J. Membrane Biol. 185, 257–263 (2002)
DOI: 10.1007/s00232-001-0128-8

The Journal of
Membrane
Biology

Sodium Transport in Triads Isolated from Rabbit Skeletal Muscle

P. Donoso,[1] **C. Hidalgo**[1,2]
[1]Instituto de Ciencias Biomédicas, Facultad de Medicina, Universidad de Chile, Casilla 70005, Santiago 7, Chile
[2]Centro de Estudios Cientificos, Valdivia Chile

Received: 24 May 2001/Revised: 5 October 2001

Abstract. Triads and transverse tubules isolated from mammalian skeletal muscle actively accumulated Na^+ in the presence of K^+ and Mg-ATP. Active Na^+ transport exhibited a fast single-exponential phase, lasting 2 min, followed by slower linear uptake that continued for 10 minutes. Valinomycin stimulated Na^+ uptake, suggesting it decreased a pump-generated membrane potential gradient (V_m) that prevented further Na^+ accumulation. At the end of the fast uptake phase transverse tubule vesicles incubated in 30 mM external [Na^+] attained a ratio $[Na^+]_{in}/[Na^+]_{out} = 13.4$. From this ratio and the transverse tubule volume of 0.35 μl/mg protein measured in this work, $[Na^+]_{in} = 400$ mM was calculated. Determinations of active K^+ transport in triads, using $^{86}Rb^+$ as tracer, showed a 30% decrease in vesicular $^{86}Rb^+$ content two minutes after initiating the reaction, followed by a slower uptake phase during which vesicles regained their initial $^{86}Rb^+$ content after 10 minutes. Transverse tubule volume increase during active Na^+ transport—as shown by light scattering changes of isolated vesicles—presumably accounted for the secondary Na^+ and $^{86}Rb^+$ uptake phases. These combined results indicate that isolated triads have highly sealed transverse tubules that can be polarized effectively by the Na^+ pump through the generation of significant Na^+ gradients.

Key words: Sodium pump — Potassium transport — Active transport — Transverse tubules — Sarcoplasmic reticulum

Correspondence to: P. Donoso; email:pdonoso@machi.med.uchile.cl

Introduction

In skeletal muscle, the action potential generated at the endplate propagates along the surface of the fiber and through the transverse tubules (T-tubules). The transient depolarization of the T-tubule membranes opens the calcium release channels located on the terminal cisternae of the neighboring sarcoplasmic reticulum (SR). The ensuing increase in free [Ca^{2+}] produces contraction. The precise molecular mechanisms underlying the coupling between T-tubule depolarization and SR calcium release (Ríos & Pizarro, 1991), also known as excitation contraction coupling, are not fully understood at present.

Triad fractions isolated from skeletal muscle, formed by two sealed membrane fragments derived from SR terminal cisternae joined together through a sealed T-tubule vesicle, provide a suitable vesicular system to study the molecular events underlying excitation contraction coupling. The terminal cisternae can be loaded with calcium via the action of the SR Ca^{2+} pump and, simultaneously, the associated T-tubules can be repolarized through the action of the Na^+-K^+-ATPase. Subsequent depolarization of the repolarized T-tubules, by changing the extravesicular ionic composition, can induce calcium release in the millisecond time range (Ikemoto, Antoniu & Kang, 1992; Ikemoto et al., 1994; Yamaguchi, Igami & Kasai, 1997). Because calcium release is abrogated when an inhibitor of the Na^+ pump is included during active calcium-loading, in most of these studies the authors have assumed that T-tubules were effectively repolarized. Whereas the generation of a membrane potential by the Na^+ pump has been measured in triads following partition of a slowly equilibrating anion (Ikemoto et al., 1992), the actual Na^+ gradient generated by active transport has not been measured. The aim of this work was to investigate the Na^+ transport properties of T-tubules that form part of triads and to compare them with the

Na^+ transport activity of purified T-tubule vesicles (Lau et al., 1979).

Methods and Materials

Isolation of Membrane Fractions

Transverse tubules and triads were isolated from rabbit fast skeletal muscle as described elsewhere (Hidalgo, González & Lagos, 1983; Hidalgo et al., 1993).

Passive Na^+ Influx

Triads (0.5 mg/ml) or T-tubules (0.2 mg/ml) were incubated at 25°C in a solution containing (mM) 150 K-gluconate, 15 Na-gluconate, 0.1 $CaCl_2$, 5 $MgSO_4$, 1 DTT, 5 phosphoenolpyruvate(tris)sodium salt (Na_3PEP), 20 MOPS-Tris, pH 7.0. Either ^{24}Na or ^{22}Na were added as tracers to final specific activities of 1 mCi/mmol or 0.6 mCi/mmol, respectively. At different times, 50-μl fractions were rapidly filtered (Millipore, HA 0.45 μm) and washed with 5 ml of (mM) 150 K-gluconate, 15 Na-gluconate, 0.5 $MgSO_4$, 0.4 $CaCl_2$, 2 EGTA (to give pCa 7) and 20 MOPS-Tris, pH 7.0. The radioactivity remaining in the filters was determined in a liquid scintillation counter.

