Histol Histopath (1991) 6: 37-42

Histology and Histopathology

Effects of hypothyroidism on the ultrastructure of rat pancreatic acinar cells: a stereological analysis

A. Blanco-Molina[1], J.A. González-Reyes[2], J. Torre-Cisneros[3], J. López-Miranda[3], M. Nicolás[3] and F. Pérez-Jiménez[3]

[1]Servicio de Medicina Interna, Hospital «Valle de los Pedroches», Pozoblanco, [2]Departamento de Biología Celular, Universidad de Córdoba and [3]Departamento de Medicina Interna, Hospital Regional «Reina Sofía», Córdoba, Spain

Summary. The morphological and stereological characteristics of the exocrine pancreas subcellular organelles from healthy and thyroidectomized rats have been studied. The acinar tissue from hypothyroid rats showed an interstitial edema and evidence of degenerative processes. Stereological parameters of zymogen granules were significantly reduced in thyroidectomized rats. The hypothyroidism induced degenerative changes in the pancreatic acinar cells as well as a decrease in the number and size of the zymogen granules. These modifications probably cause functional alterations.

Key words: Hypothyroidism, Exocrine pancreas, Ultrastructure, Stereology

Introduction

Hypothyroidism is a condition resulting from decreased thyroidal hormone production and a subnormal serum thyroidal hormone concentration. The typical pathological finding in this disease is the accumulation of glycosaminoglycans, primarily acid hyaluronic, in the interstitial tissue due to an increased synthesis (Lund et al., 1986). The hydrophilic properties of hyaluronic acid lead to the development of mucinous edema. In fatal cases, high concentrations of hyaluronic acid and mucinous edema are found in the interstitial tissue of almost all body organs, which causes functional abnormalities (Douglas et al., 1957).

In the gastrointestinal system the hypothyroidism induces tongue enlargement, decreased gastric and intestinal motility, and malabsorption of various nutrients (Javitt, 1978). However, the influence of hypothyroidism on the pancreatic function and morphology still remains unknown. In some isolated cases, an increase in serum amylase levels due to a chronic pancreatic during severe hypothyroidism has been described (Macleand et al., 1973). Recently, several papers have been published, using immunohistochemical methods (Leduque et al., 1985) as well as radioimmunoassay analysis (Wolf et al., 1984), in which high concentrations of TRH were found in pancreatic ß cells of Langerhans islets from hypothyroid rats.

In a previous work we have shown a decreased stimulated secretion of pancreatic lobules from thyroidectomized rats (Torre-Cisneros et al., 1988). Furthermore, an absence of inhibitory TRH effect on the stimulated secretion in lobules from hypothyroid animals was found (Blanco-Molina et al., 1986).

This paper describes ultrastructural morphological changes and variations of stereological parameters of subcellular organelles in pancreatic acinar cells from hypothyroid rats.

Materials and methods

Animals used were male rats of the Wistar strain weighing 100-120 g. Four rats were surgically thyroidectomized and injected with I^{131} (100 µCi) within a week after the operation as described elsewhere (Obregón et al., 1980). Other animals were kept untreated as control. In both groups, rats were regularly weighed and sacrificed six weeks after the operation, when the thyroidectomized rats stopped gaining weight. The intensity of hypothyroidism was attested by undetectable T_4 and T_3 plasma, and the highest TSH plasma levels were determined by radioimmunoassay (Weeke and Orskov, 1978).

Electron microscopy

After an overnight fast, samplings were started at 08:00 a.m., and three pancreatic fragments of about 1 mm^3 were obtained from each animal and quickly fixed in a mixture of 2.5% glutaraldehyde-2% paraformaldehyde

Offprint requests to: A. Blanco-Molina, Servicio de Medicina Interna, Hospital «Valle de los Pedroches», Pozoblanco (Córdoba), Spain

in 0.1mM cacodylate buffer during 6 h at 4° C. After rinsing pieces were posftixed in 1% osmium tetroxide in the same buffer. Specimens were dehydrated in ethanol and embedded in Epon. Thin sections were obtained and stained for 20 min with aqueous uranyl acetate (4%) and Reynold's lead citrate. Sections were viewed and photographed in a Jeol 200 (Japan) electron-microscope.

