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Characteristics of a Ca^{2+}-ATPase activity measured in islet homogenates

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Ca^{2+}-ATPase activity was measured in rat islet homogenates, in a medium of low ionic strength containing a low concentration of Ca^{2+} and Mg^{2+} and devoid of K^+. The enzyme activity was highly sensitive to inhibition by compound 48/80 (a calmodulin inhibitor), stimulated by 120 nM calmodulin and slightly affected by 10 mM NaN_3. The addition of Mg^{2+} to the assay medium promotes the disappearance of apparent Ca^{2+}-ATPase activity. Ouabain (0.1 mM) did not modify this ATPase activity. The enzyme showed two kinetic components for Ca^{2+} as well as for ATP: one with high apparent affinity and low maximum velocity and the other with low apparent affinity and high maximum velocity. Incubation of islet homogenates in this assay medium with [γ-^{32}P]ATP in the presence of proteolytic inhibitors, results in the appearance of a single labelled band of 130 kDa, identified by gel electrophoresis. The incorporation of ^{32}P into this band was similar in the presence of either 2.8 or 50 μM Ca^{2+} and susceptible to hydroxylamine attack. The results indicate that, under the conditions described above, the Ca^{2+}-ATPase activity evidenced in the islet homogenates had characteristics resembling those of the enzyme which catalyzes the outward Ca^{2+} transport. On the other hand, the method could provide a useful tool to test the effect of different agents which affect insulin secretion upon the islet plasma membrane Ca^{2+}-ATPase activity.

Introduction

We have previously shown [1] that the Ca^{2+}-ATPase activity exhibited by whole rat islet homogenates has characteristics similar to those obtained for the enzyme in partially purified membrane preparations from rat pancreatic islets, i.e., Ca^{2+} dependence, susceptibility to calmodulin inhibitors and inhibition of its activity by vanadate. We describe here the characteristics of the Ca^{2+}-ATPase activity from rat islet homogenates measured in a medium of low ionic strength containing a low concentration of Ca^{2+} and Mg^{2+} and devoid of K^+. This procedure, which allows the measurement of Ca^{2+}-ATPase activity directly in a crude islet homogenate, could provide a useful tool to test the transient effect of different insulin secretagogues and blockers on the enzyme activity.

Abbreviations: TLCK, *Nα-p*-tosyl-L-lysine chloromethyl ketone; PMSF, phenylmethylsulfonyl fluoride.

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Material and Methods

Materials

Collagenase was obtained from Serva, Heidelberg; Trasylol® (100 000 KIU), was kindly provided by Bayer Argentina. [γ-^{32}P]ATP was pre-

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pared according to the procedure of Glynn and Chappell [2], except that no unlabelled orthophosphate was added to the incubation mixture. ^{32}P-labelled orthophosphate was provided by the Comisión Nacional de Energia Atómica (Argentina). Calmodulin was purified from bovine brain as described by Kakiuchi et al. [3]. Ouabain, ATP, compound 48/80, protein inhibitors (TLCK and PMSF), electrophoresis reagents, enzymes and the cofactors used for the synthesis of [γ-^{32}P]ATP, were obtained from Sigma (U.S.A.). SDS was obtained from Fluka, XAR-5 X-ray film from Kodak and Hi-Plus enhancer screens from Ilford.

Methods

Animals and preparation of islets. Pancreases from fed male Wistar rats (approx. 200 g body weight) were used to obtain isolated islets by collagenase digestion [4].

Enzyme preparation. Groups of 400–500 islets were placed in a microhomogenizer (tissue grinder, Potter-Elvehjem, Teflon, size 18, rod o.d. 1/8 inch, 0.5 ml capacity, from Kontes Scientific Glassware Instruments) and washed twice at 4°C with 0.3 M sucrose/10 mM Tris-HCl (pH 7.24). The islets were then homogenized in 0.15 ml of the same buffer by 200 excursions of the plunger. This crude homogenate preparation was used in all the experiments described in this paper.

