied by this means were: (a) essentially complete inhibition by 10^{-4} M ouabain, (b) inhibition by [Na]o, (c) activation by [K]o according to a rectangular hyperbola in the absence of [Na]o, (d) linear activation by [Na]i over a wide range in concentration, (e) zero or undetectably low pumping rate as [Na]i → 0, (f) the number of Na ions actively transported per K ion actively transported is 1.4-1.7 normally and 1.1 in the presence of Ba.

INTRODUCTION

When frog striated muscles, in the presence of [K]o, recover from Na enrichment, there occurs a 1:1 exchange of Na for K (Steinbach, 1940; Desmedt, 1953; Adrian and Slayman, 1966). Although the properties of Na efflux under these conditions have been studied extensively (Keynes, 1954, 1965; Edwards and Harris, 1957; Mullins and Frumento, 1963; Horowicz et al., 1970; Sjodin, 1971; Beaugé and Ortiz, 1970, 1972), only some of the properties of K influx under the same conditions have been investigated. Potassium influx is rather insensitive to cardioactive steroids in normal fresh muscle. If [Na]i is elevated, an extra K influx occurs that is abolished by ouabain or strophanthinidin (Harris, 1957; Keynes, 1965; Sjodin and Beaugé, 1968).
The increased $K$ influx is correlated with the net gain which occurs when Na-enriched muscles extrude Na in the presence of $[K]_o$.

The present investigation is concerned with the mechanism for the increased $K$ influx which accompanies an increase in the rate of Na pump operation. There are two ways in which $K$ influx could be linked to Na efflux in muscle fibers: an active transport process for $K$ ($K$ pump) could be coupled in some way to the Na pump, or an increased passive $K$ influx may occur in response to an electrogenic or current-producing Na pump (Kernan, 1962; Frumento, 1965; Mullins and Noda, 1963; Mullins and Awad, 1965; Adrian and Slayman, 1966). Because the muscle fiber membrane has a high conductance for $K$ ions, some of the electrical type of linkage will always occur whenever the Na pump contributes to the muscle fiber membrane potential. A significant fraction of net $K$ entry during recovery of muscles from Na enrichment could occur in this way. The presence of linkage via a $K$ pump must be inferred from cases in which $K$ reaccumulation occurs when the internal fiber membrane potential is less negative than the $K$ equilibrium potential (Keynes and Rybová, 1963; Cross et al., 1965).

Until now it has not been possible to reliably estimate the component of the $K$ influx attributable to the electrogenic pump. The purpose of this investigation is to try to eliminate passive $K$ movements with Ba ions to better resolve pumped $K$ movements. Clearly, an agent which reduced the $K$ permeability severalfold while not greatly affecting the pumped fluxes would enable one to examine the $K$ pump with better resolution. Barium ions reduce $K$ conductance, permeability, and exchange in sartorius muscles (Sperelakis et al., 1967; Henderson and Volle, 1972).

METHODS

All experiments were performed on whole sartorius muscles from *Rana pipiens*. Flux and membrane potential measurements were made at 20°C.

*Flux Measurements*

Potassium influx was measured using $^{42}K$. Measurement procedures were identical to those previously described (Sjodin and Henderson, 1964; Sjodin and Beaugé, 1968). In some cases, $^{42}K$ efflux and $^{22}Na$ efflux were also measured. Rate constants were obtained from plots of the logarithm of the radioactivity remaining in the muscle against time. When $^{42}K$ efflux was measured using Na-enriched muscles, a high degree of isotopic equilibration was achieved before measurement of efflux. The procedure followed was identical to that previously reported (Sjodin and Beaugé, 1973).

*Method for Varying the Internal Na Concentration, $[Na]$*

Muscles with varying elevated Na concentrations were prepared by placing freshly dissected muscles in K-free Ringer solution for varying periods of time. The time-
course of Na gain by muscles under these conditions has been previously reported in detail (Sjodin and Beaugé, 1973). For each [Na], desired, the required time of incubation in K-free Ringer solution was determined from the published curves. For the highest [Na], employed, muscles were stored for 24–36 h in K-free Ringer solution at 4°C.

**Determination of Intracellular Cation Contents**

Intracellular Na and K were determined by a modification of the method previously described (Sjodin and Beaugé, 1973). Before preparation for flame photometric analysis, muscles were washed in a series of MgCl₂-substituted Ringer solutions. The schedule of rinses was; 1, 2, 2, 5, and 10 min in 5-cm³ tubes. This procedure gave values for intracellular cation content which agreed within errors with the more complete method previously described. After washing, wet weight was determined and muscles were then dried in an oven at 105°C and reweighed to determine dry weight. Muscles were ashed and the ash dissolved and assayed for Na and K as previously described (Sjodin and Henderson, 1964; Sjodin and Beaugé, 1968).

**Membrane Potentials**

Membrane potentials of muscle surface fibers were measured with microelectrodes filled with 3 M KCl using a DC preamplifier with grid current of 10⁻¹² A or less, an oscilloscope, and a digital voltmeter (See Sjodin and Beaugé, 1973).

