Thyroid hormone as a biological amplifier of differentiated trophoblast function in early pregnancy

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Abstract. Direct effects of T₃ or T₄ on the trophoblast function were investigated in vitro using an organ culture system of human placental tissues. Explants of trophoblastic tissues obtained from normal early and term placentas were cultured with or without graded doses of T₃ or T₄ for 5 days in a serum-free condition. Addition of T₃ (10⁻⁸ mol/l) resulted in the maximum increase in daily secretion of progesterone, estradiol-17β as well as hCGα, hCGβ, hCG and hPL by cultured early placentals. Increases in progesterone and estradiol-17β secretion caused by the addition of T₃ were further augmented in response to concomitant addition of pregnenolone and testosterone, respectively, suggesting that T₃ (10⁻⁸ mol/l) enhances 3β-hydroxysteroid dehydrogenase and aromatase activity in the placenta. These stimulatory effects of T₃ (10⁻⁸ mol/l) on the trophoblast endocrine function were also found with the use of T₄ (10⁻⁷ mol/l). Addition of higher or lower concentrations of T₃ or T₄ gave attenuated effects. These results suggest that the optimal concentration of thyroid hormone is needed for it to exert its maximally stimulatory action on trophoblast endocrine function. Unlike early placental tissues, cultured term placental tissues did not respond to the addition of T₃ or T₄ with increased endocrine activity. Thus, the frequent occurrence of spontaneous abortion in early pregnancy during the state of hypothyroidism or hyperthyroidism may represent a direct consequence of inadequate thyroid hormone availability at the level of placental trophoblasts, followed by diminished expression of trophoblast endocrine function.

It is clinically evident that hypothyroidism is one of the known causes of the habitual abortion (1-3). Although this clinical evidence suggests a possible involvement of thyroid hormone in the maintenance of pregnancy, the mechanism of action of thyroid hormone in pregnancy maintenance remains unclear. Since the placenta occupies a unique position and function for the maintenance of pregnancy, a possible relationship between thyroid hormone and placental function is assumed to exist. Thus, in order to characterize the nature of the possible action of thyroid hormone on the placenta, direct effects of thyroid hormone on trophoblast endocrine function were investigated in vitro using an organ culture system of human placental tissues.

Early placental tissues cultured in vitro in a serum-free condition have been found to maintain their viability for a 6-day culture period when determined by the cellular uptakes of ³H-labelled thymidine and uridine (4). Human chorionic gonadotropin (hCG) and its subunits synthesized by cultured early placental tissues have been demonstrated to be promptly secreted into the medium without being accumulated in cultured trophoblasts (5,6). Hence, the in vitro culture system of placental explants offers a suitable model for investigating the direct action of thyroid hormone on trophoblast function.

In this report, we present that an optimal concentration of thyroid hormone exerts direct stimulatory effects on the production and secretion of progesterone and estradiol-17β as well as hCG (α, β) and human placental lactogen by early placenta, and suggest that thyroid hormone may play a physiological role in the maintenance of early pregnancy through acting as an enhancer of the trophoblast endocrine function.
Materials and Methods

First trimester placentas (N=4) were obtained from patients undergoing therapeutic abortions at 7-8 weeks of pregnancy by mechanical dilation and curettage without the use of oxytocin or prostaglandin. Term placentas (N=3) were obtained from spontaneous full-term deliveries. Informed consent for the use of the patient's placental tissues for the experiments was obtained before surgery and delivery. The placentas were immediately brought to the laboratory, rinsed thoroughly with ice-cold Hank's Balanced Solution, and placental villous tissues dissected free of fetal membranes were cut into approximately 1-mm² explants.

3,5,3'-L-triiodothyronine (T₃), L-thyroxine (T₄), pregnenolone, and testosterone were purchased from Sigma Chemical Co (St. Louis, MO). T₃ and T₄ were dissolved by adding 1 drop 1 mol/l NaOH and one drop absolute ethanol, and brought to a concentration of 1 × 10⁻⁶ mol/l with warm distilled water followed by filtration of the solution through a 0.22 micron filter. The T₃ and T₄ stock solutions were diluted in culture medium immediately before use.

Tissue culture

An in vitro culture system similar to that previously described was used (7). Briefly, the explants of early placental villous tissues (total wet weight 50 mg) or term placental villous tissues (total wet weight 150 mg) were placed on filter papers (0.45 mm; Millipore, Inc., Bedford, MA) within plastic multiwell plates, 24 mm in diameter (Becton Dickinson, Oxnard, CA), to which 2 ml McCoy's 5a medium (Gibco, Grand Island, NY) containing 100 × 10⁻⁶ U/l penicillin and 50 mg/l streptomycin were added.

