Steroid Secretion by Follicles and Cysts from the Hypothyroid, hCG-Treated Rat

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Abstract. **Hypothyroid rats develop cystic follicles after daily administration of human chorionic gonadotropin (hCG). This study was undertaken to compare progesterone, testosterone, and estradiol secretion by antral follicles (0.9–1.0 mm) and cysts (small: 1.2–2.0 mm; large: >2.0 mm) from hypothyroid, hCG-treated rats with that of antral follicles from euthyroid, saline-treated animals. After 3, 5, or 10 days of hCG injections, follicles and cysts were dissected from the ovaries, diameter determined, and incubated in minimum essential medium (MEM) for 2 hr at 37°C. Media were assayed for progesterone, testosterone, and estradiol by RIA. Progesterone secretion by antral follicles removed after 3, 5, or 10 days was similar but small cysts secreted significantly more of this steroid than did antral follicles. Large cysts secreted significantly more progesterone than all other follicles and cysts. After 10 days of treatment with hCG, antral follicles from hypothyroid, hCG-treated rats secreted two to three times more testosterone than similar follicles from euthyroid, saline-treated animals. Changes in estradiol secretion were not apparent until after 10 days of hCG treatment. Both small and large cysts from those animals secreted significantly more estradiol than all other follicle and cyst groups. These results suggest that steroid secretion by follicles and cysts could be contributing to elevated serum levels of progesterone and testosterone during the first 10 days of cyst induction. However, cysts may be contributing estradiol only during the later stages of cyst induction.**

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Hypothyroid rats given daily injections of human chorionic gonadotropin (hCG) develop large ovaries with antral follicles, corpora lutea, and numerous cystic follicles (1). Ovaries from both saline-treated euthyroid and hCG-treated hypothyroid rats have antral follicles characterized by fluid-filled antra and a multilayered wall containing layers of granulosa cells in typical stratified squamous epithelial arrangement (2, 3). The presence of normal appearing corpora lutea in the hypothyroid, hCG-treated rat ovaries suggests that some follicles ovulate. However, many follicles fail to ovulate and are characterized as cysts by reduced numbers of granulosa cell layers and large fluid-filled antra (1). The mechanisms responsible for the failure of some follicles to ovulate and the subsequent development of cystic follicles are not known. Anovulation and cyst formation in women may be associated with a chronic elevation of serum estrogens (4) and androgens (5). Cyst formation may be associated with changes in ovarian steroid and gonadotropin secretion. Elevated serum concentrations of estrone and free estradiol have been associated with increased secretion of luteinizing hormone (LH). High serum LH stimulates additional androgen production which increases substrate for aromatization to estrogens (6, 7). In addition, the reduction in serum androgen following wedge resection of the ovary often results in follicular maturation and normalization of se-

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rum LH/follicle-stimulating hormone (FSH) ratios (8). Local effects may be related to the positive action of estradiol on folliculogenesis, particularly on the early stages of development of the cystic ovary (9). This may be the impetus for abnormal steroid production. Estradiol also appears to have a role in follicular fluid formation and cellular rearrangement (10). It is possible that, in excess, estradiol may be involved in local actions such as overproduction of follicular fluid.

The mechanism responsible for cyst development in the hypothyroid, hCG-treated rat may be significantly different from human ovarian cyst formation. However, serum concentrations of testosterone and estradiol have been characterized through 40 days of hCG treatment (11, 12). Estradiol concentrations were significantly elevated as early as 48 hr after the first hCG injection and remain elevated through 40 days of hCG treatment. Thus, both testosterone and estradiol may contribute to the development of cystic follicles in the hypothyroid, hCG-treated rat.

Several studies have investigated the steroidogenic activity of the isolated cystic ovary. Bruot (13) reported that the secretion of estradiol and testosterone by the ovaries from hypothyroid, hCG-treated rats during short-term incubation was increased during the early stages of cyst development. Callard and Leathem (14) reported that fully developed cystic ovaries synthesized very little androgen and estrogen *in vitro*. However, the total conversion of androstenedione to estrogen was increased in the fully developed cystic ovary (15).

The secretion of androgens and estrogens and the resulting effects on the developing follicle may be important in the development of cystic follicles in the hypothyroid, hCG-treated rat. Serum concentrations of these hormones may not accurately reflect hormone secretion by individual follicles and cysts. However, collective secretion by these structures may make a significant contribution to the elevated serum concentrations found in this animal model. Very little is known about the steroidogenic activity of antral and cystic follicles from the hypothyroid, hCG-treated rat. Therefore, the focus of this study is to determine if the follicles and cysts of the hypothyroid, hCG-treated rat could be a source of elevated serum steroid concentrations. These results may contribute to our understanding of the events responsible for ovulatory failure and, hence, the development of cystic follicles in the hypothyroid, hCG-treated rat.

