

Properties of Sodium Pumps in Internally Perfused Barnacle Muscle Fibers

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ABSTRACT To study the properties of the Na extrusion mechanism, giant muscle fibers from barnacle (*Balanus nubilus*) were internally perfused with solutions containing tracer ^{22}Na . In fibers perfused with solutions containing adenosine 5'-triphosphate (ATP) and 30 mM Na, the Na efflux into 10 mM K seawater was $\sim 25\text{--}30$ pmol/cm 2 ·s; 70% of this efflux was blocked by 50–100 μM ouabain, and $\sim 30\%$ was blocked by removal of external K. The ouabain-sensitive and K-dependent Na effluxes were abolished by depletion of internal ATP and were sigmoid-shaped functions of the internal Na concentration ($[\text{Na}]_i$), with half-maxima at $[\text{Na}]_i \approx 20$ mM. These sigmoid functions fit the Hill equation with Hill coefficients of ~ 3.5 . Ouabain depolarized ATP-fueled fibers by 1.5–2 mV ($[\text{Na}]_i \geq 30$ mM) but had very little effect on the membrane potential of ATP-depleted fibers; ATP depletion itself caused a 2–2.5-mV depolarization. When fueled fibers were treated with 3,4-diaminopyridine or Ba^{2+} (to reduce the K conductance and increase membrane resistance), application of ouabain produced a 4–5-mV depolarization. These results indicate that an electrogenic, ATP-dependent Na-K exchange pump is functional in internally perfused fibers; the internal perfusion technique provides a convenient method for performing transport studies that require good intracellular solute control.

INTRODUCTION

Sodium-potassium (Na-K) exchange pumps control the distribution of Na and K across the plasmalemma of most animal cells (e.g., Glynn and Karlish, 1975). To determine how internal as well as external solutes affect Na transport through these pumps, it is necessary to have direct access to a cell's interior so that the cytoplasmic composition can be controlled. Internal solute control has been achieved in a few cells, most notably in red blood cells (RBCs), and giant nerve and muscle fibers.

The internal composition of RBCs can be changed by lysing them in a hypotonic solution and then resealing them in a solution of the desired intracellular composition (Hoffman, 1958). Because internal solute control is not continuous, only initial internal conditions can be set with this technique.

By microinjecting various solutes into giant nerve and muscle fibers (Caldwell et al., 1960), the intracellular composition of these cells can also be altered in a discontinuous fashion.

Another technique, internal dialysis, permits continuous control over the ion and small molecule levels in the cytoplasm of giant axons (Brinley and Mullins, 1967) and muscle fibers (DiPolo, 1972). The cytoplasmic fluid composition of these large cells is controlled by dialysis against a solution flowing continuously through a porous capillary on which the axon or muscle fiber is impaled. Internal dialysis has two main drawbacks: (a) the wall of the capillary acts as a diffusional barrier across which only small solutes ($\sim 1,000$ mol wt or less) can move readily (Brinley and Mullins, 1967); and (b) diffusional delays occur because the wall of the dialysis tube is a significant distance from the plasma membrane.

The intracellular solute composition of large cells can also be controlled by simply perfusing the desired internal solution directly through the cytoplasmic space. This technique overcomes the disadvantages of internal dialysis: namely, it allows the introduction and removal of large soluble molecules and increases the rate at which the intracellular composition can be changed.

Oikawa et al. (1961) and Baker et al. (1961) demonstrated that the electrical properties of squid axons could be maintained during internal perfusion. Unfortunately, after the axoplasm has been squeezed out, axons perfused for more than 10 min become leaky to Na and, therefore, unsuitable for detailed studies of active transport (Baker et al., 1971). This leakiness has been ascribed to the mechanical damage (perhaps resulting in lysosome disruption) incurred when the axoplasm is extruded.

The giant barnacle muscle fiber is another large cell (1–2 mm in diameter and 3–5 cm long) that is suitable for both internal dialysis (DiPolo, 1972) and perfusion studies (Keynes et al., 1973; Murayama and Lakshminarayanaiah, 1977). In the present study we used the internal perfusion technique to investigate the properties of Na extrusion from barnacle muscle fibers. This technique enabled us to change intracellular Na and nucleotide concentrations ($[Na]_i$ and $[ATP]_i$, respectively) with a half time of 30–40 min (at a perfusion rate of 5 μ l/min). We were also able to introduce apyrase, an adenosine 5'-di- and triphosphatase, into the cells to reduce $[ATP]_i$ to very low levels. Many perfused fibers maintained stable Na effluxes and membrane potentials (-45 to -65 mV) for 12 h or more. We were able to demonstrate directly that Na extrusion from perfused barnacle muscle cells is (a) dependent upon internal Na and ATP and upon external K, (b) inhibited by ouabain, and (c) electrogenic. A preliminary description of some of these findings has been published (Nelson et al., 1978).

METHODS

Biological Material

Single giant muscle fibers from large specimens of *Balanus nubilus* from Friday Harbor, Washington, were prepared as described by Hoyle and Smyth (1963). Fibers not used

immediately after dissection were stored in Na seawater at 12°C, for use the following day. All experiments were completed within 48 h after dissection.

External Solutions

The compositions of representative external solutions are listed in Table I A. Ouabain (0.05–1.0 mM, Sigma Chemical Co., St. Louis, Mo.) and 3,4,-diaminopyridine (DAP; 2–5 mM, Aldrich Chemical Co., Milwaukee, Wis.) were added directly to the external solutions (see Results for details). The solution osmolalities (930–960 mosmol/kg) were determined on a vapor pressure osmometer (Wescor, Inc., Logan, Utah).

Internal Solutions

The compositions of the internal solutions are listed in Table I B. 15–30 μCi of ^{22}Na (New England Nuclear, Boston, Mass.) were added to each milliliter of internal

TABLE I
COMPOSITION OF SOLUTIONS

A. Representative external solutions						
Solution	NaCl	KCl	CaCl ₂	Tris	BaCl ₂	MgSO ₄
<i>mmol/liter</i>						
10 mM K	456	10	11	6	—	32
K-free	466	—	11	6	—	32
10 mM K + Ba	456	10	11	6	25	—
69 mM K	397	69	11	6	—	32
In addition to the solutes listed, all solutions were buffered to pH 7.8 at 20°C with maleic acid.						
B. Representative internal solutions						
Solution	Na gluta- mate	K gluta- mate	KCl	Sucrose	MgCl ₂	EGTA
<i>mmol/liter</i>						
Low Na	10	189	38	375	5.0	2.0
High Na	37	162	38	375	5.0	2.0
In addition to the solutes listed, all perfusion fluids contained 40 mM HEPES and 0.2 mM phenol red; the fluids were buffered to pH 7.3 with Tris base.						

All osmolalities ranged from 930 to 960 mosmol/kg.

perfusate, to give a specific activity of $\sim 1 \mu\text{Ci}/\text{mmol}$ of Na. In several of the ATP-free experiments, apyrase (Sigma) was added to the perfusate to give a final concentration of 1–3 U/ml. In all experiments that required ATP, an ATP-regenerating system was added to the perfusate; this system consisted of 3–5 mM ATP (Sigma), 3–5 mM phosphoenol pyruvate (Sigma), 8 $\mu\text{g}/\text{ml}$ pyruvate kinase (Sigma), and 3–5 mM MgCl₂ (Lowry and Passoneau, 1972).

Procedures

EXPERIMENTAL SETUP FOR PERFUSION EXPERIMENTS After 60–90 min of incubation in Ca-free seawater (to prevent contractions), muscle fibers with diameters of 1.3–1.7 mm were cut at the basal end. A single fiber was then placed in the experimental chamber containing Ca-free seawater (see Fig. 1). The chamber was identical to the one used in our laboratory for dialysis experiments (cf. Russell and

Blaustein, 1975; Blaustein, 1977). The cut (basal) end of the fiber was slipped over a glass cannula (1.0–1.5 mm o.d.) and tied with 7-0 surgical silk. This cannula extended to the exterior of the chamber through a silicone rubber seal. The tendon was tied to a small metal hook on the other side of the chamber.

