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Immunohistochemical mapping of neurotensin in the alpaca diencephalon

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Abstract

Introduction. The distribution of the immunoreactive cell bodies and fibers containing neurotensin in the alpaca diencephalon was determined by an immunohistochemical technique.

Material and methods. The study was carried out in four male alpacas that lived at sea level. Brains of deeply anesthetized animals were fixed by perfusion with 4% paraformaldehyde. Cryostat sections were stained by a standard immunohistochemical method.

Results. Cell bodies containing neurotensin were observed in the zona incerta and hypothalamus. A low/moderate density of these cell bodies was observed in the lateral hypothalamic area, anterior and dorsal hypothalamic areas, suprachiasmatic nucleus, periventricular region of the hypothalamus and in the ventromedial hypothalamic nucleus. In both thalamus and hypothalamus, immunoreactive fibers showed a widespread distribution. In the thalamus, a high density of these fibers was mainly found in the midline nuclei, whereas in the hypothalamus a high density was in general observed in the whole structure.

Conclusions. In comparison with other mammals, the thalamus of the alpaca showed the most widespread distribution of neurotensin-immunoreactive fibers. The widespread distribution of neurotensin through the alpaca diencephalon suggests that the peptide can be involved in many physiological actions. *(Folia Histochemica et Cytobiologica 2018, Vol. 56, No. 1, 49–58)*

Key words: camelid; *Lama pacos*; hypothalamus; thalamus; IHC

Introduction

Many immunohistochemical and radioimmunoassay studies have been carried out on the distribution of the tridecapeptide neurotensin in the mammalian central nervous system [1–11]. In the species studied (*e.g.*, rat, guinea pig, cat, minipig, monkey, man), these works have reported a widespread distribution of neurotensin in the central nervous system and this suggests that the peptide might be involved in many physiological actions. In fact, neurotensin, acting as a neurotransmitter and/or neuromodulator, has been involved in several physiological actions affecting hemodynamic, neuroendocrine, food intake, nociceptive, thermoregulatory, locomotor, respiratory, sleep-waking, gustatory, memory, auditory and glucoregulatory systems [1, 9, 12–16]. Moreover, it is known that neurotensin promotes the dendritic spine maturation and dendrite elongation [17] and modulates high-voltage-activated calcium currents [18]. The neurotensin/neurotensin receptor system is also involved in cancer (*e.g.*, neuroendocrine tumors, leukemia, pancreatic adenocarcinoma) [19–21]. Despite the data on the distribution of neurotensin in the mammalian central nervous system (CNS) [1–11],

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the presence of neurotensin in camelids has not been reported, with the exception of alpaca (*Lama pacos*), in which its presence in the brainstem was demonstrated by immunohistochemistry [12]. The alpaca is a camelid very important for some countries in South America (*e.g.*, Peru). Due to the economic importance of the wool production, several studies have been published on the reproductive mechanisms of these animals [22–26]. Moreover, since 2007, the chemical neuroanatomy of neuropeptides in the alpaca CNS has increased considerably. Using immunohistochemical (IHC) techniques, the distribution of calcitonin gene-related peptide, somatostatin-28 (1-12), leucine-enkephalin, adrenocorticotropic hormone, beta-endorphin (1-27), alpha-melanocyte-stimulating hormone and alpha-neo-endorphin has been reported in the alpaca's CNS [27–32]. However, to date no data are currently available in the literature detailing the location of either fibers or cell bodies containing neurotensin in alpaca's diencephalon, a CNS region involved in many important functional mechanisms. Therefore, the aims of this study were to examine the distribution of immunoreactive structures containing neurotensin in the diencephalon of alpaca using immunohistochemistry.

Material and methods

Animals. Four male adult alpacas (*Lama pacos*) (Huacaya race) (5–8 years; 70–80 kg) were obtained from the Peruvian University Cayetano Heredia (Faculty of Veterinary Medicine and Animal Sciences, Lima, Peru). The experimental design, protocols, and procedures of this work were performed under the principles of laboratory animal care and under the guidelines of the ethical and legal recommendations of Peruvian and Spanish legislation. This work was also approved by the research commission of the Peruvian University Cayetano Heredia (Lima, Peru). The animals were kept all their life at the altitude of 0 m under standard conditions of light (lights on at 06:00 and off at 20.00 h) and temperature (26° C), and had free access to food and water.

Tissue preparation. As previously reported [27–32], animals were deeply anesthetized with ketamine (10 mg/kg) and xylazine (4 mg/kg) (both intravenously), heparinized, and perfused *via* the carotid artery with 3 l of cold 0.9% NaCl. This pre-rinse was immediately followed by infusion of the fixative: 5 l of cold 4% paraformaldehyde in 0.15 M phosphate-buffered saline (PBS) (pH 7.2). The diencephalons were dissected out and post-fixed overnight in the latter solution and cryoprotected by immersion in increasing concentrations of sucrose solution (10–30%) until they sank. Using a cryostat, 50-mm frontal sections were cut, collected in PBS and kept at 4° C.

