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THE PITUITARY CELLS OF INFANTILE RATS AFTER THYROXINE TREATMENT

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This work mainly deals with cytological changes of thyrotropic (TSH), gonadotropic (GTH), and somatotropic (GH) pituitary cells in infantile rats treated with thyroxine during the early postnatal period.

Ten 3-day-old male Wistar rats were treated with tnyroxine (DL-T_4) in doses of 50 μg per animal, every other day until 13 days old. The glycoprotein and growth hormone-producing pituitary cells were examined by light and electron microscopy. Histological structure of the thyroid glands was used as a parameter for assessing thyroxine action at the pituitary level.

Infantile rats neonatally treated with thyroxine showed a decrease in body, adrenal and testicular weight. In the pituitary, clusters of nondifferentiated and slightly granulated, so called Ambiguous cells, were observed. Normal TSH and GTH cells were seldom identified. Numerous investigated cells were in different stages of regression. Many of the GH cells were degranulated. The glandular epithelium of thyroid gland follicles was low prismatic or squamous.

Our present findings led us to conclude that the administration of multiple doses of T_4 to neonatal rats slow-down differentiation of TSH, GTH and GH cells. The ultrastructure of glycoprotein producing cells was altered and most of them appeared as regressive cells.

INTRODUCTION

Thyroid hormones are necessary for normal growth and development in birds and mammals (Falconer, 1971; Ringer, 1976). Harvey (1983) concluded that thyroid hormones inhibit the release of growth hormone (GH) in birds, while thyroidectomy leads to an increase in basal concentrations of GH. Wilkins et al. (1974) reported that thyroid hormones act directly at

the level of GH-synthesis in hypothyroid rats. Exogenous thyroxine, surgical thyroidectomy or local thyroid irradiation inhibit or stimulate neurosecretory cell activity of hypothalamic nuclei, as well as TSH, GH and prolactin-producing (PRL) pituitary cells (Stošić et al., 1969; Stošić and Pantić, 1973; Stošić and Gledić, 1977; Stošić, 1980). Exogenous thyroxine clearly stimulates GH and PRL cells differentiation in the pituitary of intact adult male rats and regranulation and differentiation of GH and PRL cells in thyroidectomized rats (Stošić et al., 1981).

The interaction between thyroid and sex hormones has not been widely examined. Triiodothyronine (T_3) and thyroxine (T_4) are more active in inducing growth in males than in females (May, 1980; Leung et al., 1985). Thyroid hormones play an important part in brain maturation in the postnatal period. The rat brain acquires sensitivity to thyroid hormones in the first 10—14 days of postnatal life, i.e. in the period when microneurons differentiation and glia cells divisions occur (Nicholson and Altman, 1972 a, b; Amur et al., 1984; Kawada et al., 1988).

The interaction between thyroid hormones and pituitary cell structure during the infantile period in mammals has been poorly documented. For that reason, the purpose of the present investigations was to examine the effects of an excess of neonatal thyroxine on differentiation and cytological properties of pituitary TSH, GTH and GH cells in infantile rats.

MATERIAL AND METHODS

The experiments were performed using 20 neonatal male, Wistar rats. The rats were kept under controlled conditions of temperature, humidity and nutrition. Ten 3-day-old male rats, with an average body weight of 5 g, were injected subcutaneously with thyroxine (DL-T_4, Roche) in doses of 50 μg per animal, every other day until 13 days old. The intact and treated rats were sacrificed 24 hours after the last injection of T_4. Pituitary and thyroid glands were fixed in Bouin-Holland sublimate and Bouin solution and stained using Alcian blue-PAS-orange G and hematoxylin-eosin methods respectively. Pieces of adenohypophysis were fixed in 4% glutaraldehyde in a cacodylate buffer, postfixed in veronal-buffered 1% osmic acid and embedded in Araldite. Semithin sections, 1 μm thick, were stained with methylene blue or aniline blue and azure II in combination, while ultrathin sections were contrasted with saturated solutions of uranyl acetate and lead citrate. The ultrathin sections were examined using an electron microscope.

