Thyroid Hormones (T_4, T_3, and rT_3) in Serum of Rats Injected with Relaxin, Progesterone And/ Or Estradiol Benzoate

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ABSTRACT

An experiment was undertaken to investigate the effect of relaxin, progesterone and/or estradiol benzoate and their combinations on circulating thyroid hormones in ovariectomized rats. Blood samples were obtained after 20 days of daily subcutaneous injection. Serum samples were analyzed by radioimmunoassay for thyroxine (T_4), 3,5, 3'-triiodothyronine (T_3) and 3, 3', 5'-triiodothyronine (rT_3). Analysis of variance showed a significant decrease ($P < .01$) in body weight in response to estradiol benzoate (EB), progesterone (P), the combination of two hormones and all three hormones ($p < .01$), but there was no difference with relaxin. Serum T_4 was significantly decreased ($P < .01$) from control in response to estradiol benzoate, relaxin and the combination, but there was no significant difference with progesterone ($p > .05$). Serum T_3 was significantly increased ($p < .01$) with estradiol benzoate, progesterone, and the combination, but there was no significant change due to relaxin. Reverse T_3 increased with progesterone ($p < .05$) and decreased with relaxin ($p < .05$), but did not change with estradiol benzoate and progesterone or the combination of all three hormones. Changes in serum rT_3 were thought to reflect peripheral conversion at the target cell level from T_4. Progesterone appeared to increase the conversion rate while relaxin decreased it.

INTRODUCTION

The relationship between ovarian functions and the hypothalamic-pituitary-thyroid axis has been studied in animals and humans. Most reports considered the effect of estradiol benzoate (EB) on thyroid hormone concentration of ovariectomized rats. Administration of estrogens was associated with a decrease in circulation of T_4 (1, 2). The effect of estrogens on the secretion of pituitary thyrotropin (TSH) in the rat has been varied in different studies. Turner and Cupps (3) found a decrease in TSH secretion in untreated female ovariectomized rats, while D'Angelo (4) reported a decreased TSH secretion in female rats treated with estradiol benzoate. Chen and Wal-

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fish (2) observed the concentration of T_3 was double that of controls, while T_4 levels were lower than those of controls when rats were treated with estradiol benzoate (EB). Boado and Zaninovich (5) found EB induced a marked drop in intrapituitary TSH by decreasing synthesis. There was no change in the circulating TSH. They also found EB enchanced the peripheral conversion of T_4 to T_3.

Recent studies on thyroid hormones and pregnancy have shown no apparent changes in thyroid hormone indices of the mother (6, 7, 8). Since progesterone is the dominant hormone of pregnancy, these findings suggest a minor role for progesterone in affecting thyroid hormones or vise versa.

A paucity of research investigation has been evident in persuing the literature concerning effects of relaxin upon thyroid hormones. Therefore, this investigation was undertaken to determine the changes which occurred in serum of T_4, T_3 and reverse T_3 in response to estradiol, progesterone and/or relaxin in ovariectomized rats injected daily for 20 days.

MATERIALS AND METHODS

Rats **(Charles River)** of 190 to 200 g body weight were purchased as ovariectomized animals. All had access to the same diet (Purina Rat Chow) and water ad libitum. There were eight groups with six rats each. The source of estradiol and progesterone was Sigma Chemical Co., St. Louis, MO. Relaxin (CM_a, 3000 U/mg) was supplied by Dr. O.D. Sherwood, University of Illinois, School of Medicine, Urbana, IL. All groups were injected each day with 0.1 ml oil subcutaneously. The first group was the control injected with sunflower seed oil; the second group was injected daily with 1 ug estradiol benzoate (EB); the third group with 3 mg progesterone (P); the fourth group was injected with 1 ug estradiol benzoate (EB) and progesterone (3 mg); the fifth group was injected with 10 GPU relaxin (R); the sixth group was injected with EB and R; the seventh group was injected with P and R; and the last group was injected with a combination of progesterone, estradiol benzoate and relaxin. All rats were injected daily for 20 days.

