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SHORT COMMUNICATION

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INFLUENCE OF L-TRIIODOTHYRONINE ON RAT SOMATOTROPH CELLS

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Summary: The L-Triiodothyronine (L-T_3) has a direct influence on the population of somatotrophs in rat pituitary gland. This effect is dose-dependent and induces both proliferation of somatotrophs and striking changes in the synthesis and secretion of growth hormone (GH). Daily injections of 5µg L-T_3 for 7 days increased significantly the synthesis and storage of GH in pituitary gland, but the GH release was partially blocked. By contrast, injections of 10 µg L-T_3 promote rapid synthesis and secretion of GH with removal of the cytoplasmic stores of the hormone and a consequent rise of serum levels. A close correlation was found between levels of stimulation and proliferation or retrogression of lactotroph cell population.

Key words: somatotroph, cell proliferation, RIA, morphometry, electron microscopy

Endocrine glands are constituted by several secretory cell lines, the populations of which vary greatly and proportionally to the level stimulation of their hormonal secretions. A sustained stimulation of a specific endocrine cell population is usually followed by a remarkable cell proliferation to expand the secretory competence of the gland. By contrast, cessation of the stimulus activates programmed cell death of surplus cells, to level the population to prestimulation values [1, 2, 3].

It is now well established that thyroid hormones are important factors in the regulation of synthesis and secretion of the growth hormone (GH) [4, 5]. In rat pituitary gland, L-Triiodothyronine (L-T_3) stimulates the synthesis of GH. This effect is triggered by the binding of L-T_3 to a nuclear associated receptor[6] and interrelated to an increased synthesis of pituitary rGH mRNA[7, 8].

In the present communication we are reporting the behavior of the somatotroph population in rats submitted to different doses of L-T_3. The changes introduced by L-T_3 in the pituitary were monitored by ultrastructural morphometry and immunocytochemistry and correlated to the levels of GH in serum and pituitary gland.

Adult male rats, Wistar strain, were raised in this laboratory under controlled light (14h dark/ 10h light) and temperature conditions. The rats were divided into 3 experimental groups of 11 rats and subjected to the following treatments: Group 1, injected intraperitoneally (i.p.) with 5µg L-T_3 in saline per 100g body weight/day for 7 days. Group 2, treated as group 1 but with 10 µg L-T_3. Group 3, injected with the solvent, served as control. All rats were killed by decapitation, 24h after the last injection, and the blood from head and trunk collected for RIA. Then the pituitary gland was rapidly excised and split by a medial section with a razor blade into two halves. One hemipituitary was processed for electron microscopy, morphometry and immunocytochemistry as described earlier [9]. The other half was frozen at -20°C until GH radioimmunoassay was performed [3].

The treatment with L-T_3 induced a differential and dose-dependent response in the population of somatotrophs and in the synthesis and secretion of GH. The administration of 7 daily injections of 5µg L-T_3 increased almost 2-fold the GH content in the pituitary but the serum concentra-

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tions of the hormone declined significantly. In contrast, 10µg L-T_3 depleted the GH pituitary stores and raised the levels of the hormone in serum (Fig. 1). Both treatments provoked a remarkable proliferation of somatotrophs, which was confirmed morphometrically by estimation of the volume density (Vv) and of net the counting of somatotrophs (cell density) (Fig. 2). L-Triiodothyronine caused additional alterations of the fine structure of somatotrophs. There was a striking hypertrophy of the cytoplasmic organelles involved in protein synthesis, including the rough endoplasmic reticulum and the Golgi complexes. However, the net numbers of secretory granules reacted differently to these two L-T_3 treatments, while 5µg enhanced the packaging and storage of secretory granules (Fig. 3); 10µg depleted substantially GH inclusions. The few remaining secretory granules often appeared aligned along the cellular plasma membrane or undergoing exocytosis (Fig. 4 and inset) . In pituitaries from rats treated with 10µg L-T_3, a number of somatotrophs with evident signs of involution were detected by electron microscopy and immunocytochemistry.