The placental explants were cultured for 5 days at 37°C in an atmosphere of 95% air-5% CO₂ in the presence or absence of various concentrations of T₃ or T₄ with or without pregnenolone or testosterone. The medium was changed every day and replenished with fresh medium containing various concentrations of T₃ or T₄. Each set of culture was performed in quadruplicate. When the cultures were terminated, the medium was collected and stored at -20°C until analysed.

Radioimmunoassays

Immunoreactive hCGα, hCGβ and hCG in the media were determined by homologous double-antibody RIAs (8). The hCGα (CR-119), hCGβ (CR-119-2) and hCG (CR-119) used as standard preparations in the assays were gifts from the Center for Population Research of the NICHD and Dr R. E. Canfield, Columbia University (New York, NY). The specific antisera to hCGα, hCGβ and hCG used were generated by Dr C.-C. Chang and Dr Y. Y. Tsong, the Population Council (New York, NY).

The human placental lactogen (hPL) concentrations in the media were determined using hPL RIA Kit (Daiichi Radioisotope Inc, Tokyo).

The concentrations of progesterone and estradiol-17β in the media were determined using Progesterone RIA Kit (Green Gross Inc, Tokyo) and Estradiol RIA Kit (Green Cross Inc, Tokyo), respectively. The cross-reactivity of pregnenolone in the Progesterone RIA Kit was less than 0.12%. The cross-reactivity of estrone and testosterone in the Estradiol RIA Kit was less than 0.5 and 0.01%, respectively.

Other determinations

Aliquots from the placental tissue homogenates were diluted to 0.5 ml with 0.4 mol/l NaOH, samples being taken for the determination of protein and DNA. Protein was measured by the method of Bradford (9) using reagents from Bio-Rad Laboratories and bovine γ-globulin as standard. DNA was measured by the method of Burton (10) as modified by Giles & Myers (11).

Statistical analysis

Each experiment was repeated at least twice and comparable results were obtained. Results from a representative experiment are presented.

Data were analysed by use of a one-way analysis of variance (ANOVA) followed by Dunnert’s multiple comparison test to compare all means with the control. Differences at p<0.05 were considered significant.

Results

Effect of thyroid hormone on progesterone and estrogen secretion by cultured early placental tissues.

Early placental tissues were exposed to various T₃ concentrations throughout the 5-day culture period. Fig. 1 shows the time course of the daily secretion level of progesterone from cultured early placental tissues. Addition of T₃ (10⁻₈ mol/l) resulted in the maximum increase in the daily secretion of progesterone relative to that in control cultures. The increase in progesterone secretion caused by the addition of 10⁻₈ mol/l T₃ became apparent between 3 and 5 days of culture. Addition of higher or lower concentrations of T₃ gave attenuated effects, and treatment with an excessive concentration of T₃ (10⁻³ mol/l) resulted in a significant decrease in progesterone secretion.

Fig. 2 shows the daily progesterone secretion by early placental tissues cultured in the presence of graded doses of pregnenolone. The increase in progesterone secretion caused by the addition of 10⁻₈ mol/l T₃ was further augmented in response to the concomitant addition of pregnenolone and became apparent between 1 and 3 days of culture. Treatment with an excessive concentration of T₃
Fig. 1.
Effect of various concentrations of T₃ on progesterone secretion by cultured early placental tissues. The results represent the mean ±sd of four determinations. *: p<0.05; **: p<0.01 vs control cultures.

$10^{-3}$ mol/l) caused a remarkable decrease in progesterone secretion by cultured early placental tissues.

Fig. 3 shows the time course of the daily secretion levels of estradiol from cultured early placental tissues. Addition of $10^{-8}$ mol/l T₃ resulted in the max-

Fig. 2.
Effect of various concentrations of T₃ on progesterone secretion by early placental tissues cultured in the presence of pregnenolone. The results represent the mean ±sd of four determinations. *: p<0.05; **: p<0.01 vs control cultures.
Effect of various concentrations of T₃ on estradiol secretion by cultured early placental tissues. The results represent the mean ±sd of four determinations. *: p<0.05; **: p<0.01 vs control cultures.

Fig. 4
Effect of various concentrations of T₃ on estradiol secretion by early placental tissues cultured in the presence of graded doses of testosterone. The increase in estradiol secretion caused by the addition of T₃ was further enhanced in response to the concomitant addition of testosterone and became apparent at day 1. The greatest increase in estradiol secretion in response to T₃ was found between 1 and 3 days of culture in the concomitant presence of testosterone as the substrate. In contrast, treatment with an excessive concentration of T₃ (10⁻³ mol/l) caused a striking decrease in estradiol secretion by early placental tissues.

Similar effects on steroid hormone secretion
were also attained by treatment with various doses of T₄, 10⁻⁷ mol/l T₄ exerting the most pronounced increase in progesterone and estradiol secretion (data not shown).

