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PERMEABILITY OF RECONSTITUTED SARCOPLASMIC RETICULUM VESICLES

RECONSTITUTION OF THE K^+, Na^+ CHANNEL

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Summary

Permeability properties of reconstituted rabbit skeletal muscle sarcoplasmic reticulum vesicles were characterized by measuring efflux rates of [^{3}H]inulin, [^{3}H]choline$^+$, $^{86}Rb^+$, and $^{22}Na^+$, as well as membrane potential changes using the voltage-sensitive probe, 3,3′-dipentyl-2,2′-oxacarbocyanine. Native vesicles were dissociated with deoxycholate and were reconstituted by dialysis. Energized Ca^{2+} accumulation was partially restored. About 1/2 of the reconstituted vesicles were found to be 'leaky', i.e., permeable to choline$^+$ or Tris$^+$ but not to inulin. The remaining reconstituted vesicles were 'sealed', i.e., impermeable to choline$^+$, Tris$^+$ and inulin. Sealed reconstituted vesicles could be further subdivided according to their K^+, Na^+ permeability. About 1/2, previously designated Type I, were readily permeable to K^+ and Na^+, indicating the presence of the K^+, Na^+ channel of sarcoplasmic reticulum. The remaining sealed vesicles (Type II) formed a permeability barrier to K^+ and Na^+, suggesting that they lacked the K^+, Na^+ channel. These studies show that the K^+, Na^+ channel of sarcoplasmic reticulum can be solubilized with detergent and reconstituted with retention of activity. Furthermore, our results suggest that part or all of the decreased Ca^{2+}-loading efficiency of reconstituted vesicles may be due to the presence of a significant fraction of leaky vesicles.

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Abbreviations: Hepes, *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid; EGTA, ethylene glycol bis(β-aminoethyl ether)-*N*,*N*′-tetraacetic acid; Pipes, 1,4-piperazinediethanesulfonic acid.

Introduction

The sarcoplasmic reticulum membrane of skeletal muscle is an ideal model for analyzing the molecular mechanisms of ion transport because of its relatively simple structure, function and composition, as well as the ease with which large amounts of material can be obtained in high purity. A membrane-bound, Mg^{2+}-dependent, Ca^{2+}-stimulated ATPase (Ca^{2+}-ATPase), the Ca^{2+}-transport protein of sarcoplasmic reticulum, accounts for up to 90% of the total membrane protein. The Ca^{2+}-ATPase has been purified and reconstituted into Ca^{2+}-transporting vesicles, and its structure, partial reaction steps and interaction with membrane lipid have been extensively studied (for reviews, see Refs. 1 and 2).

Recently, independent evidence has been obtained for a K^+, Na^+ permeable channel by experiments using isotope exchange and membrane potential techniques on isolated native sarcoplasmic reticulum vesicles [3,4] and experiments in which vesicles were fused with planar lipid bilayers [5]. In the bilayers, the channel displayed a voltage-dependent K^+ conductance. Sarcoplasmic reticulum provides us, therefore, with the opportunity to isolate and characterize the molecular properties of a biological ion channel.

In this paper we have investigated the permeability properties of reconstituted sarcoplasmic reticulum vesicles. We found that the K^+, Na^+ channel, like the Ca^{2+}-ATPase [6], can be solubilized with deoxycholate and reconstituted into membranous vesicles by removing detergent by dialysis.

Materials and Methods

Reagents. The fluorescent dye, 3,3′-dipentyl-2,2′-oxacarbocyanine (diO-C_5-(3)), was the generous gift of Dr. Alan Waggoner (Amherst College, Amherst, MA). The dye, Antipyrylazo III (ICN Pharmaceutical, Plainview, NY), was used without further purification. Other reagents used were reagent grade.

Preparation of native sarcoplasmic reticulum vesicles. Sarcoplasmic reticulum vesicles used in this study have been characterized previously [7]. Vesicles of 'intermediate' bouyant density were prepared by zonal centrifugation of rabbit skeletal muscle homogenates and were subsequently washed in 0.45 M KCl, 15% sucrose for 1 h at 0°C.