Stereological techniques

Fifty photographs (x 15,000) containing whole acinar cells were obtained for each group. Selection of cells was made on the basis of showing a clear basal-nucleus zymogen granule-apical polarization which ensures a relatively medial section plane. A point-counting method (Weibel, 1979) using a simple square-lattice test system of 6 mm spacing (equivalent to 0.4 µm) was applied to obtain the volume density (Vv) of every subcellular organelle.

The numerical density of mitochondria, lysosomes and zymogen granules were calculated using the formula (Weibel and Gómez, 1962)

$$Nv = \frac{k}{\beta} \frac{Na^{3/2}}{Vv^{1/2}}$$

where «Na» is the numerical profile density, «k» size distribution coefficient and «ß» is the shape coefficient. These factors were calculated for every organelle following the methods described by Weibel (1979). Values calculated for k and ß are indicated in the corresponding table and were similar to those found previously by other authors (Carneiro and Sesso, 1987).

Statistical evaluation

Morphometrical and stereological evaluations were made using a Semiauthomatic Image Analysis (IBAS; Kontron, Hannover, FRG). Data were statistically evaluated using the Student t-test programme written for the IBAS computer console.

Results

Pancreatic acinar cells from control rats showed a typical morphology: there was an axial polarization of these cells in which the nucleus was located in a basal position while secretory granules were located in apical zones of the cells near the centroacinar lumen (Fig. 1a). The other cellular organelles showed a distribution pattern similar to that described in exocrine gland cells. The centroacinar lumens were filled with an electron-dense and homogeneous material (Fig. 2a).

Visual examination of pancreatic tissues fixed 42 days after the thyroidectomy, led us to detect several morphological differences when comparing them with control animals. Nuclear envelopes showed swollen perinuclear spaces with an irregularly shaped nucleus. In the cytoplasm evident modifications with respect to the control were found. The cisternae of endoplasmic

Table 1. Volume densities ($\mu m^3/\mu m^3$) of acinar pancreatic cytoplasmic components from healthy control and hypothyroid rats. Data are expressed as mean ± SEM.

	CONTROL	HYPOTHYROID
Mitochondria	0.094 ± 0.029	0.081 ± 0.013
Golgi apparatus	0.029 ± 0.004	0.032 ± 0.007
Lysosomes	0.013 ± 0.003	0.016 ± 0.002
Zymogen granules	0.206 ± 0.019	0.063 ± 0.013*
Nucleus	0.140 ± 0.012	0.167 ± 0.017
Rough endoplasmic reticulum	0.580 ± 0.025	0.640 ± 0.035

* $p < 0.01$

Table 2. Numerical densities ($n^o/\mu m^3$) (mean ± SEM), size distribution (k) and shape (ß) coefficients of cytoplasmic components in pancreatic acinar cells from control and hypothyroid rats.

	CONTROL	HYPOTHYROID	k	ß
Mitochondria	0.189 ± 0.094	0.131 ± 0.018	1.22	1.95
Lysosomes	0.015 ± 0.003	0.053 ± 0.013**	1.08	1.38
Zymogen granules	0.442 ± 0.063	0.277 ± 0.070*	1.10	1.38

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

Table 3. Morphometric (planimetric) parameters obtained for zymogen granules from control and hypothyroid rats. Data are expressed in indicated units ± SEM.

