Effect of oxygen concentrations on sodium iodide symporter expression and iodide uptake and hCG expression in human choriocarcinoma BeWo cells

Li H, Landers K, Patel J, Richard K, Mortimer RH. Effect of oxygen concentrations on sodium iodide symporter expression and iodide uptake and hCG expression in human choriocarcinoma BeWo cells. Am J Physiol Endocrinol Metab 300: E1085–E1091, 2011. First published March 15, 2011; doi:10.1152/ajpendo.00679.2010.—Normal human fetal development requires an adequate supply of thyroid hormone from conception. Until about 16 wk gestation this is supplied entirely by placental transfer of maternal hormone. Subsequently, the fetal thyroid synthesizes thyroid hormones, requiring a supply of maternal iodide. Trophoblast iodide transfer is mediated by the apical sodium iodide symporter (NIS). Placental oxygen levels are low in early pregnancy (~1%), rising with placental vascularisation to a plateau of ~8% at about 16 wk. Although the impact of these changing oxygen levels on placental implantation is well recognized, effects on trophoblast materno-fetal exchange are less understood. We investigated expression of the NIS regulator hCG, NIS mRNA expression, and I\textsuperscript{125} uptake in choriocarcinoma BeWo cells (a model of the trophoblast) cultured in 1 and 8% oxygen and in room air (21% oxygen). Expression of NIS and hCG mRNA and protein was low at 1% oxygen but rose significantly at 8% and at 21%. This was reflected in significant increases in I\textsuperscript{125} uptake. Desferrioxamine, an iron chelator and hypoxia mimic, decreased NIS and hCG expression and I\textsuperscript{125} uptake in BeWo cells. NIS expression and I\textsuperscript{125} uptake in cells grown at 1% oxygen were not increased by addition of hCG (2,500 IU/l). We infer that placental NIS mRNA and protein expression are regulated by oxygen, rising with vascularization of the placenta in the late first trimester, a time when fetal iodide requirements are increasing.

human chorionic gonadotropin; placenta; hypoxia; thyroid hormone

NORMAL FETAL DEVELOPMENT IS DEPENDENT ON an adequate supply of thyroid hormone (TH) throughout gestation. Before the human fetal thyroid begins to secrete TH at about 16 wk gestation, the fetus must derive its TH from the maternal circulation. Subsequently, although materno-fetal TH transport appears, to some degree, to continue throughout pregnancy (10, 61), the fetus synthesizes TH from maternally derived iodine. This can be drawn from placental deiodination of maternal TH and by placental transport of maternal iodide (21). Iodine deficiency is the greatest single cause of preventable brain damage (9). When compounded by the physiological increases in iodine requirements of pregnancy, even mild iodine deficiency can result in impaired fetal production of TH (45, 51, 65).

Iodide transport into the thyroid follicle and across the placenta share many features. Within the thyroid gland, iodide is actively taken up by thyocytes via the basal cell membrane

sodium iodide symporter (NIS) (14, 39, 57). It is then discharged into the thyroid follicle through the apical cell membrane anion transporter pendrin. Pendrin is an anion exchanger (37, 50, 53, 55) and is activated by high concentrations of intracellular iodide (63). The pituitary glycoprotein TSH upregulates NIS expression and iodide uptake (5, 29), whereas iodide has the opposite effect (15). In placenta, materno-fetal transfer of iodide involves iodide uptake through the apical trophoblast cell membrane and efflux through the basal cell membrane. NIS is expressed in the apical trophoblast cell membrane, which faces the maternal circulation. Conversely, Pendrin is expressed in the basal trophoblast cell membrane, and the two iodide transporters mediate iodide transfer in placenta (7, 37, 40). Trophoblasts synthesize and secrete large amounts of human chorionic gonadotropin (hCG), which has significant homologies with TSH. hCG has autocrine effects on trophoblast cells via the hCG-leutinizing hormone receptor, resulting in the upregulation of NIS expression. Iodide downregulates both NIS and hCG expression in trophoblast (4, 33).

