

## Thyroid Hormones in Rats with Hyperluteinized Ovaries

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The findings of research work showed that animals with hyperluteinized ovaries (HL) obtained after mechanical lesions in the posterior hypothalamus, the circulatory patterns of pituitary and ovarian steroid hormones were disturbed. In these animals the pre-ovulatory surge of luteinizing hormone (LH) was lower and prolactin (Prl) was higher, while in early proestrus estradiol was higher, and progesterone in late proestrus was lower when compared to the intact controls. It is recognized that there is a close relationship between ovarian function and thyroid hormones, measured the serum content of total tri-iodothyronine (T<sub>3</sub>) and thyroxine (T<sub>4</sub>) in animals with hyperluteinized ovaries obtained after mechanical lesions in the posterior hypothalamus. There were no significant differences in the circulatory concentrations of these hormones between rats with HL and intact controls. Histological analysis also showed that there were no significant differences in structure of thyroids between the two groups of animals, although the weights of the thyroid glands in the lesioned animals were significantly heavier than in intact controls. These results showed that lesions in the posterior hypothalamus, which result in a disturbance of the hypothalamo-pituitary-ovarian axis inducing HL, did not bring about changes in the hypothalamo-pituitary-thyroid axis.

**Key words:** Thyroid hormones, hyperluteinized ovaries, rats

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## Introduction

The relationship between thyroid glands and ovaries has been of fundamental interest for a long time, both *in vitro* and *in vivo* studies. The thyrotropic activity of a number of glyco-protein hormones, due to the structural similarity among them, has been widely studied in several *in vivo* and *in vitro* systems (Carayon *et al.*, 1980; Pekonen and Weintraub, 1980; Nisula and Ketelslegers, 1974). The results of Carayon *et al.* (1980) indicate that the chorionic gonadotropin molecule has a thyrotropic action on the human thyroid. Leatham, (1958) showed that thyroidectomy of the rat blocks the ripening of ovarian follicles and that these thyroidectomized, thiouracil-treated animals required much larger amounts of gonadotropic hormones to induce the process of luteinization than required in normal animals. A series of data concerning various animal species as well as humans show that treatment with thyroid suppressive pharmacological preparations results in ovarian atrophy, blocking the ripening of follicles (Folley, 1938; Krohn, 1951; Williams, *et al.*, 1944). Ovulatory dysfunction with formation of cysta in primary hyperthyroidism may be due to the number of mechanisms including altered metabolism of estrogens and hypothalamic pituitary dysfunction. Fitko *et al.* (1994) and Fitko and Szlezzyngier, (1994) showed *in vivo* that the thyroid - gonadal interaction is based on the formation of gonadotropic receptors in ovarian tissue. In hyperthyroid animals, the amount of these receptors was reduced. Most authors agreed that the anterior part of the median eminence and tuber cinereum have a specific role in the regulation of TSH in thyroid system. Lesions of these areas depress TSH secretion and releases (Palkovits, 1982). We have shown that the mechanical lesions in the posterior hypothalamus induced the appearance of hyperluteinized ovaries and changes in the secretion pattern of glyco-protein hormones from the anterior pituitary. In these animals preovulatory surge of LH was lower and Prl was higher when compared to the intact controls. (Ivanisevic-Milovanovic *et al.*, 1999). This research work was therefore, conducted to investigate, if the disturbed secretion pattern of the above mentioned hormones of hyperluteinized rats have an effect on the functioning of the thyroid gland in these animals.

## Materials and Methods

Female rats of a Wistar strain were used and The hyperluteinization of ovaries was achieved as described by (Martinovitch *et al.*, 1968). Briefly, animals not older than 12 hours after birth, were subjected to mechanical lesions in the region of the (posterior hypothalamus) with a fine steel knife. The lesions were aimed into the posterior mamillary body covering the antero-posterior planes between  $A_{3.4} - A_{3.8}$  according to the De Groot rat brain stereotaxic atlas (1959). The pups were then returned to their mothers after the operation and kept under standard conditions in the animal room (temperature 22°C and lights on from 7:00 to 19:00 hours). The young animals were weaned at the age of 1 month. At the age of 4 months, the animals were laparatomized to confirmed the presence of hyperluteinized ovaries (HL) and sacrificed in estrus at the age of 7 months by decapitation, when the ovaries show an extreme number of corpora lutea (HL have over 40 corpora lutea per ovary, in contrast to intact controls where in our strain the average number of corpora lutea is 7, but never more than 18 per ovary). Intact female rats of the same age and the estrus phase served as controls. Blood was taken for hormone assays and thyroids for histological analysis.

