ELSEVIER

Biochimica et Biophysica Acta 1564 (2002) 325–332

BIOCHIMICA ET BIOPHYSICA ACTA
BBA

www.bba-direct.com

Calcium uptake and calcium transporter expression by trophoblast cells from human term placenta

Robert Moreau [a], Georges Daoud [a], Renée Bernatchez [a], Lucie Simoneau [a], André Masse [b], Julie Lafond [a,*]

[a] *Laboratoire de Physiologie materno-foetale, Département des Sciences Biologiques, Université du Québec à Montréal, C.P. 8888, Succursale "Centre-Ville", Montreal, Quebec, Canada H3C 3P8*
[b] *Department of Obstetrics and Gynecology, CHUM, Montreal, Quebec, Canada H3C 3P8*

Received 31 December 2001; received in revised form 26 April 2002; accepted 24 May 2002

Abstract

Placental transfer of maternal calcium (Ca^{2+}) is a crucial step for fetal development although the biochemical mechanisms responsible for this process are largely unknown. This process is carried out in vivo by the placental syncytiotrophoblast layer. The aim of this study was to define the membrane gates responsible for the syncytiotrophoblast Ca^{2+} entry, the first step in transplacental transfer. We have investigated the basal Ca^{2+} uptake by primary culture of human term placenta syncytiotrophoblast. Kinetic studies revealed an active extracellular Ca^{2+} uptake by cultured human syncytiotrophoblast. We demonstrated by Northern blot the presence of transcript for calcium transporter type 1 (CaT1) in cultured human syncytiotrophoblast and CaT1 expression was further confirmed by reverse transcription polymerase chain reaction (RT-PCR). In addition, the expression of calcium transporter type 2 (CaT2) was revealed by RT-PCR in cultured human syncytiotrophoblast. It has been reported that the activity of this family of Ca^{2+} channels is voltage-independent, and is not sensitive to L-type Ca^{2+} channels agonist and antagonist. Interestingly, modulation of membrane potential by extracellular high potassium concentration and valinomycin had no effect on the basal Ca^{2+} uptake of human syncytiotrophoblast. Moreover, the addition of L-type Ca^{2+} channel modulators (Bay K 8644 and nitrendipine) to the incubation medium had also no effect on the basal Ca^{2+} uptake, suggesting that the process is mainly voltage-independent and does not involved L-type Ca^{2+} channels. On the other hand, we observed that two known blockers of CaT-mediated Ca^{2+} transport, namely extracellular magnesium (Mg^{2+}) and ruthenium red, dose-dependently inhibited Ca^{2+} uptake by cultured human syncytiotrophoblast. Therefore, our results suggest that basal Ca^{2+} uptake of human syncytiotrophoblast may be assured by CaT1 and CaT2.

Keywords: Placenta; Syncytiotrophoblast; Calcium uptake; CaT1 channel; CaT2 channel; L-type channel

1. Introduction

Mammalian fetal nutrition during gestation is dependent on the transfer of maternal ions and nutrients by the placenta [1–4]. Throughout gestation, the fetus acquires all of its Ca^{2+} from the maternal circulation for its development. This ion is actively transported across the placenta from the maternal to the fetal circulation, and consequently the total Ca^{2+} concentration in the fetal plasma over a wide range of species of mammals exceeds that in the mother especially during the late part of the gestation [5,6]. This active Ca^{2+} transfer is carried out in vivo by the placental syncytiotrophoblast layer [7]. In vitro, the cytotrophoblasts isolated from human term placenta undergo spontaneous syncytiotrophoblastic-like morphological and biochemical differentiation, and are thought to reflect in vivo syncytiotrophoblast. Although the cell isolation procedure of Kliman et al. [8] has greatly improved the investigation of placental nutrient exchanges, the placental Ca^{2+} transfer mechanisms remain to be established. Particularly, the membrane gate for the troph-

Abbreviations: DMEM, Dulbecco's modified Eagle medium; HBSS, Hank's balanced salt solution; PBS, phosphate-buffered saline; FBS, fetal bovine serum; hCG, human chorionic gonadotropin; CRF, corticotrophin-releasing factor; RT-PCR, reverse transcription polymerase chain reaction; CaT1 and CaT2, calcium transporter type 1 and 2; ECaC, epithelial Ca^{2+}channel; TRP, transient receptor potential channel; bp, base pair

* Corresponding author. Tel.: +1-514-987-6592; fax: +1-514-987-4647.
E-mail address: lafond.julie@uqam.ca (J. Lafond).

0005-2736/02/$ - see front matter © 2002 Elsevier Science B.V. All rights reserved.
PII: S0005-2736(02)00466-2

oblast Ca^{2+} entry, which constitutes the first step in transcellular placental transfer, is not resolved. Primary cultures of syncytiotrophoblast from human term placenta have not been used until now to resolve this aspect and these cells may represent numerous advantages in regard to the in vivo situation.

A limited number of membrane channels could be considered in the entry of Ca^{2+} into the cell. Ca^{2+} channels with pharmacological characteristics of L-type Ca^{2+} channels have been functionally identified in trophoblasts in relation to the regulation of hormonal secretions [9–12]. These characteristics include voltage dependence of activation and sensitivity to dihydropyridine and phenylalkylamine organic Ca^{2+} channel modulators. Moreover, capacitative Ca^{2+} current via store-operated channels could also contribute to the ion entry since we have recently reported the involvement of such conductance in corticotrophin-releasing factor (CRF) secretion by trophoblast cells [13]. Other candidates for the trophoblast Ca^{2+} entry are members of a subfamily related to the transient receptor potential (TRP) family of nonselective, Ca^{2+}-permeable, cation channels. This subfamily is composed of novel highly selective Ca^{2+} channels that have been recently cloned and shown to be mainly expressed in epithelium involved in Ca^{2+} transfer. Calcium transporter protein type 1 or epithelial Ca^{2+} channels 2 (CaT1 or ECaC2) have been reported in intestinal epithelium [14–16] and placental tissue (CaT-like) [17]. Calcium transporter protein type 2 or epithelial Ca^{2+} channels 1 (CaT2, ECaC1 or ECaC) have been cloned from rabbit [18], rat [19] and human [20] renal epithelium.

