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SERUM GONADOTROPIN CHANGES IN THYROIDECTOMIZED RATS FOLLOWING ORCHIDECTOMY OR LRH CHALLENGE[1]

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Ching-Fong Liao and W. Chia-Mo Wan (1983) Serum gonadotropin changes in thyroidectomized rats following orchidectomy or LRH challenge. *Bull. Inst. Zool., Academia Sinica* **22**(1): 49-56. Serum gonadotropin (GTH) levels in response to orchidectomy or luteinizing hormone-releasing hormone (LRH) challenge were examined in hypothyroid rats. Young adult male Long-Evans rats were thyro-parathyroidectomized (Tx) or sham Tx at 60 days of age, and the testes were then removed 4 weeks later. Blood samples were collected by heart puncture weekly, started at the time of thyro-parathyroidectomy for 8 weeks. Serum TSH, LH and FSH were measured by specific radioimmunoassays. One week after thyro-parathyroidectomy, the serum level of TSH was found 6 times that of control and remained in *a* plateau thereafter. Orchidectomy had no effect on serum TSH in Tx rats, but lowered that in sham Tx group. Serum levels of GTH were not changed after thyro-parathyroidectomy, but increased significantly in all rats following orchidectomy. Moreover, the Tx rats showed even higher GTH levels than those of sham group. A median dose of LRH (30 ng/100 g B.W., i.p.) gave a significant increment of serum LH in Tx rats 15 minutes after injection, but not in sham Tx rats. As compared with sham Tx rats, a high dose of LRH (90 ng/100 g B.W.) induced a much higher elevation of GTH in Tx rats. From these studies, we conclude that the response of pituitary GTH release following orchidectomy or stimulation of LRH is enhanced in hypothyroid rats.

It has been reported that hypothyroidism results in reproductive abnormality in many species. In female rats, thyro-parathyroidectomy causes prolonged estrous cycle with irregular duration of diestrus (Contopoulos and Koneff, 1963; Wan and Hwang, 1973), reduces the number of ova (Hagino, 1971) and corpora lutea (Choong and Wan, 1975) and decreases peak of LH surge (Freeman *et al.*, 1976). There is no apparent indicator, such as estrous cycle in female, to help to reveal the malfunction in reproduction of male rats caused by thyroidectomy. However, some data do indicate that hypothyroidism affects the reproductive hormones. Pituitary content of gonadotropins (GTH) decreases in thyro-parathyroidectomized (Tx) male rats, as determined either by bioassay (Contopoulos *et al.*, 1958; Wan and Chen, 1974) or radioimmunoassay (RIA) (Aranda *et al.*, 1976; Suzuki *et al.*, 1978). Upon castration, the Tx or propylthiouracil (PTU) treated animals show more elevation of serum LH and FSH than euthyroid ones (Bruni *et al.*, 1975; Kalland *et al.*, 1978).

In regard to basal serum GTH in hypothyroid animals, the findings have been conflicting: both LH and FSH decreased (Bruni *et*

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al., 1975); LH decreased but not FSH (Baksi, 1973); or unchanged (Kalland *et al.*, 1978; Jea *et al.*, 1981). On LRH challenge experiments, Kalland *et al.* (1976) found higher responsiveness in PTU or I-131 treated rats whereas Jea *et al.* (1981) found no difference between Tx and control rats. The inconsistency may be due to different duration of hypothyroidism and different doses of LRH. Thus, in our investigation, weekly changes of serum GTH before and after orchidectomy in Tx rats have been examined and three dose levels of LRH have been used to study the pituitary responsiveness in hypothyroid rats.

MATERIALS AND METHODS

Animals

Male Long-Evans rats, purchased from National Laboratory Animal Resources, National Taiwan University, Taipei, were maintained in a temperature-controlled room (22±1°C) with 12-hour lighting schedule (6:00 AM–6:00 PM). The rats were fed with purina chow and tap water *ad libitum*, and their body weights were recorded throughout the studies.

