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Sex-specific response of the vasopressin-reacting neurons of the paraventricular nucleus of the rat hypothalamus following chronic administration of met-enkephalin

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Summary. Using the peroxidase-antiperoxidase immunocytochemical technique, a morphometric study of the magnocellular neurons of the Paraventricular nucleus of the rat hypothalamus, reactive to specific anti-vasopressin rabbit serum, was made. Following systemic and chronic administration of met-enkephalin the number of immunoreactive neurons was higher, especially in females. Additionally, in the females, it was possible to observe an increase in the immunoreactivity and the presence of well-stained fibres. These findings suggest, especially in females, a blockage in the release of vasopressin, facilitating its immunocytochemical visualization.

Key words: Vasopressin, Met-Enkephalin, Paraventricular Nucleus, Rat

Introduction

It is well known that opiates modulate the release of vasopressin (VP). Indeed, pharmacological and physiological studies have provided evidence for an inhibitory role on VP release (Clarke et al., 1979; Van Wimersma Greidanus et al., 1979, 1981; Lightman et al., 1982).

Recently, our group demonstrated a sex-specific effect of methionine-enkephalin (Met-Enk) on the VP-immunoreactive neurons of the rat supraoptic nucleus (SON) when the substance was administered intraventricularly (Blanco et al., 1989).

In the last two decades different authors have highlighted the possible role of opiate drugs when administered systemically. Different effects on the release of some adenohypophyseal hormones such as prolactin have been demonstrated (Meites et al., 1979; Spies et al., 1980). However, there are no morphometric references concerning the possible effect of systemic and chronic administered Met-Enk on the VP-producing neurons of the paraventricular nucleus (PVN).

On the basis of the foregoing, in the present study, our aim was to elucidate the possible effect of Met-Enk, administered through a systemic route, on the VP-reactive magnocellular neurons of the PVN.

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Materials and methods

Subjects

Animals were housed in standard laboratory conditions: seasonal light-dark cycle, temperature 22±2°C, relative humidity 50±5%, and food and water ad libitum.

Thirty male and female Sprague-Dawley rats were used, divided into three groups: 1) Normal animals (n=10, 5 per sex); 2) Control rats (n=10, 5 per sex) that received a daily dose of physiological saline intramuscularly (100 µl) over 14 days; 3) 10 rats (5 per sex) that received a daily (14 days) intramuscular injection of Met-Enk (20 µg/100 µl distilled water, as in previous papers (Blanco et al., 1989). All experiments in the female rats were performed in the pro-oestrus phase as determined by vaginal smears.

Sample processing

After sacrifice by decapitation, the hypothalamic-hypophyseal block was fixed by immersion in Bouin-Hollande solution and embedded in paraffin. Serial 5 µm sagittal sections were obtained with a microtome.

Immunocytochemistry

After inhibiting endogenous peroxidase by passing the samples through a methanol H_2O_2 bath (Streefkerk, 1972), the sections were pretreated with normal swine serum (DAKO). Following this, the PAP immunocytochemical method was applied (Sternberger et al., 1970).

As primary serum, anti-VP serum was employed (kindly supplied by Dr. Sánchez-Franco, Endocrinology Service, Ramón y Cajal Hospital, Madrid) (Negro-Vilar et al., 1979; Sánchez-Franco et al., 1986) at a dilution of 1/1000. The rest of the sera (anti-rabbit immunoglobulin swine serum and PAP soluble complex) were supplied by DAKO. A battery of immunocytochemical controls was carried out: absorption of the primary serum with VP (SIGMA) at a proportion of 10 nmol per 0.1 ml of diluted primary serum produces a total abolition of the immunoreactivity. Studies were also performed in which the primary serum was substituted by washing buffer of normal rabbit serum.

Morphometry

As in a previous paper (Sánchez et al., 1990), the number of cells was calculated by analyzing all the VP-immunoreactive cells. Only the cells in which the nucleus and nucleolus were present were considered. Calculation of the total number of stained magnocellular cells in each subdivision of the PVN was made following the protocol proposed by Rhodes et al. (1981) using a correction factor of 0.333, according to the formula of Abercrombie (1946),