	DIAMETER (µm)	VOLUME (μm^3)
CONTROL	0.78 ± 0.01	0.25 ± 0.01*
HYPOTHYROID	0.70 ± 0.01	0.20 ± 0.01*

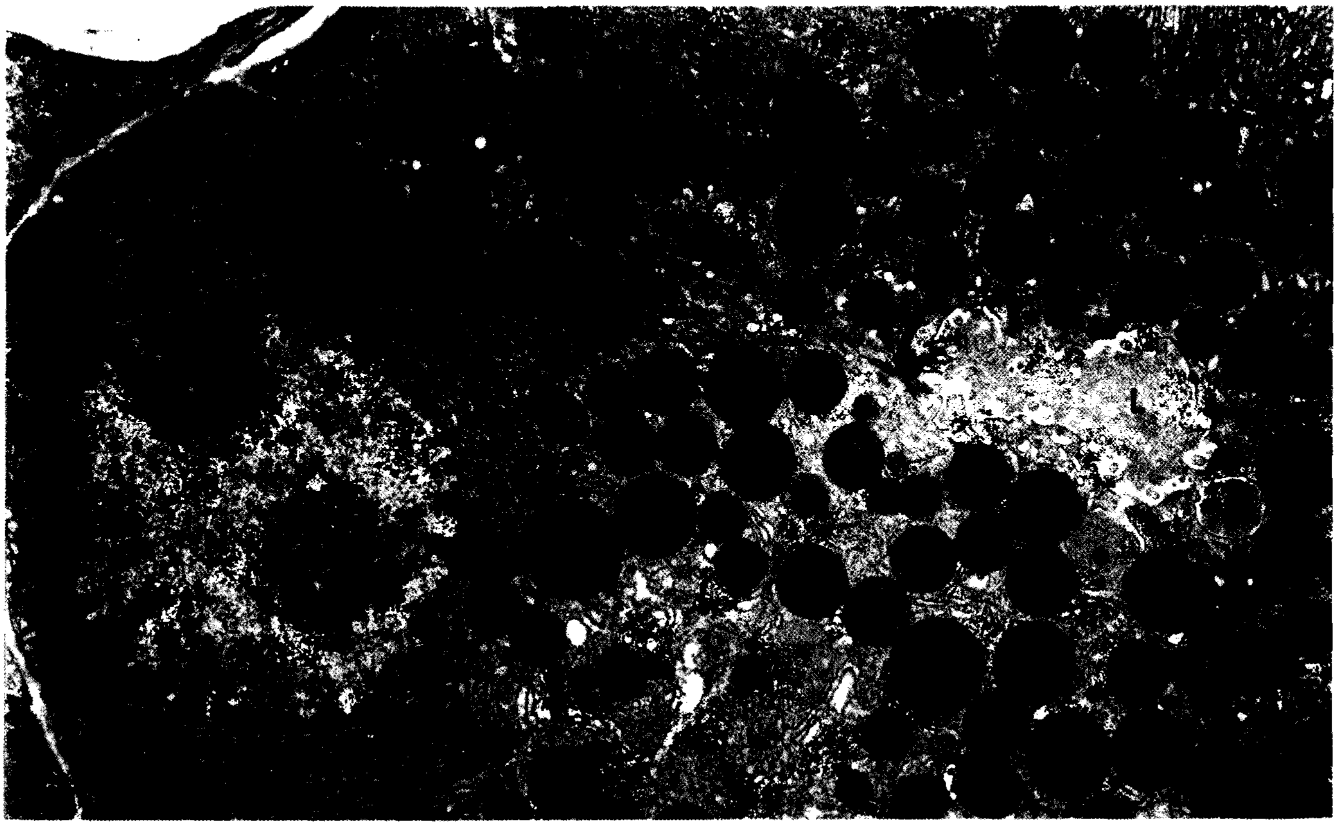
* $p < 0.01$

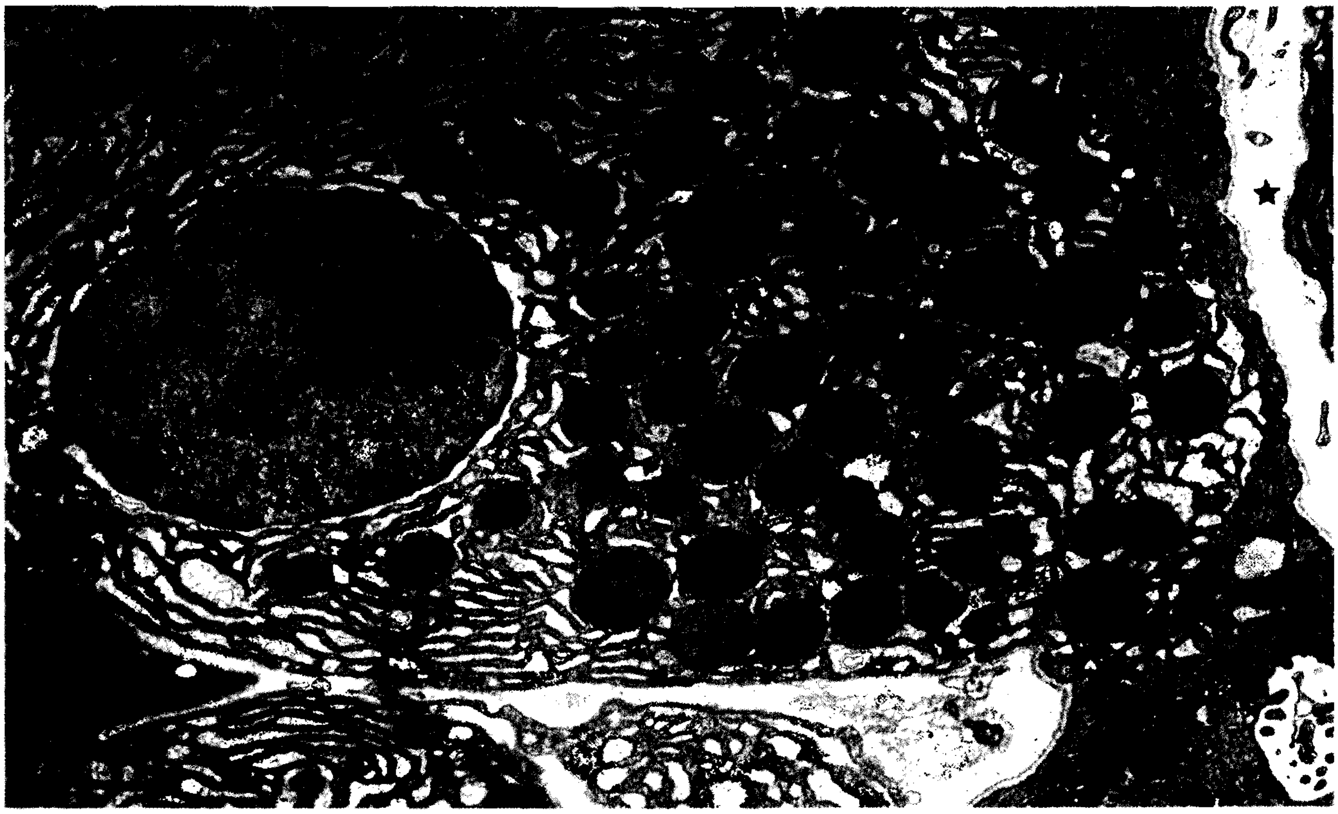
Fig. 1A. Portion of a pancreatic acinar cell from a normal rat. Nucleus (N) is placed at a basal position opposite secretory-zymogen granules (Z) which are located in the apical zone near the centroacinar lumen (L). Note some zymogen granules at the final stage of the secretory process (*). ER: Endoplasmic reticulum, M: Mitochondria, G: Golgi complex. × 10,500