**Effect of thyroid hormone on hCG (α, β) and hPL secretion by cultured early placental tissues**

Addition of T₃ (10⁻⁷ ~ 10⁻⁹ mol/l) resulted in a significant increase in the daily secretion of hCGα, hCGβ and hCG from cultured early placental tissues relative to those in control cultures. The maximally stimulatory effects on hCGα, hCGβ and hCG secretion were observed with the use of 10⁻⁸ mol/l T₃. The increase in hCGα caused by the addition of T₃ was apparent between 1 and 3 days of culture (Fig. 5), whereas the increase in hCGβ and hCG caused by the addition of T₃ became apparent only between 3 and 5 days of culture (Fig. 6).

On the other hand, the daily secretion of hPL from cultured early placental tissues was also augmented by the addition of T₃ (10⁻⁷ ~ 10⁻⁹ mol/l), with 10⁻⁸ mol/l T₃ exerting the most pronounced increase (Fig. 7). The increase in hPL secretion caused by the addition of T₃ was apparent between 1 and 3 days of culture.

Similar effects on hCG (α, β) and hPL secretion were also observed in response to the treatment with T₄ (10⁻⁶ ~ 10⁻⁸ mol/l), with 10⁻⁷ mol/l producing the most pronounced increase in hCG (α, β) and hPL secretion (data not shown).

These experiments with early placental tissues obtained at different ages of gestation were repeated at least two times with similar results.

**Effect of thyroid hormone on progesterone, estradiol, hCG (α, β) and hPL secretion by cultured term placental tissues**

Term placental tissues were exposed to various T₃ concentrations throughout the 5-day culture period. In cultures of term placental tissues, there were no significant differences in the daily secretion levels of progesterone, estradiol, hCG (α, β) and hPL between T₃-treated cultures and control cultures (data not shown).

**Effect of thyroid hormone on protein and DNA content of cultured early placental tissues**

During the 5-day culture period, there were no significant differences in the protein and DNA content in the cultured early placental tissues between T₃ (10⁻⁸ mol/l)-treated cultures and control cultures (data not shown).

**Discussion**

We have recently demonstrated that thyroid hormone exerts a direct effect on the ovary and acts as a biological amplifier of the actions of follicle-stimulating hormone in the functional differentiation of ovarian granulosa cells, stimulating morphological differentiation, gonadotropin receptor forma-

![Fig. 5](image.png)

*Fig. 5.*

Effect of various concentrations of T₃ on hCGα secretion by cultured early placental tissues. The results represent the mean ±sd of four determinations. *: p<0.05; **: p<0.01 vs control cultures.
tion, and steroidogenic enzyme (3β-hydroxysteroid dehydrogenase and aromatase) induction (12,13).

The present in vitro study demonstrates that thyroid hormone exerts a direct action on the placenta and that an optimal concentration of thyroid hormone stimulates trophoblast endocrine function, including progesterone and estradiol secretion as well as hCG (α,β) and hPL secretion by cultured early placental tissues. The stimulatory effect of thyroid hormone on trophoblast function was maximally exerted at the concentration of $10^{-8}$ mol/l T₃ or $10^{-7}$ mol/l T₄, and treatment with either higher or lower concentrations of T₃ or T₄ gave attenuated effects. It should be noted that treatment with an excessive concentration of thyroid hormone ($10^{-3}$ mol/l T₃) resulted in a striking inhibition of progesterone and estradiol secretion. These results suggest that the optimal concentration of thyroid hormone is crucial for its facilitative action on trophoblast endocrine function.

Fig. 6.
Effect of various concentrations of T₃ on hCGβ secretion by cultured early placental tissues. The results represent the mean ±sd of four determinations. *: p<0.05; **: p<0.01 vs control cultures.

