Expression of the genes for 3β-hydroxysteroid dehydrogenase type 1 and cytochrome P450scc during syncytium formation by human placental cytotrophoblast cells in culture and the regulation by progesterone and estradiol

C Beaudoin1, C H Blomquist3, M Bonenfant1 and Y Tremblay1,2

1Laboratory of Molecular Endocrinology, CHUL Research Center and 2Department of Physiology, Laval University, Centre Hospitalier de l’Université Laval, Sainte-Foy, Québec, Canada G1V 4G2 and 3Department of Obstetrics and Gynecology, HealthPartners, St Paul-Ramsey Medical Center, St Paul, Minnesota 55101, USA

Abstract

We have investigated the expression of cholesterol side-chain cleavage cytochrome P450 (P450scc) and 3β-hydroxysteroid dehydrogenase (3β-HSD) type 1 genes during human trophoblast differentiation in culture and the modulation of their steady-state mRNA levels by steroids. During the first 24 to 48 h after plating, mononucleated cells aggregated, forming colonies. After 60 h in culture, cell diameters were increased and nuclei appeared centrally distributed within large cells, consistent with syncytiotrophoblast formation. During these striking morphological changes in culture the expression and activity levels of 3β-HSD type 1 and P450scc increased significantly as isolated cytotrophoblasts progressed to a differentiated state, with P450scc and 3β-HSD type 1 mRNAs activities being more abundant in cells cultured for 48 to 72 h. In the same culture, however, the amount of 3β-HSD protein decreased during the first 12 to 24 h by 50% compared with freshly isolated trophoblasts but remained at these levels throughout the culture period. The specific activity of the 3β-HSD as determined with pregnenolone or dehydroepiandrosterone was similar but increased with time as syncytiotrophoblast was formed in vitro. These observations provide additional evidence that the expression of these two progesterone-synthesizing enzymes is coincident and that they reach their maximum steady-state mRNA levels at a time when syncytium formation occurs in vitro. Incubation of trophoblast cells with progesterone or estradiol increased the abundance of P450scc and 3β-HSD type 1 mRNAs but had no significant effect on the amount of 3β-HSD protein. These observations of the regulation of 3β-HSD type 1 mRNA levels by steroids suggest a complex relationship of the mechanisms regulating transcription/mRNA processing and transduction of the 3β-HSD type 1 gene.


Introduction

Among the steroidogenic enzymes found in the human placenta, the synthesis of progesterone depends principally on cholesterol side-chain cleavage cytochrome P450 (P450scc) (EC 1.14.15.67) and 3β-hydroxysteroid dehydrogenase (EC 1.1.1.145)/A5-A4 isomerase (EC 5.3.3.1) (3β-HSD). The mitochondrial P450scc system in the placenta is analogous to the machinery used by the gonads and the adrenals. It is composed of cytochrome P450scc and an NADPH-dependent flavoprotein dehydrogenase coupled to an iron–sulfur-dependent protein for electron transfer (Miller 1988). Human P450scc cDNA has been cloned (Chung et al. 1986) and is encoded by a single gene termed CYP11A1 (Nebert et al. 1989) localized on chromosome 15q23–q24 (Sparkes et al. 1991). 3β-HSD is an NAD-dependent membrane-bound enzyme. In the human, two highly homologous 3β-HSD type 1 and type 2 genes have been characterized. The 3β-HSD type 1 gene is expressed in placenta, skin and breast, whereas the type 2 gene encodes an enzyme that differs from type 1 in 12 amino acids and predominates in gonads and adrenals (Labrie et al. 1992). Both genes lie on chromosome 1p13 (Bérubé et al. 1989).

