Sex-related differences in pituitary GH-expressing cells induced by hypothyroidism following treatment with methimazole

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Summary

An immunocytochemical and morphometric study of pituitary GH-expressing cells was carried out in adult rats following chronic treatment with methimazole. The morphological results were correlated to the basal GH serum levels. In both sexes, hypothyroidism induced by methimazolee produced a significant decrease of GH serum levels in relation to untreated animals ($p<0.05$). Morphometrically, methimazole induced significant decreases in the nuclear area ($p<0.05$) and in the numerical density of immunoreactive cells ($p<0.05$). In males, these modifications were accompanied by a significant decrease in cellular and cytoplasmic size ($p<0.05$), but this was not seen in females. Our results confirm the existence of a physiological stimulatory role of thyroid hormones on the activity of pituitary somatotroph cells that must be modulated by gonadal steroids because the effects of hypothyroidism were more evident in male than in female rats.

Key words: Pituitary – GH-cells – Hypothyroidism – Immunocytochemistry

Introduction

The hypothalamic-pituitary-thyroid axis influences pituitary GH secretion. TRH fibers contacting with arcuate GHRH neurons have been described (Shioda et al., 1987) that could regulate, by synaptic mediation, GHRH secretion and TRH is able to stimulate, sinergically with GHRH, GH pituitary secretion (Lapiere et al., 1987; Strollo et al., 1988). Despite this, several authors have suggested that TRH could have an inhibitory effect on GHRH-stimulated release of GH (Zanoboni et al., 1988).

Although the inhibition of the GHRH-stimulated release of GH by T3 or TRH has been described (Scanes and Harvey, 1989), in situ hybridization studies in rats suggest a thyroid hormone-induced increase in GH-mRNA levels in the pituitary gland (Nyborg et al., 1984; Yaffe and Samuels, 1984) and these mRNA levels decrease in hypothyroidism (Martinoli and Pelletier, 1989).

The above findings are in concordance with the observed decreases in serum and pituitary GH levels in hypothyroidism (Peake et al., 1973; Martial et al., 1977; Seo et al., 1977; Spindler et al., 1982) and the presence of T3-receptors in the chromatin of GH-expressing cells.

Combined immunocytochemical and morphometric studies have proved to be a good approach to determining the cellular activity in GH-expressing cells (Carretero et al., 1990). However, no analysis of these cells in hypothyroidism has been carried out. The aim of this work was to analyse the cellular activity of adenohypophyseal GH-expressing cells from methimazole-induced hypothyroidism in a morphometric study.

Material and Methods

Forty adult Sprague-Dawley rats (20 per sex) were employed. They were divided into 2 groups: Group 1.- Adult untreated animals (10

Submitted: January 15, 2004
Accepted: April 2, 2004

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per sex). Group 2.- Adult rats treated along 21 days with methimazole according to the procedure of Stoll et al. (1978), as described in a previous work (Carretero et al., 1989). All animals were kept under standard stable conditions (temperature: 20+2ºC, relative humidity: 50+5%, lights on 8.00 to 20.00h, a Panlab balanced diet and water ad libitum). The animals were handled according to guidelines of the European Communities Council directive (86/609/EEC) and current Spanish legislation for the use and care of laboratory animals (BOE 67/8509-12, 1998).

Animals were sacrificed by decapitation under fluorane anaesthesia and blood samples were taken for serum GH levels determination by RIA according to the protocol provided by the National Pituitary Hormone Distribution Program (NIAMDD, Bethesda, Md) by double antibody RIA using the NIH rat hormone kit. The animals were sacrificed at 11.00 hours, based on the secretory patterns of GH (Tannenbaum and Martin, 1976).

After sacrifice, the pituitaries were carefully removed and fixed in 15% picric acid and 4% paraformaldehyde in phosphate buffer (0.1M, pH 7.4). The glands were embedded in paraffin and 5 μm coronal serial sections were obtained and processed for immunocytochemical study using the peroxidase-antiperoxidase method. Endogenous peroxidase activity was blocked with 0.24% H_2O_2 in methanol and the samples were saturated with normal swine serum (Dako, diluted 1:30). GH-expressing cells were detected using rabbit polyclonal anti-GH serum (Dako, diluted 1:1000) overnight at 4ºC, swine anti-rabbit Ig G (Dako, diluted 1:100) and rabbit soluble PAP complex (Dako, diluted 1:100). TRIS-saline buffer (0.05M, pH 7.4) plus 0.8% NaCl was used for dilutions and washes. Negative results were obtained following substitution of the primary antibody by non-immune rabbit serum or preabsorption with GH (hGH, UCB and rGH, NIH). The specificity of anti-GH serum was measured by RIA and proved to be 100% for GH, 1% for prolactin and less than 1% for PL, FSH, TSH or LH.