At 60 days old, 200–230 gm of body weight, the rats were Tx or sham Tx. Four weeks later (Week 4), rats were either castrated or subjected to LRH injection. Blood samples were collected weekly by heart puncture, immediately before the thyroid operation (Week 0) and thereafter for 8 weeks. Sera were obtained and stored at −20°C until hormone assay. All the operations and blood samplings were performed under light ether anesthesia.

LRH challenge

LRH was administered intraperitoneally (i.p.), in single injection to Tx and sham Tx rats with 3 dose levels of 10, 30 and 90 ng/0.1 ml/100 g B.W. Saline which served as a vehicle was injected in the same manner in control groups. There were 5 or 6 rats for each dose. Blood samples were collected at 0, 15, 30 and 90 minutes after the administration of LRH or saline.

I-131 uptake

Radioiodine uptake was done at the end of experiments. The procedure has been described previously (Wan *et al.*, 1981). Briefly, each rat was injected with I-131 (0.2 μCi/0.1 ml, i.p.). Twenty-four hours later, the rats were sacrificed by abdominal aorta exsanguination. Serum, thigh muscle, trachea and/or thyroid gland were removed and weighed. Their radioactivities were counted in a gamma scintillation spectrometer (Gamma 8000, Beckman Instruments, Inc.). The percent tissue uptake was calculated as follows.

$$\%\text{Uptake}=\frac{\text{cpm/g tissue}-\text{cpm/g muscle}}{\text{cpm}_{\text{total injected}}-\text{cpm}_{\text{background}}}\times 100$$

Rats with thyroidal I-131 uptake of more than 1% (*i.e.* incomplete thyroidectomy) were eliminated from the Tx group.

Hormone assays

Serum TSH, LH and FSH were measured by specific double-antibody RIA with kits from NIAMDD (The National Institute of Arthritis, Metabolism and Digestive Diseases, USA). Its instructions for hormone assay were followed except that the volume of each component was reduced to 1/10. All incubations were carried out in duplicate and in 4×50 mm pyrex tubes; and solutions were added with Hamilton syringe and dispenser (Follett *et al.*, 1972). The results were expressed as TSH-RP-1, LH-RP-1 and FSH-RP-1. Data calculation and quality control for assays were according to the description of Rodbard *et al.* (1970). Specificity of antihormone antisera were tested by incubating the antisera with I-125-labeled TSH, LH and FSH respectively, and the cross-reactivity was found to be less than 1%. The coefficients of variance of within assays and between assays were 8.4%, 7.5% for TSH; 12.8%, 8.4% for LH and 10.7%, 7.9% for FSH respectively.

Statistics

All the data that deviated from the means of groups by two standard deviation were regarded as abnormal and rejected (Wang *et al.*, 1975). Consequent statistical analyses were processed according to the procedures of Sokal and Rohlf (1969).

RESULTS

The weekly body weight change is shown in Fig. 1. Sham Tx group continued to have body weight gain, while that of Tx rats ceased 1 week after operation. Highly significant difference between Tx and sham Tx rats was noted from week 2 and thereafter.

Serum TSH elevated sixfold in one week after thyroidectomy and then remained in a plateau. Orchidectomy did not result in any further TSH change. In sham Tx rats, the serum TSH remained unchanged for four weeks but decreased significantly following castration (Fig. 2).

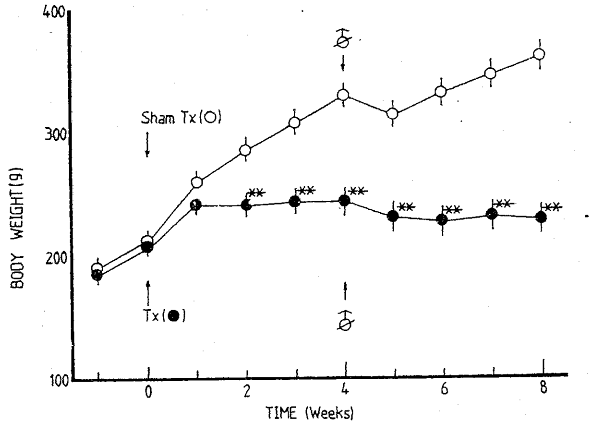

Fig. 1. Body weight changes in Tx and sham Tx rats. Thyroid operations were performed at week 0, and orchidectomy (♂) at week 4. Closed and open circle correspond to Tx and sham Tx respectively. Each point represents the mean of 8 rats. The vertical bar represents the SEM.
** $p<0.01$ (Tx compared to sham Tx)

