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COMPARISON OF THE PROPERTIES OF ACTIVE Ca^{2+} TRANSPORT BY THE ISLET-CELL ENDOPLASMIC RETICULUM AND PLASMA MEMBRANE

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The properties of active or ATP-dependent calcium transport by islet-cell endoplasmic reticulum and plasma membrane-enriched subcellular fractions were directly compared. These studies indicate that the active calcium transport systems of the two membranes are fundamentally distinct. In contrast to calcium uptake by the endoplasmic reticulum-enriched fraction, calcium uptake by islet-cell plasma membrane-enriched vesicles exhibited a different pH optimum, was not sustained by oxalate, and showed an approximate 30-fold greater affinity for ionized calcium. A similar difference in affinity for calcium was exhibited by the Ca^{2+}-stimulated ATPase activities which are associated with these islet-cell subcellular fractions. Consistent with the effects of calmodulin on calcium transport, calmodulin stimulated Ca^{2+}-ATPase in the plasma membranes, but did not increase calcium-stimulated ATPase activity in the endoplasmic reticulum membranes. The physiological significance of the differences observed in calcium transport by the endoplasmic reticulum and plasma membrane fractions relative to the regulation of insulin secretion by the islets of Langerhans is discussed.

Introduction

Calcium concentrations in the β-cell are thought to play a central role in the regulation of stimulus-secretion coupling in a manner analogous to the role of calcium in excitation-contraction in the muscle fiber [1]. Cellular calcium concentrations may be regulated by altering the rate of calcium entry or the rate of calcium efflux at the plasma membrane, as well as, by changes in the handling of calcium by cellular organelles. Secretagogue-induced insulin secretion is thought to be accompanied by the opening of voltage-dependent calcium channels which rapidly increases the rate of calcium entry into the β-cell [2]. Calcium efflux from the β-cells may occur through Na^+-Ca^{2+} exchange [3] as well as by active calcium extrusion by the plasma membrane calcium pump [4]. Various subcellular organelles including the endoplasmic reticulum, mitochondria, and secretory granules [5] may be involved in the dynamic regulation of β-cell calcium levels.

Studies of calcium fluxes in intact islets have shown that glucose-stimulated insulin secretion is accompanied by complex alterations in the rate of calcium influx and efflux, suggesting that glucose stimulation changes calcium handling both at the plasma membrane and at subcellular sites [1,6]. Studies using intact islets, however, are restricted in their ability to elucidate the precise control mechanisms which are involved in the regulation of cellular calcium concentrations.

We have therefore developed a technique for simultaneous preparation of islet-cell subcellular fractions which are enriched in endoplasmic reticulum or plasma membrane [7,8] to directly

Abbreviations: EGTA, ethyleneglycol bis(β-aminoethyl ether)-*N*,*N*′-tetraacetic acid; Mes, 2-(*N*-morpholino)ethanesulfonic acid; Pipes, piperazine-*N*,*N*′-bis(2-ethanesulfonic acid).

evaluate the active calcium transporting systems of these membranes. The calcium transport system of the β-cell endoplasmic reticulum has been recently characterized [8]. Previous communications have also described calcium uptake into inside-out vesicles of plasma membrane and the plasma membrane $(Ca^{2+} + Mg^{2+})$-ATPase activity believed to be the enzymatic representation of this process [4]. One major difference between the active calcium transport systems of the endoplasmic reticulum and the plasma membrane is that neither calcium transport, nor the calcium-stimulated ATPase activity associated with the endoplasmic reticulum, is stimulated by calmodulin [10], whereas both of these processes in the plasma membrane are stimulated by this calcium binding protein [4,9]. Additionally, calcium chelated by EGTA (Ca-EGTA), which has been shown to stimulate red blood cell plasma membrane Ca^{2+} transport and $(Ca^{2+} + Mg^{2+})$-ATPase activity in a manner similar to calmodulin [11,12], stimulates these processes in the islet-cell plasma membrane without affecting calcium transport in the endoplasmic reticulum [13,14]. The experiments described herein have taken advantage of the capability of obtaining cell fractions enriched in both types of membranes from the same preparations of islets to further directly compare and contrast the calcium transport properties of the islet-cell endoplasmic reticulum and plasma membrane.