Expression of Ca^{2+} channels of the CaT/ECAC family has been shown in human placental tissue. Barley et al. [14] have identified transcripts from human placental tissue that hybridize with partial cDNA of CaT1/ECaC2 and Peng et al. [16] have reported by Northern blot the expression of CaT1/ECaC2 in human placental tissue. Moreover, Hoenderop et al. [18] have demonstrated in the same tissue transcripts that hybridize with cDNA of the rabbit ECaC and Müller et al. [20] have reported the presence in the human placenta of mRNA that corresponded to CaT2/ECaC1. Accordingly, Peng et al. [21] have reported expression of both type of Ca^{2+} channels and reported by quantitative real-time PCR that CaT1 is highly expressed compared to CaT2 in placental tissue. In view of the functional investigation of the role of CaT/ECaC family, extracellular Mg^{2+} and ruthenium red have been recently reported to be effective inhibitors of CaT-mediated Ca^{2+} uptake [22].

There is no information about the expression of CaT/ECaC and their involvement in Ca^{2+} uptake in cultured human trophoblast cells isolated from human term placenta. In the current study, we have evaluated the expression of Ca^{2+} channels from the CaT/ECaC family in primary culture of human syncytiotrophoblast and investigated its role in basal Ca^{2+} uptake.

2. Materials and methods

2.1. Materials

Dulbecco's modified Eagle medium (DMEM), newborn calf serum and TriZol™ were purchased from Life Technologies (Burlington, Ontario, Canada). Hank's balanced salt solution (HBSS), trypsin, DNAse, Percoll, ruthenium red, $MgCl_2$ and Triton X-100 were obtained from Sigma (Oakville, Ontario, Canada). The 24-well plates were purchased from Sarstedt (Montreal, Quebec, Canada). Fetal bovine serum (FBS) and enzyme-linked immunosorbent assay (ELISA) for human chorionic gonadotropin (hCG) were obtained from Medicorp (Montreal, Quebec, Canada). The fluorescein isothiocyanate (FITC)-conjugated monoclonal antibody against cytokeratin 7 was purchased from Accurate Chemical and Scientific (Westbury, NY, USA). Bovine serum albumin (BSA), [ethylenebis(oxyethylenenitrilo)]-tetraacetic acid (EGTA), 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), the random hexamer primers were obtained from Roche Molecular Biochemicals (Laval, Quebec, Canada). Radiolabeled Ca^{2+} ($^{45}CaCl_2$) was purchased from ICN Biomedicals (Irvine, CA, USA). The bicinchoninic acid (BCA) reagent was obtained from Pierce (Brockville, Ontario, Canada). Omniscript™ RT and Taq PCR core kits were purchased from Qiagen (Mississauga, Ontario, Canada). The restriction enzymes (*Xho*I and *Sac*I) were purchased from Amersham Pharmacia Biotech (Baie d'Urfé, Québec, Canada). The RNA millennium™ size markers for Northern blot were purchased from Ambion (Austin, TX, USA). The thermal cycler GeneAmp PCR system 2400 was obtained from Perkin Elmer (Markham, Ontario, Canada).

2.2. Human placental trophoblast isolation and purity evaluation

Cytotrophoblasts were isolated from human term placentas according to the procedure of Kliman et al. [8]. The placentas were obtained from normal deliveries at the Pavilion St-Luc of the Centre Hospitalier Universitaire de Montréal (Quebec, Canada) and were immerged in DMEM for no more than 1 h after delivery. Briefly, fetal membranes and maternal decidua were removed and villous tissue was cut into approximately 1-in. cubes and washed extensively with saline in order to remove blood. The tissue was then incubated three times in HBSS containing 1.5–1.6 mg/ml trypsin and 0.2 mg/ml DNAse at 37 °C for 30 min. After each incubation, the supernatant was removed and replaced by fresh digestion media. The supernatant was layered on to newborn calf serum and centrifuged at $1215 \times g$ for 15 min. Pellets were resuspended in DMEM, deposited on top of a discontinuous 5% to 40% Percoll gradient, and centrifuged at $507 \times g$ for 20 min. Cytotrophoblast layers were removed and washed in DMEM. Cells were seeded at approximately

1.7×10^6 cells/well in 24-well plates. The medium was refreshed daily with DMEM containing 10% FBS.

The purity of the cytotrophoblast preparations was evaluated by flow cytometry using FITC-conjugated monoclonal antibody against cytokeratin 7 following the procedure described by Campbell et al. [23]. It has been reported that anti-cytokeratin 7 antibody was specific for trophoblast cells [24,25]. Approximately 500 000 cells from the preparations were fixed in methanol at −20 °C for 20 min, washed in phosphate-buffered saline (PBS) and then incubated in PBS containing FBS (dilution 1:50) for 30 min to eliminate nonspecific binding. Thereafter, cells were washed with PBS and incubated with mouse monoclonal anti-human cytokeratin 7 in PBS containing 0.2% BSA for 45 min at room temperature in the dark. Controls were performed by omitting the antibody. Cells were then washed in PBS and analysed by flow cytometry using FACScan system (Becton Dickinson, San Jose, CA, USA) with WinMDI software.

