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Morphometric changes in GH-immunoreactive adenohypophyseal cells induced by intraventricular administration of colchicine to adult rats

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Summary. In order to elucidate whether the gender differences observed in the somatotropic cells of adult rats are mediated by hypothalamic neuropeptides, a morphometric analysis was made of the GH-immunoreactive cells of adult rats treated intraventricularly with colchicine. The morphometric and morphological findings obtained were correlated to the basal serum levels of GH at the time of sacrifice. Treatment with colchicine was seen to increase serum GH levels; this increase was accompanied by an increase in the intensity of the reaction of the GH-cells and, morphometrically, an increase in their size due to an increase in the nuclear area, but with no significant changes in the cytoplasmic area. The results suggest that in the absence of somatostatin and GRF the basal release of GH is elevated in a similar fashion in both sexes, in turn suggesting that gonadal steroids might act at hypothalamic level on the release of somatostatin and, indirectly, on the intracellular pool of GH and hormonal secretion.

Key words: Rat, Hypophysis, GH-cells, Immunocytochemistry, Morphometry

Introduction

The existence of different sex-dependent secretory patterns of growth hormone in rats (Saunder et al., 1976; Eden, 1979; Jansson et al., 1985; Martin et al., 1985; Millard et al., 1986) leads to differences in the morphology of the somatotropic cells. These are more pronounced in male than in female rats (Gross, 1980; Schultte et al., 1980; Carretero et al., 1988, 1990a) and after ovariectomy the cells show a greater reaction intensity (Gross, 1980) accompanied by an increase in cell size due to a corresponding increase in cytoplasmic area (Carretero et al., 1990b).

Such differences seem to be due to the effect of gonadal steroids. Reports have been made of a stimulatory effect of androgens (Martin et al., 1985; Wehrenberg et al., 1985; Millard et al., 1986; Smals et al., 1986; Akira et al., 1988) and estrogens (Eriksson and Jansson, 1985; Moll et al., 1986; Quabbe, 1986; Breier et al., 1988; Eriksson et al., 1988; Papas et al., 1988) on the release of GH, whose levels decrease after ovariectomy (Mobbs et al., 1985; Eriksson et al., 1988; Carretero et al., 1990b).

Combined immunocytochemical, morphometric and analytical studies have been shown to be an adequate approach to the assessment of the consequences of different experimental hypophyseal states. In order to check whether the effects of gonadal steroids on the morphology of somatotropic cells are mediated by the hypothalamic neuropeptides Somatostatin (SRIF) and Growth Hormone Releasing Factor (GRF), an immunocytochemical and morphometric study was carried out on the GH-immunoreactive cells of adult rats treated intraventricularly with colchicine, correlating the findings with the serum levels of the hormone at the time of sacrifice.

Materials and methods

Animals and treatments

In the present study 20 Sprague-Dawley rats of both sexes with a weight range of 150-200 g were used. The animals were divided into two groups of ten rats each (5 per sex).

Untreated animals

These were stabled under standard conditions. They were housed in a daylight regime (8.00-20.00 h) at a temperature of 20±2 °C and a relative humidity of

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50±5% with a standard balanced diet and water ad libitum.

Colchicine-treated animals

These received 90 μg of colchicine intraventricularly, under ketamine anaesthesia, in a similar way to that described in a previous work (Sánchez et al., 1988). On the first day of the experiments all the females employed were in the pro-estrus phase, as confirmed by vaginal smears.

Sample processing

At 24 h after intraventricular administration of the colchicine the animals were sacrificed from 11.30 a.m.-12.30 a.m. to obtain the basal levels of GH, in accordance with the secretory cycles reported by Tannenbaum and Martin (1976), Eden (1979) and Sato et al. (1989). Following sacrifice blood samples were obtained for hormone determination by RIA according to the protocol provided by the National Pituitary Distribution Program (NIAMDD, Bethesda, Md) by double antibody RIA using the NIH rat hormone kit. The pituitaries were removed and fixed in Bouin-Hollande fluid for 5 days and subsequently embedded in paraffin and cut in serial frontal sections for immuno-cytochemical analysis.