RESULTS

Thyroxine administration results in retarded development of treated rats compared to control animals. The body weight of 13-day-old treated rats was reduced by 32%, the weight of the adrenals by 13.5%, and of the testes by 18% (Figure 1). The weight of the pituitary was not affected.

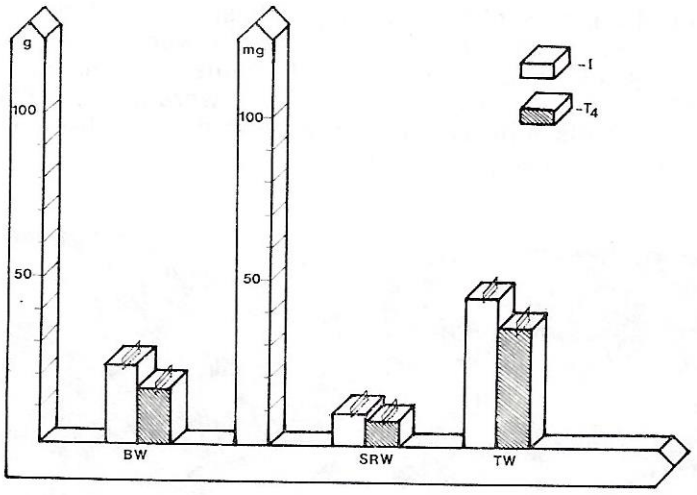


Figure 1. Body (BW), suprarenal (SRW) and testicular (TW) weight of intact (I) and 13-day-old rats treated with thyroxine (T_4).

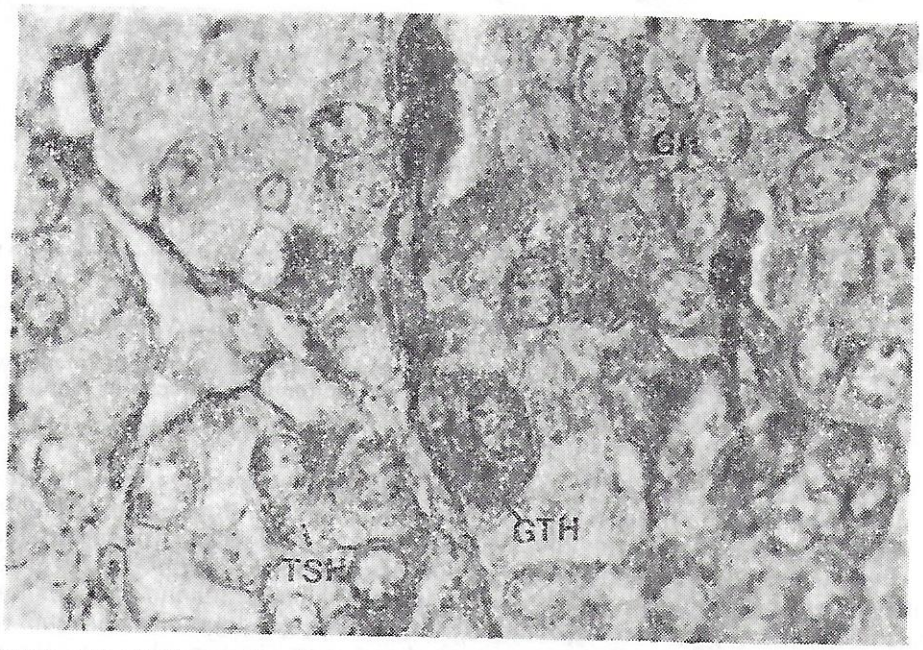


Figure 2. The pituitary cells of an intact infantile, 13-day-old rat. Bouin-Holland sublimate. Alcian blue-PAS-orange G. x 1200. TSH — thyrotropic cell; GTH — gonadotropic cell; GH — growth hormone synthesis cell.