A 0.5-ml gas-tight syringe (Hamilton Co., Inc., Reno, Nev.) containing the internal perfusate was then attached to an infusion pump (Harvard Apparatus Co., Inc., Millis, Mass.). The fluid was infused at a rate of 5 $\mu\text{l}/\text{min}$ through a polyethylene

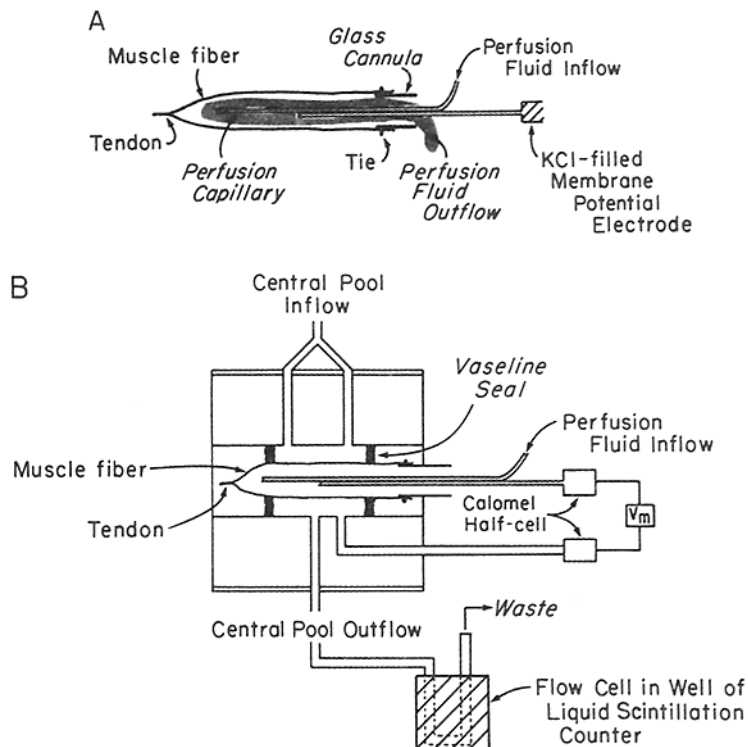


FIGURE 1. Experimental arrangement for barnacle muscle perfusion. (A) Internal perfusion arrangement for giant barnacle muscle fibers. The shaded area in the fiber indicates the path of the perfusion fluid flow through the sarco-plasmic space. (B) Arrangement of vaseline seals around the muscle fiber in the perfusion chamber.

tube and into a glass capillary 6.5–7.0 cm long and 125–150 μm o.d. (Fig. 1 A). This glass capillary was attached to a second glass capillary (75–100 μm o.d.) filled with 3 M KCl. The KCl capillary, which was 9–10 mm shorter than the perfusion tube, served as an electrical bridge between the interior of the cell and a calomel half-cell (Fig. 1 B). These tubes (with the perfusion fluid flowing) were then guided (under direct visual control) into the glass end-cannula and along the central axis of the fiber to the tendon end; this operation usually took 60–90 min. In this configuration, the perfusion fluid (stippling in Fig. 1 A) flows out the end of the longer capillary, into the cytoplasmic space of the cell near the tendon end; it then flows back along the outside of the capillaries to the cut base of the muscle fiber where it exits through the

glass cannula. This fluid (effluent) could be collected and assayed for Na, Ca, and ATP.

When the open tip of the perfusion capillary was positioned near the tendon end of the fiber, the fiber was lowered into a slot in the chamber. The cannulated and tendon ends were isolated from the central portion of the fiber by vaseline seals, as shown in Fig. 1 B.

Once the fiber was sealed in place, the central segment of the fiber was superfused with standard (10 mM K) Na seawater (see Table I). The membrane potential was then checked. If the membrane potential was > 45 mV (cytoplasm negative to the external reference solution), ^{22}Na and, in appropriate cases, ATP and the ATP-regenerating system were introduced into the perfusate. The radioactive tracer then diffused throughout the cytoplasmic space; some of it was transported across the plasma membrane into the extracellular fluid. The extracellular fluid superfused the sealed-off central portion of the fiber at a rate of 1.4 ml/min; 0.2 ml/min was diverted through the two guard outflows, adjacent to the vaseline seals, and discarded (See Fig. 1 in Russell and Blaustein, 1975). The remaining extracellular fluid (1.2 ml/min) passed through the main outflow: $\sim 80\%$ (i.e., ~ 1 ml/min) was channeled through an anthracene-filled flow cell set in the well of a liquid scintillation counter (β -Mate II, Beckman Instruments, Inc., Fullerton, Calif.), as indicated in Fig. 1 B. The counts were recorded on a digital print-out (DDP-7 panel printer, Datel Systems, Inc., Canton, Mass.). In addition, the output of the β -Mate II was passed through a Beckman linearizer and continuously monitored on a two-channel strip chart recorder (Houston Instrument Div., Bausch and Lomb, Inc., Austin, Tex.). The detailed methodological aspects of this on-line counting system have been published elsewhere (Blaustein, 1977).

The membrane potential of the cell was monitored with a pair of calomel half-cell electrodes. One half-cell was in contact with the cell's interior via the 3 M KCl capillary, and the reference electrode made contact with the extracellular fluid via one of the guard outflows. The membrane potential was displayed digitally, and also recorded as an analog signal on the strip chart recorder.

CALCULATION OF FLUXES Giant barnacle muscle fibers have somewhat irregular shapes that can be approximated by elliptic cylinders (cf. Brinley, 1968). Because the irregular shapes limit the accuracy of even the elliptic cylinder calculations, we employed the simpler circular cylinder approximations (based on mean fiber thickness measured at several places along each fiber) to estimate the surface area of the fibers.

A more serious difficulty arises as a result of the fact that barnacle muscle fibers are invaginated by deep, branching clefts into which numerous transverse tubules open (Selverston, 1967; Hoyle et al., 1973). Thus, the actual surface area of the sarcolemma may be some 15–20-fold larger than that calculated by the method described in the preceding paragraph (cf. Selverston, 1967). In the present work, all flux measurements are reported in terms of the cylindrical surface area approximation (see Discussion).

EXPERIMENTAL PROCEDURE FOR NONPERFUSED FIBERS The experimental arrangement was essentially identical to that used for the internally perfused fibers. The only difference was that a single 3 M KCl filled capillary (75 μm o.d.), instead of the double-barrel (perfusion and KCl) capillary, was inserted down the length of the fiber to the region of the central pool. The nonperfused fibers were used to determine the effects of ouabain and DAP upon the cell membrane potential.

ATP MEASUREMENTS Perfused and intact fibers were prepared for ATP measurements by the "HCl-methanol" extraction method (cf. Lowry and Passoneau, 1972, p. 124). The tendon and basal ends were removed and discarded, and only the central portions of the fibers were used. The fiber segment was gently blotted on filter

paper and weighed (samples ranged from 20 to 40 mg). The tissue was then homogenized in 0.1 N HCl in methanol (2 μ l/mg wet tissue wt). Perchloric acid (10 μ l of 0.3 N solution per mg wet tissue wt) was then added to the mixture. This mixture was centrifuged in a Microfuge (Beckman Instruments, Inc., Spinco Div., Palo Alto, Calif.) for 5 min. The supernate was transferred into a test tube and neutralized with a solution containing 0.25 M KOH, 0.15 M imidazole, and 0.15 M KCl (12 μ l/mg wet tissue wt). All solutions were kept at 0°C.

The luciferin-luciferase method (Karl and Holm-Hansen, 1976) was used to determine ATP levels in the perfusion fluid effluents and in tissue extracts (see preceding paragraph) from intact and perfused muscle fibers. Aliquots (300 μ l) of a 25 mg/ml suspension of firefly extract (Sigma) were mixed with 40 μ l of 5.0 mg/ml luciferin (Sigma). This mixture was stored on ice in the dark. Aliquots (34 μ l) of this firefly extract-luciferin mixture were mixed with 460 μ l of 0.15 M Tris-HCl buffer (pH = 7.5) in a 0.5-ml cuvette. An appropriately diluted aliquot (5 μ l) of the sample to be measured was then added to the cuvette. The cuvette was placed in the well of a photometer (American Instrument Co., Silver Spring, Md.), and light emission (at 5°C) was recorded with an analog signal on a strip-chart recorder. These readings were then compared to a standard concentration curve of ATP. Duplicate samples contained an internal standard consisting of a known concentration of ATP.