Active Na^+ Transport

To measure active Na^+ influx, 10 units/ml of pyruvate kinase (PK) and 5 mM ATP (Na_2ATP) were added to vesicles equilibrated with Na^+ and 50-μl fractions were filtered at different times and washed as above. The radioactivity remaining in the filters was determined in a liquid scintillation counter. To investigate the effect of chloride on Na^+ transport, Na-gluconate was replaced by an equivalent amount of NaCl. In some experiments, 2 μm valinomycin was added during active Na^+ transport to clamp the membrane potential to the K^+ equilibrium potential. To inhibit the Na^+ pump, digoxin was added to a final concentration of 1 mM from a concentrated stock ethanol solution. The final ethanol concentration was 0.01%. To dissipate rapidly the Na^+ gradient formed, vesicles actively loaded with Na^+ were diluted at 25°C in a solution at pCa 7 containing (mM) 150 K-gluconate, 0.47 $CaCl_2$,2 EGTA, 20 MOPS-Tris, pH 7.0 and 2 μM monensin, a Na^+ ionophore.

Passive Rb^+ Equilibration and Active Transport of Rb^+

We used $^{86}Rb^+$ as a tracer for K^+ (Sweadner & Goldin, 1975). Triads or T-tubule vesicles were incubated as for passive Na^+ equilibration or active Na^+ transport. The amount of $^{86}Rb^+$ taken up by the vesicles was measured by filtering 50-μl fractions at different times, as described above for Na^+ experiments.

Determination of the Intravesicular Volume of T-Tubule Vesicles

The intravesicular volume of T-tubule vesicles was determined by measuring the distribution volume at equilibrium of [^{14}C]-sucrose or ^{22}Na. Both radioactive labels equilibrated rather slowly across the T-tubule membrane, providing enough time to wash the vesicles in order to eliminate the extravesicular radioisotope without losing intravesicular label. The distribution volume at equilibrium of [^{14}C]-sucrose was determined at 25°C by incubating vesicles (0.25 mg/ml) for 2.5 hr in (mM): 150 KCl, 15 NaCl, 20 MOPS-Tris, pH 7.0, 10 μCi/ml of ^{14}C-sucrose (specific activity: 5 mCi/mmol). After reaching equilibrium, 0.1-ml fractions were rapidly filtered (Millipore, HA 0.45 μm) and washed 3 times with 3 ml of ice-cold non-radioactive solution. To determine the distribution volume of Na^+ at equilibrium, vesicles (0.36 mg/ml) were incubated for 3 hr at 25°C with 4 different concentrations of Na-gluconate and K-gluconate, such that Na-gluconate + K-gluconate = 150 mM, in 20 mM MOPS-Tris, pH 7.0, using $^{22}Na^+$ as tracer (specific activity: 1–137 (μCi/nmol). Samples (50 μl) were rapidly filtered in triplicate through Millipore filters (HA 0.45 μm) and were washed twice with 5 ml of ice-cold non radioactive solution. The radioactivity remaining in the vesicles was determined in a liquid scintillation counter.

Determination of Vesicular Volume Changes

Osmotically induced changes in T-tubular vesicle size during active Na^+ transport were monitored in a SX.18MV fluorescence stopped-flow spectrometer from Applied Photophysics Ltd. (Leatherhead, U.K.). One volume of T-tubule vesicles (1.0 mg/ml) equilibrated in (mM): 150 K-gluconate, 15 NaCl, 0.1 $CaCl_2$, 1 DTT, 20 MOPS-Tris, pH 7.0, was mixed with 10 volumes of three different solutions. For hypertonic or hypotonic conditions, vesicles were diluted in 0.75 M sucrose plus 20 mM MOPS-Tris, pH 7.0 or only in 20 mM MOPS-Tris, pH 7.0, respectively. To measure volume changes during active Na^+ transport, vesicles were diluted in a solution containing (mM): 5.5 Na_2ATP, 5.5 $MgSO_4$,150 K-gluconate, 15 NaCl, 5 Na_3PEP, 1 DTT, 20 MOPS-Tris, pH 7.0, plus 10 units/ml PK. The light scattered at 450 nm at a right angle to the incoming light beam was measured to follow volume changes as a function of time.