Solubilization and reconstitution of sarcoplasmic reticulum vesicles. Sarcoplasmic reticulum vesicles were solubilized and reconstituted using the reconstitution method of Meissner and Fleischer [6]. Vesicles (7 mg protein/ml) were suspended at 0°C in 1 ml of a 10 mM Tris-HCl buffer, pH 7.9, containing 15% (w/v) glycerol, 0.4 M KCl, 1.5 mM Mg^{2+}, 1 mM EDTA, and 0.1 mM Ca^{2+}. Vesicles were rapidly solubilized by the addition of 50—60 μl of 7.25% deoxycholate. The detergent concentration used resulted in a nearly, but not completely, clear solution. Remaining membranous fragments were removed by centrifugation for 90 min at 200 000 × *g*. An aliquot of 0.8 ml of the resulting clear supernatant (5—6 mg protein/ml) was placed into a dialysis bag and dialyzed at 15—20°C for 24 h against 1 l of a 2.5 mM Hepes buffer, pH 7.3, containing 20% (w/v) glycerol, 0.4 M KCl, 1.5 mM Mg^{2+}, 1 mM EDTA

and 0.1 mM Ca^{2+}. To reduce further the deoxycholate content as well as to fill the vesicles with potassium in the presence of a membrane-impermeant anion (see below), reconstituted vesicles were diluted with 30 ml of 440 mosM K^+-Pipes or K^+-gluconate buffer, pH 7.0, and incubated for 3—5 h on ice, unless otherwise indicated. After centrifugation for 45 min at 135 000 × *g*, pellets were resuspended in the above incubation medium at a protein concentration of about 10 mg/ml. In a control, vesicles were solubilized with deoxy[^{14}C]cholate (10^5 cpm/mg deoxycholate) and analyzed after reconstitution to confirm that greater than 99% of the detergent was removed by the above procedure. Vesicles were stored at —65° C before use.

Assays. Protein was estimated by using the method of Lowry et al. [8] using bovine serum albumin as a standard.

Apparent isotope spaces and efflux rates of reconstituted vesicles to [^{3}H]-choline$^+$, $^{22}Na^+$ and $^{86}Rb^+$ were determined by Millipore filtration as previously described [9]. Membrane potentials were generated by dilution of vesicles containing permeant ions into media of the same osmolarity but containing differing concentrations of the permeant ions. The potentials (negative inside) were visualized with the use of the fluorescent dye diO-C_5-(3) [10] as previously described [4]. The sign of the membrane potential is reported according to standard convention, i.e., reference (ground) is extravesicular. Fluorescence assays were carried out at 15° C under stirring in a Farrand Model 801 Fluorometer. Excitation was at 470 nm and emission was recorded at 495 nm. Slits used resulted in a half-band width of 2.5 nm. Vesicle concentrations (approx. 15 μg protein/ml or 15 nmol phospholipid/ml) were used which produced negligible perturbation of the fluorescence emission during dilution with incubation medium.

Ca^{2+}-loading rates were measured at 20° C with a GCA/McPherson spectrophotometer using the dye Antipyrylazo III for the determination of free Ca^{2+}. A buffer solution containing 10 mM Hepes, pH 7.3, 0.1 M KCl, 5 mM $MgCl_2$, 5 mM oxalate, 3 mM ATP and 0.1 mM Antipyrylazo III was added to sample and reference cuvettes. Ca^{2+} was added to the sample cell at a final concentration of 100 μM and the change in absorbance at 651 nm was recorded. The sample (10—40 μg protein/3 ml) was rapidly added to the sample cell and the decrease in absorbance at 651 nm was monitored as a function of time.

Ca^{2+}-stimulated ATPase activity was determined at 20° C in a buffer solution containing 10 mM Hepes, pH 7.3, 0.1 M KCl, 5 mM $MgCl_2$, 5 mM oxalate, 3 mM ATP and 0.1 mM $CaCl_2$. Ca^{2+}-stimulated ATPase activity was corrected for 'basic' ATPase activity which was measured under identical conditions except that 1 mM EGTA instead of 0.1 mM Ca^{2+} was added to the buffer. ATPase activities were measured at 1, 2 and 3 min. The enzyme concentrations used resulted in less than 10% hydrolysis of ATP. P_i was determined by using the procedure of Fiske and Subbarow [11] with Elon as a reducing agent.

