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Study of h-TSH immunoreactive adenohypophyseal cells following treatment with methymazole

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Summary. The TSH-producing adenohypophyseal cells of Wistar rats were studied after treatment with orally administered methymazole by the PAP immunocytochemical method. These cells were compared with those of normal animals. The effects of methymazole were different in the females to those observed in males. In both sexes, the numerical density (number of TSH cells per 1000 μm^2) increased after treatment. The cellular and cytoplasmic areas increased in the females while they decreased in the males. The nuclear area and the nuclear area/cytoplasmic area ratio increased in the males and decreased in the females following treatment with methymazole. This treatment caused the appearance of large, intensely stained cells, with an eccentric nucleus and cytoplasmic processes accompanied by weakly stained cells that were situated close to blood vessels.

Key words: Hypophysis, TSH-cells, Methymazole, Immunocytochemistry, Morphometry

Introduction

It is accepted that antithyroid substances lead to hypertrophy of the TSH-producing cells, this may in turn lead to the appearance of cells similar to those observed following thyroidectomy, and both phenomena are due to an excessive secretion of TSH as a response to a lack of the hormone (Stoll and Maraud, 1963; Nadler et al., 1970; Stoll et al., 1978; Foord et al., 1986).

However, although the effects on the thyroid gland and, in analytical terms, on the hypophyseal gland, have been analyzed in depth, no immunocytochemical or morphometric studies have been conducted to confirm such findings. Thus, in the present work we were prompted to analyze the effect of methymazole on TSH immunoreactive cells of the rat hypophysis, performing a descriptive analysis of the cell morphology observed and a quantitative morphometric and planimetric evaluation of such cells.

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Materials and methods

Animals

Twenty adult Wistar rats of both sexes were used. The animals were divided into two groups: 1- normal animals kept in standard stable conditions (10 animals, 5 per sex). 2- animals treated orally with 0.25 mg/day of methymazole following the protocol established by Stoll et al. (1978) over a period of 21 days (10 animals, 5 per sex).

Processing of samples

Following treatment, under ketamine anaesthesia, the animals were perfused transcardially with phosphate saline buffer (0.01M, pH = 7.4) plus 0.8% NaCl and 5 mg of heparin over 20 seg at a controlled pressure of 130 mm Hg. After perfusion, the hypophysis was carefully removed and postfixed in the same fixative solution for 5 days in darkness at room temperature. Following dehydration in ethanol, the glands were embedded in paraffin and 5 µm serial sections obtained and processed for immunocytochemical studies.

Immunocytochemistry

The samples obtained were processed according to the enzymatic PAP immunocytochemical method (Sternberger et al., 1970). After inhibition of endogenous peroxidase (Streefkerk, 1972) and treatment of the samples with normal swine serum (Dako Z-180) at a dilution of 1:30, the samples were incubated with anti-h-TSH serum (Dako A-574) at a dilution of 1:1200 overnight at 4ºC. The reaction was then continued with anti-rabbit swine serum (Dako-Z

196) at a dilution of 1:100 and PAP soluble complex (Dako Z-113) at a dilution of 1:100. The reaction was visualized with 3-3'-DAB (Sigma) according to the procedure of Graham and Karnovsky (1966). TRIS-saline buffer (0.05 M, pH = 7.5, plus 0.08% NaCl) was used for the washing and dilutions of the sera. The process included a prior absorption test with TSH (Sigma, T-9265) at 10 nm per 0.1 ml of diluted anti-TSH serum (Forssmann et al., 1981) and substitution of the specific serum by the washing buffer and normal rabbit serum.

Morphometry

Using an Apple digital planimeter connected to a RCA video system, we calculated the cellular, nuclear and cytoplasmic areas, together with the nuclear area/cytoplasmic area ratio of 100 reactive cells chosen randomly from each of the pars distalis regions of the hypophysis (lateral, intermedia and medial) according to the procedure described by Carretero et al. (1988). Twenty-four parasagittal sections of the gland (8 from the lateral region, 8 from the intermedia region and 8 from the medial zone) per animal were also examined, calculating the total number of reactive cells per section and their numerical density per 1000 μm^2.

