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# Neuropeptides in the cat diencephalon: II. Hypohalamus

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## SUMMARY

We have reviewed the distribution and functions of neuropeptides in the cat hypothalamus. Our review focuses in the cat hypothalamus on the following points: 1) the distribution and coexistence of neuropeptides; 2) the anatomical relationships among the different neuropeptides; 3) the peptidergic pathways (afferences and efferences); 4) comparison of the distribution of neuropeptides in the mammalian hypothalamus; and 5) the physiological functions of neuropeptides. Although at present the distribution of many neuropeptides in the hypothalamus of the cat is known, there is little information about other aspects of neuropeptides in the same diencephalic region. Thus, in order to know more the distribution and functions of neuropeptides in the cat hypothalamus in detail, in the future appropriate methodologies must be applied in order to determine, for example, the distribution of the neuropeptide receptors, the distribution of neuropeptidases, the peptidergic synaptic connections, the coexistence of neuropeptides and the physiological actions of the neuropeptides in the cat hypothalamus.

**Key Words:** Neuropeptides – Hypothalamus – Diencephalon – Cat

## INTRODUCTION

The hypothalamus is part of the diencephalon. Along the animal scale, it is one of the most preserved zones of the central nervous system (CNS). This shows that the findings observed in the hypothalamus of animals used for experimental research can be extrapolated to humans. It is a centre that is connected with the limbic system, which is involved in autonomic and homeostasis functions. In this sense, the hypothalamus has been considered as the "great ganglion" of the autonomic nervous system, since it integrates autonomic function at central level (Kupfermann, 1981). In addition, the hypothalamus has been implicated in a large number of very important functions, such as drinking, food intake, thermoregulation, neuroendocrine control of the hypophysis (by means of releasing and inhibiting factors), defence (immunoregulation), circadian rhythms, blood pressure, emotions, stress, reproduction, aggressive behaviour, sexual orientation, as well as in the production of neurohormones (vasopressin, oxytocin,...) (see Swaab, 1997). In sum, it is a small centre in the CNS, but is involved in very important functions. Thus, it has been indicated that lesions in the hypothalamus could elicit several diseases/ alterations: depression, anorexia nervosa, bulim-

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ia, changes in sexual orientation, diabetes insipidus, Cushing's disease, alterations in sleep and temperature, sudden-infant-death-syndrome, Wolfram's syndrome, Prader-Willi's syndrome, malignant syndrome, aggressive behaviour, alterations in the emotions, as well as alterations in the release of hormones into the hypophysis. Moreover, hypothalamic modifications have been reported in neurodegenerative diseases, such as Alzheimer's, Parkinson's, Huntington's, multiple sclerosis,… (see Swaab, 1997).

Moreover, the hypothalamus is connected to a large number of other CNS centres (Carpenter, 1980; Saper, 1990). Thus, the hypothalamus receives inputs from the hippocampus, the amygdala, the cerebral cortex, the retina, the spinal cord, the ventrolateral medulla, the nucleus of the solitary tract, the parabrachial nucleus, the locus coeruleus and the raphe, whereas hypothalamic neurons send projections into the amygdala, the periaqueductal gray, the reticular formation of the mesencephalon, the thalamus, the hypophysis, the nucleus of the solitary tract, the parabrachial nucleus, the locus coeruleus, the nucleus ambiguus, the area postrema, the spinal cord, the nucleus accumbens and the median eminence.

As mentioned in a previous article (Part I: Thalamus) (Coveñas et al., 2001), a large number of neuroanatomical, neurophysiological, neuropharmacological and behavioural data have been reported for the cat. However, until the eighties research on the distribution of neuropeptides in the cat diencephalon has received little attention. Here, our aim is to review, in the cat, currently available morphological and physiological data concerning neuropeptides that have emerged over the past eighteen-twenty years concerning one of the most important functional areas of the CNS: the hypothalamus. We also compare the results obtained on neuropeptides in the cat hypothalamus with those found in the same diencephalic area of others mammalian species (e.g., rat, monkey, human).

