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Immunohistochemical-morphometric study of the LH-adenohypophyseal cells following chronic treatment with met-enkephalin

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Summary. In order to test the possible effect of chronic treatment with met-enkephalin upon the LH-adenohypophyseal cells, an immunohistochemical-morphometric study was carried out in rats of both sexes receiving a daily dose of 40 µg of met-enkephalin intramuscularly over 15 days. Following the administration of the opioid, a drastic decrease in the cellular, cytoplasmic and nuclear areas when compared to the normal and control animals was detected. Morphologically, the main finding in males was the appearance of irregularly-shaped pseudovacuolated cells. On the other hand, in females a decrease in the intensity of reaction was found. These results strongly suggest a decrease in the activity of the LH-adenohypophyseal cells following chronic administration of met-enkephalin.

Key words: LH, Adenohypophysis, Met-enkephalin, Rat

Introduction

Nowadays, it is well known that brain opioid peptides are involved in the control of several neuroendocrine processes. They interact with specific binding sites present in the brain (Pfeifer and Herz, 1982; Leadem and Yagenova, 1987).

To date there exists evidence which suggests that brain opioids participate in the control of gonadotropin secretion (Petraglia et al., 1984; Piva et al., 1986) acting directly (Sánchez-Franco and Cacicedo, 1986) or indirectly on the hypophysis by affecting the action of other neurotransmitters (Cicero et al., 1977, 1985; Rotsztejn et al., 1978; Adler and Crowley, 1984).

However, the results obtained with the administration of different opioids and opioid antagonists suggest that brain opioids may either inhibit luteinizing hormone or stimulate the same (Meites et al., 1979; Cicero et al., 1980; Motta and Martini, 1982; Sylvester et al., 1984; Piva et al., 1986). Finally, it seems clear that the effects of opioids and their antagonists on gonadotropin secretion might be modulated by the endocrine status at the time of sacrifice, especially by the steroid environment (Bhanot and Wilkinson, 1983a,b, 1984; Petraglia et al., 1984; Van Vugt et al., 1984).

Despite all the studies carried out, there are very few works that have examined the morphological changes taking place in the LH-immunoreactive hypophyseal cells following chronic administration of opioids. Thus, the aim of the present study was to test the morphological-morphometric characteristics of these cellular types after chronic administration of met-enkephalin to male and female rats.

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Materials and methods

Subjects

A total of 30 adult male and female Sprague-Dawley rats were employed (15 per sex). The animals were divided into 3 groups. *Normal animals*: This group was formed by 10 animals (5 per sex) that were kept under standard stabling conditions (temperature 20±2 °C, controlled light regime (8:00-20:00 h), relative humidity 50±5%, and a balanced diet and water *ad libitum*). *Control animals*: This group comprised 10 animals (5 per sex) that were treated with 50 µl of physiological saline intramuscularly over 15 days (one dose/day at 11:00 h). *Treated animals*: This group was formed of 10 animals (5 per sex) that were treated with 40 µg of met-enkephalin in 50 µl of distilled water intramuscularly over 15 days (one dose/day at 11:00 h). All the females employed in the study were in the proestrus phase on the first day of the experiments as was checked by vaginal smears.

Sample processing

Following treatment, at 6 h after the last administration, with a view to avoid the acute effect of the drug, the animals were sacrificed and the hypophyses carefully dissected out. The glands were fixed in Bouin-Hollande fluid for 5 days and embedded in paraffin to obtain serial sections of 5 µm for the immunocytochemical study.

Immunocytochemistry

The samples obtained were processed according to the enzymatic PAP immunohistochemical method (Sternberger et al., 1970), using anti-LH serum (Dako, A-527) at a dilution of 1:1200, overnight at 4 °C, anti-rabbit swine serum (Dako Z-196) at a dilution of 1:100 and soluble PAP complex (Dako, Z-113) at a dilution of 1:100. The reaction was visualized using a 0.05% solution of 3,3'-diaminobenzidine in Tris-HCl buffer containing a 0.01% hydrogen peroxide. For the washings and dilutions of the sera, Tris-saline buffer (0.05M, pH 7.4) with 0.8% NaCl was used. Preabsorption, with r-LH (NIH), and substitution tests, with normal rabbit serum, were carried out. No residual staining was found.

Morphometry

Using an Apple digital planimeter connected to an RCA video system, the cellular, nuclear and cytoplasmic areas of 100 reactive cells chosen randomly from the adenohypophysis of each animal were calculated according to the protocol described by Carretero et al. (1991) measuring a total of 500 cells per group and sex. Table 1 shows the mean values (±SD) obtained in each of the groups studied. The differences observed were analyzed using the ANOVA test, values of $p<0.05$ for Fisher PLSD and Scheffe-F jointly were considered as significant.

Results

Morphological findings

In normal females LH-immunoreactive cells showed a variable morphology, with a predominance of oval or polygonal shapes. The reaction intensity of the cytoplasm permitted the differentiation of two cell types: the first comprised cells intensely immunostained, with a cytoplasm that had reacted homogeneously; the second type, less abundant, comprised weakly-stained elements with a granular irregularly-stained cytoplasm. In both, the nucleus was generally rounded or oval and large and was located in the centre of the cell or displaced towards one of the sides. No vacuolated cells were observed (Fig. 1).

