

INFLUENCE OF L-TRIIODOTHYRONINE ON RAT SOMATOTROPH CELLS**MÓNICA BONATERRA, CRISTINA A. MALDONADO, AGUSTIN AOKI, ALICIA I. TORRES***Centro de Microscopía Electrónica, Facultad de Ciencias Médicas, Universidad Nacional de Córdoba*

Summary: The L-Triiodothyronine (L-T₃) 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-T₃ 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-T₃ 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-T₃) stimulates the synthesis of GH. This effect is triggered by the binding of L-T₃ to a nuclear associated receptor⁶ 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-T₃. The changes introduced by L-T₃ 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-T₃ in saline per 100g body weight/day for 7 days. Group 2, treated as group 1 but with 10 µg L-T₃. 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 earlier⁹. The other half was frozen at -20°C until GH radioimmunoassay was performed³.

The treatment with L-T₃ 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-T₃ increased almost 2-fold the GH content in the pituitary but the serum concentra-

Received: 27-XII-1995

Accepted: 24-IV-1996

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tions of the hormone declined significantly. In contrast, 10µg L-T₃ 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-T₃ 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-T₃, a number of somatotrophs with evident signs of involution were detected by electron microscopy and immunocytochemistry.

The administration of L-T₃ 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-T₃ increases the syn-

thesis 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-T₃ 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-T₃ accelerated the cell renewal process to maintain required levels of GH production. Changes in thyrotroph and somatotroph populations were suggested previously in experimental hypothyroidism¹¹; 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 population³.

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.

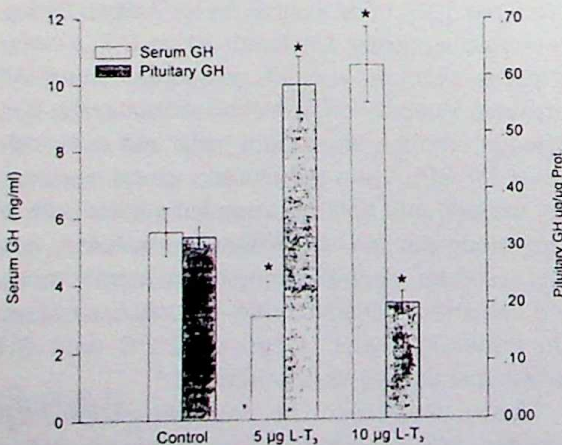


Fig 1: Serum and pituitary GH contents in control and rats treated with L-T₃. Bar= Mean ± SEM. *p<0.05 vs control, à p<0.05 vs 5 µg. ANOVA followed by Tukey test.

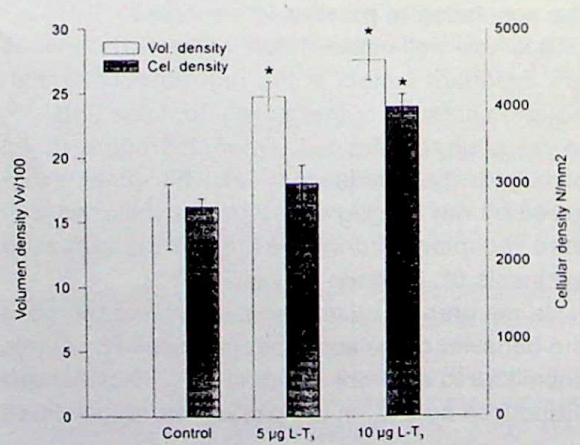


Fig 2: Somatotroph cell volumen density (Vv) and number/area in control and L-T₃ treated rats. Bar=Mean ±SEM. *p<0.05 vs control. ANOVA followed by Tukey test.