Immunocytochemistry

The samples obtained were studied with the PAP immunocytochemical enzyme method (Sternberger et al., 1970) after inhibiting endogenous peroxidase activity (Streefkerk, 1972), using rabbit anti-human GH serum (Dako A-570) diluted at 1:1000, swine anti-rabbit serum (Dako Z-196) diluted at 1:100 and rabbit PAP-soluble complex (Dako Z-113) diluted at 1:100; following a protocol similar to that reported previously (Carretero et al., 1990b). The specificity of the primary antiserum was measured by RIA and was calculated to be 100% for GH, 1% for prolactin and less than 1% for PL, FSH, LH and TSH.

Table 1. Analytical (ng/ml) and morphometric (μm^2) values (mean ± SE) observed in the different groups of animals studied.

	MALES		FEMALES	
	Untreated	Colchicine	Untreated	Colchicine
GH levels	4.03±1.43	20.30±2.36[a]	7.65±3.90[b]	22.49±2.97[c]
Cellular A.	117.19±3.13	130.98±2.69[b]	82.78±4.90[a]	92.86±4.41[d]
Cytopl. A	87.15±2.73	92.68±1.95	54.66±2.93[e]	55.72±3.03[e]
Nuclear A.	30.04±1.58	38.30±1.22[a]	28.12±0.96[b]	37.14±1.66[d]

Cellular A.: cellular area; Cytopl. A.: cytoplasmic area; Nuclear A.: nuclear area; [a]: $p<0.01$ with respect to untreated males; [b]: $p<0.05$ with respect to untreated males; [c]: $p<0.01$ with respect to untreated females; [d]: $p<0.05$ with respect to untreated females and $p<0.01$ with respect to colchicine-treated males; [e]: $p<0.05$ wtih respect to males.

Morphometry

For the morphometrric study, frontal sections were taken from the distal lobe of the hypophysis. An Apple digital planimeter connected via an RCA video system to a Leitz Dialux EB-20 microscope was used to calculate the cellular, nuclear and cytoplasmic areas of 100 GH-immunoreactive cells chosen randomly from each animal. Thus, 500 cells per group and sex were examined. The results obtained were evaluated statistically applying an ANOVA test for the contrast of hypotheses, considering p values of less than 0.05 for the Fisher PLSD and Scheffe-F tests as being significant. The values found (±SE) are shown in Table 1.

Results

Serum GH levels

In the untreated animals the basal serum GH levels were higher in the females than in the males ($p<0.05$). Following colchicine administration the basal serum GH levels rose ($p<0.01$) in both sexes and no differences between the sexes were observed (see Table 1).

Morphological findings

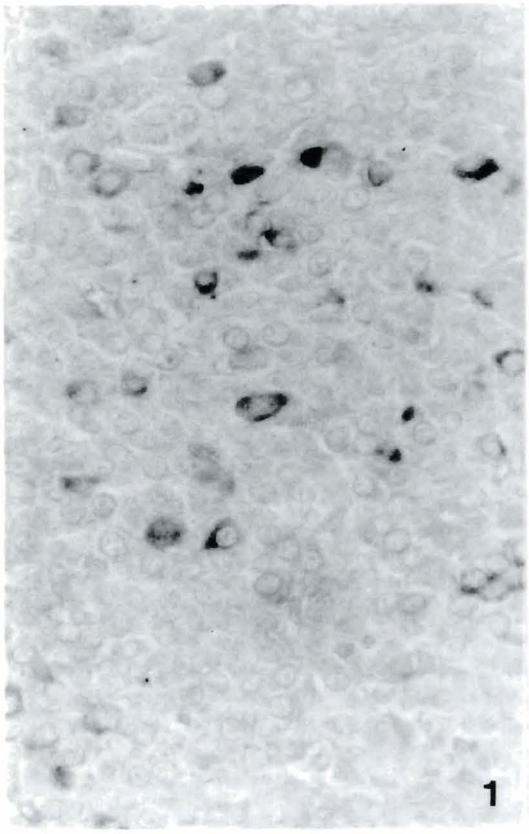
In the untreated females the cytoplasm of the GH-immunoreactive cells had a granular aspect and was irregularly immunoreactive. There was a rounded central nucleus (Fig. 1). These cells were either isolated or formed small clumps. The morphology was varied, with a predominance of oval or polygonal shapes. The GH-immunoreactive cells of the untreated males had polygonal shapes, were grouped, and had a larger cytoplasm than that of the females (Fig. 2).