Ca^{2+}-ATPase assay. ATPase activity was measured in 0.5 ml of 50 mM Tris-HCl (pH 7.24 at 37°C), 0.1 mM ouabain, 1 mM [γ-^{32}P]ATP, 1 mM EGTA and several concentrations of $CaCl_2$ and other additions as shown in Results. Of the tissue homogenate, 0.05 ml containing 5–6 μg of protein (equivalent to 10 islets) was added to each assay tube. Ca^{2+}-ATPase activity was expressed as the difference between the activity measured in the above medium and that measured in the same medium without calcium. After 60 min of incubation at 37°C, the tubes were transferred to an ice/water bath and after 1 min each tube received 0.6 ml of isobutanol followed by 0.75 ml of 0.5% (w/v) ammonium molybdate in 5% (v/v) perchloric acid. After 15 s at 37°C, the mixture was vigorously stirred for 20 s and then spun down for 3 min at 1700 × *g* (4°C). The radioactivity was measured in an aliquot of the organic phase by liquid scintillation and the amount of inorganic phosphate liberated from ATP was calculated from this value. All determinations were performed in triplicate. Under these experimental conditions, no more than 4% of the ATP in the reaction mixture underwent enzymatic hydrolysis and the rate of appearance of ^{32}P remained constant up to 90 min of incubation time.

Free calcium was measured with a Ca^{2+}-sensitive electrode [5]. Protein was measured according to the method of Lowry et al. [6].

Phosphorylation procedure. Phosphorylation was carried out at 4°C in a medium containing 50 mM Tris-HCl (pH 7.20 at 4°C) and different reagents as described in the legend of each figure. The reaction was started by the addition of [γ-^{32}P]ATP (10 μM final concentration) under vigorous stirring and after 30 s was stopped by the addition of an ice-cold solution of 50 mM inorganic phosphate in 50% trichloroacetic acid. The tubes were then spun down at 3000 × *g* for 5 min and the precipitates obtained were then washed three times in the same solution and later processed for SDS-PAGE. For this purpose, they were dissolved in a medium containing 50 mM Tris-HCl (pH 7.0 at 37°C), SDS 5%, dithiothreitol 5%, glycerol 10% and Bromophenol blue, with or without the addition of proteolytic inhibitors as described in the legends of figures and incubated for 15 min at 37°C. Immediately after this procedure, the samples were transferred to an ice-cold bath until they were placed into the wells of the stacking gel. Electrophoresis was performed at pH 6.5 in discontinuous 1.5 mm thick slab gels. They were casted from a 5.6% acrylamide/0.2% bisacrylamide solution with 0.1 M sodium phosphate, 0.1 or 0.2% SDS, 0.05% Temed and 0.15% ammonium persulfate. Stacking gels had a similar composition, except that acrylamide concentration was 4.6%. The reservoir buffer contained 0.1 M sodium phosphate (pH 6.5) with 0.1–0.2% SDS. Migration of the sample components took place at 12°C, with a current of either 30 mA (0.1% SDS gels) or 40 mA (0.2% SDS gels) and was continued until the tracking dye reached a distance of about 8 cm from the top of the gel. Gels were stained overnight, in a cold room, with 0.05% Amido Black 10 B in methanol/acetic acid/water (4.5 : 1 : 4.5, v/v). Gels were then partially destained with methanol/acetic acid/water

(1.5 : 1 : 7.5, v/v) and dried under vacuum at room temperature. Radioactivity was visualized in Kodak XAR-5 X-ray films, each backed by an intensifying screen, and exposed at −70°C for 1–10 days.

Protein standards used for molecular weight estimation were soybean trypsin inhibitor, carbonic anhydrase, ovalbumin, bovine serum albumin and phosphorylase *b* from Bio-Rad Laboratories and bovine serum albumin dimer cross-linked with dimethylsuberimidate. Theoretical equations were adjusted to the experimental results by least-squares nonlinear regression using the procedure of Gauss-Newton with optional damping. It was assumed that concentration values were affected by negligible error, whereas the variable velocity was considered homoscedastic. The program was run on a microcomputer with 15-digit precision (Rossi, R.C. and Garrahan, P.J., unpublished data).

