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ADENOHYPOPHYSEAL GONADOTROPHIN CONCENTRATION IN THYROIDECTOMIZED FEMALE RATS[1]

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ABSTRACT

Mei-Yoong Choong and W. Chia-Mo Wan (1975). *Adenohypophyseal Gonadotrophin Concentration in Thyroidectomized Female Rats.* Bull. Inst. Zool., Academia Sinica, 14(2): 49-53. Measurements of adenohypophyseal LH and FSH concentrations were performed in female rats at 9:00 to 10:00 AM on the day of proestrus 2 months after thyroidectomy. An apparent decline of LH was demonstrated, but no FSH variations could be obtained as compared to normal controls. Possible reasons were discussed.

The effect of hypothyroidism on gonadotrophins of rats were reported repeatedly[3~5,8,14,15]; all agreed with the decreased concentration of LH in pituitaries of both male and female rats. Nevertheless, disagreement in FSH concentration of pituitaries after thyroidectomy in female rats was reported[4]; the variation was suggested to be influenced by the time of pituitary removal in different stages of the estrous cycle. In order to avoid this possible endogenous variation of FSH content, the present study is designed to observe the FSH and LH change after thyroidectomy with a fixed time of pituitary removal at 9:00 to 10:00 AM of the proestrus[2,9,12].

MATERIALS AND METHODS

Animals: Eighty to 90 days old female rats of Sprague-Dawley strain reared in this laboratory in a controlled condition (room temperature at 23±1°C; light on 6:00 AM-6:00 PM) were utilized for the present investigation. The rats were given chicken feed (Taiwan Sugar Corp Taipei) and tap water *ad lib.* Seventy-six rats selected for this study all demonstrated a regular 4-day cycle for at least 2 cycles. Thirty-eight rats were subjected for surgical thyroid-parathyroidectomy (Tx), and 30 μC of ^{131}I per rat (Tsing-Hua University, Hsing Chu, Taiwan) was administrated intraperitoneally 30 days later. Calcium gluconate (0.5%) was supplied in drinking water to rats after surgical Tx for one week.

Organ collection: Estrous cycle inspection by vaginal smear in both control and Tx rats was conducted 2 months after the surgery. On the proestrous day, the rats were sacrificed by exsanguination through dorsal aorta under light ether anesthesia at the time of 9:00 to 10:00 AM.

The pituitaries were removed within 3 min. after anesthesia. The adenohypophyses were

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dehydrated in the precooled acetone (−20°C), which was refreshed twice within 2 days. The adenohypophyses were dried in a desiccator overnight, weighed, and homogenized in 0.9% normal saline before assay. Uteri and ovaries were dissected carefully and weighed. Ten paris of ovaries selected at random were fixed in Bouin's solution and stained by hemotoxylin and eosin for corpus luteum counts after serial sections with a thickness of 7 μ.

Bioassay:

FSH: FSH potency was determined by the method of rat ovarian weight augmentation of Steelman and Pohley[13]. Twenty to 22 days old Sprague-Dawley female rats were used for assay. Standard reference (NIH-FSH-S_1 with 0.1 mg and 0.2 mg total dosage) and test materials (with 2 and 4 adenohypophyses equivalents) were each divided into 6 injections, twice a day for three days to an assay animal subcutaneously. Human Chorionic Gonadotrophin (HCG, Pregnyl, Organon) 20 I. U. per rat served for augmentation. Five replicates were done for each dose level. Relative potency and 95% confident limit were calculated[1].

LH: Ovarian ascorbic acid depletion method of Parlow[10] was employed for LH measurement. Twenty-five to 27 days old immature female rats of Sprague-Dawley strain reared in this laboratory were used. The assay animal preparation has been described previously[8,15] and 50 I. U. of pregnant mare's serum (PMS, Gestyl. Organon) and 50 I. U. of HCG were used. The standard reference material (NIH-LH-B_7, with the dose level of 0.4 μg and 1.6 μg) and adenohypophyses (1/16 and 1/4 equivalent) were administrated to assay animal through the tail vein. Ovarian ascorbic acid concentrations were obtained through optical density reading from the supernatent of 2.5% metaphosphoric acid treated ovarian tissue. 2, 6-dichlorobenzene-1-indophenol served as the indicator.

RESULTS

The observation of estrous cycle of Tx rats demonstrated a prolongated diestrus period. Even the vaginal inspection was started 7 weeks after Tx, the range was still within the interval of 5–10 days. This results were in agreement with the findings of this laboratory reported previously[15]. No complete statistical result was presented for this investigation.

Adenohypophyseal FSH concentration of Tx rats did not show significant difference from that of controls, but LH concentration indicated a definite decrease after the operation (Table 1).

TABLE 1

Effect of Thyroidectomy (Tx) on Gonadotrophin Concentrations of Adenohypophyses in Female Rats.[a]

	Control	Tx	C/Tx
FSH[b] (μg/mg)	25.82[d] (22.46–29.65)	27.67 (20.37–35.89)	1:1
LH[c] (μg/mg)	19.04 (10.69–33.92)	7.57 (5.09–11.25)	1:0.35

a. Measurements were done on the pooled homogenate of 38 control (C) and 36 Tx rats adenohypophyses obtained at the time of 9:00 to 10:00 AM at the proestrus.
b. Expressed as NIH-FSH-S_1 by ovarian weight augmentation method of Steelman and Pohley (1953).
c. Expressed as NIH-LH-B_7 by ovarian ascorbic acid depletion method of Parlow (1958).
d. The relative potence 95% confident limit, the significance was tested by t-test between C and Tx groups. LH: $P<0.05$; FSH: $P>0.05$. λ: all less than 0.18.