**Solutions**

The standard Ringer solution used for dissection and preliminary storage was of the following composition (mM): NaCl, 115; CaCl₂, 2; KCl, 5; Tris buffer, 1. The pH was adjusted to 7.35 with HCl. The storage solution for Na enrichment procedures was (mM): NaCl, 120; CaCl₂, 2; Tris buffer, 1; pH = 7.35. The K-free and Na-free rinse solution for washing out extracellular ions was of the following composition (mM): MgCl₂, 86.3; CaCl₂, 2; Tris buffer, 1; pH = 7.35. The osmotic pressure of the MgCl₂-rinsing Ringer solution was equal to 230 mosmol/liter which was also the osmotic pressure of the first two solutions. The experimental solutions were of the following compositions (mM): NaCl, 105; KCl, 5; CaCl₂, 2; Tris, 1; pH = 7.35. NaCl, 105; KCl, 5; CaCl₂, 5; CaCl₂, 2; Tris, 1; pH = 7.35. The corresponding Na-free media had the NaCl replaced by an osmotic equivalent of Tris-Cl and undissociated Tris formed by neutralizing 90% of the total Tris present with HCl. The compositions of the Na-free media were (mM): Tris (90% neutralized), 112.5; KCl, 5; CaCl₂, 2; pH = 7.35. Tris (90% neutralized), 112.5; KCl, 5; BaCl₂, 5; CaCl₂, 2; pH = 7.35. The slight difference in osmotic pressure between control and Ba media had no effect on the results, as shown by comparison with experiments in which the tonicity of the control medium was increased with additional Na such that it was the same as that of the Ba medium. In experiments performed at higher [K]o, an increase in the tonicity was tolerated in order that [Na]o would be kept constant.
RESULTS

Recovery of Muscles from the Na-Enriched State Under Conditions of a Reduced Permeability to K Ions

To confirm that Ba ions also reduce $P_K$ in Na-enriched muscle cells, K efflux and influx were measured in such muscles in the presence and absence of $[\text{Ba}]_o$ using $^{42}\text{K}$. In some of the experiments, $10^{-4}$ M ouabain was present in the solutions to remove any active K influx. The averaged results of such measurements are presented in Table I. In the absence of ouabain, K influx was considerably greater than K efflux in the Na-enriched muscles, as would be expected during the recovery process. Barium ions reduced both K efflux and influx. Though the fractional reduction in K efflux was much greater than that for influx, in the absence of ouabain, the absolute magnitude of the flux reduction was about the same for both influx and efflux. It is noteworthy that, in the presence of Ba, K efflux is negligible in magnitude compared with K influx to within experimental error. When Ba is present, the measurement of K influx in Na-enriched muscles provides a good approximation of the net K influx occurring during recovery. Also worthy of emphasis is the fact that, in the presence of $[\text{Ba}]_o$, essentially all of the K influx is abolished by ouabain. Ouabain produced a small but significant increase in K efflux in the presence of 5 mM Ba. The direction of this flux change is consistent with the expected effect of membrane depolarization (Table III) on a passive K efflux. In the presence of $[\text{Ba}]_o$ and ouabain, both K efflux

| Experimental conditions                        | Rate constant for $^{42}\text{K}$ loss | K efflux $^*$ | K influx | ± SE (N) observations |
|-----------------------------------------------|---------------------------------------|--------------|---------|-----------------------|
| Normal 5 mM K Ringer solution                  | 0.114±0.002 (4)                       | 6.8±0.4 (4)  | 23.3±1.0 (5) |                       |
| Normal Ringer solution + 5 mM Ba++             | 0.006±0.0013 (4)                      | 0.36±0.08 (4)| 15.9±1.0 (5)|                       |
| Normal solution + 5 mM Ba++ + $10^{-4}$ M Ouabain | 0.009±0.0013 (4)                      | 0.54±0.08 (4)| 0.71±0.10 (5)|                       |

*Potassium efflux is calculated by applying the measured rate constants to the average measured internal K ion content. The average K content of the muscles used in the experiments was 60.2 ± 2.4 μmol/g and the average Na content was 28.8 ± 3.0 μmol/g. The cation contents are initial values estimated from flame analyses of muscles exposed to ouabain where no recovery occurs.
and influx were reduced to approximately the same low value, indicating that Ba reduces $P_K$ in Na-enriched muscles as in normal muscles.

It is clear that muscles recovering from the Na-rich state in the presence of $[\text{Ba}]_o = 5$ mM must engage in a Na:K interchange with a very much reduced passive net K influx. To discover the effect of reduced $P_K$ on recovery, net Na and K fluxes were measured flame photometrically using paired Na-enriched muscles. One muscle of a pair was analyzed for Na and K at the end of the period of Na enrichment to provide an estimate of initial cation contents; the other muscle was permitted to recover for a given time interval in 5 mM K, 105 mM Na Ringer solution either in the presence or absence of 5 mM Ba. Experiments were performed for different time intervals of recovery. The results plotted in Fig. 1 show that Ba had little effect on the net Na and K fluxes measured during recovery from Na loading. Using

![Figure 1](image-url)