Results

Ca^{2+}-loading efficiency

Purified sarcoplasmic reticulum vesicles were solubilized with deoxycholate

TABLE I

ENZYMATIC PROPERTIES OF NATIVE AND RECONSTITUTED SARCOPLASMIC RETICULUM VESICLES

Ca^{2+} loading and Ca^{2+}-ATPase activities were determined in the presence of 5 mM oxalate at 20°C as described in Materials and Methods. The data are the average of four preparations. Standard errors were ±25% or less.

	Sarcoplasmic reticulum vesicles	
	Native	Reconstituted
Ca^{2+}-loading capacity (μmol Ca^{2+}/mg protein)	7.5	4.2
Ca^{2+}-loading rate (μmol Ca^{2+}/mg protein per min)	2.1	0.7
Ca^{2+}-ATPase (μmol P_i/mg protein per min)	1.35	1.4
Ca^{2+}-loading efficiency (Ca^{2+} : ATP)	1.55	0.5

and centrifuged at 200 000 × *g* for 90 min to remove small amounts of remaining insoluble material. Previous studies have indicated that the centrifuged solubilized sample is non-membranous as judged by electron microscopy and contains the Ca^{2+}-ATPase in a low aggregation state, most likely in the form of monomers, dimers and/or tetramers [6,12]. Removal of the detergent by dialysis was previously found to result in reformation of membranous vesicles which consist mainly of the Ca^{2+}-ATPase (greater than 90% of the protein) and phospholipid and resemble native vesicles in size [6]. Reconstituted vesicles were again capable of accumulating Ca^{2+} in the presence of ATP (Table I), as previously reported [6,13].

The Ca^{2+}-loading efficiency of the vesicles was approximated by determining rates of initial Ca^{2+} loading and ATP hydrolysis. Both activities were determined under identical conditions in the presence of 5 mM oxalate. The Ca^{2+}-precipitating agent, oxalate, was added to increase the Ca^{2+}-transporting capacity of the vesicle [1]. Reconstituted vesicles accumulated Ca^{2+} at a slower rate than native vesicles, yet both vesicle preparations had similar Ca^{2+}-dependent ATPase activities (Table I). Native vesicles took up about 1.5 Ca^{2+} per ATP hydrolyzed, in reasonable agreement with a value of 2 determined by Weber et al. [14]. The lower Ca^{2+}-loading efficiency of about 0.5 for reconstituted vesicles was most likely in part due to the presence of leaky vesicles, as discussed below.

Isotope efflux measurements

The integrity of the reconstituted vesicles was studied by determining the ability of the vesicles to retain various ions and by comparing the rates of ion movement across the membranes of native and reconstituted vesicles. Whether Rb^+ and Na^+ could be retained by vesicles was used as an indication of the presence or absence of the K^+, Na^+ channel and was quantitated by the intravesicular space. [^{3}H]Choline$^+$, $^{86}Rb^+$ and $^{22}Na^+$ efflux measurements using Millipore filtration revealed similar rates of ion transport for native and reconstituted vesicles (Fig. 1), indicating that reconstituted vesicles have restored normal permeability properties.