Cytological analysis of the adenohypophysis of treated rats showed rows of nondifferentiated cells. Numerous cells were poorly granulated, so called Ambiguous cells, with individual minute specific granules (Figures 3, 4). GH cells were degranulated and some were in regression (Figure 4). TSH and GTH cells differentiation was slowed down. Normal TSH and GTH cells were occasionally encountered.

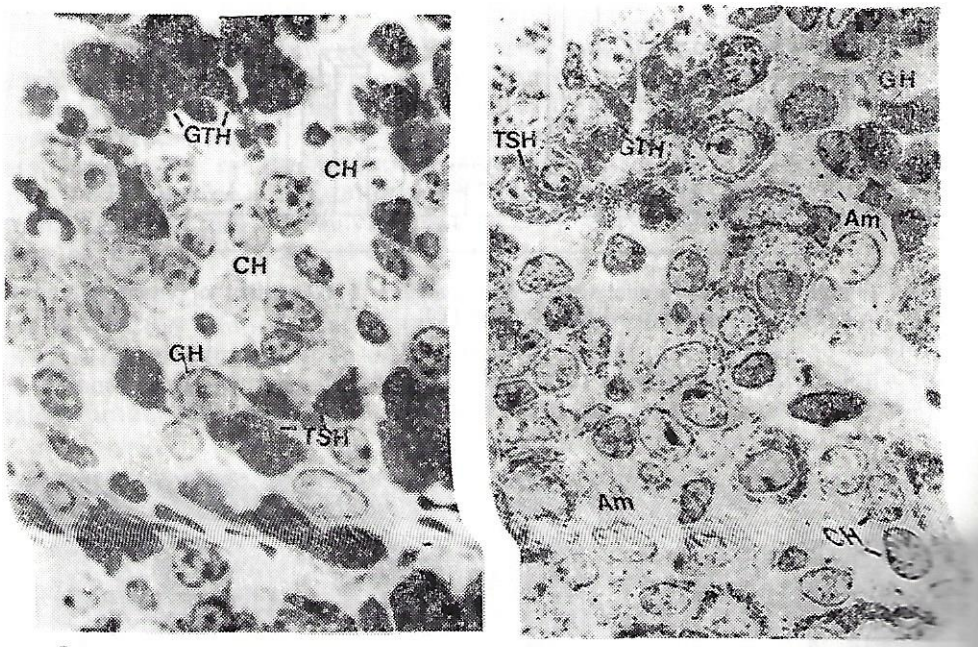


Figure 3. The pituitary cells of thyroxine treated infantile rats. 4% glutaraldehyde. Methylene blue (A); Aniline blue and azure II(B). x 1200.

A — nondifferentiated cell (CH); growth hormone synthesis cell (GH); thyrotropic (TSH) and gonadotropic (GTH) cells in regression;

B — nondifferentiated cell (CH); Ambiguous cells (Am); growth hormone synthesis (GH), thyrotropic (TSH) and gonadotropic (GTH) cells in regression.

In a certain number of TSH cells, signs of regressive changes were visible. These cells were rare, smaller, of irregular shape and contained elongated nuclei with intensely stained nuclear envelopes. These changes in the nucleus were followed by progressive condensation of chromatin into irregular granules and ribbons that gradually coalesced in pycnotic mass. Specific cytoplasmic granules formed larger accumulations and the cells gradually transformed into regressive forms (Figure 3). In a number of TSH cells, the rough endoplasmic reticulum was poorly developed. Secretory granules, 100—200 nm in diameter, were reduced in number from