## Neuropeptides in the cat hypothalamus

The hypothalamus can be divided according to topographic criteria into several regions (Kupfermann, 1981): mamillar, periventricular, medial, lateral and preoptic (see Table 1). In this review, we use the terminology of the hypothalamic nuclei according to the stereotaxic atlas of the diencephalon of the cat carried out by Jasper and Ajmone-Marsan (1966). Table 1 shows the distribution of fibers and cell bodies containing neuropeptides in the cat hypothalamus, using immunocytochemical methods. In general, as can be seen in the table, the immunoreactive structures (fibers and cell bodies) containing the neuropeptides studied showed a widespread distribution throughout the cat hypothalamus. At present, the distribution of thirteen neuropeptides has been fully studied in the cat hypothalamus. In this sense, the distribution has been studied of methionine-enkephalin (Micevych and Elde, 1980; Coveñas et al., 1988; Yoshimoto et al., 1989), substance P (Burgos et al., 1988; Yoshimoto et al., 1989), neurotensin (Hu et al., 1988; Yoshimoto et al., 1989; de León et al., 1991a), somatostatin-28 (1-12) (de León et al., 1991b), neuropeptide Y (Ueda et al., 1986; Hu et al., 1987; Léger et al., 1987), β-endorphin (1-27) (Coveñas et al., 1996a), β-endorphin (1-31) (Micevych and Elde, 1982), α-melanocyte-stimulating hormone (Micevych and Elde, 1982; Rao et al., 1987; Coveñas et al., 1996b), adrenocorticotropin hormone (Kitahama et al., 1984, 1986; Rao et al., 1986; Coveñas et al., 1996c), luteinizing hormone-releasing hormone (Barry and Dubois, 1975; Belda et al., 2000), neurokinin A (Velasco et al., 1993), delta sleep-inducing peptide (Charnay et al., 1990) and vasoactive intestinal polypeptide (Obata-Tsuto et al., 1983, 1984) (see Figure 1). Also, there are very few data concerning the distribution of another seven neuropeptides (not shown in Table 1): galanin, corticotropin-releasing factor, somatostatin-14, vasopressin, oxytocin, cholecystokinin octapeptide and thyrotropin-releasing hormone (Micevych and Elde, 1980; Kawata et al., 1982; Graybiel and Elde, 1983; Wahle and Albus, 1985; Caverson et al., 1987; Yoshimoto et al., 1989). Thus, immunoreactive cell bodies containing galanin were observed in the hypothalamus ventromedialis and in the regio praeoptica; those containing corticotropin-releasing factor in the hypothalamus posterior, hypothalamus lateralis, nucleus supraopticus, nucleus periventricularis hypothalami and in the regio praeoptica; those containing thyrotropin-releasing hormone in the regio praeoptica and around the anterior hypothalamic nucleus; those containing vasopressin in the nuclei periventricularis hypothalami, suprachiasmaticus and supraopticus, hypothalamus lateralis and in the regio praeoptica; those containing oxytocin in the hypothalamus dorsomedialis, hypothalamus lateralis, regio praeoptica and in the nuclei periventricularis hypothalami and supraopticus, and those containing cholecystokinin in the nuclei arcuatus and periventricularis hypothalami, as well as in the hypothalamus lateralis. In addition, somatostatin-14-, galanin-, corticotropin-releasing factor-, and thyrotropin-releasing hormone-immunoreactive fibers were observed in the hypothalamus posterior, whereas fibers containing vasopressin and oxytocin have been described in the nuclei periventricularis hypothalami and supraopticus.

Furthermore, using a microdissection technique combined with radioimmunoassay the

**Table 1.-** Distribution of neuropeptides in the nuclei of the cat hypothalamus.

| | MET-E | | SP | | NT | | SOM | | NPY | | β-END | | α-MSH | | ACTH | | LH-RH | | NKA | | β-END (1-31) | | DSIP | | VIP | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | F | CB | F | CB | F | CB | F | CB | F | CB | F | CB | F | CB | F | CB | F | CB | F | CB | F | CB | F | CB | F | CB |
| **MAMILLAR REGION** | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Ml | + | + | - | + | - | - | - | - | + | - | + | - | + | - | - | - | + | - | + | + | | | + | - | | |
| Mm | - | + | - | + | - | - | - | - | + | - | + | - | RIA + | - | - | - | + | - | - | - | | | + | - | | |
| **PERIVENTRICULAR REGION** | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Arc | + | + | + | + | + | + | + | - | + | + | + | + | RIA + | + | + | + | + | + | + | - | + | + | + | + | + | + |
| **MEDIAL REGION** | | | | | | | | | | | | | | | | | | | | | | | | | | |
| aHd | + | + | + | + | + | + | + | + | + | - | + | - | RIA + | + | + | - | + | - | + | + | - | - | + | - | - | |
| Ha | - | + | + | - | + | - | - | + | + | - | + | - | RIA + | - | + | - | + | + | + | - | | | + | + | - | |
| Hdm | - | + | - | - | + | - | + | + | + | - | + | - | + | + | + | - | - | - | + | + | + | - | - | - | | |
| Hp | + | + | + | + | + | + | + | + | + | - | + | - | RIA + | - | + | - | - | - | + | + | - | - | - | - | - | - |
| Hvm | + | + | + | + | + | + | + | - | + | + | + | + | RIA + | + | + | + | + | - | + | + | + | - | + | - | - | - |
| PVH | - | + | + | + | + | - | - | + | + | - | + | - | RIA + | - | + | - | + | - | + | + | - | - | + | - | + | + |
| Sch | + | + | + | + | + | + | + | + | + | - | + | - | RIA + | - | + | - | + | - | + | - | - | - | + | + | + | + |
| **LATERAL REGION** | | | | | | | | | | | | | | | | | | | | | | | | | | |
| HL | + | + | + | + | + | + | + | - | + | - | + | - | + | + | + | - | + | - | + | + | - | - | + | - | + | + |
| So | + | + | + | + | + | + | + | + | + | - | + | - | RIA + | - | + | - | + | + | + | - | - | - | + | + | + | + |
| **PREOPTIC REGION** | | | | | | | | | | | | | | | | | | | | | | | | | | |
| RPO | + | + | - | + | - | + | + | + | + | - | + | - | RIA + | - | + | - | + | + | + | - | - | - | + | + | | |
| **TRACTS** | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Fx | + | - | + | - | - | - | - | - | - | - | + | - | + | - | + | - | + | - | - | - | - | - | + | - | | |
| MFB | - | - | + | - | - | - | - | - | - | - | - | - | RIA + | - | - | - | + | - | - | - | | | - | - | | |
| TMT | + | - | - | - | + | - | - | - | + | - | - | - | RIA - | - | + | - | + | - | - | - | - | - | - | - | | |