Native vesicles can be divided into those which contain the Na^+, K^+ channel

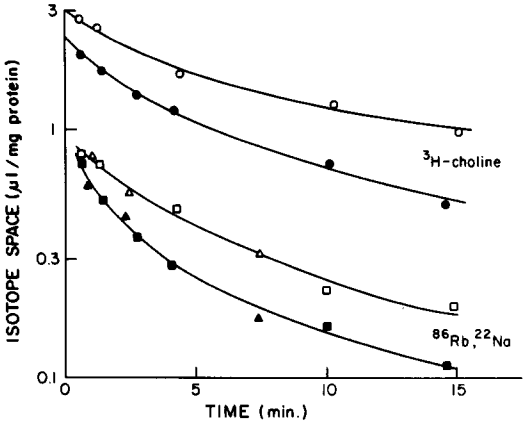

Fig. 1. Measurement of [^{3}H]choline$^+$, $^{22}Na^+$ and $^{86}Rb^+$ ion efflux rates and isotope spaces. Native (open symbols) or reconstituted (closed symbols) sarcoplasmic reticulum vesicles (approx. 10 mg protein/ml) were incubated in the following medium with [^{3}H]choline$^+$ and either $^{86}Rb^+$ (□, ■) or $^{22}Na^+$ (△, ▲) added: 0.3 M sucrose, 25 mM choline chloride, 25 mM RbCl, and 25 mM Na^+-Pipes, pH 7. After incubation for 6 or 16 h at 0°C, vesicles were diluted 100-fold at 0°C into unlabeled incubation medium. 1 ml aliquots were collected on 0.45 μm Millipore filters and rinsed. Radioactivity remaining with the vesicles on the filters was determined in a liquid scintillation counter [9].

(Type I) and those that do not (Type II) by their permeability. [^{3}H]Choline$^+$ does not pass through the channel, thus it gives a measure of the entire vesicle population. As previously reported [3,4], native sarcoplasmic reticulum enclosed an apparent [^{3}H]choline$^+$ space of 3 μl/mg protein (Table II). In the

TABLE II

APPARENT ISOTOPE SPACES AND FLUORESCENCE SIGNALS OF NATIVE AND RECONSTITUTED SARCOPLASMIC RETICULUM VESICLES

The inulin space of reconstituted vesicles was obtained by adding [^{3}H]inulin (10^5 cpm/ml) to the solubilized sample and dialysis buffer. After dialysis for 24 h, 25-μl aliquots were placed on 0.45 μm Millipore filters and rinsed with dialysis buffer for 10, 30 or 60 s. Similar amounts of radioactivity remained with the filters. [^{3}H]Choline$^+$, $^{86}Rb^+$ and $^{22}Na^+$ isotope spaces extrapolated to zero time were determined as described in the legend of Fig. 1. Fluorescence assays were performed as described in Fig. 2 using Tris-gluconate or Na^+-gluconate (+valinomycin) dilution media. Fluorescence decreases extrapolated back to the time of vesicle addition are indicated. The data are the average of four preparations. Standard errors were ±20% for radioactive isotope efflux measurements and ±15% for fluorescence measurements.

	Sarcoplasmic reticulum vesicles	
	Native	Reconstituted
Radioactive isotope	Space (μl/mg protein)	
[^{3}H]inulin	—	4.2
$^{45}Ca^{2+}$	5.5	3.2
[^{3}H]choline$^+$	3.0	2.3
$^{86}Rb^+$, $^{22}Na^+$	0.95	1.05
Type I/Type I + II	0.68	0.54
	% fluorescence decrease	
Type I	29	18
Type II	18	14
Type I/Type I + II	0.62	0.56

presence of $^{22}Na^+$ or $^{86}Rb^+$, the smaller type II vesicle space (0.95 μl/mg protein) was quantitated; Type I vesicles were not observed due to the rapid intravesicular loss of these ions through the channel.

In studying the properties of reconstituted vesicles, [^{3}H]inulin could be used in addition to the above ions, since it can be incorporated during membrane reconstitution whereas it essentially does not permeate a native vesicle. The apparent [^{3}H]inulin space of reconstituted vesicles ranged between 4 and 5 μl/mg protein (Table II). Essentially the same space was obtained when the specific activity of [^{3}H]inulin was lowered 10-fold by the addition of unlabeled inulin. In contrast, the apparent space of [^{3}H]choline$^+$ was only 2—2.5 μl/mg protein, suggesting that a significant portion (equal to 50%) of reconstituted vesicles was permeable to choline$^+$ but not inulin. These vesicles are designated leaky, while the remaining population that retained both choline$^+$ and inulin is referrred to as sealed vesicles. Of the sealed vesicle population, the $^{86}Rb^+$ and Na^+ space was half the [^{3}H]choline$^+$ space (Table II), suggesting that half of these vesicles contained the K^+, Na^+ channel, as supported below. In control experiments, it was found that leakiness of reconstituted vesicles was most likely not due to the small amounts of deoxycholate (1—3 μg/mg protein) remaining with the vesicles. Addition of an excess of deoxycholate to native vesicles (20 μg/mg protein) did not significantly affect their permeability to [^{3}H]choline$^+$ and $^{22}Na^+$.