Materials and Methods

Materials

Male Wistar rats (200–300 g) were purchased from Charles Rivers Laboratories (Wilmington, MA). Collagenase (CLS IV) was obtained from Worthington, $^{45}CaCl_2$ from New England Nuclear, [γ-^{32}P]ATP from Amersham, filters (pore size 0.22 μm) from Millipore (Bedford, MA), tissue culture medium CMRL 1066 from Gibco Laboratories (Grand Island, NY), calmodulin from Calbiochem (La Jolla, CA), and vanadate-free ATP and other chemicals were obtained from Sigma Chemical Company (St. Louis, MO).

Isolation of membrane fractions

For each subcellular fractionation, islets from 30–40 rats were isolated by collagenase digestion [15] and harvested following purification on Ficoll gradients [16]. Isolated islets (4–7 mg total protein) were then cultured overnight in CMRL 1066 at 24°C in an atmosphere of 95% air/5% CO_2. Subcellular fractionation was performed by the method of Naber et al. [7], with modifications as previously described [8]. The endoplasmic reticulum-enriched fraction contained a 2–3-fold enrichement of endoplasmic reticulum marker enzymes (NADH-cytochrome *c* reductase and glucose-6-phosphatase). Although there was some contamination of this fraction with plasma membrane marker (5′-nucleotidase), this fraction contained virtually no secretory granules or mitochondria [8]. The plasma membrane-enriched fraction contained 5–11-fold enrichment of plasma membrane markers (5′-nucleotidase and bound ^{125}I-wheat germ agglutinin), and had no contamination with secretory granules or mitochondria. Both endoplasmic reticulum and plasma membrane-enriched fractions were rehomogenized (Kontes No. 19 AAA homogenizer with teflon pestle) in isotonic buffer (50 mM Mes, 250 mM sucrose, pH 7.2) prior to assay for calcium transport. Calcium uptake assays were performed on the same day as the fractionation, while ATPase assays were performed on frozen membranes, resuspended in hypotonic buffer, that had been sonicated (for 5 s at 19 watt, Branson sonifier) and were transport incompetent. Protein was measured with fluorescamine [17] using bovine serum albumin as standard.

Measurement of calcium uptake

The standard assay medium (100 μl) for the endoplasmic reticulum contained 50 mM Tris (pH 6.8 at 37°C), 100 mM KCl, 5 mM $MgCl_2$, 0.1 mM EGTA, 0.01–0.12 mM $CaCl_2$ (1–2 μCi $^{45}Ca^{2+}$/tube), with or without 1.25 mM ATP [8]. Unless otherwise indicated, for incubations employing the plasma membrane, the Tris buffer was replaced with 50 mM Tris-Pipes (pH 7.5 at 37°C) and KCl was replaced with 200 mM sucrose. Although K^+ stimulated calcium uptake by the endoplasmic reticulum, it does not affect active calcium uptake by the plasma membrane [8]. Incubations were initiated by addition of 2–3 μg membrane protein and terminated by filtration over 0.22 μm Millipore filters. The filters were

immediately washed twice with 5 ml of 250 mM sucrose, 40 mM NaCl (endoplasmic reticulum) or 300 mM sucrose (plasma membrane), air dried, and processed for liquid scintillation counting.

Calcium-stimulated ATPase

Ca^{2+}-ATPase activity was measured by a modification [9] of the technique of Seals et al. [18], which measures liberation of $^{32}P_i$ from [γ-^{32}P]ATP. Briefly, the standard assay medium (100 μl) contained 50 mM Tris (pH 6.8 at 37°C for the endoplasmic reticulum) or 50 mM Tris-Pipes (pH 7.5 at 37°C, for the plasma membrane), 20 mM NaN_3, 0.2 mM EGTA, 0.1–0.5 mM $CaCl_2$, and 0.25 mM ATP (1.0 μCi ^{32}P per tube). The reaction was initiated by addition of 0.5–1 μg membrane protein and terminated after 10–30 min by the addition of 50 μl 3% SDS. Ca^{2+}-stimulated ATPase activity was defined as the ATP hydrolyzed in the presence of Ca^{2+}, minus the hydrolysis which occurred in the presence of 0.2 mM EGTA alone.