In the control females, cellular morphology was similar to that observed in the normal females, although they sometimes exhibited small cytoplasmic prolongations conferring them a more irregular aspect (Fig. 2).

Following treatment with met-enkephalin, the weakly-stained cells were as numerous-or more so- as the well-stained ones; the nucleus was round and was seen to occupy a large part of the cell. The morphology of these cells was highly variable and was occasionally irregular (Fig. 3).

The LH-immunoreactive cells of the normal males were generally large with a rounded and usually eccentric nucleus. The cytoplasm was homogeneous and well-stained, although less so than those observed in normal females. In this sex, the vacuolated cells, similar to «ring-seal»-shaped cells or «castration cells» were frequently seen (Fig. 4).

In the control males, the LH-immunoreactive cells were similar to those observed in normal males, although they showed weaker intensity of the reaction (Fig. 5).

Following chronic treatment with met-enkephalin,

Fig. 1. Female rats. Normal group: Note the presence of intensely-stained cells together with weakly-stained cells. x 400

two cellular types were clearly observable: well-stained cells, in lower number, and weakly-stained cells which were very numerous. Both cellular types had a granular aspect and an irregular morphology, which varied from one cell to another. Some cells exhibited cytoplasmic vacuolizations, although no sealed-ring cells were observed (Fig. 6).

Morphometrical findings

Table 1 shows the mean values (μm^2) together with the standard error of all the parameters analyzed in the different groups of animals studied.

Analysis of these results shows that there was a marked and statistically significant difference between the male and female animals ($p<0.05$) with respect to the size of the LH-immunoreactive cells; in this sense, the cellular area was greater in males than in females in all the experimental groups. This difference was mainly due

Table 1. Morphometric mean values (±SD), expressed in μm^2 of Cellular (CA), Cytoplasmic (CytA) and Nuclear (NA) areas observed in normal (Nor), control (Cont) and met-enkephalin (Met-Enk)-treated female and males rats.

	CA	CytA	NA
Females			
Nor	125.23±35.46	96.50±32.41	28.73±5.58
Cont	125.58±32.43	97.24±30.92	28.34±3.90
Met-Enk	103.94±14.57*	80.44±13.43*	23.93±4.76##
Males			
Nor	154.79±34.41**	123.74±30.54**	31.06±7.30
Cont	155.31±47.32**	124.65±45.35**	30.42±6.53
Met-Enk	123.67±39.89#	98.50±36.58#	25.17±6.39###

*: $p<0.05$ with respect to normal and control males; **: $p<0.05$ with respect to female rats; #: $p<0.05$ with respect to normal and control males and met-Enk-treated females; ##: $p<0.01$ with respect to normal and control females; ###: $p<0.01$ with respect to normal and control males, and $p<0.05$ with respect to met-Enk-treated females.

Fig. 2. Female rats. Control group: Similar characteristics to those observed in the normal group. x 400

Fig. 3. Female rat. Met-enkephalin-treated group: Higher presence of weakly immunoreactive cells. x 400

to different sizes of the cytoplasmic area, since the nuclear area showed no significant variations between the two sexes. In both sexes, animals considered as control had morphometric values almost identical to those of the normal animals.

Morphometrically, in both sexes treatment with met-enkephalin produced a decrease ($p<0.05$) in the cellular area, accompanied by a significant decrease in the cytoplasmic ($p<0.05$) and the nuclear ($p<0.01$) areas when compared to normal and control animals.

Discussion

The administration of morphine or opioids (including met-enkephalin) depresses the basal levels (Bruni et al., 1977) and the preovulatory LH-peak (Ieiri et al., 1980). By contrast, the administration of opioid antagonists raises LH levels (Johnson and Crowley, 1984; Sylvester et al., 1984; Nazian, 1992).

The findings of the present study seem to confirm, in morphological terms, the analytical results described previously. Based on the experiments of Tang and Tang (1979), Louglin et al. (1984), Dada et al. (1984) and Watanabe (1986) a working hypothesis is that the existence of a decrease in cellular area, accompanied by a reduction of the cytoplasmic and nuclear areas, points to a state of cellular hypoactivity with a decrease in the synthesis and release of LH (Batten and Wigham, 1984; Wendelaar et al., 1984; Carretero et al., 1988). Accordingly, the morphometric alterations observed by us after chronic treatment with met-enkephalin are indicative of a decrease in LH synthesis and release which would confirm the generally accepted inhibitory role of opioids in the regulation of LH secretion (Meites et al., 1979; Ferrin et al., 1984), which morphologically becomes manifest as the appearance of numerous weakly reactive cells with irregular morphologies.