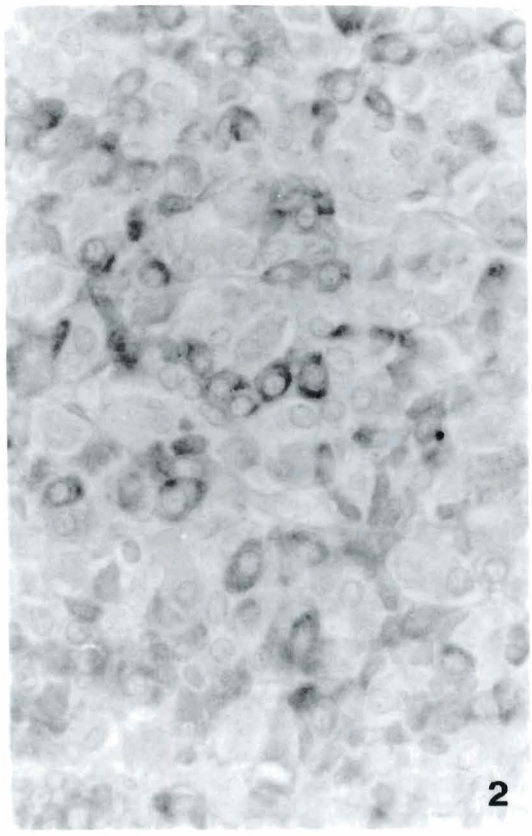
Following colchicine treatment, in both sexes the somatotropic cells were uniformly reactive, with a greater staining intensity than in the untreated animals. In both sexes, the cells were predominantly polygonal in shape, with a large clear nucleus situated in the centre of the cells and surrounded by a well-stained cytoplasmic halo (Figs. 3, 4). As in the untreated animals, they were more abundant in the males than in the females.

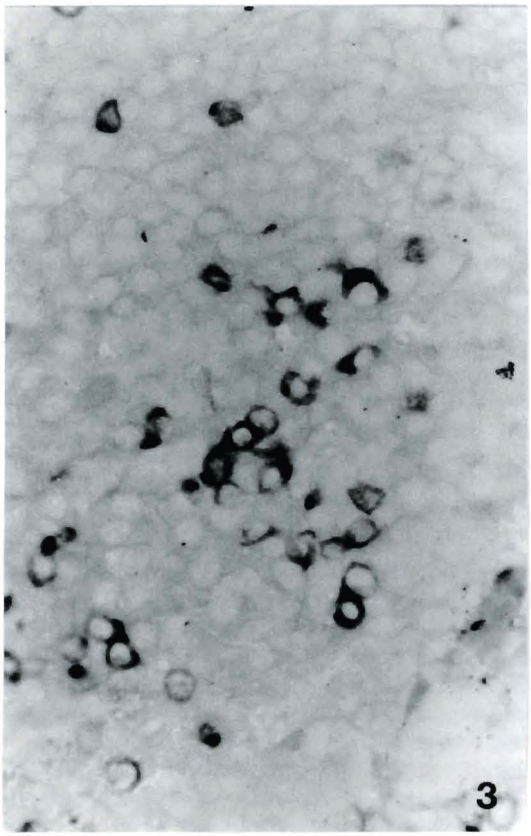
Morphometric findings

Morphometrically, the cells of the untreated males were larger than those of the females. Following treatment with colchicine, in both sexes, the cellular size increased ($p<0.05$) due to an increase in nuclear area ($p<0.01$) since although the cytoplasmic area increased slightly this increase was not significant (see Table 1).