It is becoming increasingly apparent that expression of many placental genes and the development of the placenta itself are linked to changing oxygen (O\textsubscript{2}) concentrations (44). Early in pregnancy the placenta is relatively avascular, and O\textsubscript{2} concentrations in the placental villi (the exchange units of the placenta) are very low, ~20 mmHg (1–3%). By 16 wk gestation the villi are vascularized, and O\textsubscript{2} tensions rise to about ~60 mmHg (8%), which in normal pregnancies is maintained until birth (19, 23, 49). These physiologically relevant low O\textsubscript{2} concentrations are much less than the 21% O\textsubscript{2} concentrations of room air, in which many placental experiments have usually been conducted. Numerous studies have reported the intrinsic role of hypoxia (~5% O\textsubscript{2}) in regulating placental implantation and function (19, 44, 46), suggesting that several different molecular mechanisms may be involved. However, only a small number of studies have focused on low O\textsubscript{2} concentrations and placental transport function. Nelson et al. (41) have demonstrated downregulation in the expression and function of system A amino acid transporters in isolated term human trophoblast cells cultured under hypoxic conditions. Another study has observed the upregulation of glucose transporters by hypoxia in the BeWo choriocarcinoma cell line (6).

In this study, we have investigated the expression of NIS and interactions with hCG under O\textsubscript{2} levels similar to those of the developing placenta (1, 8, and 21% O\textsubscript{2} of room air) and evaluated the roles of O\textsubscript{2} in the regulation of NIS gene expression and iodide uptake in the established BeWo human choriocarcinoma cell line, previously used by us as a model of placental iodine transport (33, 37).
**MATERIALS AND METHODS**

Cell culture and treatments. Choriocarcinoma cells (BeWo; CCL 98, passage 195) were obtained from the American Type Culture Collection (Rockville, MD) and cultured in Nutrient Mixture F-12 (Ham) medium. Media were supplemented with 10% (vol/vol) FBS (Invitrogen), 500 U/ml penicillin, 500 U/ml streptomycin (Invitrogen), and 5 μg/ml plasmocin (Integrated Sciences) in a conventional cell culture incubator with 21% oxygen. Cells were passaged twice/wk and media changed on alternate days. All experiments were performed on cells between passage 12 and 18 from original stock. Viability of cells was estimated by Trypan blue exclusion and measurement of lactate dehydrogenase (LDH) release.

For all experiments, BeWo cells were trypsinized and plated into six-well plates with 80–90% confluence after overnight incubation. The cells were cultured in either 1 or 8% O2 in a Heraeus HeraCell (Thermo Scientific, Langenselbold, Germany) incubator and measurement of lactate dehydrogenase (LDH) release.

For electrophoresis, 30 μg of membrane samples was prepared by addition of NuPAGE SDS sample buffer with reducing agent (NuPAGE, Novex; Invitrogen) and incubated at 70°C for 10 min. The samples were loaded in NuPAGE 4–12% Bis-tris Gel (Invitrogen) and transferred to a 0.45-μm nitrocellulose membrane (Bio-Rad Laboratories). The membrane was blocked with 5% skim milk in Tris-buffered saline (20 mM Tris-Cl, pH 7.4, 137 mM NaCl, 0.05% Tween-20 (TBST)) for 45 min at room temperature. The blots were then probed with monoclonal anti-NIS antibody diluted 1:400 (Chemicon) in blocking buffer overnight at 4°C or monoclonal anti-β-actin antibody diluted 1:2,000 (Sigma) for 1 h at room temperature. After washing three times with TBST, the membrane was probed with horseradish peroxidase-conjugated secondary antibody 1:2,000 (ImmunoPure peroxidase-conjugated goat anti-mouse IgG; Pierce) in blocking buffer and washed as before. The signals were visualized with Super Signal West Femto Maximum Sensitivity Substrate (Pierce) and detected by FujiFilm Luminescence Image Analyzer LAS-4000 (FujiFilm, Tokyo, Japan). The amount of chemiluminescence of NIS and β-actin bands was measured using FujiFilm Multi Gauge Version 3.0 software. Data are presented as ratio of density of NIS to β-actin.

Radioiodide uptake. Radiolabeled iodide (125I) as NaI, carrier-free in NaOH, was obtained from PerkinElmer (Glen Waverley, New South Wales, Australia). 125I uptake assay was performed as described previously (37). Briefly, BeWo cells were incubated in 2 ml of serum-free DMEM with 125I (1 kBq/ml) for 1 h at 37°C and then solubilized in 1 ml of 1 M NaOH and the wells rinsed with an additional 1 ml of NaOH. The level of 125I uptake was quantified by measuring 125I in 2 ml of solubilized cell extract in a γ-counter.