Results

Mg^{2+}-dependence of the enzyme activity

Fig. 1 shows the Mg^{2+}-dependence of Ca^{2+}-ATPase activity measured in crude islet homogenates, washed with 1 mM EGTA with or without the addition of 120 nM of exogenous brain calmodulin. It is clear that the apparent Ca^{2+}-ATPase activity decreases, either in the presence or absence of calmodulin as a function of the increase in the total magnesium concentration up to 60 μM $MgCl_2$. Greater Mg^{2+} concentrations even produce negative values of the apparent Ca^{2+}-ATPase activity (data not shown), probably due to an inhibitory effect of Ca^{2+} on Mg^{2+}-ATPase activity. On the other hand, the Mg^{2+}-ATPase activity increases as a function of the increment in the concentration of magnesium. It is worthwhile to point out that both Mg^{2+}- and Ca^{2+}-ATPases were active, even without the addition of $MgCl_2$ [7]. Nevertheless, it has been reported that the $K_{0.5}$ for Mg^{2+} during ATP hydrolysis by Ca^{2+}-ATPase is in the micromolar range [8]. Hence, virtually Mg^{2+}-free solutions could contain enough Mg^{2+} to activate the splitting of ATP by Ca^{2+}-ATPase. According to the data of Mg^{2+}-ATPase activity shown in Fig. 1, under our conditions the incubation media would contain at least 3–6 μM Mg^{2+}.

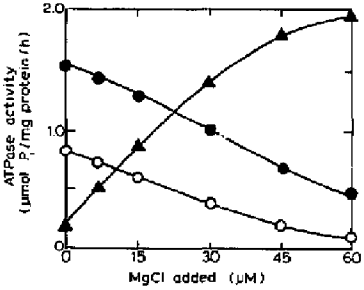

Fig. 1. Effect of Mg^{2+} on the Ca^{2+} and Mg^{2+}-ATPase activities. Ca^{2+}-ATPase activity measured in the presence (●) or the absence (○) of 120 nM calmodulin and Mg^{2+}-ATPase (▲) activity is represented as a function of the $MgCl_2$ added to the assay medium. For the measurement of the Ca^{2+}-ATPase activity, the concentration of free Ca^{2+} in the assay medium was adjusted with EGTA to 1.1 μM.

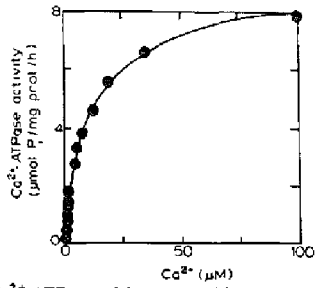

Fig. 2. Ca^{2+}-ATPase activity measured in a crude homogenate preparation of rat pancreatic islets, is represented as a function of the Ca^{2+} concentration. The continuous curve is the representation of Eqn. 1 with values for $K_{m1} = 0.600$ μM; $K_{m2} = 13.76$ μM; $V_1 = 1.090$, $V_2 = 7.890$ μmol P_i/mg protein per h.

Ca^{2+}-dependence of the enzyme activity

Fig. 2 represents the Ca^{2+}-ATPase activity of islet homogenates as a function of Ca^{2+} concentration in the range of 0.1 to 100 μM. The curve that fits the experimental points is described by the following equation, which represents the sum of two Michaelis-like equations:

$$V = V_1/(1 + K_1/[Ca^{2+}]) + V_2/(1 + K_2/[Ca^{2+}]) \quad (1)$$

where V_1 and V_2 are the maximum velocities and K_1 and K_2 the apparent affinities of each component for Ca^{2+}. The best-fitting values for these parameters were $V_1 = 1.090$ μmol P_i/mg protein per h, $V_2 = 7.890$ μmol P_i/mg protein per h, $K_1 = 0.600$ μM and $K_2 = 13.76$ μM. Fig. 2 shows that the enzyme has two components for Ca^{2+}, one with high apparent affinity and low maximum velocity and the other with low apparent affinity and high maximum velocity. An attempt to adjust the experimental points of Fig. 2 to a single Michaelis-like curve produces a bias in the fitting of the experimental points corresponding to the lower range of Ca^{2+} concentrations (0.3 to 5 μM). The presence of two components for Ca^{2+} in the Ca^{2+}-ATPase activity have also been reported in islet homogenates [1,9] or purified plasma membranes [10]. It is not clear whether these components belong to a single Ca^{2+}-ATPase or are the expression of two different enzymes. To overcome this difficulty, Ca^{2+}-ATPase activity was measured at Ca^{2+} concentrations up to 1.1 μM, where the contribution of the low-affinity component to the total activity is almost negligible.