Calcium-stimulated ATPase activity was measured with 9 μM magnesium (i.e., endogenous concentration as determined by atomic absorption spectrophotometry) and at 100 μM magnesium. Magnesium was added to the assay to approximate conditions where calcium uptake could be measured [8]. However, higher concentrations of magnesium (> 100 μM) increased the background Mg^{2+}-ATPase activity in both membrane preparations to a level that often obscured the calcium-stimulated activity.

Ionized calcium levels were calculated as previously described [8] after the method described by Schatzmann [19]. The stability constant for Ca-EGTA was taken to be $10^{10.56}$ with protonization values α_1 and α_2 of $10^{9.38}$ and $10^{8.77}$, respectively. The following association constants were also used: $[Mg \cdot ATP^{2-}]/[Mg^{2+}][ATP^{4-}] = 10^{4.94}$ and $[Ca \cdot ATP^{2-}]/[Ca^{2+}][ATP^{4-}] = 10^{4.5}$ [20]; $[Mg \cdot oxalate]/[Mg^{2+}][oxalate] = 10^{2.55}$, and $[Ca \cdot oxalate]/[Ca^{2+}][oxalate] = 10^{2.3}$ [21]. Calcium stock solutions were standardized by atomic absorption spectrophotometry. The accuracy of the calculated free calcium concentrations was confirmed with a calcium selective electrode (Orion Model 93-20; Ref. 22). Theoretical free calcium concentrations in the presence of EGTA fell on a line which was extrapolated from calcium standards measured in the absence of EGTA.

Statistical analysis was by analysis of variance or Student's t analysis, as appropriate. Unless stated otherwise, 'N' refers to the number of individual tissue preparations which were used for a particular experiment.

Results

Active calcium uptake

The pH optimums for active calcium uptake by the endoplasmic reticulum and the plasma membrane-enriched fractions were directly compared at saturating calcium concentrations in the absence of EGTA. As shown in Fig. 1, calcium uptake by the endoplasmic reticulum demonstrated a sharp pH optimum at pH 6.8; the rate of calcium uptake by the endoplasmic reticulum declined pre-

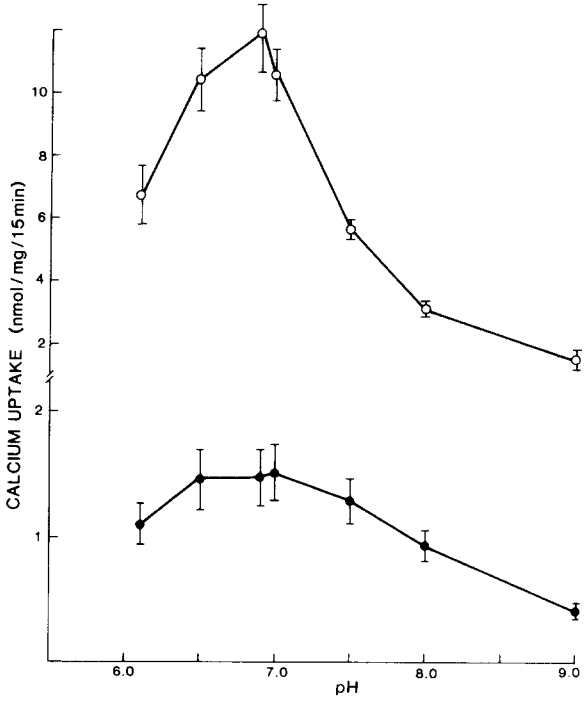

Fig. 1. pH optimum of calcium uptake in islet-cell endoplasmic reticulum (○) and plasma membrane (●)-enriched fractions. Calcium uptake assays were performed using the islet-cell fractions as described in Methods. 15-min assays were conducted at saturating calcium concentrations (40 μM) in the absence of EGTA. The pH was maintained as indicated on the abscissa by 50 mM Tris-Pipes. Data represent the mean ± S.E. of three experiments each performed in triplicate.

cipitously at pH greater than 7.0. In contrast, calcium uptake by the plasma membrane had a much broader pH optimum, and the rate of calcium uptake by the plasma membrane fraction declined significantly only at pH > 8.0. For these reasons, and in order to minimize the contribution of contaminating membranes in the respective assays, calcium uptake by the endoplasmic reticulum and plasma membrane fractions was studied at pH 6.8 and 7.5, respectively.

