Biochimica et Biophysica Acta, 944 (1988) 308–314
Elsevier

BBA 74135

Ca^{2+} transport by rat liver plasma membranes: the transporter and the previously reported Ca^{2+}-ATPase are different enzymes

Mark A. Birch-Machin and Alan P. Dawson

School of Biological Sciences, University of East Anglia, Norwich (U.K.)

(Received 22 February 1988)
(Revised manuscript received 2 June 1988)

Key words: Calcium ion transport; ATPase, Ca^{2+}-; Enzyme comparison; Plasma membrane; (Rat liver)

A rat liver plasma membrane fraction showed an ATP-dependent uptake of Ca^{2+} which was released by the ionophore A23187. This activity represents a plasma membrane component and is not due to microsomal contamination. The Ca^{2+} transport displayed several properties which were different from those of the high-affinity Ca^{2+}-ATPase previously observed in these membranes (Lotersztajn et al. (1981) J. Biol. Chem. 256, 11209–11215; Birch-Machin, M.A. and Dawson, A.P. (1986) Biochim. Biophys. Acta 855, 277–285). These observations have shown that Ca^{2+}-ATPase does not require added Mg^{2+} whereas we have demonstrated that, in the same membrane preparation, Ca^{2+} uptake required millimolar concentrations of added Mg^{2+}. The Ca^{2+}-ATPase has a broad specificity for the nucleotides ATP, GTP, UTP and ITP while Ca^{2+} uptake remains specific for ATP. Ca^{2+} uptake also displayed different affinities for free Ca^{2+} and MgATP compared to Ca^{2+}-ATPase activity, with apparent K_m values of 0.25 μM Ca^{2+}, 0.15 mM MgATP and 1.0 μM Ca^{2+}, 4 μM MgATP respectively. The apparent maximum rate of Ca^{2+} uptake was about 150-fold less than Ca^{2+}-ATPase activity. These features suggest that the high-affinity Ca^{2+}-ATPase is not the enzymic expression of the ATP-dependent Ca^{2+} transport mechanism.

Introduction

In liver, the intracellular calcium concentration is in the range of 0.1–0.2 μM [1]. This low concentration is maintained in part by a plasma membrane Ca^{2+} pump system. The best studied plasma membrane Ca^{2+} pump is that of erythrocytes from which a Ca^{2+}-stimulated ATPase has been purified [2]. Its ATP-dependent Ca^{2+} pumping activity has been demonstrated by reconstituting the purified enzyme into artificial liposomes [3]. In plasma membranes of some other tissues, smooth muscle for example, a calmodulin-stimulated ATPase has properties which reflect those of the ATP-dependent Ca^{2+} pump [4]. This concept has been extended by implication to plasma membranes derived from a variety of different cell types including the liver [5–7].

However, evidence for the existence and nature of Ca^{2+} pumps is generally indirect or incomplete, particularly in the case of liver plasma membranes where technological limitations apply to fractionation procedures. Contaminating organelles which are known to actively transport calcium (i.e., en-

Abbreviation: Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulphonic acid; EGTA, ethylene glycol bis(β-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid.

Correspondence: M. A. Birch-Machin, Department of Pharmacology, The Medical School, University of Newcastle, Newcastle-upon-Tyne, NE1 7RU, U.K.

doplasmic reticulum and mitochondria) may prohibit the unequivocal assignment of Ca^{2+} transport activity to the plasma membrane. Indeed, recent investigations have demonstrated that in many cells the relationship between high-affinity Ca^{2+}-ATPase activity and ATP-dependent Ca^{2+} transport is complex. For example, Minami and Penniston [8] have shown that corpus-luteum plasma membranes, in the absence of Mg^{2+}, contained a high-affinity Ca^{2+}-ATPase with properties rather different from those of the Ca^{2+} transport which is observed only in the presence of added Mg^{2+}. A similar inconsistency has been observed in neutrophil plasma membrane vesicles [9] and rat liver plasma membranes [10,11].