$$\frac{\text{section thickness}}{\text{object length + section thickness}}$$

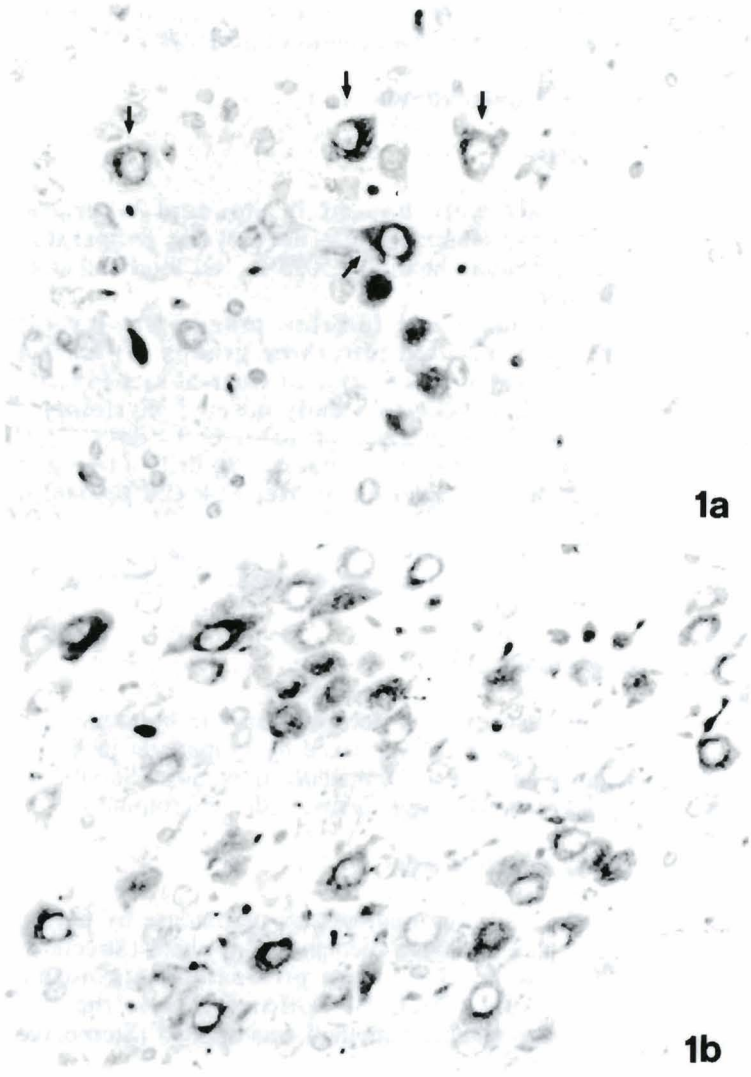
Calculation of the number of cells was carried out with an Apple digital planimeter connected to an RCA video system.

Statistical test

The values of the parameters obtained were compared statistically using an ANOVA test. Values of $p<0.05$ were considered significant.

Results

In the present analysis we considered two



Figs. 1. Normal animals. **a.** Male. Only a few scattered VP-reacting neurons can be seen in the commissural subdivision (arrows). **b.** Female. Most VP-reacting neurons are situated in the posterior subdivision. x 200

Table 1. Number of VP-reacting cells in the commissural and posterior magnocellular subdivisions.

GROUP	COMMISSURAL		POSTERIOR	
	MALES	FEMALES	MALES	FEMALES
NORMAL	40±14	38±13	1215±237	1195±219
CONTROL	35±12	34±12	1180±220	1201±221
TREATED	50±17	64±16*	1380±243	1601±253*

*: P<0.05 when compared to the rest of the groups.

magnocellular subdivisions: «commissural» (following Peterson, 1966; fairly superimposable to the anterior and medial magnocellular subdivisions proposed by Swanson and Kuypers in 1980); and «posterior» (following Swanson and Kuypers, 1980). This division of the magnocellular component was used in previous papers (Sánchez et al., 1988, 1990, 1992; Alonso et al., 1992).