Materials and Methods

Animals. Long-Evans rats weighing 90–120 g, approximately 30–35 days old, were housed in well-ventilated rooms with a 12:12-hr light:dark cycle. All animals received rat chow and water *ad libitum*. Hypothyroidism was induced by adding propylthiouracil (PTU; Sigma Chemical Co., St. Louis, MO) to the drinking water at a concentration of 0.04%.

Experimental Design. The animals were given either tap water or PTU water for up to 20 days. On day 11, euthyroid and hypothyroid rats were given daily subcutaneous injections of 0.1 ml saline or 10 IU hCG (Ayerst Laboratories, Inc.) dissolved in 0.1 ml saline, respectively. Animals were sacrificed with anesthetic overdose after 3, 5, and 10 days of saline or hCG treatment.

Incubations. The ovaries were removed and immediately placed in ice-cold Eagle's minimum essential medium (MEM; Sigma Chemical Co.). After removal of fat and connective tissue, the ovaries were preincubated in 0.33% collagenase-MEM for 20 min in a Dubnoff metabolic shaker (60 cycles/min) at 37°C to facilitate dissection of the follicles and cysts (16, 17, 18). Preliminary studies have shown that 0.33% collagenase does not affect basal steroid secretion from follicles and cysts (2).

Healthy antral follicles similar to those characterized by Szoltys and colleagues (19) as preovulatory (diameter ranging from 0.9–1.0 mm) were removed from saline-treated euthyroid animals irrespective of the day of the estrus cycle and hCG-treated hypothyroid animals. Small (1.2–2.0 mm) and large (>2.0 mm) cystic follicles were also removed from hypothyroid, hCG-treated animals. We found no follicles greater than 1 mm in diameter in the euthyroid, saline-treated animals. Preliminary experiments have shown that follicular fluid does not contribute progesterone, testosterone, or estradiol to the incubation media with the exception of testosterone from large cysts. However, disregarding the amount contributed by the follicular fluid, the large cysts still secreted more testosterone than all other follicles and slightly more than small cysts.

Follicles and cysts were incubated one per flask in a gently shaking water bath maintained at 37°C in 2 ml of MEM for a previously determined optimal incubation time of 2 hr. The atmosphere in the incubation chamber was saturated with a 95% O_2-5% CO_2 gas mixture. At the end of the incubation the media were removed and stored at −20°C until analyzed for progesterone, testosterone, and estradiol by radioimmunoassay.

Hormone Analysis. The secretion of progesterone, testosterone, and estradiol into the incubation medium was determined by radioimmunoassay as previously reported (13). The steroids were measured directly without extraction. The interassay and intraassay variability were 11% and 7% for the progesterone assay; 6% and 3% for testosterone and 8% and 4% for estradiol, respectively.

Statistical Analysis. Significant differences were determined using a two-way analysis of variance and Student Newman-Keuls pairwise comparison test. Differences were considered significant if the P value was less than 0.05. All results are presented as the mean ± SE.

Results

Progesterone secretion by antral follicles from euthyroid, saline-treated and hypothyroid, hCG-treated groups was similar on all days tested (Fig. 1). Secretion by small cysts from all groups did not differ significantly from each other. However, secretion from small cysts was significantly greater than that for antral follicles in all treatment groups except those from hypothyroid rats that received 5 days of hCG injections. In contrast, large cysts secreted significantly more progesterone than all other follicles and small cysts. Large cysts from animals treated with hCG for 10 days secreted nearly five times more progesterone than small cysts, and twenty times more than antral follicles from similarly treated animals. Progesterone secretion by large cysts significantly increased with time. After 10 days of hCG treatment, progesterone secretion by large cysts was 3-fold greater when compared with that of large cysts after 3 or 5 days of treatment.

Testosterone secretion by antral follicles from euthyroid, saline-treated animals was similar after 3, 5, and 10 days of treatment (Fig. 2). Likewise, testosterone secretion by antral follicles and small cysts from hypothyroid, hCG-treated animals were similar. However, after day 10, follicles and small cysts from hypothyroid, hCG-treated rats secreted significantly more testosterone than did follicles and small cysts from animals given hCG for 3 and 5 days. Large cysts secreted significantly more testosterone after 3 days. This was significantly greater than all other follicles and cysts.