Previous studies had demonstrated that the addition of oxalate, as a permeant anion, to the incubation medium was essential to maintain linearity (> 30 min) of the rate of active calcium uptake by the islet-cell endoplasmic reticulum in incubations which exceed 5 min [8]. The effect of oxalate on calcium uptake by the islet-cell plasma membrane had not previously been investigated.

Fig. 2 shows that the rate of ATP-stimulated calcium uptake by islet-cell plasma membrane vesicles was linear with time for 15 min (upper panel). Calcium uptake by these vesicles was not affected significantly by the addition of 10 mM oxalate. In contrast, the rate of ATP-stimulated calcium uptake by the endoplasmic reticulum (lower panel, Fig. 2) was augmented by the addition of oxalate ($P < 0.05$, 15 min). In the absence of oxalate, net calcium accumulation by the endoplasmic reticulum did not increase significantly after 5 min incubation at 37°C.

The affinities of the two transport systems for ionized calcium were determined in parallel 15-min incubations. Although oxalate was not required to maintain the rate of calcium uptake by the islet-cell plasma membrane fraction, it was included in incubations for both tissues, such that assay conditions would be similar. The dependence of the rate of calcium uptake by the endoplasmic reticulum and plasma membrane on medium Ca^{2+} concentration was found to differ markedly (Fig. 3). The apparent K_m for calcium uptake in the plasma membrane fraction was 0.05 ± 0.003 μM ionized calcium with a maximal velocity of 3.7 ± 0.5 nmol calcium/mg protein per 15 min. In the same three experiments, calcium uptake in the endoplasmic reticulum fraction had a maximal velocity of 8.7 ± 1.2 nmol calcium/mg protein per 15 min with half maximal velocity occurring at 1.8 ± 0.1 μM ionized calcium. Thus, the affinity of the plasma membrane uptake system for calcium was more than 30-fold greater than that of the islet-cell endoplasmic reticulum.

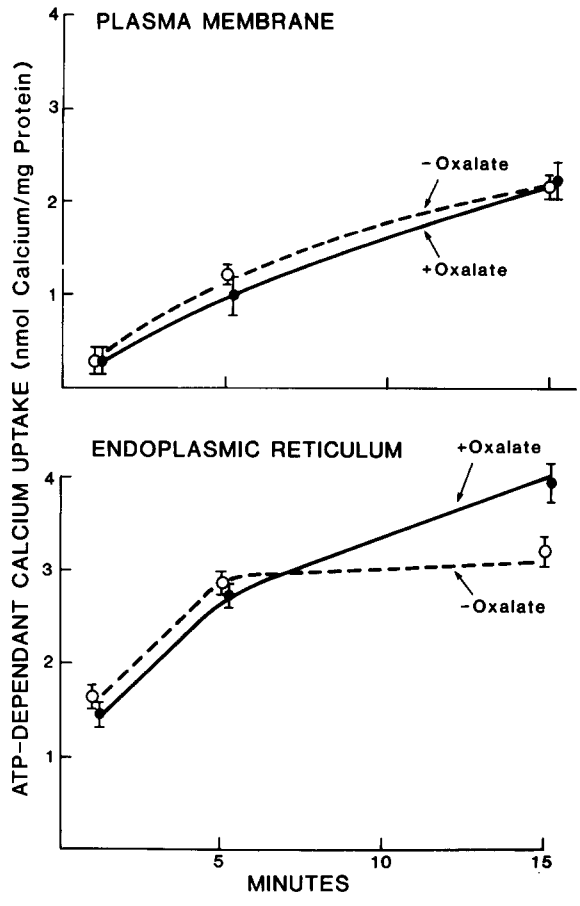

Fig. 2. The effect of oxalate on calcium uptake by the islet-cell endoplasmic reticulum and plasma membrane enriched fractions. Calcium uptake was measured as described in Methods. Incubations at 37°C were performed with (●———●) or without (O------O) 10 mM oxalate. Assays employed the plasma membrane (upper panel) or endoplasmic reticulum (lower panel) enriched fractions from the same four islet preparations. Data show the mean ± S.E. for triplicates of one experiment. Similar results were obtained in the other three experiments employing this protocol.