Materials

All reagents used were of analytical grade. Ionophores were obtained from Calbiochem (Layolla, CA) and protease inhibitors (Leupeptin, Pepstatin A, benzamidine and phenylmethylsulfonyl fluoride) were obtained from Sigma (St. Louis, MO). Both $^{24}Na^+$ and $^{86}Rb^+$ were obtained as chloride salts from the Comisión Chilena de Energía Nuclear, but in some experiments, $^{86}Rb^+$ obtained from DuPont-New England Nuclear was also used. All other radiochemicals were obtained from DuPont-New England Nuclear.

Results

Passive Na^+ Equilibration in Triads

In the presence of extravesicular [Na^+] = 30 mM, isolated triads reached at equilibrium a Na^+ content of 16.4 ± 1.4 nmol/mg protein, as illustrated in Fig. 1. The average value obtained from six independent determinations indicates that vesicles equilibrated with 17.7 ± 7.8 nmol Na^+/mg protein (mean ± SEM.). Equilibrium was reached with a halftime of 18 minutes at 25°C. Partial replacement of gluconate by chloride in the equilibration solution did not affect the amount of Na^+ passively equilibrated into triads, as illustrated in Table 1.

Table 1. Determination of passive and active Na^+ uptake in triad vesicles

	Gluconate	Chloride
Passive vesicular Na^+ content (nmol/mg)[#]	17.7 ± 7.8 (4)	14.4 ± 2.1 (3)
Net vesicular Na^+ increase (nmol/mg, after 2 min of transport)	23.5 ± 4.2 (6)	19.9 ± 1.3 (4)
Net vesicular Na^+ increase (nmol/mg, after 10 min of transport)	35.1 ± 6.9 (6)	31.4 ± 3.0 (4)
Active transport rate constant, min^{-1}	3.0 ± 1.6 (4)	2.0 ± 1.1 (6)

[#]For these determinations, vesicles were incubated in 30 mM external [Na^+]. Data are given as mean ± SEM. The number of determinations is given in parentheses. For further details, *see* Methods.

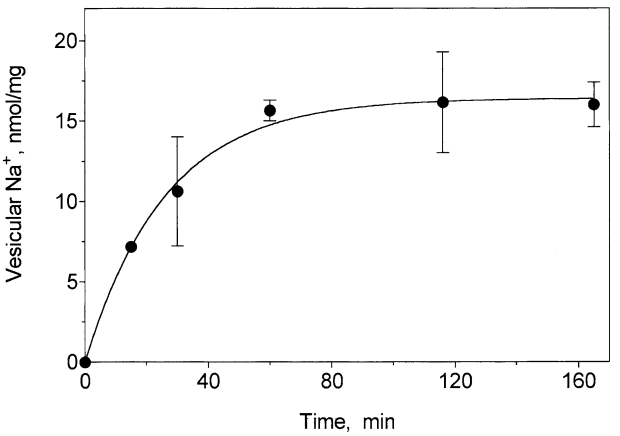

Fig. 1. Passive equilibration of Na^+ in triads. Triads (0.5 mg protein/ml) were incubated in (mM) 150 K-gluconate, 15 Na-gluconate, 0.1 $CaCl_2$, 5 $MgSO_4$, 1 DTT, 5 Na_3PEP and 20 MOPS-Tris, pH 7.0, using ^{24}Na as tracer. The amount of Na^+ remaining in the vesicles was determined as described in detail in the Methods section. Symbols represent mean ± SD of 4 determinations.

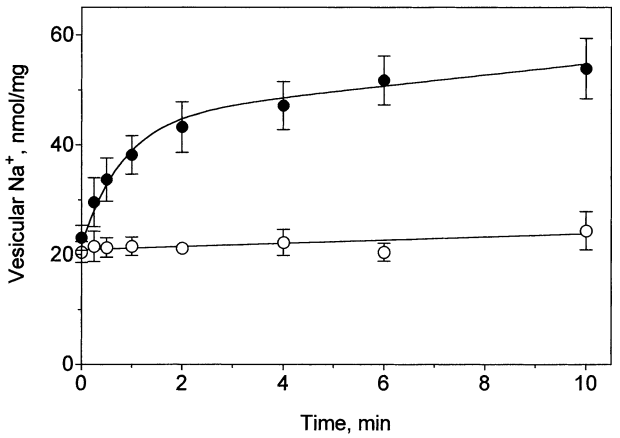

Fig. 2. Active Na^+ transport in triads. To vesicles pre-equilibrated with Na^+, 5 mM Na_2ATP and 10 Units/ml pyruvate kinase were added. The amount of Na^+ accumulated in the vesicles was determined as described in detail in the Methods section. Filled circles: control. Empty circles: 0.1 mM digoxin was added to the incubation solution.