The main aim of our work has been to compare the specific properties of plasma membrane Ca^{2+} transport with those of the previously reported Ca^{2+}-ATPase [5,12–14] to try to establish whether the latter enzyme is the enzymic expression of the Ca^{2+} pump. The evidence shows that the Ca^{2+} transport represents a plasma membrane component and is not due to microsomal contamination. Furthermore, a comparison of the in vitro properties of this Ca^{2+} pump with those of the high-affinity Ca^{2+}-ATPase, determined in the same sinusoidal plasma membrane preparation, suggests that they are probably different enzymes.

Experimental

Materials

Percoll was purchased from Pharmacia Fine Chemicals, Uppsala, Sweden. Membrane filters (0.2 μm and 0.45 μm) came from Sartorius Instruments, Belmont, U.K. ITP, UTP and ATP (including the special quality type, divalent metal-free) came from BCL, Lewes, U.K. $^{45}Ca^{2+}$ (37 MBq/μmol) was from Amersham International, Amersham, U.K. All other chemicals were from Sigma or were of equivalent analytical grade.

Membrane preparations

The plasma membrane fraction was prepared as described previously [14], which is based on the method of Epping and Bygrave [15].

The microsomal fraction was prepared essentially as described by Dawson [16] using a procedure based on that of Reinhart and Bygrave [17]. The protein concentration of both fractions was determined by the method of Lowry et al. [18] using bovine serum albumin as a standard.

Ca^{2+} uptake assay

Ca^{2+} uptake was measured as described by Dawson [16] (which is based on the filtration method used by Taylor et al. [19] and Moore et al. [20]). The modificiations were as follows: the incubation mixture contained, in a total volume of 0.5 ml: 100 mM KCl, 20 mM Hepes/KOH buffer (pH 7.0), 10 mM $MgCl_2$ (providing a free concentration of 7.5 mM), various amounts of $CaCl_2$ (to produce a free concentration of 0.5 μM) and EGTA to give the required free metal ion concentration, $^{45}Ca^{2+}$ (final specific activity, 74 kBq/μmol), and a final protein concentration of 0.5 mg/ml. Routinely an EGTA concentration of 0.2 mM was used unless otherwise stated in the text. Ruthenium Red (2 μM) was included for microsomal Ca^{2+} uptake experiments. Uptake was started by the addition of 2.5 mM ATP. Filters with 0.2 and 0.45 μm pore size were used to filter plasma membranes and microsomes, respectively. Counts were recorded on the ^{45}Ca channel of an LKB-WALLAC 1214 (80% efficient). The results presented are for a representative experiment, unless stated in the text. For the study of Ca^{2+} transport as a function of ATP concentration, an ATP-regenerating system was used consisting of 1.5 mM phospho*enol*pyruvate and 6 units/ml of pyruvate kinase.

Standard ATPase assay

The ATPase activity of the plasma membrane fraction was determined as described previously [14] which is based on the Fiske and SubbaRow [21] determination of inorganic phosphate. For the study of ATPase activity as a function of ATP concentration, the inorganic phosphate was determined by the highly sensitive method of Carter and Karl [22].

Total Ca^{2+} and Mg^{2+} content of the assay mixtures and reagents was measured, after deproteinising with trichloroacetic acid, by atomic absorption spectrophotometry. Computation of free ion concentrations and the dissociation constants used were precisely as described in Ref. 14.

Results and Discussion

General properties of Ca^{2+} uptake

Incubation of the plasma membrane fraction with 5 mM $MgCl_2$ and 2.5 mM ATP caused a marked accumulation of Ca^{2+} (Fig. 1). In the absence of ATP, a low level of Ca^{2+} associated with the membranes was seen which did not vary significantly with time. This 'passive uptake' of Ca^{2+} may be accounted for by residual binding of Ca^{2+} to the membranes and filters after the washing procedure. In subsequent experiments, Ca^{2+} binding in the absence of ATP is routinely subtracted from those values in the presence of ATP, thereby giving a true representation of ATP-dependent Ca^{2+} uptake. Addition of the bivalent-cation ionophore A23187 caused rapid and complete release of Ca^{2+}, indicating that the uptake was indeed an active transport against its concentration gradient.

