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CALCIUM TRANSPORT BY PIGEON ERYTHROCYTE MEMBRANE VESICLES

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Summary

Membrane vesicles from pigeon erythrocytes show a rapid, ATP-dependent accumulation of $^{45}Ca^{2+}$. Ca^{2+} accumulation ratios greater than or approximately equal to 10^4 are readily attained. For ATP-dependent Ca^{2+} uptake, V is 1.5 mmol · l^{-1} · min^{-1} at 27°C (approx. 0.9 nmol · mg^{-1} protein · min^{-1}), $[Ca^{2+}]_{1/2}$ is 0.18 μM, $[ATP]_{1/2}$ is 30—60 μM, the Ca^{2+} uptake rate depends on $[Ca^{2+}]^2$ and the dependence of uptake rate on ATP concentration implies strong ATP-ATP cooperativity. The Arrhenius activation energy is 19.1 ± 1.4 kcal/mol and the pH optimum is approx. 6.9.

Introduction

Sarcoplasmic reticulum and several eukaryote plasma membranes actively transport Ca^{2+}. This transport is energized by the hydrolysis of ATP. Most studies on active Ca^{2+} transport by plasma membranes have been made with erythrocyte membranes. In both the sarcoplasmic reticulum and erythrocyte systems, Ca^{2+} transport is very fast, $[Ca^{2+}]_{1/2}$ can be in the micromolar range, passive Ca^{2+} permeability is low, very high accumulation (or expulsion) ratios can be attained, and transport involves a cyclic phosphorylation and dephosphorylation of the Ca^{2+} porter. The Ca^{2+}/ATP stoichiometry has been reported as 2 for the sarcoplasmic reticulum system [1,2] and either 2 [3,4] or 1 [5] for erythrocytes by different investigators. The porter is thought to be an oligomer

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Abbreviations: EGTA, ethylene glycol bis(β-aminoethyl ether)-*N*,*N*'-tetraacetic acid; TES, *N*-Tris-[hydroxymethyl]methyl-2-aminoethanesulfonic acid; SDS, sodium dodecyl sulfate.

of protomers of approx. 100 000 daltons [6,7] and 140 000 daltons [8—10] for the sarcoplasmic reticulum and erythrocyte systems, respectively.

We describe a strongly accumulating, ATP-dependent Ca^{2+} transport by a pigeon erythrocyte membrane vesicle system. This system appears to be a useful addition to those available for the study of Ca^{2+} transport by plasma membranes. We were easily able to obtain linear Lineweaver-Burk plots of (Ca^{2+} uptake rate)$^{-1}$ vs. $[Ca^{2+}]^{-2}$, obtain a precise value for $[Ca^{2+}]_{1/2}$ (0.18 μM) and observe a dependence of Ca^{2+} uptake rate on ATP concentration implying strong ATP-ATP cooperativity. $[ATP]_{1/2}$ is between 30 and 60 μM. Analogous measurements were difficult or impossible to make with previously described mammalian erythrocyte systems.

Materials and Methods

$^{45}CaCl_2$ was obtained from ICN Life Sciences Group, 2727 Campus Drive, Irvine, CA. [*Me*-^{3}H]Choline chloride was from Amersham/Searle, Arlington Heights, IL. The scintillation counting cocktail, 3a70B, was from Research Products International, Elk Grove Village, IL. A23187 was kindly provided by Eli Lilly, Indianapolis, IN. Pyruvate kinase was from Boehringer Mannheim, New York, NY. All other biochemicals were obtained from Sigma Chemicals, St. Louis, MO. Inorganic chemicals were analytical reagent grade or better. All materials were used without further purification. All water was double deionized and glassware was rinsed with double-deionized water.

The procedure for preparing pigeon red cell membrane vesicles was similar to that described previously [11], except that just before the second sonication at setting 5 the loose pink upper layer of the pellet was gently suspended in a few ml of cold buffered KCl (143 mM KCl, 4.8 mM KH_2PO_4, 5.2 mM K_2HPO_4) and transferred with a Pasteur pipet to the sonication cup. The suspension was sonicated in 8-ml portions at setting 5 for three 30-s intervals, then centrifuged for 10 min at 3000 $\times g$. The supernatant was further processed as described before [11]. All operations were done in the cold. Membrane preparations had 43.6 ± 2.8 mg protein/g wet weight (n = 9, method of Lowry et al. [12]).