2.3. hCG secretion of trophoblast

Secretion of hCG by primary culture of trophoblast was evaluated by ELISA as described previously [12]. For the everyday follow-up of hormone release or secretion under treatments, the incubation media were retrieved, centrifuged and supernatants were frozen at −20 °C until measurements.

2.4. Ca^{2+} uptake studies

Ca^{2+} uptake studies were performed on cells from 4-day trophoblast cultures. Briefly, cells were washed twice with the Ca^{2+} uptake buffer (HBSS containing 1.26 mM $CaCl_2$, 10 mM HEPES and 0.1% BSA) and allowed to equilibrate in the same buffer (250 μl) for 10 min. Thereafter, cells were incubated at 37 °C for different intervals of time following the addition of 250 μl of uptake buffer containing $^{45}CaCl_2$ (2–4 μCi/well). The incubation was stopped by aspiration of the uptake buffer. The cells were washed three times with 500 μl of ice cold PBS containing 4 mM EGTA (to eliminate the nonspecific component of the uptake [26]), and then solubilized in PBS containing 1% Triton X-100. The cell-associated radioactivity was measured by a β-scintillation 1400^{TM} Wallac counter. The cellular protein content of each well was evaluated by spectrophotometric quantification using the BCA reagent with BSA as standard. The Ca^{2+} uptake is expressed as nanomoles of Ca^{2+} (from specific activity) per milligram of cellular proteins.

2.5. Cell expression of Ca^{2+} channels from the CaT/ECaC family

Northern blotting was performed with total RNA isolated from 4-day cultured cells using TriZol™ according to the manufacturer instructions. RNA was separated by electrophoresis on 1% agarose gel and thereafter transferred to Hybond N nylon membrane. Membrane was hybridized in the presence of 50% formamide at 42 °C overnight. A cDNA (785 bp) amplified using specific primers for CaT1 exon 15 (accession numbers AF304463; sense primer, 5′-GCTCTGAGGATTTGGACAAAGACTC-3′; antisense primer, 5′-TCAGGGATCCGAAAACGACTT-3′) was ^{32}P-labeled and used as probe. The presence of transcripts for CaT1 and CaT2 (accession number AF304464) in 4-day trophoblast cultures was investigated by PCR using specific primers for CaT1 (sense primer in exon 1, 5′-CTCTGCCTATGGAGCAAGTTCTGC-3′; antisense primer in exon 7, 5′-GAGAGTCGAGGTCAGTGGTCC-3′), and for CaT2 (sense primer in exon 9, 5′-GGCCTATGAGACACGTGAAGATATC-3′; antisense primer in exon 13, 5′-ATAGAATTGCCCCAGACTGGT-3′). Isolated RNA was reversed transcribed using random hexamer primers and Omniscript™ RT kit. PCRs were performed using Taq PCR core kit in a thermal cycler GeneAmp PCR system 2400. The specificity of the amplicon was evaluated with restriction enzymes.

2.6. Statistical analysis

Statistical analyses were performed using unpaired Student's *t*-test. Differences were considered significant when P values were <0.05. Data were expressed as the mean ± S.E.

3. Results and discussion

3.1. Purity of the cytotrophoblast preparations

Following the isolation of cytotrophoblast cells from human term placenta, the purity of the cell preparations were ascertained by flow cytometry using mouse monoclonal antibody against human cytokeratin 7 (trophoblast specific). Fig. 1 shows representative data where more than 96% of the cells were positive for cytokeratin 7. This percentage of purity was considered acceptable by other investigators [8,23,25]. Subsequent investigations of Ca^{2+} uptake and channel expression would be representative of the trophoblast characteristics.

3.2. Hormonal status of the primary cultures of human trophoblasts

In order to verify the differentiation of isolated cytotrophoblast cells into functional syncytiotrophoblast, we measured over a period of 7 days the secretion of hCG, a well known marker of this process [8]. Fig. 2 shows a profile of the relative secretion of hCG by freshly isolated cytotrophoblasts from term placentas (37–40 weeks of pregnancy). The hCG secretion was very modest over the first 24 h of culture. The secretion then increased reaching a peak at 4 days of culture (typical value of 1456 mU/well/24 h for a 38-week placenta), and declined thereafter. These results

