Histol Histopath (1991) 6: 101-105

Histology and Histopathology

Morphometric study of the LH-immunoreactive gonadotrophic cells of rats following treatment with methoclopramide

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Summary. The LH-immunoreactive cells of the adult rat hypophysis were studied morphometrically after chronic treatment with methoclopramide. The morphological features of these cells showed modifications in both male and female rats, after treatment. Additionally, morphometric changes revealed a significant decrease ($p < 0.05$) in both cytoplasmic area, which was more evident in the female rats, and nuclear area, with respect to the normal and control animals. These findings suggest that chronic inhibition of the dopaminergic system in rats atrophies LH-immunoreactive gonadotrophic cells of rats.

Key words: Hypophysis, LH-cells, Methoclopramide, Immunocytochemistry, Morphometry

Introduction

The dopamine content of the neural endings of the median eminence changes during the estrous phase and may be altered by endocrine manipulation, leading to changes in the secretion of gonadotrophic hormones (Fuxe and Hökfelt, 1967).

Both testosterone in male and estradiol in female animals, increase dopaminergic turnover in the tuberoinfundibular neurons (Fuxe et al., 1969; Kalra and Kalra, 1985); this fact decreases rapidly before the preovulatory LH peak (Fuxe and Hökfelt, 1969).

Intraventricular administration of dopamine increases the basal secretion of LH, while LH and FSH secretion decrease after dopamine and norepinephrine depletion (Brown, 1971; Dickey and Marks, 1971) or after halloperidol administration (Bhattarcharga et al., 1972). Among other mechanisms, this action could be due to a stimulating effect of LHRH release (Schneider and McCann, 1969; Bennet et al., 1975; Rotsztejn et al., 1977; Negro-Vilar and Ojeda, 1978) since «in vitro» studies have been unable to demonstrate alterations in gonadotroph hormone secretion (Schneider and McCann, 1969).

However, some dopaminergic agonists have been shown to inhibit LH release during the follicular phase (Lachelin et al., 1977), mainly when serum oestrogen levels are at a peak.

The present work was undertaken to verify the effect of chronic inhibition of the dopaminergic system on LH-immunoreactive adenohypophyseal cells, by chronic treatment of adult rats of both sexes with methoclopramide.

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Materials and methods

Animals and treatment

In the present study thirty Sprague-Dawley rats of both sexes with a weight range between 200 and 250 g were used. The animals were divided into three groups of ten rats each (5 per sex):

1).- Normal animals: These were stabled under standard conditions (a daylight regime, 8:00 to 20:00 h; temperature of 20 ± 2° C and R.H.: 50 ± 5%, with a balanced diet and water ad libitum).

2).- Control animals: These received a daily (10:00 h) intramuscular dose (100 µl) of saline serum during 15 days.

3).- Treated animals: These recieved a daily intramuscular dose of Methoclopramide (20 mg/kg body weight) at 10:00 h during 15 days. The animals of groups 1 and 2 were sacrificed six hours after the last administration. On the first day of the experiment, all the female rats were in proestrous phase as determined by vaginal smears.

Processing of samples

After decapitation, the hypophyses were carefully removed and fixed in Bouin-Hollande fluid for 5 days,

embedded in paraffin and cut into 5 μm serial sections for immunocytochemical study.

Immunocytochemistry

The samples obtained were studied with the PAP immunocytochemical enzymatic method (Sternberger et al., 1970) using anti-LH serum (Dako, A-572), obtained from rabbits, at a dilution of 1:1200; swine anti-rabbit immunoglobulin (Dako, Z-196), diluted at 1:100, and PAP soluble complex, obtained from rabbits (Dako, Z-113) and diluted at 1:100; following a protocol similar to that described in a previous work by Carbajo et al. (1988). The process included a pre-absorption test with LH (100% cross-reaction) and FSH (8% cross-reaction) (LH and FSH of rat, NIH, kindly supplied by Dr. Sánchez-Franco and Dr. Cacicedo, Ramón y Cajal Hospital, Madrid, Spain) and substitution of the specific serum by the washing buffer and normal rabbit serum (the reaction was negative). Tris saline buffer (0.05M, pH:7.4, with 8% of NaCl) was also used for the washing and dilution of sera.

