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Hypoxia alters expression and function of syncytin and its receptor during trophoblast cell fusion of human placental BeWo cells: implications for impaired trophoblast syncytialisation in pre-eclampsia

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Abstract

The fundamental process of placental trophoblast cell fusion (syncytiotrophoblast formation or syncytialisation) which is a characteristic of this tissue is poorly understood. Pre-eclampsia is associated with placental hypoxia and suppressed syncytiotrophoblast formation. We therefore have studied the effect of low-oxygen tensions on the rate of cell fusion, relative abundance of mRNAs encoding syncytin and its receptor, amino acid transport system B^0, which are thought to be involved in trophoblast cell fusion (as well as the activity of amino acid transport through this system) in a cell model of syncytialisation (BeWo cells following forskolin treatment). Forskolin-induced cell fusion (determined by a quantitative flow cytometry assay) was reversibly suppressed in 2% oxygen compared to 20% oxygen. This was associated with suppressed secretion of human chorionic gonadotropin. Forskolin stimulated relatively less syncytin mRNA (determined by reverse transcription–polymerase chain reaction) in 2% than in 20% oxygen and there was no stimulation after 48 h in 2% oxygen. There was a spontaneous, time-dependent increase of amino acid transporter B^0 mRNA in vehicle, which was suppressed by 2% oxygen and by forskolin treatment in 20% oxygen. Forskolin-induced changes in amino acid transport system B^0 function were not seen in cells cultured for 48 h in 2% oxygen. These observations suggest that under conditions of low ambient oxygen, dysregulation of expression of syncytin and of its receptor may suppress the normal process of cell fusion necessary for syncytiotrophoblast formation and contributes to syncytiotrophoblast abnormalities characteristic of pre-eclampsia.
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Keywords: Syncytiotrophoblast; Cell fusion; Oxygen; Placenta; BeWo cell

1. Introduction

The trophoblast surface of the human placenta forms a syncytium (the syncytiotrophoblast) which is built from the underlying cellular cytotrophoblast. The syncytiotrophoblast layer, in direct contact with maternal blood, plays an important role throughout pregnancy because it is the site of many placental functions, including nutrient exchange and the synthesis of steroid and peptide hormones which are required for normal fetal growth and development. However, it is remarkable that little is known about the trophoblast cell fusion process (syncytiotrophoblast formation or syncytialisation). Mi et al. [1] suggested that syncytin, a protein encoded by an envelope gene of the recently identified human endogenous retrovirus-W (ERV-W) [2], may mediate placental cytotrophoblast fusion in vivo, and thus be important in human placental morphogenesis. In primary culture of isolated cytotrophoblast cells, the transcript levels of syncytin increase with the differentiation and fusion of cytotrophoblasts into syncytiotrophoblasts [3]. Blond et al. [4] also showed that the ERV-W envelope gene encodes a highly fusogenic membrane glycoprotein that can induce syncytium formation upon interaction with the type D mammalian retrovirus receptor. Recently, the type D mammalian retrovirus receptor has been identified as amino acid transporter B^0 (ASCT2) [5]. Our recent experiments showed that under normoxic conditions (20% O_2), relative mRNA abundance for syncytin is enhanced and for its

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receptor, amino acid transport system B^0 (ASCT2), is suppressed in BeWo cells during syncytialisation by forskolin [6]. It has been reported that placental syncytin mRNA expression level is reduced in pregnancies complicated with pre-eclampsia [7] and its extreme form, hemolysis, elevated liver enzymes and low platelets syndrome [8]. Pre-eclampsia is a disorder associated with maternal hypertension, reduction in placental blood flow and placental hypoxia [9]. The histological abnormalities of placentae in pregnancy associated with underperfusion and hypoxia are characterised by cytotrophoblast prominence and abnormalities in syncytiotrophoblast formation [10]. It also has been demonstrated that trophoblast cell fusion and differentiation are inhibited by hypoxia [11–13]. These observations have raised the possibility that low oxygen may, via regulation of syncytin gene expression, suppress trophoblast cell fusion. To address this proposal, we now have studied in parallel the effect of low-oxygen tensions on the rate of trophoblast cell fusion and on the expression of mRNAs encoding syncytin and its receptor (amino acid transport system B^0 (ASCT2)) together with functional changes in amino acid transport through this system using BeWo cells as a cell model of syncytialisation.

2. Materials and methods

2.1. BeWo cell culture

BeWo cells were maintained at 37 °C as monolayers in Ham's F-12 medium supplemented with 10% fetal bovine serum (FBS), 2 mM L-glutamine, 100 U ml^{-1} penicillin and 100 U ml^{-1} streptomycin in a humidified atmosphere of 5% CO_2 and 95% air. Cells were subcultured by treating with 0.05% trypsin in Ca^{2+}- and Mg^{2+}-free phosphate buffer saline (PBS) containing 0.02% ethylenediaminetetraacetate, seeded in 35-mm plastic culture dishes and grown for 2–3 days to the stage of 50% confluence in a humidified atmosphere of 5% CO_2 and 95% air. At 50% confluency, the medium was changed to one containing 100 μM forskolin or vehicle (dimethyl sulfoxide) and incubated for the indicated times at 37 °C in a humidified atmosphere of either 95% air and 5% CO_2 (20% O_2) or 2% O_2, 93% N_2 and 5% CO_2 (Invivo$_2$ Hypoxia Workstation, Ruskinn Technology, Leeds, West Yorkshire, UK). At the end of the incubation period, cells were more than 95% viable as assessed by trypan blue dye exclusion. Cultures were conducted in triplicate for each set of experiments to assess reproducibility. The conditioned medium was collected and centrifuged at $3000 \times g$ at 4 °C for 10 min to remove cellular debris and stored at − 70 °C until use.