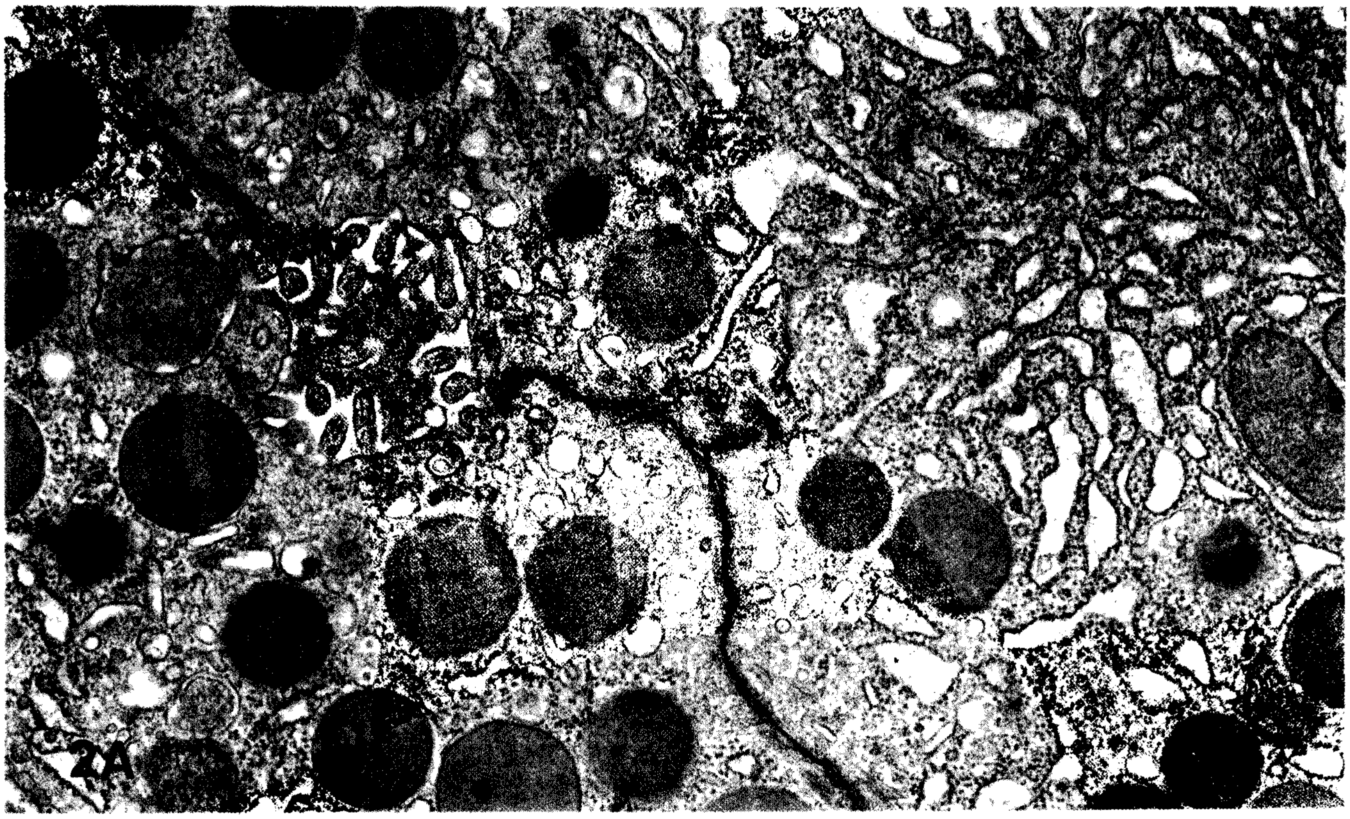
Fig. 1B. Portion of a pancreatic cell from hypothyroid animal. By visual examination, the main difference with the control concerns the swollen cisternae of ER and modifications at the nuclear envelope (arrowhead). Interstitial edema (*) is also regularly found in these preparations. In most of the photographs, changes in zymogen granules were found after a more detailed quantitative analyses. Abbreviations as in Fig. 1A. ×11,500