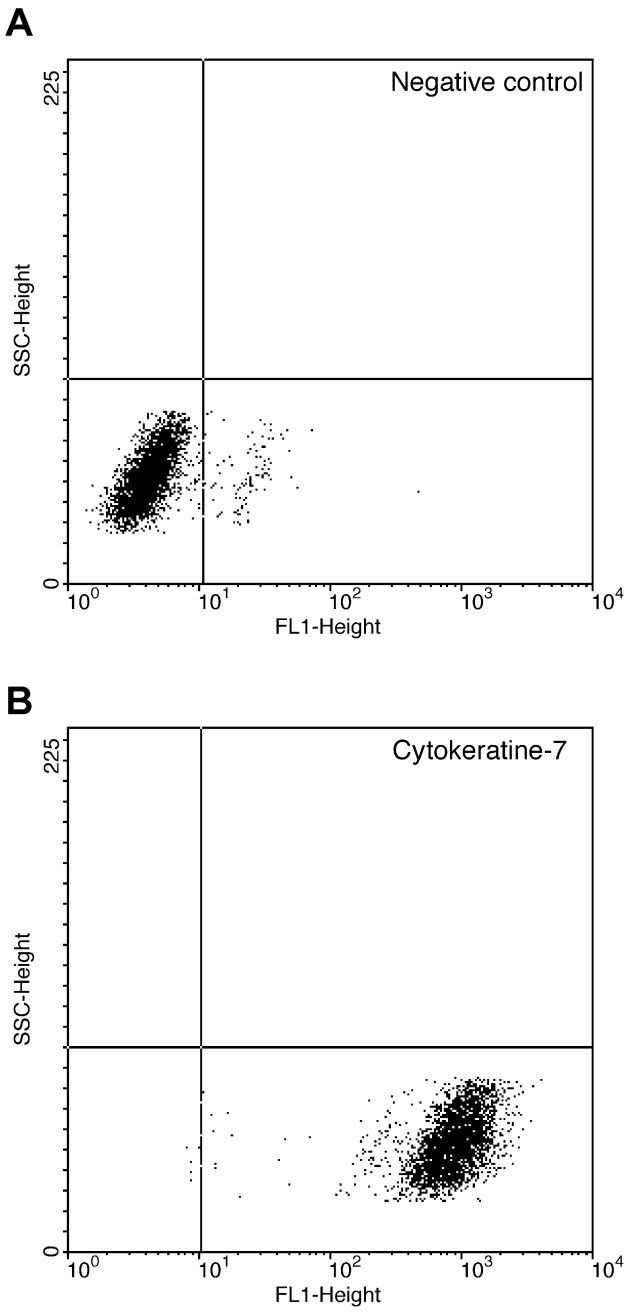

Fig. 1. Flow cytometry data of a representative preparation of cytotrophoblast cells from human term placenta incubated with PBS alone (A) or with monoclonal antibody against cytokeratin 7 (B). FL1-height corresponds to arbitrary intensity of FITC staining and SSC-height corresponds to side scatter an arbitrary evaluation of cell size.

suggest that our cytotrophoblast preparations were highly purified, without syncytial fragments, since hCG secretion over the first 24 h was barely detectable. These cells differentiated into syncytiotrophoblast during 4 days of culture as indicated by the increase of hCG secretion, confirming what was previously described [8]. The following decrease of hCG secretion has been observed previously [24,27] but explanation remains obscure although apoptosis could explained such decrease in hCG secretion [28].

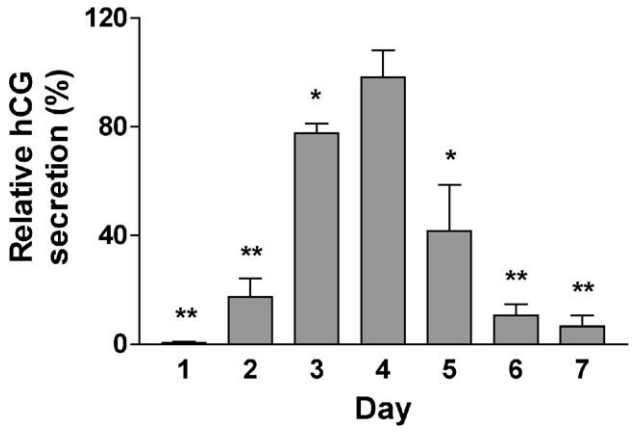

Fig. 2. hCG secretion of trophoblast primary culture from human term placenta in function of culture time. The data are expressed as relative secretion of hCG ± S.E. of four cell preparations from placentas of 37–40 weeks of pregnancy. * $P<0.025$, ** $P<0.001$.

Therefore, we performed subsequent experiments with 4-day cultured trophoblasts.

3.3. Ca^{2+} uptake by primary culture of human trophoblasts

The cells exhibited an active extracellular Ca^{2+} uptake (Fig. 3) with a rapid uptake during the first 2 min with an initial velocity (V_i) of 6.02 ± 0.29 nmol/mg/min, which gradually reached a plateau (14.55 ± 1.82 nmol/mg protein at 30 min of incubation). This uptake was proportional to the concentration of Ca^{2+} in the incubation medium (Fig. 4). The kinetic of Ca^{2+} uptake by syncytiotrophoblast has similar shape compared to basal Ca^{2+} uptake by undifferentiated cytotrophoblast cell line BeWo [26] with, however, a higher V_i (compared to 4.17 ± 0.25, $P<0.005$) and a higher level of cell-associated $^{45}Ca^{2+}$ at the plateau (compared to 8.65 ± 0.84 nmol/mg protein, $P<0.025$). The Ca^{2+} uptake studies using whole cell are a reflection of the dynamic homeostasis of cellular Ca^{2+} where mechanisms of Ca^{2+} entry and exit coexist, resulting in the

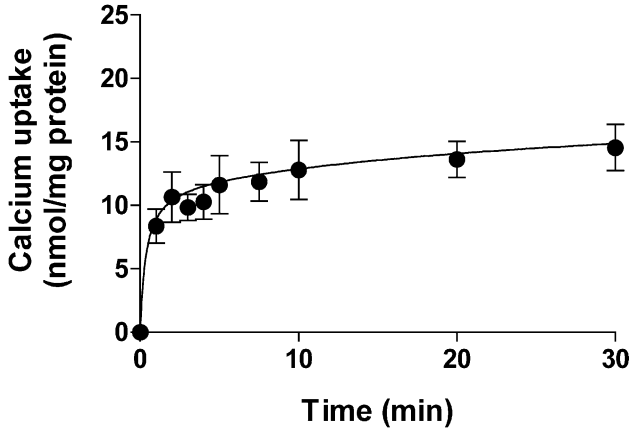

Fig. 3. Time-course of Ca^{2+} uptake by 4-day trophoblast culture cells. The results represent the mean ± S.D. of experiments performed in duplicates with cell preparations from three to five placentas.