Another relevant ion in comparing native and reconstituted membrane properties in $^{45}Ca^{2+}$. Its rate of efflux was similar to that of choline$^+$ in both membranes (not shown). Ca^{2+}, however, was retained by native and reconstituted vesicles at a level greater than choline$^+$ (Table II). The presence of sarcoplasmic reticulum membranes displaying both high- and low-affinity Ca^{2+} binding [15] most likely accounted for most of this increase. Native vesicles contained in addition calsequestrin, a high-capacity Ca^{2+}-binding protein [15,16]. Because of Ca^{2+} binding, the Ca^{2+} space enclosed by reconstituted vesicles could be less well defined than those for the other ions.

Membrane potential experiments

The ability of sarcoplasmic reticulum vesicles to generate and maintain membrane potentials in the presence of an ion gradient is an effective means of distinguishing vesicles that contain the K^+, Na^+ channel (Type I) from those that do not (Type II) [4]. Membrane potential experiments may give an indication of the surface area of polarized vesicles [17]. Vesicles filled with 440 mosM K^+-gluconate were diluted into Tris or Na^+-gluconate media, in the presence or absence of the K^+ ionophore, valinomycin (Fig. 2). The fluorescent dye diO-C_5-(3) was used to visualize K^+-induced membrane potentials (negative inside). In native sarcoplasmic reticulum vesicles, only the K^+, Na^+ permeable vesicle fraction formed a negative membrane potential when diluted from K^+ into Tris$^+$ medium (trace I in Fig. 2). The impermeability of Type II vesicles to K^+ and Tris$^+$ prevented formation of a potential in about one-third of all vesicles. Addition of valinomycin to the Tris$^+$ medium made K^+, Na^+ impermeable (Type II) vesicles permeable to K^+ and increased the dye signal, since it enabled development of a membrane potential in the entire vesicle population, i.e., Type I + Type II vesicles (trace I + II in Fig. 2). A negative potential

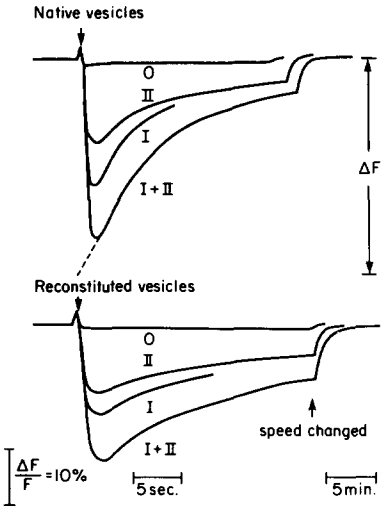

Fig. 2. Time course of fluorescence emission changes of diO-C_5-(3) to K^+ gradients across K^+, Na^+ permeable (Type I) and K^+, Na^+ impermeable (Type II) vesicles. Excitation was at 470 nm and fluorescence emission was recorded at 495 nm. At the time indicated by the arrows, native (upper panel) or reconstituted (lower panel) sarcoplasmic reticulum vesicles present in 440 mosM K^+-gluconate medium, pH 7, were diluted 60-fold into an isoosmolal K^+- or Na^+-gluconate medium (curve O), Na^+-gluconate medium plus 0.5 μM valinomycin (II), Tris-gluconate medium (I), or Tris-gluconate medium plus 0.5 μM valinomycin (I + II). All dilution media contained 1.5 μM diO-C_5-(3). In curves I, only Type I vesicles are expected to be polarized; in II, only Type II; in I + II, both Types I and II; and in O none.

could be generated exclusively in K^+, Na^+ impermeable vesicles by transferring sarcoplasmic reticulum vesicles from K^+ to Na^+ medium, containing the K^+-selective ionophore valinomycin (trace II in Fig. 2). In Na^+ dilution medium, no membrane potential was formed in K^+, Na^+ permeable vesicles, since these vesicles rapidly exchanged all of their K^+ for Na^+ within 1—2 s, the experimental limit of detection. In all cases, the fluorescence signal slowly returned to its initial intensity. This breakdown was most likely due to the slow inward diffusion of the relatively impermeable $Tris^+$ or Na^+.