Results

Morphological findings

The normal females showed irregular cells diffusely distributed throughout the hypophysis with different

Table 1. Mean values (± S.D.) obtained after calculating the global and regional numerical densities of the different groups studied. (F: females, M: males, NORM: normal animals, MET: methymazole-treated animals) *: $p<0.05$ with respect to normal females, **: $p<0.01$ with respect to normal animals and $p<0.05$ with respect to the lateral region of the treated animals ●: $p<0.01$ with respect to the normal animals.

NUMERICAL DENSITY

(number of TSH-cells per 100 μm^2)

	TOTAL	LATERAL	INTERMEDIA	MEDIAL
F NORM	0.22 ± 0.018	0.13 ± 0.003	0.25 ± 0.04	0.24 ± 0.01
F MET	1.22 ± 0.036**	1.38 ± 0.006**	0.76 ± 0.10●	1.42 ± 0.003**
M NOR	0.41 ± 0.008*	0.55 ± 0.002*	0.27 ± 0.002	0.48 ± 0.02*
M MET	1.13 ± 0.066**	1.40 ± 0.08**	0.77 ± 0.04●	1.33 ± 0.08**

Table 2. Mean values (± S.D.) in μm^2 obtained after calculating the cellular, cytoplasmic and nuclear areas and the nucleus/cytoplasm ratio (overall and regional) of the different groups studied (F:females M: males, NORM: normal animals, MET: methymazole-treated animals). *: $p<0.05$ with respect to normal animals; ●: $p<0.01$ with respect to normal animals.

MORPHOMETRY

	TOTAL	LATERAL	INTERMEDIA	MEDIAL
CELLULAR AREA				
F NORM	127.31 ± 40.13	140.26 ± 44.64	119.62 ± 33.81	122.05 ± 41.96
F MET	162.08 ± 58.58*	121.65 ± 50.06	174.82 ± 53.39*	189.76 ± 72.30●
M NORM	159.41 ± 59.63	145.94 ± 45.70	173.69 ± 54.73	158.59 ± 78.48
M MET	131.49 ± 31.43*	130.10 ± 30.07	134.98 ± 32.43*	129.40 ± 31.81*
CYTOPLASMIC AREA				
F NORM	93.50 ± 38.59	100.90 ± 40.38	84.79 ± 34.25	94.82 ± 41.16
F MET	129.25 ± 54.34*	93.96 ± 45.26	138.75 ± 48.67*	155.05 ± 69.11●
M NORM	130.55 ± 56.23	120.45 ± 42.79	141.05 ± 50.75	130.15 ± 75.17
M MET	99.11 ± 29.05*	98.83 ± 27.20*	101.26 ± 30.29*	97.24 ± 29.66*
NUCLEAR AREA				
F NORM	33.81 ± 9.77	39.36 ± 15.03	34.83 ± 7.30	27.23 ± 6.98
F MET	32.82 ± 8.27	27.69 ± 8.15*	36.06 ± 9.96	34.71 ± 6.80*
M NORM	28.86 ± 8.43	25.49 ± 8.44	32.64 ± 8.01	28.44 ± 8.83
M MET	32.38 ± 7.40*	31.27 ± 8.41*	33.71 ± 6.67	32.16 ± 7.13*
NUCLEAR AREA/CYTOPLASMIC AREA RATIO				
F NORM	0.43 ± 0.22	0.45 ± 0.26	0.49 ± 0.24	0.34 ± 0.17
F MET	0.29 ± 0.11●	0.32 ± 0.12●	0.28 ± 0.09●	0.26 ± 0.11*
M NORM	0.25 ± 0.11	0.24 ± 0.12	0.25 ± 0.09	0.27 ± 0.12
M MET	0.36 ± 0.15*	0.35 ± 0.19*	0.36 ± 0.12●	0.36 ± 0.14●

staining intensities (Fig. 1); some cells appeared with a granular-like aspect, with small vacuoles while others exhibited a well-stained cytoplasm and cytoplasmic prolongations, in contact or not with blood vessels (Fig. 2).