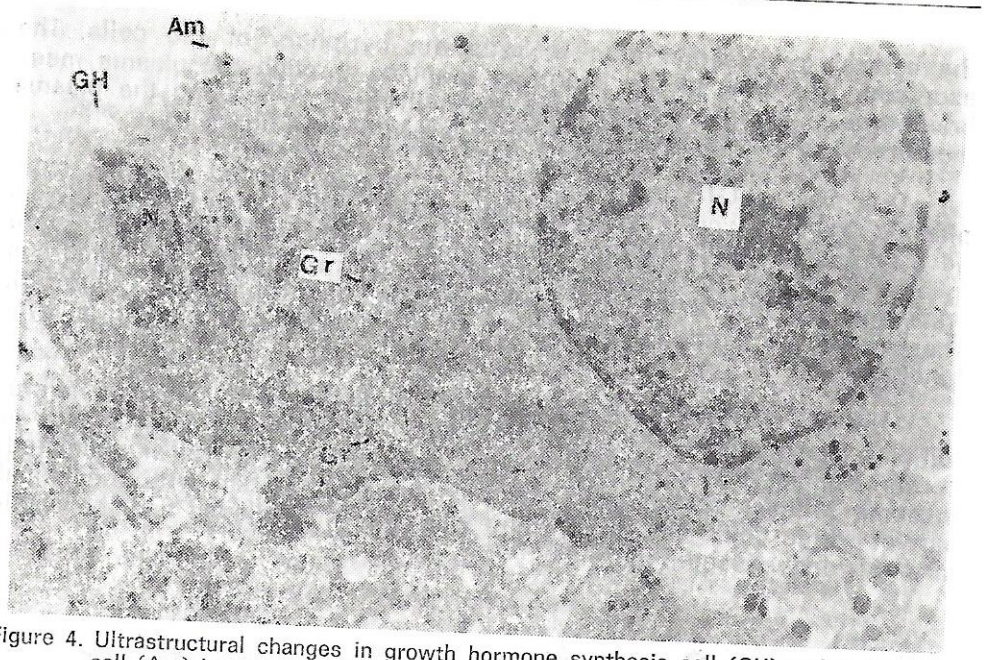


Figure 4. Ultrastructural changes in growth hormone synthesis cell (CH) and Ambiguous cell (Am) in pituitary of treated infantile rat. 4% glutaraldehyde. Uranyl acetate and lead citrate, x 9750.
N — nucleus; Gr — specific granules.