2.2. Cell fusion analysis

The rate of cell fusion (syncytialisation) was measured as described previously by flow cytometry [14]. Cloned BeWo cells expressing either a fusion protein of green fluorescent protein and human histone H2B (H2B-GFP) or a fusion protein of red fluorescent protein and the mitochondrial targeting sequence from subunit VIII of human cytochrome *c* oxidase (Mit-DsRed2) were mixed at 50% and grown to the stage of 50% confluence. At 50% confluency, the medium was changed to one containing 100 μM forskolin or vehicle (dimethyl sulfoxide), and incubated for the indicated times at 37 °C in a humidified atmosphere of either 95% air and 5% CO_2 (20% O_2) or 2% O_2, 93% N_2 and 5% CO_2, or in 2% O_2 for the first 24 h and then in 20% for the time indicated. Cells were harvested by trypsinisation, fixed and the number of single fluorescent (green or red) positive cells (i.e., non-fused or non-detectably fused cells) and double fluorescent (green and red) positive cells (i.e., detectably fused cells) were counted using a fluorescence-activated cell sorting (FACS) (EPICS Altra, Beckman Coulter, High Wycombe, UK). Twenty thousand cells were analysed on each sample.

2.3. Human chorionic gonadotropin (hCG) secretion

hCG secretion was determined by measuring its concentrations in the conditioned medium by an immunoassay kit which specifically detects β-chain of hCG.

2.4. Thymidine, uridine and leucine incorporation

To estimate DNA, RNA and total protein synthesis in BeWo cells, the degree of thymidine, uridine and leucine incorporation into acid-insoluble material was measured by the incubation of cells with culture medium containing [^{3}H]thymidine (1 μCi ml^{-1}), [^{3}H]uridine (1 μCi ml^{-1}) and L-[^{3}H]leucine (1 μCi ml^{-1}), respectively. After incubation for 1 h, cells were fixed to the dish and subsequently washed three times with ice-cold 10% trichloroacetic acid. The cells were then made soluble with 0.1 M NaOH and 0.1% SDS and an aliquot was taken for scintillation counting.

2.5. RNA extraction and reverse transcription–polymerase chain reaction (RT-PCR) analysis

Relative mRNA abundance of syncytin and amino acid transporter B^0 was analysed by semi-quantitative RT-PCR using glyceraldehyde 3-phosphate dehydrogenase (GAPDH) as an internal standard. Total RNA was extracted from cultured cells with QuickPrep Total RNA Extraction Kit according to the manufacturer's protocol. RNA samples were treated with DNase I before RT-PCR to remove any contaminating DNA. The primers used in the subsequent RT-PCR were as follows: syncytin [1], forward, 5′-AGGAGCTTCGAAACACTGGA-3′ and backward, 5′-GTGAGCTAAGTTGCAAGCCC-3′; amino acid transporter B^0 [15], forward, 5′-GGCTTGGTAGTGTTTGCCAT-3′ and

backward, 5′-GGGCAAAGAGTAAACCCACA-3′; GAPDH [16], forward, 5′-CGGGAAGCTTGTGATCAATGG-3′ and backward, 5′-GGCAGTGATGGCATGGACTG-3′. The expected sizes of the PCR products are 494 bp for syncytin, 205 bp for amino acid transport system B^0 and 358 bp for GAPDH. One microgram RNA was reverse transcribed into cDNA using oligo(dT)$_{12-18}$ primer. The reverse transcription reaction, containing 500 μM deoxynucleotide 5′-triphosphate, 25 μg ml^{-1} oligo(dT)$_{12-18}$ primer, 10 unit μl^{-1} M-MLV reverse transcriptase, 3 mM $MgCl_2$, 75 mM KCl, 10 mM dithiothreitol and 50 mM Tris–HCl (pH 8.3), was sequentially incubated at 25 °C for 10 min, at 42 °C for 50 min and at 70 °C for 15 min and cooled on ice. As a negative control for the absence of exogenous DNA contamination, reactions run without RNA or with RNA in the absence of the reverse transcriptase revealed no amplified product (data not shown). The synthesised cDNA (0.05 μg equivalent to RNA) was used for PCR amplification in a reaction mixture containing 200 μM dNTP, 1 μM forward and backward primers, 0.05 unit μl^{-1} Taq DNA polymerase, 1.5 mM $MgCl_2$, 50 mM KCl and 20 mM Tris–HCl (pH 8.4). The PCR conditions were: 94 °C for 3 min, 60 °C for 1 min and 72 °C for 2 min; then 22 cycles (for syncytin and amino acid transporter B^0) and 20 cycles (for GAPDH) of 94 °C for 1 min, 60 °C for 1 min and 72 °C for 2 min; followed by a 10-min final extension at 72 °C. The amount of template cDNA and the number of cycles were determined experimentally so that quantitative comparison could be made during the exponential phase of the amplification process for both target and reference gene. PCR products were separated on a 2% agarose gel. Gels were stained with ethidium bromide. A single band for each gene was observed at the expected size. The intensity of either the target or GAPDH band for each sample was quantitated using a gel documentation and analysis system (GDS8000, Ultra-Violet Products, Cambridge, UK) and the ratio of the two was used as a normalised value for expression of each target gene. All assays were conducted in triplicate.

