Simultaneous presence of two distinct endoplasmic-reticulum-type calcium-pump isoforms in human cells

Characterization by radio-immunoblotting and inhibition by 2,5-di-(t-butyl)-1,4-benzohydroquinone

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Phosphorylation, immunoblotting, limited proteolysis and drug-sensitivity analysis were used to characterize the sarco-endoplasmic-reticulum Ca^{2+} ATPases in a variety of human cell types. In platelets, several megakaryoblastoid and lymphoblastoid cell lines two distinct autophosphorylated forms of these ATPases with molecular mass of 100 and 97 kDa could be observed, whereas in several other cell types the 97 kDa form was absent. On immunoblots the 97 kDa species was specifically recognized by an inhibitory monoclonal antibody raised against the Ca^{2+} pump of platelet internal membranes, yielded on trypsinolysis a major fragment of 80 kDa, exhibited a distinct electrophoretic migration pattern as compared with the skeletal-, cardiac- and smooth-muscle Ca^{2+} pumps, and its autophosphorylation was strongly inhibited by the Ca^{2+} -mobilizing agent 2,5-di-(t-butyl)-1,4-benzohydroquinone (tBHQ). The 100 kDa species reacted with an antibody specific for the cardiac- and smooth-muscle Ca^{2+} pumps, yielded on trypsinolysis fragments of 55 and 35 kDa, and its autophosphorylation was much less sensitive to tBHQ inhibition. These findings indicate the simultaneous presence of two different endoplasmic-reticulum Ca^{2+} pumps in a variety of human cell types, and may explain the previously observed differences in the Ca^{2+} -handling characteristics of different intracellular Ca^{2+} pools and cell types.

INTRODUCTION

Ca^{2+} -transporting ATPases (Ca^{2+} pumps) translocate Ca^{2+} ions against a steep concentration gradient through cellular membranes. Ca^{2+} fluxes between the cytoplasm, intracellular Ca^{2+} -storage organelles and the plasma membrane play a key role in cellular homeostasis and signal transduction. Upon stimulation of a wide variety of receptors, Ca^{2+} enters the cytoplasm from intracellular pools as well as from the extracellular compartment through Ca^{2+} channels [1–4]. In a reversible activation event, Ca^{2+} is thereafter resequenced in intracellular storage pools and eliminated from the cell through the plasma membrane. Ca^{2+} -transport ATPases thus play an important role in the control of cell activation.

Two types of Ca^{2+} pumps have been described in human tissues, the plasma-membrane-type (PMCA), and the sarco-endoplasmic-reticulum-type (SERCA) Ca^{2+} -transport ATPases. Several genes are known for both types, which upon alternative splicing give rise to various mRNA species and protein isoforms. The expression of these isoforms is tissue-specific and developmentally regulated. The SERCA 1a and 1b alternatively spliced isoforms are expressed in skeletal muscle. The SERCA 2a isoform is found in cardiac muscle, whereas the 2b isoform is found predominantly in smooth muscle [5–13]. These isoforms are identical, except the C-terminal tetrapeptide part, present in the 2a isoform, which is replaced by a 49-amino-acid sequence in the 2b isoform [12]. In non-muscle tissues, mRNA studies indicate the presence of the SERCA 2b isoform [12,14]; moreover, RNA corresponding to a third gene (SERCA 3) has also been detected [13]. However, the corresponding protein has not yet been identified. Previously we and others found that human platelets