The Ca^{2+} transport activity in Fig. 1 appears to represent a plasma membrane component and is not due to microsomal contamination. First of all, a ratio of 5'-nucleotidase (plasma membrane marker) to glucose-6-phosphatase (endoplasmic reticulum marker) of 52 indicates a very low level of endoplasmic reticulum contamination in the plasma membrane preparation.

Secondly, a pH profile for Ca^{2+} transport in the plasma membrane fraction (Fig. 2) shows a broad pH optimum of 7.0–7.4 which contrasts

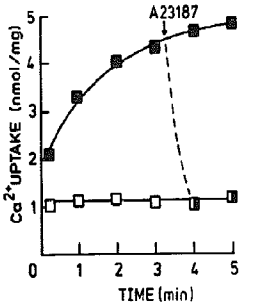

Fig. 1. Ca^{2+} uptake by liver plasma membranes: time course. Ca^{2+}-uptake was measured under the conditions described in the Experimental section in the absence (□) and presence (■) of 2.5 mM ATP. Ca^{2+} uptake was measured at the appropriate times for a period of 5 min. 1 μM A23187 was added after 3.25 min. Total Ca^{2+} was 0.13 mM.

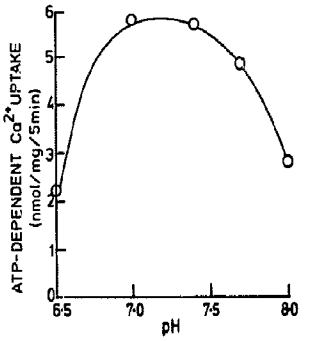

Fig. 2. pH optimum of ATP-dependent Ca^{2+} transport in liver plasma membranes. The Ca^{2+} uptake medium was adjusted to the required pH with KOH. Ca^{2+} uptake was measured as described in the Experimental section. The reaction was started by the addition of ATP (adjusted to the required pH) and a sample removed after 5 min. Due to the pH-dependence of the association constant for Ca^{2+} and EGTA, the total Ca added to the assay was increased from 0.035 mM at pH 6.5 to a concentration of 0.19 mM at pH 8.0 in order to keep the concentration of free Ca^{2+} constant at 0.5 μM.

with a distinct optimal value of 6.8 for the microsomal fraction [16].

Thirdly, the stimulation of microsomal Ca^{2+} uptake by the intravesicular Ca-precipitating anion, oxalate [16,23], was not observed in the plasma membrane fraction (results not shown).

Finally, plasma membrane and microsomal Ca^{2+} uptake may be discriminated from each other by a clear difference in the concentration-dependent inhibition of Ca^{2+} transport by vanadate. Fig. 3 shows that half-maximal inhibition of the liver microsomal Ca^{2+} pump requires a 4-fold higher vanadate concentration (i.e., 1 μM). A similar observation has been reported by Schanne and Moore [24] in rat liver.

Ca^{2+} uptake in the plasma membrane fraction was also not due to mitochondrial contamination, as a concentration of Ruthenium Red (10 μM) which produces maximal inhibition of mitochondrial Ca^{2+} transport [25] had no effect on plasma membrane Ca^{2+} accumulation.