The administration of L-T_3 to adult male rats results in striking changes in the fine structural organization of somatotroph and in the GH synthesis and secretion. However, these effects are not uniform or proportional to the degree of stimulation. A low dose (5µg) of L-T_3 increases the synthesis of GH and the storage of the hormone in secretory granules. Apparently the release of GH is partially blocked at the cell membrane level as it can be inferred by the absence of exocytotic figures and the depression of the hormone concentrations in serum. The injection of a higher L-T_3 dose (10µg) is followed by a significant increase in GH release, which in turn uplifts the GH circulating in blood. These data are in keeping with an earlier study of Coiro et al.[10]. The depletion of secretory granules in these rats is compatible with the low concentrations of the hormone detected in pituitary gland. This suggests a rapid turnover of GH in highly stimulated somatotrophs. On the other hand, the presence of degenerating somatotrophs in these rats reveals that 10µg L-T_3 accelerated the cell renewal process to maintain required levels of GH production. Changes in thyrotroph and somatotroph populations were suggested previously in experimental hypothyroidism[11]; however, no specific techniques for identification of each cell type were applied in this study. The immunocytochemical techniques developed for the present report appear to be crucial for quantification of the somatotroph population[3].

The changes described in somatotrophs are closely interrelated with stimulation levels of trophic hormones and provide additional information on the kinetics of replication and renewal of pituitary cells.

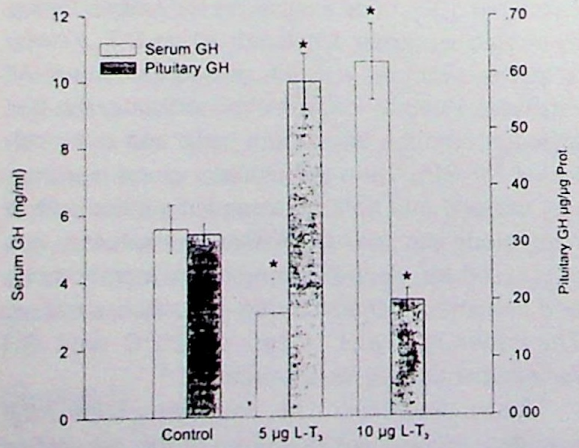


Fig 1: Serum and pituitary GH contents in control and rats treated with L-T_3. Bar= Mean ± SEM. *p<0.05 vs control, à p<0.05 vs 5 µg. ANOVA followed by Tukey test.

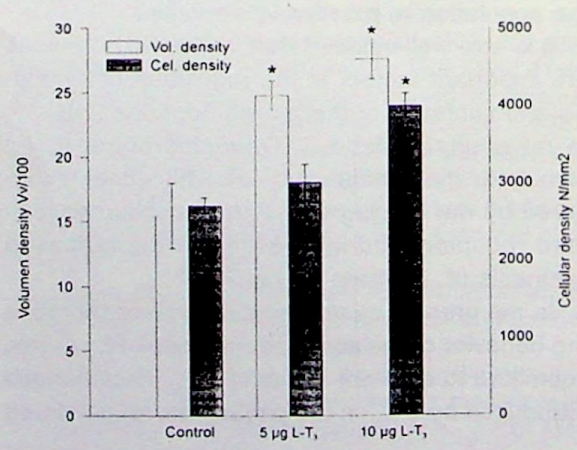
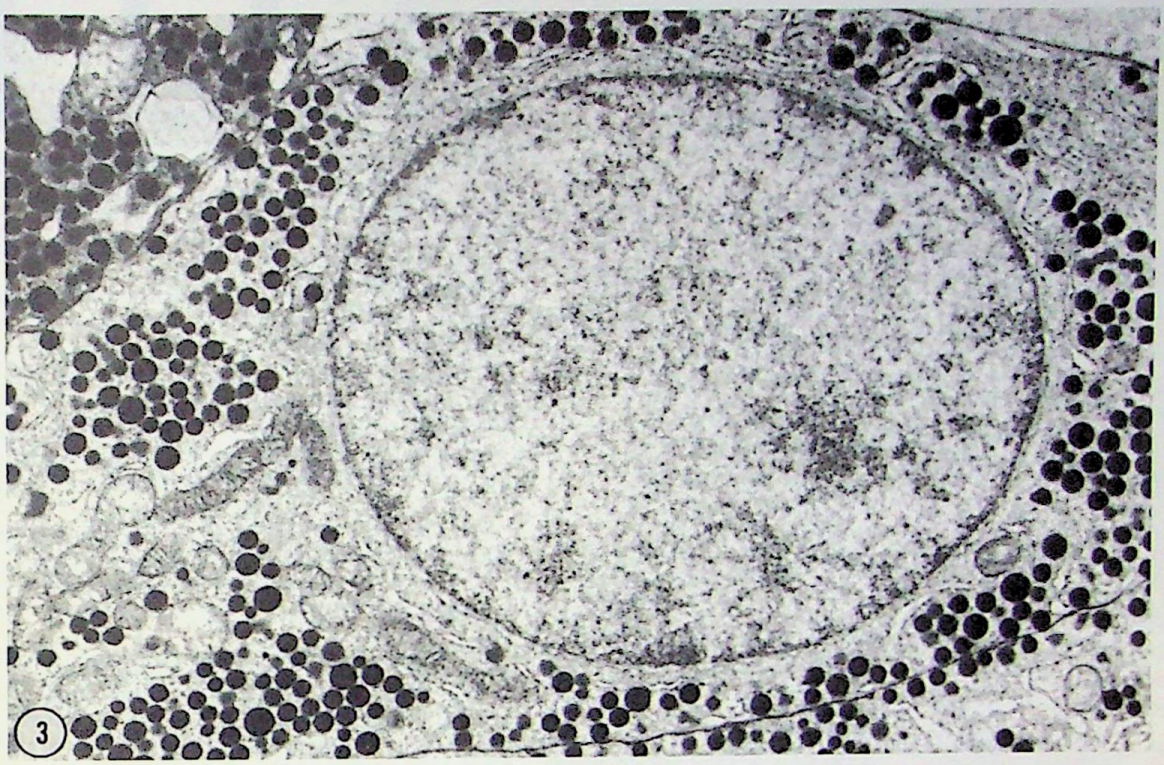