Using an IMCO-10 (Microm, MIP-2) automatic image analyzer, the cellular, cytoplasmic and nuclear areas of 500 randomly chosen GH-expressing cells from all regions of the pituitary gland per group and sex were measured interactively. The numerical density of GH-expressing cells (number of GH cells per 10,000 μm^2) was calculated in 500 fields per group of treatment and sex. All measurements were carried out on sections separated from each other by 250 μm.

Data from individual experiments were subjected to ANOVA, followed by the Scheffeé F test for multiple comparisons, accepting $p<0.05$ as significant. The results are expressed as arithmetical means ± standard errors of the mean.

RESULTS

Basal GH levels in untreated rats were higher in females than in males ($p<0.05$). In both sexes, after treatment with methimazole significant

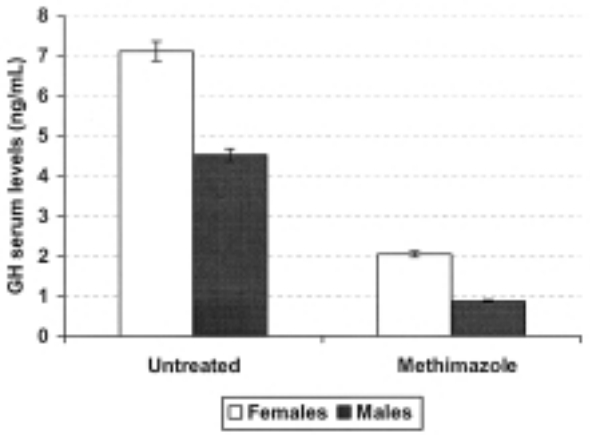

Fig. 1. Plot showing basal GH serum levels (ng/ml) found in the different groups of animals studied

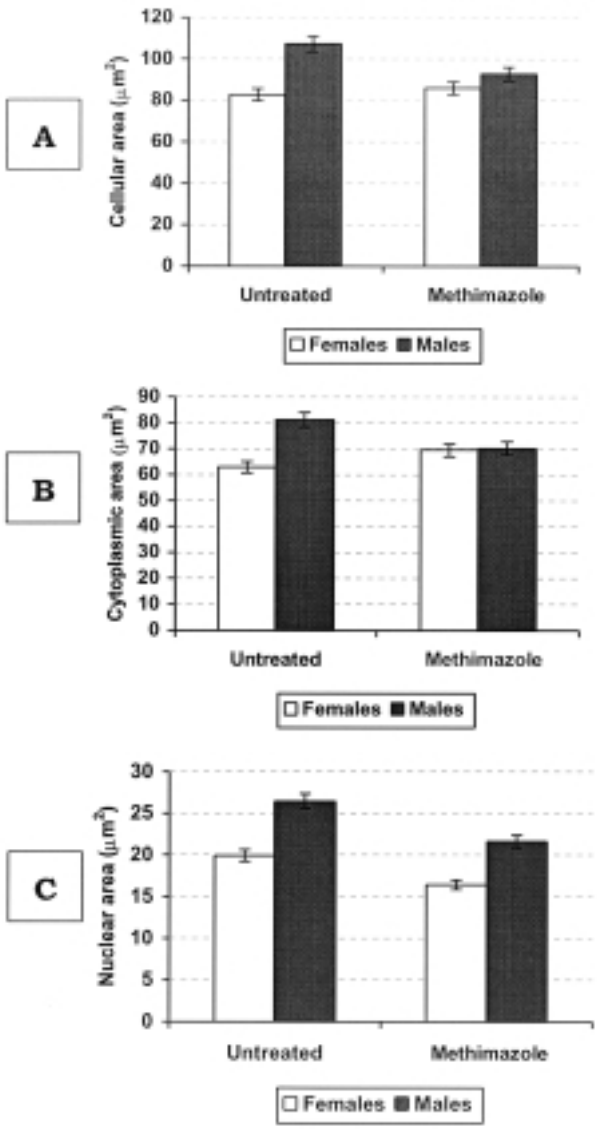

Fig. 3. Plot showing the morphometric values found int he different groups of animal studied. (**A:** cellular area; **B:** Cytoplasmic area; **C:** Nuclear area).