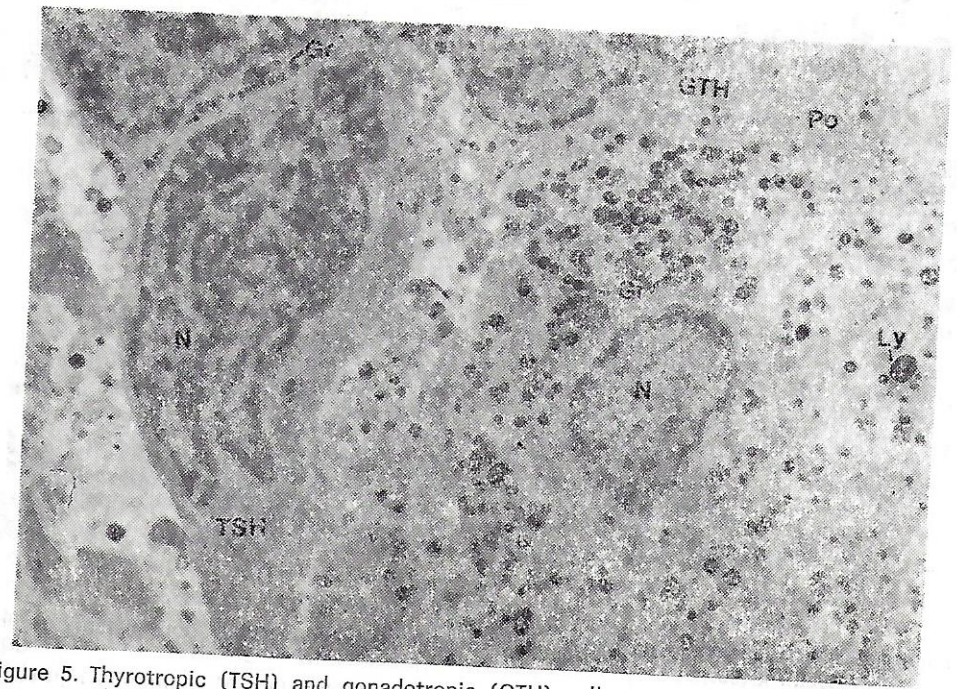
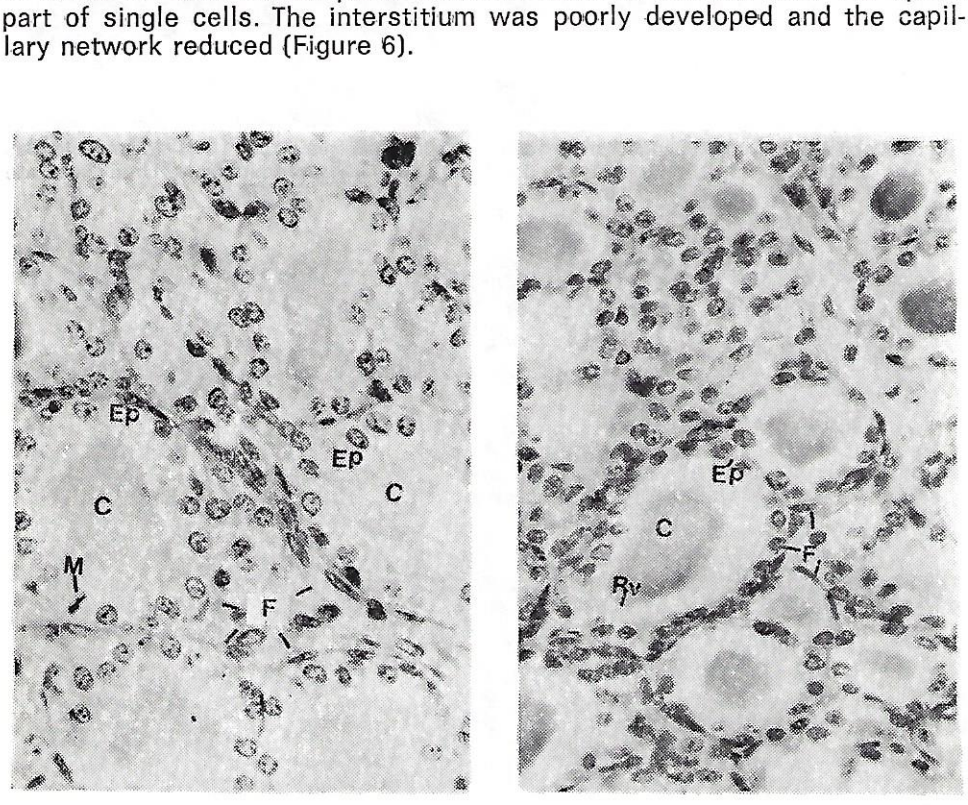


Figure 5. Thyrotropic (TSH) and gonadotropic (GTH) cells in regression in the pituitary of treated infantile rat. 4% glutaraldehyde. Uranyl acetate and lead citrate. x 9750.
N — nucleus; Gr — specific granules; Po — polysome; Ly — lysosome.

the central cytoplasmic area towards the periphery of the cells. The exocytotic transport of the secretory granules through the plasma membrane was changed and they were distributed in rows along the plasma membrane. Numerous free polysomes were present (Figure 5).

The size of GTH cells and their nuclei was reduced. Specific cytoplasmic granules were intensively stained using the Alcian blue-PAS-Orange G method. In semithin and ultrathin sections nuclei of some cells were in regression and membranous organelles were rare, except for numerous lysosomes. Specific granules varied in size and electron density (Figure 5). These cells exhibited cytological signs of suppressed function or dysfunction.