**ACTH**: adrenocorticotropin hormone (18-39); **DSIP:** delta sleep-inducing peptide; **β-END**: β-endorphin (1-27); **β-END (1-31):** β-endorphin (1-31); **LH-RH**: luteinizing hormone-releasing hormone; **MET-E**: methionine-enkephalin; **α-MSH**: α-melanocyte-stimulating hormone; **NKA**: neurokinin A; **NPY:** neuropeptide Y; **NT**: neurotensin; **RIA**: radioimmunoassay; **SOM:** somatostatin-28 (1-12); **SP**: substance P; **VIP:** vasoactive intestinal peptide. For the nomenclature of the hypothalamic nuclei, see list of abbreviations. **CB:** immunoreactive cell bodies; **F**: fibres; **+**: presence; **-**: ausence; **no sign** : no studied.

concentration of α-melanocyte-stimulating hormone has been measured in several regions of the cat hypothalamus (O'donohue et al., 1979), and the concentration of neurotensin and kassinin in the whole cat hypothalamus has also been measured (Goedert and Emson, 1983; Hunter et al., 1985). Finally, the localization of calcitonin-, and neurokinin-1 binding sites has been demonstrated in the cat hypothalamus (Guidobono et al., 1987; Yao et al., 1999).

Except for β-endorphin (1-31) and vasoactive intestinal polypeptide, the other neuropeptides studied (eleven) in the cat hypothalamus showed a widespread distribution in this diencephalic region (see Table 2). Thus, in all the hypothalamic nuclei of the cat, immunoreactive fibers and/or cell bodies containing methionine-enkephalin, neuropeptide Y, β-endorphin (1-27) and α-melanocyte-stimulating hormone were observed, whereas substance P-, luteinizing hormone-releasing hormone- and neurokinin A-immunoreactive fibers and/or cell bodies were found in twelve of the thirteen hypothalamic nuclei and in eleven of the thirteen nuclei fibers and/or cell bodies containing neurotensin, somatostatin-28 (1-12), adrenocorticotropin hormone and delta sleep-inducing peptide.

Finally, in all the hypothalamic nuclei of the cat, seven or more neuropeptides were described (Table 3). The hypothalamic nucleus in which the highest number of neuropeptides (thirteen) was observed in fibers and/or cell bodies was the nucleus arcuatus, whereas in other nuclei, such as the hypothalamus ventromedialis, periventricularis hypothalami, suprachiasmaticus, hypothalamus lateralis and supraopticus, twelve neuropeptides were seen in each of them.

## COEXISTENCE OF NEUROPEPTIDES IN THE CAT HYPOTHALAMUS

As indicated in the previous chapter, the presence of at least seven neuropeptides in each of the hypothalamic nuclei has been reported. This indicates a possible interaction among such neuropeptides and an elaborate modulation of functions in which the hypothalamic nuclei are involved. In addition, the localization of several different neuropeptides in the same hypothalamic nuclei indicates the possibility that two or more of them may coexist in the same neuron. This research line must be developed in the future, since only a few data showing the coexistence of neuropeptides in the neurons of the cat hypothalamus are available. Thus, Micevych and Elde (1982) reported that α-melanocyte-stimulating hormone and β-endorphin coexist in neurons located in the cat nucleus arcuatus, whereas the coexistence of delta sleep-inducing peptide and luteinizing hormone-releasing hor-