It is well established that under a variety of widely used culture conditions, mononuclear cytotrophoblast cells aggregate and undergo morphologic changes consistent with syncytiotium formation (Kliman et al. 1986, Douglas & King 1990). As pregnancy progresses, cytotrophoblasts are scattered throughout the placenta which becomes largely
dominated by syncytiotrophoblasts, the end-stage of cytotrophoblast differentiation. Concurrent with but not necessarily dependent upon these morphologic changes (Douglas & King 1989), there are increases in the expression of a variety of genes, including those for aromatase and human chorionic gonadotropin (Ringler & Strauss 1990). We have recently demonstrated that although 17β-HSD type 1 and type 2 mRNA and enzymatic activity are present at a high level in term placental villi, their relative levels differ markedly in freshly isolated cytotrophoblasts and during the course of syncytium formation in vitro as does their susceptibility to regulation by steroid hormones (Beaudoin et al. 1995). These observations led us to propose that the expression of the genes for these two isoforms of 17β-HSD is regulated by specific and distinct mechanisms in vitro.

A similar approach was taken in this investigation to compare the patterns of expression of the genes for P450scc and 3β-HSD type 1 in terms of mRNA and protein levels and enzymatic activity in cytotrophoblasts cultured under conditions leading to aggregation and syncytium formation.

Materials and Methods

Chemicals

Trypsin and DNase I were obtained from Sigma Co. (St Louis, MO, USA). Dulbecco’s modified Eagle’s medium with high glucose (DMEM), calcium- and magnesium-free Hank’s balanced salt solution (HBSS), Hepes, and glutamine were obtained from Gibco (Grand Island, NY, USA). Fetal calf serum (FCS) was obtained from Hyclone (Logan, UT, USA). [1,2,6,7-3H]pregnenolone (25 Ci/mmol), and [1,2,6,7-3H]dehydroepiandrosterone (DHEA) (89 Ci/mmol) were obtained from Amersham (Arlington Heights, IL, USA). [32P]Á-deoxy–CTP, [7,3H]Á-glycerol, and [3H]Á-deoxy–CTP (25 Ci/mmol), and [1,2,6,7-3H]Á-dehydroepiandrosterone (DHEA) (89 Ci/mmol) were obtained from Amersham (Arlington Heights, IL, USA). Unlabeled steroids were purchased from Steraloids (Wilton, NH, USA).

Cell cultures

JEG–3 cells (Kohler & Bridson 1971, Kohler et al. 1971), and JAR (Pattillo et al. 1971) were obtained from the American Type Culture Collection (Rockville, MD, USA) and cultured as reported (Tremblay et al. 1989). Experiments with primary cultures of cytotrophoblasts were repeated two or three times (see each Figure legend for the details). Autoradiograms from Northern blots from each experiment were densitometrically scanned in a linear range using an appropriate exposure, and the abundance of mRNA adjusted to the 18S hybridization and relative to the control value, which was arbitrarily fixed at 1.0, is presented in the histograms. Each bar represents the mean ± S.D.

Isolation of cytotrophoblasts

Consent forms were signed by patients from whom tissues were obtained. Term placentae (38–42 weeks) were obtained after normal delivery or uncomplicated Cesarean section. Initial processing of the tissue was performed as described (Hall et al. 1977) except that relatively pure populations of cytotrophoblasts were achieved by adding a Percoll gradient centrifugation step (Kliman et al. 1986, Beaudoin et al. 1995). Briefly, villous tissue was dissected from areas free of calcification or infarction, membranes and cord vessels were removed and the tissue minced in HBSS, pH 7.4, containing 25 mm Hepes and subjected to three 30-min digestions with 0.125% trypsin and 0.2 mg/ml DNase I at 37 °C. Dispersed cells were collected and layered onto an FCS cushion and centrifugated at 1000 g. The cell pellets were resuspended in DMEM–Hepes and applied to a 5–70% discontinuous Percoll gradient. Cell populations enriched in cytotrophoblasts were collected at a density of 1.048 to 1.062 g/ml and diluted in DMEM–Hepes, containing 50 µg/ml streptomycin, 100 IU/ml penicillin, 2·2 g/l NaHCO3 and 20% heat-inactivated FCS (DMEMS) and cultured in humidified 5% CO2–95% air at 37 °C. Cytotrophoblast cells were plated at a density of 5 × 105 cells/ml in DMEMS for RNA isolation (10 ml/dish) and at 2 × 105 cells/ml in DMEMS for enzymatic activity (2 ml/dish) and protein analysis (5 ml/dish). The medium was changed twice during the next 2 days, after which the experiments were started. The experiments with steroids were performed in similar medium but supplemented with 2% (v/v) dextran-coated charcoal-treated and heat-inactivated FCS the day before adding the steroids.