ATP-dependence of the enzyme activity

Ca^{2+}-ATPase activity was measured as a function of ATP concentrations (1 to 1900 μM), in a medium with 7 μM Ca^{2+} (Fig. 3). The curve that fits the experimental points can be adjusted by the sum of two Michaelis-Menten equations:

$$V = V_1/(1+[ATP]/K_{m1}) + V_2/(1+[ATP]/K_{m2}) \quad (2)$$

where V_1 and V_2 are the maximum velocities and K_{m1} and K_{m2} are the Michaelis constants for the ATP high- and low-affinity components, respectively. The best-fitting values for these parameters were $V_1 = 1.92$ μmol P_i/mg protein per h, $V_2 = 4.21$ μmol P_i/mg protein per h; $K_{m1} = 1.90$ μM and $K_{m2} = 120$ μM. This biphasic pattern is common to other transport ATPases, like endoplasmic reticulum and plasma membrane Ca^{2+}-ATPases.

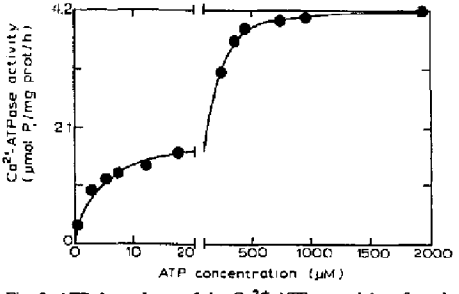

Fig. 3. ATP-dependence of the Ca^{2+}-ATPase activity of crude islet homogenates. The actual concentration of Ca^{2+} in the assay medium was measured at each ATP concentration and adjusted to 7 μM. The curve that fits the filled circles represents Eqn. 2 (see Results) with values for $K_{m1} = 1.90$ μM, $K_{m2} = 120$ μM, $V_1 = 1.92$ and $V_2 = 4.21$ μmol P_i/mg protein per h.

TABLE I

EFFECTS OF DIFFERENT DRUGS UPON Ca^{2+}-ATPase ACTIVITY

The values represent the mean ± S.E. Number of cases in parentheses. Ca^{2+} concentration was adjusted to 1.1 μM with EGTA.

Effector	Ca^{2+}-ATPase activity (μmol P_i/mg protein per h)
Control	1.095 ± 0.019 (4)
NaN_3 (10 mM)	0.938 ± 0.018 (4) $P < 0.005$
Calmodulin (120 nM)	1.895 ± 0.015 (3) $P < 0.001$
Compound 48/80 (50 μg/ml)	0.544 ± 0.020 (3) $P < 0.001$

Effect of different drugs upon Ca^{2+}-ATPase activity

Table I shows the effect of different drugs and effectors upon the Ca^{2+}-ATPase measured in pancreatic islet homogenates. The addition of 10 mM NaN_3 to the incubation medium does not significantly affect the activity of Ca^{2+}-ATPase. Since NaN_3 is an effective blocker of mitochondrial activity [11], these results indicate that, under our experimental conditions, ATP hydrolysis consecutive to the mitochondrial activity is poorly expressed.

The addition of 120 nM of purified brain calmodulin, significantly increases the Ca^{2+}-ATPase activity up to 180% of the control value. Table I also shows the effect of compound 48/80, described as the most specific calmodulin inhibitor [12], upon the enzyme activity. In the presence of 50 μg/ml of compound 48/80, Ca^{2+}-ATPase activity drops to near 50% of its control value and to 28.7% of the activity measured in the presence

of calmodulin. Ouabain (0.1 mM) does not modify the Ca^{2+}-ATPase activity measured in any of the experiments shown in Table I.

In a similar medium as described in Materials and Methods, but with the addition of 120 mM NaCl, 30 mM KCl, 0.1 mM EGTA and 1 mM $MgCl_2$, the Na^+/K^+-ATPase activity, measured as the difference between the activity in a medium with or without 0.1 mM ouabain, was 0.529 ± 0.054 μmol P_i/mg protein per h for five independent preparations. Hence, another enzyme activity related to the plasma membrane can be measured in crude pancreas islet homogenates.