The hypophysis of methymazole-treated females, showed an increase in the number of reactive cells (Fig. 3); they showed two cells types: some large and irregular cells with a large, intensely stained cytoplasm (arrows, Fig. 3) and others, also large, with a polarized nucleus

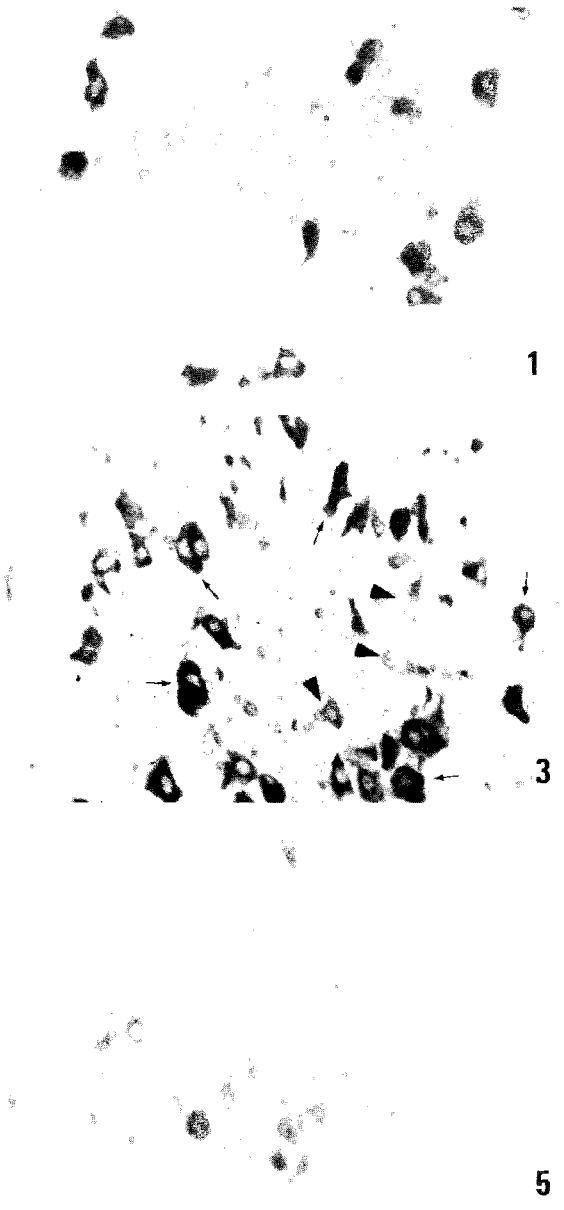

Fig. 1. Micrograph from a normal female. Note low number of reactive cells appearing isolated throughout the gland. × 1,000

Fig. 2. TSH immunoreactive cells from a normal female. Note situation close to blood vessels, towards which they project cytoplasmic prolongations. × 2,500

Fig. 3. Typical image of hypophysis from a female rat treated with methymazole. Note numerous reactive cells, among which there are two types: some are intensely stained (arrows) while others are weakly stained (arrow-head). × 1,000