K^+-induced membrane potentials could be generated in reconstituted vesicles in a manner similar to that of native vesicles (Fig. 2). As in native vesicles, the slow breakdown of potentials may be ascribed to inward diffusion of the relatively impermeable cations $Tris^+$ or Na^+ (cf. Fig. 1). However, the magnitudes of the signals were only about 50—75% of those elicited by native vesicles (Table II). Since the magnitude of the dye signal was dependent on the fraction of vesicles which formed a membrane potential [4], it appeared that a significant fraction of reconstituted vesicles (approx. 25—50%) was readily permeable to $Tris^+$. The ratio of the dye signals assigned to Type I and Type II vesicles was about the same for native and reconstituted vesicles, suggesting that the two preparations contained a similar proportion of sealed K^+, Na^+ permeable and impermeable vesicles.

Table III shows that the dye signal, and therefore the membrane potential generated in Type I vesicles, was greatly reduced when the K^+ gradient formed by dilution was lowered 10-fold by increasing the K^+-gluconate concentration in the assay medium from 7.3 to 73 mosM. Na^+, ethanolamine$^+$, and dimethyl-

TABLE III

EFFECT OF VARIOUS CATIONS ON FLUORESCENCE SIGNALS OF DiO-C_5-(3) ELICITED BY K^+, Na^+ PERMEABLE (TYPE I) SARCOPLASMIC RETICULUM VESICLES

Fluorescence assays were carried out as described in the legend of Fig. 2 with the following modifications. Type I vesicles were examined in Tris medium in the absence of valinomycin (see Fig. 2). Native or reconstituted sarcoplasmic reticulum vesicles present in 440 mosM K^+-gluconate medium were diluted 60-fold into isoosmolal Tris-gluconate medium containing the gluconate salts of the indicated cations at a concentration of 73 mosM. K^+ of the vesicle medium served to establish an initial 60-fold K^+ gradient throughout the experiments. Fluorescence decreases were obtained by extrapolation to the time of vesicle addition (cf. Fig. 2). Results are expressed as percent decrease in fluorescence.

Cation	Sarcoplasmic reticulum vesicles	
	Native	Reconstituted
$Tris^+$ (control)	29	19
K^+	12	9
Na^+	12	9
Ethanolamine$^+$	12	10
Dimethylaminoethanol$^+$	12	10
Trimethylaminoethanol$^+$ (choline$^+$)	29	19

aminoethanol$^+$ behave like K^+ in that they attentuated the dye signal in native and reconstituted vesicles in a similar manner. Trimethylaminoethanol$^+$ (choline$^+$) was without effect, indicating that it behaved like an impermeant cation. In previous studies, K^+, Na^+, ethanolamine$^+$ and dimethylaminoethanol$^+$ were found to pass rapidly through the channel, while choline$^+$ and $Tris^+$ were impermeable [4]. Similar behavior of these cations in native and reconstituted vesicles supported our contention that the K^+ permeability of sealed reconstituted vesicles was not simply due to vesicles leaky to K^+, but was rather due to the presence of the K^+, Na^+ channel of sarcoplasmic reticulum.

Discussion

This study has shown that reconstituted sarcoplasmic reticulum vesicles are permeable to small molecules in a complex manner. Ion flux and membrane potential measurements indicated that roughly 1/2 of the reconstituted sarcoplasmic reticulum vesicles were leaky, i.e., permeable to compounds smaller than inulin ($M_r \sim 5000$). These vesicles probably represented faulty or imperfect reconstitution and were not further studied. The remaining 1/2 of the reconstituted vesicles were relatively impermeable to choline$^+$ and $Tris^+$ and are referred to as sealed vesicles. Sealed vesicles were composed of two types which differed in their permeability to K^+ and Na^+. About 1/2 of the sealed vesicles, Type I, appeared to contain the K^+, Na^+ channel, since they were readily permeable to K^+ and Na^+. The remainder of the reconstituted sealed vesicles (Type II) seemed to lack the cation channel, since K^+, Rb^+ and Na^+ permeated across their membranes as slowly as choline$^+$ or $Tris^+$.