**Table 1. PCR Primer sequences**

<table>
<thead>
<tr>
<th>Gene</th>
<th>Primer Sequence</th>
<th>GeneBank ID</th>
<th>PrimerBank ID</th>
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</thead>
<tbody>
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<td>NIS Forward</td>
<td>5′ TGGGGGACTTTTGACATCATTT</td>
<td>NM000453</td>
<td>4507035a1</td>
</tr>
<tr>
<td>NIS Reverse</td>
<td>5′ TGGAGATTAATCCGCTGAGCA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>hCG Forward</td>
<td>5′ GTTGAGCAATACCGCGAT</td>
<td>NM033142</td>
<td>1545175a1</td>
</tr>
<tr>
<td>hCG Reverse</td>
<td>5′ GAGTCGCGGTAGACATGGGA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>β2M Forward</td>
<td>5′ GGCTATCCAGCGTACTCCCCA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>β2M Reverse</td>
<td>5′ GCACGGCAATCAGTGCTTTT</td>
<td>NM 004048</td>
<td>4757826a1</td>
</tr>
</tbody>
</table>

NIS, sodium iodide symporter; hCG, human chorionic gonadotropin; β2M, β2-microglobulin.
RESULTS

Viability of BeWo cells in all treatment groups, judged by Trypan blue exclusion, LDH release assay secretion, and total cell count per well, was not significantly different between treatment groups (data not shown). BeWo cells grew well to confluence in 1% oxygen and with DFO treatment, with no evidence of reduced viability or cell necrosis.

To investigate NIS and hCG expression response to O\(_2\) concentrations, BeWo cells were incubated in 1, 8, and 21% O\(_2\) for 24 h. NIS mRNA was increased significantly as O\(_2\) rose from 1 and 8% to 21% (Fig. 1A) and was mirrored by increased membrane NIS protein (Fig. 1B) and \(^{125}\)I uptake (Fig. 1C). Addition of DFO (a hypoxia mimetic) to BeWo cells incubated in 21% O\(_2\) significantly reduced NIS mRNA expression in a time-dependent manner (Fig. 2A). These reductions were reflected in significant falls in membrane NIS protein and \(^{125}\)I uptake after 24 and 48 h of DFO treatment (Fig. 2, B and C).

To exclude a direct effect of DFO on NIS functional iodide uptake, \(^{125}\)I uptake was also examined in BeWo cells after 4-h incubation of DFO, which showed no difference in uptake compared with 21% O\(_2\) (data not shown).

hCG mRNA also increased in parallel with NIS mRNA with increasing O\(_2\) concentrations (Fig 1A). The increased hCG mRNA was accompanied by increases in hCG protein secretion. The concentration of hCG in the culture medium was 95.27 ± 2.18 IU/l in 1%, 412.23 ± 66.02 IU/l in 8%, and 539.36 ± 74.46 IU/l in 21% O\(_2\) (Fig. 3A). A time course study showed that the fold decrease of NIS mRNA was 4.25 ± 0.72-(\(P < 0.001\)), 6.7 ± 0.82-(\(P < 0.001\)), and 15.45 ± 0.98-fold (\(P < 0.001\)) after 8, 24, and 48 h, respectively, in 1% O\(_2\) compared with 21% O\(_2\), whereas expression of hCG mRNA was 1.8 ± 0.42-(\(P < 0.05\)), 3.3 ± 0.82-(\(P < 0.01\)), and 2.6 ± 0.78-fold (\(P < 0.05\)), respectively (Fig. 4). Expression of hCG mRNA and protein secretion were reduced significantly by DFO but only after 24 h of incubation (Figs. 2A and 3B).

To determine the hCG effect on NIS expression at changing O\(_2\) concentrations, BeWo cells were treated with or without 25,000 IU/l of hCG for 4, 8, and 24 h in 1 or 8% O\(_2\). No difference in NIS expression was observed at 1% O\(_2\) culture after hCG treatment compared with cultures without hCG treatment (Fig. 5A). However, hCG increased NIS mRNA expression in BeWo cells incubated in 8% O\(_2\), with statistical significance (\(P < 0.05\)) at 24 h (Fig. 5A). There was no measurable increase in \(^{125}\)I uptake at either O\(_2\) concentration after 48-h hCG treatment (Fig. 5B).