Energy-dependent calcium uptake in both systems showed a specific requirement for ATP. Thus, neither *p*-nitrophenyl phosphate (0.1–16 mM) nor UTP or GTP (1–4 mM) could substitute for ATP in experiments employing both the endoplasmic reticulum and plasma membrane fractions (data not shown). The dependence on ATP was similar for both uptake systems with half maximal activa-

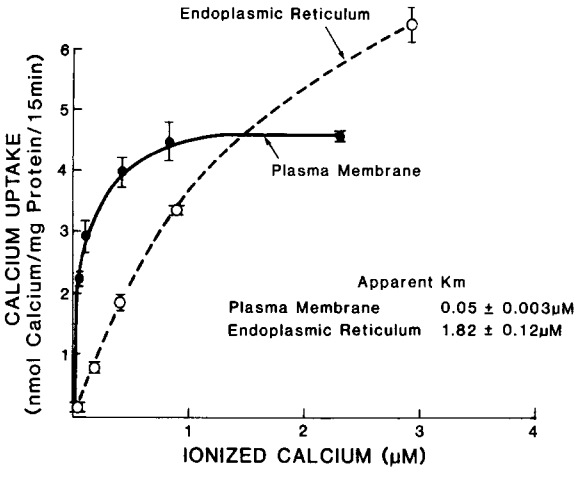

Fig. 3. Calcium concentration dependency of calcium uptake. Calcium uptake was measured in 15-min assays at 37°C as described in Methods. Free calcium concentrations were varied using 100 μM EGTA. Data represent mean ± S.E. of triplicate determinations for a representative experiment ($N = 3$). The average ± S.E. apparent K_m for the three experiments is shown in the insert.

tion occurring at 26 ± 6 μM and 27 ± 10 μM in the endoplasmic reticulum and plasma membrane fractions, respectively ($N = 3$, 0.025–1.0 mM ATP, data not shown).

We had demonstrated previously that increasing total EGTA and calcium concentrations in the incubation media, while maintaining constant free Ca^{2+} levels as verified by calcium ion electrode measurements, dramatically increased the velocity of the islet-cell plasma membrane ($Ca^{2+} + Mg^{2+}$)-ATPase as well as active Ca^{2+} uptake [13,14]. In contrast to the plasma membrane, both active Ca^{2+} uptake and Ca^{2+}-stimulated ATPase activities by the islet-cell endoplasmic reticulum were unaffected when assayed under identical conditions [14].

To characterize further the marked sensitivity to EGTA of active Ca^{2+} transport by the plasma membrane in contrast to the endoplasmic reticulum, experiments were performed to compare directly the rates of calcium uptake at saturating free Ca^{2+} (endoplasmic reticulum > 10 μM; plasma membrane > 3 μM) at various concentrations of EGTA. In three experiments, an increase of EGTA concentration (0, 0.5, 1, and 2 mM) produced a concentration-dependent increase in active calcium uptake by the plasma membrane fraction without significantly altering the rate of active calcium uptake by the endoplasmic reticulum fraction (Table I). The dose-dependent increase in plasma membrane active Ca^{2+} transport (maximum increase of 10-fold at 2 mM EGTA) is in agreement with results obtained previously showing a similar dose-dependent enhancement of the plasma membrane ($Ca^{2+} + Mg^{2+}$)-ATPase with increasing total EGTA and Ca^{2+} concentrations [14].

Calcium-stimulated ATPase

The dependence of the rate of calcium-stimulated ATPase activity in the endoplasmic reticulum fraction on ionized medium calcium was markedly different than that of the ($Ca^{2+} + Mg^{2+}$)-ATPase activity associated with the plasma membrane. While the enzyme activity associated with the endoplasmic reticulum fraction demonstrated a single affinity for calcium (3 ± 1 μM), the plasma membrane enzyme had both a low (52 ± 16 μM) and high (0.3 ± 0.2 μM) affinity for ionized calcium ($N = 3$) as presented in Table II. These kinetic properties were not changed when the assays were performed with 9 or 100 μM mag-

