ABSTRACT

**Purpose:** The localization and distribution of neuropeptide expression in the cat visual pathway can provide information about the function of that pathway.

**Method:** Study of optic pathway in eight cats. Following extraction of the brain, slices were prepared using a microkeratome. The slices were examined by indirect immunocytochemistry using antimeutenkephalin as antibody to determine the presence or absence of this pentapeptide in the visual pathway.

**Results:** Met-enkephalin receptors in both cortical and subcortical regions of the brain were detected. This suggests that met-enkephalin could be involved in the visual mechanism.

**Conclusions:** The presence of met-enkephalin receptors in both cortical and subcortical regions of the brain suggests that this pentapeptide could be involved in the visual mechanism (Arch Soc Esp Oftalmol 2009; 84: 245-250).

**Key words:** Substance P, optic nerve, visual pathway, neurotransmitter, neuroprotection.

RESUMEN

**Objetivo:** Conocer la localización y distribución de neuropéptidos en la vía óptica, concretamente, la determinación de inmunorreactividad a met-encefalina, clave para la funcionalidad de dicha vía.

**Método:** Se analizó la vía óptica de ocho gatos. Tras extracción quirúrgica; cortes con microkeratomo y procesamiento mediante inmunocitoquímica indirecta, utilizando como anticuerpo la antimeutenkefalina, con el fin de detectar la presencia o ausencia del pentápeptido en la vía óptica del gato.

**Resultados:** Se detectaron receptores a met-encefalina en áreas del encéfalo tanto corticales como subcorticales. Así, se considera la posible implicación de dicho pentápeptido en la funcionalidad de la vía óptica.

**Conclusiones:** La presencia de receptores a met-encefalina en áreas del encéfalo tanto corticales como subcorticales, muestra la posible implicación de dicho neurotransmisor en la funcionalidad de la vía óptica.

**Palabras clave:** Sustancia P, vía óptica, neurotransmisor, neuroprotección.
INTRODUCTION

Neuropeptides and neuromodulation have been known for over 30 years. Neuropeptides not only release hormones into blood but also form synapses over the surface of other cells. Peptides comprise a number of characteristics which differentiates them from typical neurotransmitters, including receptor activation at much lower concentrations and a much longer duration than conventional neurotransmitters (1). In addition, neuropeptides are characterized by coexistence, as a single synapse can release two or more neurotransmitters (peptide and classical) at the same time (1-3). They are also characterized by co-transmission, i.e., the regulation of a peptide or classical neurotransmitter by another peptide-type neurotransmitter (4). To date over 50 neuropeptides have been described and several classifications thereof have been made.

Methionine-enkephalin was one of the first endogenous opiates to be isolated, having analgesic and euphoria-inducing properties similar to morphine. It is characterized by its high selectivity and stereospecificity (5). It can be deactivated by any structural change and it can act as an opiate antagonist. There is a high number of receptors in vertebrates (4).

The distribution map of the opiate receptor in the brain matches the paleo-spinal-thalamic pathway of pain. It is also found in the amygdala, the striated body and the hypothalamus. The variability of locations matches the diversity of functions. Its distribution matches quite closely that of exogenous as well as endogenous opiate receptors, mainly for enkephalin. Opiate receptors are divided in µ receptors, in turn subdivided in delta and kappa receptors (6).

The presence of opiate receptors in the brain led to the proposition that said receptors could be utilized by specific endogenous substances. To this end, the presence of enkephalin was proved in rats (7). Subsequently, it was verified that the neurotransmitters of specific neuronal systems located in the brain participate in the integration of sensory information related to pain and emotional behavior (8).

The opiate peptides derived from proopiomelanocortin (POMC), proenkephalin (from which methencephalon is derived) and from prodynorphin (9).

Methencephalon is a molecule that behaves as a neurotransmitter and it also is a neuromodulator because it modulates the action of classical neurotransmitters through histological coexistence and physiological co-transmission (1). It also behaves as a hormone (at a distance) and has neuroendocrine activity because it acts on the hypophysis modifying the release of some hormones, stimulating the secretion of ACTH, GH and prolactin in the anterior lobe, of MSH in the intermediate lobe and ADH in the posterior lobe, while inhibiting the secretion of TSH, LH and FSH. Accordingly, on the basis of this multiplicity of functions, we can consider methencephalon as a molecule capable of interconnecting different brain systems.

Methencephalon is involved in different behaviors and physiological actions, ranging from the perception and modulation of pain to the response to stress, learning, energy balance, motor activity, food intake, sexual behavior, sleep and thermal and respiratory regulation (4,12,15).

SUBJECTS, MATERIAL AND METHOD

The experimental surgery and animal facility of our centre was utilized.

Experimental resource: eight male adult cats were utilized, having weights between 2.5 - 4 kg.

Laboratory and biological material: antibody 1 (anti-methionin enkephalin); antibody 2 (rabbit anti Ig H+L joined to a peroxidase); normal sheep serum.