contain two different endoplasmic-reticulum-type Ca^{2+} -pump species of molecular masses about 100 and 97 kDa and identified the 100 kDa form as the SERCA 2b isoform [15–19]. The other pump form, of approx. 97 kDa, although recognized by an anti-(skeletal-muscle Ca^{2+} pump) antiserum similarly to the 2b isoform, exhibited several special characteristics: it failed to react with a SERCA 2b-specific antibody, its phosphoenzyme intermediate formation was enhanced by La^{3+} and it was less sensitive toward thapsigargin inhibition than the 2b isoform. Moreover, whereas the 2b isoform gave 55 and 35 kDa autophosphorylatable fragments upon trypsinolysis, the 97 kDa form yielded a 80 kDa autophosphorylatable fragment [15,17]. Ca^{2+} pumps can be specifically labelled in their intact membrane environment by $[\gamma\text{-}^{32}\text{P}]\text{ATP}$ by the Ca^{2+} -dependent phosphoenzyme-formation reaction. After electrophoresis in an acidic gel system to preserve the phosphoenzyme intermediate, Ca^{2+} pumps can be detected after immunoblotting by autoradiography and simultaneous immunostaining [15,20,21]. In order to characterize the 97 kDa Ca^{2+} -pump species found in platelets, in the present work using this system we studied Ca^{2+} pumps in membranes prepared from platelets, as well as from several human cell lines including cells of megakaryocytic, T- and B-lymphoid origin. We studied the effect of 2,5-di-(t-butyl)-1,4-benzohydroquinone (tBHQ), a recently described Ca^{2+} -mobilizing agent [22,23], on the phosphorylation of the 100 and 97 kDa Ca^{2+} -pump forms, compared the electrophoretic migration, inhibition by tBHQ and tryptic fragmentation pattern of the 97 kDa form with those of smooth-, cardiac- and skeletal-muscle sarcoplasmic-reticulum Ca^{2+} -pump isoforms. In addition, we studied the differential recognition of the simultaneously expressed 100 and 97 kDa intracellular Ca^{2+} pumps by antibodies

Abbreviations used: tBHQ, 2,5-di-(t-butyl)-1,4-benzohydroquinone; E-P, Ca^{2+} -pump aspartyl phosphate intermediate; SERCA, sarco-endoplasmic-reticulum-type Ca^{2+} -transport ATPase; PMCA, plasma-membrane-type Ca^{2+} -transport ATPase.

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raised against the SERCA 2 gene products and against the Ca^{2+} -pump protein present in platelet intracellular membranes.

MATERIALS AND METHODS

Materials

The IID8 and PL/IM 430 anti- Ca^{2+} -pump monoclonal antibodies were gifts from Dr. G. White, Chapel Hill, North Carolina, U.S.A., and Dr. N. Crawford, London, U.K., respectively. IID8 is a mouse monoclonal antibody raised against dog cardiac-muscle sarcoplasmic-reticulum Ca^{2+} pump by K. P. Campbell and colleagues [24]. This antibody recognizes a shared epitope present on cardiac-muscle (i.e. SERCA 2a) as well as on smooth-muscle (SERCA 2b) Ca^{2+} pumps [25]. PL/IM 430 was obtained by immunizing with highly purified human platelet internal membranes. This monoclonal antibody recognizes the Ca^{2+} pump of platelet internal membranes and inhibits the active Ca^{2+} -pumping activity of these membranes [26]. The anti-mouse-IgG-peroxidase conjugate was purchased from Sigma.

Cell-culture reagents were from Boehringer Mannheim or Gibco BRL. tBHQ was from Aldrich and was added in the assays as a 100-fold concentrated stock solution in dimethyl sulphoxide. Trypsin (type XIII), soybean trypsin inhibitor, Bowman-Birk trypsin-chymotrypsin inhibitor, leupeptin, phenylmethanesulphonyl fluoride and aprotinin were from Sigma. The other reagents were as specified in [15].

The HS-Sultan B-lymphoid, Y-79 retinoblastoma, SK-MEL-1 melanoma, DU-4475 breast cancer and the KATO III gastric cancer cell lines were obtained from the American Type Culture Collection and were grown as described in [27]. The Dami [28] and MEG 01 [29] megakaryoblastoid cell lines were generously given by Professor J. Peries, U 107 INSERM, Paris, and were grown in RPMI medium supplemented with 10% heat-inactivated fetal-calf serum plus 2 mM-glutamine, 1 mM-sodium pyruvate, antibiotics and Minimum Essential Medium (Eagle) non-essential amino acids at 37 °C in a humidified atmosphere of air/ CO_2 (19:1) in a cell-culture incubator. Jurkat, MOLT 4 T-lymphoblastoid, MG-63 osteosarcoma and the megakaryoblastoid HEL cells [30–32] were grown similarly. Smooth-muscle cells obtained from pig and rat aorta were grown as described in [33] in HAM F10 medium supplemented with 2 mM-glutamine, antibiotics and 10% fetal-calf serum.