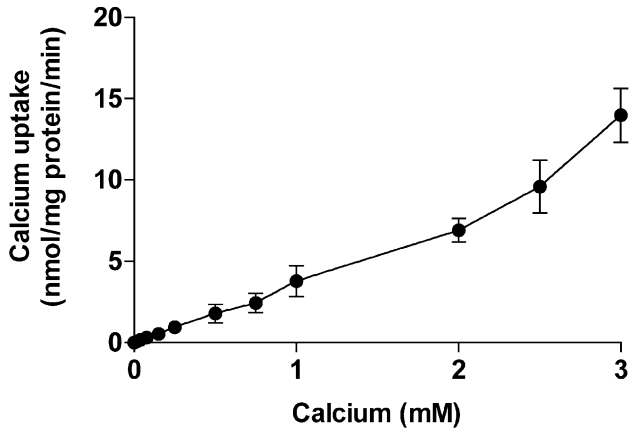

Fig. 4. Ca^{2+} uptake in function of different Ca^{2+} concentrations. Cells were incubated for 1 min at 37 °C in modified HBSS (without calcium) containing increasing amount of Ca^{2+} (0.0325–3 mM) with $^{45}CaCl_2$. The results represent the mean ± S.E. of experiments performed in duplicates with cell preparations from three placentas.

maintenance of an adequate intracellular Ca^{2+} level. The first portion of the kinetic curve (first 5 min) more likely represents Ca^{2+} uptake mechanisms and our results indicated higher Ca^{2+} uptake capacity of syncytiotrophoblast compared to BeWo cells (according to respective V_i). The establishment of a gradual plateau around 5 min of incubation has been generally viewed as an equilibrium of the radiolabel between the medium and the intracellular space. The level of cell-associated $^{45}Ca^{2+}$ observed at the plateau also showed the extend of intracellular Ca^{2+} retention that depends on factors such as the interaction of Ca^{2+} with intracellular proteins or its sequestration in intracellular membrane compartments. The intracellular Ca^{2+} retention capacity seems higher in the trophoblast cells compared to BeWo cells (according to the difference in the level of cell associated $^{45}Ca^{2+}$ at the plateau). In renal and intestinal epithelium, the Ca^{2+} transfer is modulated by the expression of specific Ca^{2+} binding proteins (calbindins). The expression of such calbindins in primary culture of trophoblasts of human term placenta has not been investigated until now although calbindin expression has been reported in placental tissue [20].

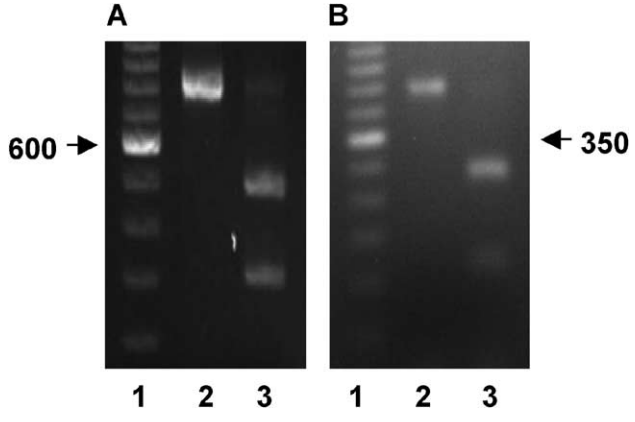

Fig. 5. Evaluation of the expression of Ca^{2+} channels of the CaT/ECaC family in primary culture of trophoblast from human term placenta. (A) PCRs were performed with specific primers for CaT1 (lane 2) and the amplicon was digested with *Sac*I (lane 3). Lane 1: 100-bp ladder. (B) PCRs were performed with primers for CaT2 (lane 2) and the amplicon was digested with *Xho*I (lane 3). Lane 1: 50-bp ladder.

3.4. Expression of Ca^{2+} channels of the CaT/ECAC family in human trophoblast cells

The expression of CaT1 and CaT2 was evaluated by reverse transcription polymerase chain reaction (RT-PCR) in 4-day trophoblast cultures using specific primers. Fig. 5A shows that an amplicon of 795 bp corresponding to the expected size was intensely amplified with total RNA from trophoblasts cultured for 4 days using another set of primers for CaT1. Specificity of the amplicon was demonstrated following digestion with *Sac*I (fragments of 490 and 305 bp, lane 3). Fig. 5B shows that an amplicon of 448 bp corresponding to the expected size was intensely amplified with total RNA from 4-day cultured trophoblast using specific primers for CaT2. Specificity of the amplicon was demonstrated following digestion with *Xho*I (fragments of 282 and 166 bp, lane 3). Expression of CaT1 was also investigated in cultured human syncytiotrophoblast by Northern blotting. A cDNA probe (785 bp) was generated by PCR with primers specific to CaT1 (Fig. 6A). The expression of CaT1 as transcript of approximately 3 kilobase (kb), of size expected according to the sequence of GenBank, was revealed by Northern blot in cultured syncytiotrophoblast (Fig. 6B). However, expression of CaT2 could not be revealed by Northern blot with PCR-generated CaT2 specific cDNA probe and may be due to low sensi-

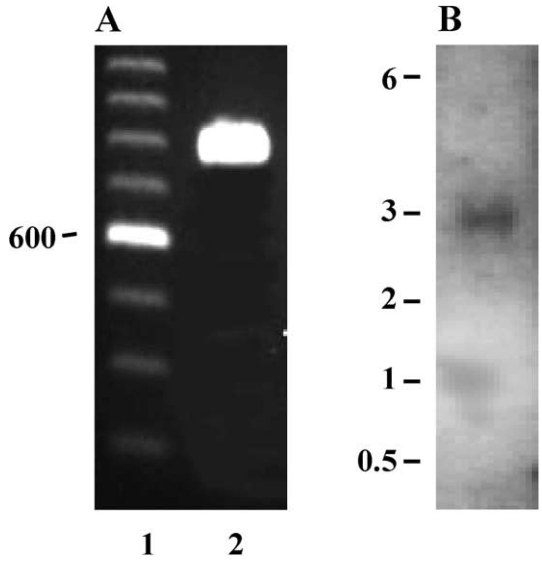

Fig. 6. Representative Northern blot of cultured human syncytiotrophoblast RNA with CaT1 probe. (A) cDNA amplified from total RNA and used as CaT1 probe. Lane 1: 100-bp ladder. (B) Total RNA (10 μg) was hybridized with cDNA CaT1 probe. Size markers are indicated (kb).