Examination of the phosphorylated intermediates

The autoradiogram represented in Fig. 4 shows the phosphorylated species formed in a preparation of homogenized islets incubated at 4°C for 30 s with 10 μM labelled ATP and subsequently separated by electrophoresis in gels containing 0.1% SDS. Phosphorylation was carried out in the presence of EGTA 20 μM (Ca^{2+} concentration < 0.1 μM), with the addition of 20 μM $MgCl_2$ (lanes 1 and 2) or with 50 μM Ca^{2+} (EGTA-Ca buffer without further addition of Mg^{2+}, lanes 3 and 4). Under the former condition, the phosphorylated species of 105, 61 and 46 kDa show greater intensity than in the presence of high Ca^{2+}/low Mg^{2+}. These phosphorylated species present characteristics of acyl phosphate intermediates, namely acid stability and, as presented later on, susceptibility to hydroxylamine cleavage [13]. The Na^+ does not modify the distribution of the radioactive intermediates (cf. lanes 1 and 2). Hence, these intermediates could originate from the Mg^{2+}-ATPase or the Ca^{2+}-ATPase activity but would not be derived from the Na^+/K^+-dependent enzyme. Labelled intermediates of 98, 80 and 55 kDa were present in higher amounts when the islet phosphorylation was performed in the presence of Ca^{2+} (lanes 3 and 4) but the overall distribution of radioactive bands was qualitatively similar to the previous one. These patterns, in turn, resemble those described by Enyedi et al. [14] for platelets submitted to partial proteolysis by trypsin. Since the islet homogenization and the subsequent procedures were, in this case, performed in the absence of proteolysis inhibitors and the ATPases can be degraded by lysosomal enzymes, the 105 to

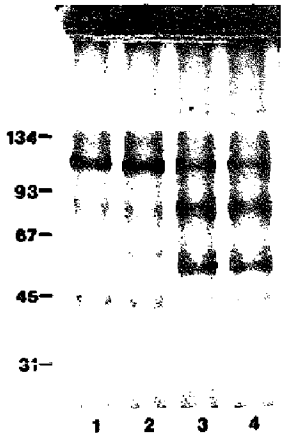

Fig. 4. Autoradiogram of the phosphoenzyme intermediates (EP) of the Ca^{2+}-pump generated after its phosphorylation in a medium devoid of proteolysis inhibitors. Phosphorylation was carried out with 100 μg islet protein at 4°C for 30 s in the following media:

	Lanes:	1	2	3	4
Tris-HCl (pH 7.4)	50 mM	+	+	+	+
KCl	50 mM	−	−	−	+
NaCl	50 mM	−	+	−	−
Choline-Cl	50 mM	+	−	+	−
$MgCl_2$	20 μM	+	+	−	−
Ca^{2+} free	< 0.1 μM	+	+	−	−
EGTA-$CaCl_2$ buffer					
Ca^{2+}-free	50 μM	−	−	+	+
EGTA	20 μM	+	+	−	−

After this procedure, aliquots of each sample were submitted to gel electrophoresis in the presence of 0.1% SDS, and the radioactive bands were visualized by exposing the dried gel slab to the X-ray film for 10 days.

46 kDa species could also represent degradative products [14]. However, most of the radioactivity did not enter the gel when the electrophoresis was performed in 0.1% SDS, giving rise to dark spots on top of the autoradiogram. It should be pointed out that lengthy exposure in autoradiography was necessary to reveal the existence of the degradation products. On the other hand, a single band of 130 kDa was observed when the phosphorylation procedure was performed in the presence of proteolytic inhibitors and the electrophoresis was car-

ried out in 0.2% SDS (Fig. 5, lanes 4 and 5). No phosphorylated intermediate appeared when islet homogenates were incubated with [γ-^{32}P]ATP in the absence of Ca^{2+} (lanes 1 and 2). A similar lack of radioactive intermediates was noted when the islet phosphorylation was performed in the presence of Ca^{2+} plus hydroxylamine (lane 3), suggesting the existence of acyl phosphate groups in the labelled species. The presence of K^+ decreased the level of phosphorylation (cf. lanes 4 and 5).

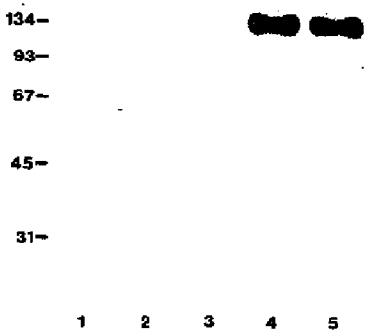

Fig. 5. Autoradiogram of the phosphoenzyme intermediates (EP) of the Ca^{2+}-pump generated after its phosphorylation in a medium with proteolysis inhibitors (1 mM PMSF and TLCK) and hydroxylamine. Phosphorylation was carried out with 100 μg/ml islet protein at 4°C for 30 s in the following media:

	Lane:	1	2	3	4	5
Tris-HCl (pH 7.2)	50 mM	+	+	+	+	+
KCl	50 mM	–	–	–	–	+
NaCl	50 mM	–	+	+	–	–
Mg^{2+}	< 10 μM	–	–	–	+	+
$MgCl_2$	20 μM	+	+	–	–	–
NH_2OH	100 mM	–	–	+	–	–
EGTA-$CaCl_2$ buffer						
Ca^{2+}-free	50 μM	–	–	+	+	+
EDTA	100 μM	+	+	–	–	–

Following the phosphorylation procedure, aliquots of each sample were submitted to gel electrophoresis in the presence of 0.2% SDS and the radioactive bands were visualized by exposing the dried gel slabs to the X-ray film for 2 days.