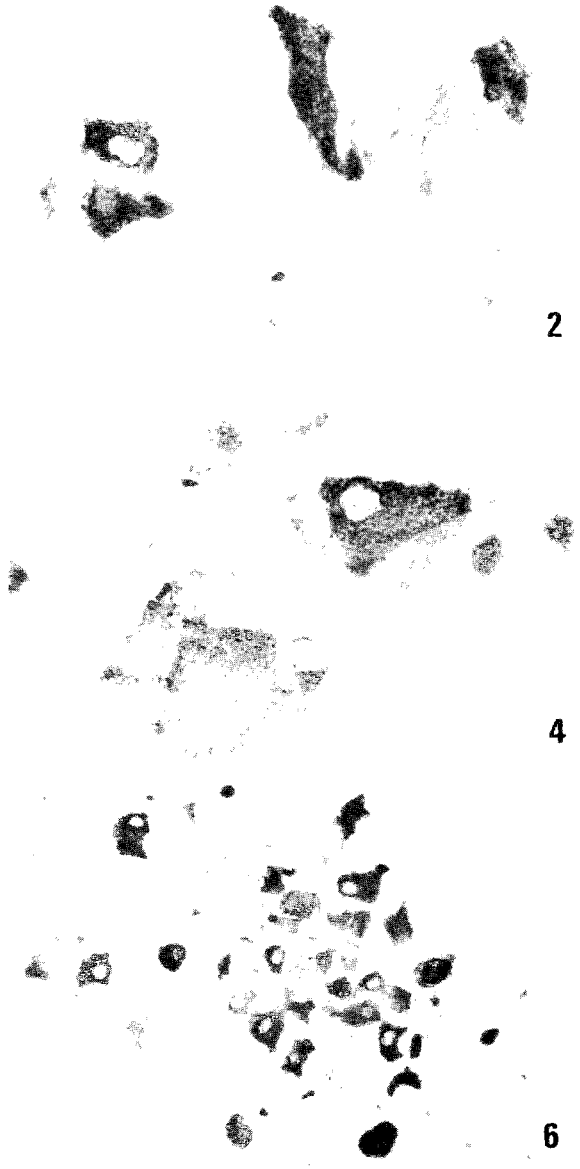

Fig. 4. Micrograph showing, at higher magnification, TSH-cells after treatment with methymazole. Note strog cytoplasmic reaction, granular aspect, large cell size and generally eccentric disposition of the nucleus. × 2,500

Fig. 5. Image of the hypophysis from a normal male. Note the low number of reactive cells, dispersed throughout the gland and their weak reaction intensity. × 1,000

Fig. 6. Image obtained from a male animal treated with methymazole. Note similar characteristics to those described for the females in Fig. 3. × 1,000

and weakly-stained cytoplasm (arrows heads, Fig. 3). In general, there was a predominance of the first kind of cell with abundant cytoplasm and a granular aspect (Fig. 4).

The normal males had TSH of similar characteristics to those of the normal females (Fig. 5), although they were less intensely stained. They were very polymorphic, with an eccentric nucleus and cytoplasmic prolongations towards the blood vessels. The changes observed following treatment with methymazole were also similar to those observed for the females receiving the same treatment (Fig. 6) and were characterized by the appearance of large cells with two staining intensities.

Morphometric findings

The TSH cells showed clear differences with respect to the sex of the animals; the number of TSH-reactive cells was greater in the males than in the females. This difference was clearly significant ($p<0.05$) in the total hypophyseal gland and in the lateral and medial regions of the gland but not in the intermedia region, where the values found were similar for both sexes (Table 1).

Following treatment with methymazole, in both the male and female animals an increase was seen in the number of reactive cells. This increase was much more pronounced in the lateral and medial regions than in the intermedia one, whose values were approximately half those found in the other two regions (see Table 1).

Morphometrically, differences appeared with respect to the sex of the rats in the response to methymazole. Considering the hypophysis overall, there was an increase in the cellular and cytoplasmic areas in the females while a decrease was observed in the males. By regions, the findings observed were identical to those seen throughout the gland, with the exception of the lateral region of the female rats, where the behaviour was the same as in the males.

The values for the nuclear area increased in the males, mainly in the lateral and medial regions. In the females, there were no overall modifications owing to the variability of the response obtained in the different regions; this increased in the medial region, while it decreased in the lateral and no modifications were observed in the intermedia region (Table 2).

The findings show that following treatment with methymazole a decrease occurs in the nuclear area/cytoplasmic area ratio in the females rats while in the males an increase is apparent (Table 2).

Discussion

In mammals, there are very few TSH cells and these only comprise between 2 and 3% of the adenohypophyseal cell population. Most of them are situated in the ventromedial area of the gland and are also scattered throughout the lateral and medial regions (Girod, 1984).