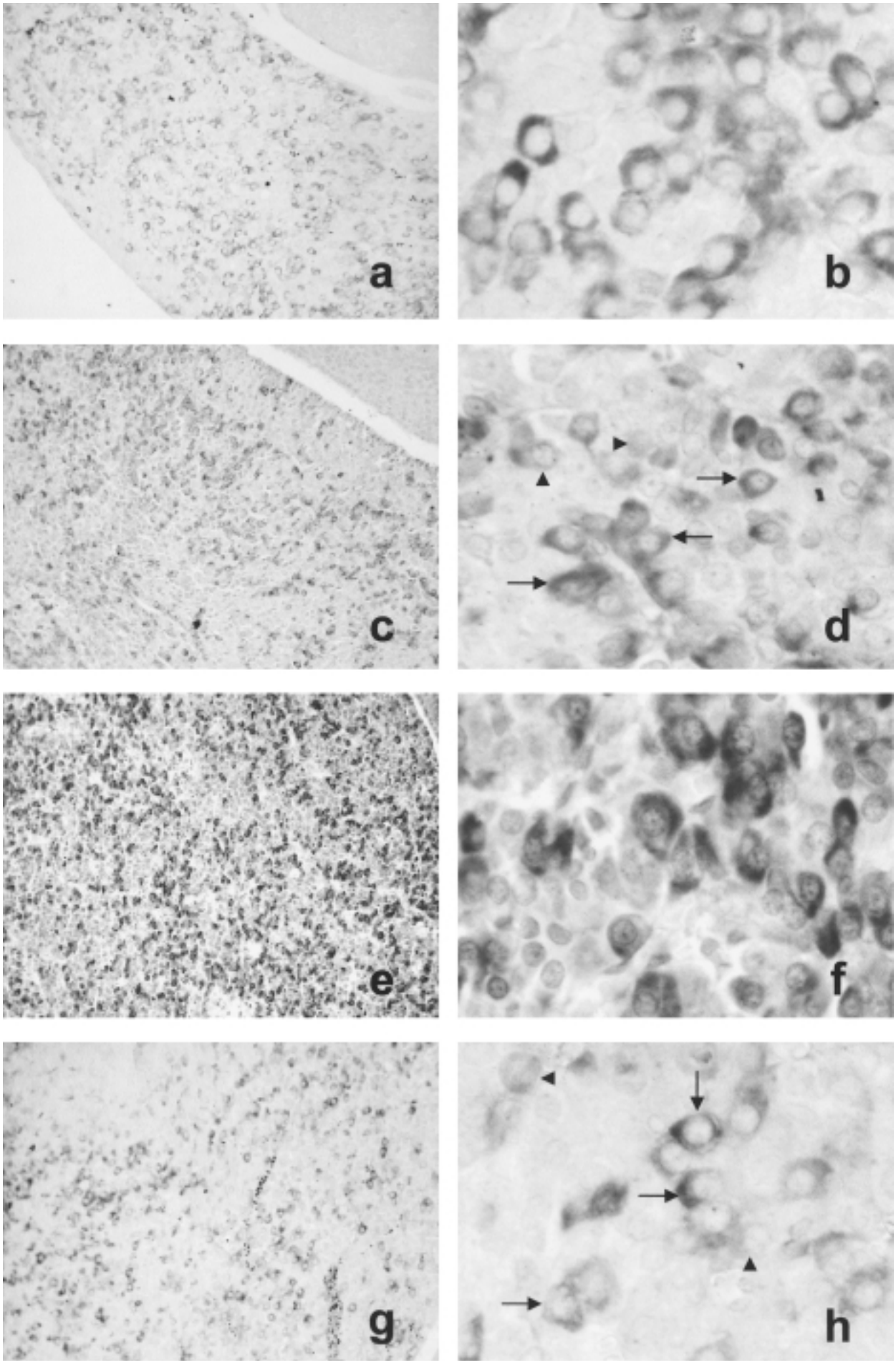

Fig. 2. Micrographs of GH-positive cells in untreated female rats **(a, b)**, methimazole treated female rats **(c, d)**, untreated male rats **(e, f)** and methimazole treated male rats **(g, h)**, a, c, e, g: x 100; b, d, f, h: x 500.

decreases in basal GH serum levels were found ($p<0.05$). However, the differences observed between male and females rats in untreated animals were not modified by hypothyroidism (Fig. 1).

In the untreated females (Figs. 2a, 2b), the immunocytochemical reaction of GH-expressing cells was more irregularly distributed than in male rats (Figs. 2e, 2f). In both sexes, the cells were either isolated or, more frequently, formed small clumps and were adjacent to blood vessels. Their morphology was varied, with a predominance of oval or polygonal shapes.