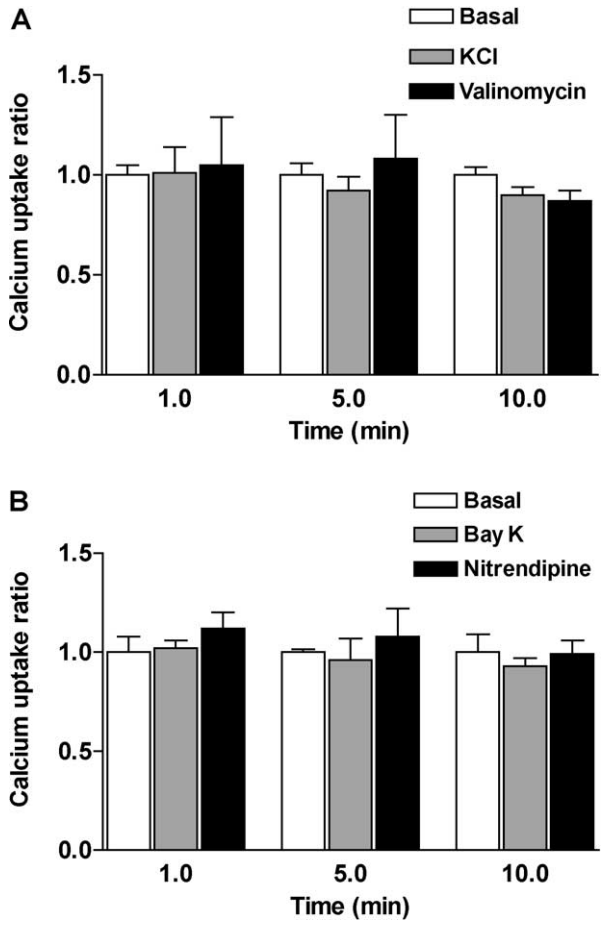

Fig. 7. Effect of cell membrane potential and L-type Ca^{2+} channel modulators on the Ca^{2+} uptake of trophoblast cells. The cells were incubated in the presence of (A) 40 mM KCl, 10 μM valinomycin or in control medium (Basal), and in the presence of (B) 2 μM nitrendipine, 10 μM Bay K 8644 or in control medium (Basal). The results are expressed as the ratio of the Ca^{2+} uptake of treated cells compared with cells incubated in control conditions (vehicle or isoosmotic medium). Data represent the mean ± S.E. of experiments performed in duplicates with cell preparations from three placentas.

tivity of the technique for low level of expression of CaT2 as reported by Peng et al. [21].

3.5. Effect of cell membrane potential and L-type Ca^{2+} channel modulators on the Ca^{2+} uptake

Basal Ca^{2+} uptake of trophoblast cultures was not modulated by cell depolarisation with high potassium (40 mM) in the incubation medium or by hyperpolarisation with valinomycin (Fig. 7A). Furthermore, modulators of the activity of L-type Ca^{2+} channels (namely Bay K 8644 as an agonist and nitrendipine as an antagonist) had no effect on the basal Ca^{2+} uptake of trophoblast cultures (Fig. 7B). Cell treatments with KCl and Bay K 8644 were, however, effective to stimulate hCG secretion (57.8 ± 12.3 and 62.2 ± 13.8 mU/well/12 h for KCl and Bay K 8644, respectively compared to 34.2 ± 8.7 mU/well/12 h for a 40-week placenta) as previously reported [10]. Nevertheless, such Ca^{2+} uptake that trigger hCG secretion may be minimal and localized, and would not be of major impact on global cell Ca^{2+} uptake. Recently, we have reported similar insensitivity of basal Ca^{2+} uptake by BeWo cells [26], indicating that voltage-sensitive Ca^{2+} channels are minor contributor in the basal Ca^{2+} uptake. For short incubation period (1 and 5 min) where Ca^{2+} uptake is likely to depend principally on membrane transfer, the various treatments (KCl, valinomycin, Bay K 8644 and nitrendipine) had not effect on this initial uptake. This insensitivity of Ca^{2+} uptake under these conditions argues well with the minor contribution of L-type Ca^{2+} channels in basal Ca^{2+} uptake by cultured trophoblast cells. For longer incubation period of 30 min, KCl (13.45 ± 1.39 nmol/mg protein), valinomycin (14.86 ± 0.90 nmol/mg protein), Bay K 8644 (12.01 ± 0.98 nmol/mg protein) and nitrendipine (14.97 ± 1.00 nmol/mg