Specific properties of plasma membrane Ca^{2+} transport: A comparison with Ca^{2+}-ATPase activity

It has been proposed [7] that the rat liver plasma membrane high-affinity Ca^{2+}-stimulated

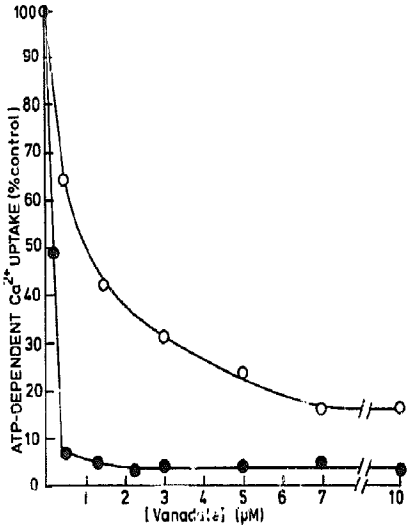

Fig. 3. Effect of vanadate on ATP-dependent Ca^{2+} uptake by plasma membranes and microsomes. Ca^{2+} uptake by plasma membranes (●) and microsomes (○) was measured as described in the Experimental section at varying concentrations of vanadate. Ca^{2+} accumulation was measured 1 min after the addition of ATP. Total Ca concentration was 0.13 mM. The control rates of Ca^{2+} uptake (i.e., with vanadate absent) for the microsomal and plasma membrane fractions were 8.2 and 1.88 nmol Ca^{2+}/mg protein per min, respectively.

ATPase is the enzymic expression of the Ca^{2+} pump. We investigated this by comparing specific properties of Ca^{2+} transport with those of the high-affinity Ca^{2+}-ATPase which we have previously measured in the same plasma membrane preparation [14].

Sensitivity to inhibition by vanadate

Fig. 3 shows that Ca^{2+}-transport activity is 50% inhibited by 0.25 μM vanadate. In contrast, Ca^{2+}-ATPase activity is unaffected by at least 100 μM vanadate even in the presence of Mg^{2+} (Table I) which aids the binding of vanadate to ATPases [26].

Mg^{2+}-dependence of enzyme activity

Initial rates of plasma membrane Ca^{2+} uptake were measured as a function of the Mg^{2+} concentration (Fig. 4). ATP-dependent Ca^{2+} uptake is observed at Mg^{2+} concentrations greater than 1.5 mM. This Ca^{2+} transport increases with Mg^{2+} concentrations reaching a maximum between 5 and 10 mM, although there is evidence [6] that there is a decline in rat liver plasma membrane Ca^{2+} transport above 10 mM Mg^{2+}. By contrast, our previous studies [14] have shown that the Ca^{2+}-ATPase does not require exogenously added Mg^{2+} in the presence of free Ca^{2+} and ATP concentrations similar to those used in Fig. 4. Furthermore, studies on the Ca^{2+}-ATPase activity are complicated by the fact that under the standard Ca^{2+} transport assay condition, i.e., in the presence of millimolar amounts of exogenously added Mg^{2+}, we have previously shown that there is no measurable Ca^{2+}-dependence of ATPase activity.

TABLE I

EFFECT OF VANADATE ON Ca^{2+}-ATPase ACTIVITY

Enzyme activity was assayed as described in the Experimental section. Ca^{2+}-ATPase activity represents the difference in ATPase activity measured in the absence (2 nM free Ca^{2+}) and presence (0.5 μM free Ca^{2+}) of Ca^{2+}. Experimental conditions were: $-Mg^{2+}$ = 0.28 μM free Mg^{2+}; $+Mg^{2+}$ = 5 mM free Mg^{2+}; + vanadate = 100 μM total added. Results are the mean values ± S.D. (n = 4).

Vanadate	Mg^{2+}	Ca^{2+}-ATPase activity (μmol P_i/10 min per mg)
−	−	2.60 ± 0.30
+	−	2.74 ± 0.14
−	+	0.22 ± 0.02
+	+	0.21 ± 0.03

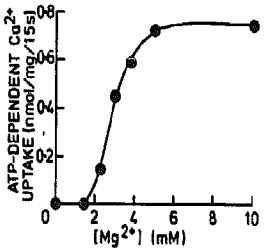

Fig. 4. Dependence of the initial rates of plasma membrane ATP-dependent Ca^{2+} uptake on magnesium concentration. Ca^{2+} uptake was measured as described in the Experimental section 15 s after the addition of ATP. Total Mg^{2+} was varied as indicated. The data are typical of three separate experiments. Total Ca concentration was varied from 0.15 mM to 0.13 mM as total Mg was increased to maintain the free Ca^{2+} at 0.5 μM.

