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BIOLOGICAL ACTIVITY OF LUTEINIZING HORMONE IN ADENOHYPOPHYSES OF OVARIECTOMIZED-THYROIDECTOMIZED RATS

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P. Shyi-Gang Wang and W. Chia-Mo Wan (1978) Biological activity of Luteinizing hormone in Adenohypophyses of Ovariectomized-thyroidectomized rats. *Bull. Inst. Zool, Academia Sinica* 17(2): 81-84. Biological activity of LH in adenohypophyses (Ad) of ovariectomized (Ovax) rats following thyroidectomy (Tx) were determined by ovarian ascorbic acid depletion method (OAAD). Adult female rats 35 days following Ovax were Tx. Twenty-one or 63 days after Tx, Ad were removed for LH determination. There is no significant difference between Ovax-Tx and Ovax groups, both groups showed significant higher in Ad LH as compared with intact controls. A lack of thyroid hormones seems have no influence on Ad LH content in Ovax rat, thus, it is possible that the declination of Ad LH found in Tx rats could not be mainly due to thyroid hormone deficiency. The hypothesis that the manfacture of TSH at the expense of LH in Tx rats seems not possible.

Radioimmunoassayable hypothalamic gonadotrophin releasing hormone (GnRH), adenohypophyseal (Ad) and serum LH and FSH have been reported to be varied following thyroidectomy (Tx) or induced hypothyroidism[2,5,6,7,10~14]. However, there are controversies on serum LH and FSH level of rats after Tx[2,6,11]. It was suggested that the effect of gonadal steroid feedback and tissue response in hypothyroidism make the difference[2]. In addition, it was proposed that the synthesis of TSH is at the expense of LH in Tx rats[9]

It remains to be seen what effect of thyroid hormone on the biological activity of Ad LH in the absence of ovarian hormone. That may also manifest the direct effect of thyroid hormone on Ad LH activity. In the present studies, the biological activity of Ad LH of ovariectomized (Ovax) rats superimposed by Tx were determined.

MATERIALS AND METHODS

Female adult rats of Sprague-Dawley strain, at age of 80-90 days, reared in this laboratory in regulated environment (room temp. 23±1°C, light on 6 AM-6 PM) were subjected for the present investigations. The rats were given chicken feed (Taiwan Sugar Corp., Taipei) and tap water *ad lib.* Sixty rats, after 2 regular estrous cycle were randomly subdivided into the following groups: 1). Normal intact control (C), the rats were killed at the day of proestrus in the afternoon between 1 PM-3 PM. 2). Overiectomized group (Ovax), the rats were sacrificed 35 days following operation. 3). Ovariectomized-thyroidectomized group (Ovax-Tx). Thyroparathyroidectomy were performed 35 days following Ovax, 1% of $CaCl_3$ were then given in drinking water to these rats for 3-4 days. Thirty μC of ^{131}I were injected within 1 week after

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Tx, and the rats were sacrificed 21 or 63 days after Tx, which designated as ovax-Tx (21) or ovax-Tx (63). 4). Ovax-Tx with thyroxine replacement (ovax-Tx-T_4) group. L-thyroxine (T_4) (2.2 μg/100 g b. w./day) L-thyroxine were injected subcutaneously, up to 21 or 63 days following Tx. This groups were designated ovax-Tx-T_4 (21) or ovax-Tx-T_4 (63).

Under light ether anesthesia the rats were killed by exsanguination. Ad were collected with 3 min following, and dehydrated in precooled acetone and stored in −20°C. The acetone were refreshed three times in 48 hours. Acetone dried Ad from same group were homogenized in physiological saline, and the supernatant were obtained by centrifuging at 4°C for 20 min in 2500 RPM (1000×g) and lyophilized for LH determiniation.

Biological activity of LH in Ad were measured by ovarian ascorbic acid depletion method (OAAD), as introduced by Parlow[8], with modification as mentioned previously[5]. PMS (50IU, Gestyl Organon) and HCG (35IU, Pregnyl, Organon) were used to obtain fully luteinized ovaries in 23-days-old immature rats. Bovine LH (NIH-LH-B7) were used as standard preparation. The dose response curve were of 4-point design[1].

RESULTS

As in Table 1, with Ovax-Tx, the Ad wet weight increased slightly, but only in Ovax-Tx (63) group showed a significant increase. Uterine weight decreased significantly. Without T_4 replacement, in both groups of rats, 21 or 63 days following Tx, the adrenal weight of Ovax rat decreased significantly as compared with normal or with T_4 replacement.

The LH potency in experimental groups, all demonstrated significant increase, however, there is a tendency of, but not significant, lowering of LH activity in Ads of Ovax-Tx (Table 2).