2.6. Amino acid influx studies

After aspirating culture medium, each dish was washed twice with pre-warmed (37 °C) PBS and cells were depleted of intracellular amino acids by incubating in PBS at 37 °C for 30 min to minimize any *trans* effects. The influx of amino acid was initiated by replacing this with pre-warmed PBS containing 2 μM L-[^{3}H]alanine, followed by further incubation at 37 °C. Other additions are described in the figure legends. Following aspiration of isotope solution, cells were quickly washed with ice-cold PBS with 10 mM unlabelled L-alanine. Then, 0.1 M NaOH and 0.1% sodium dodecyl sulfate solution were added for solubilisation and aliquots were taken for liquid scintillation counting and protein determination. The carrier-mediated L-alanine influx rate was determined by subtracting the diffusional component from the total influx rate. The diffusional component was determined by measuring the influx of 2 μM L-[^{3}H]alanine in the presence of 50 mM unlabelled L-alanine. The system B^0 mediated influx rate was defined as the difference between the influx in the presence of α-(methylamino)isobutyric acid and the influx in the presence of α-(methylamino)isobutyric acid plus 2-aminobicyclo-(2,2,1)-heptane-2-carboxylic acid.

2.7. Protein estimation

Protein concentration of the cell extract was determined by the method of Lowry et al. [17] using bovine serum albumin as a standard.

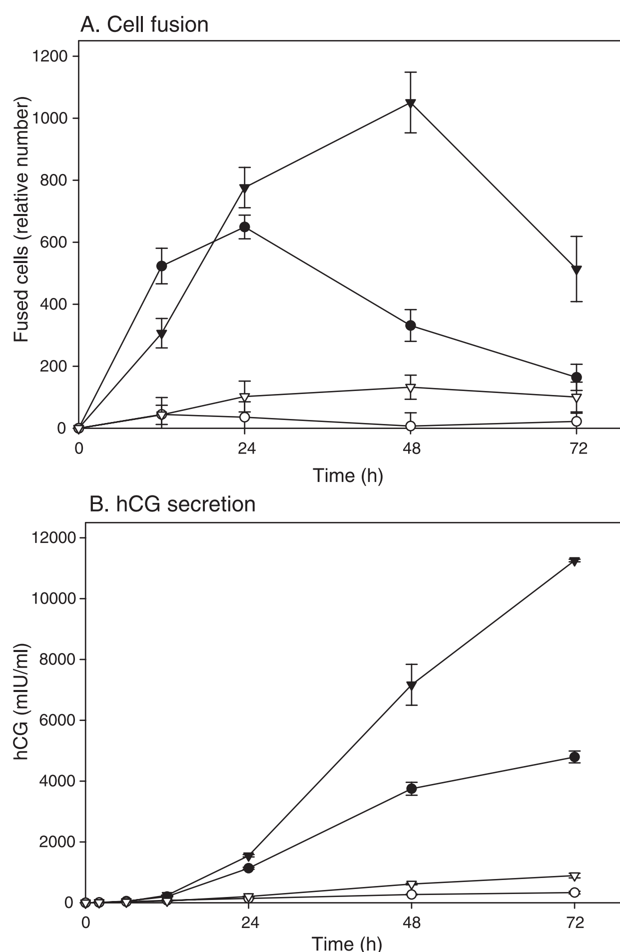


Fig. 1. Effect of oxygen tension on cell fusion and hCG secretion in BeWo cells. BeWo cells expressing either H2B-GFP or Mit-DsRed2 were mixed and cultured with 100 μM forskolin (filled symbols) or vehicle (open symbols) in 20% O_2 or 2% O_2 for the time indicated. Cell fusion was analysed on a FACS (A) and hCG concentrations in the conditioned medium were measured by immunoassay (B) as described in Materials and methods. ▾, ▿, 20% O_2; ●, ○, 2% O_2. Data represent the mean ± S.D. of three separate experiments.

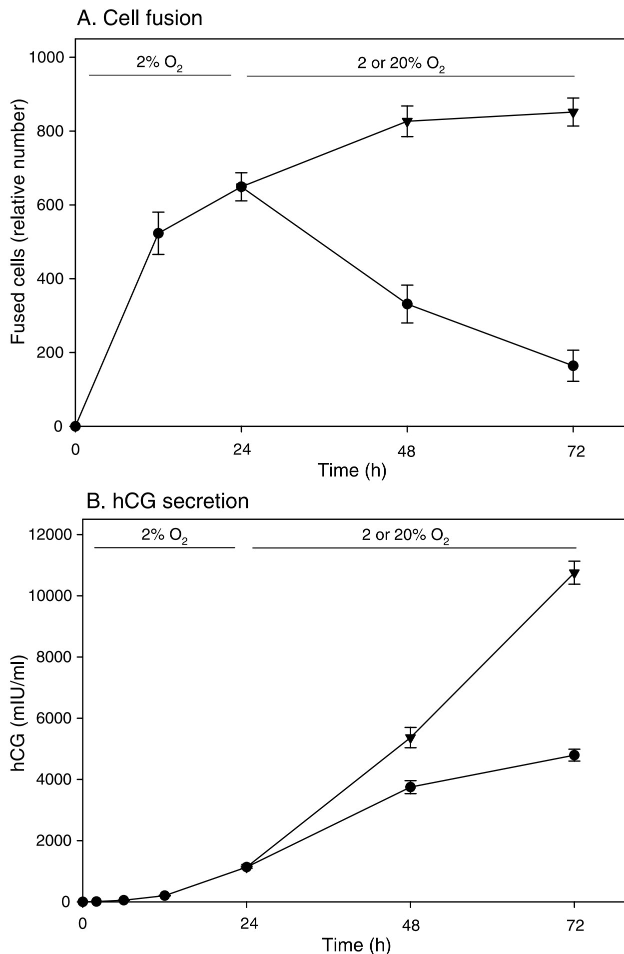


Fig. 2. Effect of shifting oxygen tension on cell fusion and hCG secretion in BeWo cells. BeWo cells expressing either H2B-GFP or Mit-DsRed2 were mixed and cultured with 100 μM forskolin in 2% O_2 for the first 24 h and then in 2% or 20% O_2 for the time indicated. Cell fusion was analysed on a FACS (A) and hCG concentrations in the conditioned medium were measured by immunoassay (B) as described in Materials and methods. ▾, 20% O_2; ●, 2% O_2. Data represent the mean ± S.D. of three separate experiments.