Following membrane solubilization and reconstitution, membrane proteins may dissociate into subunits and lose their asymmetric orientation within the bilayer. The fact that we could reconstitute a functional channel suggested

that the channel is monomeric, that it is composed of nondissociating subunits, or that the dissociated subunits assemble during removal of detergent so that reconstituted membranes become permeable to small monovalent cations in a manner similar to the native membrane. Orientation of the channel protein(s) was not assessed in the present study. One promising approach would be to fuse reconstituted vesicles with a planar bilayer, thereby rendering both sides readily accessible to probing. Using the bilayer technique, Miller [5] could show that the *'cis'* (external) side of the channel contains a divalent cation blocking site.

The studies reported here with reconstituted vesicles were in accord with the presence of a limited number of K^+, Na^+ channels in sarcoplasmic reciulum. Studies with native sarcoplasmic reticulum vesicles of varying size and derived from different parts of the reticulum structure have suggested that sarcoplasmic reticulum contains approx. 50 randomly distributed K^+, Na^+ channels per μm^2 of surface area [4]. As a consequence, during homogenization, only a portion of the vesicle population contains the K^+, Na^+ channel. On the other hand, data obtained with vesicles fused with planar bilayers have been interpreted to indicate 30 or more channels per sarcoplasmic reticulum vesicle, i.e., more than 1000 channels/μm^2 [5]. Reconstituted vesicles in our preparation were 0.07—0.15 μm in diameter [6], which corresponded to roughly 50 vesicles/μm^2. Assuming that reconstitution of the K^+, Na^+ channel is a random process and that there are 50 channels/μm^2 reconstituted surface area, it can be calculated that about 1/3 of the vesicles contained no channel (Type II) while the remainder had one or more channels (Type I). In good agreement with this estimate, potential measurements and ion flux experiments indicated a ratio of about 1 : 1 for reconstituted Type I/Type II vesicles. This ratio was similar to that of native vesicles and corresponded to an average of about one channel per reconstituted vesicle.

The Ca^{2+}-transport ATPase of sarcoplasmic reticulum that represents about 90% of sarcoplasmic reticulum membrane protein is a large amphiphilic molecule ($M_r \sim 115\,000$), of which the polar end is believed to protrude into the outer, more aqueous phase of the membrane in native vesicles (for review, see Ref. 18). When the detergent-solubilized ATPase is reconstituted into the membrane by dialysis, it loses this characteristics asymmetric arrangement, as indicated by freeze-fracture or negative-staining electron microscopy [19,20]. About half of the ATPase molecules are therefore incorrectly oriented for Ca^{2+} transport. The decreased Ca^{2+}-loading rate we have observed in reconstituted vesicles agreed qualitatively with this prediction , however, no decrease was observed in the ATP hydrolytic activity. A rate of ATP hydrolysis greater than that expected may be explained by the presence of leaky vesicles which most likely allowed ATP to enter and react with ATPase molecules protruding toward the lumen as well. However, the present study did not rule out the possibility that an altered protein or phospholipid orientation in reconstituted vesicles may have partially 'uncoupled' the Ca^{2+}-transport system. In this regard, it is of interest that the Ca^{2+}-ATPase has been reported to be present in an oligomeric form in native membranes [1]. To establish unambiguously the reason for the low Ca^{2+}-loading efficiency by reconstituted vesicles, the Ca^{2+} and ATP permeability of reconstituted leaky vesicles must be known.

The physiological function of the K^+, Na^+ channel has not yet been established. Our present hypothesis is that the K^+, Na^+ channel indirectly facilitates rapid movement of Ca^{2+} during muscle contraction and relaxation, thereby alleviating formation of a membrane potential. Our ability to solubilize and reconstitute the channel should allow us to isolate the channel so that its molecular properties and precise role in muscle function can be determined.

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