*Figure 1.* Net changes in cation contents of Na-enriched muscles recovering in the presence of 5 mM K at 20°C are plotted against time in contact with the recovery solution. Results were obtained using paired sartorii, one muscle of the pair being analyzed initially to provide initial values of $[\text{Na}]_i$ and $[\text{K}]_i$. All experiments were performed in Na-Ringer solution. Circles refer to measurements made in the absence of barium ions. Triangles refer to measurements made when 5 mM Ba$^{++}$ was added to the recovery media. Each point is the average of six or more experiments ±1 SE (vertical bars). The average value of $[\text{Na}]_i$ initially as obtained for control muscles was 41.4 ± 1.1 μmol/g wet weight or 66.8 ± 1.8 mmol/liter fiber water.
initial slopes to estimate the magnitude of the net fluxes, the initial net flux amounts to about 25 μmol/g h. for both Na and K in the presence or absence of [Ba]. The initial slope for K net gain in the presence of Ba is somewhat smaller than the control slope but the difference is not statistically significant. The average initial Na content of the Na-enriched control muscles was 40 μmol/g. The results show that Ba does not significantly affect the rate of the net interchange of Na and K that occurs during recovery.

K Sensitivity of Na Extrusion in the Presence of Ba Ions

Barium ions may affect both the Na pumping rate and the rate of inward Na leakage in such a way that the net Na efflux remains unaltered. It seemed important, therefore, to discover any effect that Ba might have on the Na pump.

The K sensitivity of the Na pump in the presence of Ba was measured by methods previously reported (Sjodin and Beaugé, 1968; Sjodin, 1971). The efflux of tracer Na from 22Na-loaded muscles was measured in a K-free solution and in a solution containing 5 mM K. Experiments were performed on paired sartorii in the presence and absence of 5 mM Ba. The initial intracellular Na content of muscles was obtained from the known specific activity of 22Na by extrapolation of tracer washout curves. The results in Table II show that Ba has a slight slowing action on the Na pump. The difference in the rate constant for 22Na loss in the presence of 5 mM K is significant at the level 0.001 < P < 0.01. Sodium efflux under K-free conditions was also reduced in magnitude by Ba. The rate of the K-activated portion of the Na

### Table II

| Rate constants for 22Na loss | 105 mM Na Ringer solution | 5 mM Ba, 105 mM Na Ringer solution |
|-----------------------------|--------------------------|----------------------------------|
|                             | K-free        | 5 mM K | K-sensitive | K-free        | 5 mM K | K-sensitive |
|                             | h⁻¹           | h⁻¹    | h⁻¹        | h⁻¹           | h⁻¹    | h⁻¹         |
| 0.51                        | 1.17          | 0.66   | 0.45       | 1.07          | 0.62   |             |
| 0.63                        | 1.27          | 0.64   | 0.50       | 1.18          | 0.68   |             |
| 0.57                        | 0.98          | 0.41   | 0.30       | 0.97          | 0.47   |             |
| 0.97                        | 1.38          | 0.41   | 0.96       | 1.31          | 0.55   |             |
| 0.94                        | 1.77          | 0.83   | 1.01       | 1.66          | 0.65   |             |
| 0.96                        | 1.98          | 1.02   | 0.58       | 1.74          | 1.16   |             |

Mean ± SE  0.76±0.09  1.42±0.15  0.66±0.10  0.67±0.10  1.32±0.13  0.65±0.11

Rate constants were measured on paired muscles, one muscle being in normal Ringer solution with and without K⁺ and one muscle being in media with 5 mM Ba⁺⁺ added. The average internal sodium content of the muscles used was 38.8 ± 2.6 μmol/g muscle as measured by 22Na equilibration.
pump was not significantly affected by Ba. It is concluded that Na ions are extruded at a rate about 7% lower in the presence of 5 mM Ba. As the net Na efflux during recovery was not influenced significantly by Ba (Fig. 1), the results on \(^{22}\)Na efflux suggest that the rate of inward Na leakage was also affected by Ba.

Activation of the K Pump in Muscle by External K Ions

To resolve a component of K influx that approximates the true pumped K influx, K influx was measured as a function of \([K]_o\) in the presence of 5 mM external Ba using \(^{40}\)K as a tracer. The results are plotted in Fig. 2 for both 105 mM Na and Na-free Ringer solutions each containing 5 mM Ba. The low base-line K influx occurring in the presence of 5 mM Ba and \(10^{-4}\) M ouabain is evident. For \([K]_o = 5\) mM and above, about 95% of the K influx occurring in the presence of 5 mM Ba is abolished by \(10^{-4}\) M ouabain in Na-enriched muscles. This is in marked contrast to results obtained when ouabain or strophanthidin is applied to Na-enriched muscles in the absence

![Diagram](image-url)

**Figure 2.** Potassium influx measured unidirectionally with \(^{40}\)K ions in the presence of 5 mM Ba\(^{++}\) is plotted against \([K]_o\). The muscles were all enriched with Na by storage in K-free Ringer solution at 4°C for 24 h. Triangles refer to experiments performed in Na-free, Tris-substituted Ringer solution. Solid circles refer to experiments performed in Na-Ringer solution. Open circles refer to experiments performed in the presence of \(10^{-4}\) M ouabain and 5 mM Ba\(^{++}\). The upper two curves were obtained using paired muscles. Each point is the average value for four muscles except for \([K]_o = 20\) mM where the points refer to individual experiments. Vertical lines refer to \(\pm 1\) SE.
of Ba, in which K influx is reduced only by about 50% (Sjodin and Beaugé, 1968).