Active Na^+ Transport in Triads

Addition of 5 mM ATP to triads equilibrated with Na^+ produced rapid accumulation of Na^+ inside the vesicles (Fig. 2). Active Na^+ uptake exhibited two phases: a fast single-exponential phase lasting 2 min followed by a slower linear phase that did not level off 10 min after initiating Na^+ uptake. In the experiment illustrated in Fig. 2, in the presence of gluconate, vesicles increased their Na^+ content from 22 to 45 nmol/mg protein during the fast uptake phase, actively accumulating 23 nmol Na^+/mg protein. The total amount of Na^+ taken up by the vesicles after 10 min was 55 ± 5.1 nmol/mg protein.

Addition of digoxin produced complete inhibition of ATP-dependent Na^+ transport, as illustrated in Fig. 2. After 10 min in the presence of digoxin, vesicles had only 24.2 ± 3.4 nmol/mg protein, a value not significantly different from that obtained for passive Na^+ equilibration in the triads given in Table 1.

Following 20-fold dilution of triad vesicles, actively loaded with Na^+ for 10 min, in a solution containing 2 μM of the Na^+ ionophore monensin plus (mM) 150 K-gluconate, 15 Na-gluconate, 0.47 $CaCl_2$, 2 EGTA (pCa 7), 20 MOPS-Tris, pH 7.0, rapid loss of vesicular Na^+ was observed. Thus, 15 sec after dilution in the solution containing monensin, luminal Na^+ decreased to zero, indicating that the pump effectively transported Na^+ to the vesicular lumen (*data not shown*).

Partial replacement of 15 mM gluconate by 15 mM chloride in the transport solution did not change significantly the amount of Na^+ taken up during the fast or the slow phase, or the rate of active transport (Table 1).

Effect of Valinomycin on Active Na^+ Uptake

Addition of valinomycin produced a substantial increase in the amount of Na^+ actively taken up by triads. As illustrated in Fig. 3, valinomycin stimulation of Na^+ transport in triads was evident after the first minute of uptake and produced a 2-fold increase in the total amount of Na^+ taken up after 10 minutes, from 46 to 98 nmol Na^+/mg protein.

Sodium Transport in T-Tubules

Figure 4 shows that active Na^+ transport into T-tubule vesicles exhibited the same kinetic behavior as observed in triads. A fast phase was observed, during which

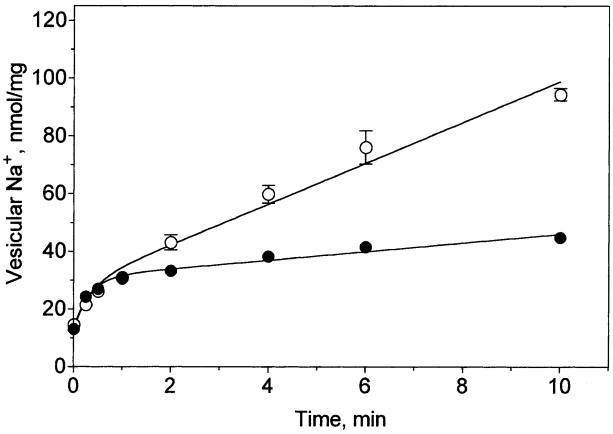

Fig. 3. Effect of valinomycin on active Na^+ transport in triads. Vesicles (0.5 mg protein/ml) were incubated in (mM) 150 K-gluconate, 15 NaCl with ^{24}Na added as tracer, 0.1 $CaCl_2$, 5 $MgSO_4$, 1 DTT, 5 Na_3PEP, 5 Na_2ATP, 20 MOPS-Tris, pH 7.0, plus 10 Units/ml of pyruvate kinase. Filled symbols: control. Empty symbols: plus 2 μM valinomycin.

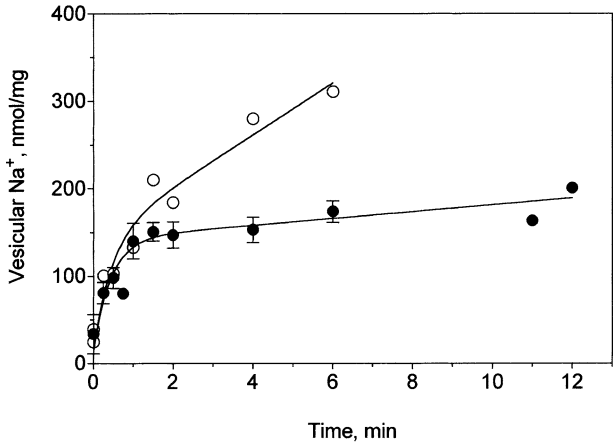

Fig. 4. Active Na^+ transport in T-tubules. Vesicles (0.2 mg protein/ml) were incubated in (mM): 150 K-gluconate, 15 Na-gluconate witli ^{24}Na added as tracer, 0.1 $CaCl_2$, 5 $MgSO_4$, 1 DTT, 5 Na_3PEP, 5 Na_2ATP, 20 MOPS-Tris, pH 7.0, plus 10 Units/ml pyruvate kinase. Filled symbols: control. Empty symbols: plus 2 μM valinomycin.