**Hormone assays and histological analysis:** After obtaining the blood, serum samples were prepared by centrifuging the blood at 3000 rpm for 15 min. The serum was stored at -20°C until

assayed for total  $T_3$  and  $T_4$  hormones using radioimmunoassay kits supplied by the manufacturer, Laboratory for Isotopes of the Institute for Nuclear Sciences "Vinca". All samples were assayed in duplicate in a single lot for each hormone, according to instructions supplied with the kits. The intra-assay variation was less than 10%. Both assay systems employ hormones labeled with  $^{125}I$ . The antibodies were obtained from rabbit serum and bound and unbound fractions after an incubation period of 18 hours were separated with polyethylene glycol. The lowest detectable limit for  $T_3$  and  $T_4$  was 0.3 and 20n mol<sup>-1</sup> respectively. This method of hormone assay was similar to that described in Pierzchala *et al.*, 1983. After the animals were sacrificed and bled, the thyroid glands, ovaries and uteri were removed and cleaned of surrounding tissues and their weights measured. The thyroid glands were fixed in 10% neutral formalin solution and embedded in paraffin. The 5µm thick sections were stained with the hematoxylin and eosin method and viewed under a light microscope (Gosney, 1985).

The values are expressed as the mean ± S.E.M. For statistical analysis the Student "t" test was used and the statistical significance was set at  $p < 0.05$  (Alder and Roessler, 1962).

## Results

Body weights at the time of sacrifice between the animals with hyperluteinized ovaries (244.8 ± 10.4 g) and intact controls (249.2 ± 4.6 g) do not differ significantly. On the other hand, as expected from the accumulation of an extreme number of corpora lutea, the HL (152.7 ± 19.6 mg) were significantly heavier ( $p < 0.01$ ) than ovaries of control rats (59.9 ± 3.3 mg). However, the uteri of rats with HL (320.3 ± 36.2 mg) have significantly lower weights ( $p < 0.05$ ) as compared to uteri of intact controls (396.1 ± 24.7 mg). The thyroid glands were significantly heavier ( $p < 0.05$ ) in animals with HL (16.5 ± 0.9 mg) than in intact controls (14.7 ± 0.4 mg) (Table 1).

In Table 2 the ratios of ovarian and thyroid weights against body weight in rats with HL as compared to the controls was increased 160 and 14% respectively. A reduction of 18% in the ratio of uterine weights against body weight was determined in rats with HL when compared to the intact controls.

Analyzing the serum content of  $T_3$  and  $T_4$  it is evident that there was no significant difference in the circulatory concentration of these hormones between rats with HL ( $T_3$  and  $T_4$  0.61 ± 0.07, 36.99 ± 6.36nmol L<sup>-1</sup> respectively) and intact controls ( $T_3$  0.81 ± 0.06,  $T_4$  37.20 ± 3.32nmol L<sup>-1</sup> respectively) Fig. 1 and 2. Histological analysis of thyroid glands also showed that there was no significant difference in structure of these glands between the rats with HL and intact controls. In both groups of animals the characteristic arrangement of various-sized spherical follicles containing eosinophilic colloid identifying the thyroid gland were observed. In all the animals, the follicles were lined with simple cuboidal epithelium.

## Discussion

Previously described that mechanical lesions placed in the posterior hypothalamus, more precisely, in the region of the posterior mamillary body of newborn rats induce the appearance of HL which are responsible for numerous physiological changes in the exchange of information between the hypothalamus and pituitary gland, leading to a reduced secretion of the adrenocorticotrophic hormone (ACTH) into the circulation (Ivanisevic-Milovanovic *et al.*, 1992 a) and elevated pre-ovulatory concentrations of prolactin (PRL), a reduction of pre-ovulatory release of the luteinizing hormone (LH), but with no effect on circulatory concentrations of the follicular stimulating hormone (FSH) (Ivanisevic-Milovanovic