The enhancing effect of Na-free conditions on K influx in Fig. 2 must be due to a direct effect on the K pump. Sodium-free conditions in the external medium thus similarly affect the Na and K pumps in muscle membrane. The curves relating rate of K pump operation to [K]o are similar in shape to curves obtained for the K activation of Na pump (Sjodin, 1971). As the curve obtained for the pumped K influx in the absence of [Na]o appears to be hyperbolic, it was of interest to plot the data reciprocally. A modified Lineweaver-Burk plot was used in order to avoid grouping of data points near the origin, as often occurs on conventional double-reciprocal plots. The equation for the modified plot is:

\[
\frac{[K]_o}{v} = \frac{1}{V_{\text{max}}} \frac{[K]_o}{k_m} + \frac{k_m}{V_{\text{max}}} \cdots, \tag{1}
\]

where \(v\) refers to the K pump rate, \(V_{\text{max}}\) refers to the maximal rate of the K pump at very high \([K]_o\), and \(k_m\) is a constant denoting the reciprocal of the affinity of the K pump sites in the membrane for K. In Fig. 3, the data are plotted as \([K]_o/v\) versus \([K]_o\). For the case \([Na]_o = 0\), a straight line is obtained and Eq. 1 is obeyed with \(k_m = 3.5\) mM and \(V_{\text{max}} = 42.5\) μmol/g/h. With \([Na]_o = 105\) mM, Eq. 1 is not obeyed at low \([K]_o\). It is of interest to compare these results with those obtained for the Na pump. In the absence of \([Na]_o\), Na efflux follows a hyperbolic relationship when plotted against

![Graph](image)

**Figure 3.** For the same data used to construct Fig. 2, the quotient of \([K]_o\) divided by the value of K influx is plotted vs. \([K]_o\). Potassium influx has the same units as in Fig. 2. Solid circles refer to measurements made in Na-Ringer solution. Open circles refer to data obtained in Na-free media. The bars denote ±1 SE. The results obtained in the presence of 10⁻⁴ M ouabain are not shown in the figure.
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For Na extrusion, $k_m = 3.3 \text{ mM}$ and the maximal rate constant for pump operation was 2.5 $h^{-1}$. These results apply to muscles with an initial Na content of around 30 $\mu\text{mol/g}$. Potassium influx measurements provide an average value for influx over an initial 20–30-min period. The average Na content over this period is somewhat less than 30 $\mu\text{mol/g}$ and is close to 25 $\mu\text{mol/g}$. To get a comparable value of $V_{\text{max}}$ for Na, the maximal rate constant of 2.5 $h^{-1}$ was applied to the Na content of 25 $\mu\text{mol/g}$ to estimate $V_{\text{max}}$ for Na extrusion to be 62.5 $\mu\text{mol/g h}$. The ratio of $V_{\text{max}}^{\text{Na}+}$ to $V_{\text{max}}^{\text{K}+}$ is calculated to be 1.47 which may be taken as the pump stoichiometric ratio as estimated from maximal flux data.

Dependence of the Rate of the K Pump on $[\text{Na}]_i$

To discover the kinetics of K pump activation by $[\text{Na}]_i$, the rate of the K pump was measured as a function of $[\text{Na}]_i$ by the previously described technique which makes use of Ba. The results are plotted in Fig. 4. Both normal K influx and influx measured in the presence of 5 mM Ba rise linearly with increasing $[\text{Na}]_i$ with approximately the same slopes. The Ba-sensitive K influx (difference between normal values and the values measured in the presence of Ba) declines at a moderate rate with increasing $[\text{Na}]_i$. These

![Diagram](image_url)

Figure 4. Unidirectional potassium influx determined using $^{40}$K ions is plotted against the intracellular Na content in the presence and absence of 5 mM Ba$^{++}$. The intracellular Na content was varied as described in the text. The value for $[\text{Na}]_i$ was taken as the value obtained for a control group of muscles analyzed for Na after being incubated in K-free Ringer solution for the same time intervals employed to produce the muscles used in the experiments. Each point is the average value obtained for four to eight measurements. Vertical bars denote $\pm 1$ SE. The magnitude of $[\text{K}]_o$ was 5 mM for all experiments. The curve marked Ba$^{++}$-sensitive influx was obtained as the difference between values measured in the presence and absence of barium ions. Open circles refer to measurements made in the presence of $10^{-4}$ M ouabain and 5 mM Ba$^{++}$. 
results indicate that K influx in the presence of Ba extrapolates to the origin at [Na]_i = 0. In this limiting case, there is no activation of the K pump by [Na]_i. A corollary of this observation is that the control K influx and the Ba-sensitive K influx extrapolate to the same value at [Na]_i = 0. The data show that essentially all of the passive K influx is sensitive to Ba and that the K pump rate increases linearly with increasing [Na]_i.