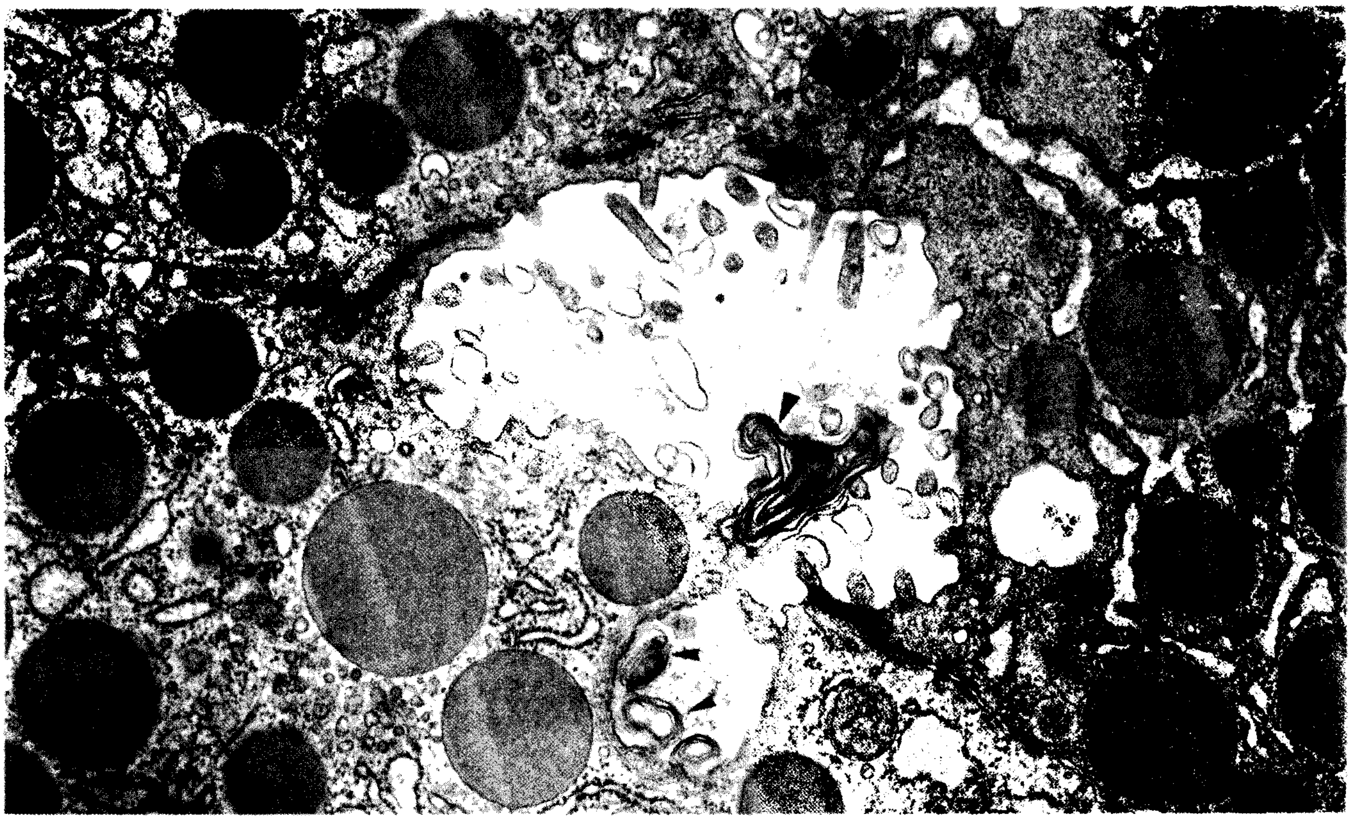
Fig. 2A. Portion of a pancreatic acini from control rat. Centroacinar lumen is occupied by a homogeneous and highly electron-dense material. × 24,000

Fig. 2B. Portion of a pancreatic acini from hypothyroid animal. Centroacinar lumen shows a scarce amorphous material different to that found in control (see Fig. 2A). Myelin-like figures (arrowheads) are also observed at this zone in hypothyroid group. × 25,000






2A



reticulum were dilated, secretory granules seemed to be more scarce, myelin-like figures were also present in the cytoplasm and inside the centroacinar spaces, which contained scanty heterogeneous opaque material (Figs. 1b, 2b). Finally, interstitial edema could easily be found in the intercellular tissue.