mRNA preparation and cDNA probes

Tissue (100 mg) or 107 cells were homogenized or lysed in 1 ml Tri Reagent, a monophase solution containing phenol and guanidino thiocyanate (Molecular Research Center, Cincinnati, OH, USA). Separation of DNA/RNA and protein was achieved by adding 200 µl chloroform. RNAs were precipitated with 500 µl isopropanol, glyoxalized, and electrophoresed as described (Tremblay et al. 1988). A rat 18S rRNA oligonucleotide end-labeled probe was used for the amount of RNA loaded in each lane (Clements et al. 1988, Tremblay & Beaudoin 1993). Blots were probed with the purified full-length (1·8 kb) human P450scc cDNA (Chung et al. 1986) and with human 3β-HSD type 1 EcoRI/PvuII cDNA fragment (1038 bp; hp3BHSD–36). The probes were labeled with random primers (Feinberg & Vogelstein 1983).

Protein extraction and immunoblot analysis

Trophoblast cells were washed with Dulbecco’s PBS and scraped into ice-cold buffer containing 1·0 mm EDTA.
and 20% (v/v) glycerol in 40 mm potassium phosphate, pH 7-0. Samples were homogenized, centrifuged at 1000 g to remove cell debris and at 105 000 g for 1 h. The pellets were saved as the microsomal fractions and kept at −80 °C until assayed for 3β-HSD content. Western blotting was performed as previously described (Tremblay et al. 1994). 3β-HSD protein levels were estimated with a rabbit polyclonal antihuman 3β-HSD serum diluted 103-fold (Luu-The et al. 1989), followed by exposure to horseradish peroxidase-labeled donkey antirabbit immunoglobulin G. The antigen–antibody complex was detected by enhanced chemiluminescence with luminol and hydrogen peroxide (ECL; Amersham).

Enzymatic assay of trophoblast cultures

Trophoblast cells were cultured for 12, 36, and 60 h before 3β-HSD activity was assayed. Activity was determined in 1 ml DMEMS containing tritiated pregnenolone (0·36 Ci) and pregnenolone (1 µm), or tritiated DHEA (0·36 Ci) and DHEA (1 µm). Incubations were for periods of 3, 6, 9, 12, and 18 h and performed in triplicate. Enzymatic reaction was stopped by adding 50 µl acetic acid (0·5 M). Activity values were obtained by calculating the conversion of pregnenolone to progesterone or DHEA to androstenedione, estrone, and estradiol. Steroids were extracted with diethyl ether, applied to silica gel-coated TLC plates, and developed in a toluene-acetone mixture (80:20, v/v). Radioactivity was quantified with a Berthold digitalized autoradiograph (Weldbrod, Germany) coupled to a Dar Signal Analyzer Imaging System (San Diego, CA, USA). The identities of substrates and newly produced steroids were determined by co-migration of HPLC-purified labeled steroids and samples. At the end of the experiment, the DNA content in each well (triplicates) for each time-point was assayed and used to calculate specific activity (nmol product formed/µg DNA; n=3) (Beaudoin et al. 1995). Specific activity at each time-point (3, 6, 9, 12, and 18 h), i.e. nmol/µg DNA per h, for each culture (12, 36, and 60 h after plating) was also calculated and statistically compared. Values represent the mean ± s.d. (n=5) for each culture (see Table 1). Statistical comparisons were performed by a one-way ANOVA (n=5) followed by post-hoc pairwise comparisons with a Fisher’s probability of least significant difference test. P<0·05 was considered as significant (Table 1).