The rats were killed on the twenty-first day by cervical dislocation and the blood was collected by the drain-out method. Serum analyses of T_4, T_3 and rT_3 were by RIA (Serono Diagnostics, Inc., Braintree, MA). Statistical analyses of the data were by ANOVA and a multiple range test (9).

RESULTS

Table 1 shows the results of body weights obtained in rats. Administration of 1 ug estradiol benzoate (EB), progesterone (3 mg), relaxin (10 GPU), EB and P, EB and R, P and R, and a combination of the three hormones (EB, P and R) during 20 days resulted in significant decreases in body weight ($p < .01$) by EB or progesterone or the combination, but no decrease by relaxin injections.

Table 2 shows the results of the effect of administration of estradiol benzoate, progesterone, relaxin, and the combination on the concentrations of T_4 in serum. There was a significant decrease in T_4 ($p < .01$) with EB alone, with R alone and combinations of EB and R and P. However, there was no change from progesterone alone.

Table 3 shows the results of T_3 concentration in serum of rats that were injected with estradiol benzoate, progesterone, relaxin, and all combinations of the three. All

Table 1 — Effect of 20 Days Treatment with Estradiol Benzoate (EB), Progesterone (P), Relaxin (R), and All Combinations on Body Weights in Ovariectomized Rats.

	Body Weight, Grams (mean ± S.E.)		
Control	292 ± 8	R	289 ± 8
EB	205 ± 8★	EB and R	242 ± 8★
P	252 ± 8★	P and R	226 ± 10★
EB and P	212 ± 8★	EB and P and R	234 ± 8★

★ Significantly different from control ($P < .01$).

Table 2 — Effect of 20 Days Treatment with Estradiol Benzoate (EB), Progesterone (P), Relaxin (R), and All Combinations on Concentration of Thyroxine (T_4) in Serum of Rats.

	T_4, ng/ml Serum (mean ± S.E.)		
Control	59 ± 4	R	44 ± 4★
EB	40 ± 4★	EB and R	42 ± 5★
P	69 ± 4	P and R	30 ± 4★
EB and P	39 ± 4★	EB and P and R	35 ± 4★

★ Significantly different from control ($P < .01$).

Table 3 — Effect of 20 Days Treatment with Estradiol Benzoate (EB), Progesetrone (P), Relaxin (R), and All Combinations on Concentration of Triiodothyronine (T_3) in Serum of Rats.

	T_3, ng/ml Serum (mean ± S.E.)		
Control	0.67 ± .06	R	0.68 ± .06
EB	1.13 ± .06★	EB and R	1.05 ± .07★
P	0.94 ± .06★	P and R	1.03 ± .06★
EB and P	1.15 ± .06★	EB and P and R	1.07 ± .06★

★ Significantly different from control ($P < .01$).

except relaxin alone showed a significant increase in T_3 ($p < .01$).

Table 4 shows the concentration of reverse triiodothyronine (rT_3) in serum of rats. There was a significant increase with progesterone ($p < .05$) and a significant decrease from relaxin ($p < .05$), but no significant difference with the estradiol benzoate and progesterone, estradiol benzoate and relaxin, progesterone and relaxin, and the combination of all three hormones.

Table 4 — Effect of 20 Days Treatment with Estradiol Benzoate (EB), Progesetrone (P), Relaxin (R), and All Combinations on Concentration of Reverse Triiodothyronine (rT_3) in Serum of Rats.

	rT_3, pg/ml Serum (mean ± S.E.)		
Control	101 ± 9	R	72 ± 9 *
EB	115 ± 9	EB and R	116 ± 10
P	126 ± 9*	P and R	107 ± 9
EB and P	109 ± 9	EB and P and R	101 ± 9

* Significantly different from control ($P < .01$).

DISCUSSION

The present data indicate that estradiol benzoate affects the body weight and the concentrations of thyroid hormones in serum of female rats. Body weight was significantly decreased with estradiol benzoate, progesterone, and all combinations, which included either or both of these steroid hormones.

Plasma T_4 was decreased in the present study, an observation previously reported in pregnant rats (1) and in estrogen-treated female rats (2). The low circulating T_4 in the presence of an accelerated thyroidal function may have resulted from an increase in metabolic clearance of this hormone, as it has been reported to occur in pregnant rats and in estrogen treated rats (10). In addition, there may be a faster conversion of T_4 to T_3 as reported by Chen and Walfish (2).