RESULTS

Rate of Equilibration of ^{22}Na in Perfused Muscle Fibers

The efficacy of perfusion methods for controlling intracellular solute concentrations depends upon solute wash-in and wash-out times. For this purpose, the time-course of ^{22}Na equilibration was determined. When ^{22}Na was added to perfusion fluid flowing at a rate of 5 μ l/min, the radioactive tracer concentration in the fluid emerging from the cannula at the basal end of the fiber (see Fig. 1 A) reached a steady level with a half time of \sim 12 min. The ^{22}Na efflux (i.e., ^{22}Na in the superfusion fluid; see Fig. 1 B) rose to a steady level with a half time of \sim 40 min.

When ^{22}Na was removed from the perfusion fluid, the concentration of radioactive isotope in the fluid emerging from the end-cannula fell with a half time of \sim 20 min, while the efflux of ^{22}Na declined with a half time of \sim 50 min.

The exchange times depended upon the perfusion rate. Slowing the perfusion rate from 5 to 2.5 μ l/min significantly lengthened these exchange times. On the other hand, the exchange times were reduced by \sim 50% when the perfusion rate was increased fivefold (to 25 μ l/min).

ATP Levels in Intact and Perfused Muscle Fibers

Sodium pump activity depends not only upon internal Na, but also upon internal ATP (De Weer, 1975). It was therefore necessary to determine how well the ATP levels could be manipulated in perfused fibers. As a base line, ATP concentrations were determined in fresh, intact muscle fibers. The result, 3.6 ± 0.6 mmol ATP/kg wet wt (mean \pm SD, $n = 8$), is comparable to the previously reported value of 4.3 ± 0.5 mmol/kg wet wt for *Balanus aquila* (DiPolo and Caputo, 1977). Moreover, we found that intact muscle fibers,

attached to the shell, which were incubated in Na seawater at 10°C for 24 h after dissection, had ATP levels that were virtually identical to those of fresh fibers.

Many experiments were conducted with 1–5 mM (usually 3 mM) concentrations of ATP in the perfusion fluids. For ATP-depletion experiments, the fibers were perfused with ATP-free fluids; in some instances apyrase was added to these fluids to increase the rate of ATP depletion.

Internal ATP levels in perfused fibers were monitored in three ways. (a) The ATP concentration of whole, perfused fibers was measured after 1–2 h of perfusion with ATP-free solutions. As indicated in Fig. 2 (■), the ATP

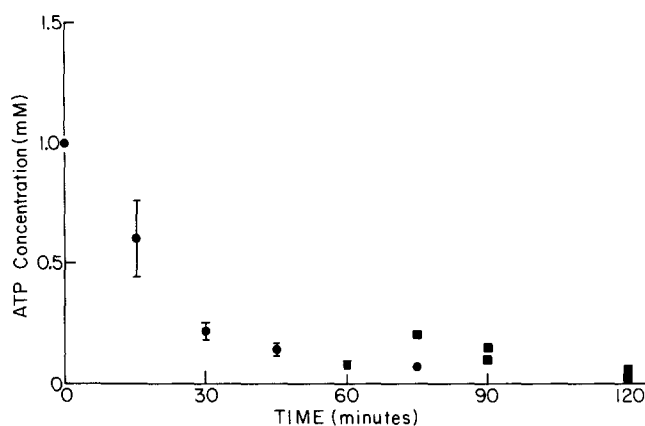


FIGURE 2. ATP concentrations in whole, perfused fibers (■) and in perfusion effluent (●) vs. the time the fibers were perfused with ATP-free solutions. Each circle indicates the mean \pm SE of the ATP concentration in the effluent from four perfused fibers. The squares indicate the ATP concentrations in the sarcoplasm of single (whole) perfused fibers. Since the insertion of the perfusion capillary down the axis of the fiber takes from 60 to 90 min, the perfusion fluid initially contained 1 mM ATP to assure a uniform base line (at time = 0).

concentrations in the middle segments of these fibers fell to 25–75 $\mu\text{mol/kg}$ wet wt after < 2 h of perfusion with ATP-free solutions. (b) The ATP concentration in the fluid emerging from the end cannula, after passage through the sarcoplasmic space, was measured (see Fig. 1 B). The ATP concentration in this fluid (the effluent) reflects its concentration in the central core of the fiber. When ATP-free fluid replaced fluid containing ATP as the perfusate, the ATP concentrations in the end-cannula effluent fell with a half time of \sim 20 min (Fig. 2; ●). Conversely, when ATP was perfused into ATP-depleted fibers, the ATP concentrations in the effluent rose with a half-time of \sim 12 min (data not shown). These half times are identical to the corresponding half times for ^{22}Na exchange mentioned above. (c) For transport studies, it is desirable to monitor ATP concentrations and concentration changes at the cell membrane. Since the Na pump is known to be fueled by ATP, we could, indirectly, monitor changes in the ATP level at the plasma membrane by measuring changes in ^{22}Na efflux in response to changes in the ATP

concentration in the perfusion fluid. Fig. 3 shows data from a representative experiment in which ATP was added to the perfusion fluid of an ATP-depleted fiber; after the introduction of ATP, the ^{22}Na efflux rose with a half time of ~ 60 min. This half time (60 min) is only slightly longer than the half time for ^{22}Na efflux to reach a steady level after adding the isotope to the perfusion fluid.

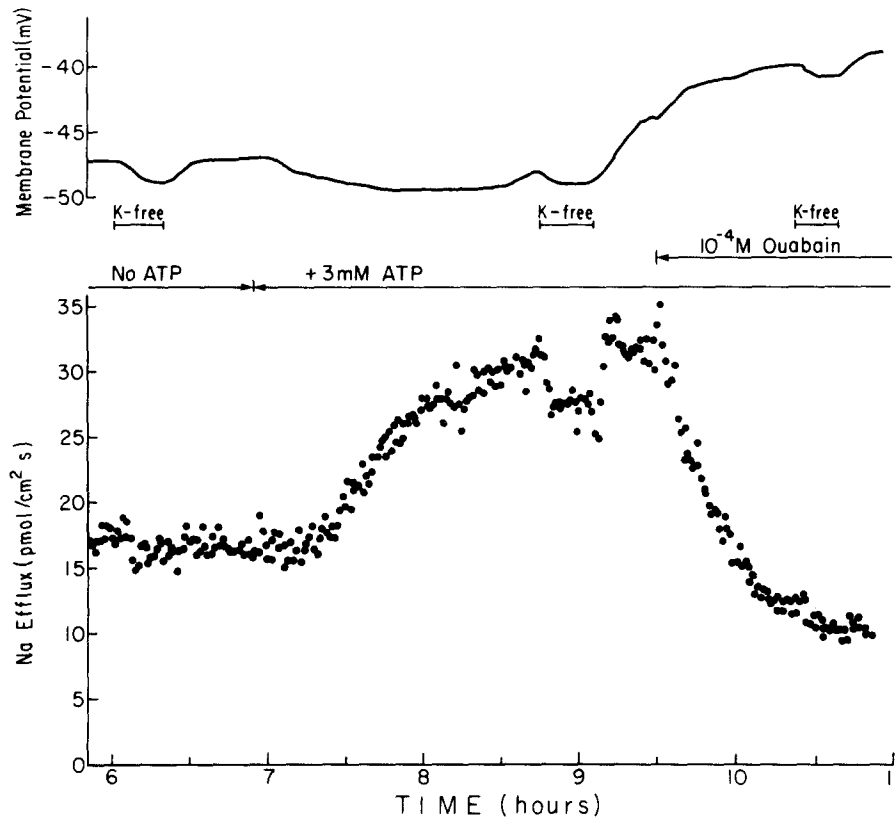


FIGURE 3. Effects of external K and ouabain and internal ATP on the membrane potential and ^{22}Na efflux from an internally perfused barnacle muscle fiber. Internal perfusion with ^{22}Na -containing solution was begun 350 min before the data shown here were obtained. ATP (3 mM) was added to the perfusion fluid at 420 min. The fiber was continuously exposed to 10 mM K seawater, except for the three periods indicated, when K-free seawater was used. Ouabain (10^{-4} M) was added to the 10 mM K solution after 580 min of perfusion. $[\text{Na}]_i$ was kept constant at 32 mM. Temperature = 16°C . Fiber diameter = 1.5 mm.