128 nmol of Na^+ per mg of protein were transported into the vesicles, followed by a slower linear phase that lasted the 12 min of the experiment. At the end of the slower phase, close to 200 nmol of Na^+ per mg protein had been taken into the T-tubule vesicles (Fig. 4). Valinomycin did not change the amount of Na^+ taken up during the fast phase and again, as in triads, it produced a significant stimulation of uptake during the slower phase, increasing 2-fold the amount of Na^+ taken up in 6 minutes, from 174 to 320 nmol Na^+/mg protein. As in triads, digoxin completely abolished active Na^+ transport in T-tubules (*not shown*).

Potassium Transport

We measured the changes in intravesicular K^+ concentration during active Na^+ transport using $^{86}Rb^+$ as tracer (*see* Methods). The halftime for passive equilibration of $^{86}Rb^+$ into triads, 11.3 min (Fig. 5), was significantly shorter than the halftime for the passive equilibration of Na^+. Upon addition of 5 mm ATP, the intravesicular $^{86}Rb^+$ content decreased 30% during the first 2.5 min of active transport (Fig. 6). At longer times, however, there was a reversion of this efflux and 10 minutes after ATP addition, the vesicular $^{86}Rb^+$ content was equal to the amount present before ATP addition. The time course of the $^{86}Rb^+$ -efflux phase was coincident with the fast phase of Na^+ uptake in triads and T-tubules (Figs. 2, 3 and 4) while the subsequent period of slow uptake of $^{86}Rb^+$ followed the same time course as the slow Na^+ uptake phase.

Determination of the Intravesicular Volume of T-tubule Vesicles

Under basal conditions, i.e., before initiating active Na^+ transport, we determined the intravesicular volume of T-tubule vesicles. An average value of 0.35 ± 0.03 μl/mg protein was obtained, as shown in Table 2. This value was obtained from [^{14}C]-sucrose-distribution volume at equilibrium (0.35 ± 0.04 μl/mg) and from the average value of the distribution volume of four different Na^+ concentrations under passive equilibrium conditions (0.35 ± 0.02 μl/mg).

Intravesicular Volume Changes During Active Transport

To detect volume changes during active Na^+ transport, we measured the light-scattering properties of T-tubule vesicles as a function of time, after initiating active transport by rapidly mixing vesicles with an ATP-containing solution. As illustrated in Fig. 7, light scattering remained relatively constant during the initial 2-min period after ATP addition. After this time, there was a steady decrease in the light scattered, indicating an increase in vesicular volume at the same time scale as the slow increase in Na^+ accumulation depicted in Fig. 4. This change in light scattering was not modified by addition of 2 μm valinomycin to the vesicle-containing solution (*data not shown*).

Discussion

Sodium-Gradient Generation by the Na^+-K^+-ATPase

Depolarization-induced calcium release in isolated triads requires prior repolarization of their T-tubule component to remove voltage-sensor inactivation (Rios & Pizarro, 1991). Due to the electrogenic nature of the Na^+-K^+-ATPase, a membrane potential

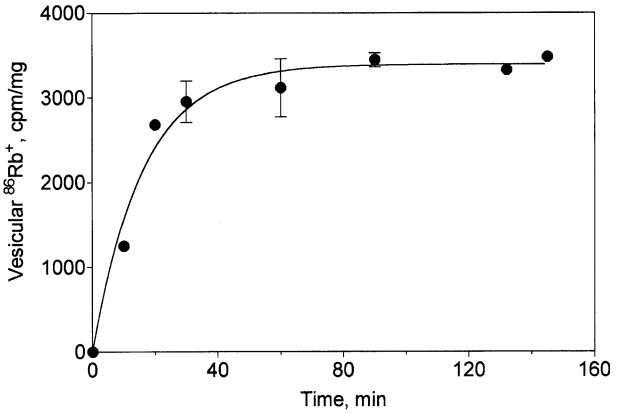

Fig. 5. Time course of passive equilibration $^{86}Rb^+$ in triads. Vesicles (0.5 mg protein/ml) were incubated in (mM) 150 K-gluconate plus $^{86}Rb^+$ added as tracer, 15 NaCl, 0.1 $CaCl_2$, 5 $MgSO_4$, 1 DTT, 5 Na_3PEP, 5 Na_2ATP, 20 MOPS-Tris, pH 7.0, plus 10 Units/ml pyruvate kinase. Radioactivity associated to the vesicles was determined as detailed in Methods.

