Circulating Hormone Concentrations in Hypothyroid Rats with Induced Polycystic Ovaries (43312)

MONG-TING LEE, WALTER C. ADAMS, AND BRENT C. BRUOT[1]
Department of Biological Sciences, Kent State University, Kent, Ohio 44240

Abstract. **The induction of polycystic ovaries in hypothyroid rats by human chorionic gonadotropin (hCG) has been studied for many years. A complete understanding of this phenomenon requires information regarding the circulating levels of the hormones of the hypophyseal-gonadal axis. In this study, serum prolactin (PRL), luteinizing hormone (LH), estradiol, testosterone, and progesterone were measured by radioimmunoassay at intervals during the 40-day period in which large ovarian cysts were induced in hypothyroid rats by daily injections of hCG. After 20 injections, ovaries increased in weight 10-fold, and well-developed ovarian cysts were present, accompanied by lutein tissue; cyst development continued for the subsequent 20 days of hCG. Both PRL and LH rose during the first 5 days of treatment and were maintained at high levels from day 20 on. The pattern of change of gonadal steroids showed greater increases with hCG in hypothyroid than in euthyroid rats. Levels of estradiol in hypothyroid, hCG-injected rats increased in parallel to ovarian hypertrophy, whereas progesterone was high in initial stages and then declined. Testosterone increased in both euthyroid and hypothyroid animals, with no clear pattern coincident with cyst formation. The data suggest that the formation of polycystic ovaries in the hypothyroid rat is associated with high levels of PRL and LH followed by elevations of estradiol, which may serve to maintain continuous PRL, as well as LH, stimulation of the ovary.**

[P.S.E.B.M. 1991, Vol 198]

When given daily injections of human chorionic gonadotropin (hCG) for 20 days, hypothyroid rats develop polycystic ovaries, which are characterized histologically by large follicular cysts denuded of granulosa and by interstitia containing large corpora lutea (1). Several endocrine changes are known to be associated with the ovarian cyst development induced by this method, and Leathem and Adams (2) suggested that estrogen plays an essential role in the induction of these cysts, since administration of the antiestrogen MER-25 prevented their formation. Moreover, prolactin (PRL) is elevated during ovarian cyst development in hypothyroid rats (3), PRL administration augments cyst formation (4), and the dopamine agonist bromocriptine interferes with cyst induction in conjunction with its suppression of circulating PRL (3). These results suggest that PRL and estrogens play a central role in the induction of ovarian cysts in hypothyroid rats.

Lee *et al.* (5) reported an elevation in progesterone and testosterone, as well as estradiol and PRL, during the early stages (12–48 hr) of cyst development in hypothyroid rats. However, with the exception of PRL, it is not known whether these hormones remain elevated during the later stages of cyst development. The present study was undertaken to assess the levels of reproductive hormones during later stages of ovarian cyst development in hypothyroid rats and to provide a comparison of the endocrine state of these animals with euthyroid animals during the growth phase of these structures.

Materials and Methods

Animals. Female Long-Evans rats, 30–35 days of age and weighing 90–110 g, were housed in well-ventilated rooms at 24 ± 1°C with a 14:10-hr light:dark cycle. All rats were fed *ad libitum* a semisynthetic diet

[1] To whom correspondence and requests for reprints should be addressed at Department or Biological Sciences, Kent State University, Kent, OH 44240.

Received June 4, 1990. [P.S.E.B.M. 1991, Vol 198]
Accepted June 6, 1991.

0037-9727/91/1982-0737$3.00/0

of 20% casein and 27% fat (6). Hypothyroidism was induced by incorporating thiouracil (ICN Pharmaceuticals, Inc., Cleveland, OH) at a concentration of 0.5% into the diet. The rats were assigned to either a euthyroid or hypothyroid group and fed diets with or without thiouracil for up to 50 days. On Day 10 of the experiment, at which time the vaginae were open, the euthyroid and hypothyroid groups were each subdivided into two groups. One group was injected subcutaneously daily at 0900 hr with 10 IU of hCG in 0.1 ml of saline, while the other group was injected with saline. Animals were sacrificed between 0800 and 0900 hr by decapitation on Days 15, 30, 40, or 50 of the experiment. Trunk blood was collected, allowed to clot at 25°C, and centrifuged to obtain serum. Ovaries and thyroids were removed, dissected free of connective tissue, and weighed to the nearest tenth of a milligram.