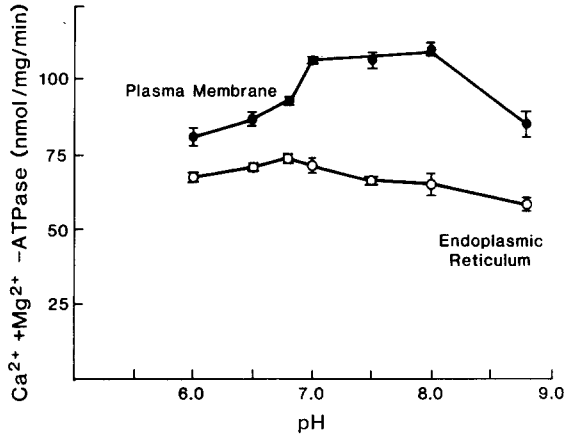

Fig. 4. pH optimum of Ca^{2+}-ATPase activity in endoplasmic reticulum and plasma membrane-enriched fractions. Ca^{2+}-stimulated ATPase activity was measured in 30-min assays as described in Methods. Free calcium concentrations were maintained at saturating levels (> 100 μM) for both enzyme activities with 2.1 mM $CaCl_2$ and 2.0 mM EGTA. The pH was maintained as indicated with 50 mM Tris-Pipes. Under these conditions the plasma membrane enzyme exhibits a single high affinity for free Ca^{2+}. Data represent mean ± S.E. of three experiments.

TABLE I

EFFECT OF Ca-EGTA ON ATP-DEPENDENT Ca^{2+} UPTAKE IN ISLET-CELL FRACTIONS AT SATURATING FREE CALCIUM CONCENTRATIONS

The rate of active calcium uptake was measured in 15-min assays at 37°C. Various concentrations of EGTA were used to maintain free Ca^{2+} concentrations which were 2.7–3.4 μM Ca^{2+} for the plasma membrane assays and 7–12.5 μM Ca^{2+} for assays employing the endoplasmic reticulum. In the absence of EGTA, free Ca^{2+} was 20 μM. Thus, all assays contained saturating concentrations of free Ca^{2+} (see also Fig. 3). Results indicate the mean ± S.E. ($N = 3$) ATP-dependent Ca^{2+} uptake. Calcium binding in the absence of ATP was not detectably altered by increasing concentrations of Ca-EGTA (not shown).

EGTA (mM)	Ca^{2+} uptake (nmol/mg per 15 min)	
	Plasma membrane	Endoplasmic reticulum
0	2.2 ± 0.4	15.6 ± 2.8
0.5	6.7 ± 0.8	18.7 ± 2.8
1.0	11.2 ± 2.4	20.4 ± 2.2
2.0	15.5 ± 3.3	23.1 ± 3.3

nesium. Similar differences were also observed when both enzyme activities were measured both at pH 6.8 and 7.5 ($N = 3$, data not shown).

In order to compare the pH optimum for Ca^{2+}-ATPase activities in the two fractions, simultaneous assays utilizing the endoplasmic reticulum and plasma membrane fractions were performed with 2.1 mM $CaCl_2$ buffered with 2 mM EGTA. This concentration of Ca-EGTA converts the plasma membrane enzyme to a single high affinity for Ca^{2+} and thus assures that both the endoplasmic reticulum enzyme and the high affinity plasma membrane activity are assayed at saturating free Ca^{2+} concentrations at all pH conditions studied [13,14]. Under these conditions, the plasma membrane enzyme exhibited a broad pH optimum with maximal activity occurring between pH 7 and 7.8. The endoplasmic reticulum enzyme also did not show a sharp pH optimum, but the activity declined above pH 7.0 (Fig. 4).

Vanadate (1–100 μM) did not significantly alter the Ca^{2+}-ATPase activity localized to either the plasma membrane or the endoplasmic reticulum. Vanadate was ineffective whether present during a 5-min preincubation, or when added directly to 10 min ATPase assays employing both islet-cell fractions, with or without 100 mM KCl.