It is of interest that the Ba-sensitive K influx shows a decline as [Na]_i is elevated. The Ba-sensitive K influx represents a passive process due to permeation or to a K:K exchange. As [Na]_i rises, the factors that could affect passive K movements are an increased membrane potential due to electrogenic hyperpolarization or a decrease in [K]_i. Membrane hyperpolarization could reduce P_K and so decrease the Ba-sensitive component of K influx, or a reduced [K]_i could result in a decreased K:K exchange. On either basis, the behavior shown in Fig. 4 would be expected.

**Muscle Fiber Membrane Potential During Recovery**

Since part of the net K influx during recovery is a passive ion movement, it is important to consider the muscle fiber membrane potential in the analysis of the K fluxes. Membrane potentials of Na-enriched muscle fibers were measured in a K-free solution at 20°C and subsequently in various recovery media at 20°C. Potentials were recorded after 10 min in the recovery solution over a 15-min interval. The recorded membrane potentials were stable over approximately a 20-min period. The measured potentials are presented in Table III. The results show that when [K]_o = 5 mM, the active K influx

| Solution | [K+]_o mM | [Na+]_o mM | [Ba++]_o mM | Ouabain | V_m ± SE mV | (N) Observations | V_K mV |
|----------|-----------|------------|-------------|----------|-------------|----------------|--------|
| 0        | 120       | 0          | 0           | —        | -90.9±2.1   | (2)           | —      |
| 0        | 105       | 0          | 0           | —        | -110.3±1.8  | (28)          | —      |
| 5        | 105       | 0          | 0           | —        | -93.5±1.7   | (8)           | -72.6  |
| 5        | 105       | 0          | +           | —        | -73.9±2.7   | (3)           | -72.6  |
| 5        | 105       | 5          | 0           | —        | -96.1±1.6   | (8)           | -72.6  |
| 5        | 105       | 5          | +           | —        | -76.9±1.6   | (6)           | -72.6  |

* Measured at 4°C in Na-enrichment solution.

Each membrane potential entry is the average potential for the number of sartorius muscles indicated in parentheses. Each individual muscle value used was the average value of the membrane potential of 8 to 12 surface fibers. The K equilibrium potential was calculated using the average [K]_i obtained in muscles subjected to the same 24-h Na-enrichment period employed in the membrane potential experiments. Membrane potentials were measured at a temperature of 20°C except for the single case indicated in which the temperature was 4°C which was the same temperature used during Na enrichment to obtain the highest internal sodium concentrations.
and the net passive K influx during recovery are in the same direction, both in the presence and in the absence of Ba, and that the passive driving forces for inward K movement are about the same in the presence and in the absence of 5 mM Ba. It is clear that the electrogenic ion pumps involved do not compensate for a greatly reduced $P_K$ by generating a much higher driving force. The data support the conclusion that a considerable fraction of the net K inward movement during recovery in the presence of Ba occurs by way of an active K pump.

**Fractionation into Pump and Passive Components of Flux for K and Na**

The exact fractions of active K movements must be estimated from calculations of the passive K flux. A minimum value for the active K flux measured in a medium containing Ba can be arrived at in a straightforward manner. The largest value that passive net K influx can have in the absence of Ba is 100% of the total net K influx during recovery. From the data in Fig. 1, this value is 25 μmol/g h. From the data in Table I, Ba reduces K permeability by about 20-fold. The calculated value for passive net K influx in the presence of Ba is thus 1.25 μmol/g h, and one concludes that over 90% of net K influx in the presence of Ba is via a K pump.

It is impossible to say more about the fraction of K influx that is pumped either in the absence or presence of Ba without basing arguments on calculated values of passive K influx, which depend upon the assumptions applied. Two additional methods of calculation were applied: the Ussing equation for the passive flux ratio, and a method based on electrodiffusion combined with an electrogenic pump (see Appendix). The results of the calculations are summarized in Table IV where it is seen that the estimates are in reasonable agreement with little reason to choose between them considering the assumptions that have to be made to obtain them. The utility of using Ba to

| TABLE IV |
| --- |
| CALCULATED PASSIVE NET K FLUXES IN THE PRESENCE AND ABSENCE OF Ba |

| Barium | Flux ratio method* | From membrane potential and net passive Na flux† |
| --- | --- | --- |
| Absent | 8.8 | 5.1 |
| Present | 0.6 | 0.5 |

* Calculated from the equation $\frac{\phi_o}{\phi_i} = e^{(V-V_K)F/RT}$ where $\phi_o$ is passive K efflux, $\phi_i$ is passive K influx, $V$ is the membrane potential, and $V_K$ is the potassium equilibrium potential. The symbols $F$, $R$, and $T$ have their usual significance. Values for passive K efflux were taken from Table II and values for $(V-V_K)$ were taken from Table III. Passive K influx, $\phi_i$, was calculated from the equation and the net K flux was obtained as $\phi_i - \phi_o$.

