Energy-dependent Calcium Transport in Endoplasmic Reticulum of Adipocytes*

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DAVID E. BRUNS,$ JAY M. MCDONALD,$ AND LEONARD JARETT

From the Division of Laboratory Medicine, Departments of Pathology and Medicine, Washington University School of Medicine, and Barnes Hospital, St. Louis, Missouri 63110

The endoplasmic reticulum from isolated rat adipocytes has the ability to actively accumulate calcium. The calcium uptake was characterized using the 20,000 x g supernatant (S1 fraction) of total cellular homogenate. Endoplasmic reticulum vesicles isolated from the S1 fraction as a 160,000 x g microsomal pellet prior to testing demonstrated little ability to accumulate calcium. The calcium uptake in the S1 fraction was localized to the endoplasmic reticulum vesicles by morphologic appearance, by the use of selective inhibitors of calcium uptake, and by high speed sedimentation of the accumulated calcium. The uptake was MgATP- and temperature-dependent and was sustained by the oxalate used as the intravesicular trapping agent. Uptake was linear with time for at least 30 min at all calcium concentrations tested (3 to 100 μM) and exhibited a pH optimum of approximately 7.0. The sulfhydryl inhibitor p-chloromercuribenzenzene sulfonate produced a dose-dependent inhibition of calcium uptake with total inhibition at 0.01 μmol/mg protein. Ruthenium red and sodium azide inhibited less than 5% of the uptake at concentrations (5 μM and 10 mM, respectively) which completely blocked calcium uptake by mitochondria isolated from the same cells. The K_m for calcium uptake was 12 μM total calcium which corresponded to approximately 3.6 μM ionized calcium in the assay system. The maximum velocity of the uptake was 5.0 nmol (mg of microsomal protein)^-1 (min)^-1 at 24°C under the assay conditions used and exhibited a Q_10 of 1.8. The uptake activity of the endoplasmic reticulum vesicles in the S1 fraction exhibited a marked time- and temperature-dependent lability which might account in part for the lack of uptake in the isolated microsomal fraction. This energy-dependent calcium uptake system would appear to be of physiologic importance to the regulation of intracellular calcium.

Calcium ions have been proposed as critical regulators of intermediary metabolism (1–8). Direct studies of the control of cellular calcium metabolism are complicated by its high degree of compartmentalization, with estimated calcium concentrations of 10^{-8} to 10^{-7} M in cytosol (5) and 10^{-1} M in mitochondria (10) and endoplasmic reticulum (11) in a variety of cells, compared to extracellular calcium concentrations typically near 10^{-6} M. This has made the interpretation of calcium fluxes with intact cells difficult, and has necessitated the use of subcellular fractions. Such studies have shown that mitochondria from many cell types accumulate calcium actively (12) and that sarcomplasmic reticulum rapidly accumulates calcium and plays a central role in the relaxation-contraction cycle (11). The importance of active calcium uptake by endoplasmic reticulum has been investigated only recently in other cells (13, 14).

Rat adipocytes contain an extensive smooth endoplasmic reticulum (15) which has been shown to contain dense deposits of calcium by the pyronin-methylene blue technic (16). This has suggested a function in calcium homeostasis analogous to that of sarcoplasmic reticulum but there has been no demonstration of a "calcium pump" to maintain the calcium gradient suggested by morphologic investigation.

The present study is part of a series of investigations designed to elucidate the roles of the various subcellular organelles in calcium homestasis in adipocytes and describes energy-dependent calcium uptake by endoplasmic reticulum. The uptake has been characterized using the 20,000 x g supernatant (S1) of total cellular homogenate containing endoplasmic reticulum vesicles. Virtually no calcium uptake was present in these vesicles isolated as a microsomal fraction. The characteristics of the uptake were analogous to those in sarcoplasmic reticulum. The uptake was MgATP- and ATP-dependent, saturable, azide-insensitive, blocked by sulfhydryl inhibitor, and sustained by oxalate. The K_m was approximately 3.6 μM ionized calcium, which suggests a significant role at physiologic concentrations of cytosol calcium. Characterization of this uptake allows further investigation of the role of calcium as a regulator of adipocyte metabolism.
EXPERIMENTAL PROCEDURES