Morphometry

An Apple digital planimeter connected through an RCA video system, to a Leitz Dialux EB-20 microscope was used to calculate the cellular (CA), nuclear (NA) and cytoplasmic (CytA) areas of 500 reactive cells (100 per animal) of each group and sex, chosen randomly from the different regions of hypophyses, according to the procedure described by Carretero et al. (1988, 1989). The results obtained were evaluated statistically, applying ANOVA test, considering values of $p < 0.05$ as significant. The values obtained are shown in Table 1.

Results

Morphological findings

In normal females, LH-immunoreactive cells were polymorphic, mainly rounded and polygonal. Two kinds of cells could be distinguished, according to the intensity of immunoreaction: one stained intensely, with a homogeneous reaction; the other stained weakly with granular type of immunoreaction (Fig. 1). These cells were in close proximity to blood vessels and were grouped at the craneal pole of the gland, forming two bands (dorsal and ventral) in the medial regions. Scattered cells also appeared throughout the hypophysis.

The control females showed a cellular distribution similar to those of the normal ones, though their cells were more irregular, with evident cytoplasmic prolongations, mainly in medial regions; a weaker cytoplasmic reaction was observed (Fig. 2).

After methoclopramide treatment, the LH-immunoreactive cells showed heterogeneous morphological

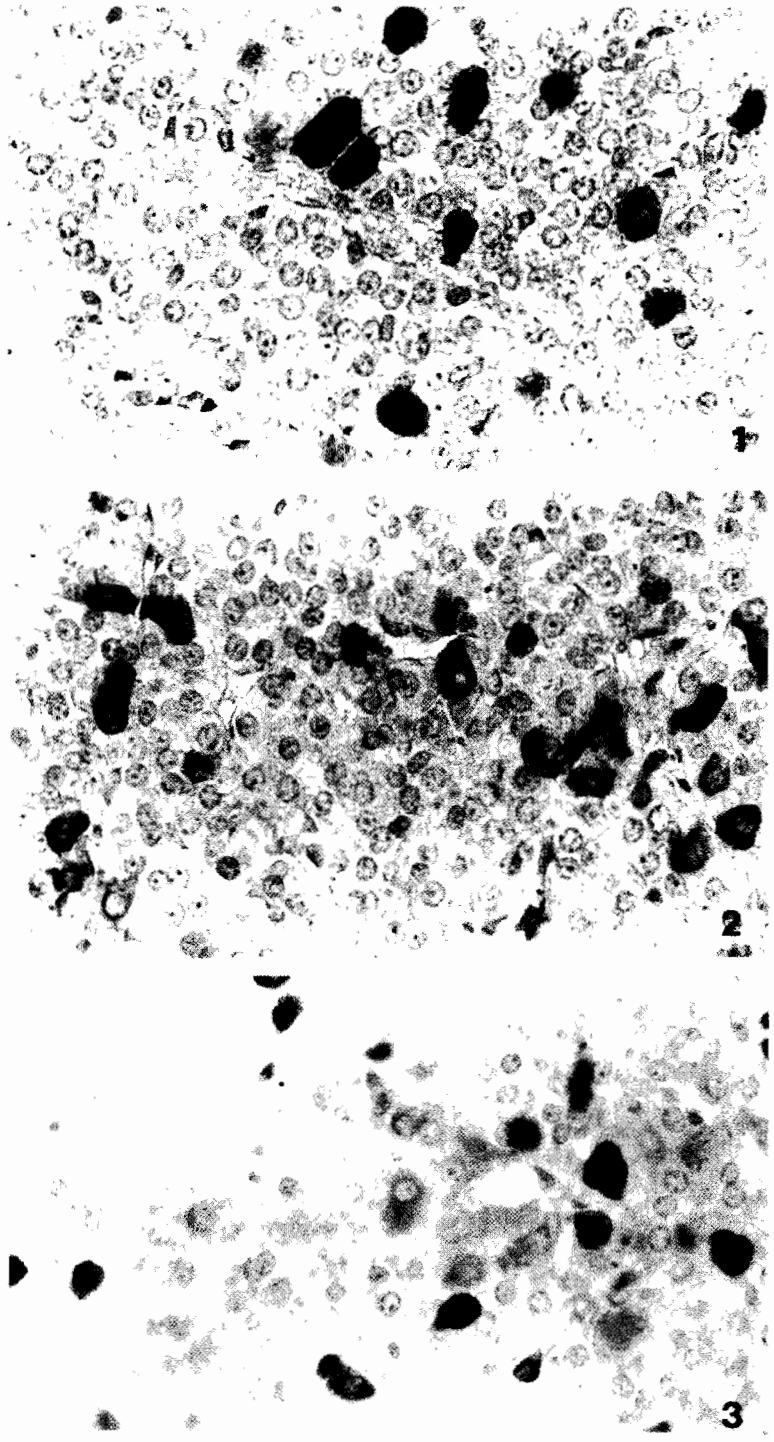

Fig. 1. LH-immunoreactive cells of normal female. × 1,800

Fig. 2. LH-immunoreactive cells of control female. × 1,800

Fig. 3. Micrograph taken from methoclopramide-treated female. × 1,800

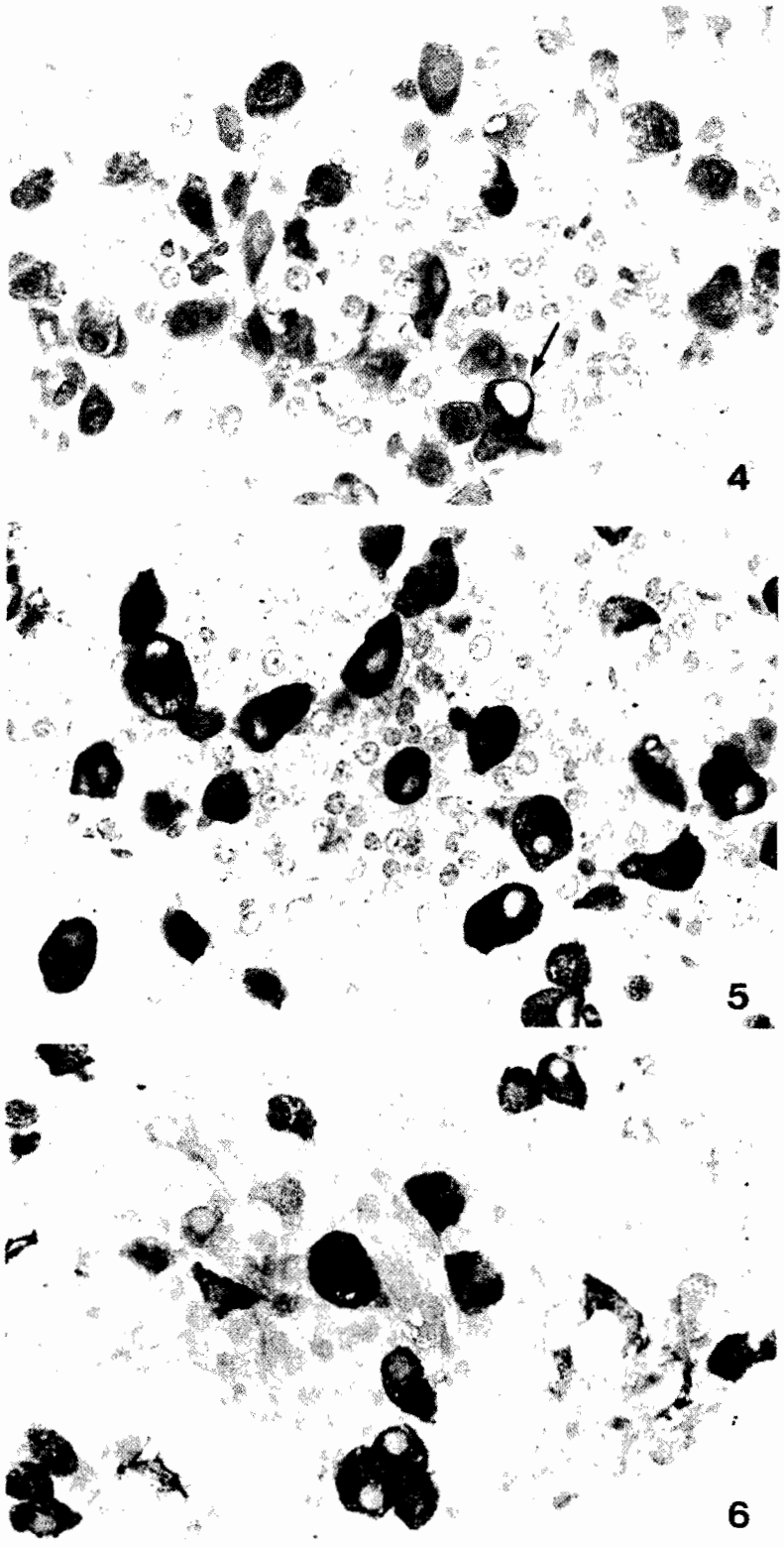

Fig. 4. Image taken from normal male showing LH-immunoreactive cells (arrow: «ring-seal»-shaped cell). × 1,800

Fig. 5. Micrograph of control male showing LH-reactive cells. × 1,800

Fig. 6. LH-immunoreactive cells from methoclopramide-treated male. × 1,800

features: the intensity of immunoreaction was different from one cell to another; there were large and small cells, with or without cytoplasmic processes and with or without negative image of Golgi complex. None of these characteristics were determinant or kept relation among themselves (Fig. 3). The LH-cells in the dorsal band appeared less stained than in the rest of the hypophysis.

In the normal males, LH-immunoreactive cells were large, rounded, and showed a homogeneous and intensely stained cytoplasm. Some of these cells, practically absent in the female rats, exhibited a large vacuole, this resembling «the ring cells» observed following castration (arrow in Fig. 4). Their distribution within the hypophysis was nearly the same as that described for normal females.

The cells of the control males were rounded or polygonal, with short prolongations. The cytoplasm appeared well stained, with a granular aspect (Fig. 5) and small vacuoles. As in the normal males «ring cells» were also observed. The hypophyseal distribution was similar to the other groups described.

Following treatment with methoclopramide, the male rats showed two cellular types: one was large, rounded and intensely stained, with homogeneous cytoplasm exhibiting vacuoles and sometimes «ring cells». The other type was round or polygonal, weakly stained, with a granular aspect and accompanied by small vacuoles. The nucleus was oval or rounded and eccentric in both cellular types (Fig. 6). The localization within the hypophysis was similar with respect to the normal and control animals.

Morphometric findings

Morphometrically, the cellular and cytoplasmic areas of the LH-immunoreactive cells were significantly increased ($p < 0.05$) in the normal male, as compared to the normal female rats.

The stress induced by the administration of physiological saline did not modify the morphometric values observed in the control animals and were very similar to those observed in the normal animals. When male and female rats in this group were compared, the males maintained a significantly ($p < 0.05$) greater CA and CytA than females.