Na Efflux from Muscle Fibers Perfused with ATP

EFFECTS OF K-FREE MEDIUM AND CARDIOTONIC STEROIDS In barnacle muscle, as in many other types of cells, "active" Na extrusion mediated by the sodium (Na-K exchange) pump depends upon external K and is inhibited by cardiotonic steroids. In microinjected barnacle muscle fibers, the average Na

efflux (with $[Na]_i \approx 19$ mmol/kg wet wt) was 39 pmol/cm²·s; this flux was reduced by ~ 50% by removal of external K, and by ~ 80% when the fibers were treated with 10–30 μ M ouabain (Brinley, 1968). In dialyzed barnacle muscle fibers, Na effluxes of 35 pmol/cm²·s ($[Na]_i = 15$ mM; Brinley, 1969), 30 pmol/cm²·s ($[Na]_i = 32$ mM; DiPolo and Caputo, 1977), and 23 pmol/cm²·s ($[Na]_i = 16$ mM; Russell and Blaustein, 1975) have been reported. Removal of external K reduced the Na efflux by ~ 50%, and treatment with ouabain reduced the efflux by ~ 70% in dialyzed fibers (Brinley, 1969).

Similar properties can be demonstrated in the perfused muscle fibers. For example, when the standard (10 mM K) superfusion fluid was replaced by a nominally K-free fluid, the Na efflux from ATP-fueled, perfused fibers with $[Na]_i > 30$ mM, fell by ~ 30%, and the membrane hyperpolarized by ~ 2 mV; these effects are illustrated in Figs. 3, 4, 6, and 7. A hyperpolarization of 2 mV would be expected to reduce passive Na efflux by < 5%, as determined by the constant field equation (i.e., < 1 pmol/cm²·s), and therefore cannot account for the observed reduction in Na efflux (~ 6–8 pmol/cm²·s).

In ATP-fueled fibers with $[Na]_i = 30$ mM, Na efflux declined by 50–80% when a cardiotonic steroid (50 μ M or more of ouabain, dihydro-ouabain, or dihydrodigitoxigenin) was added to the standard (10 mM K) external medium (see Figs. 3, 4, 7, and 8). The inhibition of Na efflux by these steroids appeared to be irreversible. As one would expect, removal of external K after treatment with ouabain failed to alter the Na efflux (see Fig. 3)—an indication that the cardiotonic steroid had already completely blocked the Na-K exchange pumps. The nature of the difference between the magnitudes of the K_o-dependent and ouabain-sensitive Na effluxes will be addressed in the Discussion.

EFFECT OF $[Na]_i$ ON Na EFFLUX Na efflux depends upon the concentration of intracellular Na, $[Na]_i$. Data from an ATP-fueled muscle fiber that illustrate the effects of ouabain and of changing $[Na]_i$ and $[K]_o$ are presented in Fig. 4. When $[Na]_i$ in this fiber was increased from 16 to 32 mM, the Na efflux rose from 16 to 27 pmol/cm²·s with a half-time of 30 min; the K_o-dependent Na efflux approximately doubled, from ~ 1.5 to 3.0 pmol/cm²·s. The addition of 100 μ M ouabain to the standard superfusion fluid late in the experiment (with $[Na]_i = 32$ mM) inhibited the Na efflux by > 60%.

The total Na efflux from perfused fibers increased on the average from 10 to 25 pmol/cm²·s when the internal Na concentration was raised from 15 to 25 mM. When $[Na]_i$ was increased further, to 57 mM, the Na efflux rose to 30–40 pmol/cm²·s (data not shown).

Fig. 5 A and B shows that as $[Na]_i$ was increased from ~ 15 to ~ 55 mM, the ouabain-sensitive and K_o-dependent Na effluxes were activated in almost identical, sigmoidal fashion. Both fluxes were half-maximal at $[Na]_i \approx 20$ mM, and nearly maximal at $[Na]_i = 30$ mM. The sigmoid nature of the curves fitted to the data indicates that more than one internal Na ion may be required to activate the Na-K exchange pump. Hill plots of the data from Fig. 5 A and B have slopes (Hill coefficients) of ~ 3.5 (Fig. 5 C), which is consistent with the idea that the cooperative action of about three Na ions may be needed to promote Na extrusion.

The ouabain-insensitive and ATP-independent (“leak”) Na efflux increased

in a linear fashion with increasing $[Na]_i$ (data not shown) in agreement with observations of Brinley (1968) made on intact barnacle muscle fibers.

Na Efflux from ATP-depleted Fibers

As already mentioned, when fibers were fueled with ATP, a K_o -dependent (and ouabain-sensitive) Na efflux could be readily demonstrated, provided that $[Na]_i$ was > 10 mM. If ATP was subsequently removed from the perfusion

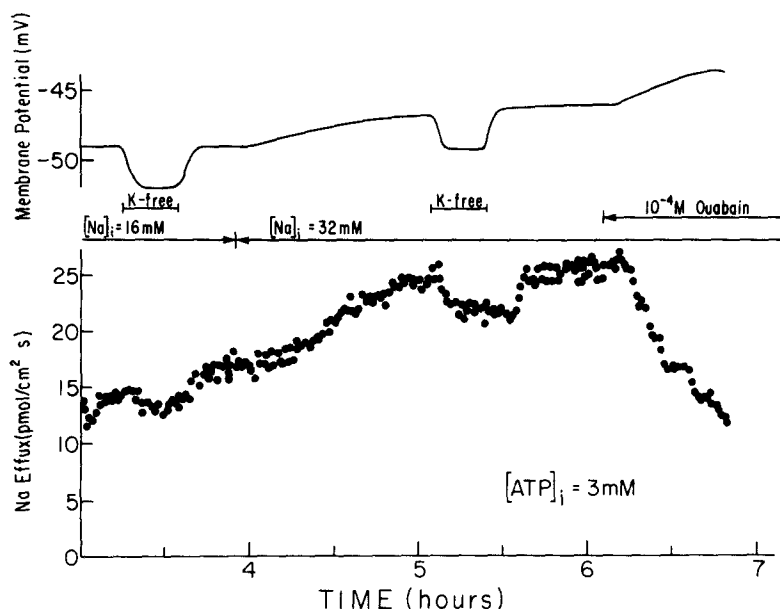


FIGURE 4. Effects of external K and ouabain and internal Na on the membrane potential and ^{22}Na efflux from an internally perfused barnacle muscle fiber. Internal perfusion with ^{22}Na , ATP, and an ATP-regenerating system was begun 180 min before the data shown here were obtained. After 240 min, $[Na]_i$ was raised from 16 to 32 mM by the addition of concentrated NaCl; the ^{22}Na concentration was also doubled to maintain a constant specific activity. The observed net depolarization could be a reflection of increased internal Cl^- . The fiber was continuously exposed to 10 mM K seawater, except during the two periods indicated, when the seawater contained no K. After 370 min, 10^{-4} M ouabain was added the 10 mM K seawater. Temperature = 16° . Fiber diameter = 1.5 mm.

fluids and apyrase added to speed the depletion of ATP, the Na efflux declined to a new steady level with a half time of ~ 45 min. The residual Na efflux was then no longer sensitive to removal of external K (see Fig. 6) or to the application of ouabain (data not shown). These observations are a good indication that $[ATP]_i$ was reduced sufficiently to shut off most of the Na-K exchange pumps.

In fibers that were perfused initially with ATP-free fluids for a sufficient

period of time (> 1.5 h) to lower the ATP levels to $< 50 \mu\text{M}$, the Na efflux was $10.8 \pm 2.5 \text{ pmol/cm}^2 \cdot \text{s}$ ($n = 5$ with $[\text{Na}]_i = 30 \text{ mM}$). In comparison, the ouabain-insensitive (+ATP) Na efflux was $10.2 \pm 3.4 \text{ pmol/cm}^2$ ($n = 6$ with $[\text{Na}]_i = 30 \text{ mM}$). The ATP-independent Na efflux was insensitive to the removal of external K (Fig. 3), indicating that the Na-K exchange pumps were not operating in these ATP-depleted fibers. When, subsequently, 3 mM ATP was added to the perfusion fluid, the Na efflux approximately doubled, with a half time of ~ 60 min. This ATP-stimulated efflux could be inhibited by the removal of external K (Fig. 3), and completely eliminated by the application of ouabain (Fig. 3). These data clearly indicate that the ouabain-sensitive, K_o -dependent Na efflux is fueled by ATP in barnacle muscle fibers.

Na-K Pump and the Membrane Potential (V_m). Is the Na-K Pump Electrogenic?