Materials—Male Wistar rats, weighing 120 g, were purchased from National Laboratory Animal Co., O’Fallon, Mo. Chemicals were obtained from the following sources: collagenase (Type I from Clostridium), ATP (disodium salt), and bovine serum albumin (Cohn Fraction V), Sigma Chemical Co., St. Louis, Mo.; Omnifluor and 8CaCl, (approximately 1 mCi/mmol), New England Nuclear, Boston, Mass.; Instagel and sample solubilizer (Soluene), Packard Instrument Co., Downers Grove, Ill. All other materials were reagent grade quality and purchased from standard sources. All solutions were prepared with water deionized by a double-chambered, mixed bed ion exchange system (Culligan, Inc., Northbrook, Ill.) and filtered through a 0.25-μm pore size filter (Ultipor, PTFM Corp., Cortland, N.Y.). The calcium contents of the assay reagents were measured by atomic absorption spectroscopy and the concentrations found were included in all calculations of the experimental data. Type HAWP membrane filters with a 0.45-μm pore size were obtained from Millipore Corp., Bedford, Mass., and were soaked in 0.25 M KCl prior to use.

Adipocyte Fractionation—Fat cells were isolated from the epididymal fat pads by a modification (17) of the method of Rodbell (18) using 0.5% collagenase/ml. The cells were homogenized in 0.25 M sucrose containing 10 mM Tris/HCl, pH 7.4 at 4°C (Tris/sucrose) and fractionated as previously described (17, 19) except that EDTA was omitted throughout. Omission of EDTA did not affect the protein distribution or purity of the fractions used in this study as assessed by measurement of marker enzmys, 8-nucleotidase, ouacine dehydrogenase, cytochrome c reductase, and Mg2+ATPase performed as previously described (19-21). The total cell homogenate was centrifuged for 15 min to sediment mitochondria, plasma membranes, nuclei, and cellular debris. Mitochondria were isolated from the pellet as previously described using differential and gradient centrifugation (10). The supernatant, containing the microsomal fraction and cytosol, was termed S1. The microsomal fraction was further purified by centrifugation at 300,000 × g for 1 h and was resuspended in Tris/sucrose. The microsomal fraction was an enriched endoplasmic reticulum preparation containing less than 3% mitochondria (19) and less than 10% plasma membranes (19, 22). The contamination of endoplasmic reticulum in S1 by plasma membranes and mitochondria can be taken to be no greater than these values since the high speed centrifugation used to prepare the microsomes would certainly sediment these organelles present in S1. The fractions were assayed for calcium uptake activity immediately or quick frozen in dry ice/ethanol or liquid nitrogen and stored at -70°C for in this manner retained active calcium transport for several weeks but variations of stability among preparations necessitated careful monitoring.

Assay of Calcium Uptake by Filtration—Calcium accumulation was investigated using the microsomal, S1, and mitochondrial fractions as well as all assays were performed using a filtration technique (14, 20) with minor modification. Standard incubations were performed in polystyrene tubes with constant shaking for 20 min at 24°C. The assay was initiated by addition of 20 to 80 μg of protein to the incubation medium containing 0.1 mM KCl, 5 μM MgCl2, 5 mM ATP, 10 mM oxalate, and 15 to 25 mM Tris/HCl, pH 7.0 at 24°C, 1 to 100 μM CaCl2, and 0.25 to 0.50 μCi of 8CaCl2 in a total volume of 300 or 500 μl. The uptake was terminated by membrane filtration of 250- or 400-μl aliquots, respectively, and immediate washing of the filters with three 5-ml volumes of 0.25 M sucrose. The filters were dried and 8Ca measured by liquid scintillation counting. In certain experiments the S1 fraction 5-fold higher concentrations of protein were used to allow introduction of an amount of microsomal protein comparable to that used in studies with isolated microsomes. In such experiments iso-osmolality was approximated by reducing the concentration of KCl to 0.05 M to allow for the osmolar contribution of sucrose in S1. Calcium uptake rates measured by this modification were identical with those using the lower protein concentrations. In all assays of calcium uptake, appropriate control tubes without protein were included to correct for nonspecific calcium binding to the filters (routinely less than 1% of total). The specific activity of calcium was determined by counting aliquots of incubation media dried on filter discs.