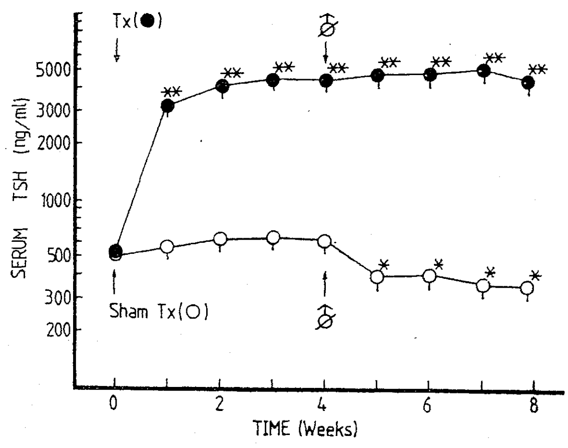

Fig. 2. Serum TSH levels in response to Tx and orchidectomy.
* $p<0.05$ (week 5, 6, 7 or 8 compared to week 4 in sham Tx group)
** $p<0.01$ (Tx compared to sham Tx)
See legend to Fig. 1 for details.

TABLE 1

Completed Anova with regression on the serum LH (A) and FSH (B) profiles following orchidectomy in Tx rats (from week 4 to week 8)

A.

Source of variation	df	SS	MS	F
Among weeks	4	1428332	357083	37.715**
Linear regression	1	1362681	1362681	62.268**
Deviations from regression	3	65651	21884	2.311 n. s.
Within weeks	35	331374	9468	

Regression Line $Y=-445.625+130.513X$; regression coefficient=130.513 ** $p<0.01$

B.

Source of variation	df	SS	MS	F
Among weeks	4	20107905	5026976	44.024**
Linear regression	1	18085167	18085167	26.822*
Deviations from regression	3	2022738	674246	5.905**
Within weeks	35	3996511	114186	

* $p<0.05$ ** $p<0.01$

TABLE 2
Completed Anova with regression on the serum LH (A) and FSH (B) profiles following orchidectomy in sham Tx rats (from week 4 to week 8)

A.

Source of variation	df	SS	MS	F
Among weeks	4	571811	142953	15.424**
Linear regression	1	561460	561460	162.720**
Deviations from regression	3	10351	3450	0.372 n. s.
Within weeks	35	324389	9268	

Regression Line $Y=-281.571+83.775X$; regression coefficient=83.775 ** $p<0.01$

B.

Source of variation	df	SS	MS	F
Among weeks	4	7718694	1929674	47.957**
Linear regression	1	7218011	7218011	179.386**
Deviations from regression	3	500683	166894	4.148*
Within weeks	35	1408304	40237	

* $p<0.05$ ** $p<0.01$

In both Tx and sham Tx groups, basal level of serum LH and FSH did not change for 4 weeks after thyroid operations. In response to castration, serum GTH elevated and the Tx group had significantly more increment than that of sham Tx rats (Figs. 3 and 4).

The changes of serum GTH following castration were analyzed by linear regression. We found that the increase of LH could fit into a linear equation (Table 1A and 2A) and the regression coefficient of the serum LH of Tx rats was greater than that of sham Tx group (Table 3). Instead, the increase of FSH could not be simply expressed by linearity (Table 1B and 2B).