In addition to its inhibitory effect on Na efflux, ouabain depolarized the perfused, ATP-fueled fibers by an average of 1.7 mV (Table II, and see Figs. 3 and 4). This change in potential is comparable to the (average) 1.8-mV depolarization observed when intact (nonperfused) fibers were treated with ouabain (Table II). This depolarization may be explained in three ways. (a) Ouabain-sensitive Na extrusion is electrogenic, as in many other types of cells (e.g., Thomas, 1972). That is, depolarization reflects the inhibition of the efflux of net positive charge (e.g., if three Na ions are extruded per two K ions taken up). (b) Ouabain has a direct effect upon the membrane conductance. (c) Na pump inhibition results in significant extracellular K accumulation, thereby depolarizing the cell. A number of experiments were carried out to distinguish between these possibilities.

If the ouabain-induced depolarization is, in fact, a consequence of turning off the Na-K pumps, and not the result of a direct effect of ouabain upon the membrane conductance, other manipulations that shut off the pumps, such as depletion of (internal) ATP, should also cause depolarization. Indeed, as illustrated in Fig. 6 (also Table II), a depolarization of 3 mV occurs when internal ATP is depleted. Conversely, as shown in Fig. 3, reintroduction of ATP into a muscle fiber that had previously been depleted of ATP causes the fiber to hyperpolarize by ~ 2 mV. The magnitudes of these membrane potential changes are very similar to the ouabain-induced depolarizations mentioned above (and Table II).

If the depolarization observed with either ATP-depletion or ouabain treatment is the result of Na-K pump inhibition, ouabain should not have any effect on the membrane potential of ATP-depleted fibers (in which the pumps are not operating). On the other hand, if the ouabain-induced depolarization is the consequence of a change in ionic conductance, the steroid should also depolarize ATP-depleted fibers. Our experimental observations show that ouabain has little effect upon the membrane potential of ATP-depleted fibers (Table II), implying that Na pump inhibition is responsible for the ouabain-induced depolarization in ATP-fueled fibers.

Since Na-K pumps normally mediate an obligatory exchange of K for Na (e.g., Hodgkin and Keynes, 1955), a third way to inhibit the pumps is to remove external K (e.g., see Figs. 3, 4, 6 and 7). Unfortunately, the effects of

changing $[K]_o$ are complicated by the fact that the barnacle muscle plasma membrane is moderately permeable to K ions (Hagiwara et al., 1964); a change in the $[K]_o/[K]_i$ ratio (and the potassium equilibrium potential, E_K) would be expected to alter the membrane potential, V_m , directly. If the Na-K

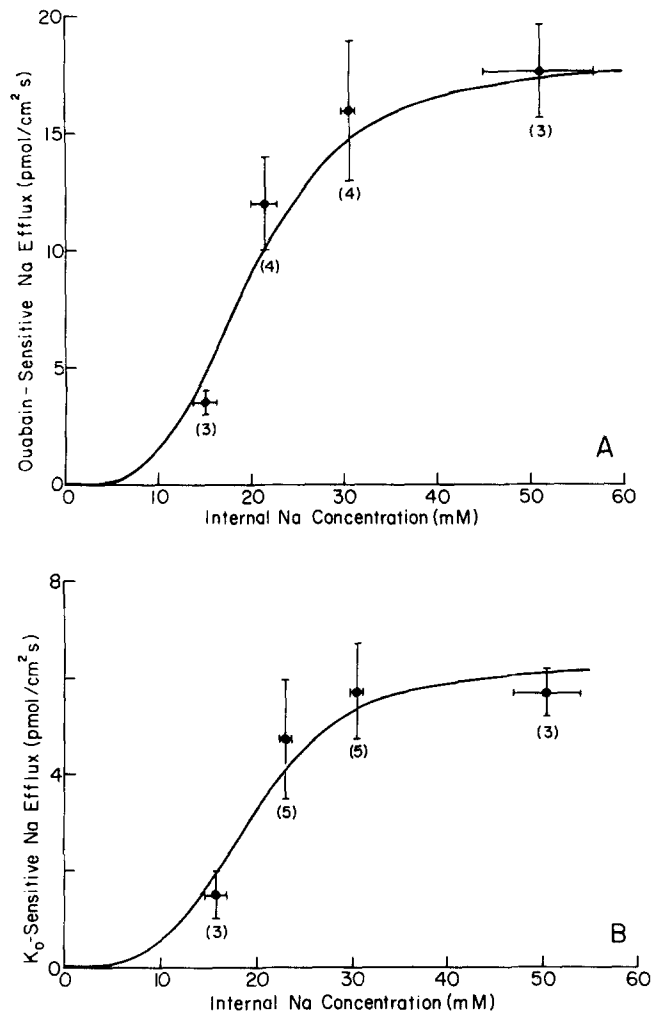


FIGURE 5

pumps are electrogenic, the net effect on V_m of reducing $[K]_o$ should be the sum of the hyperpolarization from the change in E_K and the depolarization from the inhibition of the pumps. Under these circumstances, removal of external K should hyperpolarize ATP-depleted or ouabain-treated fibers (i.e., with Na-K pumps already inhibited) to a greater extent than unpoisoned, ATP-fueled fibers (with pumps operating). Indeed, as shown in Table II, removal of external K did hyperpolarize the ATP-depleted fibers by 2.7 mV

and the ATP-fueled fibers by only 1.6 mV, on the average. The 1.1-mV difference presumably represents the contribution of the Na-K pumps (in the fueled fibers) that is abolished by the removal of external K. Note that both this K_o -dependent potential change (1.1 mV) and the K_o -dependent Na efflux

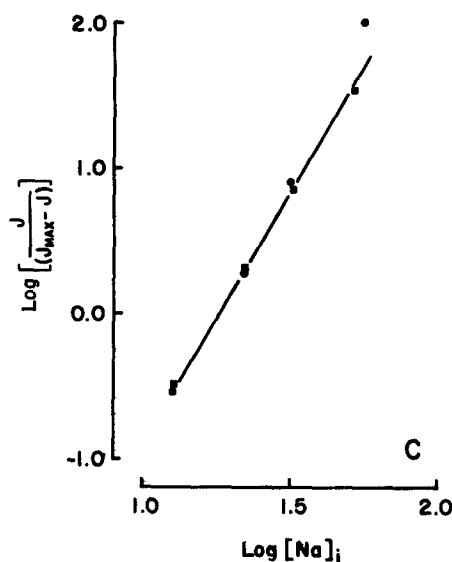


FIGURE 5. (A) Ouabain-sensitive Na efflux and (B) external potassium (K_o)-dependent Na efflux graphed as a function of internal $[Na]_i$ in internally perfused barnacle muscle fibers. The horizontal bars indicate the range of Na concentrations. The vertical bars indicate the standard errors for the Na effluxes averaged from the numbers of fibers shown in parenthesis. The curves were drawn to fit the Hill equation (Hill, 1910):

$$J = \frac{J_{max}}{1 + \left(\frac{K_{Na}}{[Na]_i}\right)^n},$$

where J = Na efflux, J_{max} = maximal Na efflux, $K_{Na} = [Na]_i$ that gives half-maximal Na efflux, $[Na]_i$ = internal Na concentration, n = Hill coefficient. For both curves $K_{Na} = 20$ mM and $n = 3.5$; n is the slope of the Hill plot shown in C. In C, the squares are the means of the ouabain-sensitive Na fluxes (from A); the circles are the means of the K_o -dependent Na fluxes (from B).

(~ 30% of the total efflux) are approximately one-half as large as the ouabain-induced depolarization (1.7–1.8 mV) and the ouabain-sensitive Na efflux (60–80% of the total efflux), respectively. A plausible explanation for these differences is that ouabain can diffuse into the spaces enclosed by the surface invaginations to block all of the Na-K pumps; on the other hand, K efflux from the sarcoplasmic space into the clefts tends to maintain a finite K

concentration in these clefts, even when K is omitted from the superfusion fluid, allowing some of the pumps to operate.