Assay of Calcium Uptake by Centrifugation—Calcium uptake by the S1 fraction was also determined by a centrifugation technique for comparison to the filtration method described above. Calcium uptake was terminated by sedimentation of the microsomes by centrifugation at 160,000 × g for 1 h at 4°C and aliquots of both the supernatant and the solubilized pellet were counted to determine the uptake. The uptake rates determined from either 8Ca2+ accumulation in the pellet or loss of 8Ca2+ from the supernatant agreed with each other within 5%. The close agreement between the filtration and centrifugation assays (see "Results") indicated that soluble protein in S1 did not affect the filtration assay (24).

Electron Microscopy Technique—Samples of microsomes sedimented at 160,000 × g and of the fraction of S1 retained by 0.45-μm filters were examined by electron microscopy. The mitochondrial pellet was examined immediately after resuspending in Tris/sucrose. The fraction of S1 retained on the Millipore filter was recovered by gentle shaking in Tris/sucrose. The samples were negatively stained for 1 min with 1% ammonium molybdate in 2% ammonium acetate, pH 7.4, and examined on Formvar carbon-coated grids using a Philips 200 electron microscope.

Protein Determination—Protein was determined by the method of Lowry et al. (25), using bovine serum albumin as standard.

Calculations—Free calcium concentrations were calculated as described by Katz et al. (26). All data are expressed as mean ± SE.

RESULTS

Microsomes

Initial studies of active calcium uptake by endoplasmic reticulum were performed using the microsomal fraction prepared from isolated adipocytes.

ATP Dependency of Calcium Uptake by Isolated Microsomes—The passive binding of calcium to adipocyte microsomes has been characterized previously (27) in a system containing 0.1 M KCl, Tris buffer, and 8CaCl2. Addition of ATP, magnesium, and oxalate to this system resulted in a 2- to 3-fold increase in the amount of calcium associated with the particulate material (not shown) suggesting the presence of an energy-dependent calcium uptake system in the microsomal fraction. Both ATP and magnesium were required to demonstrate this calcium accumulation which was enhanced by the presence of oxalate as an intravesicular "trapping agent" for calcium (23). Calcium uptake in this system continued for at least 30 min in contrast to the rapid passive binding of calcium (27).

Effect of Mitochondrial Inhibitors on Microsomal Calcium Uptake—The inhibitors of mitochondrial calcium uptake, oxydazole (10 mM), and ruthenium red (5 μM), each blocked more than 65% of the ATP-dependent microsomal calcium uptake, indicating that two-thirds of the uptake was attributable to the mitochondria. Therefore, the endoplasmic reticulum vesicles which represented the majority of organelles in the microsomal fraction accounted only for a small amount of calcium uptake.

S1 System

The above studies were performed on a microsomal fraction isolated by ultracentrifugation from S1 and assayed for calcium uptake by the membrane filtration assay. It appeared reasonable that direct filtration of S1 would accomplish isolation of the endoplasmic reticulum vesicles. Thus, calcium uptake studies were performed using the S1 (rather than the microsomal fraction) directly in the assay.

Comparison of Calcium Uptake Activities in S1 and Microsomal Fractions—Calcium uptake using S1 in the assay was compared to uptake by microsomes which had been isolated from S1 (Fig. 1). Both uptakes were calculated on the basis of microsomal protein which was 8.8 (±0.6)% of total S1 protein (n = 11). S1 demonstrated an active uptake of calcium which...
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was linear for 20 to 30 min at all calcium concentrations tested (3 to 100 μM). The calcium uptake at 20 min using S1 was approximately 100 times that seen using the microsomes. Addition of microsomal supernatant (“cytosol” fraction) to the isolated microsomes to produce a “reconstituted” S1 fraction did not increase the calcium uptake by the isolated microsomes and routinely produced a slight depression of the uptake. The cytosol fraction itself showed no calcium uptake.