Steroid measurements

Frozen tissues (whole placenta (n=6) and villous tissues free of membranes and decidua (n=12)) were homogenized in methanol and the extracts were separated on LH–20 columns before RIA measurements (Bélanger et al. 1980). The results are shown as the means ± s.d. of duplicate determinations of individual samples (Table 2).

Results

This investigation was undertaken to characterize and compare the expression patterns of the genes for 3β-HSD type 1 and P450scc during cytotrophoblast differentiation in culture. 3β-HSD type 1 and P450scc mRNAs were quantitated in washed fragments of villous tissue, in freshly
isolated cytotrophoblasts (t=0), and in cytotrophoblasts cultured for up to 72 h under conditions that support syncytium formation (Kliman et al. 1986) (Fig. 1). Two mRNAs present on Northern blots as transcripts of 1.7 and 2.0 kb indicate that the 3β-HSD type 1 and P450scc mRNAs are present at high and similar levels in villous tissue (Fig. 1A and B, lane 3). The time-dependence of gene expression in trophoblast cells in culture was also studied. Isolated cytotrophoblasts expressed both genes after 12 to 24 h of culture, mRNA levels gradually increased and were maximal after 48 to 60 h of culture (Fig. 1A and B, lanes 4–10), with 3β-HSD type 1 mRNA being more abundant. No message was detected in freshly isolated cytotrophoblasts (t=0). The choriocarcinoma cell lines JEG-3 (Tremblay & Beaudoin 1993) and JAr, known to contain 3β-HSD and P450scc mRNAs, were used as positive controls. This 48- to 60-h interval represents the time required for syncytium formation in vitro as already reported (Ringler & Strauss 1990) and under the culture conditions used here (Fig. 2). Indeed 12 h after plating, cells were mostly mononucleated (Fig. 2A). At 24 h and 48 h in culture, aggregates composed of individual cells were identified (Fig. 2B and C). At 60 h in culture (Fig. 2D), the morphological appearance of this group of cells was quite different; this group of cells have a centrally positioned nuclei with no intervening membranes visible, and they appeared to be, therefore, syncytial groups. Residual mononuclear cells were still present. Again, this striking change in cellular morphology observed with time in culture is consistent with the differentiation of cytotrophoblasts in vitro.

In our studies, cytotrophoblasts are isolated following a standard trypsin-DNase dispersion method and recovered from a discontinuous Percoll gradient step by collecting

Figure 2 Appearance of isolated trophoblast cells after different times in culture. Cells were plated in DMEM/F12 medium and cultured for (A) 12 h, (B) 24 h, (C) 48 h, and (D) 60 h. Phase-contrast and unfixed cells; magnification × 200.
cells distributed between densities of 1.048 to 1.062 g/ml. To ensure that the majority of the cells collected were trophoblasts, we also isolated the mRNA from the cellular material from the rest of the gradient. Although high levels of rRNAs were detected in this material, expression of 3β-HSD type 1 and P450scc was undetectable (Fig. 1, lane 11). Western blotting of 3β-HSD type 1 protein in trophoblasts from the same cultures as those used for mRNA analysis revealed a unique and abundant 42 kDa band present throughout the 72 h in culture. Protein levels of 3β-HSD decreased by about 50% within the first 12 to 24 h of culture but remained at a relatively constant level for up to 72 h (Fig. 3, lanes 1–7). To understand this apparent discrepancy between the progressive increase in 3β-HSD type 1 mRNA and the apparently stable protein level better, we measured the capacity of the trophoblasts to metabolize pregnenolone and DHEA after 12, 36, and 60 h in culture (Fig. 4). Specific activity in terms of nmol progesterone (Fig. 4A) or androstenedione, estrone, and estradiol (Fig. 4B) formed per µg DNA was determined at intervals up to 18 h. 3β-HSD activity with each substrate was quite similar (Fig. 4 and Table 1) The rates of product formation were approximately linear for 18 h. Comparison of trophoblast cells after 12, 36, and 60 h indicated that 3β-HSD activity increased with time in culture with both substrates and was quantitatively higher in cells cultured for 60 h (Table 1). These results indicate that 3β-HSD protein is stable in culture and that maximum 3β-HSD activity occurs in trophoblast cells cultured long enough for syncytium formation in vitro.