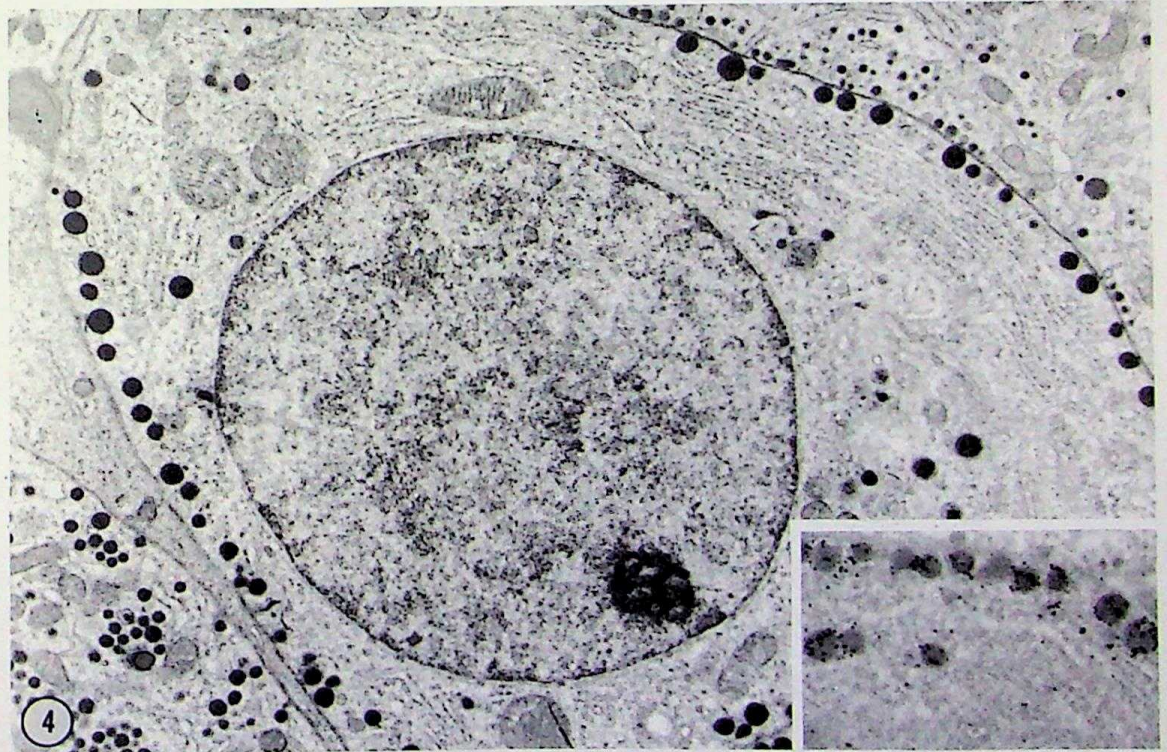
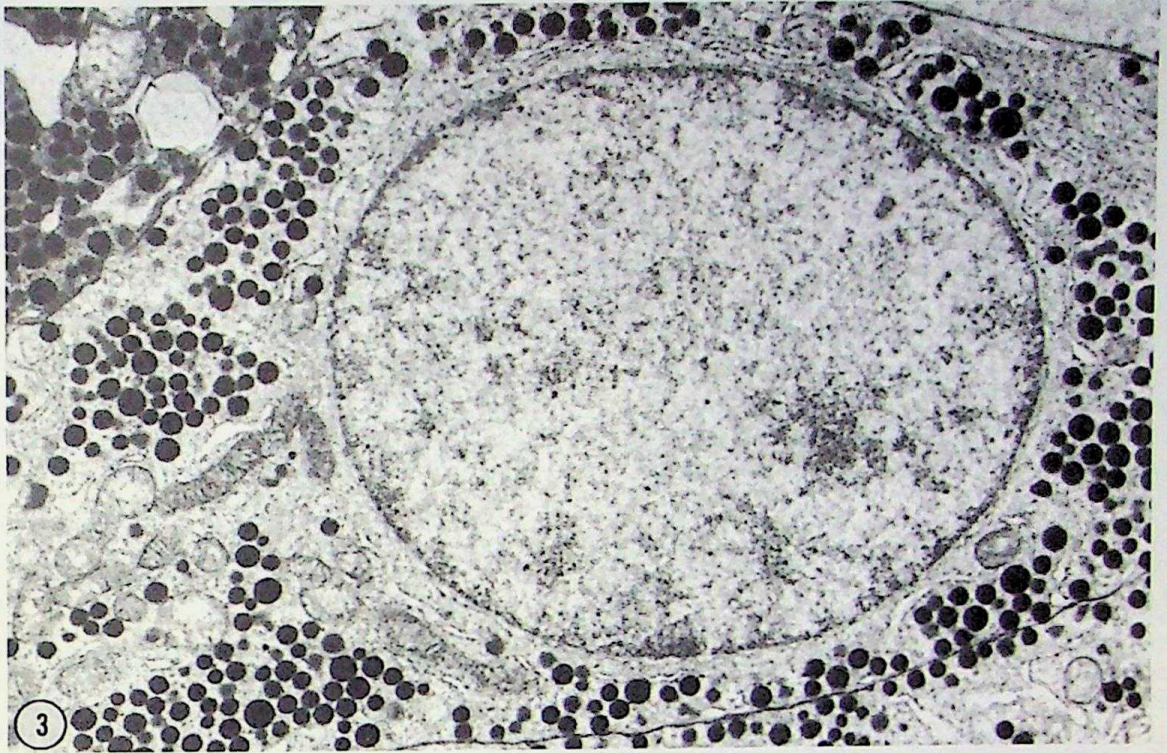


Fig 3. Electron microscopy of a somatotroph from a 5 µg L-T₃ treated rat.. There are well developed organelles involved in protein synthesis and a remarkable storage of secretory granules. Degenerated somatotroph in upper left corner. X 15000

Fig 4. Somatotroph after treatment with 10 µg L-T₃. 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-T₃ 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-T₃ promueven una rápida síntesis y secreción de GH, con movilización 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.

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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)

citado en *Nature* 1996; 380: 495