The thyroid glands of thyroxinized animals were made up of microfollicles predominantly. The epithelium was low prismatic or squamous in certain follicles. The nuclei of low prismatic cells were oval or elongated. In some follicles reabsorptive vacuoles were in contact with the apical part of single cells. The interstitium was poorly developed and the capillary network reduced (Figure 6).



I T_4

Figure 6. Thyroid glands of intact (I) and thyroxine (T_4) treated rats. Bouin. Hematoxylin-eosin. x 480.
F — follicles; C — colloid; Ep — glandular epithelium; M — mitotic figure.

DISCUSSION

In thyroxinized, 13-day-old rats, body weight was reduced by 32% compared to controls. In the pituitary of the same animals GH cell differentiation was slowed down while degranulation of certain cells war clear.

The proportion of GH-secreting cells to the total number of the other types increases after partus and reaches its peak at 15—20 days of age (Smets et al., 1989). Stepien and Pawlikowski (1977) reported that thyroid hormones act directly at the level of GH cells, while Samuels and Tsai (1973) identified the receptors for thyroid hormones on GH_1 cells in vitro. Thyroxine stimulates GH synthesis in a clonal strain of tumour cells in vitro (Ishikawa et al., 1976). According to our previous examinations the activity of GH and PRL cells is inhibited in the pituitaries of thyroidectomized mature, male rats. Thyroxine subsequently injected into these thyroidectomized rats stimulates regranulation and GH and PRL cells differentiation (Stošić et al., 1981). At the same time T_4 was reported to have a marked stimulatory effect on differentiation of GH and PRL cells in the pituitary of intact rats (Stošić et al., 1981). In hypothyroid rats GH_1 cells respond to T_3 by a decrease in the number of nuclear receptors for T_3 (Yaffe and Samuels, 1984). The reduction of catecholamines in the hypothalamus is primarily responsible for reducing GH secretion (Sonntag et al., 1980; 1982). Recently, Morimoto et al. (1988) reported that catecholamines which stimulated growth hormone-releasing factor synthesizing neurons, plays an important role in renewing GH secretion in mature, male rats.

Our cytological and ultrastructural analysis of the pituitary cells in this study showed that, besides GH cells, multiple injections of T_4 during the early postnatal period in rats results in impaired, slowed-down, differentiation and alteration of GTH cells. The mechanism of T_4 inhibited GTH cell activity is still unclear.

Thyrotropic, LH- and FSH-secreting cells represent 10% of immunostained pituitary cells respectively at day 18 postconception. These proportions remained high until postnatal day 10, after which they decreased gradually to 2%, 2% and 2—7% respectively in adult animals (Smets et al., 1989). It has previously been shown that T_4 and T_3 modulate secretion of pituitary hormones. Carr et al. (1985) indicate that T_3 affects transcription of pituitary hormone genes. Both, alpha and beta TSH subunit mRNA synthesis were suppressed by T_3. A single injection of T_3 to hypothyroid rats modifies transcriptional activity for TSH and GH (Carol et al., 1987). Repeated injections of T_3 result in possible changes in nuclear receptor number for T_3 (Yaffe and Samuelson, 1984; Franklyn et al., 1986). However, we should emphasize the possibility of T_4 conversion to T_3 and reports that biological activity and lifespan of these two hormones are similar (Astier, 1973; Assenmacher, 1973; Assenmacher, 1973; Davison, 1978). In thyroidectomized mature male rats we found a reduced number of GH cells and an enriched population of thyrotrophs compared to intact animals. Replacement with T_4 in the same animals caused a sharp increase in the population of GH cells and concurrent decrease in TSH cells (Stošić et al., 1981).

