Role of thyroid hormone in controlling the concentration of luteinizing hormone/human chorionic gonadotropin receptors in rat ovaries

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Little is known about the mechanism by which thyroid hormones influence ovarian function, especially in gonadotropin receptor formation. In this study the concentration of luteinizing hormone (LH)/human chorionic gonadotropin (hCG) receptors in the ovaries of hypo- and hyperthyroid rats was estimated. Rats were made experimentally hypothyroid by thyroidectomy (N = 10) and hyperthyroid by injections of 40 μg of i-thyroxine daily for 21 days (N = 14). After 3 weeks the ovaries were excised, weighed, immersed in liquid nitrogen and then, after 24 h of incubation with 125I-labeled hCG (CR-121), the concentration of receptors (cpm) for one ovary and 1 mg of tissue was counted in their respective homogenates. The ovaries of the hyperthyroid group were diminished in size and consequently the level of receptors per ovary also was reduced when compared with control animals. The number of receptors per ovary and per milligram of tissue of hypothyroid rats was three times higher than in the control. In hyperthyroid animals a significant decrease in these values was noted when compared with hypothyroid rats, especially in the calculation of receptor concentration per ovary. It may be concluded that thyroid function may affect the size of the gland and also the number of LH/hCG-binding sites in rat ovaries. These data may be useful for interpretation of the pathophysiology of polycystic ovary syndrome in women and animals.

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The concentration of gonadotropin receptors in the ovaries varies depending on the stage of the estrus cycle, development of the follicles and actual hormonal stimulation. Generally, the number of receptors decreases with the differentiation and growing process of follicles. Some evidence suggests that the concentration of gonadotropin receptors in the ovaries is regulated by gonadotropins—FSH, LH (self-regulation, stimulation or “down-regulation”), progesterone, estrogen, androgens—and by some local hormonal factors.

Despite the large bulk of evidence demonstrating the role of thyroid hormones in ovary function, the exact mechanism of its action in this organ is, hitherto, obscure. Early evidence (1–3) suggests that in female and male hypothyroid animals there exists some hypersensitiviy of the gonads to gonadotropin action (polycystic ovary syndrome), and in hyperthyroid animals there is an attenuation of this response. Furthermore, it has been established (1, 2, 4) that exogenous gonadotropins exert a “thyrotropic” and “hypophyseotropie” action (enlargement of the organs, proliferative changes) in rats, ewes and chickens. It has been found also that the “thyrotropic” action of gonadotropins depends on the presence of the ovaries in the animals and does not exist in male rats (5). More recent in vitro and in vivo evidence (6–12) has confirmed previous observations on the “thyrotropic” action of exogenous gonadotropins. According to Ballabio et al. (7), the “TSH-like activity” observed in pregnant women depends on some isofoms of human chorionic gonadotropin (hCG).

The lack of clear information on the role of the thyroid in gonadotropin receptor formation in the ovary, except for some recent in vitro findings (13–15), is visible from the above data. Therefore, the aim of this work was to estimate the concentration of LH/hCG receptors in hypo- and hyperthyroid rat ovaries.

Material and methods

Animals and experimental groups

The experiment was performed on 34 Wistar/Han. female rats aged 3 months. At all times rats were maintained in accordance with the NIH Guidelines for the Care and Use of Laboratory Animals. Ten rats were made hypothyroid by previous thyroidectomy 7 days before the experiment (Tr-X) and 14 were made
hypothyroid (T₄) by daily injections for 21 days of 40 μg of i-thyroxine (Reanal, Hungary) dissolved in alkaline saline. Ten rats served as a control (injection of saline). Rats were housed in a room with a 12-h lighting period (07:00−19:00 h) and controlled temperature (21−23°C) and fed standard rat chow (Muirgrain, Motycz, Poland) and water ad libitum. After 3 weeks the rats were sacrificed by exsanguination and the ovaries were weighed quickly and immersed in liquid nitrogen.

Receptor estimation

Ovarian receptors were estimated according to the procedure of Pomp et al. (16). After thawing, pairs of ovaries were homogenized in 2.0 ml of cold PBS (0.01 mol/l) at 4°C with a Polytron homogenizer (35 000 rpm, two 6–8-s bursts) and then incubated with ¹²⁵I-labeled hCG in polystyrene tubes at 22°C for 24 h with continuous shaking. Purified hCG (CR-121; 13.45 × 10⁹ IU/kg) was supplied to Professor Dr A Zieck (Agrotechnology and Veterinary Sciences, PAS, Olsztyn, Poland) by the Center for Population Research of the National Institute of Child Health and Human Development, USA. The hCG was labeled by the chloramine T method using Na ¹²⁵I (Amersham International, Amersham, UK). Specific activity of labeled hCG was determined by self-displacement analysis in the radioligand receptor assay and varied in two assays from 33 to 40 cpm/pg. The corrected specific activity was 42−51 μCi/μg. The level of binding was 30%. The interassay coefficient of variation was 7%. The incubation mixture consisted of 0.1 ml of PBS containing 5 mmol/l MgCl₂ and 0.1% BSA (incubation buffer, pH 7.2), 0.1 ml of incubation buffer containing 100 000 cpm ¹²⁵I-labeled hCG and 0.25 ml of ovarian homogenate. The concentration of hormone receptors (cpm) was estimated per ovary and per milligram of wet ovarian tissue (LKB 1271, Clinigamma).