Cell membrane preparation

For this, cells grown in suspension were harvested by centrifugation at 150 g for 10 min at room temperature and resuspended in wash buffer consisting of 160 mM-KCl, 17 mM-K-Hepes, pH 7.0, and 50 μM -EGTA at 4 °C. After centrifugation at 1500 g for 5 min at 4 °C, the supernatant was discarded and the cell pellet was frozen in liquid nitrogen. To the pellet corresponding to approx. $(0.5\text{--}2.5) \times 10^9$ cells, 10 ml of freshly prepared ice-cold lysis buffer (10 mM-KCl, 10 mM-K-Hepes, pH 7.0, 100 μM -dithiothreitol, 50 μM -EDTA, 50 μM -EGTA, 50 μM -phenylmethanesulphonyl fluoride, 100 μg of soybean trypsin inhibitor/ml, 100 μg of Bowman-Birk trypsin-chymotrypsin inhibitor/ml, 100 μg of aprotinin/ml, 100 μg of leupeptin/ml) was added and the cells were thawed at 4 °C with vortex mixing. The cell suspension was homogenized in a Teflon-glass homogenizer with 50 strokes at 4 °C and then sonicated on ice in a Sonics Vibracell sonicator at setting 3 for 20 s in a fume hood to avoid aerosol inhalation. The suspension was then centrifuged at 16000 g for 10 min at 4 °C and the resulting supernatant was further centrifuged at 170000 g at 4 °C for 1 h. The mixed microsomal pellet was resuspended in 10 mM-KCl/10 mM-K-Hepes, pH 7.0, containing 100 μM -dithiothreitol,

divided into portions, frozen in liquid nitrogen and stored at -80 °C. Cells grown in monolayer were rinsed with wash buffer in the culture flasks, scraped into lysis buffer at 4 °C and thereafter processed as above for membrane preparation.

Platelet microsomal membrane vesicles were prepared as in [17,34]. Cardiac- and skeletal-muscle sarcoplasmic-reticulum microsomal fractions were isolated as described in [35,36].

Trypsin treatment of membrane vesicles

This was performed at 4 °C in 160 mM-KCl/17 mM-K-Hepes, pH 7.0, containing 500 μg of membrane protein and 50 μg of trypsin/ml. The reaction was terminated for mild trypsinolysis at 5 min, and for more excessive digestion at 20 min, by adding a 10-fold excess of soybean trypsin inhibitor, and the membrane vesicles were processed for Ca^{2+} -pump radiolabelling.

Ca^{2+} -pump radiolabelling

The specific radiolabelling of the Ca^{2+} pumps in cell membrane preparations by [γ - ^{32}P]ATP, electrophoresis in an acidic 7% polyacrylamide-gel system, transfer on to nitrocellulose and autoradiography were carried out as in [15].

Immunostaining

Nitrocellulose membranes after electroblotting were incubated under slow agitation with quenching solution (10 mM-Tris, 170 mM-NaCl, 0.1% Tween-20, 5% non-fat dry milk, pH 7.4) for 1 h, followed by incubation overnight in quenching solution supplemented with 10% heat-inactivated human plasma. Blots were then rinsed with quenching solution and incubated for 2 h with the first antibodies (IID8 or PL/IM 430) freshly diluted 2000-fold in the same buffer. Thereafter the blots were rinsed five times and further incubated for 2 h with an anti-mouse IgG-peroxidase conjugate, diluted 2000-fold in quenching solution. After a washing step as above, peroxidase staining was done in 10 mM-sodium phosphate (pH 6.0) containing 100 mM-NaCl, 10% (v/v) methanol and 0.1 mg of *o*-dianisidine/ml supplemented with 1/10000 vol. of 30% H_2O_2 .

Measurement of total Ca^{2+} -pump phosphoenzyme levels

After the labelling reaction and trichloroacetic acid precipitation as described in [15], the precipitate was filtered through a Whatman GF/C glass microfibre filter. The filter was rinsed with 2×5.0 ml of the trichloroacetic acid precipitating solution and counted for radioactivity in a liquid-scintillation counter. In tBHQ inhibition experiments the membrane suspension was incubated for 10 min at 37 °C with the inhibitor (added as a 100-fold concentrated solution in dimethyl sulphoxide), thereafter placed on ice for an additional 10 min and then the phosphoenzyme-formation reaction was done as above. Dimethyl sulphoxide (1%) alone did not interfere with the assays.