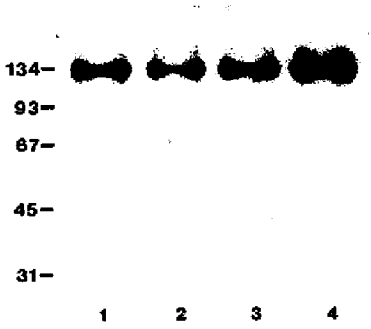

Fig. 6. Effect of ionic strength and low Ca^{2+} concentration on the appearance of phosphoenzyme intermediates (EF) of the Ca^{2+}-pump. Phosphorylation was carried out with 100 μg/ml islet homogenates at 4°C for 30 s in the following medium with proteolysis inhibitors (1 mM PMSF and TLCK).

	Lanes:	1	2	3	4
Tris-HCl (pH 7.2)	50 mM	+	+	+	+
KCl	50 mM	–	–	+	–
NaCl	50 MM	+	–	–	–
Choline-Cl	50 mM	–	+	–	–
$MgCl_2$	20 μM	+	+	–	–
Ca^{2+} (contam.)	2.8 μM	+	–	–	–
EGTA-$CaCl_2$ buffer					
Ca^{2+}-free	50 μM	–	+	+	+

After phosphorylation procedure, aliquots or each sample were treated as described in Fig. 5.

When islets homogenates were incubated with [γ-^{32}P]ATP under different conditions, but keeping constant a medium of low ionic strength, only a single band, of 130 kDa, appeared in the chromatograms (Fig. 6, lanes 1–4). The radioactivity of this band was larger when the islet incubation was performed with 50 μM Ca^{2+} in the absence of either NaCl or KCl (compare lane 4 with 1, 2 and 3). Otherwise, the [γ-^{32}P]ATP incorporation into this band was similar in the presence of either 2.8 μM (lane 1) or 50 μM Ca^{2+} (lanes 2 and 3). Hence, these results would suggest that only one protein fraction is phosphorylated, with a high apparent affinity for Ca^{2+}. They would also suggest that both the high- and low-affinity calcium sites, evidenced by the Ca^{2+}-ATPase, give rise to a common phosphorylated intermediate.

Discussion

Using a low-ionic-strength medium (with low concentration of Mg^{2+} and K^+) and slightly alkaline pH, it was possible to measure in crude islet homogenates a Ca^{2+}-ATPase activity. In such a medium, Na^+/K^+-ATPase is not detected, probably due to the high requirement of this enzyme for Mg^{2+} to express its activity. In addition, the presence of ouabain in the assay medium further blocks such activity. The slight effect of NaN_3 upon Ca^{2+}-ATPase activity measured in the islet homogenates makes the participation of the mitochondrial activity in the ATP hydrolysis a remote possibility.

