Thyroid-stimulating hormone receptor and thyroid hormone receptors are involved in human endometrial physiology

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Objective: To study the expression, distribution, and function of thyroid-stimulating hormone receptor (TSHR) and thyroid hormone receptors (TR) α1, α2, and β1 in human endometrium.
Design: Experimental clinical study.
Setting: University hospital.
Patient(s): 31 fertile women.
Intervention(s): Endometrial biopsy samples obtained throughout the menstrual cycle.
Main Outcome Measure(s): Real-time reverse transcriptase polymerase chain reaction, immunohistochemistry and Western blot to study the expression of TSHR, TRα1, TRα2, and TRβ1 messenger RNA (mRNA) and proteins in human endometrium.
Result(s): We found TSHR, TRα1, TRα2 and TRβ1 mRNA and proteins expressed in human endometrium. Immunostaining for TSHR in the luminal epithelium and TRα1 and β1 in the glandular and luminal epithelium increased statistically significantly on luteinizing hormone (LH) days 6 to 9, coinciding with appearance of pinopodes. Endometrial stromal and Ishikawa cells expressed mRNA for TSHR, TR, and iodothyronine deiodinases 1–3. After 48 hours, TSH significantly increased leukemia inhibitory factor (LIF) and LIF receptor (LIFR) messenger RNA (mRNA) in endometrial stromal cells, but decreased their expression in Ishikawa cells. Glucose transporter 1 mRNA was up-regulated by TSH in Ishikawa cells. We found that TSH statistically significantly increased secretion of free triiodothyronine (T_3) and total thyroxin (T_4) by Ishikawa cells compared with nonstimulated cells.
Conclusion(s): Thyroid hormones are directly involved in endometrial physiology. (Fertil Steril® 2011;95:230–7. ©2011 by American Society for Reproductive Medicine.)

Key Words: Deiodinases, human endometrium, thyroid hormone receptor, thyroid hormones, TSH receptor

Thyroid hormones are related to various aspects of human reproduction. Both hypothyroidism and hyperthyroidism are known to affect the metabolism of sex steroids and ovarian function in women (1) and are associated with a wide range of reproductive disorders, from abnormal sexual development to menstrual irregularities and infertility (2). A significantly higher prevalence of menstrual irregularities in hypothyroid and hyperthyroid patients compared with euthyroid women has been reported (3, 4). The relative risk of female infertility was significantly increased in women positive for thyroid peroxidase antibodies and, in particular, with infertility related to endometriosis (5). This suggests that direct thyroid hormone receptor contact in disseminated endometrial cells may play a role in the pathogenesis of infertility. In a registrar study, we found that women on thyroid medication early in pregnancy had a history of longer infertility and a higher recurrent miscarriage rate than the healthy population (6).

There are two major thyroid hormone receptor (TR) isoforms, TRα and TRβ (7), which bind triiodothyronine (T_3) and mediate thyroid hormone (TH)-regulated gene expression. Alternative splicing of the TRα gene generates two mature messenger RNAs (mRNAs) encoding two proteins: TRα1 and TRα2. Thyroid hormone receptor α2 cannot bind T_3 and cannot transactivate TH-responsive genes (8). There are two TRβ isoforms derived from the TRβ gene: TRβ1 and

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TRβ2. Shahrara et al. (9) reported the expression of TR mRNAs and proteins in several human tissues including the heart, brain, placenta, lung, liver, skeletal muscle, kidney, and pancreas. There is, to our knowledge, only a single report on the expression of TRα1, TRα2, and TRβ1 transcripts in human endometrium, in which progesterone regulation of TR was suggested (10).

The main control over T_3 and thyroxin (T_4) secretion is mediated by the short-term and long-term actions of thyroid-stimulating hormone (1). Thyroid-stimulating hormone (TSH) is the primary regulator of thyroid hormone release and secretion, and it has a critical role in thyroid growth and development. It binds to the thyroid-stimulating hormone receptor (TSHR), which is a transmembrane receptor and is abundant in human thyroid tissues (11). There is also evidence for extrathyroidal TSHR expression: TSHR mRNA and protein have been reported to be expressed in a variety of cell types, including kidney, thymus, cardiac muscle, and adipose tissue cells (12). We have recently demonstrated the expression of TSHR mRNA and protein in human ovarian tissue (13), and its transcript was detected in human endometrium (10).

Important genes involved in the activation or inactivation of thyroid hormones are peroxidase enzymes, called iodothyronine deiodinases. Type 1 iodothyronine deiodinase (DIO1) and type 2 iodothyronine deiodinase (DIO2) catalyze the activation of the prohormone T_4 to the active hormone T_3, and type 3 iodothyronine deiodinase (DIO3) catalyzes the inactivation of T_4 and T_3 (14, 15). The transcript for DIO2 was detected in endometrium throughout the menstrual cycle (10, 16), indicating the possibility of generating T_3 from T_4 (17). Also, DIO3 is expressed in human placenta, maternal decidua, and endometrial glands of the nonpregnant human uterus (14, 15).