RESULTS

Specific inhibition of the phosphoenzyme-intermediate formation of the 97 kDa platelet Ca^{2+} pump by tBHQ

Ca^{2+} pumps were labelled with [γ - ^{32}P]ATP by Ca^{2+} -dependent acyl phosphate intermediate formation in platelet mixed microsomal membrane vesicles at increasing tBHQ concentrations. After electrophoresis and blotting, Ca^{2+} -pump proteins were detected by autoradiography (see the Materials and methods section). Two closely migrating labelled bands were obtained, having apparent molecular masses of approx. 100 kDa ($\blacktriangleright$) and 97 kDa ($\triangleright$) (Fig. 1, lane 1). tBHQ selectively inhibited the phosphoenzyme formation of the 97 kDa form ($\triangleright$). Inhibition occurred in the 0.1–1 μM range, and complete inhibition

Fig. 1. Specific inhibition of the phosphoenzyme formation of the 97 kDa platelet Ca^{2+} pump by tBHQ

Ca^{2+} pumps in platelet microsomes were labelled with $[\gamma\text{-}^{32}\text{P}]\text{ATP}$ at increasing tBHQ concentrations. tBHQ inhibited the autophosphorylation of the 97 kDa ($\blacktriangleright$) Ca^{2+} pump, but had no effect on the autophosphorylation of the 100 kDa ($\blacktriangleleft$) pump protein. Inhibition occurred in the 0.1–1 μM -tBHQ concentration range (lanes 2 and 3), and over 5 μM -tBHQ the inhibition was complete (lane 4).

Fig. 2. Comparison of (a) the electrophoretic migration of the different Ca^{2+} -pump isoforms and (b) the tryptic fragmentation of different Ca^{2+} pumps and inhibition of the 80 kDa platelet Ca^{2+} -pump fragment by tBHQ

(a) Ca^{2+} pumps in platelet (lane 1), smooth-muscle (lane 2), cardiac-muscle (lane 3) and skeletal-muscle (lane 4) microsomal vesicles were labelled by $[\gamma\text{-}^{32}\text{P}]\text{ATP}$, resolved by electrophoresis, blotted and autoradiographed. All isoforms migrated to approx. 100 kDa ($\blacktriangleleft$), except the second platelet pump protein ($\blacktriangleright$), which exhibited clearly higher electrophoretic mobility. (b) Platelet (lane 5), smooth-muscle (lane 7), cardiac-muscle (lane 8) and skeletal-muscle (lane 9) microsomes were submitted to mild trypsin proteolysis and resolved by electrophoresis after radiolabelling of the Ca^{2+} -pump fragments with $[\gamma\text{-}^{32}\text{P}]\text{ATP}$. The smooth-, cardiac- and skeletal-muscle Ca^{2+} pumps gave rise to 55 kDa autophosphorylatable fragments. With platelets, an additional 80 kDa fragment was also formed (lane 5). Addition of 10 μM -tBHQ into the autophosphorylation reaction of trypsin-treated platelet membranes abolished the phosphorylation of the 80 kDa pump fragment (lane 6).

was seen with 5 μM -tBHQ in both the absence and the presence of 1 mM- Mg^{2+} . Hydroquinone, a related compound lacking the two tertiary butyl groups, had no inhibitory effect on phosphoenzyme formation of platelet Ca^{2+} pumps, whereas tBHQ had no effect on the Ca^{2+} -pump autophosphorylation in red-cell inside-out membrane vesicles, which contain only PMCA-type Ca^{2+} pumps [7,20,21].