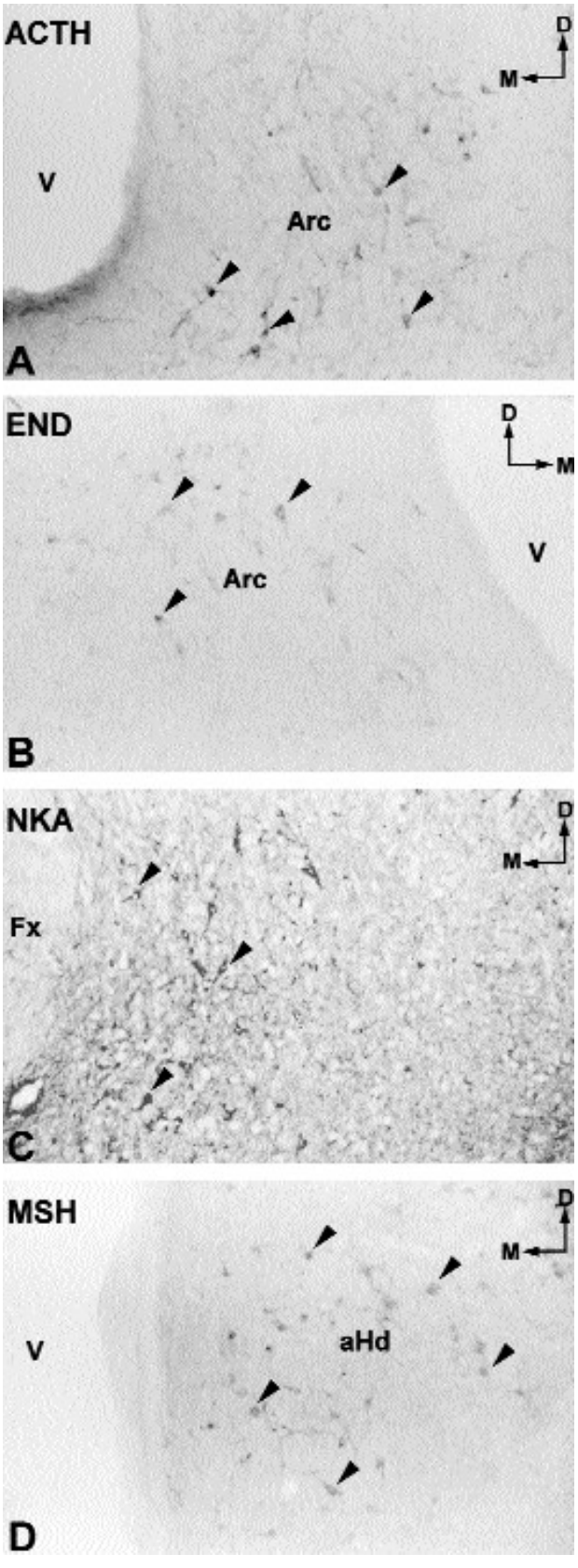


**Fig. 1.-** Neuropeptides in the cat hypothalamus. **A:** Immunoreactive fibers and cell bodies (arrowheads) containing adrenocorticotropin hormone (ACTH) in the nucleus arcuatus (Arc). D: dorsal; M: medial; V: ventricle. x 125. **B:** Immunoreactive cell bodies (arrowheads) containing β-endorphin (1-27) (END) in the nucleus arcuatus (Arc). D: dorsal; M: Medial; V: ventricle. x 160. **C:** Neurokinin A (NKA)-immunoreactive fibers and cell bodies (arrowheads) located around the fornix (Fx). D: dorsal; M: medial. x 100. **D:** Immunoreactive cell bodies (arrowheads) containing α-melanocyte-stimulating hormone (MSH) in the area hypothalamica dorsalis (aHd). D: dorsal; M: medial; V: ventricle. x 100.

mone has also been described in the cat hypothalamus (Charnay et al., 1990). Moreover, the possible coexistence of neurotensin and α-melanocyte-stimulating hormone can be suggested for cell bodies located in the nucleus arcuatus because the morphological characteristics of perikarya containing α-melanocyte-stimulating hormone are similar to the neuronal population containing neurotensin (de León et al., 1991a; Coveñas et al., 1996b). By contrast, in the cat nucleus arcuatus, the existence of cell bodies containing substance P, neuropeptide Y and neurotensin has been described (Léger et al., 1987; Burgos et al., 1988; de León et al., 1991a). However, although there is an identical localization of immunoreactive perikarya containing such neuropeptides and β-endorphin (1-27)-immunoreactive cell bodies in the nucleus arcuatus of the cat (Coveñas et al., 1996a), the possible coexistence of β-endorphin (1-27) with some of the three above-mentioned neuropeptides can not be suggested in this nucleus since the morphological characteristics of β-endorphin (1-27)-immunoreactive perikarya are quite different from those shown by the neuronal populations containing substance P, neuropeptide Y or neurotensin.

## Anatomical relationships among neuropeptides in the cat hypothalamus

There is a close anatomical relationship among the neuropeptides in the cat hypothalamus, since as shown in Table 4, the lowest level found was 76.92%. This value indicates that in 76.92% of the cat hypothalamic nuclei, for example, substance P-immunoreactive fibers and/or cell bodies and neurotensin-immunoreactive fibers and/or cell bodies have been observed. The percentage was calculated taking the total number of the cat hypothalamic nuclei (thirteen) as 100%. In addition, this Table also indicates that in all the hypothalamic nuclei of the cat (100%) methionine-enkephalin and neuropeptide Y-immunoreactive structures were observed, and this was also the case for methionine-enkephalin and β-endorphin, methionine-enkephalin and α-melanocyte-stimulating hormone, neuropeptide Y and β-endorphin, neuropeptide Y and α-melanocyte-stimulating hormone and β-endorphin and α-melanocyte-stimulating hormone.

## Peptidergic pathways in the cat hypothalamus

Yoshimoto et al. (1989) have reported the following afferent peptidergic pathways to the cat posterior hypothalamus:

1. From the medial preoptic area, medial amygdaloid nucleus and nucleus of the stria terminalis to the ventrolateral/dorsolateral posterior hypothalamus: containing galanin.