The data presented thus far support the idea that the Na-K pumps make a "direct" contribution to the membrane potential. However, these data do not prove that the pumps are electrogenic. An alternative possibility is that

TABLE II
EFFECTS OF VARIOUS TREATMENTS ON BARNACLE
MUSCLE MEMBRANE POTENTIAL

Treatment*	Change in membrane potential [‡]	
	A. Intact fibers	
	<i>mV</i>	
Ouabain	+1.8 ± 0.4 (6)	
Ouabain + DAP§	+4.5 ± 0.3 (6)	
Ouabain + Ba [†]	+4.0 ± 0.4 (5)	
	B. Perfused fibers	
	+ ATP	ATP-free
	<i>mV</i>	<i>mV</i>
Depletion of ATP	—	+2.6 ± 0.6 (2)
Addition of ATP	-2.0 ± 0.2 (3)	—
Ouabain	+1.7 ± 0.3 (8)	+0.4 ± 0.1 (4)
K-free	-1.6 ± 0.3 (8)	-2.7 ± 0.1 (6)
Ouabain + DAP§	+6.1 ± 0.1 (2)	—
Ouabain + Ba [†]	+3.0 (1)	—

* [Na]_i = 30–32 mM in all perfused fibers; ouabain concentration = 50–200 μM when present.

‡ Positive values indicate depolarization, negative values indicate hyperpolarization. Values are means ± SEM; the numbers of fibers per determination are given in parentheses.

§ DAP concentration = 3–5 mM.

† Ba concentration = 25 mM.

the pumps are electroneutral and that pump inhibition by ouabain or the lack of ATP results in significant K accumulation in the clefts and tubules. The observed depolarization could then be explained by the decrease in E_K . In order to distinguish between these two alternatives, ouabain-induced membrane potential changes of nonperfused and of perfused fibers were measured in the absence and presence of the potassium channel blockers, 3,4-diaminopyridine (DAP; Kirsch and Narahashi, 1978) and Ba²⁺ (Hagiwara et al., 1974). Fibers superfused with seawaters containing DAP (2–4 mM) or Ba²⁺ (25 mM) should have increased membrane resistances as a consequence of the reduction in potassium conductance (g_K).

If ouabain depolarizes the muscle fibers primarily by raising [K]_o (and decreasing E_K), ouabain should have a smaller effect on V_m in DAP- or Ba²⁺-

treated (low g_K) fibers than in untreated fibers. On the other hand, if ouabain's primary effect is to inhibit electrogenic Na-K pumps, it should have a greater effect on V_m in the DAP- or Ba^{2+} -treated fibers than in untreated fibers, because the former have a higher R_m (so that a larger $I_P \times R_m$ drop is expected). The experimental data (Table II) clearly show that the ouabain-

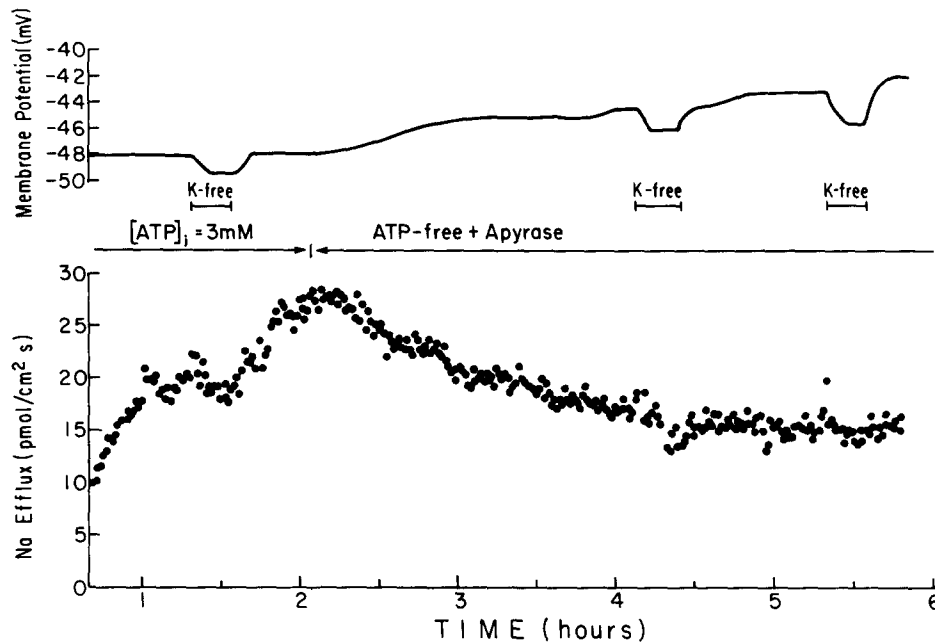


FIGURE 6. Effects of external K and internal ATP on the membrane potential and ^{22}Na efflux from an internally perfused barnacle muscle fiber. Internal perfusion with ^{22}Na , ATP, and an ATP-regenerating system was begun 30 min before the data were obtained. After 130 min, the fiber was perfused with ATP-free solution that contained apyrase (3 U/ml) and no ATP-regenerating system. The fiber was exposed to 10 mM K seawater, except for the three indicated periods. $[\text{Na}]_i = 30$ mM. Temperature = 16°C . Fiber diameter = 1.6 mm.

induced depolarization in DAP- or Ba^{2+} -treated fibers (4.0–4.5 mV) was more than twice that observed in untreated fibers (~ 1.8 mV).

Although the aforementioned results seem straightforward, it is important to demonstrate that Ba^{2+} and DAP do not affect the Na-K pumps, and that they do, indeed, reduce g_K . Data from one such control experiment are illustrated in Fig. 7: note that 25 mM Ba^{2+} had little effect upon the total, K_o -dependent and ouabain-sensitive Na effluxes. This indicates that the enhancement of ouabain-induced depolarization caused by Ba^{2+} (Table II) cannot be attributed to an increase in Na-K pump activity.

Fig. 8 shows data from an experiment in which the effects of DAP, K-rich media (69 mM K) and ouabain were tested; it illustrates several important points. (a) Raising $[\text{K}]_o$ from 10 to 69 mM depolarized the fiber (by reducing

E_K) and stimulated Na efflux. The latter effect is presumably due primarily to further activation of the Na-K pumps, since 10 mM K may not be sufficient to saturate the external K-binding sites (cf. Baker et al., 1969). This view is supported by the fact that, after treatment with ouabain, raising $[K]_o$ did not affect the Na efflux. (b) DAP did not affect the Na efflux into 10 mM K

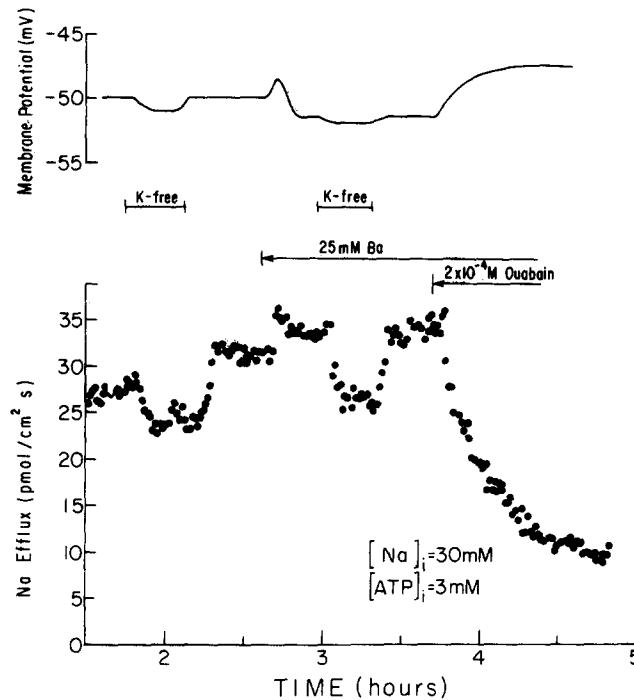


FIGURE 7. Effects of external K, barium, and ouabain on membrane potential and ^{22}Na efflux from an internally perfused barnacle muscle fiber. Internal perfusion with ^{22}Na , ATP, and an ATP-regenerating system was begun 90 min before the data shown here were obtained. The fiber was exposed to 10 mM K seawater, except for the two periods noted, when the seawater contained no K. After 165 min, the fiber was superfused with seawater containing 25 mM Ba, and after 230 min, ouabain was added to the seawater. $[\text{Na}]_i = 30 \text{ mM}$. Temperature = 16° . Fiber diameter = 1.7 mm.

seawater, or the increment in efflux due to raising $[K]_o$. This is evidence that DAP does not alter Na-K pump activity. (c) DAP reduced the magnitude of the depolarization induced by changing $[K]_o$ from 10 to 69 mM. The fact that, in the presence of DAP, the membrane potential response to changes in E_K is reduced is evidence that DAP reduces g_K . (d) Ouabain depolarized this DAP-treated fiber by $\sim 6 \text{ mV}$. The effect of ouabain on the membrane potential in the K-rich DAP solution may be estimated from the difference (5 mV) between the V_m in 69 mM K solution with DAP (-29 mV) and 69 mM K solution with DAP plus ouabain (-24 mV). This 5-mV "ouabain-

induced depolarization" cannot be attributed to a change in E_K (due to K accumulation in the clefts), because inhibition of the Na-K pumps may be expected to have a negligible effect on E_K when the superfusion fluid already