Morphometric and stereological studies of pancreatic acinar cells showed several quantitative differences between both experimental situations. Thus, volume density of zymogen granules decreased significantly in the hypothyroid group, while the other cellular organelles remained quantitatively unaltered (Table 1).

On the other hand, numerical density of zymogen granules was lower in the thyroidectomized animals. However, lysosomes showed the highest values for this parameter in the acinar cells from hypothyroid rats (Table 2).

Finally, a planimetric study showed that zymogen granules were significantly bigger in control specimens (Table 3).

Discussion

We report here the ultrastructural differences between pancreatic acinar cells from healthy and hypothyroid rats. The animals used were male rats starved for 12 h, and every experimental group was sacrificed at the same time (about 8 a.m.) because important variations in numerical and volume densities of cytoplasmic organelles has been described to occur throughout the day (Uchiyama and Saito, 1982).

In the thyroidectomized specimens, as has been previously reported in other organs from hypothyroid animals (Lund et al., 1986) interstitial pancreatic edema was found. In addition, several morphological modifications in nucleus and cytoplasm of acinar pancreatic cells from thyroidectomized rats were also found, which is in agreement with a typical degenerative change (Dustin, 1969). Since thyroid hormones have been shown to play an essential role in aminoacid and electrolite transport across the plasma membrane (Shambaugh, 1978), a close relationship between low T_4 availability and morphological changes can be propossed.

One of the most important findings reported in this paper, concerns zymogen granules. While secretory granules were found in a high number in normal pancreas, hypothyroid acinar cells showed a significantly decreased number. This phenomenon may be due to an increased liberation of granule content to the centroacinar lumen. However, in an «in vitro» study, differences in basal enzyme secretion were not detected between control and thyroidectomized rats neither in pancreatic isolated lobules nor dissociated acini (Torre-Cisneros et al., 1988). These findings, taken together with those reported here, suggest the interference of hypothyroidism with early events in the secretory process; probably with protein synthesis. This hypothesis is supported by the proposed role that thyroid hormones seem to play in transcriptional control of exocrine pancreatic cellular secretion (Burgi, 1986).

The response of mature tissue to thyroid hormones does not seem to be uniform: tissues such as the liver, kidney, muscle and salivary glands respond to T_4 by increasing oxygen consumption; but other tissues, including the gonads and spleen, are unaffected by thyroid hormone. The question why some tissues respond to the thyroid hormone and others are resistant has been clarified by the identification of receptor proteins in the tissues that respond to these hormones (Shambaugh, 1978).

Recently, it has been shown that both thyroid hormones and glucocorticoids play an important role in the exocrine pancreas development (Githens, 1986). The administration of T_4 to 5-10-day-old rats, induced an increase in amylase (Takeuchi et al., 1977), DNA and RNA content, as well as in the number of zymogen granules in pancreatic acinar cells (Sesso, 1962). In addition to the results reported in this paper, a decrease in amylase, lypase and total protein content, was previously observed in pancreas of hypothyroid animals (López-Segura, 1989). All these observations seem to situate the exocrine pancreas as a thyroid hormone responsive tissue. However, further research should be performed in order for a better understanding of all these matters.

References

Blanco-Molina A., Jiménez-Murillo L. and Pérez-Jiménez F. (1986). Evidence for a direct effect of TRH on the rat exocrine pancreas. Digestion 35, 9.

Burgi H. (1986). The thyroid gland. In: Clinical endocrinology: theory and practice. Labhart A. (ed). Springer-Verlag. Berlin. pp 181-348.

Carneiro S.M. and Sesso A. (1987). Morphometric evaluation of zymogen granule membrane transfer to Golgi cisternae following exocytosis in pancreatic acinar cells from suckling newborn rats. J. Submicrosc. Cytol. 19, 19-33.