While vanadate affected neither enzyme activity under these conditions, the effect of calmodulin on the two fractions differed markedly. As previously reported [9], the addition of calmodulin to the plasma membrane fraction increased calcium-stimulated ATPase activity in a concentration-dependent manner. In contrast, calmodulin did not stimulate the calcium-stimulated ATPase activity

TABLE II

DIFFERENCES IN ACTIVE CALCIUM TRANSPORT AND ATPase ACTIVITY BETWEEN ISLET-CELL FRACTIONS

This table constitutes a summary of the major differences in the properties of calcium transport and Ca^{2+}-ATPase activity in isolated islet-cell endoplasmic reticulum and plasma membrane-enriched fractions. Data are from this communication or are discussed elsewhere: (a) Ref. 10; (b) Ref. 4; (c) Ref. 9; (d) Ref. 8.

Experimental parameters	ATP-dependent Ca^{2+} transport		Ca^{2+}-ATPase	
	Endoplasmic reticulum	Plasma membrane	Endoplasmic reticulum	Plasma membrane
K_m (ionized Ca^{2+})	1.5–2 μM	0.04–0.07 μM	Single affinity 2–3 μM	Two affinities 0.1–0.3 μM (high) 37–55 μM (low)
Stimulated by calmodulin	no [a]	yes [b]	no	yes
Stimulated by Ca^{2+}-EGTA	no	yes	no [c]	yes [c]
Oxalate requirement	yes	no	–	–
K^+ (20 mM)	stimulation [d]	no effect [d]	stimulation [a]	inhibition [b]
K_m (ATP)	27 μM	26 μM	–	Two affinities for [b] ATP 2.3 and 70 μM

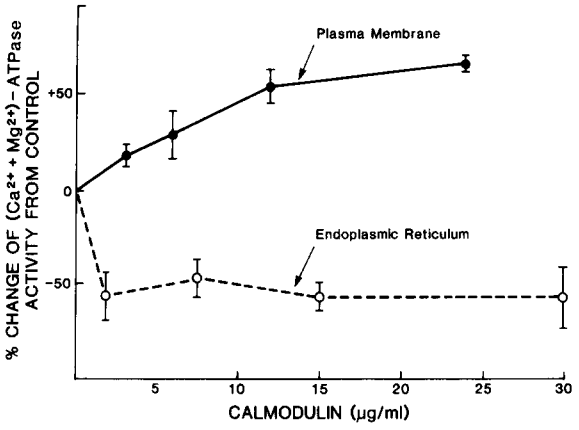

Fig. 5. The effect of calmodulin on calcium-stimulated ATPase activity in islet-cell subcellular fractions. The rate of Ca^{2+}-ATPase activity in islet-cell fractions enriched in plasma membrane or endoplasmic reticulum was determined as a function of the concentration of calmodulin. ATPase assays (15–30 min) were performed according to the protocol described in Methods at 100 μM magnesium and 1–1.8 μM free calcium. Data show the mean ± S.E. % change in the calcium-stimulated component of ATPase activity in four experiments.

in the endoplasmic reticulum (Fig. 5), but rather increased the magnesium or background ATPase activity. The effect of calmodulin on the blank or magnesium-ATPase activity was more pronounced in some preparations of the endoplasmic reticulum and was greater at higher concentrations of calmodulin. This effect served to reduce the measured calcium-stimulated ATPase activity in this fraction (Fig. 5).

Discussion

Direct comparison of active calcium transport by the islet-cell endoplasmic reticulum and plasma membrane subcellular fractions emphasized several basic differences between these two transport systems which are summarized in Table II. These differences, as well as others previously described, are consistent with the specific characteristics of endoplasmic reticulum and plasma membrane derived from other tissues, and may reflect the specific functions of the two transport systems.

As previously reported [8], oxalate was required as an intravesicular calcium trapping agent in order to linearize the rate of calcium uptake by the islet-cell endoplasmic reticulum with time. Oxalate had similar effects on calcium uptake by the endoplasmic reticulum at pH 6.8 and 7.5 (data not shown). The ineffectiveness of oxalate on calcium uptake by the islet-cell plasma membrane fraction, is consistent with the lack of effect of oxalate on calcium transport by sarcolemmal membranes [23–25]. The differential effect of oxalate on calcium uptake may reflect a lower permeability of the plasma membrane to oxalate in comparison to the endoplasmic reticulum [24,26]. Also, it should be noted that calcium accumulated by the sarcoplasmic reticulum is capable of rapidly inhibiting further uptake by a process known as 'back inhibition' [27]; this is theoretically avoided when the build up of vesicular free calcium is controlled by the intravesicular precipitation of calcium oxalate, allowing calcium uptake to proceed. Whereas 'back inhibition' may be envisioned as an important control mechanism for regulating the degree of calcium uptake by the endoplasmic reticulum, such a mechanism would be of less value at the plasma membrane where calcium must be extruded against a larger gradient into an essentially constant extracellular environment.