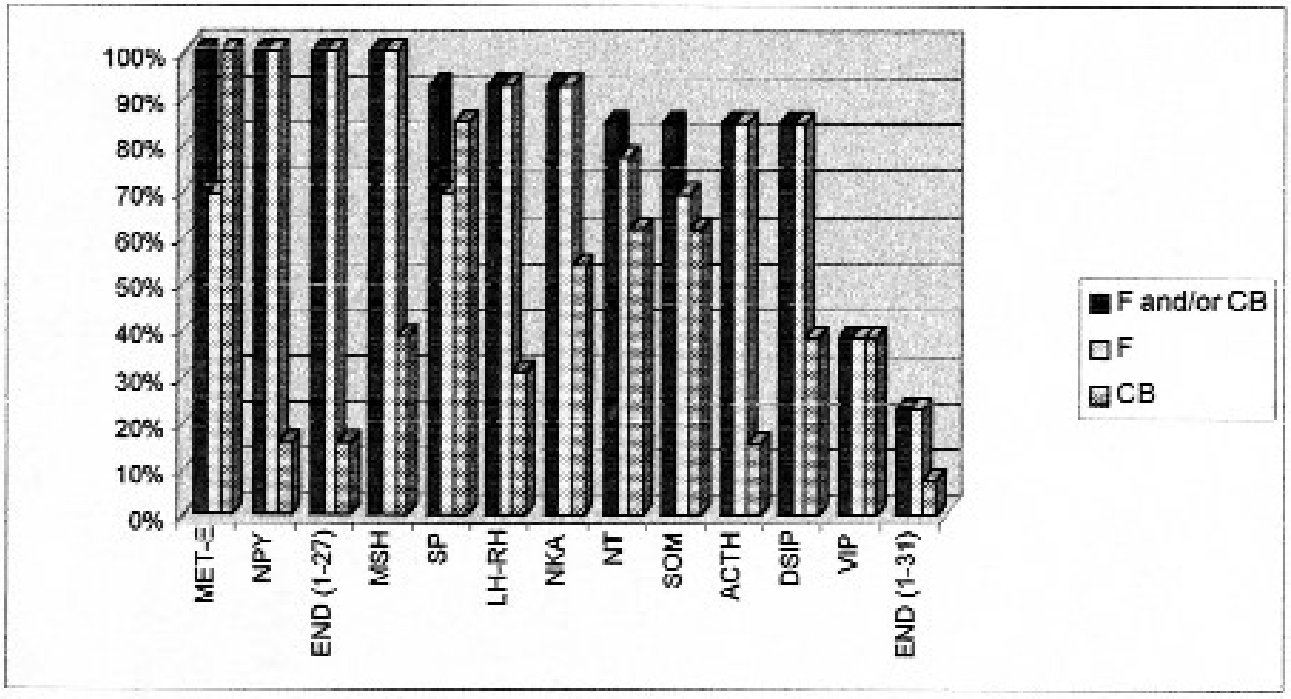


**Table 2.-** Percentanges of hypothalamic nuclei of the cat that contain a neuropeptide located in fibers and/or cell bodies (F and/or CB), in fibers (F) or in cell bodies (CB).
For a nomenclature of the neuropeptides, see Table 1. The total number of hypothalamic nuclei is: 13.

**Table 3.-** Presence of neuropeptides in the cat hypothalamic nuclei and tracts.

| | | |
|---|---|---|
| Arc | 13/13 | 100% |
| Hvm, PVH, Sch, HL, So | 12/13 | 92.30% |
| Ha, RPO | 11/12 | 91.66 % |
| aHd | 11/13 | 84.61% |
| Hp | 10/13 | 76.92 % |
| Hdm | 9/12 | 75% |
| Ml | 8/11 | 72.72 % |
| Mm | 7/11 | 63.63 % |
| Fx | 7/12 | 58.33% |
| TMT | 6/12 | 50 % |
| MFB | 3/11 | 27.27 % |

For nomenclature of the hypothalamic nuclei, see list of abbreviations. For example, 10/13: indicates that of 13 neuropeptides studied, 10 were found in fibers and /or cell bodies in the Hp. Their percentages are also indicated.

**Table 4.-** Anatomical relationships between the neuropeptides in the cat hypothalamus.

| | % |
|---|---|
| MET-E/NPY; MET-E/ β-END; MET-E/α-MSH NPY/β-END; NPY/α-MSH; β-END/α-MSH | 100 |
| MET-E/SP; MET-E/LH-RH; MET-E/NKA; SP/NPY; SP/β-END; SP/α-MSH; SP/LH-RH; NPY/LH-RH; NPY/NKA; β-END/LH-RH; β-END/NKA; α-MSH/LH-RH; α-MSH/NKA | 92.30 |
| MET-E/NT; MET-E/SOM; MET-E/ACTH; SP/NKA; NT/SOM; NT/NPY; NT/β-END NT/α-MSH; NT/ACTH; NT/NKA; SOM/NPY SON/β-END; SOM/α-MSH; SOM/ACTH; SOM/NKA; NPY/ACTH; β-END/ACTH; α-MSH/ACTH; ACTH/NKA; LH-RH/NKA | 84.61 |
| SP/NT; SP/SOM; SP/ACTH; NT/LH-RH; SOM/LH-RH; ACTH/LH-RH | 76.92 |

For a nomenclature of the neuropeptides, see Table 1.

2. From the anterior hypothalamus and medial preoptic area to the posterior hypothalamus: containing corticotropin-releasing factor.
3. From the medial preoptic area, dorsal hypothalamic nucleus and ventrolateral posterior hypothalamus to the posterior hypothalamus: containing neurotensin.
4. From the medial preoptic area and the mamillary region to the posterior hypothalamus: containing methionine-enkephalin.
5. From the medial amygdaloid nucleus, medial preoptic area and lateral/dorsal and posterior hypothalamic areas to the posterior hypothalamus: containing substance P.
6. From the medial preoptic area and around the anterior hypothalamic nucleus to the posterior hypothalamus: containing thyrotropin-releasing hormone.
7. From the nucleus of the stria terminalis to the posterior hypothalamus: containing substance P or methionine-enkephalin.

In addition, in the cat Yanagihara and Niimi (1989) have described a projection containing substance P from the posterior hypothalamus to the hippocampal formation. Kitahama et al.