Various experimental evidence suggests that the Ca^{2+}-ATPase activity measured belongs to the islet plasma membrane. (a) The two Ca^{2+}-components with different affinity for the cation have values quite close to those reported for the Ca^{2+}-ATPase measured in purified plasma membrane preparation of rat islets [10]. (b) The enzyme also shows two different kinetic sites for ATP, one with high affinity and low maximum velocity and the other with low apparent affinity and high maximum velocity for ATP. This behavior is similar to that reported for the Ca^{2+}-transport ATPase [15]. (c) The activity is enhanced by calmodulin, as occurs with the membrane Ca^{2+}-ATPase [16], and is decreased by compound 48/80, a highly specific calmodulin inhibitor [12]. (d) As previously reported [1], this Ca^{2+}-ATPase activity is highly sensitive to vanadate inhibition. (e) In islet homogenates phosphorylated with [γ-^{32}P]ATP in a medium of composition similar to that used for ATPase assay, it was possible to identify by SDS-PAGE, an acid-stable single radioactive band of 130 kDa. A similar molecular mass was reported for the Ca^{2+}-ATPase of plasma membrane from different cells [15]. The 80 and 55 kDa as well as the 105 kDa radioactive intermediates, obtained when the above-mentioned method was performed in the absence of proteolitic inhibitors, have the same molecular masses as those reported by digestion of purified Ca^{2+}-ATPase from red cell membranes and liver plasma membranes, respectively [14]. (f) The contrast to the results obtained with the Ca^{2+}-ATPase measured in endoplasmic reticulum, K^+ inhibits the incorporation of ^{32}P into the 130 kDa intermediate. (g) The appearance of this 130 kDa intermediate was completely blocked by hydroxylamine and 70% inhibited by 25 μM vanadate when enough Mg^{2+} and K^+ were added to the phosphorylation media (data not shown). Altogether, these results reinforce the concept that we are dealing with a plasma membrane rather than with a Ca^{2+}-ATPase from the endoplasmic reticulum [17]. Although we cannot be absolutely sure that the total activity measured in the homogenates belongs to the islet plasma membrane, we can safely suggest that, if any, only a minor proportion of this activity would correspond to the endoplasmic reticulum fraction.

Regardless of the above-mentioned characteristics, we cannot rule out the possibility of measuring a Ca^{2+}-ATPase activity similar to the one described by Enyedi et al. [18] in the rat myometrium, which is apparently not involved in Ca^{2+}-transport.

The procedure currently described and performed with only 10 islets could provide an instrument to test the transient effect of different agents which affect insulin secretion on the plasma membrane Ca^{2+}-ATPase activity. These facts, as well as the uncertainty of the role of Ca^{2+}-ATPase in the mechanism of insulin secretion, prompt us to use this method in the search of experimental evidence capable of answering this question.

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References

1 Gronda, C.M., Rossi, J.P.F.C. and Gagliardino, J.J. (1987) Acta Physiol. Pharmacol. Lat. 37, 245–256.
2 Glynn, I.M. and Chappell, B.J. (1964) Biochem. J. 90, 147–149.

3 Kakiuchi, S., Sobue, K., Yamasaki, R., Kambayashi, J., Sakon, M. and Kosaki, G. (1981) FEBS Lett. 126, 203–207.
4 Lacy, P. and Kostianovsky, M. (1967) Diabetes 16, 35–39.
5 Kratje, R.B., Garrahan, P.J. and Rega, A.F. (1983) Biochim. Biophys. Acta 131, 40–46.
6 Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) J. Biol. Chem. 193, 265–275.
7 Allen, B.G., Katz, S. and Roufogalis, B.D. (1987) Biochem. J. 244, 617–623.
8 Caride, A.J., Rega, A.F. and Garrahan, P.J. (1986) Biochim. Biophys. Acta 863, 165–177.
9 Kasson, B.G. and Levin, S. (1981) Biochim. Biophys. Acta 662, 30–35.
10 Pershadsingh, H.A., McDaniel, M.L., Landt, M., Bry, C.G., Lacy, P.E. and McDonald, J.M. (1980) Nature (Lond.) 288, 492–495.
11 Kemmler, W., Löffler, G. (1977) Diabetologia 13, 235–238.
12 Gietzen, K., Adamczyk-Engelman, P., Wuthrich, A., Konstantinova, A. and Bader, H. (1983) Biochim. Biophys. Acta 736, 109–118.
13 Knauf, P.A., Proverbio, F. and Hoffman, J.H. (1974) J. Gen. Physiol. 63, 324–336.
14 Enyedi, A., Sarkadi, B., Foldes-Papp, Z., Monostori, S. and Gardos, G. (1986) J. Biol. Chem. 261, 9558–9563.
15 Rega, A.F., and Garrahan, P.J. (1986) The Ca^{2+}-pump of Plasma Membrane, CRC Press, Boca Raton, FL.
16 Kotagal, N., Patke, C., Landt, M., McDonald, J.M., Colca, J., Lacy, P. and McDaniel, M.L. (1982) FEBS Lett. 137, 249–252.
17 Colca, J.R., Kotagal, N., Lacy, P. and McDaniel, M.L. (1983) Biochem. J. 212, 113–121.
18 Enyedi, A., Minami, J., Caride, A. and Penniston, J.T. (1988) Biochem. J., in press.