Fig 2: Somatotroph cell volumen density (Vv) and number/area in control and L-T_3 treated rats. Bar=Mean ±SEM. *p<0.05 vs control. ANOVA followed by Tukey test.



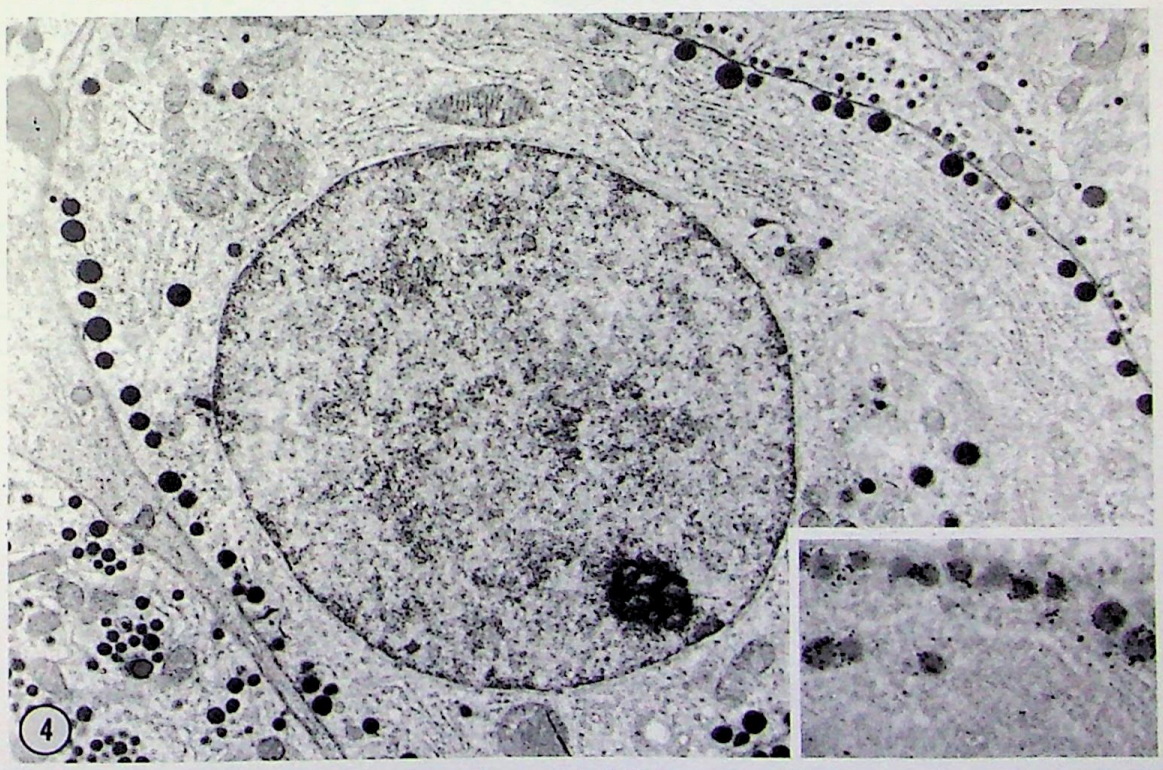


Fig 3. Electron microscopy of a somatotroph from a 5 µg L-T_3 treated rat.. There are well developed organelles involved in protein synthesis and a remarkable storage of secretory granules. Degenerated somatotroph in upper left corner. X 15000