We believe these are fairly pure cytoplasmic membrane vesicles. The vesicles show erythrocyte cytoplasmic membrane properties including Na^+-dependent glycine accumulation ([13], and unpublished data), ATP-dependent [^{3}H]-ouabain binding (Lee, J.W. and Vidaver, G.A., unpublished data), Cl^- 'permeability' producing complete loss of $^{36}Cl^-$ within 20 min at 0—4°C (Sorensen, E.N., Lee, J.W. and Vidaver, G.A., unpublished data) and SDS-polyacrylamide gel electropherograms very similar to those reported for hen erythrocyte membrane preparations [14,15]. For reasons given earlier [16], we presume the cells lack intracellular membranes other than nuclear membranes. We estimate from its DNA content (Jones, S.W. and Vidaver, G.A., unpublished data) that our membrane preparation contains approx. 0.16% nuclear membrane contamination. The DNA/nuclear membrane protein ratio of nuclei was obtained from Jackson's [14] data. A calibration curve was made by mixing pigeon erythrocyte nuclei with plasma membrane, both prepared as described by Jackson [14], in a range of proportions and sonicating and washing the mixtures by our preparative procedure but without separating the upper and lower layers of

pellet. DNA was lost during washing. Our calculation assumed no nuclear membrane was lost and so gives an upper limit for nuclear membrane contamination.

'Sidedness' of membrane vesicle fractions from bovine serum albumin density gradients (Table I) was estimated by the neuraminidase-accessible sialic acid method [17] (modified by incubation at pH 6.2 instead of pH 5.7). This method gave values of 30—39% (median, 36%, five experiments) for inaccessible sialic acid in the main dense fraction and 0—24% (median, 7%, five experiments) in the least dense fraction.

Membrane vesicles were 'annealed' by incubating vesicle suspensions 17 min at 41°C in 136 mM KCl, 5.0 mM KH_2PO_4, 5.0 mM K_2HPO_4 (or 10 mM TES in some experiments), 2.5 mM $MgCl_2$ and 1.0 mM [^{3}H]choline chloride (4 μCi/ml) (as a marker for 'trapped space'). The membrane suspension was chilled 2 min, diluted with 10 ml of ice-cold 154 mM KCl and centrifuged for 20 min at 30 900 $\times g$. The pellets were washed once with 154 mM KCl, then centrifuged 20 min at 30 900 $\times g$.

$^{45}Ca^{2+}$ uptake by membrane vesicles was measured by incubating vesicles (16—20 mg, wet weight) in 0.5 ml 141 mM KCl, 10 mM TES (usually pH 6.9), 1.5 mM $MgCl_2$ and $^{45}Ca^{2+}$ with or without MgATP (usually 2.5 mM). For initial rate measurements, incubation was for 2 min at 27°C. $^{45}Ca^{2+}$ uptake was stopped by chilling the suspensions 2 min, immediately diluting with 9.5 ml 154 mM KCl and promptly centrifuging for 20 min at 30 900 $\times g$. Pellets were washed once with 10 ml KCl, the tubes were drained and the insides wiped dry. Pellets were weighed, dissolved at room temperature for 1 h in 1.00 ml 1% (w/v) SDS, 1 mM $CaCl_2$ and 5 mM choline chloride, mixed with 10 ml 3a70B and counted. Samples of medium and ^{45}Ca and ^3H standards were prepared and counted under the same conditions. All samples were equally quenched so quench corrections were unnecessary. Spill corrections were made using the spill ratios of the standards.

Vesicle space was ^3H cpm (pellet)/^3H cpm/ml (annealing medium). Total vesicle space was used in calculating 'uptake/ml' values. In most cases, the choline space of unincubated vesicles was used for all samples as 5—20% of the choline was released during 2—20-min incubations.

For some experiments (trace Ca^{2+}) radioactive Ca^{2+} (usually 0.1 μCi/ml, approx. 17 Ci/g) plus 20 μM unlabelled Ca^{2+} were added to the incubation media. These solutions are assumed to also contain 10—30 μM Ca^{2+} arising from reagents, water and glassware as reported earlier [18]. Where lower controlled Ca^{2+} was needed, 1.0 mM EGTA was added as a mixture of CaEGTA and MgEGTA. The free Ca^{2+} and Mg^{2+} were calculated (Vidaver, G.A. and Ting, A., unpublished data) as a function of total EGTA, total Ca^{2+}, total Mg^{2+}, pH and temperature. 1—5 μCi$^{45}Ca^{2+}$/ml was used with the Ca^{2+}-buffered systems.