Our study investigated the presence of thyroid hormone receptors in endometrial tissue samples in different phases of the menstrual cycle from healthy fertile women and correlated the cell type-specific expression of the receptors with endometrial ultrastructure, assessed by the development of pinopodes (18). The potential involvement of thyroid hormones in endometrial function and the possibility of endometrial cells to be a site of T_4 production were investigated.

RESULTS

Hormone Analyses

All of the women had regular ovulatory cycles, and their TSH and free T_4 serum concentrations were within the normal range: 1.6 $\pm$ 0.15 mIU/L and 14.3 $\pm$ 0.7 pmol/L, respectively. Their hormone concentrations did not differ between menstrual cycle phases and did not correlate with the TSHR, TRα1, or TRβ1 immunostaining intensities in human endometrial tissue when assessed on either individual or group level (data not shown).

Presence and Distribution of TSHR, TRα1, TRα2, and TRβ1 Proteins in Normal Human Endometrium

The TSHR immunostaining was found in the cytoplasm of all cell types of normal human endometrium (Fig. 1A, E, I, M). There was a statistically significant increase of TSHR staining in the luminal epithelium on days LH +6 to +9, coinciding with the appearance of pinopodes and the supposed period of uterine receptivity for blastocyst implantation in our healthy research participants (Table 1). The TSHR immunostaining in the glandular epithelium was strongest in the proliferative phase. The stromal endometrial cells showed faint TSHR staining throughout the menstrual cycle (see Table 1).

We found that TRα1 protein was present in all cell types of human endometrium throughout the menstrual cycle (see Fig. 1B, F, J, N). Perinuclear immunostaining in luminal and glandular epithelium was stronger than that in the stromal cells. Strong TRα1 apical staining of luminal epithelial cells and pinopodes in the midsecretory phase was statistically significantly more intense than that in early secretory endometrium. In glandular epithelium, TRα1 immunoreactivity was most intense during the middle and late secretory phases (see Table 1).

In both epithelial and stromal endometrial cells, TRβ1 was expressed (see Fig. 1C, G, K, O). A granular pattern of staining was restricted to cytoplasm with little nuclear staining present. Apical staining of luminal epithelial cells and pinopodes was observed. Staining for TRβ1 was most intense in midsecretory phase luminal epithelium (see Table 1). The expression of TRβ1 in glandular epithelium was statistically significantly higher during the middle and late secretory phases when compared with that seen in the early secretory phase (see Table 1). The TRβ1 expression in endometrial stroma was similar throughout the menstrual cycle (see Table 1).

We did not find TRα2 immunoreactivity in any of the human endometrial samples. However, Western blotting of the endometrial biopsy samples showed the presence of all four receptors studied: TRα1 (mol wt = 48 kd), TRα2 (mol wt $\sim$ 60 kd), TRβ1 (mol wt = 55 kd), and TSHR (mol wt = 56 kd) (see Figure 1T).

Endometrial Surface Ultrastructure

Scanning electron microscopy showed that all samples obtained from women during days LH +6 through +9 had pinopodes at different developmental stages (see Fig. 1D, H, L, P). Endometrial biopsy samples from the proliferative phase and from days LH +0 to LH +4 and LH +10 to LH +13 did not show any signs of pinopodes.

TSHR, TRα1, TRα2, and TRβ1 mRNA Expression in Normal Human Endometrium

The QRT-PCR analysis revealed the presence of TRα1, TRα2, and TRβ1 mRNA in the endometria of healthy fertile women throughout the menstrual cycle, n = 3 in each phase (Fig. 2). Relative expression of TRα1 mRNA was statistically significantly ($P<.001$) higher during the midsecretory phase when compared with that in the proliferative and late secretory phases (see Fig. 2A), but the opposite was seen for TRα2 ($P=.004$; see Fig. 2B). The highest levels of TRβ1 mRNA were seen in the early secretory phases of the cycle ($P=.001$; see Fig. 2C).

The QRT-PCR analysis detected TSHR mRNA expression throughout the menstrual cycle (n = 3 in each phase; see Fig. 2D), with a statistically significant ($P=.05$) decrease in the midsecretory phase compared with the proliferative phase, which probably, together with cycle variations in TR transcripts' expression, creates a balanced maintenance of endometrial TH system. Ishikawa cells and isolated endometrial stromal cells expressed mRNA for TSHR, TRα1, TRα2, and TRβ1 (data not shown).

Deiodinase mRNA Expression in Normal Human Endometrium

The presence and relative expression of DIO1, DIO2, and DIO3 mRNA were analyzed in endometrial biopsy samples throughout menstrual cycle (n = 3 in each phase) and in endometrial stromal and epithelial (Ishikawa) cells. The mRNA for all three DIOs were expressed in endometrium, with DIO2 being the most abundant (see Fig. 2E). Expression of all three mRNAs was statistically significantly lower in the midsecretory phase of the cycle compared