The total body weight and the weight of ovaries and uteri demonstrated a similiar declination after thyroidectomy, but not the pituitary dry weight. Estimation of the number of corpora lutea in pairs of ovaries also indicated a decrease after Tx (Table 2).

DISCUSSIONS

Adenohypophyseal LH concentration of Tx female rats at the proestrus was approximatley

TABLE 2
Body and organ weights of female rats after thyroidectomy

	Control	Thyroidectomized
Body weight (g)[a]	212.5±2.3[b]	178.7±2.7
Adenohypophyseal dry weight (mg)	1.58±0.05	1.56±0.05
Uterine weight (mg)	473.35±10.98	289.53±13.19**
Ovarian weight (mg) (pair)	62.62±1.25	36.36±0.87**
Number of corpora lutea per pair of ovaries	23.5±5.61	8.6±3.34**

a. No. of observations: control: 38, thyroidectomized: 36.
b. Mean±S. E.
** $P<0.01$ as compared with control.

1/3 of that of the control group, while no significant change was found in FSH concentration post-Tx (Table 1).

Investigations on the initiation of follicular growth in hypophysectomized rats stimulated by the pituitary tissue from Tx donors showed a declination of FSH in pituitaries of Tx rats[4,5]. The discrepancy might stem from the time for collecting pituitaries during the estrous cycle and the time interval after operation. It is known that both FSH and LH in the pituitary reach the highest level in the morning of the proestrus[2,9,12]. Thus, in the present investigation, the rats were all killed at 9:00 to 10:00 AM on that day. In this case, the possible hormonal variation caused by the estrous cycle *per se* may be avoided.

An experiment designed to observe the influence of the time lapse after Tx on gonadotrophin contents in adenohypophysis demonstratted a FSH concentration recovery in the pituitary as the postoperative time elongated until 4 weeks postoperation (Wan, in preparation). Owing to the elongated estrous cycle, the time for emergence of the proestrus was irregular. Therefore, it is impossible for the present study to have the rats sacrificed exactly at the same day after Tx. A duration of about 1 week for sacrificing all the proestrous rats post-Tx was needed. Since no significant defference in pituitary FSH concentration between normal control rats and 4 or 8 week post-Tx rats observed in the abovementioned experiment, it is believed that one week interval for sacrificing all the proestrous Tx rats might not introduce any sufficient variations.

The decrease of LH concentration is in agreement with our previous reports in males and proestrous females after Tx[8,14,15]. Furthermore, on account of the observations of the prolonged estrous cycle, decreased uterine and ovarian weights and decreased number of corpora lutea after Tx, it is presumed that the circulating LH also declined. Histological observation of corpora lutea leads to the presumption that the accumulation of LH in the pituitary may be in progress after Tx, and the expected appearance of estrus indicated an ovulation. In addition, variation in FSH concentration in the pituitary after Tx is non-significant. Therefore, it seems not totally true to presume that the altered reproductive functions after Tx are only caused by lowering of total metabolism due to thyroid hormone deficiency.

(Note added in proof: A report by Peppler, Hess and Dunn (1975)[17] indicated that the number of ova shed per ovary is significantly decreased after Tx. This is in agreement with our finding in corpora lutea counts of Tx female rats.)

It was proposed that the priority of TSH secretion after Tx might be the reason for the lowering LH level in the pituitary[11]. It is

possible that the alpha subunit of LH may be shifted to the manufacturing of TSH in case of thyroid hormone deficiency. However, since alpha subunits in FSH and LH are similar, both subunits should be shifted based on this explanation. Our results of FSH concentration after Tx seems not support this proposal of TSH priority.

After introducing PMS to Tx rats, Hagino[7] observed that the thyroxine treatment will restore the time for initiation of ovulation and the number of ova. He suggested that the regulation of initiation of ovulation and the number of ova depends upon the adequent amount of thyroxine present in CNS. The destruction of arcuate nucleus and medial eminence prevented the hypertrophy of uterus as induced by thyroxine administration[6]. In another way, the restoration of irregular estrous cycle to normal in Tx rats by intravenous injection of LRH (Wan *et al.* in preparation) seems to suggest a declination of LRH after Tx. In addition, it was indicated that the release of FSH and LH in response to LRH administration follow a completely different pattern[16]. Accordingly, the different response of pituitary FSH and LH after Tx obtained in our results is further supported.

It is rather difficult at present to put forward any explanation for all the phenomena. Nevertheless, the changes in total metabolic rate in hypothyroidism seems not influence the pituitary FSH concentration but, at present, the possibility still can not be totally excluded.

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甲狀腺切除對雌鼠腦下腺前葉激性腺素之影響

鍾 美 雲　　萬 家 茂

甲狀腺切除後至少兩個月，將雌鼠之腦下腺前葉在動情前期早上 9:00 至 10:00 間取出。以維生素丙消竭法測其 LH 含量；以卵巢重量增進法測其 FSH 含量。結果顯示 LH 明確下降，但 FSH 則與正常者相同。其可能之原因在正文中曾加討論。