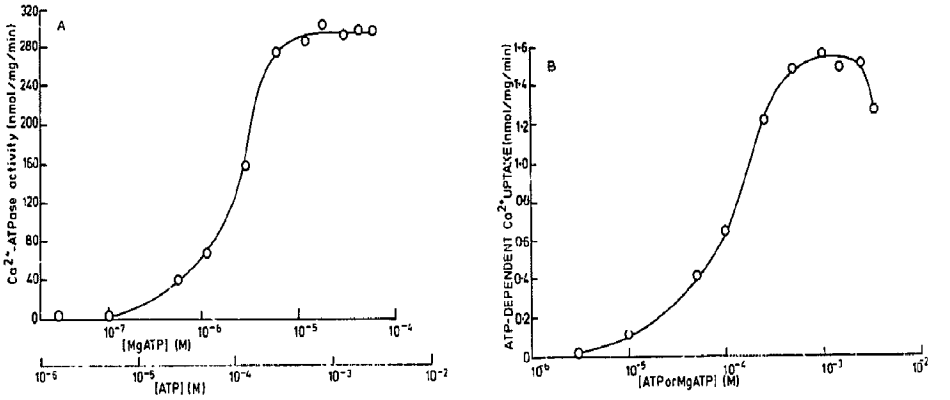

Fig. 5. The dependence of Ca^{2+}-stimulated ATPase and Ca^{2+} uptake activities on MgATP and ATP concentration. Ca^{2+}-ATPase (A) and Ca^{2+} uptake (B) activities were assayed as described in the Experimental section. Using the computer program described previously [14], calculated MgATP values can be substituted for corresponding total ATP concentrations on the abscissa. Fig. 5 shows data which are typical of four (A) and three (B) separate experiments. (A) Ca^{2+}-ATPase activity represents the difference in ATPase activity measured in the absence and presence of Ca^{2+}, which are defined as low and high free Ca^{2+} concentrations, respectively. At the low, 2 nM, free Ca^{2+} concentration (corresponding to endogenous, 2.5 μM, total Ca), free Mg^{2+} was maintained at 2 μM by varying total Mg from 4 μM to 57 μM as ATP was increased. At the high, 0.5 μM, free Ca^{2+} concentration (corresponding to 0.14 mM total Ca), free Mg^{2+} was maintained at 2 μM by varying total Mg from endogenous, 1 μM, to 40 μM. Free Ca^{2+} and Mg^{2+} concentrations were buffered by 0.2 mM EGTA and protein concentration was 0.10 mg/ml. (B) The concentration of free Mg^{2+} was maintained at 7.5 mM by varying total Mg from 7.5 mM to 11 mM with increasing ATP. (Total Ca concentration remained at 0.13 mM). Ca^{2+} accumulation was measured 1 min after the addition of ATP.

Apparent affinity for MgATP

A result of the relatively high Mg^{2+} concentration required for Ca^{2+} transport activity is that essentially all of the ATP in the reaction mixture is complexed with Mg^{2+}. Under these conditions therefore, the concentrations of total ATP and MgATP can be regarded as identical. This is important if MgATP is considered as a substrate [5] rather than an addition to ATP [27]. If one considers total ATP as the only substrate, Ca^{2+}-ATPase (Fig. 5A) and Ca^{2+} transport (Fig. 5B) show similar affinities, namely 0.20 ± 0.03 (S.D.) and 0.15 ± 0.02 mM ATP, which is in agreement with other studies [6,28]. However, if MgATP is considered as the only substrate a reassessment of the data in Fig. 5 (with MgATP now along the abscissa) shows that while Ca^{2+} transport retains a K_m of 0.15 mM, the Ca^{2+}-ATPase shows a much lower value of 4 ± 0.6 (S.D.) μM MgATP. However, the possibility that either MgATP or ATP can serve as a substrate should not be ignored. Although both profiles show a maximal response at approx. 0.5–0.6 mM ATP there is an inhibition of Ca^{2+} accumulation at higher total ATP concentrations, which contrasts with the data on Ca^{2+}-ATPase activity. The situation may be complicated further by the claim that in the absence of Mg^{2+}, i.e., under the standard Ca^{2+}-ATPase assay, a CaATP complex may act as substrate [29].