In characterizing the S1 system, it was necessary to confirm that the uptake seen was attributable to endoplasmic reticulum and not to the small amount of contaminating organelles. This was investigated using three independent approaches: comparative morphologic examination of the vesicles isolated by centrifugation and filtration; measurement of calcium uptake using isolation of endoplasmic reticulum by the usual high speed centrifugation to terminate the uptake; and the use of selective inhibitors of calcium uptake.

Electron Microscopy of Material Obtained from S1 by Centrifugation and by Filtration—Electron microscopic examination of the structures isolated from S1 either by high speed centrifugation (microsomes, Fig. 2A) or by filtration (Fig. 2B) revealed morphologically similar, variably sized, smooth vesicles without visible mitochondria. No large vesicles with the invaginations characteristic of plasma membrane vesicles were seen. Examination of material recovered from membrane filters after carrying S1 through the assay procedure revealed these same vesicles.

Comparison of Centrifugation and Filtration Systems in Assay of Calcium Uptake by S1—Aliquots of S1 were incubated for 10 or 20 min in standard assay medium and calcium uptake was determined by isolating the vesicles either by filtration or by high speed centrifugation. The amounts of calcium uptake measured using these two separation techniques were essentially identical (Table I).

Effects of Selected Inhibitors on Calcium Uptake—The uptake in S1 was decreased by less than 5% by ruthenium red and by less than 3% by sodium azide at concentrations which blocked over 99% of mitochondrial calcium uptake (Table II). These effects of mitochondrial inhibitors on the S1 uptake are in contrast to their effects on the isolated microsomes. The amount of uptake in S1 blocked by the mitochondrial inhibitors was consistent with the small mitochondrial contamination of the fraction and clearly indicated that the uptake was primarily non-mitochondrial. The sulfhydryl inhibitor p-chloromercuribenzenesulfonate produced a dose-dependent inhibition of the S1 calcium uptake with 50% inhibition at a concentration of 10 μM.

**Table I**

| Length of incubation (min) | Centrifugation | Filtration |
|---------------------------|---------------|------------|
| 10                        | 34            | 32         |
| 20                        | 83            | 82         |

**Fig. 1.** Time course of calcium uptake using S1 (○) and microsomes (□). Calcium uptake was initiated by addition of protein to the standard assay medium containing 11 μM calcium. The assay was terminated by filtration as described under “Experimental Procedures.” The S1 was used immediately after preparation. Microsomes isolated from an aliquot of S1 by centrifugation were resuspended to the original volume in Tris/sucrose and assayed immediately. In both cases, the uptake was expressed as nanomoles of calcium/mg of microsomal protein.

**Fig. 2.** Electron microscopic appearance of material isolated from S1 by centrifugation (A) and by Millipore filtration (B). The vesicles were resuspended and examined using negative staining as described under “Experimental Procedures.” × 27,000.
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Microsomal calcium uptake was measured in the standard assay in the presence and absence of the indicated concentrations of inhibitors at 5 to 10 μM calcium. Calcium uptake by isolated mitochondria was measured as described under "Experimental Procedures" using 5 μM calcium. Results are expressed as per cent of calcium uptake in the absence of inhibitors. Control values for mitochondrial calcium uptake averaged 31.1 (±0.5) nmol of calcium/mg of protein/20 min. Each value represents the mean of at least triplicate determinations.

| Inhibitor | Calcium uptake | Mitochondria |
|-----------|----------------|--------------|
| Ruthenium red (5 μM) | 95.1 | 0.3 |
| Sodium azide (10 mM) | 97.2 | 0.8 |
| p-Chloromercuribenzoate sulphone (50 μM) | 1.1 | 3.0 |

The calcium uptake demonstrated in S1 was associated with the endoplasmic reticulum as judged by the morphologic appearance, sedimentation properties, and the characteristic inhibition pattern. This uptake was characterized in further studies.