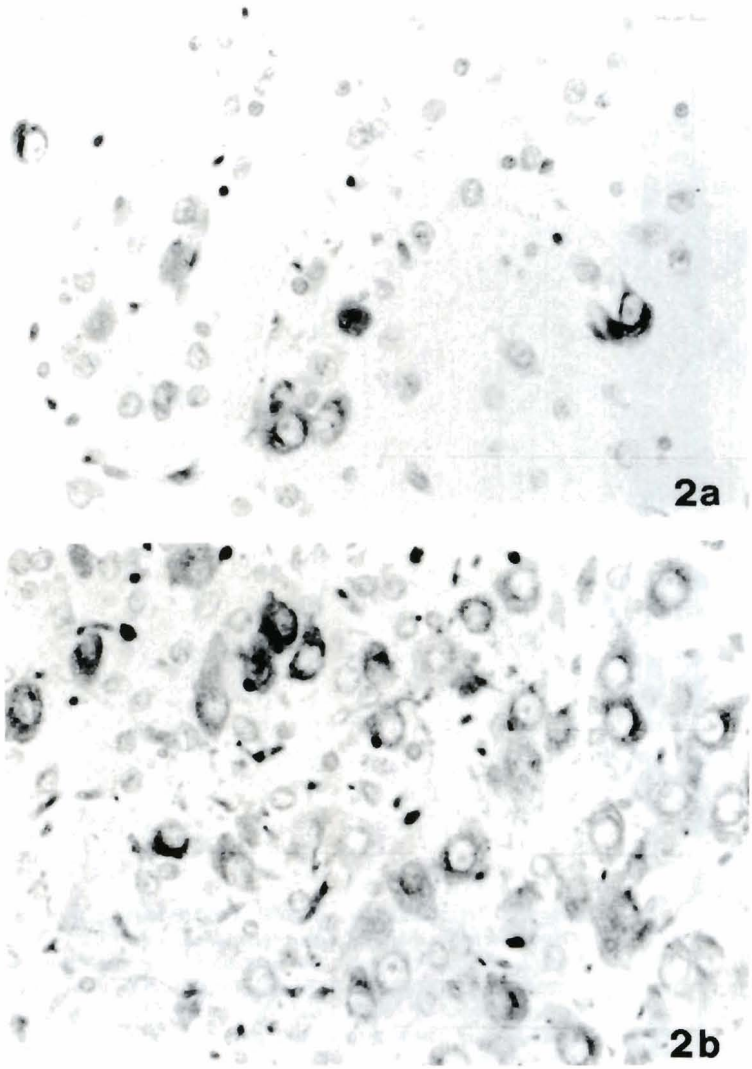
Normal animals

Only a few reacting cells were seen in the posterior part of the commissural subdivision (Fig. 1a). In both males and females, most VP-reacting neurons were observed in the posterior subdivision (Fig. 1b). In both subdivisions considered, the distribution of immunoreactive material in the cytoplasm was homogeneous, although different neurons showed a variable intensity of staining (Fig. 1b).

In the morphometric parameters, no significant statistical differences were found between the male and female animals ($p>0.05$) in any of the subdivisions studied (Table 1).

Control animals

The morphological characteristics observed were fairly similar to those of the latter group (Figs. 2a,b). In the morphometric study no statistical differences were



Figs 2. Control animals. Note the same morphological characteristics as in the previous group. Commissural (**a.** Male) and posterior (**b.** Female) subdivisions. x 200

found when compared to the normal animals (Table 1).

Treated animals

In males, no morphological differences were found when compared to the previous groups (Fig. 3a). After Met-Enk treatment immunostaining was more intense, especially in the females, with well-stained axons, mainly located in the posterior subdivision (Fig. 3b). The rest of the morphological characteristics observed were similar to the other groups.