Hormone Analysis. Serum samples were measured by radioimmunoassay for prolactin (4–5 animals/group), progesterone (6–13 animals/group), testosterone (5–13 animals/group), and estradiol (5–13 animals/group), as described previously (5). The interassay and intra-assay variabilities (n = 10) were: for the prolactin assay, 17% and 11%, respectively; for progesterone, 13% and 7%; for testosterone, 13% and 10%; and for estradiol, 10% and 10%. Recovery of estradiol after addition of 37 and 146 pg was 113% and 97%, respectively, and estradiol levels were measured in ovariectomized (15 pg/ml) and male (<14 pg/ml) rats. Serum luteinizing hormone (LH) (6–15 animals/group) was measured by radioimmunoassay, as described by Bruot and Clemens (7). The interassay and intrassay variabilities for this assay were 11% and 6%, respectively. The lower limit of sensitivity of the assay was 0.025 ng. At 50% displacement of the tracer, LH antibody cross-reacted less than 1% with hCG. All samples were analyzed in duplicate. Due to limited serum volumes, all hormones were not measured in each animal. However, an average of four hormones were measured in the serum from each rat.

Statistical Analysis. All results were analyzed using a three-way analysis of variance and Newman-Keuls pair-wise comparison test (8). Differences were considered significant if the P-value was less than 0.05. All results are presented as the mean ± SE.

Results

Body, Thyroid, and Ovarian Weights. The effects of thiouracil and hCG on body weights and organ weights are shown in Table I. The thyroid glands of rats fed the thiouracil diet were significantly larger than those of control animals, whereas hCG had no significant effect on thyroid weights. Body weights were, in general, reduced by hypothyroidism, and euthyroid animals receiving hCG were heavier than all other groups in each treatment period.

Ovarian weights, relative to body weight, were not affected by hypothyroidism alone, but were significantly increased by hCG in both euthyroid and hypothyroid rats at all treatment periods. In euthyroid rats, ovarian weights reached their maximum after 20 days of hCG treatment (Day 30) and declined thereafter, whereas in hypothyroid rats, ovaries continued to enlarge throughout the 40 days of hCG injection, reaching 2–4 times the size of ovaries in euthyroid rats (Table I and Fig. 1).

Circulating Hormone Levels. Neither the addition of thiouracil to the diet nor hCG administration to euthyroid animals affected serum PRL (Fig. 1), whereas daily injections of hCG in hypothyroid animals significantly increased serum PRL concentration to its highest level after 20 days, with only a slight decline thereafter. Serum LH concentrations were not altered by thiouracil. Although consistently elevated to a slight extent in euthyroid animals injected with hCG, LH was not significantly different from control levels. However, in hypothyroid rats, serum LH was some 10-fold greater in hCG-injected rats than in controls, with the highest concentration reached at 40 days of hCG (Day 50).

Induction of hypothyroidism alone had no effect upon serum estradiol levels (Fig. 1). Daily injections of hCG increased estradiol significantly in both euthyroid and hypothyroid rats to peak serum concentrations of 187 ± 43 and 372 ± 70 pg/ml, respectively, at Day 40 (30 days of hCG). Thus, the magnitude of the increase in hypothyroid rats was nearly twice that of euthyroid animals.

Administration of hCG resulted in significant increases in serum testosterone concentration, from 4 to 6 times control levels at the four experimental time periods (Fig. 1). In euthyroid and hypothyroid rats, similar elevations were observd at 5 and 30 days of hCG injection. However, in euthyroid rats, at 20 and 40 days, these concentrations were significantly less than those induced in the hypothyroid rats and, at 40 days, testosterone levels had declined to within the control range.