**Mg<sup>2+</sup> and ATP Dependency of Calcium Uptake** – Calcium uptake was dependent upon Mg<sup>2+</sup> and ATP (Table III). The omission of either ATP or MgCl<sub>2</sub> resulted in uptake less than 10% of control values. Substitution of NaCl for ATP (used as the disodium salt) did not support calcium uptake. The amount of calcium associated with the endoplasmic reticulum in the absence of Mg<sup>2+</sup> and ATP was comparable to that found in studies of passive calcium binding to isolated microsomes. The addition of MgCl<sub>2</sub> alone (Table III) lowered the binding, consistent with the previous demonstration of inhibition by Mg<sup>2+</sup> of passive binding of calcium by isolated endoplasmic reticulum (27). The addition of ATP alone increased the calcium associated with microsomes. The K<sub>M</sub> for MgATP in this system appeared to be below 0.5 mM since there was little variation in the rate of calcium uptake with equimolar concentrations of MgCl<sub>2</sub> and ATP of 0.5 to 10 mM (not shown).

**Dependence of Calcium Uptake on Concentration of Calcium** – The rate of calcium uptake by endoplasmic reticulum in the S1 fraction was dose-dependent and saturable (Fig. 3). Double-reciprocal analysis yielded a mean K<sub>M</sub> of 11.8 (±6.1) μM total calcium and maximum velocity of 97 (±16) nmol of calcium/mg of protein per 20 min in seven experiments at pH 6.8 or 7.0 using five preparations of S1. The K<sub>M</sub> was 3.6 ± 1.0 μM free calcium using ionized calcium concentrations calculated at the pH of the assay according to the method of Katz et al. (26) and verified by Moore et al. (14) to account for calcium formed in a complex in the presence of 5 mM magnesium and ATP. A similar K<sub>M</sub> of 1.2 μM calcium was found using Ca/EGTA buffers as described by Katz et al. at pH 6.8 (26). The K<sub>M</sub> values agreed quite well considering the variables and assumptions used in the calculations of free calcium. The apparent K<sub>M</sub> was also a function of the oxalate concentration used, as described below. Calcium uptake in this system was maximal at 50 μM CaCl<sub>2</sub>. When 5 mM rather than 10 mM oxalate was used, calcium concentrations greater than 50 μM were inhibitory. Similar inhibition of calcium uptake by suprapotential calcium concentrations has been described in detail for sarcoplasmic reticulum (28).

**Effects of Oxalate on Calcium Uptake** – Oxalate was necessary for the demonstration of sustained calcium uptake (Fig. 4). In the absence of oxalate, the calcium accumulating ability gradually diminished and the accumulated calcium was lost from the endoplasmic reticulum vesicles (Fig. 4). Similar findings have been reported for endoplasmic reticulum from other tissues including muscle (29). The calcium uptake at 20 min in the absence of oxalate averaged 5% of that in the presence of 10 mM oxalate under the conditions described in the legend to Fig. 4 (n = 5). Such stimulation of calcium uptake by oxalate has been interpreted as indicating active calcium translocation as contrasted to cation binding by membranes (23). When the oxalate concentration was varied between 2.5 and 20 mM, the calcium uptake averaged 31.1 (±0.5) nmol of calcium/mg of protein/20 min.
the rate of calcium uptake increased approximately 2-fold for each doubling of the concentration of oxalate. The linear dependence of rate of calcium uptake upon oxalate concentration was observed at all calcium concentrations tested between 6 and 100 μM.

The kinetic properties of the uptake were investigated in studies at 5 and 10 mM oxalate (Fig. 5). These concentrations were chosen to allow comparison to calcium uptake by endoplasmic reticulum from other tissues. The maximum velocity of calcium uptake using 5 mM oxalate was half that found at 10 mM oxalate and the apparent $K_m$ was slightly (20%) lower at the lower oxalate concentration (Fig. 5).