**Table 5.-** Possible peptidergic afferents to the cat hypothalamic nuclei (F: +++/++; CB: -).

| ACTH | β-END | NPY |
|---|---|---|
| aHd (+++) | aHd (++) | aHd **(+++)** |
| Ha (+++) | Ha (++) | Ha **(+++)** |
| Hdm (+++) | Hdm (++) | Hdm **(+++)** |
| HL (++) | Hp (++) | HL **(++)** |
| Hp (++) | PVH (+++) | Hp **(+++)** |
| PVH (+++) | RPO (+++) | Hp **(+++)** |
| Sch (++) | Sch (++) | Ml **(+++)** |
| **DSIP** | **LH-RH** | PVH **(+++)** |
| Hvm (+++) | Hp (++) | Sch **(++)** |
| **β-END (1-31)** | Ml (++) | So **(++)** |
| Hvm (++) | PVH (++) | **NT** |
| **α-MSH** | Sch (++) | PVH **(++)** |
| Ha (+++) | **NKA** | **SOM** |
| Hp (++) | RPO (++) | Hvm **(+++)** |
| PVH (+++) | So (+++) | |

For nomenclature of the neuropeptides and the hypothalamic nuclei, see respectively, Table 1 and list of abbreviations. **CB:** immunoreactive cell bodies (-: absence); **F:** immunoreactive fibers (+++: high density; ++: moderate density).

**Table 6.-** Possible peptidergic projecting neurons in the cat thalamic nuclei (F: -; CB: +++/++).

| MET-E | NT |
|---|---|
| Hdm (++) | RPO (+++) |
| Mm (+++) | **SP** |
| PVH (++) | Ml (++) |
| **SOM** | Mn (+++) |
| Ha (+++) | RPO (+++) |
| PVH (+++) | |

For nomenclature of the neuropeptides and the hypothalamic nuclei, see respectively, Table 1 and list of abbreviations. **CB:** immunoreactive cell bodies (+++: high density; ++: moderate density); **F:** immunoreactive fibers (-: absence).

(1984, 1986) have demonstrated that several peptidergic pathways come from the nucleus arcuatus of the cat. The peptidergic projections described, containing adrenocorticotropin hormone, coursed from the nucleus arcuatus to the midline of the thalamus, the nucleus periventricularis anterior, the locus coeruleus, the perifornical region, the zona incerta, the hypothalamus posterior, lateralis, anterior, dorsomedialis and ventromedialis and to the nuclei supraopticus, suprachiasmaticus and periventricularis hypothalami. These authors also indicated that all the peptidergic fibers and terminals containing adrenocorticotropin hormone observed in the cat central nervous system come from the cell bodies located in the nucleus arcuatus. In addition, Kitahama et al. (1986) showed that all the fibers and terminals containing adrenocorticotropin hormone in the cat nucleus ventromedialis hypothalami come from cell bodies located in the nucleus arcuatus. These data suggest that the adrenocorticotropin hormone-immunoreactive cell bodies observed later in the nucleus ventromedialis hypothalami of the cat (Coveñas et al., 1996c) would be projecting neurons. At present, in the cat central nervous system the only sites in which cell bodies containing adrenocorticotropin hormone have been described are the nucleus arcuatus (Kitahama et al., 1986; Rao et al., 1986; Coveñas et al., 1996c) and the nucleus ventromedialis hypothalami (Coveñas et al., 1996c).

Tables 5 and 6 show the nuclei of the cat hypothalamus in which peptidergic fibers but no cell bodies have been observed (Table 5), as well as the nuclei in which a moderate/high density of immunoreactive cell bodies has been found, but no immunoreactive fibers (Table 6).

These data indicate that the hypothalamic nuclei shown in Table 5 could receive peptidergic afferents arising from neurons located inside and/or outside the hypothalamus, whereas the nuclei included in Table 6 could contain projecting neurons, which could send projections to other hypothalamic nuclei and/or other parts of the central nervous system. According to these data, the following peptidergic pathways can be suggested in the cat (in order to demonstrate these pathways, the use of both immunocytochemical and tract-tracing techniques is required):

1. From the hypothalamus lateralis to the parabrachial nuclei, the locus coeruleus, the nucleus of the solitary tract, the nucleus medialis dorsalis, and to the nucleus habenularis lateraris: containing α-melanocyte-stimulating hormone. This pathway could be possible, since in the brainstem and thalamic nuclei referred to, fibers containing α-melanocyte-stimulating hormone were observed but no immunoreactive cell bodies (Coveñas et al., 1996b, 2000), whereas in the hypothalamus lateralis a high density of cell bodies containing α-melanocyte-stimulating hormone was described (Coveñas et al., 1996b). Moreover, this observation is in agreement with previous works carried out in the cat, in which, using horseradish and autoradiographic techniques, researchers have described pathways from the hypothalamus lateralis to the five brain areas mentioned above (Ono and Niimi, 1985; Holstege, 1987).
2. From the pars ventralis of the corpus geniculatum laterale to the nucleus suprachiasmaticus: containing neuropeptide Y. In this case, a moderate density of immunoreactive cell bodies containing neuropeptide Y was found in the thalamic nucleus (Coveñas et al., 1990), whereas in the hypothalamic nucleus neuropeptide Y-immunoreactive fibers, but no cell body

**Table 7.-** Comparative study of the distribution of fibers and cell bodies containing neuropeptides in the hypothalamus of mammals.

| | F | CB |
|---|---|---|
| MET-E | C = R > H | C = R > M > H |
| SP | C = R > H =M | C = R > H = M |
| NT | C = R = H | C = R = H |
| SOM | C = R = H | R = H > C |
| NPY | C = R = M = H | C = R = M = H |
| β-END | C = R | C = R |
| α-MSH | C = R | C = R |
| ACTH | C = R = H | C = R = H |
| LH-RH | C > R | M = H > C = R |

For nomenclature of the neuropeptides, see Table 1. **C:** cat; **CB:** distribution of immunoreactive cell bodies; **F:** distribution of immunoreactive fibers; **H:** human; **M:** monkey; **R:** rat.

containing this neuropeptide was observed (Hu et al., 1987). This possible pathway containing neuropeptide Y is in agreement with the results obtained by Swanson et al. (1974), since these authors demonstrated an anatomical projection from the pars ventralis of the corpus geniculatum laterale to the nucleus suprachiasmaticus.