† The method of calculation is described in the Appendix.
resolve the K pump is apparent. In the absence of Ba, estimates of passive K influx are all fairly large fractions of the total net influx (25 μmol/g h); in its presence, however, passive K influx is a small fraction of the total net influx and any error in its estimation is much less serious. To arrive at estimates of the stoichiometry of Na:K active transport, it is necessary to add the inward leakage rate of Na to the net Na extrusion rate of 25 μmol/g h (Fig. 1) to obtain the true initial rate of operation of the Na pump. As it is not possible to measure the net inward leakage rate for Na during pumping, such measurements have to be made in the presence of ouabain. A value of 3 μmol/g h was obtained for net Na influx in the presence of 10^{-4} M ouabain (Sjodin and Beaugé, 1973). The magnitude of the Na pump rate in the ab-

| Type of flux | Control (K) | Control (Na) | Presence of Ba^{2+} (K) | Presence of Ba^{2+} (Na) |
|--------------|-------------|--------------|-------------------------|--------------------------|
| Total net flux | 25          | -25          | 25                      | -25                      |
| Passive shunt | 8.8         | 3            | 0.6                     | 1.5                      |
| Pump flux     | 16.2        | -28          | 24.4                    | -26.5                    |
| Pump current (expressed as flux) | -11.8       | -2.1         |                         |                          |

All entries were measured or calculated as indicated in the text. The pump current was obtained as the difference between active Na and K fluxes. A positive value for flux indicates an inward flux.

The presence of Ba is thus 28 μmol/g h. With Ba present, the rate of inward Na leakage in 10^{-4} M ouabain was reduced by 48% (average reduction in P_{Na} obtained for six muscles). With 5 mM Ba present, the net inward leakage rate for Na was thus reduced to 1.5 μmol/g h and the calculated value for the Na pump rate in the presence of Ba is 26.5 μmol/g h. This method of calculation indicates a slight inhibiting action of Ba on the Na pump as did the tracer Na measurements reported in Table II. The results of the analysis are presented in Table V. The passive flux estimates were taken from Table IV using the values obtained with the Ussing equation.

DISCUSSION

Barium ions have provided a useful means to reduce the passive K fluxes across the muscle fiber membrane so that the K pump can be studied with better resolution. Muscles extruding Na in the presence of Ba must do so with a greatly reduced capacity to produce a passive K ion current. The possible membrane adjustments to the altered conditions are to produce extra hyperpolarization to increase the driving force as a compensation for a
reduced $P_K$, to decrease the rate of active Na extrusion, or to increase the rate of the K pump. Measurements of the membrane potential showed that an increased driving force occurred only to a very minor extent. Also, measurements of the total net Na:K interchange during recovery in the presence of Ba showed no significant change in the total net fluxes. It seems clear that the K pump in the muscle fiber membrane was able to compensate for a reduced passive K influx by producing a greater current of inwardly pumped K ions.

Some comment should be made about the electrogenic behavior of the Na:K pump observed in the presence of Ba. The data in Table III indicate that the ouabain-sensitive membrane potential is about the same in the presence or absence of Ba. This result follows from the twofold effect of Ba in reducing the Na:K coupling ratio while increasing the membrane resistance. The summary presented in Table V shows that the net effective electrogenic pump current is reduced about sixfold in the presence of Ba. The membrane resistance to K ions is increased fivefold by Ba (Sperelakis et al., 1967). If K is the main ion acting as a shunt on the electrogenic potential, the net pump current should have fallen by about the amount observed. The membrane potentials of the Na-loaded fibers both in the cold (4°C) and at 20°C are such as to predict low cellular chloride contents (4°C, 2 μmol Cl⁻/g; 20°C, 1 μmol Cl⁻/g). As muscles were warmed for 30 min before use, the lower value for the intracellular [Cl] should apply initially. These intracellular Cl contents proved to be too small to detect any shifts during the experiments reported. Any small shunt due to Cl is thus negligible in the analysis.

Some of the observed properties of the K pump in muscle are similar to those of the Na pump, and some of the behavior is apparently different. External K and Na have similar effects on active Na and K transport. Any differences in the kinetics tend to be rather minor and quantitative in nature rather than qualitative. In a Na-free solution, both Na extrusion and the K pump rate follow the Michaelis-Menten equation with the same $k_m$ to within experimental errors. An apparent difference was observed at low [Na], values. The activation of the K pump by [Na], appears to be a first-order process even at low [Na]. Sodium extrusion in muscle, on the other hand, shows higher order kinetics in this region of [Na], (Keynes and Swan, 1959; Mullins and Frumento, 1963). The apparent difference in behavior may be a real one but, unfortunately, a firm conclusion cannot be drawn for two reasons. The data in Fig. 4 obtained in the presence of Ba follow a straight line.

1 The apparent decrease in membrane conductance due to Ba is smaller than the decrease in $P_K$ deduced from $^{36}$K fluxes in the presence of Ba. Some of the difference could be due to differences in $[Ba]_o$. Also, some of the Ba-sensitive K movement may involve a K:K exchange diffusion via membrane carriers which would contribute to tracer flux but not to membrane conductance.
line to within experimental error down to the lowest \([\text{Na}]_i\) employed. The deviation required to produce nonlinear behavior in this region, however, is slight. Indeed, if the first two data points are used alone, the straight line connecting the points does not pass through the origin. Also, the residual ouabain-insensitive passive K influx should be subtracted to obtain the true active transport rate. At high values of \([\text{Na}]_i\), the correction is within errors and is negligible. At low values of \([\text{Na}]_i\), this influx may contribute significantly to the total measured influx. The magnitude of the ouabain-sensitive influx at very low \([\text{Na}]_i\), is not known with sufficient accuracy to clearly resolve the kinetics in this region. An additional problem is that Ba may influence the kinetics of Na extrusion at low values of \([\text{Na}]_i\), even though there was only a slight effect on Na extrusion in the high \([\text{Na}]_i\) region. It seems safest to conclude that the K and Na pumps behave similarly in the concentration regions where the resolution of the K pump is the best.