**Temperature Dependency of Calcium Uptake** — Calcium uptake was strongly temperature-dependent. At 4°C the uptake was comparable to the level of passive energy-independent calcium binding to microsomes (27) and was less than 3% of the uptake at the standard incubation temperature of 24°C. Peak calcium uptake was observed at 37°C and was twice that at 24°C. At this temperature, the time course of calcium uptake was essentially linear through 20 min. Double-reciprocal plots of data from experiments at 24°C and 37°C, using the same preparation of S1, demonstrated an approximate doubling of the $V_{max}$ at 37°C. Analysis of the data by Arrhenius plots resulted in a $Q_{10}$ of 1.8 in the temperature range 24–37°C.

**Dependency of Calcium Uptake on pH** — Calcium uptake was maximal at pH 7.0, being 25% decreased at pH 6.5, 15% at pH 7.5, and 80% at pH 6.0. Measurements of pH during the course of calcium uptake demonstrated a fall of less than 0.1 unit over 30 min under standard conditions at various calcium concentrations, illustrating that the buffering capacity was adequate.

**Stability of Calcium Accumulating Function** — The calcium uptake activity of microsomes in S1 was extremely labile. Fig. 6 illustrates that preincubation of S1 at 4°C, 24°C, and 37°C produced a time- and temperature-dependent loss of the calcium-accumulating function. At 24°C and 37°C the loss of activity was marked within 5 min. In contrast, calcium uptake was linear during 20 to 30 min assays at both 24°C and 37°C (e.g. Fig. 1). This suggested a protective effect on the calcium uptake system of some component(s) in the assay medium. This was investigated by preincubating S1 in the presence of various components of the assay system and assaying the residual uptake activity (Table IV). ATP alone or with magnesium did not protect the uptake. In contrast, the inclusion of oxalate in combination with magnesium and ATP consistently produced a near-total protection of the calcium-accumulating ability.
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S1 was preincubated at 24°C for 10 min in the presence or absence of combinations of MgCl₂ (5 mM), ATP (5 mM), and potassium oxalate (10 mM). After this preincubation, 50 μl of S1 were assayed for remaining calcium uptake activity in the standard assay system using 30 μM calcium (total assay volume 500 μl). The incremental amounts of Mg²⁺, ATP, and oxalate introduced into the final assay were negligible owing to the 10-fold dilution. Values are expressed as percentage of uptake observed with S1 which had not been preincubated. Each value represents the mean (±S.E.) of four experiments, each performed in triplicate.

| Additions           | Calcium uptake remaining
|---------------------|--------------------------|
|                     | % control                |
| None                | 60 ± 14                  |
| ATP                 | 72 ± 2                   |
| Mg²⁺ and ATP        | 51 ± 7                   |
| Mg²⁺, A1P, oxalate  | 92 ± 9                   |
| Oxalate             | 41 ± 8                   |

DISCUSSION

Active calcium uptake was demonstrated in the S1 (20,000 × g supernatant) fraction of adipocyte homogenates. The uptake was localized to endoplasmic reticulum contained in S1 on the basis of morphology, sedimentation, and sensitivity to inhibitors. This uptake was extremely labile and could not be demonstrated in endoplasmic reticulum vesicles isolated from S1 as a microsomal fraction. This indicates the potential for fractionation artifacts in studies of calcium uptake by the isolated endoplasmic reticulum. Absence of uptake may be misleading and the magnitude of observed uptake underestimated. The latter problem has been documented and investigated in sarcoplasmic reticulum (11, 29, 30). Defined subcellular fractions similar to the S1 of adipocytes might be useful in studies with other cells (cf. Refs. 29 and 30 for cardiac tissue).