Throughout the experiment it was necessary to ensure a constant free Mg^{2+} concentration (see Fig. 5 legend for details); in addition, the use of a

TABLE II

NUCLEOTIDE SPECIFICITY OF Ca^{2+}-ATPase AND Ca^{2+} UPTAKE

Enzyme activities were assayed as described in the Experimental section. 100% Ca^{2+}-ATPase activity was 2.40 μmol P_i/mg protein per 10 min. 100% calcium uptake was 2.85 nmol Ca^{2+}/mg protein per 3 min.

Nucleotide	%Enzyme activity	
	Ca^{2+}-ATPase	Ca^{2+} uptake
ATP	100	100
ITP	68	25
GTP	88	9
UTP	70	6

special quality ATP, which was free of divalent metal cations, contributed to a greater control of ionic conditions. Consequently there was no need to maintain a large excess of free Mg^{2+} over the concentration of MgATP which in certain studies [30] may hinder the observation of Ca^{2+}-ATPase through either a high basal Mg^{2+}-ATPase activity or a Mg^{2+}-induced inhibition.

Nucleotide specificity

The nucleotide specificities of Ca^{2+} transport and Ca^{2+}-ATPase were measured by testing whether equal concentrations (2.5 mM) of nucleotides other than ATP could support enzyme activity. Table II shows that although ITP could support a significant degree of Ca^{2+} transport the rate is only 25% of the value observed in the presence of ATP. This relative specificity for ATP contrasts greatly with the broad nucleotide specificity of the Ca^{2+}-stimulated ATPase, which suggests strongly that the Ca^{2+}-ATPase is an unlikely candidate for the Ca^{2+} transporter.

Apparent affinity for Ca^{2+}

ATP-dependent Ca^{2+} uptake was measured as a function of the free calcium concentration (Fig. 6). Initial-rate measurements were performed to reduce any interference by Ca^{2+} recycling across the vesicle membrane. The Ca^{2+} uptake exhibited

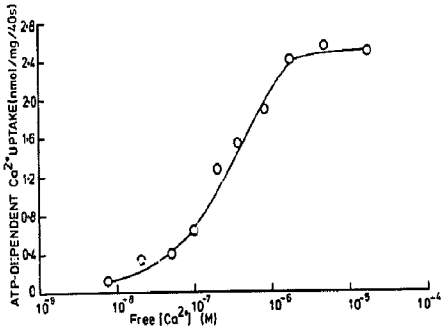

Fig. 6. Dependence of the initial rates of plasma membrane ATP-dependent Ca^{2+} accumulation on free Ca^{2+} concentration. Ca^{2+} uptake was measured as described in the Experimental section. The free Ca^{2+} range (8 nM–16 μM) corresponded to total Ca from 20 μM to 0.7 mM. The EGTA concentration was 0.7 mM. Ca^{2+} accumulation was measured 40 s after the addition of ATP. The data are typical of four separate experiments.

saturable kinetics showing a maximum at approx. 20 μM and a K_m of 0.25 ± 0.02 (S.D.) μM free Ca^{2+}. By contrast, kinetic analysis of previously reported Ca^{2+}-ATPase activity as a function of Ca^{2+} concentration [14] shows a K_m for Ca^{2+} of 1 ± 0.1 μM ($n = 5$).