TABLE 1

Organ weight of normal control, ovariectomized and ovariectomized-thyroidectomized rats

Groups	No. of rats	B. W. (g)	Adenohypophyseal wet weight (mg)	Uterus weight (mg)	Adrenals weight (mg)
Normal control	7	208	9.79±1.83	572	49.04
Ovax	7	228	8.60±1.70	86.83*	46.34
Ovax+Tx (21)	9	229	10.41±1.68	75.48*	35.51*
Ovax+Tx+T_4 (21)	10	224	10.13±2.60	83.46*	47.47
Ovax+Tx (63)	8	202	14.31±2.55*	78.21*	32.96*
Ovax+Tx+T_4 (63)	10	272	12.38±1.74	80.29*	48.20

* Indicates the significant level at $p<0.05$ as compared with normal control group.
Ovax: ovariectomy; Tx: thyroidectomy; T_4: thyroxine replacement; number in parenthesis indicated the days between Tx and sacrifice of the rats.

TABLE 2

Biological activity of adenohypophyseal LH normal control, ovariectomized and ovariectomized-thyroidectomized rats

Groups	Range of Relative potency* (μg/mg)	Lambda	Weighted mean potency (μg/mg)	0.5% Confidence Limit
Normal Control	1.26- 2.32	0.26**	1.46	1.09- 1.95
Ovax	4.65-11.63	0.34	5.53	4.09- 7.47
Ovax+Tx (21)	4.19	0.15	4.19	1.91- 4.19
Ovax+Tx+T_4 (21)	5.56- 5.94	0.29	5.73	3.09-10.68
Ovax+Tx (63)	3.81	0.29	3.81	1.59- 9.14
Ovax+Tx+T_4 (63)	4.08- 6.15	0.35	5.07	3.07- 8.37

* Ten rats pituitaries for each pool and the range obtained for at least 3 repeat measurements.
** Maxium Value.

DISCUSSION

Rats subjected by Ovax, Ovax-Tx and Ovax-Tx with T_4 supplement demonstrated at least 2-fold increase in biological activity in Ad LH as compare with normal intact groups. There are no significant difference among all treated groups.

By bioassay and radioimmunoassay the declination of Ad LH following Tx were demonstrated repeatedly[4,7,10~12]. With no thyroxine replacement, the compensatory increased Ad LH of Ovax rats showed no declination following Tx (Table 2). These indicated that the lack of thyroid hormone can not lowering the uprose LH concentration in Ad. Thus, the decreases in the synthetic activity of LH if it happened in pituitary following Tx may not be due to the decrease of metabolic rate owing to the lack of thyroid hormone.

In measuring hypothalamic GnRH concentration in Tx rats, a significant decline was observed[11,12], same as that of castrated rats[3]. In addition, it was observed that the pituitary responsiveness to exogenous GnRH is changed in rats in related to the thyroid status[15]. It is very possible, the increased gonadotrophin release in Tx rats[12] is due mainly to the increased stimulation of GnRH same as castrated rats. It may also be the reason for Ovax rats demonstrated no further increase in Ad LH following Tx. However, the differences in mechanism of GnRH between Tx and castrated rats remains unknown.

It was proposed that the compensatory increasing in TSH after Tx may utilizing the finished subunits for LH or/and FSH production, thus the activity of LH and FSH will apparently decreased following Tx. The present results seemingly disagree with such suggestion. For compensatory increased LH following Ovax showed no significant decrease superimposed with with Tx either for 21 or 63 days.

Apparently, the GnRH decrease in hypothalamus, may in turn increase the release of LH, is responsible for LH content lowering in pituitary, Presumedly, the compensatory increased secretion of TSH and LH are of different control mechamism, no speculation can be given based on the present results.

The lowering in uterine weight is apparently showing the lowering in ovarian hormone production, it was indicated earlier that ovary weight and number corpus luteum is decreased after Tx[4]. However, the Ad weight seems not really indicating the Ad function, and the changes of adrenal weight remain to be evaluated, nevertheleas, it could be functionally substitute the ovarian steroid production.

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去除甲狀腺及卵巢對腦下腺黃體生成素之影響

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本實驗之目的在研究腦下腺激素間之相互關係，是否有優先分泌之可能性，並藉以尋求甲狀腺刺激素與黃體生成素，在腦下腺中是否係獨立分泌之佐證。以 Parlow (1958) 所介紹之「卵巢維生素C消竭法」測定腦下腺中之黃體生成素，所得結果顯示，雌性大白鼠於切除卵巢 35 日後，其腦下腺中黃體生成素之濃度增爲正常雌鼠之三倍有餘。繼卵巢切除 35 日後，再切除甲狀腺，則兩個月後，其腦下腺中黃體生成素之濃度，雖有略爲減低之趨勢，但仍爲正常雌鼠之二倍餘，而與去卵巢組無顯著之差異。顯然，甲狀腺之切除，對於因切除卵巢而使腦下腺中黃體生成素濃度增高之現象，並無顯著之效果。同時亦顯示甲狀腺素之缺如，對於腦下腺中黃體生成素濃度之改變不及雌性素之缺如，所造成之影響爲大。對於甲狀腺功能過低而導致腦下腺中黃體生成素濃度之降低，似藉其對下視丘之影響所造成。