Serum progesterone levels were slightly elevated in euthyroid rats after 5 and 20 days of hCG (Fig. 1), but they were indistinguishable from those of control animals after 30 or 40 days of hCG. In contrast, serum progesterone levels in hypothyroid, hCG-treated rats were several-fold greater at all time periods than those of all other groups sacrificed at the same time. Nevertheless, progesterone levels steadily decreased in the hypothyroid, hCG-treated rats, from the high of 584 ± 86 ng/ml after 5 days of hCG to a low of 279 ± 58 ng/ml after 40 days.

Discussion

The absence of an influence of thiouracil-induced hypothyroidism upon ovarian weight and upon serum

Table I. Body Weights and Relative Organ Weights[a]

0.5% Thiouracil (days)	10 IU hCG (days)	Rats (*n*)	Body Weight (g)	Thyroid weight (mg/100 g body wt)	Ovarian Weight (mg/100 g body wt)
0	0	7	146 ± 3	10.3 ± 0.5	37 ± 1.4
15	0	8	131 ± 3	45.1 ± 0.5[b]	43 ± 6.2
0	5	8	158 ± 5	10.8 ± 0.1	79 ± 3.3[d]
15	5	8	139 ± 1	53.2 ± 0.3[c]	114 ± 5.9[d]
0	0	13	145 ± 5	10.3 ± 0.3	36 ± 3.3
30	0	13	122 ± 5	45.1 ± 0.6[b]	31 ± 1.8
0	20	13	161 ± 4	8.7 ± 0.1	135 ± 19.4[d]
30	20	13	126 ± 4	50.8 ± 0.7[c]	325 ± 24.5[c,d]
0	0	11	158 ± 5	14.6 ± 0.1	29 ± 1.6
40	0	11	147 ± 4	49.6 ± 1.3[b]	24 ± 1.3
0	30	10	183 ± 4	8.4 ± 0.2	124 ± 18.1[d]
40	30	10	138 ± 4	59.7 ± 1.7[c]	391 ± 42.6[c,d]
0	0	7	175 ± 6	11.0 ± 1.3	27 ± 3.0
50	0	7	131 ± 3	75.4 ± 3.1[b]	29 ± 1.3
0	40	7	204 ± 5	12.5 ± 0.6	102 ± 35.8[d]
50	40	8	147 ± 5	50.5 ± 4.6[c]	410 ± 56.1[c,d]

[a] Data represent the body weights and relative organ weights in rats fed thouracil-containing diets (0.5%) and injected with 10 IU hCG daily for 5, 20, 30, or 40 days. Means ± SE are presented.
[b] Significantly different ($P < 0.05$) from euthyroid, saline-injected animals.
[c] Significantly different ($P < 0.05$) from euthyroid, hCG-injected animals.
[d] Significantly different ($P < 0.05$) from hypothyroid, saline-injected animals.

concentrations of estradiol, testosterone, progesterone, PRL, and LH is consistent with earlier studies of the effects of hypothyroidism on reproductive hormone levels (3, 5).

In euthyroid rats, hCG enlarged and luteinized the ovaries and increased circulating estradiol, testosterone, and progesterone during the first 20 days. During the subsequent 20 days of hCG, ovarian weight declined slightly, while serum progesterone returned to basal levels, suggesting a refractoriness of the euthyroid ovary to continued hCG stimulation.