Chemical material: physiological serum, paraformaldehyde, Sörensen buffer, sucrose, distilled water, oxygenated water, hydrochloric acid, hydroxi-methyl-amino-methane (TRIS), triton X-100 dianaminobenzidine (D.A.B.), glycerin.

Optical microscopy material, photographic and cartographic material (map of the cat central nervous system -CNS- by Reinoso, F.).

After applying an aesthetic with ketamine, a surgical approach of the left ventricle was carried out on eight cats. Sections were cut from ocular globes and encephalic (approximately 80 µ thick) utilizing a freeze microkeratome. The sections were cut on the basis of a stereotaxic system centered along a horizontal plane containing both tragoinfraorbital axes and along a frontal plane traversing the biauditive axis, with said mean point being the stereotaxic axis 0 of the system, and the plane which contains it being plane 0.

The sections were processed by indirect immunocytochemistry, utilizing anti-methencephalon as antibody in order to detect the presence or absence of the pentapeptide.
After processing the sample with D.A.B. they were assembled and classified per region.

The presence of immunoreactivity observed at the level of anatomic structures was determined utilizing an optical microscope. After establishing the location and intensity of immunoreactivity, we extrapolated the results to Reinoso’s cat CNS topographic maps (11), taking into account that 1 cm in the map corresponds to 1 mm. The maps showed 50 sections from 30 in the anterior ones (A+30) up to 20 in the posterior ones (P-20). We only represented the sections in which some immunoreactivity was identified (A+15 to P-10).

RESULTS

The immunoreactivity findings were classified on the basis of their intensity: Low, moderate and intense.

Low immunoreactivity was found in the lateral geniculate body, the pulvinar body, the pretectum and areas 17, 18 and 19 of the cerebral cortex (table I).

The intensity rose to moderate in the upper culliculum and in the superficial, median and deep grey stratum (table 3).

No immune reactivity was found in the remaining areas of the optic pathway.

DISCUSSION

The chosen experimentation animal was the cat due to the availability of highly detailed cartographic maps (9).

Brain cartographic maps of this same pentapeptide have been described in the brains of different animals due to immunocytochemistry techniques (the technique we utilized in this study): rats (10), cats, monkeys and humans.

The highest densities of fibers and encephalinergetic terminations in the SNC (10,11) are found in the striated core, specifically the globus pallidus.

Other areas where the existence of met-enkephalin was determined were the somas of the striated body, the supra-optical and para-ventricular hypo-

<table>
<thead>
<tr>
<th>Anteriority</th>
<th>Moderate</th>
<th>Low</th>
<th>Null</th>
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<tbody>
<tr>
<td>A+15</td>
<td>Cortex: Areas 18 and 19</td>
<td>O.C.</td>
<td></td>
</tr>
<tr>
<td>A+14</td>
<td>Cortex: Areas 18 and 19</td>
<td>O.C.</td>
<td></td>
</tr>
<tr>
<td>A+13</td>
<td>Cortex: Areas 18 and 19</td>
<td>O.T.</td>
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<tr>
<td>A+12</td>
<td>Cortex: Areas 18 and 19</td>
<td>O.T.</td>
<td></td>
</tr>
<tr>
<td>A+11</td>
<td>Cortex: Areas 18 and 19</td>
<td>O.T.</td>
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</tr>
<tr>
<td>A+10</td>
<td>Cortex: Areas 18 and 19</td>
<td>O.T.</td>
<td></td>
</tr>
<tr>
<td>A+9</td>
<td>Cortex: Areas 18 and 19</td>
<td>O.T.</td>
<td></td>
</tr>
<tr>
<td>A+8</td>
<td>Cortex: Areas 18-19, L.G., P.</td>
<td>R.O.,G.M.O.T</td>
<td></td>
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<tr>
<td>A+7</td>
<td>Cortex: Areas 18-19, LGD, LGV</td>
<td>G.M., O.T</td>
<td></td>
</tr>
<tr>
<td>A+6</td>
<td>Cortex: Areas 18-19, LGD, LGV, P</td>
<td>G.M., O.T</td>
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<tr>
<td>A+5</td>
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<td>G.M.</td>
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<tr>
<td>A+4</td>
<td>Cortex: Areas 18 and 19, L.G.</td>
<td>G.M.</td>
<td></td>
</tr>
<tr>
<td>A+3</td>
<td>Cortex: Areas 18 and 19</td>
<td>G.M.</td>
<td></td>
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<tr>
<td>A+2</td>
<td>Cortex: Areas 18 and 19</td>
<td>G.M.</td>
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<td>A+1</td>
<td>Cortex: Areas 17, 18 and 19</td>
<td>G.M.</td>
<td></td>
</tr>
<tr>
<td>A+0</td>
<td>Cortex: Areas 17, and 18</td>
<td>G.M.</td>
<td></td>
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</tbody>
</table>

thalamus nucleus and nucleus of the cerebral trunk, the telencephalus fibers in some cortex regions, the lateral septum, the central nucleus of the amygdala and the striated body, the diencephalus thalamus, hypothalamus and sub-thalamus, the reticular formation of the mesencephalon, the periacueductal grey substance (SGPA) and the interpeduncular nucleus, the trunk of the reticular formation of the encephalus, the nucleus of the prominent line, the locus coeruleus, the nucleus of the solitary tract, spinal nucleus of the trigeminus, the cochlear nucleus, the parabrachial nucleus, the commissural nucleus and the cranial pairs nucleus. We also identified the presence of met-enkephalin in the cat thalamus (10).