The result of single dose LRH challenge is depicted in Fig. 5. With low dose of LRH (10 ng/100 g B.W.), slight increase ($p<0.1$) of serum LH was noted in Tx rats but no change was detected in sham Tx group. With median

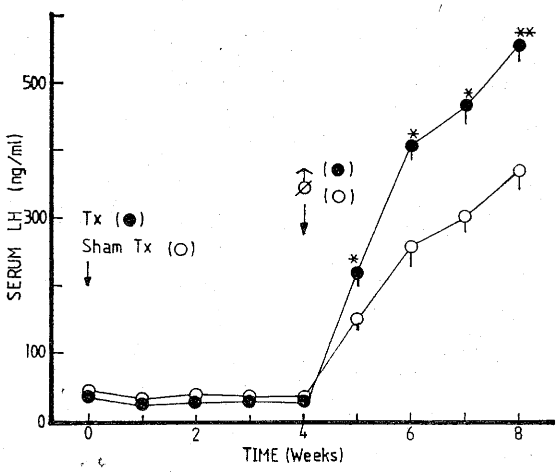

Fig. 3. Effect of Tx and orchidectomy on serum LH levels.
* $p<0.05$, ** $p<0.01$ (Tx compared to sham Tx)
See legend to Fig. 1 for details

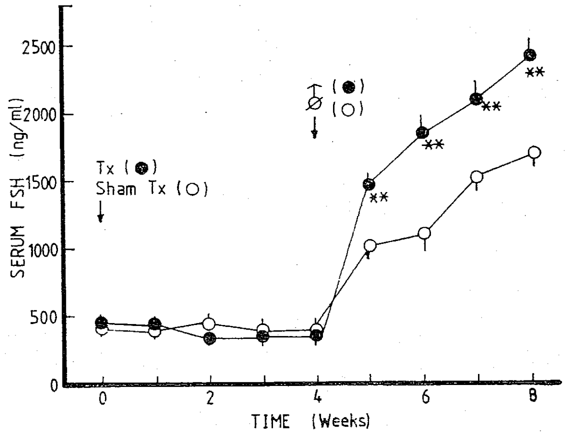

Fig. 4. Effect of Tx and orchidectomy on serum FSH levels.
** $p<0.01$ (Tx compared to sham Tx)
See legend to Fig. 1 for details

TABLE 3
F-test for difference between two regression coefficients (b) obtained from table 1A and 2A ($b_1=130.513$; $b_2=83.775$)

Source of Variation	df	SS	MS	F
Between regressions	1	87375.757	87375.757	6.898*
Within regressions	6	76001.988	12667.0	