We recently reported that a 24-h incubation with progesterone and estradiol increased 17β-HSD type 1
mRNA levels in cytotrophoblast cultures (Beaudoin et al. 1995). Thus, it was of interest to determine whether these steroids had a similar influence on 3β-HSD type 1 and P450scc mRNAs. Cytotrophoblasts were isolated and cultured for 48 h before being exposed for 24 h to progesterone, estradiol, or cortisol (Fig. 5). The steroids were added at the time when 3β-HSD type 1 and P450scc mRNAs reached their steady-state levels in cells (Fig. 1). Control and steroid-treated cells were cultured for the same interval. Progesterone at $10^{-6}$ M increased the abundance of 3β-HSD type 1 (Fig. 5A, lane 1) and P450scc (Fig. 5B, lane 1) mRNAs by 7.1- and 2.2-fold respectively, whereas $10^{-8}$ M progesterone had no effect (Fig. 5A and B, lane 2). Estradiol at $10^{-9}$ M or $10^{-8}$ M increased 3β-HSD type 1 mRNA levels (Fig. 5A, lanes 3 and 4), whereas an increase in the P450scc mRNA level was only seen with $10^{-8}$ M estradiol (Fig. 5B, lanes 3 and 4). At the two concentrations used, cortisol had no effect when compared with 18S signal hybridization (Fig. 5A and B, lanes 5 and 6). The 3β-HSD protein content of these steroid-induced cells, presented in Fig. 6, showed no significant difference from that of control. The fact that only $10^{-6}$ M progesterone elicited an increase in 3β-HSD type 1 and P450scc mRNA levels, whereas $10^{-8}$ M progesterone did not, was unexpected. Therefore, we felt it to be important to determine whether such a concentration was consistent with progesterone levels found in term placenta. To consider this question, the levels of progesterone and several steroids were measured in whole placenta and in villous tissue (Table 2). The data indicated that washed villi and whole placenta contain high levels of steroids, principally progesterone and estrogens, and that the use of $10^{-6}$ M progesterone with trophoblast cells in culture approximates the concentration present in normal term placenta.

### Table 1 Specific activity of 3β-HSD determined in whole cells after 12, 36, and 60 h in culture. Specific activities of 3β-HSD represent the means ± s.d. determined with five different time-points (see Fig. 4 legend for time-points)

<table>
<thead>
<tr>
<th>Steroid incubated [3H]</th>
<th>Time after plating (h)</th>
<th>Specific activity (n=5) (nmol product formed/μg DNA per h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pregnenolone</td>
<td>12</td>
<td>15.40 ± 2.52</td>
</tr>
<tr>
<td></td>
<td>36</td>
<td>35.42 ± 6.90 (P=0.0006)</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>47.40 ± 9.50 (P=0.0001)</td>
</tr>
<tr>
<td>DHEA</td>
<td>12</td>
<td>17.77 ± 4.31</td>
</tr>
<tr>
<td></td>
<td>36</td>
<td>30.22 ± 9.76 (P=0.0479)</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>45.34 ± 10.76 (P=0.0004)</td>
</tr>
</tbody>
</table>

$P$ values were calculated in comparison with the 12-h time-point. Errors: 95% confidence interval.