As regards the above mentioned coexistence (1) in the optic pathway, where we determined the existence of met-enkephalolin and considering the peptidic and non-peptidic coexistence (1,4), said coexistence was evidenced with other classic or non-peptidic neurotransmitter.

Met-enkephalin or P Substance is a molecule that behaves as a neurotransmitter because it fulfills the requirements to do so. In addition it is a neuromodulator and also behaves as a hormone due to having neuroendocrine activity because it acts on the hypophysis. Met-enkephalin is involved in a variety of physiological behaviors and mechanisms, ranging from pain perception and modulation to stress response and learning.

Some authors have found met-enkephalin in some structures of the optic pathway, such as the retina of turtles (18) and salamanders (19), in the pretectum of pigeons (20), the geniculate nucleus (21,22) and the upper colliculum of rats (20,21), in rabbits (23) and more recently in cats (24).

It seems that through the amacrine cells the P substance intervenes in the development and operation of the rabbit retina (28). By means of immuno-reactivity techniques, the presence of P substance has been proven in the human retina, specifically in the nervous fiber layer, but not in the internal plexiform layer. Through the amacrine cells an undefined relationship has been assumed in the formation of the human retinoblastoma (28,30).

After determining the existence of met-enkephalin in specific optic pathway structures, its possible relationship with other optic pathway structures has been demonstrated (27,28). It has been suggested that met-enkephalin plays a role in the retinal-hypothalamus tract in relation to the light-darkness cycle (29) and certain activity in the development and function of the retina (25). In this situation, we pondered the role it would play in its physiology. On the basis that said pentapeptide is an inhibiting neurotransmitter, it might inhibit the neurotransmission of said pathway, playing a role in the regulation of the nervous impulse in these structures. The activation of the optic system fibers produced by external physical stimulants (light stimulants) and/or chemical-endogenous stimulants might be antagonized or partially inhibited.

It could be thought that the biochemical-functional basis of a «pleasant vision» could be related to the release of met-enkephalin after perception which, through a direct connection with the limbic system, would produce the gratifying outcome. An additional possible mechanism is that, after releasing met-enkephalin in the cerebrospinal fluid and in the blood, said limbic system is indirectly stimulated, or both mechanisms in a joint action.

A further point to be emphasized relates to the existence of myosis and midriasis after administering endogenous opiates and due to abstinence thereof, respectively. This mechanism could be explained, at least in part, by the fact of finding immuno-reactivity to met-enkephalons in the pretectum because the pathway from the retina to the pretectum is important in the pupil reflex.

A good anatomical-physiological relationship can be assumed when determining immunoreactivity against met-enkephalin both in the pulvinar nucleus and in Areas 18 and 19 of the visual cortex (extra-striated areas). It is well known that this complex thalamic nucleus projects broadly towards extra-striated visual cortex regions and that many of these regions reciprocally project towards the Pulvinar (28).

On the basis of the sum of knowledge about the presence of met-enkephalin in the optic pathway, the following deductions can be made:

<table>
<thead>
<tr>
<th>Immunoreactivity</th>
<th>Lateral Geniculate Body (G)</th>
<th>Pulvinar (LP)</th>
</tr>
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<tbody>
<tr>
<td>Low</td>
<td>Pretectum (PTE)</td>
<td>Visual cortex (Areas 17,18 and 19)</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Immunoreactivity</th>
<th>Superior Culiculum (CL)</th>
<th>Stratum Griseum Medium (SGM)</th>
<th>Stratum Griseum Profundum</th>
<th>Stratum Griseum Superficialis</th>
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<tbody>
<tr>
<td>Moderate</td>
<td></td>
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</table>
The presence of the inhibiting neurotransmitter met-enkephalin in the cat optic pathway would behave inhibiting or slowing down neurotransmission along said pathway.

The absence of immunoreactivity to met-enkephalin in some optic pathway segments does not exclude the presence thereof in said pathway.

The presence of immunoreactivity to met-enkephalin in the cat optic pathway is a neuro-histochemical basis for the functionality of said pathway.

The existence of met-enkephalin in the cat optic pathway could contribute to the rational and scientific utilization of agonist or antagonist drugs of said peptide for treating optic pathway pathologies.

The presence of immunoreactivity to met-enkephalin in the cat optic pathway is confirmed, thus involving said pentapeptide in the function of said pathway.

The distribution of the pentapeptide met-enkephalin in the cat optic pathway is objectively determined throughout the encephalus; the cortical and sub-cortical portion.

The presence of met-enkephalin in the cat optic pathway contributes to improve the understanding of the operation and biochemistry of said pathway.

REFERENCES