* $p<0.05$

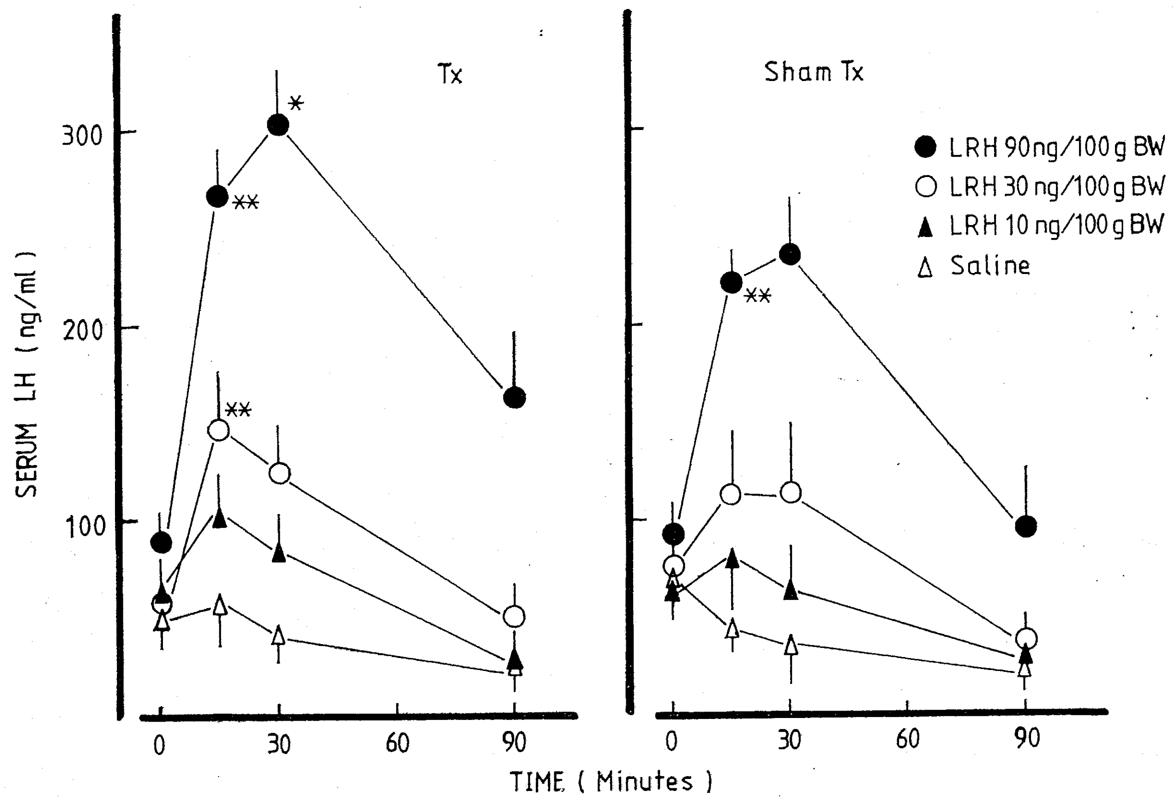

Fig. 5. Serum LH levels (mean±SEM, $n=5$ or 6) in response to LRH or saline administration. Closed circle, open circle, closed triangle and open triangle represent high, median or low dose of LRH and saline respectively.
* $p<0.05$ (Tx compared to sham Tx)
** $p<0.01$ (15 minutes post-LRH administration compared to zero time)

dose (30 ng/100 g B. W.), LH elevated significantly in Tx group but only slightly in sham Tx rats ($p<0.2$). With high dose of LRH (90 ng/100 g B. W.), both groups showed significant increase of serum LH and more increment was noted in Tx group. Saline vehicle did not increase serum LH level, however, repeated blood samplings and anesthesia probably led to the gradual decline of serum LH.

DISCUSSION

Extreme hypothyroidism profoundly affects the growth of rats, however, very small doses of thyroid hormones are sufficient to avoid the retardation of body weight gain (Evans *et al.*, 1960; Stasilli *et al.*, 1961). In this study, we have rats with incomplete thyroidectomy (as identified by I-131 uptake) which showed significant elevation of TSH but the pattern of body weight gain was similar to that of sham Tx controls. It seems that the cessation of body growth is better than serum TSH as an indicator for complete removal of thyroid gland.

Basal TSH does not change after sham Tx, however, castration resulted in significant decrease of TSH in these sham Tx rats. The finding confirms the report of Christianson *et al.* (1981), who demonstrated that absence of testosterone resulted in TSH decrease.

From the results of serum GTH levels, we notice no change of either LH or FSH for 4 weeks after Tx. This finding is consistent with those of Kalland *et al.* (1978) and Suzuki *et al.* (1978), but different from what were reported by Bruni *et al.* (1975) and Baksi (1973). The differences in age and mode of inducing hypothyroidism, duration and degree of hypothyroidism, strain of rats and anesthesia technique may be reasons for these conflicting results.