Following treatment with methimazole, GH-expressing cells were similar in both sexes (Figs. 2c, 2g). Two kinds of cellular patterns, depending on the size and intensity of the immunoreaction, were observed (Figs. 2d and 2h). One pattern displayed large and very reactive cells, polygonal in shape (thin arrows in Figs. 2d and 2h), while the other had small, irregular and lightly reactive cells, with a granular cytoplasm (arrow heads in Figs. 2d and 2h).

Morphometrically, the GH-expressing cells of the untreated animals were significantly larger in the males than in the females ($p<0.05$) due to larger cytoplasmic and nuclear areas (Fig. 3). In female rats, the hypothyroidism induced by methimazole elicited a significant decrease in nuclear areas ($p<0.05$), with no changes in cellular size. However, in males hypothyroidism decreased the cellular size ($p<0.05$) due to decreases in the cytoplasmic and nuclear areas ($p<0.05$).

The numerical density of GH-expressing cells was higher in the untreated male than in the corresponding female rats ($p<0.05$). In both sexes, hypothyroidism decreased the numerical density of these cells (Fig. 4). However, following treatment with methimazole the differences between the male and female rats were not modified ($p<0.05$).

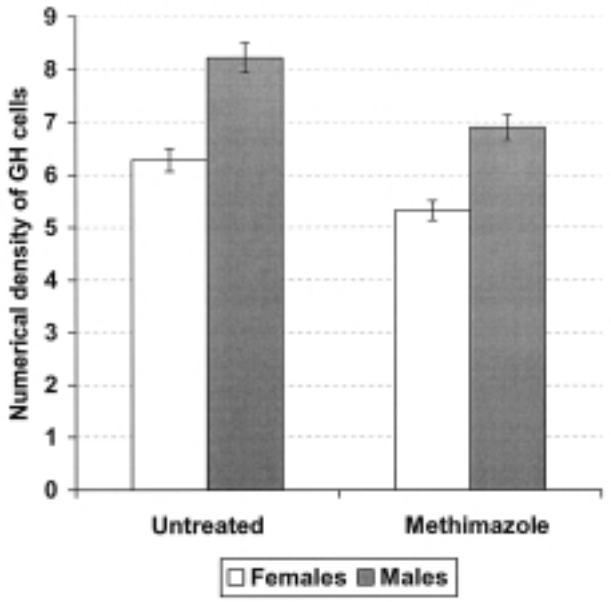

Fig. 4. Plot showing the numerical density of GH-positive cells (nº cells / 10,000 $\mu m^{2)}$ found in the different groups of animal studied.

Discussion

The decrease in nuclear area in GH-immunoreactive cells induced by a decrease in thyroid hormones in rats treated chronically with methimazole is consistent with the increase in GH-mRNA induced by thyroid hormones (Nyborg et al., 1984; Yaffe and Samuels, 1984) and with the decrease in this mRNA in hypothyroidism (Martinoli and Pelletier, 1989).

The decrease in GH serum levels accompanying the decrease in the somatotropic population and cellular size in males was reported by Peake et al. (1973) and Spindler et al. (1982).

The effect of the absence of thyroid hormones is not mediated by TRH because a stimulating role of TRH on GH synthesis and release has been described (Shioda et al., 1987; Lapierre et al., 1987; Strollo et al., 1988; Scanes and Harvey, 1989). Our findings revealed a hypoactivity of somatotroph cells in chronic hypothyroidism, manifested by decreases in the cellular and, principally, nuclear areas, and decreases in numerical density, accompanying a significant decrease in basal serum GH levels.

Our findings suggest a putative action of thyroid hormones on somatotropic cells as revealed by the decrease in GH-immunoreactive cells in methimazole-induced hypothyroidism. These effects are more evident in male than in female rats.

The sex-related differences observed in the present study could be due to a modulatory effect of gonadal steroids, because a sex-dependent secretory patterns of GH in adults rats has been reported (Tannenbaum and Martin, 1976; Martin et al., 1985; Plotsky and Vale, 1985; Millard et al., 1986) that is morphologically reflected by a greater cellular size in males than in females and that is modified by castration or following treatment with gonadal steroids (Gross, 1980; Schulte et al., 1980; Carretero et al., 1990).

Finally, our findings suggest a physiological modulatory role for thyroid hormones in the stimulation of pituitary GH secretion and synthesis of GH, modifying cellular activity, as detected by immunohistochemical and morphometric procedures. This stimulatory role is probably regulated by gonadal steroids because the effects of hypothyroidism were more evident in male than in female rats.

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