With the exception of a slight but significant increase in estradiol secretion by antral follicles from hypothyroid animals after 3 days of hCG, estradiol secretion by all follicles and cysts from hypothyroid animals after 3 and 5 days of hCG treatment were markedly similar (Fig. 3). However, a difference in the patterns of secretion between antral follicles and cysts emerged after 10 days of hCG treatment. Antral follicles from both treatment groups secreted similar amounts of estradiol. In contrast, 10 day small and large cysts secreted 2- to 3-fold more estradiol than all other follicles and cysts on any day tested.

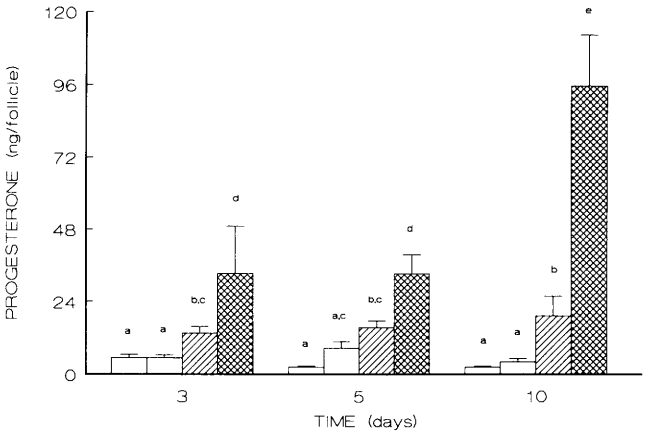

Figure 1. Effect of daily injections of saline or hCG for 3, 5, and 10 days on euthyroid (n = 16, 19, and 18, respectively) and hypothyroid (n = 19, 22, and 18, respectively) rats on progesterone secretion (ng/follicle) by antral follicles (0.9–1.0 mm), small cysts (1.1–2.0 mm) and large cysts (>2.0 mm). Progesterone secretion (ng/follicle) by antral follicles from euthyroid, saline-treated rats (n = 22, 38, and 39 follicles, respectively; open bars) and antral follicles from hypothyroid, hCG-treated rats (n = 16, 22, and 5 follicles, respectively; stippled bars), small cysts (n = 33, 35, and 19, respectively; striped bars), and large cysts (n = 4, 14, and 34, respectively; cross-hatched bars) are expressed as mean ± SE. Means with the same letters are not significantly ($P < 0.05$) different.

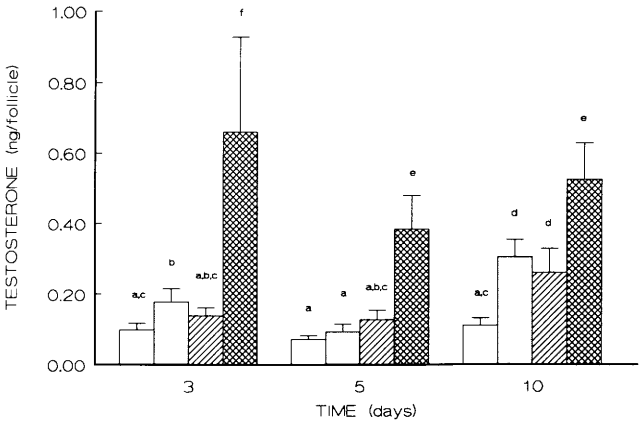

Figure 2. Testosterone secretion by antral follicles from euthyroid, saline-treated rats and antral follicles and cysts from hypothyroid, hCG-treated rats. Animal treatment and number of follicles per group are described in Figure 1. Means with the same letters are not significantly ($P < 0.05$) different.

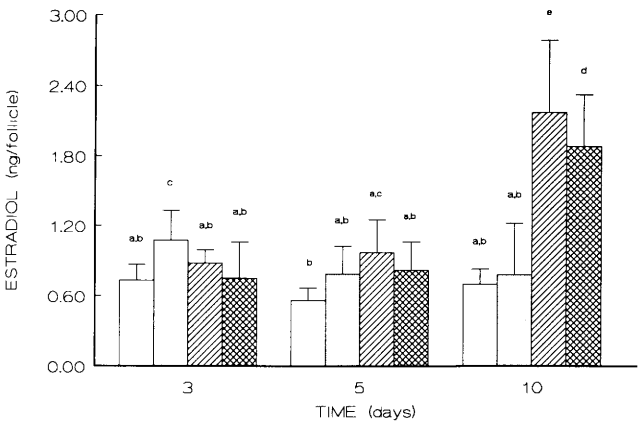

Figure 3. Estradiol secretion by antral follicles from euthyroid, saline-treated rats and antral follicles and cysts from hypothyroid, hCG-treated rats. Animal treatment and number of follicles per group are described in Figure 1. Means with the same letters are not significantly ($P < 0.05$) different.