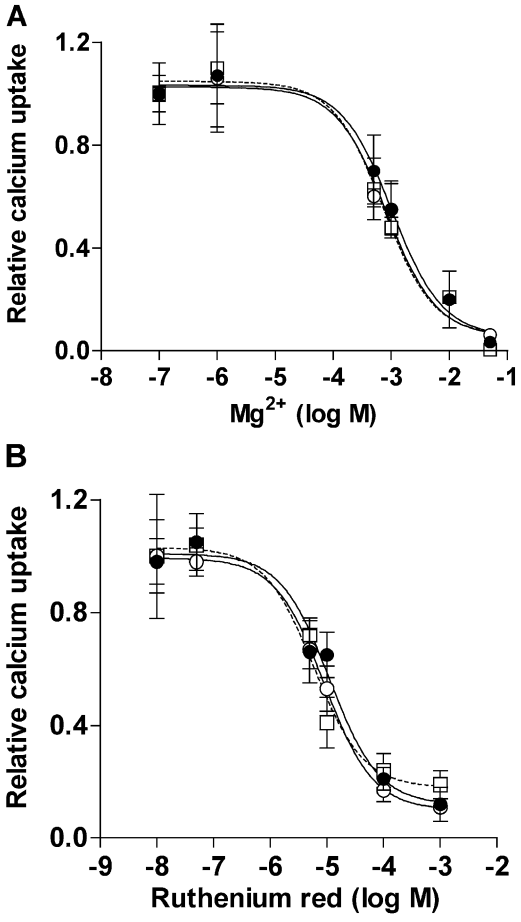

Fig. 8. Dose–response analysis for extracellular Mg^{2+} or ruthenium red inhibition of Ca^{2+} uptake by 4-day cultured trophoblasts. (A) Cells were incubated for 1 min (●), 5 min (○) and 15 min (□) at 37 °C in modified HBSS (with 100 μM Ca^{2+} and without Mg^{2+}) containing increasing amount of Mg^{2+} (0.001, 0.5, 1, 10 and 50 mM) and 2–4 μCi of $^{45}CaCl_2$. (B) Cells were preincubated with increasing concentrations of ruthenium red (0.05, 5, 10, 100 and 1000 μM) for 10 min and uptakes were performed in HBSS for 1 min (●), 5 min (○) and 15 min (□). Ca^{2+} uptake data were normalised to the corresponding uptake in the absence of Mg^{2+} or ruthenium red. The results represent the mean ± S.E. of experiments performed in duplicates with cell preparations from three to four placentas.

protein) did not induced significant effect compared to basal condition (14.23 ± 0.94 nmol/mg protein). Insensitivity of the basal placental Ca^{2+} uptake or transfer toward voltage-sensitive Ca^{2+} channel blockers was also observed by Stulc et al. [29] with perfused human placental cotyledon, by Brunette and Leclerc [30] and Kamath et al. [31] with brush border membrane vesicles. Therefore, our current results are in agreement with the insensitivity of basal placental Ca^{2+} uptake toward voltage-sensitive Ca^{2+} channel blockers. It also appears that the activity of L-type Ca^{2+} channels in trophoblast cells is of minor contribution in the Ca^{2+} uptake under basal conditions. The role of such L-type Ca^{2+} channels in trophoblast cells may reside more likely in the regulation of protein secretion (especially for hCG, placental lactogen and CRF) as previously reported [9–13].

3.6. Effect of extracellular Mg^{2+} and ruthenium red on the Ca^{2+} uptake

We performed dose-response inhibition of human syncytiotrophoblast Ca^{2+} uptake by extracellular Mg^{2+} and ruthenium red. Fig. 8A shows that increasing extracellular concentrations of Mg^{2+} dose-dependently inhibited cultured syncytiotrophoblast Ca^{2+} uptake evaluated at 1, 5 and 15 min with IC_{50} of 0.96 ± 0.09 mM. Hoenderop et al. [22] have reported IC_{50} of 1.4 mM for CaT1/ECaC2 and of 0.33 mM for CaT2/ECaC1 [32] by patch-clamp studies. Our current results are in agreement with these effective concentrations. Fig. 8B shows that ruthenium red led to a dose-dependent inhibition of cultured syncytiotrophoblast Ca^{2+} uptake evaluated at 1, 5 and 15 min with IC_{50} of 9.02 ± 1.75 μM. Similar IC_{50} of 9 μM for CaT1/ECaC2 and of 121 μM for CaT2/ECaC1 have been obtained by patch-clamp analysis [22]. Our results for ruthenium red inhibition were also in agreement with effective concentrations for CaT/ECaC-mediated Ca^{2+} uptake inhibition. Longer incubation period led to similar effect for extracellular Mg^{2+} (3.58 ± 0.39 vs. 1.22 ± 0.21 nmol/mg protein/15 min for basal compared to 50 mM Mg^{2+}, $P<0.005$) and for ruthenium red (10.58 ± 1.52 vs. 6.85 ± 1.12 nmol/mg protein/15 min for basal compared to 1000 μM ruthenium red, $P<0.005$).

Our results indicate for the first time the expression of CaT (namely CaT1 and CaT2) in primary culture of trophoblasts from human term placenta. Members of this family of Ca^{2+} channels are voltage-insensitive and are not modulated by L-type Ca^{2+} channels modulators, two characteristics of the basal Ca^{2+} uptake observed with trophoblast cells in the current study. Moreover, two potent blockers of CaT/ECaC-mediated Ca^{2+} uptake dose-dependently inhibited Ca^{2+} uptake of cultured human syncytiotrophoblast with IC_{50} similar to concentrations previously reported [22,32]. Our results suggest that Ca^{2+} channels of CaT/ECaC family are major contributors for the basal Ca^{2+} uptake of cultured human syncytiotrophoblast. Interestingly, we have previously reported the involvement of capacitative Ca^{2+} current in CRF secretion by trophoblast cells [13]. In recent studies, it was shown that the CaT1 displays the properties of the Ca^{2+}-release-activated Ca^{2+} channel [33], suggesting that CaT1 is functionally coupled to the IP_3 receptor and can be activated by depletion of the intracellular Ca^{2+} stores. Further investigations are warranted in order to ascertain the functional role of CaT1 and CaT2 in the placental physiology.