After multiple treatment with T_4 in our present study, differentiation of pituitary GTH cells of infantile rats was reduced. Numerous cells were in different phases of regression. Thyroxine applied as implant or injection reduced FSH concentrations both, in pituitary and serum and as a consequence ovarial mass was reduced, puberty postponed and fertility of the rats diminished (Bakke et al., 1974).

The alteration of TSH and GTH cells in treated rats was accompanied by alterations in corresponding target glands. The thyroid structure was impaired. The follicular epihelium was low prismatic or squamous compared to controls. In our earlier experiment we showed that in T_4 treated rats spermatogenesis was suppressed and seminiferous epithelium of 13-day-old rats contained spermatogonia only. Primary spermatocytes with typical leptotene and zygotene chromosomes were not found (Stošić, 1980).

It is well known that thyroid gland hormones are important for growth and maturation of the central nervous system. The rat brain is thyrosensitive from 8—14 days of postnatal life. The characteristic of this period is differentiation of microneurons and multiplication of glia cells prepared for the myelination process. This period is also considered to be the period of early initiation of cell differentiation as well as the period of T_4 receptors formation (Nicholson and Altman, 1972 a, b; Tsukada et al., 1977). Thyroxine stimulates the development of glia cells and myelin formation in the brain cells culture (Amur et al., 1984; Kawada et al., 1988). After treatment with T_4 from the 3rd to 13th day postpartum, the nuclear volume of hypothalamic paraventricular and ventromedial cell nuclei was reduced. These neurosecretory cells had hyperchromatic nuclei and intensively stained basophilic cytoplasm and were described as "dark" cells with decreased neurosecretion synthesis and release (Stošić, 1980).

It is clear that the devolpment of neurocontrolled mechnisms in the early infantile period of rats is altered by perinatal imbalance of thyroid hormones. It affects the development and maturation of glycoprotein and GH producing cells. It could be supposed that multiple doses of T_4 modify the rate of transcription of mRNA for TSH subunits and GTH hormones. This would result in altered development of neuroendocrine mechnisms on the one hand and an altered mechnism of hypothalamic releasing hormone activities in the pituitary on the other hand.

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ĆELIJE HIPOFIZE INFANTILNIH PACOVA PO APLIKACIJI TIROKSINA

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SADRŽAJ

U radu su, uglavnom, izučavane citološke promene tireotropnih (TSH), gonadotropnih (GTH) i somatotropnih (GH) ćelija hipofize pacova tretiranih tiroksinom, tokom ranog infantilnog perioda života. Pacovi, mužjaci, Wistar rase, su tretirani tiroksinom (DL-T_4, Roche), svakog drugog dana, u dozi 50 μg po životinji, od 3 do 13 dana života. Glikoproteinske ćelije i ćelije koje proizvode hormon rasta su izučavane primenom svetlosnog i elektronskog mikroskopa. Kao parametar promenama u hipofizi ispitivana je histološka građa štitaste žlezde.

U infantilnih pacova, neonatalno aplikovanih tiroksinom je reducirana težina tela kao i nadbubrežnih žlezda i semenika. U hipofizi su zapaženi nizovi nediferenciranih ćelija i neznatno granulisanih "Ambigous" ćelija. Normalne TSH i GTH ćelije su retko identifikovane. Brojne ispitivane ćelije su u raznim stadijumima regresije. Pojedinačne GH ćelije su degranulisane. Žlezdani epitel mikrofolikula štitaste žlezde je nizak prizmatičan ili spljošten. Apikalni deo pojedinačnih žlezdanih ćelija je u kontaktu sa reapsortivnim vakuolama koloida.

Rezultati naših ispitivanja ukazuju da višekratno tretiranje primenjenom dozom tiroksina, neonatalnih pacova usporava diferenciranje TSH, GTH i GH ćelija. Promenjena je ultrastruktura ćelija koje proizvode glikoproteinske hormone i brojne ćelije su u regresiji.