Results

Pigeon erythrocyte membrane vesicles avidly accumulate Ca^{2+} in the presence of ATP. $^{45}Ca^{2+}$ uptake from 'trace' Ca^{2+} medium can be more than two orders of magnitude greater than uptake in the absence of ATP. With higher external [Ca^{2+}] or with CaEGTA buffers the apparent ATP stimulation is somewhat less but stimulation of 1—2 orders of magnitude is readily

obtained. Replacement of phosphate buffer in the annealing solution with TES had no effect.

Fig. 1 shows that ATP dependence of $^{45}Ca^{2+}$ uptake, and the release of accumulated ^{45}Ca by the ionophore, A23187. The ATP-dependent $^{45}Ca^{2+}$ accumulation appears to be active since very high accumulation ratios are reached (e.g. Fig. 1, $[Ca^{2+}]_i/[Ca^{2+}]_o = 1.5 \cdot 10^4$ by 20 min, 27°C) and A23187 releases the accumulated $^{45}Ca^{2+}$. Fig. 1 also illustrates the time course of $^{45}Ca^{2+}$ uptake at 27°C. $[Ca^{2+}]_o$ was 0.81 μM. Since uptake vs. time plots from 'trace' Ca^{2+} media (approx. 30—50 μM $[Ca^{2+}]$) showed similar curvature, 2-min uptake values were used to approximate initial rates. Kinetic measurements were made at 27°C since at 41°C departure from linearity began sooner.

Fig. 2. shows a double-reciprocal plot, 1/(ATP-dependent $^{45}Ca^{2+}$ uptake rate) vs. $1/[Ca^{2+}]_o^2$. The uptake rate depends on $[Ca^{2+}]^2$. $[Ca^{2+}]_{1/2} = 0.18 \pm 0.01$ μM (two experiments) and $V = 1.54 \pm 0.01$ mmol $\cdot$ l^{-1} $\cdot$ min^{-1} at 27°C (approx. 0.9 nmol $\cdot$ mg^{-1} protein $\cdot$ min^{-1}).

The dependence of $^{45}Ca^{2+}$ uptake on ATP concentration was also measured. Since the membrane hydrolyses ATP, phospho*enol*pyruvate and pyruvate kinase were used to maintain the ATP concentration. Without the ATP-regenerating system, 0.2—0.5 mM ATP was required to maintain near maximal $^{45}Ca^{2+}$ uptake rates for 2 min at 27°C.

Fig. 3 shows the ATP-dependent $^{45}Ca^{2+}$ uptake vs. ATP concentration with the regenerating system present. With 0.06 mM ATP, Ca^{2+} uptake is nearly as rapid as with 0.5 mM ATP, but reducing ATP to 0.03 mM nearly abolishes Ca^{2+} uptake. This abrupt decline is not due to insufficient regenerating system; similar results were obtained with one-fifth as much regenerating system. With