The syncytiotrophoblast is believed to be the primary functional unit of the placenta producing progesterone and estradiol (Ringler & Strauss 1990). In a recent report, we...
demonstrated that 17β-HSD type 1 and type 2 mRNA expression patterns and specific activities differed from each other in freshly isolated cytotrophoblasts and during the course of syncytium formation in vitro (Beaudoin et al. 1995). In addition, the levels of activity and patterns of mRNA expression differed from those observed in term villous tissue, suggesting regulation at the level of the genome or at the level of translation levels by steroids. In support of this concept, we showed that exposure of cytotrophoblasts in culture to progesterone or estradiol stimulated formation of 17β-HSD type 1 mRNA but did not alter the level of 17β-HSD type 2 mRNA (Beaudoin et al. 1995).

This investigation was undertaken to characterize and compare the expression of the genes for 3β-HSD type 1 and P450scc in cytotrophoblasts cultured under conditions used in our study of the regulation of 17β-HSD gene expression. Our results indicated that mRNA levels for both enzymes were low in freshly isolated cytotrophoblasts, began to increase within 12 to 24 h in culture and reached maximum levels after about 48 to 60 h in culture. A similar pattern was also observed with 3β-HSD specific activity. Indeed, optimal metabolism of pregnenolone and DHEA by the 3β-HSD correlated with syncytium formation in vitro since steroid metabolism was greatest in cells cultured for 60 h when compared with cells cultured for 12 h. Our results are in agreement with results in vivo (Riley et al. 1992) and in vitro (Mason et al. 1993) that consider the syncytiotrophoblast as the principal site of 3β-HSD type 1 expression. This is in contrast with the reductase and dehydrogenase activities of 17β-HSD type 1, where no difference between cells cultured for 12 h and 60 h was observed (Beaudoin et al. 1995). A surprising result of the current study is that, in contrast to the behavior of 3β-HSD mRNA, we found that 3β-HSD protein levels decreased in early cultures but stayed fairly constant thereafter. Again, exposure of trophoblasts to concentrations of progesterone and estradiol approximating those found in term placenta (Table 2) increased 3β-HSD type 1 mRNA levels but had no significant effect on protein levels. These results suggest that 3β-HSD type 1 steady-state mRNA levels in placenta could be under post-transcriptional regulation.

These latter findings are in agreement with the results obtained by Wunsch et al. (1986) concerning progesterone and estradiol production by placental cells in culture. They observed that progesterone production increased during the 96-h culture period and that estradiol formation was stimulated by DHEA, indicative of increasing 3β-HSD activity in culture. They also found that exposure of cells to estradiol did not stimulate progesterone formation in their cultures, again consistent with the relatively constant 3β-HSD protein level reported here. Branchaud et al. (1983) also did not detect any effect of estradiol on progesterone production by placental cells in culture.

Our results, when considered with the aforementioned reports, suggest that the level of 3β-HSD type 1 mRNA in trophoblast cells in culture is subject to regulation by progesterone and estradiol, but that this regulation may not be readily apparent in terms of protein. Because the progesterone to estrogen concentration ratio stays constant throughout the second half of gestation in the human

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**Figure 6** Immunoblot analysis (50 μg) of trophoblast cells incubated with 10^-6 or 10^-8 M progesterone (lanes 1 and 2 respectively) and cortisol (lanes 5 and 6 respectively) and with 10^-8 and 10^-9 M estradiol (lanes 3 and 4 respectively). C, control cells. 3β-HSD protein revealed with specific rabbit antihuman 3β-HSD serum. Culture conditions similar to those in Fig. 5 were used. Autoradiograms for Western blots were densitometrically scanned, and the level of protein relative to control value (lane 1 fixed at 1.0) are presented in the histogram. The histogram represents the mean ± S.D. of three experiments. The arrow indicates the 42 kDa 3β-HSD type 1 protein.