Comparison of the platelet, smooth-muscle, cardiac- and skeletal-muscle Ca^{2+} pumps

In order to compare the platelet Ca^{2+} pumps with other intracellular Ca^{2+} -pump isoforms, the phosphoenzyme reaction was performed on smooth-muscle, cardiac and skeletal-muscle microsomes, and proteins were resolved by electrophoresis, blotted and autoradiographed. As shown in Fig. 2(a), whereas platelets gave rise to the usual phosphorylated doublet (lane 1) at 100 ($\blacktriangleleft$) and 97 kDa ($\blacktriangleright$), smooth muscle (lane 2), cardiac (lane 3) and skeletal muscle (lane 4) showed only single phosphorylated bands, which migrated very similarly to the 100 kDa platelet protein. Indeed, the 97 kDa platelet Ca^{2+} pump migrated clearly faster than any of the other Ca^{2+} -pump isoforms. In addition, upon limited tryptic proteolysis (i.e. after 5 min trypsin treatment), smooth-muscle (Fig. 2, lane 7), cardiac (lane 8) and skeletal-muscle (lane 9) Ca^{2+} pumps gave rise to 55 kDa, and upon more extensive digestion (i.e. after 20 min of trypsin treatment) to 35 kDa autophosphorylatable Ca^{2+} -pump fragments (results not shown), whereas in platelet vesicles (lane 5) an additional 80 kDa fragment was also formed.

When phosphoenzyme formation was carried out in the presence of tBHQ after trypsin treatment of platelet vesicles (lane 6), a preferential inhibition of the 80 kDa fragment was observed, indicating that this fragment can be assigned to the 97 kDa intact Ca^{2+} -pump isoenzyme, as suggested previously [15].

Differential sensitivities of the various SERCA-type Ca^{2+} pumps to tBHQ inhibition

The Ca^{2+} -pump phosphoenzyme reaction was performed on platelet, smooth-muscle, cardiac and skeletal-muscle microsomes in the absence (Fig. 3a) and in the presence (Fig. 3b) of 1 mM- Mg^{2+} at various tBHQ concentrations. In both cases smooth muscle, which contains predominantly the SERCA 2b isoform, appeared to be insensitive to inhibition. The pronounced inhibition observed with platelet microsomes can be assigned to the 97 kDa isoform, since the other (100 kDa) platelet Ca^{2+} pump, previously identified as the SERCA 2b isoform, is rather insensitive to tBHQ inhibition (see also Fig. 1). The cardiac and skeletal-muscle Ca^{2+} pumps were only slightly inhibited by tBHQ in the absence of Mg^{2+} (Fig. 3a); however, tBHQ inhibition was significantly enhanced by Mg^{2+} in both cases. Since Mg^{2+} facilitates the dephosphorylation of the Ca^{2+} -pump phosphoenzyme intermediate, this indicates that tBHQ interferes with the autophosphorylation step of the catalytic cycle of Ca^{2+} pumps, rather than inhibiting dephosphorylation, as observed in La^{3+} inhibition, which blocks the enzyme in the phosphorylated state [34]. However, these data taken together suggest that the different Ca^{2+} -pump isoforms have different sensitivities toward inhibition of phosphoenzyme formation by tBHQ, with sensitivity increasing in the order of smooth muscle < cardiac < skeletal muscle < platelet 97 kDa Ca^{2+} -pump form.

Differential immunostaining of the platelet Ca^{2+} -pump doublet

Ca^{2+} pumps in platelet vesicles were radiolabelled with $[\gamma\text{-}^{32}\text{P}]\text{ATP}$, resolved by electrophoresis, blotted on to nitrocellulose and autoradiographed (Fig. 4, lane 1). Thereafter the blots were immunostained with a monoclonal antibody (PL/IM 430) raised against the Ca^{2+} pump present in platelet intracellular

Fig. 3. Inhibition of formation of the phosphoenzyme intermediate (E-P) of the different Ca²⁺-pump isoforms by tBHQ

Ca²⁺ pumps in platelet (●), smooth-muscle (◆), cardiac-muscle (▲) and skeletal muscle (■) microsomes were radiolabelled with [γ -³²P]ATP in the presence of increasing concentrations of tBHQ in the absence (a) or the presence (b) of 1 mM-Mg²⁺ and the incorporated radioactivity was determined. The smooth-muscle Ca²⁺ pump was not significantly inhibited. Inhibition of cardiac and skeletal-muscle Ca²⁺ pumps was significantly enhanced by Mg²⁺. For platelet microsomes marked inhibition was obtained in the absence, as well as in the presence, of Mg²⁺.