2.8. Statistical analysis

Differences between groups were analysed using an ANOVA and results were considered statistically significant at $P < 0.05$.

2.9. Materials

BeWo cells (passage number approximately 40) were kindly given by Dr. S.L. Greenwood (Academic Unit of Child Health, St. Mary's Hospital, University of Manchester). [methyl-^{3}H]Thymidine (25.0 Ci mmol^{-1} or 925 GBq mmol^{-1}), [5-^{3}H]uridine (26.0 Ci mmol^{-1} or 962 GBq mmol^{-1}), L-[4,5-^{3}H]leucine (53.0 Ci mmol^{-1} or 1.96 TBq mmol^{-1}) and L-[2,3-^{3}H]alanine (59.0 Ci mmol^{-1} or 2.18 TBq mmol^{-1}) were purchased from Amersham Life Science (Amersham, Buckinghamshire, UK). Forskolin, α-(methylamino)isobutyric acid and 2-aminobicyclo-(2,2,1)-heptane-2-carboxylic acid were obtained from Sigma-Aldrich Chemical (Poole, Dorset,

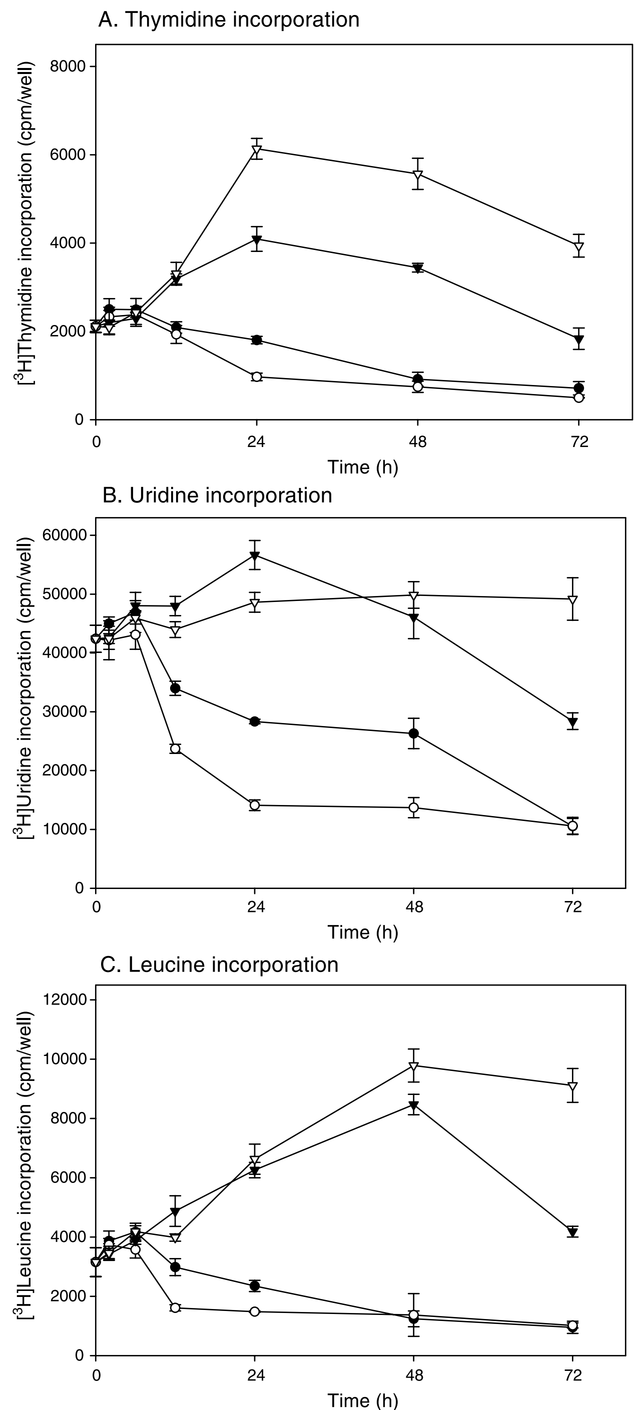
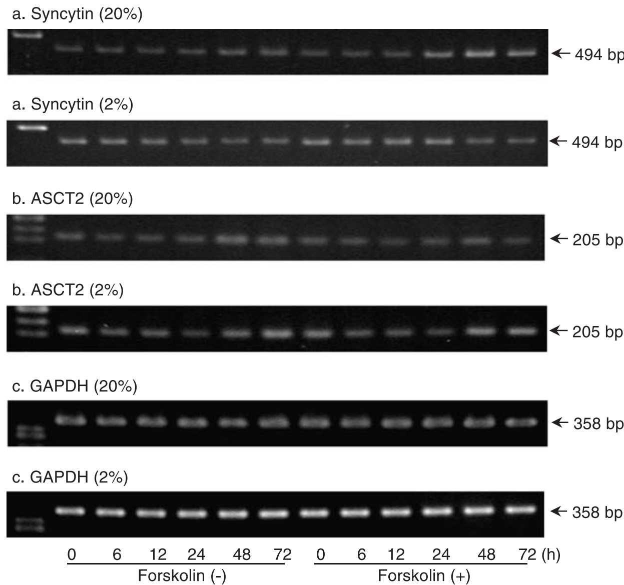