3. From the hypothalamus lateralis to the superior colliculus: containing α-melanocyte stimulating hormone. This anatomical pathway has been demonstrated previously in the cat (Rieck et al., 1986). The peptidergic pathway could be possible, since a high density of immunoreactive cell bodies and a low density of fibers containing α-melanocyte-stimulating hormone has been observed in the hypothalamus lateralis (Coveñas et al., 1996b), whereas immunoreacive fibers containing the neuropeptide were only found in the superior colliculus (Coveñas et al., 2000).

## COMPARATIVE STUDY OF THE DISTRIBUTION OF NEUROPEPTIDES IN THE MAMMALIAN HYPOTHALAMUS

The distribution of the neuropeptides in the mammalian hypothalamus has mainly been studied in humans, the monkey, cat and rat. Table 7 compares the distribution of fibers and cell bodies containing neuropeptides in the four species studied, and depicts the neuropeptides that have been most extensively studied in the hypothalamus of mammals (Barry and Carette, 1975; Hökfelt et al., 1977; Ljungdahl et al., 1978; Sar et al., 1978; Bennet-Clark et al., 1980; Finley et al., 1981a, b; Haber and Elde, 1982; Jennes et al., 1982; Micevych and Elde, 1982; Bouras et al., 1984; Johanson et al., 1984; Krukoff and Calaresu, 1984; Pelletier et al., 1984; Ronnekleiv et al., 1984; Barry et al., 1985; Chronwall et al., 1985; Mezey et al., 1985; Smith et al., 1985; Bennet-Clark et al., 1986; Bouras et al., 1986; Mai et al., 1986; Rao et al., 1986; Bouras et al., 1987; Hu et al., 1987; Léger et al., 1987; Mai et al., 1987; Rao et al., 1987; Burgos et al., 1988; Coveñas et al., 1988; Palkovits, 1988; Léger et al., 1990; de León et al., 1991a, b; Zaphiropoulos et al., 1991; Rance et al, 1994; Coveñas et al., 1996a, b, c; Belda et al., 2000). Thus, Table 7 indicates whether there is or there is not a more or less widespread distribution of the immunoreactive structures in the hypothalamus of the four species studied. In this sense, the distribution of fibers containing neurotensin, somatostatin, neuropeptide Y, β-endorphin, α-melanocyte-stimulating hormone and adrenocorticotropin hormone in the mammalian hypothalamus is in general quite similar. However, in the case of methionine-enkephalin and substance P, distribution is similar in the cat and in the rat, but is more widespread in comparison with that observed in humans (for methionine-enkephalin) and in humans and monkey (for substance P). Moreover, the distribution of fibers containing luteinizing hormone-releasing hormone in the cat hypothalamus is more widespread than that found in the rat. In general, the distribution of cell bodies in the mammalian hypothalamus containing neurotensin, neuropeptide Y, β-endorphin, α-melanocyte-stimulating hormone and adrenocorticotropin hormone shows a similar distribution. However, the distribution is more widespread in the rat and cat in comparison with the distribution found in the monkey and humans for methionine-enkephalin and substance P. In addition, the distribution of cell bodies containing somatostatin in the human and rat hypothalamus is more widespread in comparison with that found in the same diencephalic area of the cat, and the distribution of cell bodies containing luteinizing hormone-releasing hormone in the monkey and human hypothalamus is more widespread in comparison with the distribution observed for the same neuropeptide in the hypothalamus of the cat and rat.

Finally, the discrepancies found in the distribution of neuropeptides in the mammalian hypothalamus could be due to species differences and/or technical considerations (e.g., antisera used, injections of colchicine,…).

## PHYSIOLOGICAL FUNCTIONS OF NEUROPEPTIDES IN THE CAT HYPOTHALAMUS

As we have shown in Tables 3 and 4, numerous neuropeptides have been located in the same hypothalamic nuclei of the cat. This indicates that in those hypothalamic nuclei there is

an elaborate modulation of functions in which these nuclei are involved, and that in these hypothalamic nuclei a possible interaction between the neuropeptides localized in them could occur. In this sense (see Table 4), it is known that enkephalins inhibit the release of substance P from hypothalamic slices (Micevych et al., 1982) and that the opiate receptors mediate the inhibition of cholecystokinin and substance P release in the cat hypothalamus (Micevych et al., 1984). The release of methionine-enkephalin by β-endorphin has also been demonstrated (Tseng, 1989). In addition, the regulation of pro-opiomelanocortin gene expression by neuropeptide Y has been described and that neuropeptide Y may act as a melanocyte-stimulating hormone-release inhibiting factor and that neuropeptide Y modulates the release of luteinizing hormone-releasing hormone from the hypothalamus. This suggests that neuropeptide Y could be a component of the luteinizing hormone-releasing hormone pulse-generating system (Verburg-Van Kemenade et al., 1987; McDonald, 1990; de Yébenes et al., 1995).