Fig. 4. Immunostaining of the platelet-type Ca²⁺-pump doublet with anti-Ca²⁺-pump antibodies: presence of the two Ca²⁺ pumps in various cell lines

Microsomal membranes were prepared from platelets and from various cell lines. Ca²⁺ pumps were labelled with [γ -³²P]ATP, and proteins were resolved by electrophoresis, blotted and autoradiographed. Similarly to platelets (lane 1), a radiolabelled doublet at 100 kDa (▲) and 97 kDa (▷) was seen in Jurkat (T-lymphoblastoid) and HS-Sultan (B-lymphoid, plasma cell) membranes (lanes 4, 7). However, in KATO III cells only a single 100 kDa radiolabelled band was found (lane 10). Where present, the 97 kDa band was recognized by the PL/IM 430 monoclonal antibody (lanes 2, 5, 8), and the IID8 monoclonal antibody stained in all cases the 100 kDa Ca²⁺-pump protein (lanes 3, 6, 9, 12).

Fig. 5. Inhibition of formation of the acyl phosphate intermediate of the 97 kDa Ca²⁺ pump in different cell lines

Ca²⁺ pumps in microsomal membranes prepared from various cell lines were labelled with [γ -³²P]ATP in the absence and the presence of 20 μM-tBHQ. The platelet-type Ca²⁺-pump doublet was obtained in the absence of tBHQ in HS-Sultan, MOLT 4 and MEG 01 cells, and tBHQ inhibited the autophosphorylation of the 97 kDa Ca²⁺ pump (▷) in all cells.

membranes, and a specific staining of the 97 kDa (▷) phosphorylated band was seen (lane 2). On the other hand, an antibody raised against dog cardiac sarcoplasmic-reticulum Ca²⁺ pump (IID8), which is known to recognize both the SERCA 2a and 2b alternatively spliced pump isoforms [25], stained only the 100 kDa (▲) calcium pump. These data suggest that the two pump proteins bear unique antigenic epitopes, not present on the other platelet Ca²⁺-pump protein, and thus these bands represent distinct Ca²⁺-pump isoforms.

Demonstration of the two Ca²⁺ pumps in various human cell lines

In order to study whether the presence of the Ca²⁺-pump doublet is a platelet-specific feature, or whether it can be found in a wider range of cell types, microsomes were prepared from several human cell lines, including cells of megakaryocytoid and T- and B-lymphoblastoid origin. Ca²⁺-pump labelling by [γ -³²P]ATP was performed, and proteins were resolved by electrophoresis and transferred on to nitrocellulose. Upon autoradiography of the blots, the 100 and 97 kDa phosphorylated doublet was found in the megakaryoblastoid MEG 01 cells, as well as in the T-lymphoblastoid Jurkat and MOLT 4 cells and in the B lymphoblastoid HS-Sultan cells (Figs. 4 and 5). Similar results were obtained with HEL and Dami cells as well (not shown). Similarly to that found in platelets, the 100 kDa Ca²⁺ pump was recognized by the anti-(cardiac sarcoplasmic reticulum Ca²⁺-pump) antibody (Fig. 4, lanes 3, 6, 9 and 12), whereas the 97 kDa band was specifically stained by the PL/IM 430 monoclonal antibody (Fig. 4, lanes 2, 5 and 8). Both bands were stained by a polyclonal antibody raised against rat skeletal-muscle Ca²⁺ pump, having a wide recognition range [20] toward SERCA-type Ca²⁺ pumps (results not shown).

In addition, the 97 kDa Ca²⁺ pump in these cell lines was sensitive to tBHQ: addition of tBHQ in the Ca²⁺-pump phosphorylation reaction selectively inhibited the phosphorylation of the 97 kDa Ca²⁺ pump in the megakaryocytoid, as well as in the T- and B-lymphoblast, cell lines (Fig. 5). However, the presence of the 100 kDa and 97 kDa Ca²⁺-pump doublet did not

appear to be a universal feature of all non-muscle cells studied, since in KATO III cells (lane 10) only the 100 kDa pump phosphoenzyme intermediate was found. Similarly, the 97 kDa Ca^{2+} pump was lacking in Y-79 retinoblastoma, DU-4475 breast cancer, MG-63 osteosarcoma, SK-MEL-1 melanoma and normal cultured smooth-muscle cells (results not shown). The 100 kDa pump protein was recognized by the IID8 monoclonal antibody (lane 12), but no immunostaining with the PL/IM 430 antibody was observed (lane 11). When Ca^{2+} -pump phosphoenzyme intermediate formation was done in the presence of tBHQ in cells expressing both the 100 and 97 kDa calcium pumps, specific inhibition of the autophosphorylation of the 97 kDa pump was observed (Fig. 5), similarly to that observed in platelets.