The question arises as to the nature of the coupling between Na extrusion and the K pump. The results obtained do not rule out a coupled Na:K pump. The existence of similar kinetics, however, does not constitute proof for a common mechanism for the two ion pumps. Different sorts of experiments would be required to establish firmer conclusions regarding coupling of the pumps. Whatever the mechanism for the linkage of the active Na and K movements, the stoichiometric ratio of the Na to K pumping rates appears to be relatively constant over a wide range of \([\text{Na}]_i\). No obvious tendency to approach saturation was noticed when the K pump rate was plotted as a function of \([\text{Na}]_i\), up to about one half replacement of internal Na by K. Over a similar range of elevated \([\text{Na}]_i\), the Na extrusion rate also behaved as a first-order process (Sjodin, 1971). Thus, as \([\text{Na}]_i\) is elevated in muscle, it appears that the Na and K pumps keep pace with one another. This finding is in contrast to results obtained in squid giant axons where the stoichiometric ratio of Na to K pump rates appears to increase as \([\text{Na}]_i\) rises (Mullins and Brinley, 1969). Another difference between muscle fibers and squid giant axons concerns activation of pump rates by \([\text{K}]_o\). In squid giant axons, activation of the Na pump by external K follows kinetics that differ from those obtained when the K pump rate is plotted versus \([\text{K}]_o\) (Mullins and Brinley, 1969). The present experiments on muscle indicate that both activation by \([\text{K}]_o\) and the K pump rate follow similar kinetics. There is one qualification that is necessary, however: resolution of the K pump in muscle requires the use of an agent to essentially remove the passive K flux component, and the agent applied may alter the ion pumping systems. In the cases where \([\text{Na}]_i\) is considerably elevated, the Na extrusion rate was slightly decreased by the applied agent whereas the K pump rate was increased. The price one pays to resolve the K pump in muscle is to see its rate amplified somewhat. The mechanism for this is unknown but there may exist
some general relation between K pumping rate and the membrane permeability to K.

It is difficult to resolve the K pump in muscle in the absence of Ba or some other agent to reduce the passive K fluxes. If one calculates the passive K influx either by use of the Ussing flux ratio equation or by the method developed in the Appendix (Tables IV and V), the stoichiometry of the Na:K pump in the absence of Ba is in the range 1.4–1.7. In the presence of Ba, the value falls to 1.1 so that it has not been possible to resolve the K pump in muscle without a change in the coupling ratio.

**APPENDIX**

Membrane Potential with Active Na- and K-Ion Transport for Non-Steady-State Conditions

R. A. SJODIN

*Definition of Symbols*

- $\phi_j$ = the net passive flux of ion j.
- $M_j$ = the net active flux of ion j.
- $J_j$ = the total net flux of ion j such that $J_j = \phi_j + M_j$.
- $V$ = the membrane potential in the interior of the fibers relative to zero potential outside.
- $R$ = the gas constant.
- $T$ = the absolute temperature.
- $F$ = the Faraday of charge.
- $P_K$ = the permeability coefficient for potassium ions.
- $P_{Na}$ = the permeability coefficient for sodium ions.

During the recovery process, Na and K contents are not in the steady state and the Na pump contributes to the membrane potential. Equations previously employed to calculate the membrane potential of muscle fibers do not apply. An applicable equation can be derived on the following basis. Though pumped and passive-leak fluxes are out of balance for both Na and K ions, the flux components can be related as follows:

$$\phi_{Na} = -f_{Na} M_{Na},$$

and

$$\phi_K = -f_K M_K.$$  \(1a\)

The $f_j$ factors are ratios of passive net flux to pumped flux. For Na, $f_{Na}$ is always positive because the passive-leak flux of Na ions is always inward in Na-Ringer solution and the pumped net flux is outward. For K ions, however, $f_K$ may be either positive or negative depending upon the value of the membrane potential relative to the value of the K equilibrium potential. If, during recovery, the Na pump generates a potential which makes the membrane potential more negative than the K...
equilibrium potential, both passive and active net K fluxes are inward and $f_K$ is negative. By dividing the equations in Eq. 1 by each other, one obtains;

$$\frac{\phi_{Na}}{\phi_K} = \frac{f_{Na}M_{Na}}{f_KM_K}.$$  \hfill (2a)