Fig. 7.
Effect of various concentrations of T₃ on human placental lactogen (hPL) secretion by cultured early placental tissues. The results represent the mean ±sd of four determinations. *: p<0.05; **: p<0.01 vs control cultures.
The exact biochemical mechanism by which thyroid hormone exerts its effects on the placenta is unclear. The increases in progesterone, estradiol, hCG (α,β) and hPL secretion in response to the addition of thyroid hormone are not likely to be due to a general enhancement of overall protein synthesis and cell multiplication of cultured trophoblasts, since during the 5-day culture period, there were no significant differences in the protein and DNA content between T₃- or T₄-treated and control cultures of early placental tissues. Furthermore, the fact that the increase in progesterone secretion caused by addition of T₃ (10⁻⁸ ~ 10⁻⁹ mol/l) or T₄ (10⁻⁶ ~ 10⁻⁸ mol/l) was further enhanced in response to the concomitant addition of pregnenolone suggests that the optimal concentration of thyroid hormone enhances the conversion of pregnenolone to progesterone by acting at the level of 3ß-hydroxysteroid dehydrogenase in the placenta. On the other hand, the fact that the increase in estradiol secretion caused by addition of T₃ (10⁻⁷ ~ 10⁻⁹ mol/l) or T₄ (10⁻⁶ ~ 10⁻⁸ mol/l) was further augmented in response to the concomitant addition of testosterone suggests that the optimal concentration of thyroid hormone also augments the conversion of testosterone to estradiol by acting at the level of aromatase in the placenta. The possibility that thyroid hormone acts at the level of 3ß-hydroxysteroid dehydrogenase and aromatase in the placenta is supported by the fact that the lag period required for the thyroid hormone-stimulated increase in progesterone and estradiol secretion became shorter in response to the concomitant addition of pregnenolone or testosterone. Thus, the stimulatory effects of T₃ or T₄ on progesterone and estradiol secretion were found to be apparent between 1 and 3 days of culture in the presence of pregnenolone or testosterone, whereas in the absence of pregnenolone or testosterone the stimulatory effects of T₃ or T₄ in culture of early placental tissues became apparent between 3 and 5 days of culture.

On the other hand, the thyroid hormone-stimulated increase in hCGα secretion from cultured early placental tissues became apparent between 1 and 3 days of culture, whereas the thyroid hormone-stimulated increase in hCGβ and hCG secretion became apparent between 3 and 5 days of culture. These results suggest that a lag period longer than one day is needed for thyroid hormone to exert its stimulatory action on the trophoblast endocrine function. This observation is consistent with the known characteristic property of thyroid hormone that it generally requires a relatively long lag period to exert its action on target cells (14).

The present finding that optimal concentrations of thyroid hormone stimulate hCG (α,β) secretion by cultured early placental tissues suggests that the optimal concentrations of thyroid hormone may also stimulate the synthesis of hCG (α,β) by early placenta, because newly synthesized hCG (α,β) in cultured trophoblasts have been found to be secreted into the medium without being accumulated in the trophoblasts (5,6). Thus, it is likely that the difference in the lag period required for thyroid hormone-stimulated increases in hCGα and hCGβ secretion may reflect a difference in the lag period required for thyroid hormone-stimulated increases in hCGα mRNA and hCGβ mRNA in cultured early placental tissues. A similar difference in the lag period required for the increase in hCGα mRNA and hCGβ mRNA has been demonstrated in response to addition of 8-bromo-cyclic AMP to cultured human trophoblasts by Ringler and his associates (15). The latter difference seems to be related to the findings by Hoshina et al. (16,17) who demonstrated that the expression of hCGα mRNA is first initiated in less differentiated cytotrophoblasts, whereas the expression of hCGβ mRNA starts later in trophoblast differentiation.

Human placental lactogen is known to be expressed only in fully differentiated syncytiotrophoblast (16). Accordingly, production and secretion of hPL by trophoblast represent a differentiated state of trophoblast. Thus, the present finding that the cultured early placental tissues responded to thyroid hormone by increasing hPL secretion suggests a role for thyroid hormone in the induction of cellular differentiation of trophoblasts in the early placenta.

The primary effect of thyroid hormone on target cells is thought to occur through its binding to sites in the nucleus. The results obtained are consistent with those by Ashitaka and his associates (18,19) who reported that human placental trophoblasts possess nuclear T₃ receptors and that the binding capacity of nuclear T₃ receptors in early placental trophoblasts is much greater than that in term placental trophoblasts. Thus, the facilitative action of thyroid hormone on progesterone, estradiol, hCG (α,β) and hPL production by cultured early placental tissues seems to be mediated via nuclear receptors for T₃ in trophoblasts. The failure of term placental tissues to respond to the addition of thy-
roid hormone may be due to the lesser binding capacity of nuclear T3 receptors in term than in early placental trophoblasts.

Since the doses of thyroid hormones (10^{-8} mol/l T3, 10^{-7} mol/l T4) needed for the maximum enhancement of trophoblast endocrine activity are in the reported physiological range of circulating levels of thyroid hormones in human plasma, it is highly possible that thyroid hormone at the optimal concentration plays a physiological role as an enhancer of trophoblast endocrine function. Since increasing amounts of progesterone and hCG have critical roles in the maintenance of early pregnancy, our results suggest that the presence of thyroid hormone at the optimal concentration in the placenta plays a vital role in the maintenance of early pregnancy. Thus, the frequent occurrence of spontaneous abortion in early pregnancy during the state of hypothyroidism and hyperthyroidism may represent a direct consequence of inadequate thyroid hormone availability at the level of placental trophoblasts, followed by diminished endocrine activity of the early placenta.

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