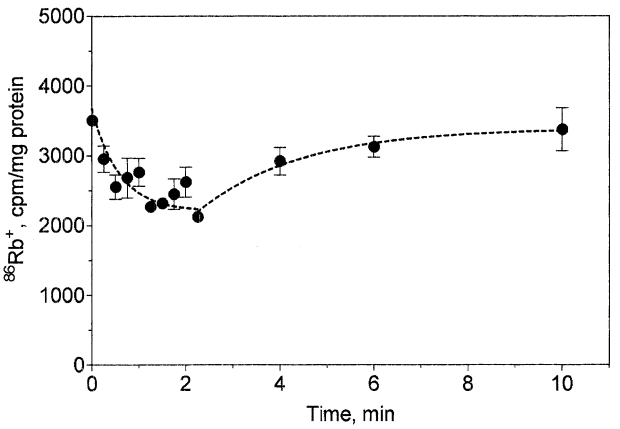

Fig. 6. Changes in triad $^{86}Rb^+$ content after ATP addition. To vesicles equilibrated with $^{86}Rb^+$, 5 mM Na_2ATP and 10 Units/ml pyruvate kinase were added. The amount of $^{86}Rb^+$ remaining in the vesicles was determined as detailed in Methods.

Table 2. Determination of Transverse Tubule Intravesicular Volume

Radioactive Tracer	mM	Distribution volume (μl/mg protein)	*n*
[^{14}C]-sucrose	2.0	0.35 ± 0.04	5
$^{22}Na^+$	1.0	0.36 ± 0.01*	2
$^{22}Na^+$	10.0	0.33 ± 0.13	3
$^{22}Na^+$	50.0	0.36 ± 0.12	3
$^{22}Na^+$	150.0	0.33 ± 0.11	3
$^{22}Na^+$	average	0.35 ± 0.02	4

Data are given as Mean ± SEM, except in the value indicated with an *, where the range is given. For further details, *see* Methods.

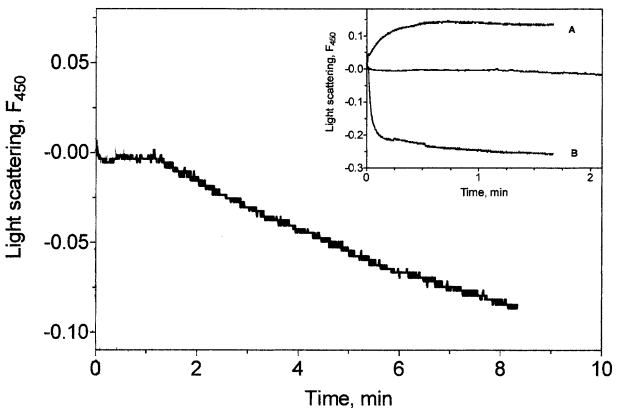

Fig. 7. Changes in light scattering during active Na^+ transport in T-tubule vesicles. Vesicles (1 mg protein/ml) were equilibrated in (mM) 150 K-gluconate, 15 NaCl, 0.1 $CaCl_2$ 1 DTT, 5.5 $MgSO_4$ and 20 MOPS-Tris, pH 7.0. At time zero vesicles were mixed with 10 volumes of (mM): 150 K-gluconate, 15 NaCl, 5.5 Na_2ATP, 5 Na_3PEP, 1 DTT, 20 MOPS-Tris, pH 7.0, plus 10 Units/ml pyruvate kinase. The light scattered at 450 nm at a right angle to the incoming light beam was measured. *Inset*: Vesicles were also mixed with *A*, a hypertonic solution containing 0.75 M sucrose plus 20 mM MOPS-Tris, pH 7.0, or *B*, a hypotonic solution of 20 mM MOPS-Tris, pH 7.0, as indicated in the Figure. For further details, *see* Methods.

will develop during active Na^+ transport (Goldshlegger et al., 1987). The Na^+ pump has been used to restore the resting polarization state of T-tubule vesicles (Ikemoto et al., 1992; Ikemoto et al., 1994; Yamaguchi et al., 1997), but the actual Na^+-concentration gradient generated by the pump in free or triad-attached T-tubules has not been measured. In this work, we found that both triads and T-tubules exhibited significant Na^+ pump activity. T-Tubules represent about 15 percent of the total membrane protein present in triads, as judged by Na^+-K^+-ATPase activity and ouabain and nitrendipine binding site-density measurements (*not shown*). In agreement with this fractional composition, triads were less efficient in actively transporting Na^+ than T-tubules, and transported within 2 min on average 23 nmol Na^+/mg of protein. This value is 5.6-fold lower than the 128 nmol Na^+/mg transported by T-tubules.