Fig 4. Somatotroph after treatment with 10 µg L-T_3. A marked hypertrophy of the RER membranes is accompanied by depletion of secretory granules. The few remaining granules seen aligned adjacent to cell membrane or being released by exocytosis. x12000 *Inset:* A somatotroph stained specifically for GH with immunogold technique. X 16200

Resumen

Influencia de la L-triiodotironina sobre las células somatotropas de la rata

La L-triiodotironina tiene una influencia directa sobre la población de células somatotropas en la glándula hipofisiaria de rata. Este efecto es dosis dependiente e induce proliferación de las células somatotropas e importantes cambios en la síntesis y secreción de la hormona de crecimiento (GH). Inyecciones diarias de 5µg L-T_3 durante 7 días incrementaron significativamente la síntesis y los depósitos de GH en la hipófisis, pero la liberación de la hormona fue parcialmente bloqueada. En contraste, inyecciones de 10µg L-T_3 promueven una rápida síntesis y secreción de GH, con mobilización de los depósitos de la hormona y una consecuente elevación de sus niveles séricos. Una estrecha correlación fue encontrada entre los niveles de estimulación y la proliferación o regresión de la población de células somatotropas.

References

1. Haggi ES, Torres AI, Maldonado CA, Aoki A. Regression of redundant lactotrophs in rat pituitary gland after cessation of lactation. *J Endocr* 1986; 111: 367-73.
2. Pasolli HA, Torres AI, Aoki A. Influence of lactotroph cell density on prolactin secretion in rat. *J Endocr* 1992, 134: 241-6.
3. Torres AI, Pasolli HA, Maldonado CA, Aoki A. Changes in thyrotroph and somatotroph populations induced by stimulation and inhibition of their secretory activity. *Histochem J* 1995; 27: 370-8.
4. Hervas F, Monrreale de Escobar G, Escobar del Rey F. Rapid effect of single small doses of L-Thyroxine and Triiodo-L-thyronine and Growth Hormone, as studied in the rat by radioimmunoassay. *Endocrinology* 1975; 97: 91-101.
5. Samuels HH, Shapiro L. Thyroid hormone stimulates *de novo* growth hormone synthesis in cultured GH_1 cells: evidence for the accumulation of a rate limiting RNA species in the induction process. *Proc Natl Acad Sci USA* 1976; 73: 3369-73
6. Wood DF, Franklyn JA, Docherty K, Ramsden DB, Sheppard MC. The effect of thyroid hormones on growth hormone gene expression *in vivo* in rats. J. Endocr, 1987, 112: 459-463.
7. Nyborg JK, Nguyen AP, Spindler SR. Relationship between thyroid and glucocorticoid hormone receptor occupancy, Growth Hormone gene transcription, and mRNA accumulation. *JBC* 1984; 259: 12377-81.
8. Yaffe BM, Samuels HH. Hormonal regulation of the Growth Hormone gene.*JBC* 1984; 259: 6284-91.
9. Maldonado CA, Aoki A. Influence of embedding media on prolactin labelling with immunogold techniques. *Histochem J* 1986; 18: 429-33.
10. Coiro V, Braverman L, Christianson D, Fang S, Goodman M. Effect of hypothyroidism and thyroxine replacement on Growth Hormone in the rat. *Endocrinology* 1979; 105: 641-6.
11. De Fesi CR, Astier HS, Surks MI. Kinetics of thyrotrophs and somatotrophs during development of hypothyroidism and L-Triiodothyronine treatment of hypothyroid rats. *Endocrinology* 1979; 104: 1172-80.

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He that wrestles with us strengthens our nerves, and sharpens our skill. Our antagonist is our helper.

El que lucha contra nosotros fortalece nuestra fibra y agudiza nuestra destreza. Nuestro antagonista es nuestro colaborador.

Edmund Burke (1729-1707)

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