**Table 2** Steroid levels in whole term placenta and term villous tissue. Each determination is the mean ± S.D. of duplicates of separate patients. Eighteen different placentas were used, six served as whole placental tissues and twelve were dissected free of membranes, vessels, and decidua and selected as whole term villi.

<table>
<thead>
<tr>
<th>Steroid measured</th>
<th>Whole placenta (n=6) (pmol/g)</th>
<th>Term villi (n=12) (pmol/g)</th>
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<tbody>
<tr>
<td>Pregnenolone</td>
<td>462 ± 68</td>
<td>433 ± 51</td>
</tr>
<tr>
<td>Progesterone</td>
<td>9683 ± 1186</td>
<td>9801 ± 902</td>
</tr>
<tr>
<td>DHEA</td>
<td>485 ± 1.15</td>
<td>376 ± 0.60</td>
</tr>
<tr>
<td>Androstenedione</td>
<td>110 ± 37</td>
<td>147 ± 22</td>
</tr>
<tr>
<td>Testosterone</td>
<td>8-14 ± 0.8</td>
<td>898 ± 1.13</td>
</tr>
<tr>
<td>Estradiol</td>
<td>322 ± 49</td>
<td>407 ± 72</td>
</tr>
<tr>
<td>Estrone</td>
<td>367 ± 59</td>
<td>305 ± 23</td>
</tr>
</tbody>
</table>

Errors: 95% confidence interval.
(Tulchinsky et al. 1972), such potential regulation of 3β-HSD type 1 mRNA levels could be of fundamental importance in vivo for placental development and function during pregnancy.

The question of the possible roles of progesterone and estradiol acting via their respective receptor-mediated mechanisms in human placenta has been of long-standing interest and remains controversial. The fact that the organ is saturated with both steroids throughout most of pregnancy and the observation that relatively high levels of progesterone and estradiol are required for in vitro effects have raised doubts about the feasibility of receptor-mediated actions under these conditions. Nonetheless, there is a growing body of evidence, including the findings reported here, that the human placenta is a progesterone and estradiol target tissue (Pepe & Albrecht 1995).

Our observation of a stimulation of 3β-HSD type 1 mRNA abundance by concentrations of progesterone and estradiol similar to the levels found in term placental tissue with no concomitant increase in enzyme protein suggests that the relationship between transcription, translation and protein turnover is complex. As shown in Fig. 1, although 3β-HSD mRNA is not detectable before 12 to 24 h in culture, enzyme protein (Fig. 3) is present and activity (Fig. 4) is detectable in these cultures. One plausible explanation for these observations is that 3β-HSD type 1 mRNA is labile and rapidly degraded during cell isolation, while membrane-bound enzyme protein is relatively stable, turning over slowly if at all during the time period of the experiment. As a result, stimulation of mRNA synthesis or stabilization of newly synthesized mRNA by progesterone or estradiol may not be reflected immediately in terms of enzyme protein. In addition, our previous observations also suggest that the level of 3β-HSD type 1 mRNA in the placenta could be post-transcriptionally regulated (Tremblay & Beaudoin 1993). We propose that, at the levels observed in term placenta, progesterone and/or estradiol could play a stabilizing role with regard to 3β-HSD type 1 mRNA. In fact, our results suggest that the primary effect of progesterone and estradiol occurs by affecting the level of 3β-HSD type 1 gene transcription and/or mRNA abundance/stabilization, rather than by affecting the rate of 3β-HSD protein synthesis. A similar mechanism was suggested in follicle-stimulating hormone-induced AG18-treated granulosa cells in culture (Orly et al. 1994). It will be of particular interest in future experiments to examine the transcriptional activation of the 3β-HSD type 1 gene versus the stability and turnover of its mRNA, and enzyme protein following exposure of trophoblast cells in culture to progesterone and estradiol alone or in combination.

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