Following treatment with methoclopramide a significant decrease was observed in CA, CytA and NA in both sexes, as compared to the normal and control animals. In these animals, the males showed a significant difference ($p < 0.05$) with respect to the females.

Table 1. Mean morphometric values (± SD) found in the different groups of animals studied.

	CA	CytA	NA
Nor F	125.23 ± 35.46	98.50 ± 32.41	26.73 ± 5.58
Cont F	125.58 ± 32.43	97.24 ± 30.92	28.34 ± 3.90
Met F	98.57 ± 35.25*	75.15 ± 31.19**	23.42 ± 3.86*
Nor M	150.79 ± 34.41·	122.74 ± 30.54·	28.06 ± 7.30
Cont M	155.31 ± 47.32#	127.65 ± 45.35#	27.42 ± 6.53
Met M	129.02 ± 48.29··	105.34 ± 47.98··	23.68 ± 9.72··

F: females, M: males, Nor: Normal animals, Cont: Control Animals, Met: Methoclopramide-treated animals. (*: $p < 0.01$ with respect to normal and control females, **: $p < 0.05$ with respect to normal and control females, ·: $p < 0.05$ with respect to normal females, #: $p < 0.05$ with respect to control females, ··: $p < 0.05$ with respect to Methoclopramide-treated females and $p < 0.01$ with respect to normal and control males.

Discussion

Our findings point to a decrease in the cellular, cytoplasmic and nuclear areas in both sexes after methoclopramide administration. Nevertheless, in the female rats the decrease in CytA was more pronounced than in the NA, while in the male rats the changes were similar. Hence, functional valuation might be, at least, partially different, and it is more evident if we consider that, in rats, there seems to be a smaller quantity of LH (Dupont and Schwartz, 1971; Döller et al., 1977), and a greater response to its release by LHRH stimulation in females than in males (Watanabe, 1986); these findings confirm our own results which showed different intensities, shapes and sizes in immunoreactive cells of the three groups studied.

It seems clear that methoclopramide is able to cause cellular atrophy, indicating that dopamine has a stimulatory role in the regulation of the synthesis and release of LH; this is consistent with earlier analytical findings (Advis et al., 1980; Negro-Vilar et al., 1982). The above-reported differences between male and female rats suggest that the stimulating effect due to dopamine is mediated by ovarian steroids, as Krieg and Sawyer (1976), attending to analytical findings have pointed out. Sex-dependent differences in the response of LH to stimulus by LHRH have also been reported (Tang and Tang, 1979; Denef and Andries, 1983; Loughlin et al., 1984; Watanabe, 1986) and it also seems clear that the function of the dopaminergic system is mediated by the LHRH system (Schneider and McCann, 1969; Bennet et al., 1975; Rotsztejn et al., 1977; Negro-Vilar and Ojeda, 1978).

The difference between both sexes was not only observed with respect to the administration of methoclopramide and hence to the regulatory role of dopamine; although to lesser extents, it was also observed in the normal and control animals, where the CA and CytA were smaller in the females than the males. This is consistent with earlier findings from our laboratory (Carbajo et al., 1987, 1989).

The polymorphism and irregular shape of the gonadotrophic cells are suggestive of cellular hypofunction, as can be inferred from previous works (Blake, 1980; Inoue and Hagino, 1984). Additionally, the decrease in the CA is related to the inhibition of the release of gonadotrophic hormones (Dube et al., 1987) since these cells exhibit a diminished response to stimulus by LHRH (Denef et al., 1982). This is even more evident when decreased in NA (Batten and Wigham, 1984) and the CytA are observed.

Acknowledgements. The authors would like to thank Dr. Sánchez-Franco and Dr. Cacicedo Egues (Endocrinology Service of the Ramón y Cajal Hospital in Madrid, Spain) for kindly providing the LH and FSH hormones (NIH) for the preabsorbtion tests. This paper was supported by CAICYT grant 3018/83 and FIS 85/913.

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Accepted August 10, 1990