Fig. 3. Effect of oxygen tension on thymidine, uridine and leucine incorporation in BeWo cells. BeWo cells were cultured with 100 μM forskolin (filled symbols) or vehicle (open symbols) in 20% O_2 or 2% O_2 for the time indicated and [^{3}H]thymidine (A), [^{3}H]uridine (B) or [^{3}H]leucine (C) incorporation was then determined as described in Materials and methods. ▾, ▿, 20% O_2; ●, ○, 2% O_2. Data represent the mean ± S.D. of three separate experiments with triplicate assay.

UK). Tissue culture supplements were from Gibco BRL (Paisley, UK) and the human chorionic gonadotropin (hCG) chemiluminescent immunoassay kit was from Euro/DPC (Llanberis, Gwynedd, UK). QuickPrep Total RNA Extraction Kit were purchased from Amersham Pharmacia Biotech (Rainham, Essex, UK). Moloney murine leukemia virus (M-MLV) reverse transcriptase, oligo(dT)$_{12-18}$ primer, deoxynucleotide 5′-triphosphate and Taq DNA polymerase were from Gibco BRL. Deoxyribonuclease I (DNase I) was from Promega (Southampton, Hampshire, UK). All chemicals were of the highest purity commercially available.



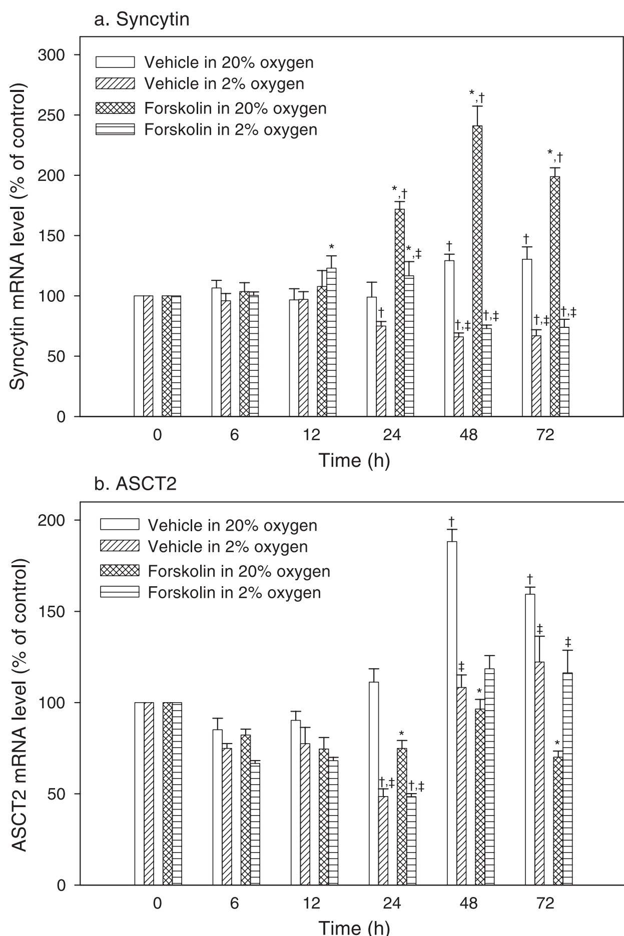


Fig. 4. Effect of oxygen tension on the relative abundance of syncytin mRNA and amino acid transporter B^0 mRNA in BeWo cells. Total RNA was extracted from BeWo cells cultured with 100 μM forskolin or vehicle in 20% O_2 or 2% O_2 for the time indicated. (A) RT-PCR. The relative abundance of syncytin mRNA(a), amino acid transporter B^0 (ASCT2) mRNA (b) and glyceraldehyde phosphate 3-dehydrogenase (GAPDH) mRNA (c) were analysed by RT-PCR as described in Materials and methods. The results presented are from a single representative experiment. (B) Relative quantitation of syncytin mRNA (a) and amino acid transporter B^0 (ASCT2) mRNA (b). The intensity of either the target gene or the GAPDH band was quantitated by using a gel documentation and analysis system of PCR products and the ratio of the two was used as a normalised relative abundance value of each target gene. Data represent the mean ± S.D. of three separate experiments, expressed as percentage of control (i.e. values at 0 h). *Significantly different from values cultured with vehicle alone ($P<0.05$). †Significantly different from values at 0 h ($P<0.05$). ‡Significantly different from values cultured in 20% O_2 ($P<0.05$).

3. Results

3.1. Effect of oxygen tension on forskolin-induced BeWo cell syncytialisation

To determine the effect of oxygen tension on the rate of cell fusion, BeWo cells were cultured in either 20% or 2% oxygen in the presence or absence of forskolin and then the number of fused cell was counted (Fig. 1A). Cell fusion was stimulated in a time-dependent manner by the presence of forskolin and peaked at 24 h in 2% oxygen and at 48 h in 20% oxygen after the initiation of forskolin treatment and declined thereafter. The number of fused cell after culturing for 48 h with forskolin in 2% oxygen was approximately one third of that in 20% oxygen. An associated increase in forskolin-induced hCG secretion by BeWo cells, a marker of syncytialisation, was more marked in 20% oxygen compared to 2% oxygen (Fig. 1B). When oxygen tension was shifted to 20% after BeWo cells had initially been cultured in 2% oxygen for 24 h, the number of fused cell was still increased (Fig. 2A). hCG secretion was also increased when oxygen tension had been shifted from 2% to 20% (Fig. 2B).