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Table 1: The Mean Values of Body Weights (g) and Weights of Endocrine Organs (gm) in Intact Controls<sup>®</sup> and Hyperluteinized (HL) Animals (Scarified at 7 Months of Age)

	C	HL	P
Body weights	249.2 ± 4.6 (210 - 346)* (38)**	244.8 ± 10.4 (162 - 380) (25)	> 0.05
Ovaries	59.9 ± 3.3 (30.0 - 105.5) (33)	152.7 ± 19.6 (55.9 - 437.0) (25)	< 0.01
Uterus	396.1 ± 24.7 (250.0 - 588.0) (16)	320.3 ± 36.2 (167.2 - 679.1) (16)	< 0.05
Thyroids	14.7 ± 0.4 (9.3 - 19.5) (38)	16.5 ± 0.9 (10.0 - 25.1) (19)	< 0.05

\* Range      \*\* Number of animals      P Level of significance

Table 2: Ovarian, Uterine and Thyroid Weights Relative to Body Weight<sup>1</sup>

	Ovarian/Body Weight	Uterine/Body Weight	Thyroid/Body Weight
Intact controls	0.024	0.159	0.006
Hyperluteinized rats	0.062	0.131	0.007
Changes <sup>2</sup>	+160	-18	+14

<sup>1</sup>Values are in %; <sup>2</sup> Increase (+) of ovarian and thyroid weights and decrease (-) of uterine weight in %

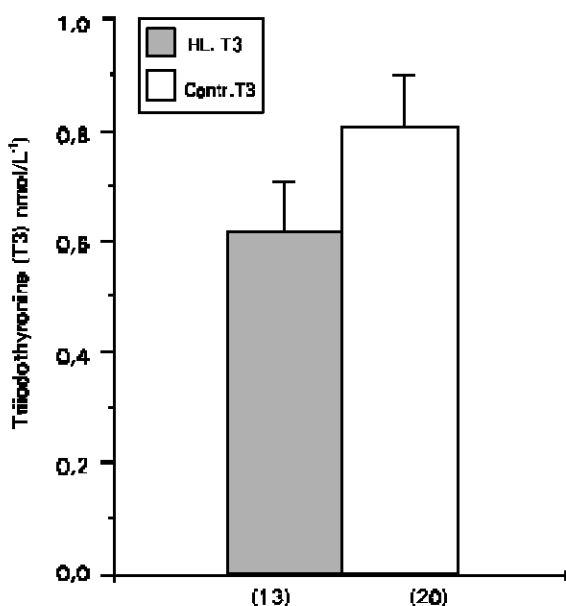


Fig. 1: Serum concentrations of tri-iodothyronine (T<sub>3</sub>) in animals with hyperluteinized ovaries (HL) and intact controls (Contr.). Columns represent the Mean ± S.E.M. ( ) N<sup>o</sup> of animals.

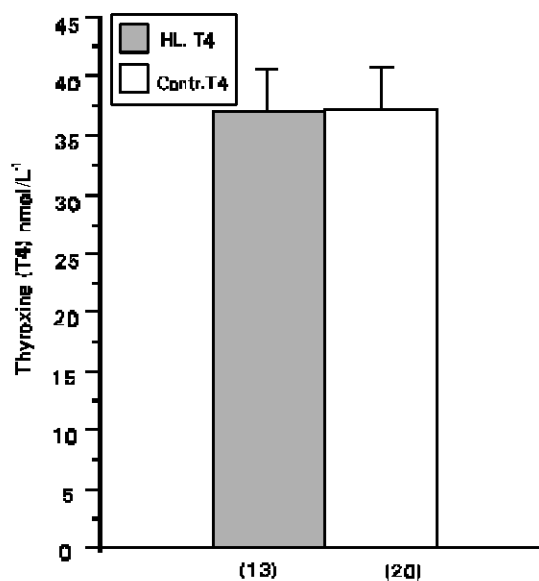


Fig. 2: Serum concentrations of thyroxine (T<sub>4</sub>) in animals with hyperluteinized ovaries (HL) and intact controls (Contr.). Columns represent the Mean ± S.E.M. ( ) N<sup>o</sup> of animals.