This effect could be mediated through a direct action

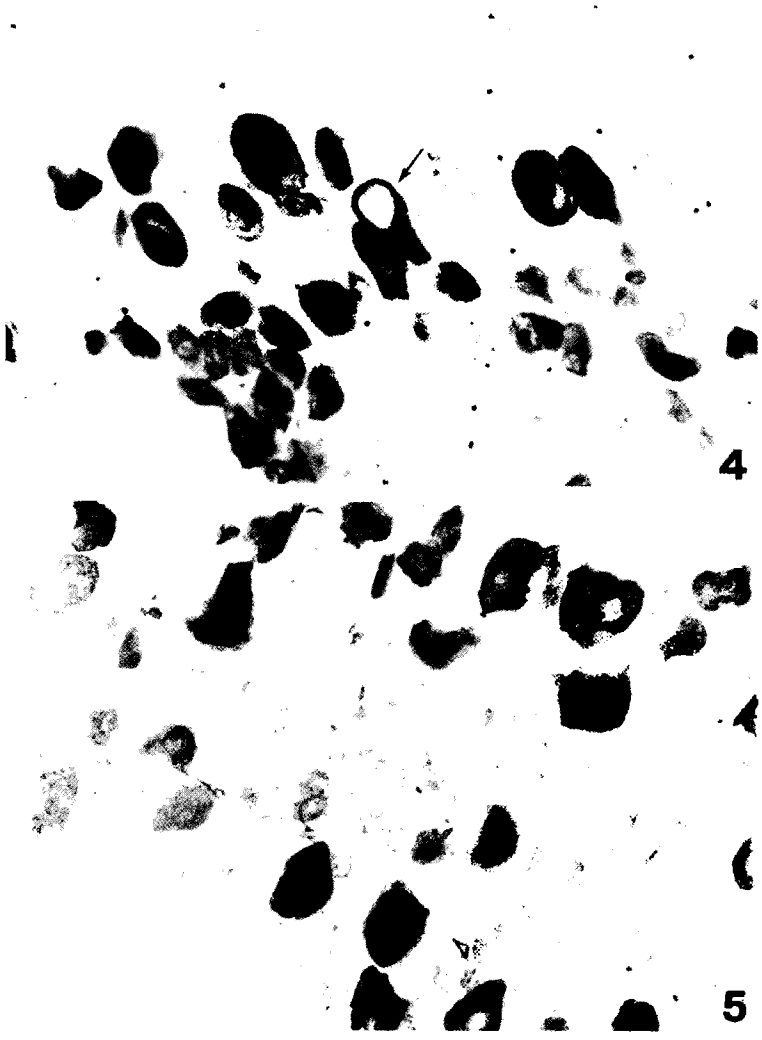

Fig. 4. Male rats. Normal group: Some vacuolated cells were present (arrow). x 400

Fig. 5. Male rat. Control group: A slight decrease in the staining is noticed. x 400

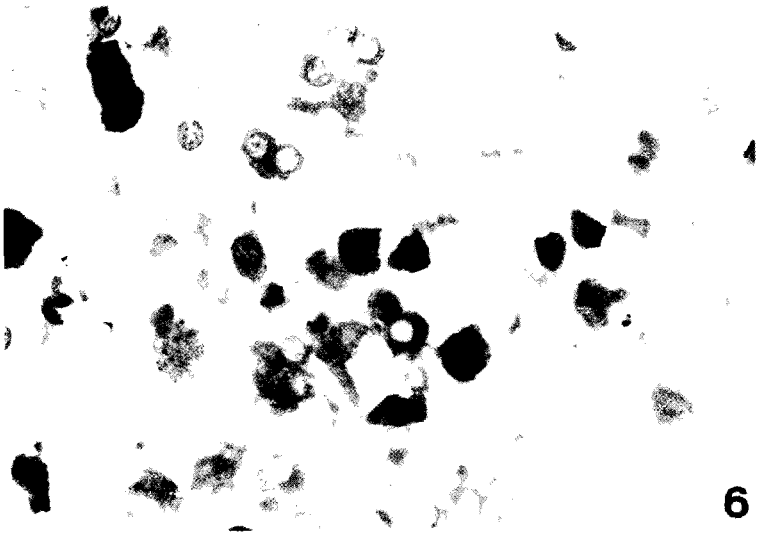

Fig. 6. Male rat. Met-enkephalin-treated group: Numerous weakly immunoreactive cells are detected. x 400

of the opioid on LH cells (Cacicedo and Sánchez-Franco, 1986; Sánchez-Franco and Cacicedo, 1986; Blank et al., 1986) or through an action on the LHRH system, or on both at the same time, as suggested by Currie and Rawlings (1987) and this effect does not seem to be affected by steroids, as pointed out by Bhanot and Wilkinson (1984), but would rather act independently, as proposed by Babu et al. (1987) and Orstead and Spies (1987). In our case it is consistent with the morphometric findings, since similar morphometric alterations were observed in both sexes. This is not so, however, in the case of the cell morphology, since in the male animals the cells had a very granular aspect while this only occurred in a few cells in the female rats.

The decrease in size, accompanied by a reduction in cytoplasmic area and nuclear area, and the decrease in the reaction intensity allows us to conclude that chronically intramuscularly-administered met-enkephalin leads to a state of cellular hypoactivity that affects both sexes, although to a different extent, and coincides with the inhibitory effects of the opioid reported earlier by other authors.

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