Effect of Valinomycin on the Generation of a Sodium Gradient by the Na^+-K^+-ATPase

Successful polarization depends not only on the activity of the Na^+-K^+-ATPase but also on the permeability of the T-tubular membrane to Na^+, K^+ and other ions that may dissipate the Na^+ gradient generated by the pump. A large component of the triadic membranes used in this work, 85–90%, is derived from terminal cisternae membranes, which are devoid of Na^+-K^+-ATPase and have a high permeability to Na^+, K^+ and Cl^- (Kometani & Kasai,

1978, García & Miller, 1978). In contrast, the isolated T-tubular component of triads has significant Na^+-K^+-ATPase activity (Hidalgo et al., 1986) with a high density of [^{3}H]-ouabain-binding sites of 170 pmol/mg (Jaimovich et al., 1986), and low permeability to Na^+ and K^+ (Sabbadini & Dhams, 1989; Donoso & Hidalgo, 1989; Hidalgo, Cifuentes & Donoso, 1991), as confirmed in this work. The present results also indicate that isolated T-tubule membranes have low chloride permeability, because Na^+ transport kinetics did not change by partial replacement of gluconate for chloride. These results indicate that isolated T-tubules did not retain the high chloride permeability they exhibit in mammalian (rat) muscle fibers (Dulhunty, 1979). This low permeability to monovalent ions should increase the efficiency of membrane-potential generation by the Na^+-K^+-ATPase of free or triad-attached isolated T-tubule vesicles. In this regard, the T-tubule Na^+-pump resembles the purified Na^+-K^+-ATPase reconstituted in phospholipid vesicles, where membrane potentials as high as 200 mV, inside positive, develop a few seconds after activation of the pump (Goldshlegger et al., 1987).

The buildup of a membrane potential, inside positive, would prevent further uptake of Na^+ in vesicles lacking significant permeability pathways for other ions, thus limiting the maximal Na^+ gradient attainable. In the absence of other significant ionic permeabilities, valinomycin, by making membranes highly permeable to K^+, should establish a membrane potential V_m equal to the K^+ equilibrium potential E_K. Thus, if the membrane potential difference generated by the pump is higher than E_K, in tightly sealed vesicles valinomycin should stimulate active Na^+ transport by decreasing V_m to E_K. The results obtained in this work show that valinomycin increased Na^+ uptake in triads and T-tubules. These results 1) confirm that T-tubule vesicles have a low basal K^+ permeability and 2) suggest that valinomycin, by setting V_m at E_K, decreased the membrane-potential difference generated by the pump, allowing further Na^+ uptake. These results contrast with previous reports showing that valinomycin does not affect Na^+ transport in T- tubules (Lau et al., 1979); however, in contrast to the T-tubules used in this work, T-tubules dissociated from triads by the French-press procedure have high chloride permeability. Hence, the French-press isolation procedure may produce T-tubule vesicles with increased K^+ permeability as well, a feature that would explain the lack of stimulation of Na^+- pump activity by valinomycin (Lau et al., 1979).

Volume Changes During Active Na^+ Transport

The intravesicular volume of T-tubules determined in this work is significantly lower than the intravesicular volume of SR vesicles, where values in the range of 2 μl/mg protein have been reported (Duggan & Martonosi, 1970; Donoso, Prieto & Hidalgo, 1995). The T-tubule vesicles did not change their volume during the first two minutes of active Na^+ uptake. After this time, vesicles exhibited a delayed increase in volume that did not level off after 8 min. This volume increase may explain the delayed component of Na^+ and $^{86}Rb^+$ uptake, because this component may simply reflect the increase in vesicular volume and not a net increase in intravesicular ion concentration. The increase in vesicular volume may be caused by the increase in intravesicular osmotic pressure caused by net Na^+ uptake, as suggested by Lau et al. (1978). Volume changes were not affected by 2 μM valinomycin, indicating that the changes in vesicular volume were not affected by increasing T-tubule K^+ permeability.

In summary, the results of this work indicate that isolated triads posses highly sealed T-tubules with low permeability to $K^+$and chloride ions that, through the generation of significant Na^+ gradients, can be polarized effectively by their endogenous Na^+ pump. These properties of triad-attached T-tubules provide a functional basis to support successful depolarization-induced calcium release in isolated triads.