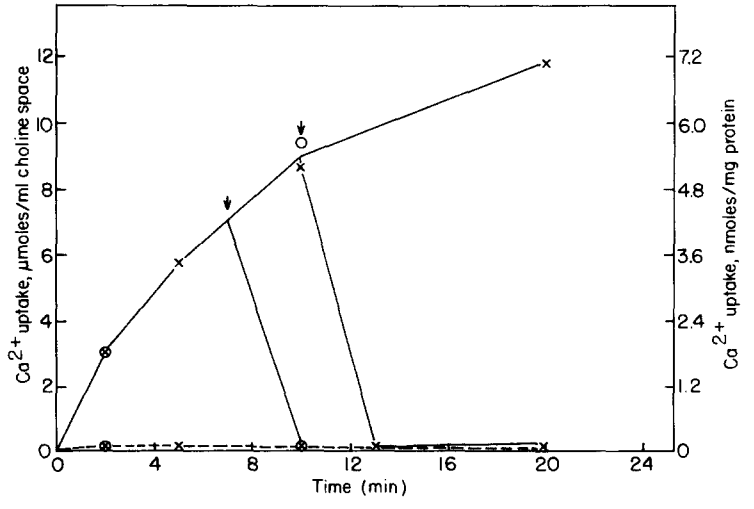

Fig. 1. The time course of $^{45}Ca^{2+}$ uptake and the release of $^{45}Ca^{2+}$ by A23187. Membrane vesicles were incubated in a medium containing 0.75 mM ^{45}CaEGTA, 0.25 mM MgEGTA, 1.5 mM $MgCl_2$, 10 mM TES, (pH 7.0), 141 mM KCl, with or without 2.5 mM MgATP for various times at 27°C. $[Ca^{2+}] = 0.81$ μM. A23187 (5 μg/ml) was added to some samples during incubation at the times indicated (arrows) and the samples were further incubated as shown. Data from two experiments are presented. ———, +ATP; - - - - -, —ATP.

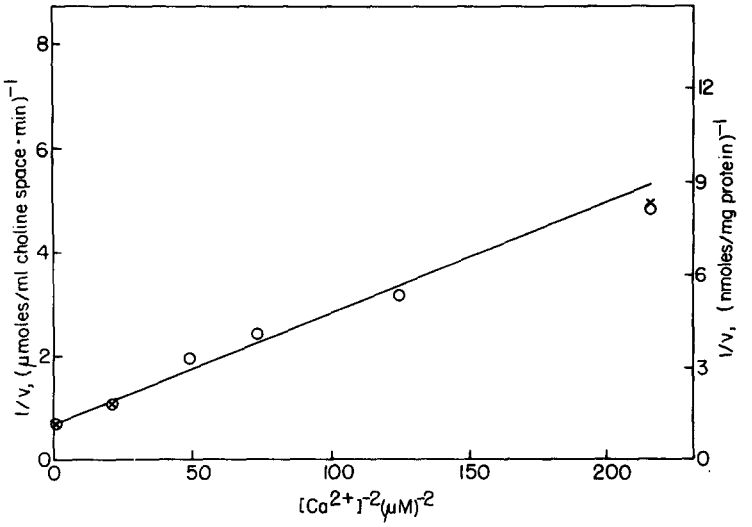

Fig. 2. The dependence of Ca^{2+} uptake rate on $[Ca^{2+}]^2$. Incubation was for 2 min at pH 7.0, 27°C. Free $[Ca^{2+}]$ was controlled * with a CaEGTA: MgEGTA buffer (1.0 mM total EGTA, 1.75—2.25 mM total Mg^{2+} (1.7—2.1 mM free $[Mg^{2+}]$); the buffer composition range here, 0.78 mM total Ca^{2+}—0.28 mM total Ca^{2+} corresponds to the free $[Ca^{2+}]$range, 0.81 μM—0.068 μM). ATP-dependent Ca^{2+} uptake rates were calculated from the differences between uptake from media with and without 2.5 mM MgATP. ATP-dependent $^{45}Ca^{2+}$ uptake is not quite zero at 0°C (Fig. 5), so to minimize $^{45}Ca^{2+}$ exposure time differences between +ATP and —ATP samples, the samples were arranged in an alternating +ATP (- - - - -) and —ATP (- - - - -) sequence. Sequential addition of $^{45}Ca^{2+}$ (at 0°C before incubation) and sequential addition of diluent (see Materials and Methods) after incubation minimized differences in ^{45}Ca exposure time between +ATP, —ATP pairs at the same free $[Ca^{2+}]$. Differences in exposure time to $^{45}Ca^{2+}$ at 0°C before incubation were compensated by differences in time at 0°C after incubation (but before dilution). Data from two experiments, shown by different symbols, were normalized to the average V.