DISCUSSION

BeWo cells, derived originally from a human choriocarcinoma, share many features with trophoblasts in primary culture. BeWo cells form a well-differentiated monolayer, undergo syncytialization, and secrete hCG (8, 35, 58). They have been used widely in placental transport studies, e.g., glucose (1, 3, 6, 13, 25, 38), amino acid (25, 26, 42), iron (12, 18), fatty acid (24, 60), and drug and toxic studies (2, 22, 36, 47).

Fig. 1. sodium iodide symporter (NIS) and human chorionic gonadotropin (hCG) regulation by oxygen in BeWo cells. A: BeWo cells were cultured in 1, 8, and 21% O\(_2\) for 24 h, and expression of NIS and hCG mRNA was measured by quantitative real-time PCR. Data are represented as ratio of target gene to the housekeeping gene, \(/\text{H}2\text{M}\). B: membrane protein (30 \(\mu\)g/lane) was examined by Western Blot (density of NIS/actin ratio). C: functional activity of NIS is measured by \(^{125}\)I uptake (counts·min\(^{-1}\)·1000 cells\(^{-1}\)) in BeWo cells cultured in 1, 8, and 21% oxygen for 48 h. mRNA data were analyzed by 1-way ANOVA with Bonferroni’s post hoc test. Comparison of 8 and 21 to 1% oxygen and \(^{125}\)I uptake by \(t\)-test. *\(P < 0.05\); **\(P < 0.01\); ***\(P < 0.001\).
BeWo cells express NIS and pendrin and demonstrate significant uptake and efflux of iodide, with kinetic and inhibitory characteristics consistent with these transporters (37). We have used these cells previously to model placental iodide handling and demonstrate iodide and hCG regulation on NIS (33, 37).

In the present study, we examined the effects of incubating BeWo cells at O2 levels observed in placental villi early in pregnancy (≈6 wk gestation, 1% O2) and from 16 wk gestation (8% O2) (19, 23, 49). BeWo cells cultured at 21% O2 were used as controls. There was a significant increase in NIS mRNA and protein expression as oxygen levels rose from 1 to 8 to 21% O2 (Fig. 1, A and B). NIS functional studies conducted by I125 uptake demonstrated increases in NIS expression (Fig. 1C). DFO is an iron chelator and mimics hypoxia by inhibiting prolyl hydroxylases, which are essential for the proteosomal degradation of hypoxia-inducible factor-1α (HIF-1α) during times of normoxia (>5% O2), resulting in the nuclear accumulation of HIF-1α and its transcriptional effects (48). DFO has been used extensively in many cell and tissue types and is recognized as the gold standard for creating a hypoxic environment under normoxic conditions (11, 48, 59). In the present study, addition of DFO significantly reduced NIS mRNA and protein expression (Fig. 2, A and B) as well as I125 uptake (Fig. 2C). Our results indicate for the first time that increasing O2 concentrations regulate NIS expression and iodide transport in BeWo cells, a model of placental trophoblasts.

Fig. 2. Effects of desferroxamine (DFO) on NIS and hCG expression. A: BeWo cells were treated with 0.2 mM of DFO for 4, 8, and 24 h, and expression of NIS and hCG mRNA was detected by real-time PCR; data are represented as the ratio of target gene to housekeeping gene (B2M). Expression of NIS membrane protein by Western blot (B) and NIS activity by I125 uptake (C) was measured in BeWo cells cultured in 0.2 mM of DFO for 24 and 48 h. Data were analyzed by 1-way ANOVA with Bonferroni’s post hoc test. *P < 0.05; **P < 0.01; ***P < 0.001.