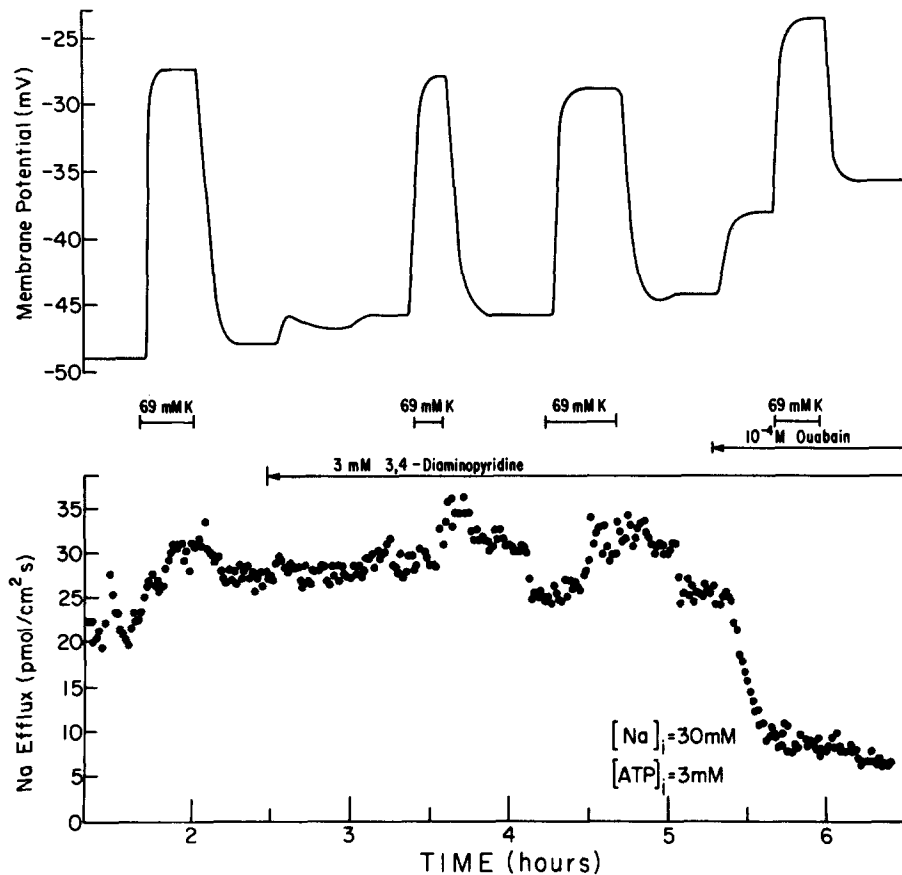


FIGURE 8. Effects of external K, ouabain, and 3,4-diaminopyridine (DAP) on the membrane potential and ^{22}Na efflux from an internally perfused barnacle muscle fiber. Internal perfusion with ^{22}Na and an ATP-regenerating system was begun 80 min before the data shown here were obtained. The fiber was exposed to 10 mM K seawater, except during the four periods indicated, when the seawater contained 69 mM K. After 150 min, DAP (3 mM) was added to the seawater, and after 310 min, ouabain (10^{-4} M) was added to the seawater. $[\text{Na}]_i = 30$ mM. Temperature = 16°C . Fiber diameter = 1.4 mm.

contains 69 mM K. Moreover, the effects of changes in $[\text{K}]_o$ (and E_K) on V_m are attenuated when g_K is reduced by DAP (see *c* above). This experiment was repeated without isotope under identical conditions and with identical results: (a) DAP reduced the 69 mM K_o -induced depolarization by ~ 5 mV; (b) ouabain depolarized the DAP-treated fiber by 6 mV; and (c) ouabain

depolarized high K_o , DAP-treated fiber by 5 mV. In sum, these observations make a strong case for the existence of electrogenic Na-K pumps in the barnacle muscle fiber.

DISCUSSION

Perfusion vs. Dialysis

The perfusion method enables us to change the internal solute composition of barnacle muscle fibers more rapidly and effectively than is possible with other techniques such as internal dialysis. For example, when fibers are perfused with ATP-free solutions, the sarcoplasmic ATP concentration falls with a half time of < 1 h, whereas fibers lose ATP at the rate of only $\sim 10\%$ per hour when dialyzed with ATP-free fluids (Brinley, 1969). Even if metabolic inhibitors such as cyanide and iodoacetate are added to the internal and external fluids, fibers must be "dialyzed extensively (> 2 h) with an ATP-free medium" to reduce ATP levels to ~ 20 – $30 \mu\text{mol/kg wet wt}$ (DiPolo and Caputo, 1977). In comparison, we achieved similar low ATP levels in unpoisoned fibers that were perfused at a rate of $5 \mu\text{l/min}$ for 2 h; this time could be cut in half by increasing the perfusion rate to $25 \mu\text{l/min}$.

The ATP depletion of the perfused muscle fibers was accomplished in a relatively short period of time without the use of metabolic poisons. Because the ATP levels can be readily controlled without poisoning, this circumvents any complicating effects that may be caused by the metabolic inhibitors themselves (e.g., Godfraind et al., 1971; De Weer, 1978).

Another important advantage of the perfusion method is that large molecules, such as apyrase, which cannot cross the walls of dialysis capillaries, can be introduced into the cytoplasmic space by perfusion. Clearly, this technique enables one to control the levels of both small (e.g., inorganic ions, nucleotides) and large (e.g., enzyme) solute molecules. A potential disadvantage, of course, is that some important large molecules (and perhaps even organelles) may be washed out in the perfusate.

The Na-K Exchange Pump Is Functional in Perfused Barnacle Muscle Fibers

The main thrust of this report is the demonstration that Na-K pumps remain functional in barnacle muscle fibers that are internally perfused for many hours; reliable quantitative data on the properties of the pumps can be obtained from these studies. The properties that we have observed are, in general, very similar to those that have been reported for Na-K pumps in a variety of cell types from both invertebrates and vertebrates. In the main, our findings show that the Na-K pumps in barnacle muscle fibers

(a) require external K; the pumps apparently mediate the exchange of Na for K (cf. Brinley, 1968). The ouabain-sensitive Na efflux into 10 mM K seawater is ~ 80 – 85% as great as the efflux into 69 mM K seawater; this implies that, under normal physiological circumstances, the external K binding sites on the pumps are nearly saturated, since the K concentration in barnacle hemolymph is about 10 mM (Keynes et al., 1973).

- (b) are completely inhibited by 50–100 μM ouabain.
- (c) are fueled by internal ATP. The ATP-dependent and ouabain-sensitive Na effluxes are nearly identical in magnitude.
- (d) have a sigmoid dependence on $[\text{Na}]_i$; the apparent half-saturation concentration for this activation is about 20 mM Na.
- (e) are electrogenic.

In the subsequent sections, several of these properties will be discussed in further detail.

Why is the Ouabain-Sensitive (and ATP-Dependent) Na Efflux Greater than the K_o -dependent Na efflux?

If the pumped efflux of Na is defined as the ouabain-sensitive (and ATP-dependent) Na efflux, we might expect all of this efflux to be external K -dependent if the pump mediates the obligatory exchange of external K for internal Na. Nevertheless, we consistently observed (also Brinley, 1968) that the magnitude of the K_o -dependent Na efflux was only 30–50% of the magnitude of the ouabain-sensitive and ATP-dependent Na efflux. It is well known (e.g., Baker et al., 1969) that Na-K pumps can also mediate a ouabain-sensitive Na-Na exchange. If a large portion of the Na efflux from our perfused barnacle muscle fibers was, indeed, a manifestation of Na-Na exchange, this could account for the observed discrepancy between the magnitudes of the K_o -dependent and ouabain-sensitive Na effluxes. However, De Weer (1976; and see Kennedy and De Weer, 1976) has demonstrated that Na-K pump-mediated Na-Na exchange requires internal ADP. Because we used an ATP regenerating system (with phospho-enol pyruvate and pyruvate kinase) to keep ADP levels very low, it seems unlikely that the aforementioned discrepancy can be accounted for by a ouabain-sensitive Na-Na exchange.