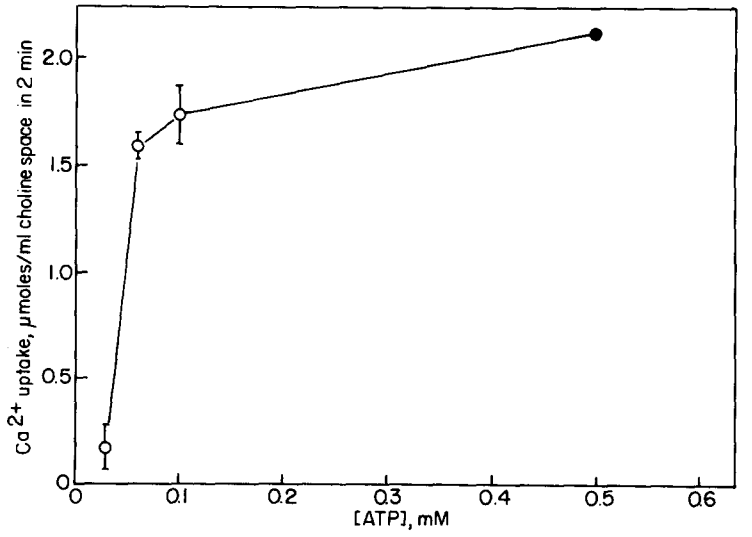

Fig. 3. The effect of ATP concentration on $^{45}Ca^{2+}$ uptake rate. Membrane vesicles were incubated for 2 min at 27°C as for Fig. 1 except that 20 mM phospho*enol*pyruvate, 130 μg/ml (26 units/ml) pyruvate kinase and various concentrations of MgATP were present. The $^{45}Ca^{2+}$ uptake of each sample was corrected by subtracting uptake by a corresponding sample which had been held at 0°C the same length of time. Ca^{2+} uptake here is total rather than ATP-dependent uptake. This correction was made to avoid possible large errors at low ATP concentrations which could arise if $[ATP]_{1/2}$ were lower at 0°C than at 27°C. Data from four experiments were normalized to the average uptake at 0.5 mM ATP (●) for presentation on the same graph. Vertical bars, ±S.E.

* The calculation procedure we used (Vidaver, G.A. and Ting, A., unpublished data) gives the same results as the more laborious procedure of Wolf [33] except that we used newer (presumably more accurate) values for association constants [34,35] and corrected these for temperature.

the latter, the 0.06 mM values fluctuated between the 0.03 mM and 0.10 mM values in different experiments. Neither enzyme nor phospho*enol*pyruvate appears to affect Ca^{2+} uptake directly: enzyme plus phospho*enol*pyruvate alone (no ADP or ATP) did not support $^{45}Ca^{2+}$ uptake; with the regenerating system, ADP and ATP were equally effective; with high enough ATP (0.5 mM or more) the regenerating system at high or low levels had no effect; and both enzyme and phospho*enol*pyruvate were required to stimulate uptake with low ATP concentrations.

The effects of temperature and pH on ATP-dependent $^{45}Ca^{2+}$ uptake were also determined. For these experiments, uptake was measured from 'trace' Ca^{2+} media rather than from CaEGTA-buffered media. This was to avoid complicating effects of temperature and pH on the EGTA buffer system. Since $[Ca^{2+}]_{1/2}$ is 0.18 μM at 27°C, 'uptake' is close to V. Fig. 4 shows the pH dependence at 27°C and Fig. 5 the temperature dependence at pH 6.9. The activation energy from the Arrhenius plot is 19.1 ± 1.4 kcal/mol. (Values of 13.6 [19] and 25 [20] have been reported for the human erythrocyte system.) Note that ATP-dependent $^{45}Ca^{2+}$ uptake is appreciable at 0°C (approx. 4.5% of the rate at 27°C).

Only part of the vesicle population accumulates Ca^{2+}. Table I shows the composition and Ca^{2+} uptake activities of discontinuous bovine serum albumin density gradient fractions. Most of the activity is in vesicles in the high-density region. These enclose about half of the total space and contain about 3/4 of the total lipid phosphorus. In the absence of bovine serum albumin the active vesicles can be preferentially sedimented at 6000 × g in 5 min ('pellet', Table I).