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Fig. 3. Effects of oxygen concentration and DFO on hCG secretion in BeWo cells. Concentration of hCG in cell culture medium was measured by immunoassay in BeWo cells cultured in 1, 8, and 21% oxygen for 24 h (A) and 0.2 mM of DFO for 4, 8, and 24 h (B). Data analysis was by 1-way ANOVA with Bonferroni’s post hoc test. ***P < 0.001.
A number of studies have demonstrated that in normal pregnancy the O2 tension in intervillous spaces of human placenta rises gradually from about 20 mmHg or 1–3% O2 at 6–12 wk gestation and peaks around 60 mmHg or 8% O2 at about week 16. This correlates with morphological descriptions of early human placenta development that document direct connections between the spiral arteries and the intervillous space after only 9 wk of gestation (23). Establishment of this well-oxygenated environment in the intercellular space is believed to play an important physiological role in stimulation of trophoblast differentiation (23, 46). Human fetal thyroid begins to concentrate iodide and synthesize thyroid hormones from around 10–12 wk of gestation and secretes thyroid hormones at about week 16 (10). In our present study, increases in NIS expression and iodide uptake were observed as O2 concentrations increased from 1 to 8%. We postulate that this in vitro model may represent the normal process of trophoblast differentiation and function during placental development. Thus the increasing maternal iodide requirements of the developing fetal thyroid are met by oxygen-induced increased trophoblast NIS expression. In abnormal pregnancies, such as those complicated by preeclampsia and intrauterine growth restriction, there is evidence of impaired placentation and vascularization. These pathologies can chronically reduce blood supply and may play a role in reducing placental exchange of key nutrients (such as iodide) to the fetus during pregnancy (56). In vitro studies have indicated that placental transport of amino acids and glucose is regulated by a hypoxic environment (6, 41, 54, 64). Furthermore, in human intrauterine growth restriction, the decrease of placental transporters of glucose and amino acids suggests that both placental metabolism and transport may be altered (34, 43, 54). Clearly, further investigation to understand the role of hypoxia in regulating cellular transport functions is warranted.

hCG upregulates NIS expression in thyroid cells and placental cell lines (4, 5, 31, 33). To examine whether the decrease of NIS expression in low O2 levels could be contributed to by low hCG levels, we examined hCG mRNA expression and protein secretion in the same samples used for NIS. hCG mRNA and hCG secretion were reduced at 1% oxygen and increased significantly as oxygen concentration rose from 1 to 8 to 21% oxygen, as was seen with increased NIS expression (Figs. 1A and 3A). Addition of DFO resulted in a decrease of both hCG mRNA expression (Fig. 2A) and protein secretion (Fig. 3B), but only after 24 h treatment, whereas NIS decreased progressively in a time-dependent manner. These data indicate that hCG production is regulated by O2. The rapid and more significant effects of low (1%) O2 concentration or DFO on NIS expression suggest that downregulation of NIS by low O2 levels may not be mediated by low hCG production. To further investigate this, BeWo cells were incubated with 25,000 IU/l of hCG in 1 and 8% O2. In our present study, the mean of endogenous hCG concentration in the medium of 1% O2 culture was 95.27 ± 2.18 IU/l and in 8% oxygen was 413.23 ± 66.02 IU/l. The concentration of added hCG was six times higher than the endogenous hCG in 8% oxygen culture. However, hCG did not increase low NIS expression or iodide uptake in cells cultured in 1% O2 (Fig. 5). We speculate that there may be two possible mechanisms of NIS regulation by oxygen. First, O2 may have an effect on the downstream pathway of hCG action on NIS. Second, O2 may have an independent effect on NIS. Hypoxia-inducible factors mediate transcriptional responses to localized hypoxia in normal tissues by altering cellular metabolism and stimulating angiogenesis (28). Translocation of HIF-1α to the nucleus in response to hypoxia...
regulates the expression of many genes, leading to decreased energy consumption and the release of proangiogenic factors subsequently changing cell function (32, 44). Changes in tissue O2 are also associated with generation of reactive O2 species and alterations in intracellular reduction/oxidation (redox) status. This also plays an important role in DNA synthesis, gene expression, enzymatic activity, and other processes (20, 30). During placental development, alteration of oxygen concentration in intervillous spaces is inevitably an alteration of redox status in trophoblast (17, 23, 62). In thyroid, NIS transcription requires Pax8 DNA binding to its promoter and enhancer. This is regulated by hCG and redox status (16, 27, 52). Although Pax8 is present in term human placenta, overexpression of Pax8 in JAR cells failed to increase NIS expression, suggesting a more complex mechanism of placental NIS regulation (16). The molecular mechanism of O2 involvement in the regulation of NIS expression and iodide transfer in placenta requires further investigation.

Maintaining normal placental iodide transfer is important for fetal thyroid hormone synthesis. The present study suggests that NIS expression and activity rise in parallel with increasing placental O2 concentrations in early pregnancy, at a time when the developing fetal thyroid requires an increased supply of maternal iodide for thyroid hormone synthesis.

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GRANTS

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DISCLOSURES

The authors have no conflicting interests.

REFERENCES


