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Functional and Structural Demonstration of the Presence of Ca-ATPase (PMCA) in Both Microvillous and Basal Plasma Membranes from Syncytiotrophoblast of Human Term Placenta

R. Marín [a,*,1], G. Riquelme [b,1], V. Godoy [b], P. Díaz [b], C. Abad [a], R. Caires [a], T. Proverbio [a], S. Piñero [a], F. Proverbio [a,1]

[a] *Laboratorio de Bioenergética Celular, Centro de Biofísica y Bioquímica, Instituto Venezolano de Investigaciones Científicas (IVIC), A.P. 21827, Caracas 1020A, Venezuela*

[b] *Laboratorio de Electrofisiología de Membranas, Fisiología y Biofísica, Instituto de Ciencias Biomédicas (ICBM), Facultad de Medicina, Universidad de Chile, Casilla 70005, Santiago 7, Chile*

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Abstract

It is known that human syncytiotrophoblast (hSCT) actively transports more than 80% of the Ca^{2+} that goes from maternal to fetal circulation. Transepithelial transport of Ca^{2+} is carried out through channels, transporters and exchangers located in both microvillous (MVM) and basal (BM) plasma membranes. The plasma membrane Ca-ATPase (PMCA) is the most important mechanism of Ca^{2+} homeostasis control in the human placenta. In this work, we reexamined the distribution of PMCA in isolated hSCT of term placenta. The PMCA activity was determined in isolated hSCT plasma membranes. A partial characterization of the PMCA activity was performed, including an evaluation of the sensitivity of this enzyme to an *in vitro* induced lipid peroxidation. Expression of the PMCA in hSCT plasma membranes and tissue sections was investigated using Western blots and immunohistochemistry, respectively. Our study demonstrates, for the first time, a correlation between the activity and structural distribution of PMCA in both MVM and BM of hSCT. It also demonstrates a higher PMCA activity and expression in MVM as compared to BM. Finally, PMCA4 seems to be preferentially distributed in both hSCT plasma membranes, while PMCA1 is shown to be present in the hSCT homogenate. However, the membrane fractions did not show any PMCA1 labeling. Our results must be taken into account in order to propose a new model for the transport of calcium across the hSCT.

Keywords: PMCA; Placenta; Syncytiotrophoblast; Microvillous plasma membranes; Basal plasma membranes

1. Introduction

It is known that Ca^{2+} plays a key role in intracellular signaling in many cells. As a consequence, the intracellular Ca^{2+} concentration should be tightly regulated [1]. This control is exerted through various mechanisms, among which the plasma membrane Ca-ATPase (PMCA) plays an important role in the maintenance of low free Ca^{2+} concentration in the cytoplasm, by extruding cytosolic Ca^{2+} from the cells [2]. This enzyme is known to be coded by four separate genes (PMCA 1–4), and due to alternative splicing, there are at least 20 variants designated by lowercase letters following the isoform number (e.g., $PMCA1_a$, $PMCA1_b$, etc.) [3,4]. PMCA 2 and 3 isoforms are found in specialized tissues while isoforms 1 and 4 are present in almost all tissues including placenta [5]. However, there is a high variability in the patterns of expression for the different splice variants of the PMCA isoforms. For instance, in placental tissues only $PMCA4_a$, $PMCA4_b$ and $PMCA1_a$ are present [6]. The different PMCA isoforms differ primarily in their regulatory regions and the modulation

* Corresponding author. Tel.: +58 2 504 1395; fax: +58 2 504 1093.
E-mail addresses: rmarin@ivic.ve, rlmh98@yahoo.com (R. Marín).
[1] R. Marín, F. Proverbio and G. Riquelme are co-senior authors of this article.

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of their activities strongly depends on the membrane composition [3,4].

The human placental syncytiotrophoblast (hSCT) is a polarized epithelium, representing the interface between the maternal and fetal circulations. This epithelium has a maternal-facing microvillous membrane (MVM) and a fetal-facing basal membrane (BM). Thus, any transport of ions and solutes to and from the fetal compartment must be performed across both MVM and BM. In particular, it is known that the syncytiotrophoblast actively transport more than 80% of the Ca^{2+} that goes from maternal to fetal circulation [7]. Transepithelial transport of Ca^{2+} is carried out by channels, transporters and exchangers located in both MVM and BM [8].