Acknowledgements

This work was supported by a grant from the March of Dimes, Saving Babies Together (USA).

References

[1] E.P. Hill, L.D. Longo, Fed. Proc. 39 (1980) 239–244, Fed. Am. Soc. Exp. Biol.
[2] H. Munro, S. Pilistine, M. Fant, Annu. Rev. Nutr. 3 (1983) 97–124.
[3] R.D.H. Boyd, Arch. Dis. Child. 62 (1987) 1205–1206.
[4] D. Shennan, C. Boyd, Biochim. Biophys. Acta 906 (1987) 437–457.
[5] C.W. Schauberger, R.M. Pitkin, Obstet. Gynecol. 53 (1979) 74–76.
[6] R.M. Pitkin, Am. J. Obstet. Gynecol. 151 (1985) 99–109.
[7] L. Dearden, C. Ockleford, in: Y. Loke, A. Whyte (Eds.), Biology of Trophoblast, Elsevier, Amsterdam, 1983, pp. 69–110.
[8] H.J. Kliman, J.E. Nestler, E. Sermasi, J.M. Sanger, J.F. Strauss III, Endocrinology 118 (1986) 1567–1582.
[9] A. Petit, N. Gallo-Payet, C. Vaillancourt, D. Bellabarba, J.G. Lehoux, S. Belisle, J. Clin. Endocrinol. Metab. 77 (1993) 670–676.
[10] S. Meuris, B. Polliotti, C. Robyn, P. Lebrun, Biochim. Biophys. Acta 1220 (1994) 101–106.
[11] B. Cemerikic, R. Zamah, M.S. Ahmed, J. Mol. Neurosci. 10 (1998) 261–272.
[12] J. Robidoux, L. Simoneau, A. Masse, J. Lafond, J. Clin. Endocrinol. Metab. 85 (2000) 3356–3364.
[13] J. Robidoux, L. Simoneau, S. St-Pierre, A. Masse, J. Lafond, Endocrinology 141 (2000) 2795–2804.
[14] N.F. Barley, A. Howard, D. O'Callaghan, S. Legon, J.R.F. Walters, Am. J. Physiol.: Gastrointest. Liver Physiol. 280 (2001) G285–G290.
[15] J.B. Peng, X.Z. Chen, U.V. Berger, P.M. Vassilev, H. Tsukaguchi, E.M. Brown, M.A. Hediger, J. Biol. Chem. 274 (1999) 22739–22746.
[16] J.B. Peng, X.Z. Chen, U.V. Berger, S. Weremowicz, C.C. Morton, P.M. Vassilev, E.M. Brown, M.A. Hediger, Biochem. Biophys. Res. Commun. 278 (2000) 326–332.
[17] U. Wissenbach, B.A. Niemeyer, T. Fixemer, A. Schneidewind, C. Trost, A. Cavalié, K. Reus, E. Meese, H. Bonkhoff, V. Flockerzi, J. Biol. Chem. 276 (2001) 19461–19468.
[18] J.G.J. Hoenderop, A.W.C.M. van der Kemp, A. Hartog, S.F.J van de Graaf, C.H. van Os, P.H.G.M. Willems, R.J.M. Bindels, J. Biol. Chem. 274 (1999) 8375–8378.
[19] J.B. Peng, X.Z. Chen, U.V. Berger, P.M. Vassilev, E.M. Brown, M.A. Hediger, J. Biol. Chem. 275 (2000) 28186–28194.
[20] D. Müller, J.G.J. Hoenderop, I.C. Meij, L.P.J. van den Heuvel, N.V.A.M. Knoers, A.I. den Hollander, P. Eggert, V. García-Nieto, F. Claverie-Martin, R.J.M. Bindels, Genomics 67 (2000) 48–53.
[21] J.B. Peng, E.M. Brown, M.A. Hediger, Genomics 76 (2001) 99–109.
[22] J.G.J. Hoenderop, R. Vennekens, D. Müller, J. Prenen, G. Droogmans, R.J.M. Bindels, B. Nilius, J. Physiol. 537 (2001) 747–761.
[23] F.M. Campbell, P.G. Bush, J.H. Veerkamp, A.K. Dutta-Roy, Placenta 19 (1998) 409–415.

[24] Y. Kato, G.D. Braunstein, J. Clin. Endocrinol. Metab. 68 (1989) 814–820.
[25] T. Haigh, C.P. Chen, C.J.P. Jones, J. Aplin, Placenta 20 (1999) 615–626.
[26] R. Moreau, L. Simoneau, J. Lafond, Placenta 22 (2001) 768–775.
[27] D.W. Morrish, D. Bhardwaj, L.K. Dabbagh, H. Marusyk, O. Siy, J. Clin. Endocrinol. Metab. 65 (1987) 1282–1290.
[28] T.M. Mayhew, L. Leach, R. McGee, W.W. Ismail, R. Myklebust, J. Lammiman, Placenta 20 (1999) 407–422.
[29] J. Stulc, B. Stulcova, M. Smid, I. Sach, Am. J. Obstet. Gynecol. 170 (1994) 162–167.
[30] M.G. Brunette, M. Leclerc, Can. J. Physiol. Pharm. 70 (1992) 835–842.
[31] S.G. Kamath, L.K. Kelley, A.F. Friedman, C.H. Smith, Am. J. Physiol. 262 (3 Pt. 1) (1992) C789–C794.
[32] R. Vennekens, J. Prenen, J.G.J. Hoenderop, R.J.M. Bindels, G. Droogmans, B. Nilius, J. Physiol. 530 (2001) 183–191.
[33] L. Yue, J.B. Peng, M.A. Hediger, D.E. Clapham, Nature 410 (2001) 705–709.