Upon castration, the Tx rats showed higher increase of serum GTH (Figs. 3 and 4) which is in agreement with all known studies either in male (Bruni *et al.*, 1975; Kalland *et al.*, 1978; Wan *et al.*, 1981) or female rats (LaRochelle *et al.*, 1974; Bruni *et al.*, 1975; Wan *et al.*, 1981) in hypothyroid condition. By linear regression, we further noted the increase of LH and FSH are of different patterns (Table 1 and 2). Bruni *et al.* (1975) used different approach in which rats were castrated first and then Tx 31 days later, they found thyro-parathyroidectomy superimposed upon castration increased both LH and FSH above the castrated control levels. Therefore, in the present study, gonadal steroid hormones changes, if any, in Tx rat is not considered as an important factor for higher serum GTH increase. Other possible explanations include the increased release of hypothalamic LRH; increased pituitary responsiveness to LRH or decreased degradation of LH and FSH. Freeman and colleagues showed the clearance rate of gonadotropins did not change in Tx-ovariectomized (LaRochelle *et al.*, 1974) or Tx female rats (Freeman *et al.*, 1975). On the other hand, pituitary gland from ovariectomized-Tx rats was reported to have greater biosynthesis (Wang *et al.*, 1980) and release (Wang *et al.*, 1982) of LH in response to LRH than ovariectomized-sham Tx rats. Kalland *et al.* (1978) used 100 ng/100 g B. W. of LRH and induced higher peaks of LH and FSH in PTU or I-131 treated rats. Therefore, we examined the pituitary responsiveness to LRH and found striking differences between Tx and sham Tx rats (Fig. 5). Moreover, we discovered a lower dose of LRH (30 ng/100 g B. W.) which significantly elevated LH in Tx rats but not in sham Tx rats. These data strongly suggest that hypothyroidism increases the pituitary responsiveness. However, these results do not rule out the possible regulatory alteration in hypothalamus or higher center following Tx as proposed by other researchers and our laboratory. (Freeman *et al.*, 1976; Kizer *et al.*, 1978; Ito *et al.*, 1977; Liu and Wan, 1982).

LH, FSH and TSH are all glycoproteins, each consisting of α and β chains. Although their α chains are essentially identical in the same species (Pierce and Parsons, 1981), it seems unlikely that higher level of any hormone measured in the present study is due to cross reaction with other hormones. The antisera used in this study is highly specific (see Materials and Methods); furthermore, we would expect parallel change of these three hormones if there were cross reactions. Nevertheless, TSH elevation upon thyroidectomy with no parallel GTH increments was observed. Likewise, with GTH increase after castration, the TSH remained the same in Tx rats or even decreased in sham Tx group (*cf.* Figs. 2, 3 and 4).

It has been demonstrated in rat that free α chain showed extensive cross-reaction with LH in the RIA system and the response was parallel to the RP-1 standard (Kalland *et al.*, 1978). In humans, it was further shown that immunoreactive α chain of LH, FSH and TSH can be released independently of the intact hormones and their release occurs in response to the same releasing hormones, i. e. LRH or TRH. (Hagen and McNeilly, 1975). Therefore, the possibility that greater amount of free α

chain was released after castration or LRH challenge in Tx rats might lead to overestimate of serum GTH can not be excluded. A more specific anti-β chain antiserum for GTH RIA or *in vitro* GTH bioassays can help to clarify this problem.

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去甲狀腺鼠睪丸切除或性釋素注射後血中促性素之變化

廖 欽 峯　　萬 家 茂

本實驗研究去甲腺鼠於睪丸切除或注射性釋素後，血中促性素之變化。年輕雄鼠分兩組於 60 日齡作甲腺切除或甲腺假手術，4 週後，再作睪丸切除。自甲腺手術起，每週由心臟採血共歷 8 週。血清中促甲素、黃體生成素及促濾泡素之濃度，以放射免疫法測定。

甲腺切除，血中促甲素於一週內卽升高 6 倍，並且一直持續；睪丸切除不再影響其促甲素濃度，但可降低假手術鼠血中之促甲素。血中黃體生成素之濃度不受甲腺手術影響，但在睪丸切除後則顯著上升，而且其增加在去甲腺鼠更為明顯。腹腔注射中等劑量性釋素（30 ng/100 克體重）15 分鐘後，去甲腺鼠血中黃體生成素顯著升高，而假手術鼠則未見變化。如注射高劑量性釋素（90 ng/100 克體重），則假手術鼠血中黃體生成素亦顯著增加，唯仍較去甲腺鼠為低。由以上結果，我們認為去甲腺鼠於睪丸切除或受性釋素刺激後，腦垂腺分泌促性素之能力增强。