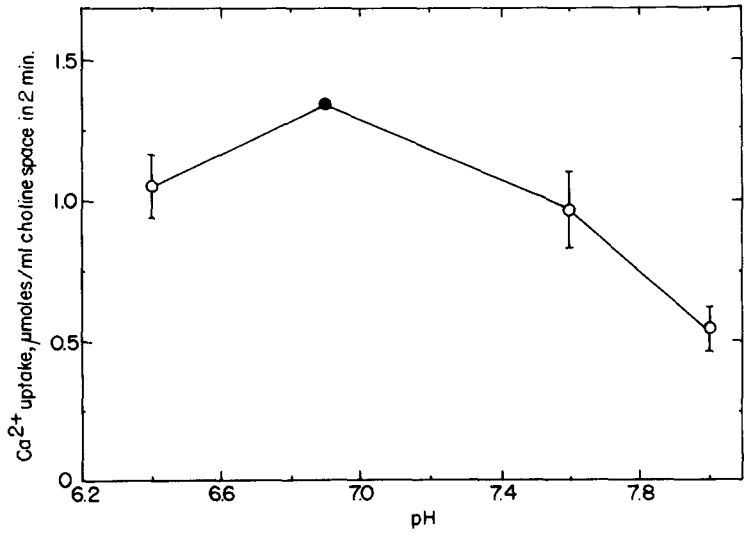

Fig. 4. The pH dependence of $^{45}Ca^{2+}$ uptake. Membrane vesicles were incubated in 'trace' Ca^{2+} media, with or without 2.5 mM MgATP for 2 min at 27°C. ATP-dependent $^{45}Ca^{2+}$ uptake is the difference between the uptake of '+ATP' and '—ATP' samples. Vertical bars, ±S.E. Data from two experiments is normalized to the uptake at pH 6.9 (●).

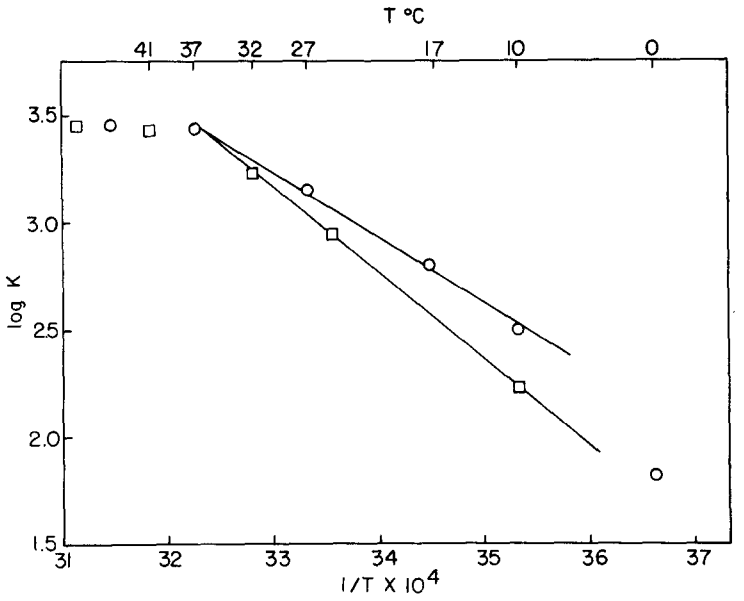

Fig. 5. Arrhenius plots of ATP-dependent $^{45}Ca^{2+}$ uptake. Membrane vesicles were incubated in a 'trace' Ca^{2+} medium (pH 6.9) with or without 2.5 mM MgATP for 2 min at various temperatures. ATP-dependent $^{45}Ca^{2+}$ uptake is the difference between the activities of '+ATP' and '—ATP' samples incubated at the same temperature. Data from two experiments are plotted separately. Some $^{45}Ca^{2+}$ uptake occurred while the samples were warming from 0°C to the bath temperature and while they were cooling to 0°C after removal from the bath. We corrected the E_a values for this as follows. Theoretical Ca^{2+} uptake values ('u') were calculated from (1) $u = \int_0^t v dt$; (2) $v = \exp(-E_a/RT)$, and (3) $T_t = T_0 + \Delta T(1 - e^{-\beta t})$ with $\Delta T = +/-(T(\text{bath})-273)$ and $\beta = 2.08$ (corresponding to a $t_{1/2}$ for temperature equilibration of tube contents of 20 s). T_t was calculated for successive 0.125 min intervals with $T_0 = 273$ K and ΔT positive, the corresponding values of v calculated and accumulated until $t = 2.25$ min. Then the sign of ΔT was reversed, T_0 was reset to T (bath), t reset to zero and v values accumulated for a further 2.25 min. Accumulated values for 4.5 min are 'u'. Sets of values for 'u' were calculated with various E_a values used in (2), Arrhenius plots made of these sets of 'u' values, and the resulting slopes plotted against the E_a values used in (2). This plot was used to correct the apparent E_a values obtained directly from the slopes. The corrected E_a values were 17.7 and 20.4 kcal/mol (average 19.1) compared to the uncorrected values of 14.1 and 18.1 (average 16.1) kcal/mol.