Following treatment with colchicine, the difference in cellular area observed between the untreated males and females persisted due to the difference in the size of the







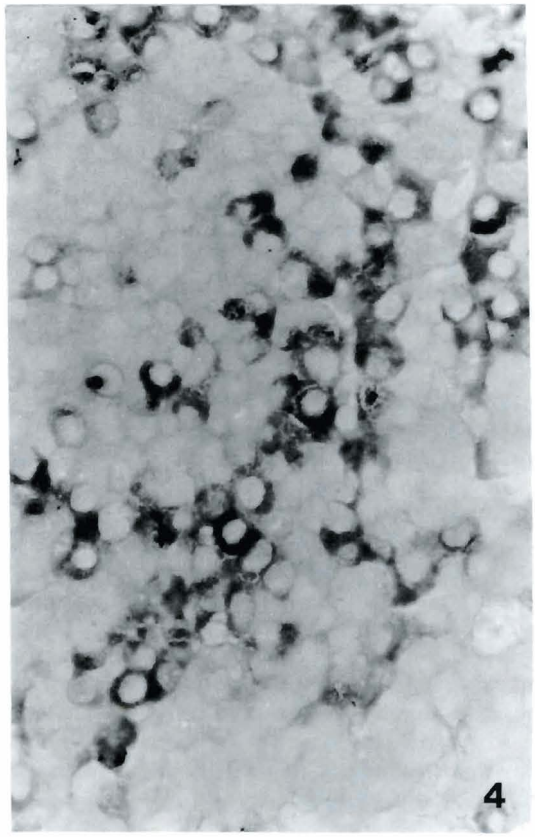


Fig. 1. Adeno-hypophyseal GH-immunoreactive cells from an untreated female. The cells appear isolated with a predominance of oval or polygonal shapes. x 450

Fig. 2. Adeno-hypophyseal GH-immunoreactive cells from an untreated male with similar shapes to those of the female but larger. x 450

Fig. 3. Adeno-hypophyseal GH-immunoreactive cells from a colchicine-treated female. They are more reactive than those of untreated females. x 450

Fig. 4. Adeno-hypophyseal GH-immunoreactive cells from a colchicine-treated male with an oval or polygonal shape and more intensely stained than those of untreated males. x 450

cytoplasmic area, since after colchicine administration both sexes displayed a similar nuclear area (see Table 1).

Discussion

The differences observed in serum GH levels and cellular, cytoplasmic and nuclear areas in the untreated animals, depending on their sex, correlate well with the descriptions of different secretory patterns of male and female rats reported by other authors (Saunders et al., 1976; Jansson et al., 1984; Tannenbaum and Ling, 1984; Plotsky and Vale, 1985). It has been observed that ovariectomy leads to an increase in hypophyseal GH concentrations and a masculine-type of secretory pattern (Akira et al., 1988) as well as changes in the cytology of the somatotropic cells (Gross, 1980; Carretero et al., 1990b). In previous works we have observed that the somatotropic cells have sex-dependent morphometric parameters (Carretero et al., 1988, 1990a,b).

This finding could be due to the fact that estradiol seems to play an antagonistic role with regards to SRIF in the regulation of GH secretion (Eriksson and Jansson, 1985) and also to the fact that under basal conditions basal SRIF levels in males are different from those in females (Akira et al., 1988). Additionally, estradiol has been shown to affect the response of GH to GRF (Silverman et al., 1988; Wehrenberg et al., 1985) and the morphology of the somatotropic cells (Gross, 1980; Carretero et al., 1990b).

Although it has been reported that in the presence of GRF SRIF essentially affects the release of GH but not its synthesis (Gick and Bancroft, 1986), the effects observed on the basal levels of GH, together with the morphometric values analyzed, could be due both to the increase in the synthesis and the release of GH owing to the abolition of the inhibitory tone of SRIF in the absence of GRF as a response to intraventricular treatment with colchicine. Thus, colchicine would hinder the transport and release of hypothalamic SRIF (Wilson, 1970; Parish et al., 1981), causing the abolition of the inhibitory tone on GH and increasing the basal release of GH in a similar way in both sexes.

The increase in cellular size due to the increase in nuclear size suggests an increase in GH synthesis following colchicine treatment. The absence of variations in the cytoplasmic area together with the increase in basal GH levels and the uniformity of the cytoplasmic reaction suggest that, after having been synthesized, GH is released into the extracellular space, accumulating inside the cell in a way different from the situation under normal circumstances.

The fact that there are no differences in the release of GH between the male and female animals following colchicine treatment suggests the existence in the adult rat of a duality, depending on the sex, in the GH secretory patterns which would essentially depend on the action of gonadal steroids on the hypothalamic SRIF secretory patterns.

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