In the rat, controversy exists among different authors; cell percentages have been described ranging from 1.71% (Siperstein, 1963) to 6.2% (Childs, 1983), and numerous intermediate values have also been described (Bogdanove, 1963; Costoff, 1973; Surks and DeFesi, 1977; Arishima et al., 1978; Takahashi and Kawashima, 1982; Dada et al., 1984).

Since we did not calculate percentages but rather the numerical density, our protocol does not allow us to decide which of the above authors best approximate to our values; however, we agree with all their data in that we were dealing with the cells that are least numerous in the hypophysis; this interpretation is based on the present and earlier findings from our laboratory (Carretero, 1984; Carbajo et al., 1987; Montero, 1987; Carretero et al., 1988).

We also found alterations in numerical density with respect to the sex of the animals. The values obtained in the males are almost twice as large as those observed in the females in overall terms, whereas on considering the different hypophyseal regions, it is clear that these cells predominate in the lateral and medial areas of the gland in the males while they are more diffusely distributed in the females. For Dada et al. (1984), the cell percentages are similar for both sexes and although Takahashi and Kawashima (1982) report a lower percentage for the males than for the females, the studies of these latter authors were made with the electron microscope (i.e. not immunocytochemically) and the differences are less clear.

Together with the differences in numerical density, we also found different morphometric values with respect to the sex of the animals, specially in the intermedia and medial regions of the gland. Regarding this, the descriptions of Dada et al. (1984) concerning the volume density of this cell type are of interest: 3.19 for the males and 2.91 for the females. These values confirm our own findings that the TSH cells have a higher cell area in males than in females. Even higher values have been reported (5.0) for males by Childs (1983). Values of 352 μm^3 have been offered for this cell type, without differentiating between males and females (Poole and Kornegay, 1982).

The different results with respect to sex could be due to sexual steroid levels in the blood. In women, pharmacological doses of estrogens are known to induce a decrease in TSH secretion, although no similar findings have been reported in physiological conditions. This seems to conflict with the observations of Labrie et al. (1979) who described an increased response to TSH at hypophyseal level owing to an increase in receptors in turn due to the action of estrogens. Further studies should attempt to evaluate this aspect in order to check the possible role of these sexual steroids.

Methymazole inhibits the production of thyroid hormones through inhibition of intracellular peroxidase; in this circumstance, free iodine is not formed from iodides and hence thyroid hormone are not synthesized (Morris and Hager, 1966; Litter, 1976; Taurog, 1976; Michot et al., 1979).

It is also known that in states of thyroid hyperactivity, the number of cells decreases and that these appear isolated and scattered throughout the hypophysis. They are reduced in size, show signs of nuclear regression and an increased immunocytochemical reaction (El Etreby

and Fath El Bab, 1978). Logically, the number of TSH-producing cells is completely altered following induction of hypothyroidism in the rat (DeFesi et al., 1979; Astier et al., 1980). Our own findings corroborate these results.

Thyroidectomy alone and in combination with stress and low temperature leads to the appearance of large cells, with a small and eccentric nucleus compared to the cytoplasm, which is sometimes vacuolated and is always large (Palomero, 1971). Severe hypothyroidism is accompanied by an increase in hypophyseal concentrations of TSH (DeVito et al., 1987). An interesting feature of hyperactivity after thyroidectomy is the appearance of reactive cells showing more than one staining intensity (Moriarty and Tobin, 1976). In hypothyroid animals the presence of different cell types has been demonstrated; post-thyroidectomy cells coexist with others that may be considered normal, together with small hyperactive cells (Leushen et al., 1978), and an increase in the number of cells.

Following treatment with methymazole in the males, all the characteristics typical of cellular hyperactivity were observed, with the exception of the decrease in cell and cytoplasmic areas, though not in the nuclear area. This suggests an increase in the synthesis and release of hormone (Leushen et al., 1978) which is more manifest in the females.

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