Statistics

Comparison of means between groups was evaluated using one-way analysis of variance (ANOVA F-test). Differences were considered significant if the p value was less than 0.05. All results have been presented as the mean ± SEM.

Results

The ovaries of hypo- and hyperthyroid rats did not show any visible signs of cyclic activity; the size of hypothyroid ovaries was not significantly different but that of the hyperthyroid rats was reduced significantly compared with the control (control: 90.0 ± 4.9 mg; Tr-X: 79.2 ± 5.0 mg; T₄: 47.3 ± 3.2 mg, respectively). Significant differences were found (Table 1) in the concentration of LH/hCG receptors (cpm) in the ovaries of both groups of experimental animals. The concentration of receptors per ovary of hypothyroid rats was three times higher (93 055 ± 11 350) in comparison to the control (30 417 ± 1704; p < 0.01). In hyperthyroid animals these values were insignificantly lower (about 30%) when compared to the control (22 337 ± 1885) and statistically significant (p < 0.001) vs the hypothyroid group. The concentration of receptors per milligram of ovarian tissue from hypothyroid animals was 3.2 times (2146 ± 245; p < 0.001) and in the hyperthyroid group only 1.5 times (1034 ± 118.4) higher than in control animals (665.2 ± 47.0). The decrease in receptor concentration in hyperthyroid rats when compared to hypothyroid rats was statistically significant (p < 0.05). It is clear that if expressed by gram or ovarian weight, i-thyroxine causes a significant decrease in receptor binding sites for labeled hCG in rat ovaries by comparison with hypothyroid animals.

Discussion

The results of the experiment clearly demonstrate that thyroid hypofunction caused a marked increase in ¹²⁵I-labeled hCG binding to rat ovarian tissue. The hyperthyroid animals, however, showed the opposite effect, i.e. a decrease in LH/hCG-binding sites in the ovaries, probably because of the reduced ovarian mass rather than a decrease per cell. This contradictory action of differing thyroid status on the concentration of LH/hCG receptors confirms the existence of an antagonistic interrelationship between the thyroid and...
ovary in gonadotropin action observed by Fitko (1, 2). It may be interpreted simply by an assumption of the theory that increased concentrations of gonadotropin receptors in ovarian tissue of hypothyroid rats cause marked sensitivity of this organ (increased weight, cyst formation) to extrapituitary gonadotropins. Hyperthyroid status (by decreasing the receptor population), however, prevents or attenuates the ovarian response to gonadotropins. The results of the experiment indicate also that thyroid–gonadotropin interaction is based on the influence of thyroid hormones on gonadotropin receptor formation in follicular walls. This has been confirmed in recent years by some experiments in vitro. Osteen et al. (15) demonstrated that thyroxine stimulates LH/hCG receptor formation in follicular granulosa cells after previous FSH action in vitro. According to Hayashi et al. (13) and Maruo et al. (14), thyroid hormones potentiated the differentiation of cells in pig granulosa culture caused by FSH, enhanced the concentration of LH receptors and intensified also steroidogenesis in these cells. Among the thyroid hormones, triiodothyronine possessed the greatest concentration of receptors in the nuclei of pig granulosa cells of small follicles.

The above-mentioned in vitro experiments are in contrast to our findings performed in living animals. In vitro thyroid hormones exert a stimulatory action on ovarian function only after previous preincubation of ovarian tissue with FSH. Thyroxine alone, without previous stimulation of ovarian tissue by FSH, failed to exert stimulatory action on granulosa cell differentiation, receptor formation or steroidogenesis. Our in vivo experiments showed the opposite effect of thyroid status on ovarian function and receptor formation, probably caused by repressor-like regulatory function of thyroid hormones in receptor protein unit formation. Thus, thyrothyroid status may increase gonadotropin receptor formation in the ovaries and hyperthyroid status may block this process. Another explanation of these findings is that, in vivo, pituitary TSH may cross-react with LH/hCG receptors in the ovaries and in this way may inhibit receptor function.

So far, little is known about the molecular mechanism of thyroid hormone action on ovarian tissue. The action could, for instance, be due to an interaction between the thyroid hormones and ovarian gonadotropin receptors, and this should be the aim of further investigations.

The molecular mechanism of the thyroid–ovarian interrelations proposed above may be useful for interpretation of the well-known hypersensitivity of the ovaries to gonadotropins in hypothyroid animals (1, 3) and its decreased sensitivity in hyperthyroid animals. It may contribute to the understanding of the pathophysiology of natural and experimental cystic ovarian disease in humans, bovinies and rats. The effect of hypothyroidism for these ovarian changes is well known (1, 3, 17–20). The main conclusion here is that hypothyroidism increases LH/hCG receptor populations in rat ovaries and hyperthyroidism decreases their concentration.

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