3.2. Effect of oxygen tension on DNA, RNA and total protein synthesis in BeWo cells

The rates of DNA, RNA and total protein synthesis in cells cultured in either 20% or 2% oxygen in the

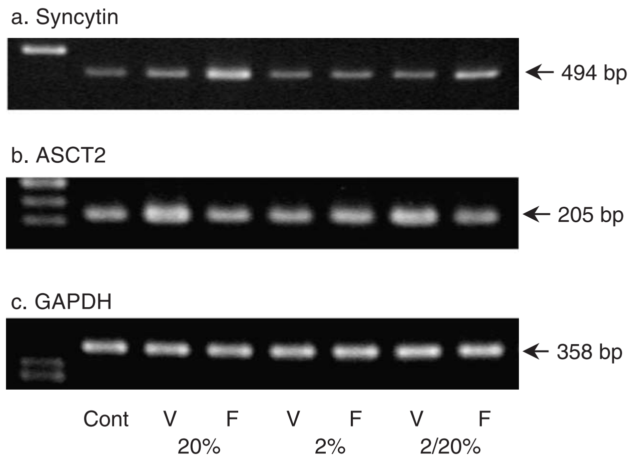


Fig. 5. Effect of shifting oxygen tension on the relative abundance of syncytin mRNA and amino acid transporter B^0 mRNA in BeWo cells. Total RNA was extracted from BeWo cells cultured with 100 μM forskolin or vehicle in 20% O_2 or 2% O_2 for 48 h or in 2% O_2 for 24 h followed by in 20% O_2 for 24 h. The relative abundance of syncytin mRNA(a), amino acid transporter B^0 (ASCT2) mRNA (b) and glyceraldehyde phosphate 3-dehydrogenase (GAPDH) mRNA (c) were analysed by RT-PCR as described in Materials and methods. The results presented are from single representative experiment. Cont, without culture; V, cultured with vehicle; F, cultured with forskolin.

presence or absence of forskolin were determined by measuring [^{3}H]thymidine, [^{3}H]uridine and [^{3}H]leucine incorporation, respectively (Fig. 3). Thymidine incorporation was significantly suppressed 12 h after the initiation of culture in 2% oxygen in either the presence or absence of forskolin. In 20% oxygen, forskolin suppressed thymidine incorporation 24 h after the initiation of culture. Uridine incorporation was also lower in 2% oxygen. It was stimulated up to 24 h and decreased thereafter in the presence of forskolin in 20% oxygen. In 2% oxygen, uridine incorporation was greater in the presence of forskolin up to 48 h. Leucine incorporation showed a similar pattern to uridine incorporation.

3.3. Effect of oxygen tension on the relative abundance of syncytin mRNA and amino acid transporter B^0 (ASCT2) mRNA in BeWo cells

The relative expression of mRNA encoding syncytin and its receptor, amino acid transporter B^0, following forskolin treatment in either 20% or 2% oxygen was studied by RT-PCR (Fig. 4). In vehicle alone, syncytin mRNA increased significantly after 48 h in 20% oxygen, whereas in 2% oxygen, the opposite change occurred, beginning at 24 h of culture (Fig. 4B,a). In 20% oxygen, forskolin elicited an earlier and greater response than vehicle alone, which peaked at 48 h (1.9-fold relative to the vehicle control). These data are similar to our earlier observations [6]. In 2% oxygen, forskolin stimulated less expression of syncytin mRNA (1.5-fold at 24 h of treatment, relative to the vehicle control) and a relative decrease at 48 and 72 h. Thus, the principle effect of culture in low oxygen was to attenuate and then reverse the marked increase stimulated by forskolin in high oxygen conditions.

In 20% oxygen, the expression level of mRNA for amino acid transporter B^0 increased time-dependently without forskolin treatment (Fig. 4B,b). Whereas forskolin stimulated the expression of syncytin mRNA in 20% oxygen, it significantly suppressed that of transporter B^0 mRNA in comparison with the vehicle control. However, compared to baseline expression, there was no consistent pattern after forskolin stimulation. In 2% oxygen, there were no differences when culture in vehicle alone was compared with culture after stimulation with forskolin. In comparison to baseline transporter B^0 mRNA declined, with or without stimulation, until then 24 h and then recovered. Two percent oxygen significantly diminished transporter B^0 mRNA expression compared with 20% oxygen but the differences were more marked and consistent in vehicle than after forskolin. In general, low oxygen had more prolonged and pronounced effects on syncytin mRNA than on transporter B^0 mRNA expression.

When cells which had initially been cultured in 2% oxygen for 24 h were shifted to a 20% oxygen environment, mRNA expression level of syncytin and amino acid transporter B^0 showed a response to forskolin similar to that observed in 20% oxygen (Fig. 5 and Table 1). GAPDH mRNA was constant for each sample.