The properties of the calcium accumulation by adipocyte endoplasmic reticulum were indicative of an energy-dependent, carrier-mediated translocation process: the uptake was temperature-dependent, required ATP and Mg²⁺, demonstrated saturation kinetics, and was sustained in the presence of oxalate. The calcium-accumulating process was clearly distinguishable from passive calcium binding to adipocyte endoplasmic reticulum since the binding was inhibited by Mg²⁺ and reached steady state within 20 min. Simple exchange of ⁴⁰Ca for endogenous calcium (as opposed to uptake) could be excluded because the V₅₅₅ of approximately 200 nmol (mg protein)⁻¹ (30 min)⁻¹ at 37°C far exceeded the calcium content (18 nmol/mg of protein) of the isolated endoplasmic reticulum (31) as well as the maximum binding capacity of 30 nmol/mg of protein for passive ⁴⁰Ca binding to isolated endoplasmic reticulum (27).

The kinetic properties of calcium uptake into adipocyte endoplasmic reticulum may be compared to those reported for other cell types. The Kₘ for calcium uptake in the present study was 12 μM total calcium and approximately 1.2 μM ionized calcium using Ca/EGTA buffers. This value was in excellent agreement with the Kₘ reported for cardiac sarcoplasmic reticulum in a study employing a system similar to that used in the present report and utilizing Ca/EGTA buffers (28). In studies of other tissues, the Kₘ reported for calcium uptake by endoplasmic reticulum has ranged from less than 1 μM ionized to approximately 25 μM total calcium (11, 13, 14). Allowing for differences attributable to the use of Ca/EGTA buffers, variations in the concentration of oxalate and ATP, etc., the Kₘ of each of these systems approximates estimated cytosol calcium concentrations (9). In contrast, a marked difference exists between the maximum velocities (V₅₅₅) reported for most sarcoplasmic reticulum preparations and those for endoplasmic reticulum from isolated adipocytes and from nonmuscular tissues. The V₅₅₅ observed in the present studies was 2 to 10 nmol (mg of protein)⁻¹ min⁻¹, similar to the values of 11 and 5.5 nmol (mg of protein)⁻¹ min⁻¹ reported for liver (14) and kidney (13), respectively. In contrast, the reported maximum velocities for sarcoplasmic reticulum range from approximately 800 to 3000 nmol (mg of protein)⁻¹ min⁻¹ (11) in comparable studies.

Strong similarities were noted between the properties of the calcium uptake and those of the passive calcium binding to the endoplasmic reticulum. The Kₘ values for ionized calcium in the transport system both in the presence (1.2 μM) and absence (3.6 μM) of Ca/EGTA buffers were similar to the Kₘ of 2 to 4 μM for passive binding of calcium to the high affinity sites of the endoplasmic reticulum (27). The V₅₅₅ observed for the adipocyte endoplasmic reticulum was approximately 2 orders of magnitude less than that reported for sarcoplasmic reticulum. This same 100-fold difference was observed between the maximum (passive) calcium-binding capacities of adipocyte microsomes and sarcoplasmic reticulum (27). Finally, the pH optimum was approximately 7.0 for both the uptake and the passive binding to the high affinity sites. These similarities suggest that the high affinity binding sites could represent the first step in the transport process and by their saturation place a limit on the rate of transport.

The data suggest that the endoplasmic reticulum of adipocytes plays an important role in cellular calcium regulation. This is supported by the previous demonstration by Hales et al. (16) that adipocyte calcium ultrastructurally appears to be concentrated in the endoplasmic reticulum network. This pool of calcium must be considered in interpreting studies of calcium fluxes using intact adipocytes with (32, 33) and without (34) hormones. In previous studies from this laboratory insulin treatment of adipocytes resulted in increased calcium binding to (27) and content of (31) the isolated endoplasmic reticulum. Preliminary data indicate an insulin-induced stimulation of the calcium uptake system (35). These findings indicate that intracellular calcium regulation by the endoplasmic reticulum is hormonally responsive. Characterization of this system will allow investigation of the role of endoplasmic reticulum in both calcium homeostasis and the mechanism of hormone action.

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