Placental Ca^{2+} transport has been reported to be inhibited by the addition of erythrosin B, a placental PMCA inhibitor [9]. In addition, Ca^{2+} transport in perfusion studies of placenta, is minimally affected when the Na^+ of the incubation medium is substituted with choline, a clear indication that the Na^+/Ca^{2+} exchanger (NCX) has a minimal role in the transplacental movement of Ca^{2+} from mother to fetus [10]. Accordingly, it has been proposed that PMCA plays an important role in Ca^{2+} homeostasis in the human placenta [11]. One of the very first studies looking for PMCA in hSCT, considered the fact of a higher concentration of calcium in the fetal circulation as compared to the maternal one, suggesting a predominant presence of such an extrusion mechanism in BM [12]. This consideration led these authors to demonstrate the presence of PMCA in membrane vesicles of BM from hSCT, without paying attention to MVM. In another set of studies, Borke et al. [13] and Strid and Powell [14], by using immunohistochemistry techniques in human placental tissue and with a monoclonal anti-PMCA antibody 5F10, found an apparent exclusive distribution of PMCA in BM. In addition, Borke et al. [13], determined from the Western blot analysis of MVM preparations that the antibody 5F10 binds poorly to the region around 140 kDa. These specific findings have been used, since then, to conclude that the PMCA, similarly to renal and intestinal epithelia, is located exclusively in BM from hSCT [8]. However, in preliminary experiments we have found: (a) the presence of a Mg^{2+}-dependent, thapsigargin-insensitive, Ca^{2+}-stimulated-ATPase activity in MVM fractions isolated from human term placenta [15]; and (b) PMCA expression in MVM fractions of placental membranes by Western blot and immunohistochemistry with specific antibodies [16,17]. These findings, together with the fact that the PMCA and the Na,K-ATPase (NKA) are colocalized in the basolateral plasma membranes of renal and intestinal epithelia, while the NKA is present in both BM and MVM from hSCT [18], led us to reexamine the distribution of PMCA in hSCT of term placenta. In this study, we tested the hypothesis that PMCA is present in both MVM and BM from hSCT. The biochemical expression of the PMCA activity was assayed and partially characterized in isolated preparations from both MVM and BM. Expression of the PMCA in isolated hSCT plasma membranes and tissue sections was also investigated using Western blots and immunohistochemistry, respectively.

2. Materials and methods

2.1. Placenta collection

Placentae obtained from normal pregnancies were collected immediately after delivery from the Maternity Hospital "Concepción Palacios" in Caracas, Venezuela and San José Hospital Maternity Unit (monitored Unit Outpatient clinic, healthy pregnancies) in Santiago, Chile and transported to the respective laboratories on ice. Any woman that, according to her medical history, was under medical treatment to control blood pressure, or if she was taking >1 g of elemental calcium per day during pregnancy, or if she had a history of hypertension, diabetes, calcium metabolism disorders, or any other chronic medical illness, was not considered for this study.

2.2. Preparation of syncytiotrophoblast plasma membranes

The human placental plasma membranes (MVM) and basal membranes (BM) were prepared from fresh placentae following a previously described method [19,20]. In brief: the maternal decidua was removed, and the central portion between the maternal and fetal surfaces was used for the preparation. Placental villous tissue (80–100 g) was chopped into small pieces, washed with 0.9% NaCl to remove blood and filtered through gauze. The purification method involved different steps: differential centrifugation, precipitation of non-microvillous membranes with magnesium ions and a sucrose gradient step. All solutions were buffered with 20 mM Tris-maleate, pH 7.4. Sucrose gradient preparation: a portion (2–3 ml) of the microvillous-enriched preparation and the basal membrane-enriched preparation were overlayed on the sucrose gradient. The band at the sucrose interface concentrations 37/45% (w/v) corresponds to the apical fraction (MVM) and the band at the sucrose interface concentrations 47/52% (w/v) corresponds to the basal fraction (BM). The two fractions were collected, diluted 10-fold with the buffer 20 mM Tris-maleate, pH 7.4, and centrifuged at 110,000 × *g* for 30 min. The final pellets were resuspended in 300 mM sucrose, 20 mM Tris-maleate, pH 7.4, and stored in liquid nitrogen or at −50 °C (freezer). The purity and enrichment of the membrane fractions were determined routinely by assaying alkaline phosphatase, an apical membrane marker; adenylate cyclase/β-adrenergic receptor (by measuring ^{3}H-dihydroalprenolol binding), basal membrane markers; cytochrome *c* oxidase/succinate dehydrogenase, mitochondrial membrane markers and glucose-6-phosphatase, endoplasmic reticulum marker [12,20].

2.3. PMCA activity

The PMCA activity was determined by measuring the quantity of inorganic phosphate liberated from the hydrolysis of ATP, according to a modification of the method described elsewhere [21]. Briefly, 180 µl of the incubation medium were preincubated for 2 min at 37 °C, and the reaction was started by addition of 20 µl of membrane suspension. After 10 min incubation, the reaction was stopped by addition of 300 µl of a cold solution containing: 2.85% ascorbic acid; 1.76% HCl; 0.48% ammonium molybdate; and 2.85% SDS. The samples were shaken and kept at 0 °C for 10 min. Then, 500 µl of 2% sodium citrate, 2% sodium arsenite and 2% glacial acetic acid solution were added to each tube, which were then rewarmed, after shaking, for 10 min at 37 °C. The absorbance of each tube was determined in a Milton Roy spectrophotometer at 705 nm. The ATPase activity is expressed as nmol Pi/mg protein min, after subtraction of a blank run in parallel under the same conditions except for the membrane suspension, which was added only after the addition of the ascorbic acid solution. The protein concentration, in all the cases, was determined according to the method of Bradford [22]. The PMCA activity was calculated as the difference in the phosphate liberated in a medium containing 250 mM sucrose; 5 mM ATP; 5 mM $MgCl_2$; 1 mM ouabain; 2 mM EGTA; 2 mM EDTA; 30 mM Tris–HCl (pH 7.2 at 37 °C); 55 mM KCl, 2 µg/ml calmodulin; 1 µM thapsigargin and 2 µM free calcium, minus the one liberated in the same medium, but in the absence of calcium. Purified bovine brain calmodulin was generously supplied by Dr. Gustavo Benaim from the Universidad Central de Venezuela.