The presence of cell bodies containing methionine-enkephalin in the nuclei periventricularis hypothalami and supraopticus suggests that such neurons could project to the median eminence and posterior pituitary, where they might regulate the release of oxytocin and vasopressin in the neurohypophysis and probably participate in cardiovascular control (Micevych and Elde, 1980). Moreover, the presence of methionine-enkephalin in the nucleus suprachiasmaticus, as well as the localization of cell bodies containing the neuropeptide in the hypothalamus dorsomedialis and in the regio praeoptica indicates that the neuropeptide could be involved in the control of circadian rhythms and/or in visual processes, as well as in controlling somatostatin release into the blood of the portal plexus (Tramu et al., 1981; Pickard, 1985; Swaab et al., 1985).

The presence of substance P in the nuclei periventricularis hypothalami, supraopticus, hypothalami ventromedialis and in the suprachiasmaticus indicates that the neuropeptide could play a role, respectively, in synaptic control of the vasopressinergic neurons of the nucleus periventricularis hypothalami, in increasing the firing frecuency of neurons located in the nucleus supraopticus, in feeding behavior, and in visual processes (Clarke et al., 1980; Heike et al., 1986). In addition, the localization of immunoreactive structures containing β-endorphin and α-melanocyte-stimulating hormone in the nuclei hypothalami ventromedialis, periventricularis hypothalami and suprachiasmaticus indicates that both neuropeptides could be involved in feeding, affective defense behavior, the regulation of thermogenesis, circadian rhymths, and in visual and stress mechanisms (Fuchs at al., 1985; Pickard, 1985; Gray et al., 1989; Preston et al., 1989). Also, the presence of immunoreactive structures containing luteinizing hormone-releasing hormone in the nuclei arcuatus, regio praeoptica, hypothalami ventromedialis, suprachiasmaticus and in the median eminence indicates that the neuropeptide could play a role in regulating the release of luteinizing hormone and follicle-stimulating hormone from the anterior pituitary, in sexual behavior, in circadian rhymths, in visual mechanisms and in the regulation of thermogenesis (Kow and Pfaff, 1988; Pan et al., 1988; Merchenthaler et al., 1989).

Moreover, in the cat, it has been described that hypothalamic vasopressin and oxytocin immunoreactive neurons are activated during the pressor reflex exercise and that vasopressin is involved in the regulation of evaporative water loss and body temperature, and it is known that the release of cholecystokinin in the cat hypothalamus is involved in nutrient and volume loading (Doris, 1982; Schick et al., 1989; Li et al., 1997).

Finally, it is known that the destruction of the preoptic area, in which cell bodies containing delta sleep-inducing peptide have been found in the cat (Charnay et al., 1990), result in a strong decrease or disappearance of both paradoxical sleep and deep slow-wave sleep (Szymusiak and McGinty, 1986; Sallanon et al., 1989). This suggests that the neuropeptide could be involved in the hypothalamic control of sleep.

## FUTURE RESEARCH ON NEUROPEPTIDES IN THE CAT HYPOTHALAMUS

As we have indicated in a previous work on the neuropeptides in the cat thalamus (Coveñas et al., 2001), in the hypothalamus there is also much to be done in order to know the distribution and the physiological functions of the neuropeptides in this diencephalic region of the cat. Thus, in addition to studying the distribution of other neuropeptides that, at the present, have not been studied in detail in the cat hypothalamus, in the future radioimmunoassay and in situ hybridization techniques should be carried out in order to compare the results obtained after using both techniques in the cat hypothalamus with those described for the distribution of neuropeptides in the same diencephalic area of the cat. In addition, however, in the future the following aspects should be explored in greater depth in the cat hypothalamus, since they have not received sufficient attention: 1) The distribution of the receptors of the neuropeptides; 2) The distribution of the neuropeptidases; 3) The location of the cell bodies that originate peptidergic afferences to the hypothalamus; 4) The

projections of the peptidergic neurons observed in the hypothalamus; 5) Knowledge of the synaptic connections; 6) The coexistence of neuropeptides and 7) The physiological functions of the neuropeptides. In sum, the application of other methodologies (combining immunocytochemical and tract-tracing methods, immunocytochemical and electron microscopic methods, immunofluorescence methods, microinjections of neuropeptides…) is required in order to gain further insight into the distribution and functions of the neuropeptides in the cat hypothalamus.

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## ABBREVIATIONS USED

**aHd:** Area hypothalamica dorsalis
**Arc:** Nucleus arcuatus
**Fx:** Fornix
**Ha:** Hypothalamus anterior
**HL:** Hypothalamus lateralis
**Hdm:** Hypothalamus dorsomedialis
**Hp:** Hypothalamus posterior
**Hvm:** Hypothalamus ventromedialis
**MFB:** Median forebrain bundle
**Ml:** Nucleus mamillaris lateralis
**Mm:** Corpus mamillare
**PVH:** Nucleus periventricularis hypothalami
**RPO:** Regio praeoptica
**Sch:** Nucleus suprachiasmaticus
**So:** Nucleus supraopticus
**TMT:** Tractus mamillo-thalamicus

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