Calcium transport by the islet-cell plasma membrane was maximally activated at a much lower calcium concentration than was the transport system of the endoplasmic reticulum. A similar phenomenon has been observed for membranes isolated from adipocytes [28], liver [29] and muscle [23–25]. The higher affinity of the plasma membrane transport system for calcium is consistent with the hypothesis that the plasma membrane calcium extrusion pump is involved in maintaining resting state calcium concentrations which may be on the order of 0.1 μM [30]. Calcium uptake by the endoplasmic reticulum would become significant during the stimulated secretory state when cellular calcium concentrations increase [1]. Recent studies using islets made permeable by electric discharge have indicated that μM concentrations of calcium are needed to elicit an insulin secretory response [31].

We have previously demonstrated that K^+ stimulated active calcium uptake by the islet-cell endoplasmic reticulum, but does not increase ATP-dependent calcium uptake by plasma membrane vesicles assayed under the same conditions [8]. Sensitivity to K^+ is known to be an intrinsic

property of the sarcoplasmic reticulum [32]. Thus, altered cellular concentrations of K^+ which occur in the stimulated β-cell [33] may regulate calcium handling by the endoplasmic reticulum [34,35].

Calcium-stimulated ATPase activity in the islet-cell plasma membrane and endoplasmic reticulum fractions, which may represent the enzymatic activity of the respective calcium pumps, showed a different dependency on medium calcium which was similar to the differences manifested by calcium uptake (Table II). Thus, the plasma membrane Ca^{2+}-ATPase was activated by lower calcium concentrations than the activity associated with the endoplasmic reticulum. Similar to plasma membrane derived from other tissues [36], the islet-cell plasma membrane Ca^{2+}-ATPase may display two affinities for calcium, whereas the endoplasmic reticulum enzyme has a single affinity for calcium.

As reported previously, calmodulin stimulated the (Ca^{2+} + Mg^{2+})-ATPase activity of the plasma membrane [9], but did not increase calcium-stimulated ATPase activity in the endoplasmic reticulum [10]. Although we have not conclusively established that this enzymatic activity in the endoplasmic reticulum represents the calcium transporting enzyme, these observations are consistent with the fact that calmodulin has been found to stimulate calcium uptake by the islet-cell plasma membrane [4], but does not affect calcium uptake by the endoplasmic reticulum [10]. In general, calcium transport by membranes derived from other tissues indicates that while calmodulin regulates the plasma membrane calcium pump [37], it does not affect calcium transport by the endoplasmic reticulum [23,25,28,38]. Consistent with these results, calcium complexed by EGTA, i.e., independent of ionized calcium in the medium, stimulates the islet-cell plasma membrane Ca^{2+}-ATPase [13,14], in a manner similar to the effect of Ca-EGTA on the red blood cell plasma membrane (Ca^{2+} + Mg^{2+})-ATPase [11,12], but does not significantly affect the endoplasmic reticulum Ca^{2+}-ATPase [13,14]. We also found that Ca-EGTA stimulated the maximum velocity of active calcium uptake by the plasma membrane vesicles in a concentration-dependent manner similar to the dose-dependent activation of the plasma membrane Ca^{2+}-ATPase, but did not significantly alter calcium uptake by the endoplasmic reticulum.

In conclusion, these studies have focused on a direct comparison of the calcium handling properties of the islet-cell endoplasmic reticulum and plasma membrane. Calcium uptake by the two systems has been found to differ in terms of pH optimum, sensitivity to oxalate, affinity for ionized calcium, and stimulation by calmodulin, Ca-EGTA, and K^+. These properties probably reflect the different functions of these membranes in vivo. Further characterization of the properties of these isolated systems should further our knowledge of the factors regulating β-cell calcium homeostasis and the changing calcium concentrations which regulate the secretion of insulin from the β-cell.

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