Discussion

The results from this study clearly demonstrate that antral follicles and cysts from hypothyroid rats secrete steroids during the 10 days of hCG treatment. The hCG treatment seems to have no significant effect on progesterone secretion by antral follicles from hypothyroid animals. As follicular cyst growth approaches the large cyst size, progesterone secretion dramatically increases. This suggests that the secretion of progesterone increases with the increasing size of the cystic follicle.

Lee *et al.* (12) reported that serum levels of progesterone were five to ten times greater in hypothyroid, hCG-treated rats than euthyroid animals injected with saline. Collectively, cysts of these ovaries and other typically hypertrophied ovarian compartments such as corpora lutea (11) may be secreting progesterone which contributes to the elevated serum levels.

These results are consistent with a recent report on progesterone secretion from large cystic follicles which form when hypophysectomized (HYPOXD) immature rats are treated *in vivo* with FSH and hCG. Progesterone accumulation in the incubation media of these cysts was significantly greater than that of follicles and cysts from control HYPOXD rats and HYPOXD rats treated with hCG for up to 14 days (20).

The changing pattern of testosterone secretion with time by follicles and cysts from hypothyroid, hCG-treated rats is quite different from that of progesterone. Antral follicles and small cysts from hypothyroid animals may not be contributing significant amounts of testosterone until later stages of development of the cystic ovary. Elevated serum concentrations of testosterone have been reported during the early stages of cyst induction in the hypothyroid, hCG-treated rat (11) and throughout 30 days of hCG treatment (12). Large cysts, which appear to be secreting dramatic amounts of testosterone as early as 3 days, collectively, may have a role in elevated serum testosterone concentrations in these animals.

The results from this experiment indicate that ovarian cysts contribute to the elevated serum levels of estradiol after the cystic condition is well established. Yet, it has been suggested that an increase in serum estradiol concentrations may play a central role in the development of ovarian cysts in this animal model (21). Furthermore, serum estradiol concentrations are elevated as early as 48 hr after the first hCG injection (11). However, additional studies are needed to determine the ovarian compartment which may be contributing to the elevation in serum estradiol during the early stages of cyst development.

The secretion of nearly eight times more estradiol than testosterone suggests that the antral follicles from the euthyroid, saline-treated group are healthy (22). A similar ratio of estradiol to testosterone after 3 and 5 days of hCG suggests the presence of healthy antral follicles in the hypothyroid animals. However, by day 10, the ratio of estradiol to testosterone secretion of antral follicles and large cystic follicles from hypothyroid, hCG-treated rats decreases to 3 and 4, respectively. It is not known whether these follicles are destined to become cysts.

This shift in steroidogenesis may be critical for continued cyst development in the hypothyroid, hCG-treated rat. Additionally, excessive steroid secretion may be intertwined with the development of cystic follicles. The role of androgens in atresia may further clarify the mechanisms that are involved in formation of the cyst from a follicular structure. Androgens have long been associated with the onset of atresia. The mechanism has not been clearly defined, but may be associated with structural changes within the granulosa cell and the activation of the degenerative processes by the direct effect of elevated androgen concentrations on the follicle itself (3). Some of the local effects of elevated estradiol secretion in the development of cysts as observed in these experiments may be related to its synergistic effects with FSH to increase granulosa cell proliferation and follicle growth (9) in addition to a possible estrogen-induced enlargement of the cystic antrum (22).

In conclusion, small cysts may be contributing to elevated serum progesterone concentrations early in the development of the cystic ovary and to testosterone during the later stages of hCG-induced cyst formation in the hypothyroid rat. Large cysts appear to secrete excessive amounts of progesterone and testosterone early in the process. In contrast, small and large cysts do not appear to secrete abnormal amounts of estradiol when compared with antral follicles until later in the development of the cystic ovary. The significant increase in testosterone secretion by normal-appearing antral follicles of the cystic ovary may also contribute to the elevated serum concentrations of testosterone during later stages of cyst formation.

The antral follicles and cysts of the hypothyroid, hCG-treated rat appear to be a site of abnormal steroidogenesis. Collectively, these structures may be contributing to the previously described elevated serum steroid concentrations.

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