The fact that removal of external K is only about half as effective as ouabain in inhibiting the contribution of the electrogenic pump to the membrane potential (see Results) also supports the view that Na-Na exchange is not a major component of the ouabain-sensitive Na efflux. Na-Na exchange appears to be electroneutral (Abercrombie and De Weer, 1978), whereas the ouabain-sensitive, but seemingly K_o -independent, Na efflux from barnacle muscle is electrogenic (cf. Table II and related discussion).

A second possibility that should be considered in attempting to account for the discrepancy between the magnitudes of the K_o -dependent and ouabain-sensitive fluxes is “uncoupled” Na extrusion through the sodium pump (Glynn and Karlsh, 1976). However, it is uncertain that uncoupled Na efflux can occur when the nominally K -free medium contains a high concentration of Na (Glynn and Karlsh, 1976).

A much more plausible explanation for the difference between the magnitudes of the ouabain-sensitive and K_o -dependent Na effluxes is that the leak of K from the sarcoplasmic space into the clefts, even when K is omitted from the superfusion fluids, allows some of the Na-K pumps to operate. On the other hand, ouabain would be able to diffuse into the clefts and tubules and inhibit all the Na-K pumps.

On the Density of Na-K Pump Sites in the Sarcolemma

As a corollary to the preceding discussion, it is useful to consider the surface density of Na-K pumps in the sarcolemma. Our data indicate that, at saturating $[Na]_i$ (cf. Fig. 5 A) and $[K]_o$ (cf. Fig. 8), the ouabain-sensitive Na efflux is about $25 \text{ pmol/cm}^2 \cdot \text{s}$. This flux value refers to muscle fibers that are treated as circular cylinders (see Methods). However, as noted above, the fibers are deeply invaginated; capacitance measurements suggest that the actual surface area of a fiber's sarcolemma may be on the order of 20 times the area calculated from the perimeter and length (Selverston, 1967). Thus, the actual pumped Na efflux is probably of the order of $1 \text{ pmol/cm}^2 \cdot \text{s}$, when referred to sarcolemma surface area. Assuming a Na-K pump turnover of $3,300 \text{ min}^{-1}$ (e.g., Kyte, 1971) and a stoichiometry of three Na ions transported per ATP hydrolyzed (see below), a flux of $1 \text{ pmol/cm}^2 \cdot \text{s}$ corresponds to about 40 pump sites per μm^2 of sarcolemma.

This Na-K pump density ($\sim 40/\mu\text{m}^2$) can be contrasted with the very low density of pumps ($< 1/\mu\text{m}^2$) in the plasma membrane of human red blood cells (cf. De Weer, 1975), and the high density of pumps ($\sim 1,000\text{--}1,500/\mu\text{m}^2$) in the axolemma of squid giant axons (cf. Fig. 3 in Baker et al., 1969; and De Weer, 1975). The difference in pump density between barnacle muscle sarcolemma and squid axolemma is not surprising in view of the fact that during depolarization of barnacle muscle most of the inward current is carried by Ca ions (Hagiwara et al., 1964), whereas Na ions carry most of the inward current in depolarized squid axons (Hodgkin and Huxley, 1952). On the other hand, the relatively high density of Na-K pumps in barnacle muscle, compared to red blood cells, may be needed to pump out the Na that enters the muscle when the Ca is extruded via Na-Ca exchange (Russell and Blaustein, 1974).

On the Relationship between $[Na]_i$ and Na Efflux

The total Na effluxes from frog skeletal muscle (Keynes and Swan, 1959; Mullins and Frumento, 1963) and mammalian cardiac muscle (Carmeliet, 1964) are sigmoid-shaped functions of $[Na]_i$ with half-maximal values at $\sim 20 \text{ mM}$ Na. Although not specifically tested in the aforementioned studies, most of the Na efflux in these preparations is, presumably, mediated by ouabain-sensitive Na-K pumps. The ouabain-sensitive Na efflux from human red blood cells is also a sigmoid function of $[Na]_i$, with a half-saturation value for Na_i of $\sim 15 \text{ mM}$ (Garay and Garrahan, 1973). The ouabain-sensitive (and K_o -dependent) Na efflux from barnacle muscle (Fig. 6 and related text) follows kinetics that are similar, if not identical, to the kinetics of Na efflux in these other preparations. The nonlinear relationships between $[Na]_i$ and the ouabain-sensitive and K_o -dependent Na effluxes can be described by expressions that assume that the Na pump contains three to four sites for internal Na. Such nonlinear kinetics are not surprising in light of other evidence that about three Na ions are transported per molecule of ATP hydrolyzed by the Na-K pump (Garrahan and Glynn, 1967).

The fact that the internal sites on the Na-K pumps are apparently half-saturated with Na^+ when $[Na]_i \approx 20 \text{ mM}$ is particularly noteworthy because

this value is very close to the normal $[\text{Na}]_i$ in intact barnacle muscle fibers (~ 19 mM; Brinley, 1968). This means that the pumps are normally poised on the steepest portion of the Na_i saturation curve.

The Na-K Pumps in Barnacle Muscle Are Electrogenic

The Na-K pumps in many types of cells contribute directly to the membrane potential by extruding more Na ions than the number of K ions taken up; the net electrical current generated by this inequality tends to hyperpolarize the cells (e.g., see the review by Thomas, 1972).

In intact barnacle muscle fibers, the average strophanthidin-sensitive Na efflux is about $32 \text{ pmol/cm}^2 \cdot \text{s}$, and the strophanthidin-sensitive K influx is about $22 \text{ pmol/cm}^2 \cdot \text{s}$ (Brinley, 1968); thus, the ratio of pump-mediated Na extrusion to pump-mediated K entry (i.e., the coupling ratio) is approximately 3:2. With this coupling ratio and a pumped Na efflux of about $30 \text{ pmol/cm}^2 \cdot \text{s}$, the net pump current I_P , is about $1 \mu\text{A/cm}^2$ [$(1/3)(30 \text{ pmol/cm}^2)(F)$, where F = Faraday's constant]. Assuming that Na-K pumps behave like constant current generators (Abercrombie and De Weer, 1978), inhibition of the pumps will result in a voltage drop (ΔV_m) that is equal to the product of I_P and the membrane resistance, R_m ($= 1\text{--}3 \text{ K}\Omega\text{-cm}^2$ in barnacle muscle; Hagiwara et al., 1968). Thus, in barnacle muscle, ΔV_m should equal $I_P \times R_m$, or $(1 \mu\text{A/cm}^2) \times (1\text{--}3 \text{ K}\Omega\text{-cm}^2) = 1\text{--}3 \text{ mV}$. This calculated value for the contribution of the Na-K pumps to the membrane potential compares favorably with our experimentally-determined values (1.7–1.8 mV; see Table II).

Treatment of the fibers with DAP or Ba^{2+} (K channel blockers) did not affect Na efflux significantly, but did increase the ouabain-induced depolarization by two- to threefold (Table II). These findings are consistent with the idea that DAP and Ba^{2+} increase R_m by reducing g_K . With I_P unchanged and R_m increased, the electrogenic pump contribution to the membrane potential, ΔV_m ($= I_P \times R_m$) will increase, as we have, in fact, observed (Table II). In quantitative terms, to account for the observed two- to threefold increase in ΔV_m , DAP and Ba^{2+} must have increased R_m by two- to threefold. Since the resting membrane is permeable to K and Cl ions, primarily, with a $\text{K}^+:\text{Cl}^-$ conductance ratio ($g_K:g_{\text{Cl}}$) of $\sim 6\text{--}7:1$ at pH 7.7 (Hagiwara et al., 1968), our data imply that DAP and Ba^{2+} reduced g_K by about two-thirds.

The conclusion from these experiments is that the ouabain-induced depolarization in barnacle muscle is the result of inhibition of electrogenic pumps, and not the secondary consequence of substantial K accumulation in the cleft and tubule spaces.

In sum, our observations demonstrate that internally-perfused barnacle muscle fibers are well suited for transport studies. The sarcolemmal Na-K exchange pumps remain functional after many hours of perfusion, and we have been able to assess, quantitatively, some kinetic and electrogenic properties of these pumps. The internally perfused barnacle muscle preparation should also be useful for studies of a variety of other solute transport systems.

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