Stoichiometry of enzyme activities

If the Ca^{2+}-ATPase is the enzymic expression of the Ca^{2+} pump one would expect a close relationship between the two enzyme activities. However, we have normally found an approx. 150-fold difference between activities of Ca^{2+} uptake and the high-affinity Ca^{2+}-ATPase with typical values ($\pm$S.D.) of 1.86 ± 0.17 ($n = 11$) nmol Ca^{2+}/mg per min and 272 ± 33 ($n = 9$) nmol P_i/mg per min, respectively. This could suggest that Ca^{2+} uptake is due to an enzyme of low activity whose ATPase activity is not detected in the presence of the higher activity Ca^{2+}-ATPase [8,31]. This difference could also be explained if the preparation was 'leaky', although since we are dealing with moderately initial rates of uptake this would suggest a very high percentage of unsealed vesicles.

In summary, for the same enzyme to be responsible for both activities it would, as Mg^{2+} was decreased, have to become uncoupled from transport, also change its nucleotide specificity, affinity for Ca^{2+} and MgATP, and its sensitivity to vanadate and increase its V_{max} for ATP hydrolysis. This seems unlikely and it is perhaps reasonable to attribute the high Ca^{2+}-ATPase activity to an enzyme separate from that responsible for Ca^{2+} transport (a conclusion which was recently reported in corpus-luteum plasma membranes [8]). This conclusion is at variance with Pavoine et al. [7] who have shown that their Ca^{2+}-ATPase can support Ca^{2+} transport. The main area of discrepancy appears to be between the apparent properties of the Ca^{2+}-ATPase which may be explained by the method that is used to isolate the plasma membrane fraction. Several studies [32,33] have argued in favour of a putative canalicular origin for the isolation procedures that are based on the popular method of Neville [34], one modification of which is used by Pavoine et al. [7]. However, the fraction in the present study is enriched in sinusoidal membranes [15] and so is likely to contain hormone receptor and ion trans-

location systems which face the blood side of the epithelium. This is important, as recent evidence [35] has shown that the characteristics of rat liver plasma membrane Ca^{2+}-ATPase activity may vary according to the membrane region in which the isolated fractions are enriched.

Other studies have indicated inconsistencies between the properties of a Ca^{2+}-ATPase and the Ca^{2+} pump activity in neutrophil [9] and kidney [36] plasma membranes. Furthermore, attempts by Lin [11] to reconstitute the purified Ca^{2+}-ATPase from rat liver plasma membranes into artificial liposomes have shown an absence of ATP-dependent Ca^{2+} transport activity.

If the Ca^{2+}-ATPase does not function as the liver plasma membrane Ca^{2+} pump, it may act as an ectoenzyme [37,38] which would regulate the extracellular ATP concentration in the vicinity of the plasma membrane. These enzymes show a broad nucleotide specificity and an insensitivity to vanadate, which are characteristic of the Ca^{2+}-ATPase (present study). Furthermore, Lin and Fain [39] have found that, in rat liver plasma membranes, the Ca^{2+}-ATPase binds to concanavalin A while the Ca^{2} pump does not, implying that the Ca^{2+}-ATPase has an external localization.

Acknowledgements

We wish to thank the SERC for their financial support and Mr. D. Fulton for his expert technical assistance.