Ovaries from hypothyroid rats treated with hCG, however, developed large cystic follicles, as well as lutein tissue (1), and enlarged to 3–4 times the size of ovaries from euthyroid rats after 5, 20, 30, and 40 days of hCG treatment. After 20 days, these rats also had serum concentrations of estradiol and progesterone, as well as of PRL and LH, greater than those of euthyroid, hCG-treated animals, although testosterone levels were not consistently different between the two types of hCG-treated animals. These results agree with the elevated serum levels of steroid hormones and PRL observed during the early stages of ovarian cyst development (5), and they confirm and extend the report by Copmann and Adams (3) of elevated PRL in hypothyroid rats after 20 days of hCG treatment and of the augmentation of ovarian cyst induction by both PRL and growth hormone (4). Thus, we have observed parallel hormonal changes in animals developing polycystic ovaries in which ovarian hypertrophy and elevation of serum estrogen and PRL concentrations occur during the first 20 days of hCG administration and are maintained during a subsequent 20 days of hCG. Immunoreactive LH remains twice as high in hypothyroid as in euthyroid animals, although the same dose of hCG was given to both.

In the hypothyroid, hCG-treated rat progesterone declines throughout the experimental period following its highest level at 5 days of hCG injection. It is possible that the initial elevation in serum progesterone results in part from stimulation of an ovary in which esterified cholesterol substrate has accumulated during 10 days of thiouracil feeding (9) and at which time the concentration of both hCG/LH and follicle-stimulating hormone receptors are elevated above that of controls (3). However, the adrenal gland is also known to secrete progesterone in response to PRL (10), and in aged (18 months) rats with polycystic ovaries induced by hCG alone, adrenal cholesterol is depleted, although the gland is of normal size and thyroid function is not deranged (11). Elevated serum progesterone may contribute to the development and maintenance of ovarian cysts as suggested by Bogovich (12), who reported that ovarian cysts developed after the administration of low doses of hCG either to immature rats with subcutaneous progesterone implants or to pregnant rats. However, Bruot (13) observed that progesterone production by incubated ovaries from hypothyroid rats after 2 days of hCG does not differ from that of euthyroid rats with the same treatment, which lends some support to the possibility of an adrenal origin of the hormone early in cyst development.

The increase in progesterone in the hypothyroid, hCG-treated rat is unlikely to have contributed to the