and Musicki, 1992. Ivanisevic-Milovanovic *et al.*, 1999.) Analyzing the serum content of T<sub>3</sub> and T<sub>4</sub>, it is evident that there was no significant difference in the circulatory concentration of these hormones between rats with HL and intact controls. It should be noted that the thyroid hormone concentrations in our experimental and control animals were measured in the estrus phase of the estrous cycle, because it has been previously shown that there are different rates of thyroid activity during the estrous cycle, the thyroid of female rats being most active during heat i.e. estrus (Nalbandov, 1975.). From Fig. 2, there is no significant difference in serum concentration of T<sub>4</sub> between the

experimental and control animals, it is however, of interest that the ratio in levels of T<sub>4</sub> against T<sub>3</sub> differ in these two groups of animals. In the animals with HL the level of T<sub>3</sub> is 60.6 times less than that of T<sub>4</sub>, while in the control animals there is 45 times less T<sub>3</sub> in relation to T<sub>4</sub>. This discrepancy in the ratio of T<sub>4</sub> and T<sub>3</sub> in the animals with HL may be either due to a reduced production of T<sub>3</sub> in the thyroid gland, the reduced conversion of T<sub>4</sub> into T<sub>3</sub>, or increased clearance of T<sub>3</sub> in the circulation.

These data confirmed the fact that ratio of T<sub>4</sub> and T<sub>3</sub> change under various physiological conditions (Vilee, 1975), which is the case with our animals with hyperluteinized ovaries where

the circulatory levels of Prl are very high (Ivanisevic-Milovanovic *et al.*, 1999.) Although the weights of thyroid glands from animal with HL are increased as compared to the intact controls, histological analysis of thyroids showed that there was no significant difference in structure of these glands between the rats with HL and intact controls. In both groups of animals the characteristic arrangement of various-sized spherical follicles containing eosinophilic colloid identifying the thyroid gland were observed. In all the animals, the follicles were lined with simple cuboidal epithelium. It has been suggested that optimal amounts of thyroid hormones are necessary for the stimulatory effect of FSH in initiating the ripening and rupture of follicles in the ovaries as well as the proliferation and differentiation of granulosa cells and the induction of receptors for LH and activation of steroid enzymes (Maruo *et al.*, 1987). This thyroid-gonadotrophic interaction is based on the effect of thyroid hormones on receptor formation in the follicle walls. Mechanisms by which thyroid hormones are regulate ovarian activity have not been yet elucidated in full, but it has been confirmed that in the sow (Maruo *et al.*, 1987), the rat (Fitko *et al.*, 1994) as well as in human females (Greenspan, 1980), thyroid hypofunction is responsible for the hypersensitivity of the ovaries, while thyroid hyperfunction is responsible for a reduced sensitivity of ovaries to gonadotropins (Warner and Meyer, 1949). According to these data, two important conditions have been fulfilled for the formation of corpora lutea in rats with hyperluteinized ovaries and they are an unchanged circulatory concentration of FSH (Ivanisevic-Milovanovic and Musicki, 1992) and optimal functioning of the thyroid glands. This is also confirmed by our histological findings of the hyperluteinized ovaries, which contain large, ruptured follicles as well as fully developed corpora lutea. These findings of thyroid hormones in rats with hyperluteinized animals confirmed the importance of an optimal status of these hormones for the development of corpora lutea. The results of this investigation, which showed that lesions in the posterior hypothalamus do not change the physiology of the hypothalamus-thyroid axis as well as previously published papers (Martinovitch *et al.*, 1968; Ivanisevic-Milovanovic and Musicki, 1992; Ivanisevic-Milovanovic *et al.*, 1992 a), indicated that the difference in the regulation of thyroid function and the ovary or adrenal glands lies in the posterior hypothalamus which participates in the regulation of ovarian and adrenal function but not involved in the regulation of thyroid activity. So, the posterior hypothalamus specifically the mamillary body does not participate in the regulation of the thyroid gland function through the thyroid releasing hormone (TRH) which as, it is known is distributed in the median hypothalamus (Palkovits, 1982) i.e. the anterior part of the tuber cinereum and anterior part of the median eminence (Soulaïrak *et al.*, 1954) as well as in the ventromedial nucleus (Brownstein *et al.*, 1974) and in the paraventricular nucleus where TRH mRNA is detected (Cizza *et al.*, 1995).

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