DISCUSSION

In this work the simultaneous presence of two distinct endoplasmic-reticulum-type Ca^{2+} pumps of molecular masses 100 and 97 kDa is shown in several human cell types by Ca^{2+} -pump specific phosphorylation, inhibition by tBHQ and immunostaining with anti- Ca^{2+} -pump antibodies.

The 97 kDa form was specifically recognized by a monoclonal antibody raised against the Ca^{2+} -pump species present in platelet internal membranes; it was strongly inhibited by tBHQ and gave a 80 kDa major tryptic fragment. This pump appeared to be different from the skeletal, cardiac and smooth-muscle Ca^{2+} -pump isoforms in its electrophoretic migration and tryptic fragmentation pattern. Since the applied antibody inhibits calcium transport [26], this specific interaction may be further used for the investigation of the different Ca^{2+} -transport systems.

The 100 kDa pump species, previously identified [15] as the SERCA 2b isoform, expressed mainly in smooth-muscle and non-muscle tissues [25], was much less sensitive to tBHQ inhibition, gave rise to 55 and 35 kDa autophosphorylatable tryptic fragments and was recognized by a monoclonal antibody specific to a common epitope of the SERCA 2a and 2b alternatively spliced Ca^{2+} -pump isoforms. However, a polyclonal anti-(sarcoplasmic reticulum Ca^{2+} pump) antiserum which has a broad recognition range toward SERCA-type pumps stained both pump isoforms on immunoblots (results not shown).

The 100 and 97 kDa Ca^{2+} -pump isoenzymes were present not only in platelets, but also in several megakaryocytoid and T- or B-lymphoid cell types. However, in several other cells studied, only the 100 kDa form was found by Ca^{2+} -pump phosphoenzyme formation and immunostaining, suggesting that the 97 kDa pump protein may be expressed in a tissue-specific manner.

Evidence is accumulating in the literature which indicates the simultaneous presence of several distinct Ca^{2+} pools within the same cell [38–44]. These pools appear to exhibit different Ca^{2+} -storage and -release characteristics. Ca^{2+} can be transferred from one pool to another, and the actual saturation level of a Ca^{2+} pool seems to be involved in the regulation of calcium influx into the cell [39,45,46]. Inhibitors of the SERCA-type Ca^{2+} pumps are increasingly used to study these processes. Thapsigargin inhibits with equal potency the already cloned SERCA-type Ca^{2+} pumps in a cell transfection system [47] and is a less potent inhibitor of the 97 kDa platelet-type Ca^{2+} pump [15]. This isoform, on the other hand, is preferentially inhibited by tBHQ, as shown here. It is also known that in the same cell active Ca^{2+} -uptake mechanisms having different thapsigargin sensitivities exist [39,48]. The findings in the present paper, demonstrating that cells may express simultaneously two distinct endoplasmic-reticulum-associated Ca^{2+} pumps with different specific features, may contribute to our understanding of the differences found in the Ca^{2+} -handling characteristics of the different intracellular Ca^{2+} pools and of different cells.

This work was supported by grants from the Ligue Nationale Contre le Cancer, from the Ministère de la Recherche et de la Technologie, from the Association pour la Recherche sur le Cancer, and by OKTA grant no. 1/3-195. The excellent technical assistance of M. Berényi and M. Marjai is greatly acknowledged. We are grateful to Anne-Marie Lompré and Jan Eggermont for help and discussions during the work, and thank J. Peries and P. Marie for supplying cell lines and G. White, K. Campbell and N. Crawford for generously giving the antibodies used in this work.

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Received 3 March 1992/29 April 1992; accepted 12 May 1992