The ability of relaxin to stimulate significant reductions in serum T_4 and rT_3 of the rat has not been reported previously. Because relaxin was effective in lowering serum T_4 alone and in combination with progesterone, the action of relaxin to lower serum T_4 is convincing. Since serum T_3 is not affected by relaxin, while rT_3 is lowered by relaxin, the mechanism whereby relaxin lowers serum T_4 is suggested as being an accelerated deiodination of T_4 in the liver and/ or kidney.

The serum concentration of T_3 increased with estradiol benzoate or progesterone alone and all combinatio of of hormones including EB and P, P and R, EB and R, and EB and P and R, presumably due to the conversion of T_4 to T_3. However, there was no effect with relaxin. These results suggest estradiol benzoate and/ or progesterone act at the peripheral target cell such as muscle to accelerate the conversion of T_4 to T_3.

Serum concentration for rT_3 was significantly higher due to estradiol benzoate or progesterone injection; this may be interpreted as an action to increase the conversion of T_4 to rT_3 in the target cells. These results are in close agreement with the study by Roti et al. (11), who found the generation of rT_3 from rats at 14 days of gestation reached a peak on the 16th day and then decreased on the 18th and 20th days of gestation. Since estradiol benzoate or progesterone increased serum rT_3 concentrations while relaxin did not, the data suggest an action by relaxin selectively to reduce serum T_4 and serum rT_3 without increasing serum T_3. This selectivity probably occurs in liver

and kidney cells. Another possibility would be fibroblasts which utilize T_3 from converted T_4 at an accelerated rate, resulting in no change in serum T_3 levels and a drop in rT_3.

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أثر حقن هرمون الريلاكسين، البروجسترون والأستروجين على تركيز هرمونات الغدة الدرقية في الفئران

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المستخلص

أجريت هذه التجربة لدراسة تأثير الحقن بهرمونات الريلاكسين، والبروجسترون والأستروجين (بنزوات الاستراديول) بصورة منفردة أو مخلوطة على مستوى تركيز هرمونات الغدة الدرقية (T_4, T_3, rT_3) في دم فئران أزيلت مبايضها. أخذت عينات دم بعد حوالى 20 يوماً من حقن الفئران تحت الجلد بالهرمونات المذكورة. وتم تحليل هرمونات الغدة الدرقية (T_4, T_3, rT_3) في مصل دم الفئران باستخدام التحليل الإشعاعي المناعي (RIA). ولقد أوضحت النتائج أن حقن هرمون الأستروجين (EB) والبروجسترون بصورة منفردة أو مخلوطة، أدى إلى انخفاض معنوي في وزن الجسم، إلّا أن حقن هرمون الريلاكسين لم يوضح هذا الأثر. عند حقن هرمون الأستروجين والريلاكسين وكذلك خليط منهما، أدى ذلك إلى انخفاض معنوي في تركيز هرمون الثايروكسين بينما لا يوجد أثر معنوي للبروجسترون على ذلك. أدى حقن هرمون الأستروجين والبروجسترون وكذلك خليط منهما الى زيادة معنوية في تركيز هرمون التراي أيودو ثايرونين ولكن لم تلاحظ أي تغيرات نتيجة حقن الريلاكسين. أوضحت النتائج أيضاً أن تركيز هرمون (rT_3) زاد بصورة معنوية نتيجة لأثر البروجسترون وانخفض نتيجة لأثر الريلاكسين إلا أنه لم يتغير نتيجة لحقن هرمون الأستروجين أو أي خليط من الهرمونات الثلاثة. هذا ويعزى سبب التغير في تركيز هرمون (rT_3) إلى امكانية تحويل هرمون الثايروكسين على مستوى سطح الخلية المستهدفة الى (rT_3). ويظهر أن هرمون البروجسترون يعمل على زيادة سرعة هذا التحويل، بينما هرمون الريلاكسين يعمل على تثبيط سرعة التحويل.