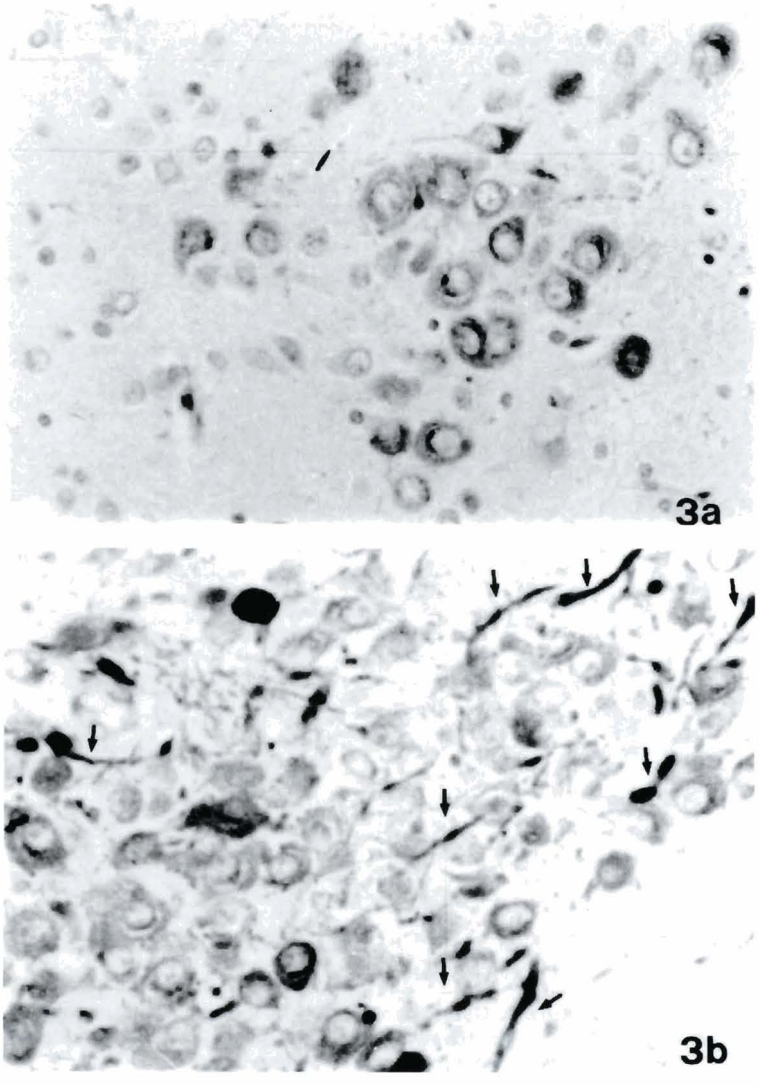
Following treatment with Met-Enk the number of immunoreactive neurons was higher, especially in females. Only in the latter the number of cells was statistically significant (Table 1).

Discussion

As has been pointed out we have previously demonstrated in the supraoptic nucleus a sex-specific inhibitory effect of Met-Enk on VP-release in the VP-reacting neurons of the SON (Blanco et al., 1989) when the opiate was administered intraventricularly. It is also well known that enkephalins regulate the release of VP, although the exact mechanism by which this occurs remains to be elucidated (Martin et al., 1983; Van Leuwen et al., 1983). However, it is accepted that magnocellular hypothalamic neurons are controlled by endogenous opiates.

Regarding the possible route of access of Met-Enk from the peripheral blood, several studies have shown that enkephalins are subject to rapid enzymatic degradation by peptidases and enkephalinases (Hambrook et al., 1976; Gorenstein and Snyder, 1980; Pardridge and Meitus, 1981; Schwartz, 1983).

It has also been shown that the half-life of Met-Enk in peripheral blood is one minute and that the cerebral uptake index is 15% (Kastin et al., 1976) since its transport across the blood brain barrier is slow both when there is a specific system (Kastin et al., 1976,



Figs. 3. Treated animals. **a.** Male: The VP-reacting neurons have the same morphological characteristics as in the former groups. A slight increase in the number of reacting cells can be observed (commissural). **b.** Female: Note the clear increase in the number of VP-reacting cells (Posterior). Additionally some well-stained fibres (arrows) and an increase in the reaction intensity can be noted. x 200

1979; Rapoport et al., 1980) and when transport occurs non-specifically (Conford et al., 1978; Zlovovic et al., 1985).

We believe that this slow uptake would not occur in an acute systemic administration, but might be possible in a case of chronic administration, as is the case of our experiment.

It has also been shown that endogenous opiates are able to stimulate the secretion of certain hypophyseal hormones when administered in both acute and systemic form (Lien et al., 1976; Bruni et al., 1977; Rivier et al., 1977; Meites et al., 1979; Spies et al., 1980). Additionally, Pechnik et al. (1987) have shown that N-methylmorphine, which is unable to cross the blood-brain barrier, is able to stimulate prolactin secretion from peripheral blood. There could be a transport system related with the choroid plexuses and the cerebrospinal fluid which, over long periods of time, would permit a central action of the opiate (Lorenzo, 1977; Begley et al., 1980; Dawson, 1982; Fenstermacher and Dawson, 1982).

The increase in the number of VP-immunoreactive neurons after Met-Enk treatment could reflect a blockage in the release of VP, facilitating its immunocytochemical visualization. This fact is fairly consistent with the increase in the immunostaining and the appearance of well-stained axons, especially in the treated females, and may indicate a clear inhibition in axoplasmic transport.

This inhibition mainly affects the female groups and hence it is likely that this action is mediated by oestrogens, although studies to elucidate the action of Met-Enk in males treated with oestrogens, for instance, should be carried out.

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