We have no accurate passive Ca^{2+} permeability value. The observed Ca^{2+} accumulation (e.g. Fig. 1) suggests a 'passive' efflux coefficient greater than or approximately equal to 0.13 min^{-1} ($[Ca^{2+}]_i/v$(entry)), but we have observed higher $[Ca^{2+}]_i$ values and have not attempted to maximize them nor to verify that steady state was reached. In two experiments at 27°C and one at 41°C, after addition of EGTA to the medium, the loss rate coefficient of previously accumulated Ca_i^{2+} was less than or approximately equal to 0.01 min^{-1}. In the absence of ATP, with $[Ca^{2+}]_0 = 0.8$ μM, the entry rate at 27°C became constant between 2 and 10 min and was 10 $\mu mol \cdot l^{-1} \cdot min^{-1}$ (v(entry)/$[Ca^{2+}]_0 \approx 12$). There is probably more than one 'passive' Ca^{2+} transport route, but disentangling the various possible routes, accounting for vesicle heterogeneity and determining ATP effects has not been attempted.

TABLE I

LIPID PHOSPHORUS, CHOLINE SPACE AND Ca^{2+} UPTAKE ACTIVITY OF MEMBRANE FRACTIONS

Membrane vesicles were prepared as described in [13] through the second sonication step. The chief difference from the procedure in Materials and Methods is that the separation of the upper from the lower layer of the pellet was done three times instead of once. After resonication, annealing was at pH 7.6 in TES buffer instead of pH 6.9. These modifications are to improve fractionation. The annealed suspension was diluted with 9 vols. 144 mM KCl, 10 mM TES (pH 7.5) and 10-ml portions centrifuged for 5 min at 6000 × *g* in 12 ml Corex tubes (Ivan Sorval) in a SS-34 rotor. The pellet is fraction P. The vesicles pelleted from the supernatant at 32 800 × *g* in 30 min are the fraction S. Fraction P was suspended with an equal volume of 17% bovine serum albumin (w/v) in KCl/TES (pH 7.5) and layered on a bovine serum albumin step density gradient (5 ml 15% bovine serum albumin/5 ml 17% bovine serum albumin) in 9/16 × 3-1/2 inch cellulose nitrate centrifuge tubes. Fraction S was suspended (approx. 100 mg/ml) in KCl/TES (pH 7.5) and layered onto an 11 ml linear 6—17% bovine serum albumin gradient [13]. Centrifugation was in a Beckman SW 41 Ti rotor for 35 min at 275 000 × *g* in a Beckman L5-65 ultracentrifuge. Fractions were withdrawn from the fraction S gradient according to the visible band patterns and the bovine serum albumin concentrations estimated from the volumes. Fractions from fraction P were those at the input/15% and 15%/17% bovine serum albumin interfaces and the pelleted material. Fractions were diluted to 12 ml with 154 mM KCl, centrifuged at 32 800 × *g* for 30 min and washed once with KCl/TES (pH 7.5). Pellets were weighed and resuspended in 5—10 times their weight of 154 mM KCl. For measurement of $^{45}Ca^{2+}$ uptake, 50-μl aliquots were mixed with 0.50 ml trace Ca^{2+} medium at pH 7.5 instead of 6.9. Suspensions were incubated for 7 min at 40°C. After centrifuging and washing, pellets were dispersed in H_2O (with carrier CaEGTA and choline chloride) and 1 ml samples counted in 12 ml Scintisol. Other 50-μl aliquots of the membrane fractions were pelleted, extracted and washed with cold 5% (w/v) trichloroacetic acid and extracted for 5 min at 90°C with 5% trichloroacetic acid. The residual pellets were digested and analyzed for P_i as described by Ames [32]. Choline space was determined from a third set of 50-μl aliquots. Fractions in Table I designated by P and S were derived, respectively, fractions P and S (above). The numbers following P or S refer to the bovine serum albumin concentration range of the fractions. This experiment is representative of results obtained in six similar experiments.