3.4. Effect of oxygen tension on L-alanine influx supported by transport system B^0 in BeWo cells

To determine the effect of hypoxia on amino acid transport system B^0 activity, L-alanine (2 μM) influx into BeWo cells was determined under initial rate condition in the presence of Na^+ before and after forskolin treatment for 48 h in either 20% (Fig. 6A,a) or 2% (Fig. 6B,a) oxygen. Data show the

Table 1
Effect of oxygen tension on the relative abundance of syncytin mRNA and amino acid transporter B^0 mRNA in BeWo cells

Oxygen	Syncytin (percentage of control)		ASCT2 (percentage of control)	
	Vehicle	Forskolin	Vehicle	Forskolin
20%	132.0 ± 6.1†	256.9 ± 12.4*,†	185.9 ± 10.3†	98.2 ± 6.4*
2%	77.7 ± 5.7†	81.6 ± 3.2†	108.9 ± 6.7	112.6 ± 8.7
2/20%	106.2 ± 7.1	192.3 ± 9.3*,†	165.4 ± 10.1†	98.7 ± 4.3*

Relative quantitation of syncytin mRNA and amino acid transporter B^0 (ASCT2) mRNA. The intensity of either the target gene or the GAPDH band was quantitated by using a gel documentation and analysis system of PCR products in Fig. 5 and the ratio of the two was used as a normalised relative abundance value of each target gene. Data represent the mean ± S.D. of three separate experiments, expressed as percentage of control (i.e. values without culture).

* Significantly different from values cultured with vehicle alone ($P<0.05$).

† Significantly different from control ($P<0.05$).

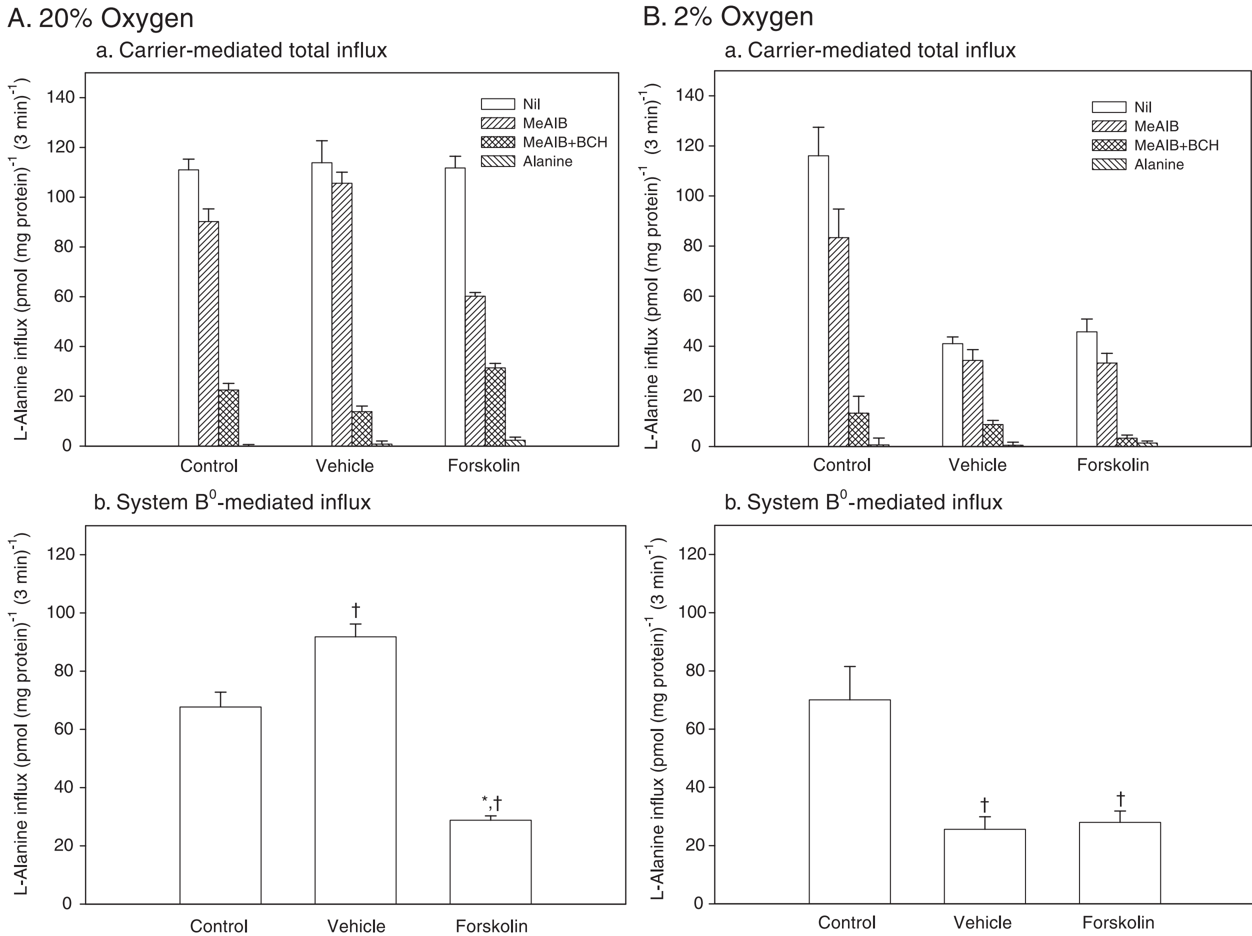


Fig. 6. Effect of oxygen tension on L-alanine influx in BeWo cells. L-Alanine influx over a 3-min period before treatment (control) and after 48 h treatment with 100 μM forskolin or vehicle in 20% O_2 (A) or 2% O_2 (B) was measured in a medium containing 2 μM L-[^{3}H]alanine with or without 20 mM unlabelled α-(methylamino)isobutyric acid (MeAIB), 20 mM MeAIB and 20 mM 2-aminobicyclo-(2,2,1)-heptane-2-carboxylic acid (BCH) or 20 mM L-alanine in the presence of Na^+. (a) Carrier-mediated influx rate defined by subtracting the diffusional component (determined by measuring the influx of L-[^{3}H]alanine in the presence of 50 mM unlabelled L-alanine) from the total influx. (b) System B^0-mediated influx rate defined as the difference between the influx in the presence of MeAIB plus BCH and the influx in the presence of MeAIB. Data represent the mean ± S.D. of three separate experiments of triplicate assay. *Significantly different from values cultured with vehicle alone ($P<0.05$). †Significantly different from control ($P<0.05$).