References

1 Murphy, E., Coll, K., Rich, T. and Williamson, J.R. (1980) J. Biol. Chem. 255, 6600–6608.
2 Niggli, V., Penniston, J.T. and Carafoli, E. (1979) J. Biol. Chem. 254, 9955–9958.
3 Niggli, V., Adunyah, E.S., Penniston, J.T. and Carafoli, E. (1981) J. Biol. Chem. 256, 395–401.
4 De Schutter, G., Wuytack, F., Verbist, J. and Casteels, R. (1984) Biochim. Biophys. Acta 773, 1–10.
5 Lotersztajn, S., Hanoune, J. and Pecker, F. (1981) J. Biol. Chem. 256, 11209–11215.
6 Chan, K.M. and Junger, K.D. (1983) J. Biol. Chem. 258, 4404–4410.
7 Pavoine, C., Lotersztajn, S., Mallat, A. and Pecker, F. (1987) J. Biol. Chem. 262, 5113–5117.
8 Minami, J. and Penniston, J.T. (1987) Biochem. J. 242, 889–894.
9 Ochs, D.L. and Reed, P.W. (1984) J. Biol. Chem. 259, 102–106.
10 Lin, S.-H. (1985) J. Biol. Chem. 260, 7850–7856.
11 Lin, S.-H. (1985) J. Biol. Chem. 260, 10976–10980.
12 Iwasa, Y., Iwasa, T., Higashi, K., Matsui, K. and Miyamoto, E. (1982) Biochem. Biophys. Res. Commun. 105, 488–494.
13 Lin, S-H., Wallace, M.A. and Fain, J.N. (1983) Endocrinology 113, 2268–2275.
14 Birch-Machin, M.A. and Dawson, A.P. (1986) Biochim. Biophys. Acta 855, 277–285.
15 Epping, R.J. and Bygrave, F.L. (1984) Biochem. J. 223, 733–745.
16 Dawson, A.P. (1982) Biochem. J. 206, 73–79.
17 Reinhart, P.H. and Bygrave, F.L. (1981) Biochem. J. 194, 541–549.
18 Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) J. Biol. Chem. 193, 265–275.
19 Taylor, W.M., Bygrave, F.L., Blackmore, P.F. and Exton, J.H. (1979) FEBS Lett. 104, 31–34.
20 Moore, L., Chen, T., Knapp, H.R. and Landan, E.J. (1975) J. Biol. Chem. 250, 4562–4568.
21 Fiske, C.H. and SubbaRow, Y. (1925) J. Biol. Chem. 66, 375–400.
22 Carter, S.G. and Karl, D.W. (1982) J. Biochem. Biophys. Methods 7, 7–13.
23 Bygrave, F.L. (1978) Biochem. J. 170, 87–91.
24 Schanne, F.A.X. and Moore, L. (1986) J. Biol. Chem. 261, 9886–9889.
25 Reed, K.C. and Bygrave, F.L. (1974) Biochem. J. 140, 143–155.
26 Simons, T.J.B. (1979) Nature 281, 337–338.
27 Gould, G.W., East, J.M., Froud, R.J., McWhirter, J.M., Stefanova, H.I. and Lee, A.G. (1986) Biochem. J. 237, 227.
28 Krauss-Friedmann, N., Biber, J. and Carafoli, E. (1982) Eur. J. Biochem. 129, 7–12.
29 Wakabayashi, S. and Shigekawa, M. (1984) J. Biol. Chem. 259, 4427–4436.
30 Lotersztajn, S., Dpand, R.M., Mallat, A. and Pecker, F. (1985) J. Biol. Chem. 259, 8195–8201.
31 Kelly, L.K. and Smith, C.H. (1987) Biochem. Biophys. Res. Commun. 148, 126–132.
32 Evans, W.H. (1977) Trends Biochem. Sci. 2, 169–171.
33 Emmelot, P., Bos, C.J., Van Hoeven, R.P. and Van Blitterswijk, W.J. (1974) Methods Enzymol. 31, 75–90.
34 Neville, D.M. (1960) J. Cell Biol. 8, 413–422.
35 Bachs, O., Famulski, K.S., Mirabelli, F. and Carafoli, E. (1985) Eur. J. Biochem. 147, 1–7.
36 Ghijsen, W., Gmaj, P. and Murer, H. (1984) Biochim. Biophys. Acta 778, 481–488.
37 Carraway, C.A.C., Corrado, F.J., Fogle, D.D. and Carraway, K.L. (1980) Biochem. J. 191, 45–51.
38 Hamlyn, J.M. and Senior, A.E. (1983) Biochem. J. 214, 59–68.
39 Lin, S.-H. and Fain, J.N. (1984) J. Biol. Chem. 259, 3016–3020.