Fraction	Lipid phosphorus (% of total)	Trapped space (% of total)	ATP-dependent Ca^{2+} uptake/ % of total
S2—6	3.8	23.7	(0.01)
S6—11	7.8	22.2	1.8
S11—15	12.3	6.7	10.1
S15—17	4.8	1.5	0.4
P8—15	1.0	3.9	0.6
P15—17	75.9	41.3	83.2
P > 17	2.0	0.7	3.9
Total	15.7 μmol	10.9 μl	approx. 200 nmol

Discussion

Our original interest in the Ca^{2+} transport process stemmed from its very high 'signal-to-noise' ratio. Because of the very large stimulation of $^{45}Ca^{2+}$ uptake by ATP, minute traces of this activity can be detected. Consequently, it is very useful for monitoring and characterizing membrane-derived systems such as reconstituted membranes (Yeung, W.K., Weisman, G. and Vidaver, G.A., unpublished data) or membrane-lipid hybrids [21]. The kinetic parameters of the original system were needed to relate the activities of the derived and original membrane systems.

The original vesicle system is also useful for other purposes. It rapidly accumulates Ca^{2+} to very high inside/outside concentration ratios (greater than

$1.5 \cdot 10^4$) without requiring Ca^{2+} precipitants or sequestrants. The system seems better suited for kinetic studies than systems previously described [3,5, 22,23]. We obtained the first ATP dependence curve for Ca^{2+} uptake, found a dependence of Ca^{2+} uptake rate on $[Ca^{2+}]^2$ and obtained a precise value for $[Ca^{2+}]_{1/2}$. The Ca^{2+} uptake rate drops precipitously between 0.1 and 0.03 mM ATP, implying positive ATP-ATP cooperativity. $[ATP]_{1/2}$ is between 30 and 60 μM. (Pigeon erythrocyte [ATP] is approx. 6 mM [24].) The ATP dependence of human erythrocyte membrane Ca^{2+}-ATPase has been variously reported as showing zero [9,25] or negative ATP-ATP cooperativity [26]. (Failure to demonstrate cooperativity, of course, does not rule out multiple ATP sites.)

The dependence of the Ca^{2+} uptake rate on the second power of $[Ca^{2+}]$ might arise from the requirement for $[Ca^{2+}]$ for activation of the Ca^{2+} porter by the Ca^{2+}-ATPase activator. Quist and Roufogalis [23] have reported that the 'activator' activates Ca^{2+}-ATPase without affecting Ca^{2+} uptake by human erythrocyte membrane vesicles, but others [27] report an approx. two-fold stimulation of Ca^{2+} uptake by hemolysate which can be partially purified by a method for purifying the Ca^{2+}-ATPase activator. A dependence of Ca^{2+} transport on $[Ca^{2+}]^2$ has been shown for the sarcoplasmic reticulum system [28] and strongly implied by data from human erythrocytes [29].

A $[Ca^{2+}]_{1/2}$ value of 0.45 μM has been reported for the high-affinity route of an erythrocyte vesicle system [23] and the value given for hemolysed and restored cells is 'less than 4 μM' [5]. Other values in the literature range from 10^{-2} M to 10^{-5} M (cited in [3]). A value for $[Ca^{2+}]_{1/2}$ for intact erythrocytes of 0.5 mM has been reported [3]. A variety of values for $[Ca^{2+}]_{1/2}$ has been reported for the Ca^{2+}-ATPase activity [9,25,26].

Part of the uncertainty of reported Ca^{2+} transport properties may arise from limitations of the systems used. Erythrocyte vesicles prepared by hypotonic vesiculation [23,24] may have altered properties since such vesicles are breakdown products of the membrane; e.g. the vesiculation procedure releases bands I, II and V [23]. The hemolysed and restored cell preparations seal poorly at low $[Ca^{2+}]$ and contain an unsealed fraction even with optimal $[Ca^{2+}]$ [5]. The limitations of the latter system prevented measurement of the real Ca^{2+} expulsion ratio (greater than 700) and $[Ca^{2+}]_{1/2}$ (greater than or approximately equal to 4 μM) [5]. With intact cells, very high expulsion ratios occur [3] and $[Ca^{2+}]_{1/2}$ has been measured [3], but experimental manipulation of intact cells is difficult.

There is probably another reason for the variability in the reported kinetic parameters of the Ca^{2+} transport and the related Ca^{2+}-ATPase systems. Ca^{2+} transport may be controlled by as yet incompletely understood mechanisms involving a Ca^{2+}-dependent activator protein [26,30,31]. Different membrane preparations may be in different control states.

Acknowledgments

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