The pump stoichiometric ratio has been defined as $r = -(M_{Na}/M_K)$ by Mullins and Noda (1963). Therefore, Eq. 2a may be rewritten as:

$$\frac{\phi_{Na}}{\phi_K} = -r \frac{f_{Na}}{f_K} = -Y,$$  \hfill (3a)

where $Y = r(f_{Na}/f_K)$. Equations relating passive fluxes to the membrane potential can be obtained from the constant-field assumption or any electrochemical flux theory that gives equations of the form

$$d_i = Pyf(V_i \cdots) \frac{\Delta C}{C_i e V_i R_i},$$  \hfill (4a)

where $C_o$ and $C_i$ refer to outside and inside concentrations and $f(V_i \cdots)$ is any function of the potential and other parameters that is the same for all cations. Substituting Eq. 4a for both Na and K ions into Eq. 3a and solving for the potential yields:

$$V = \frac{RT}{F} \ln \frac{YP_K[K_o] + P_{Na}[Na_o]}{YP_K[K_i] + P_{Na}[Na_i]}.$$  \hfill (5a)

Defining $\alpha = P_{Na}/P_K$ and $\theta = \alpha/Y$, one obtains:

$$V = \frac{RT}{F} \ln \frac{[K_o] + \theta[Na_o]}{[K_i] + \theta[Na_i]}.$$  \hfill (6a)

The equation is of use in the following way. The value of $f_{Na}$ is known from experimental data. The value of $f_K$ is unknown but can be calculated from potential data provided that the permeability ratio, $\alpha$, is known. From the fact that the total net fluxes of Na and K balance one another during recovery, $J_{Na} = -J_K$. Substitution in the previous equations gives two useful relations:

$$Y = \frac{f_{Na}(1 - f_K)}{f_K(1 - f_{Na})}.$$  \hfill (7a)

$$r = \frac{1 - f_K}{1 - f_{Na}}.$$  \hfill (8a)

This method was applied to the present data as follows. In the absence of electrogenic ion pumping,

$$V = \frac{RT}{F} \ln \frac{[K_o] + \alpha[Na_o]}{[K_i] + \alpha[Na_i]}.$$  \hfill (9a)
In the presence of $10^{-4}$ M ouabain, the resting membrane potential of fresh muscle fibers is $-87$ mV when $[K]_o = 2.5$ mM (Beaugé et al., 1973). Eq. 9 was used to calculate $\alpha = 0.016$. The inward leakage flux of Na ions was measured to be around $3 \mu$mol/g h. (Sjodin and Beaugé, 1973) and was independent of $[Na]_i$ over a wide range. The net Na extrusion rate in this work was $25 \mu$mol/g h (Fig. 3). Assuming that the leakage rate is $3 \mu$mol/g h, the Na active transport rate is $28 \mu$mol/g h. From these figures, $f_{Na}$ is calculated to be $0.11$. The membrane potential during recovery was used to obtain a value for $Y$ from Eqs. 5 and 6. Values for $f_K$ and $r$ could then be calculated from Eqs. 7 and 8. The results of the calculation when $[K]_o = 5$ mM are that $\phi_K = 5.1 \mu$mol/g h, $M_K = 19.9 \mu$mol/g h, about 80% of the net recovery influx of K is due to the K pump and the stoichiometric factor $r = 1.4$. In the presence of barium ions, the same parameters are: $\phi_K = 0.5 \mu$mol/g h, $M_K = 24.5 \mu$mol/g h, 98% of the net K influx is due to the K pump and the stoichiometric factor $r = 1.1$.

In the steady state at the normal $[Na]_i$, $f_{Na} = f_K = 1$ and $Y = r$. For these conditions, Eq. 6 becomes:

$$V = \frac{RT}{F} \ln \frac{r[K]_o + \alpha[Na]_o}{r[K]_i + \alpha[Na]_i}, \quad (10a)$$

which is identical to the result obtained by Mullins and Noda (1963). If the value of $r$ determined at high $[Na]_i$ is placed into Eq. 10, one calculates the normal resting potential when $[K]_o = 2.5$ mM in the absence of ouabain to be $-91$ mV. Further consistency of a stoichiometric factor $r = 1.4$ with flux data in fresh muscle can be arrived at as follows. From data obtained in this laboratory, the normal value for the rate of active Na extrusion in fresh muscle when $[K]_o = 2.5$ mM is $1.8$ pmol/cm²s. Under the same conditions, K influx = $6.2$ pmol/cm²s and K efflux = $7.4$ pmol/cm²s (Sjodin and Henderson, 1964). For $r = 1.4$, active K influx is $1.3$ pmol/cm²s and passive K influx is $4.9$ pmol/cm²s. The flux ratio of the passive K fluxes (efflux + influx) is then equal to 1.51 which is consistent with a membrane potential of $-91$ mV and $[K]_i = 140$ mM when $[K]_o = 2.5$ mM. This consistency depends upon the assumption of independent ion movements and equal activity coefficients for K inside and outside of the fibers. If the activity coefficient for K is lower inside the fiber than in the external solution, the stoichiometric ratio becomes greater (Mullins and Noda, 1963). Hence, any estimate of $r$ by this method is subject to uncertainty from this source. Also, these calculations do not depend very critically on the value of $r$. The method of calculating passive K influx from the Ussing equation (Table IV) gives a value of 1.7 for $r$. This value is also in agreement with the flux data given above. A value for $r$ of 2.5, however, is not in agreement with the data.

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