The authors wish to thank Dr. José Cavieres for his helpful suggestions regarding this work. This study was supported by Fondo Nacional de Investigación Cientifica y Tecnológica grant 8980009. The institutional support to the Centro de Estudios Científicos (CECS) by Fuerza Aérea de Chile, AFP Provida, Empresas CMPC, Codelco and Telefónica del Sur is also acknowledged. CECS is a Millenium Science Institute.

References

Donoso, P., Hidalgo, C. 1989. Sodium-calcium exchange in transverse tubule vesicles isolated from frog skeletal muscle. *Biochim. Biophys. Acta* **978:**8–16

Donoso, P., Prieto, H., Hidalgo, C. 1995. Luminal calcium regulates calcium release in triads isolated from frog and rabbit skeletal muscle. *Bioghys. J.* **68:**507–515

Dulhunty, A.F. 1979. Distribution of potassium and chloride permeability over the surface and T-tubule membranes of mammalian skeletal muscle. *J. Membrane Biol.* **45:**293–310

Duggan, P.F., Martonosi, A. 1970. The permeability of sarcoplasmic reticulum membranes. *J. Gen. Physiol.* **56:**147–167

García, A.M., Miller, C. 1984. Channel-mediated monovalent cation fluxes in isolated sarcoplasmic reticulum vesicles. *J. Gen. Physiol.* **83:**819–839

Goldshlegger, R., Karlish, S.J.D., Rephaeli, A., Stein, W.D. 1987. The effect of membrane potential on the mammalian sodium-potassium pump reconstituted into phospholipid vesicles. *J. Physiol.* **387:**331–335

Hidalgo, C., González, M.E., Lagos, R. 1983. Characterization of the Ca^{2+} - or Mg^{2+}-ATPase of Transverse Tubule Membranes Isolated from Rabbit Skeletal Muscle. *J. Biol. Chem.* **258:**13937–13945

Hidalgo, C., Parra, C., Riquelme, G., Jaimovich, E. 1986. Transverse tubules from frog skeletal muscle. Purification and properties of vesicles sealed with the inside out orientation. *Biochim. Biophys. Acta*. **855:**79–88

Hidalgo, C., Cifuentes, F., Donoso, P. 1991. Sodium/Calcium exchange in transverse tubule vesicles isolated from amphibian skeletal muscle. *Ann. N. Y. Acad. Sci*. **639:**483–497

Hidalgo, C., Jorquera, J., Tapia, V., Donoso, P. 1993. Triads and transverse tubules isolated from skeletal muscle contain high levels of inositol 1,4,5-trisphosphate. *J. Biol. Chem*. **268:**1511–1517

Ikemoto, N., Antoniu, B., Kang, J. 1992. Characterization of ''depolarization'' induced calcium release from sarcoplasmic reticulum in vitro with the use of membrane potential probe. *Biochem. Biophys. Res. Commun*. **184:**538–543

Ikemoto, N., Yano, M., El-Hayek, R., Antoniu, B., Morii, M. 1994. Chemical depolarization-induced SR calcium release in triads isolated from rabbit skeletal muscle. *Biochemistry* **33:** 10961–10968

Jaimovich, E., Donoso, P., Liberona, J.L., Hidalgo, C. 1986. Ion pathways in transverse tubules. Quantification of receptors in membranes isolated from frog and rabbit skeletal muscle. *Biochim. Biophys. Acta* **855:**89–98

Kometani, T., Kasai, M. 1978. Ionic permeability of sarcoplasmic reticulum vesicles measured by light scattering method. *J. Membrane. Biol*. **41:**295–308

Lau, Y.H., Caswell, A.H., García, M., Letellier, L. 1979. Ouabain binding and coupled sodium, potassium, and chloride transport in isolated transverse tubules of skeletal muscle. *J. Gen. Physiol*. **74:**335–349

Ríos, E, Pizarro. G. 1991. Voltage sensor of excitation-contraction coupling in skeletal muscle. *Physiol. Rev*. **71:**849–908

Sabbadini, R.A., Dahms, A.S. 1989. Biochemical properties of isolated transverse tubular membranes. *J. Bioenerg. Biomemb*. **21:**163–213

Sweadner, K.J, Goldin, S.M. 1975. Reconstitution of active ion transport by the sodium and potassium ion-stimulated adenosine triphosphatase from canine brain. *J. Biol. Chem*. **250:**4022–4028

Yamaguchi, N., Igami, K., Kasai, M. 1997. Kinetics of depolarization-induced calcium release from skeletal muscle triads in vitro. *J. Biochem*. **121:**432–439