Douglas R.C. and Jacobson S.D. (1957). Pathologic changes in adult myxedema. Survey of 10 necropsies. J. Clin. Endocrinol. Metabol. 17, 1354-1363.

Dustin P. (1969). Alterations celulaires elémentaires. In: Leçons d'Anatomie Pathologuique Generale. Dustin P. (ed). Presses Academic Europees Ed., Bruxelles, pp 9-61.

Guitens S. (1986). Differentiation and development of the exocrine pancreas in animals. In: The Exocrine Pancreas: biology, pathology and diseases. Go V.L., Brooks F.P., Dimagno E.P., Gardner J.D., Lebenthal E. and Scheele G. (eds). Raven Press. New York. pp 21-32.

Javitt N.B. (1978). Hypothyroidism. Gastrointestinal system. In: The Thyroid. A fundamental and clinical text. Werner S.C. and Ingbar S.H. (eds). Harper and Row. Maryland. pp 869-871.

Leduque P., Wolf B., Aratan-Spire S., Dubois P. and Czernichow P. (1985). Immunocytochemical location of Thyrotropin-releasing hormone in the ß cells of adults hypothyroid rat pancreas. Regul. Pept. 10, 281-292.

López-Segura F. (1989). Acción del péptido intestinal TRH sobre la secreción exocrina pancreática en el hipotiroidismo experimental. Thesis. Universidad de Córdoba, Córdoba, Spain.

Lund P., Horslev-Petersen K., Helin P. and Parving H.H. (1986). The effect of L-Thyroxine treatment on skin accumulation of acid glycosaminoglycans in primary myxedema. Acta Endocrinol. 113, 56-58.

Macleand D., Murisson J. and Griffiths P.D. (1973). Acute pancreatitis and diabetic ketoacidosis in accidental hypotermia and hypothermic myxedema. Br. Med. J. 4, 757.

Obregón M.J., Pascual A., Mallol J. and Morrelae de Escobar G. (1980). Evidence against a major role of L-thyroxine at the pituitary levels: Studies in rats treated with lopanoic acid (Telepaque). Endocrinology 106, 1827-1836.

Sesso A. (1962). Effet de la thyroxine et de la cortisone sur les grains de secretion, les acides nucléiques et les activites amylolytique et protéolytique du pancréas chez le jeune rat. C.R. Seances Acad. Sci. (III) 254, 569-570.

Shambaugh G.E. (1978). Chemistry and Actions of Thyroid Hormone. In: The Thyroid. A fundamental and clinical text. Werner S.C. and Ingbar S.H. (eds). 4ª edition. Harper and Row. Maryland. pp 115-124.

Takeuchi T., Ogawa M. and Sugimura T. (1977). Effects of various hormones and adrenalectomy on the levels of amylase in rat pancreas and parotid gland. Experientia 33, 1531-1532.

Torre-Cisneros J., Blanco-Molina A., López-Miranda J., Fernández de la Puebla R., Nicolás Puiggari M. and Pérez-Jiménez F. (1988). Efecto modulador de la TRH sobre la secreción exocrina pancreática. Estudio experimental. Rev. Esp. Enf. Ap. Digest. 73 (supl. 1), 57.

Uchimaya Y. and Saito K. (1982). A morphometric study of 24 hour variations in subcellular structures of the rat pancreatic acinar cells. Cell Tissue Res. 226, 609-620.

Weeke J. and Orskov H. (1978). Evaluation of thyroid function. In: Recent advances in clinical biochemistry. Alberti K.G. (ed). Churchill Livingstone. London. pp. 111.

Weibel E.R. and Gómez D.M. (1962). A principle for counting tissue structures on random sections. J. Appl. Physiol. 17, 342-352.

Weibel E.R. (1979). Stereological methods. In: Practical methods for biological morphometry. Vol I. Academic Press. London.

Wolf B., Aratan-Spire S. and Czernichow P. (1984). Hypothyroidism increases pancreatic thyrotropin-releasing hormone concentrations in adults rats. Endocrinology 113, 1334-1337.

Accepted June 26, 1990