carrier-mediated influx rate defined by subtracting the diffusional component from the total influx rate. The diffusional component was determined by measuring the influx of 2 μM L-[^{3}H]alanine in the presence of 50 mM unlabelled L-alanine. Total carrier-mediated influx was not changed following 48 h culture in 20% oxygen in either the presence or absence of forskolin. When cells had been cultured in 2% oxygen, total carrier-mediated influx was decreased with or without forskolin treatment. To separate the transport pathways contributing to total flux in the presence of Na^+, the unlabelled synthetic amino acid α-(methylamino)isobutyric acid [18], a system A specific analogue, unlabelled α-(methylamino)isobutyric acid and unlabelled synthetic amino acid 2-aminobicyclo-(2,2,1)-heptane-2-carboxylic acid [18], a system L specific analogue, or unlabelled L-alanine was added at 20 mM. System B^0-mediated influx rate was defined as the difference between the influx in the presence of α-(methylamino)isobutyric acid and the influx in the presence of α-(methylamino)isobutyric acid plus 2-aminobicyclo-(2,2,1)-heptane-2-carboxylic acid. L-Alanine influx mediated by system B^0 was decreased in cells treated with forskolin and increased without forskolin in 20% oxygen (Fig. 6A,b). In contrast, system B^0-mediated L-alanine influx was suppressed in cells cultured in 2% oxygen whether treated with forskolin or not (Fig. 6B,b).

4. Discussion

In this paper, we have demonstrated that low-oxygen conditions suppress fusion of choriocarcinoma cells as well as abnormal gene expression of syncytin and its receptor, amino acid transport system B^0. These effects were rapidly reversed if the oxygen concentration was increased from 2% to 20%. It therefore is possible that hypoxic dysregulation of the genes encoding syncytin and its receptor may lead to

abnormalities in syncytiotrophoblast formation in vivo. However, whether the effect of oxygen tension on the expression of these two genes is direct or mediated by reactive oxygen species is not clear from our study. It will be of interest to test whether oxygen tension alters expression of GCMa, a placenta-specific transcription factor, which recently has been reported to regulate expression of the syncytin gene and consequently trophoblast cell fusion [19]. Because the present study was conducted using a human choriocarcinoma cell line, it remains to be investigated whether the same phenomenon is found in freshly isolated human trophoblasts.

Suppression of trophoblast cell fusion and differentiation under hypoxic condition has also been demonstrated using isolated trophoblast cell culture [11–13] and villous explant culture [20] where the authors determined the rate of syncytialisation by means of the disappearance of immunostained E-cadherin or desmoplakin or the increased numbers of nuclei in fused cell observed, after fixation, by light microscopy. In the present study, we have applied a fluorescence-activated cell sorting-based assay for determining the rate of syncytialisation [14]. This procedure allows more precise analysis of syncytialisation and sequential quantitation in living cells.

Increased cytotrophoblast proliferation in hypoxia has been observed in isolated cytotrophoblast cell culture [21] and in villous tissue culture [20]. However, our study using the BeWo choriocarcinoma cell line showed decreased cell proliferation determined by thymidine incorporation under hypoxic conditions. This discrepancy is probably because cells studied in these references [20,21] are extravillous cytotrophoblasts while the BeWo cell line may have a villous cytotrophoblast phenotype. It is known that the effect of oxygen tension on cell proliferation and differentiation has been reported to be cell type specific [22,23].

The effects of low oxygen on syncytiotrophoblast are relevant to the maternal syndrome of pre-eclampsia, a major complication of human pregnancy, with significant morbidity and mortality. There is substantial evidence that the clinical disease may be secondary to abnormalities of placentation and the ensuing placental hypoxia owing to a relative failure of the development of the utero-placental circulation [24]. There is increased oxidative stress [25] which becomes disseminated and contributes to maternal endothelial cell activation and dysfunction [26]. In this study, low oxygen in vitro was found to impair syncytialisation of BeWo. In pre-eclampsia, associated with placental hypoxia, there is also a defect in syncytiotrophoblast formation [10]. Syncytin expression in trophoblast is reported to be reduced in pre-eclampsia and localised aberrantly to the apical syncytiotrophoblast microvillous membrane instead of on the basal cytoplasmic membrane [7]. Similarly, placental syncytin mRNA expression levels are reduced in pre-eclamptic pregnancies complicated by hemolysis, elevated liver enzymes and low platelets in the maternal circulation [8] or in pregnancies with fetal trisomy 21 [3] in which syncytiotrophoblast formation is defective. The impact of impaired syncytialisation on chorionic villous growth and maturation is unknown but it could contribute to abnormalities found in pre-eclampsia. Because the syncytiotrophoblast layer is central to nutrient and gas exchange between mother and fetus and also to the synthesis of hormones that are required for normal fetal growth and development, abnormal syncytiotrophoblast cell fusion may contribute to intra-uterine fetal growth restriction and hypoxaemia frequently associated with pre-eclampsia [27].

The present work provides some insight into mechanisms that may underlie pre-eclampsia. Much further work is needed to define the molecular basis for both syncytialisation and the consequences of placental hypoxia.

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