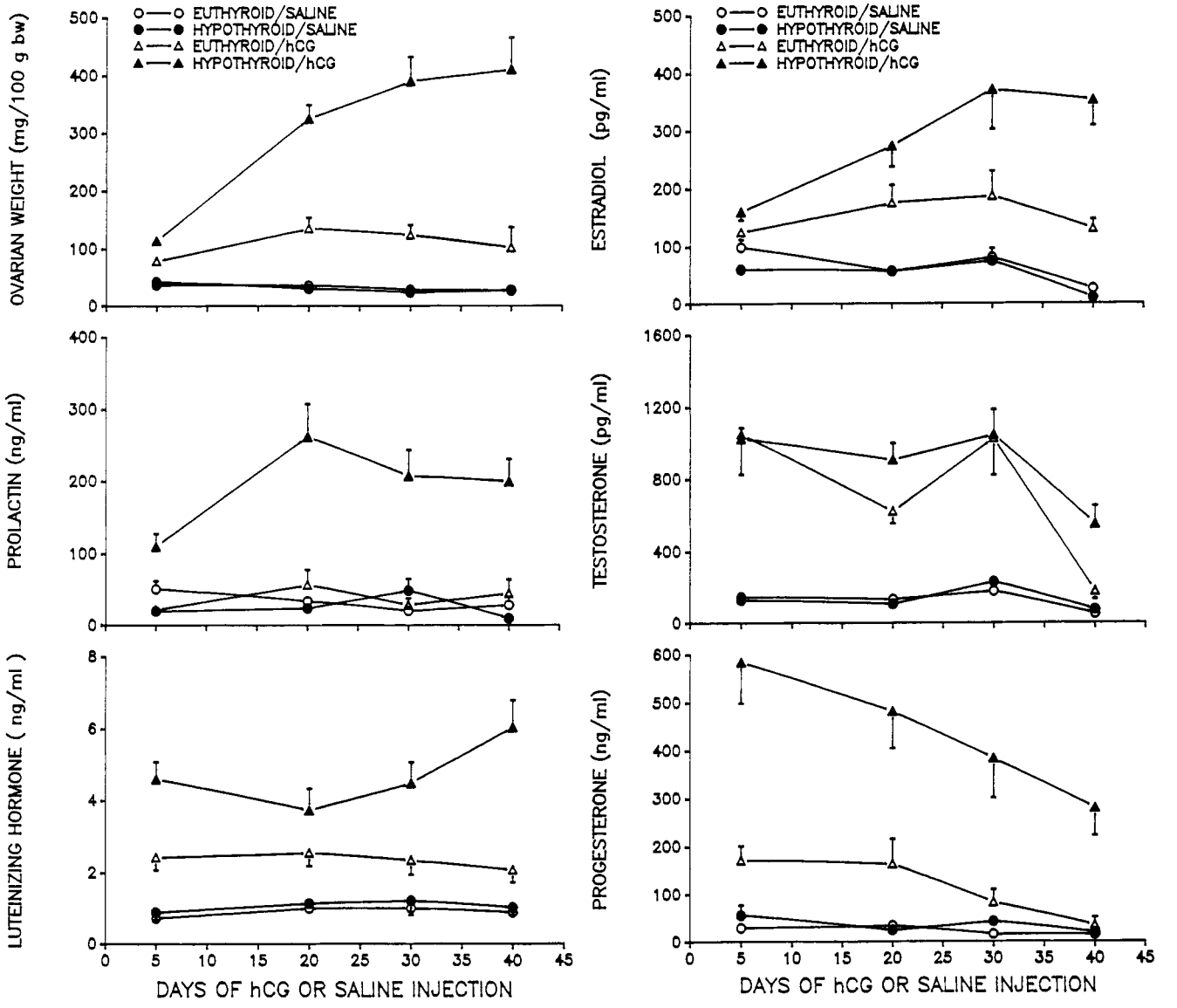

Figure 1. Ovarian weight and serum hormone levels in euthyroid and hypothyroid rats. The rats were given daily injections of hCG or saline for 5, 20, 30, or 40 days after an initial 10-day period during which control or thiouracil-containing diet was fed. Each value is expressed as the mean ± SE.

elevation in serum testosterone, since the latter was increased in both euthyroid and hypothyroid rats treated with hCG. Lee *et al.* (5) found a similar increase in serum testosterone in euthyroid and hypothyroid rats during the first 2 days of cyst induction. Khan *et al.* (14) reported that hCG stimulated the synthesis of androgens by the antral follicles and corpora lutea of pregnant rats. Thus, it is possible that hCG stimulated thecal androgen synthesis equally in euthyroid and hypothyroid rat ovaries. Elevated androgen levels are frequently associated with ovarian cysts induced by testosterone treatment (15) and in aged rats (16). Women with polycystic ovary disease also have elevated levels of serum androgens (17, 18). Thus, testosterone, while not unique to ovarian cyst development, may nonetheless be an important concomitant in concert with other factors.

The increase in circulating estradiol induced by hCG in hypothyroid animals was twice that produced in euthyroid animals. From *in vitro* studies, Adams and Senseman (19) concluded that the total conversion of androstenedione to estradiol and estrone was enhanced in cystic ovaries, and Bruot (13) reported increased estradiol synthesis either from endogenous substrate or from added androstenedione by ovaries obtained from hypothyroid, hCG-treated rats during the early stages of cyst development. In contrast, the small cystic ovaries seen in neonatally androgenized rats (20) are correlated with basal serum levels of estradiol, suggesting that this model of polycystic ovaries is distinct. Thus,

in the hCG-induced cystic ovary in hypothyroid rats, increased serum estradiol is likely a result of increased ovarian conversion from the ample supply of androgen substrate, although enhanced peripheral conversion cannot be ruled out as a contributing factor. Moreover, increasing estrogen is essential to the development of polycystic ovaries in this model, since interference with estrogen action blocks cyst induction (2).

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