Immunohistochemistry. The IHC procedure has been carried out as described previously [12]. In order to avoid possible interference by endogenous peroxidase, free-floating sections were treated with a mixture of NH_3, NaOH and H_2O_2 (Panreac, Barcelona, Spain) for 20 min. Then, sections were washed in PBS (3 × 10 min) and pre-incubated for 30 min in PBS containing 1% normal horse serum (Sigma-Aldrich, Madrid, Spain) and 0.3% Triton X-100 (Panreac, Barcelona, Spain) in order to enhance antibody penetration. Then, sections were incubated overnight at 4° C in the same phosphate buffer containing the anti-neurotensin antibody (gift of Professor Gérard Tramu), diluted 1:3,000. Sections were then rinsed extensively in PBS for 30 min and incubated for 1 h at room temperature with biotinylated anti-rabbit IgG (Vector Laboratories, Burlingame, CA, USA) diluted 1:200 in the same buffer. Thereafter, sections were incubated with Vectastain ABC reagent (Vector Laboratories) for 1 h at room temperature. After washing the sections in PBS (30 min) and Tris-HCl buffer (Panreac, Barcelona, Spain) (pH 7.6) (10 min), the tissue-bound peroxidase was developed with H_2O_2, using 3, 3'-diaminobenzidine (Sigma-Aldrich) as chromogen. Finally, sections were rinsed with PBS and coverslipped with PBS/glycerol (1/1).

Specificity of the antisera. The polyclonal anti-neurotensin antibody (obtained in the laboratory of Professor Gérard Tramu, University of Bordeaux I, France) was raised in rabbits as previously reported [12]. Moreover, the immunological properties of the anti-neurotensin antiserum have been published previously [12, 33].

In this work, the specificity of the immunostaining was checked by: 1) preabsorption of the primary antiserum with synthetic neurotensin (Sigma-Aldrich) (100 μg/mL of diluted antiserum) (Fig. 3b); 2) omitting the neurotensin antiserum in the first incubation bath (Fig. 3e); and 3) preabsorption of anti-neurotensin with an excess (10^{-7} M) of synthetic substance P, angiotensin II, beta-endorphin, somatostatin-28 (1-12), vasoactive intestinal peptide, and neuropeptide Y (Sigma-Aldrich). In all cases, the results confirmed the specificity of the antisera used in this study.

Mapping. Mapping was carried out following the frontal planes of the alpaca diencephalon that have been published in previous articles addressing the distribution of neuropeptides in alpaca's diencephalon [27–32, 34]. For each frontal plane of the alpaca diencephalon, 5–8 sections were studied. For nomenclature of the diencephalic nuclei, we used that published in the previous papers [27, 28, 32, 34]. Moreover, the brain atlas of *Lama glama* (available from the Mammalian Brain Collections of the University of Wisconsin, Madison, U.S.A.) was used. A series of sections contiguous to those reactive for the neuropeptide studied were routinely stained for Nissl substance with cresyl violet in order to identify and delineate the different nuclei of

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the diencephalon in which immunoreactive structures were found [27–32, 34].

As previously reported [27, 28], in order to determine the density of the immunoreactive fibers in alpaca's diencephalon these were graded into four categories under microscopic observation: high, moderate, low and single (a few immunoreactive fibers). This was accomplished following the protocol described by Coveñas *et al.* [35], and involved viewing the sections under bright-light illumination at constant magnification with reference to photographs in which the density (high, moderate and low) of the immunoreactive fibers had been established previously. Additionally, the length of the immunoreactive fibers was considered as short (< 90 μm), medium (90–120 μm) or long (> 120 μm) as previously described [27]. Measurement of the size of the immunoreactive cell bodies containing neurotensin studied here was carried out as previously described [35]: cell bodies with a diameter of < 15 μm were considered small; those with a diameter between 15–25 μm were medium-sized, and those with a diameter > 25 μm were large. The sizes of the immunopositive cell bodies were measured using a micrometer grid with the nuclei in the focal plane. The number of immunostained cell bodies appearing in each section was counted; a high density of cell bodies was considered when we found more than 20 cell bodies/region/section; a moderate density when we found 10–20 cell bodies/region/section, and a low density when we found fewer than 10 cell bodies/region/section [27, 28].

Photomicrographs were obtained with an Olympus DP50 digital camera (Olympus, Tokyo, Japan) attached to a Kyowa Unilux 12 microscope (Kyowa, Tokyo, Japan). To improve the visualization of the results, only the brightness and contrast of the images were adjusted, without any further manipulation of the photographs. Adobe Photoshop CS6Software was used to view the images and adjust their brightness and contrast.

Results

General considerations

The distribution of the immunoreactive fibers and cell bodies containing neurotensin in the alpaca diencephalon is shown in Table 1 and in Figures 1–4. Cell bodies containing neurotensin were found in the zona incerta and in the hypothalamus. In the latter structure, seven clusters of immunoreactive cell bodies were visualized. In all cases, a low/moderate density of these cell bodies was observed. In general, these cell bodies were round or fusiform and medium-large in size, showing 2–3 dendrites. The thalamus was devoid of cell bodies containing neurotensin. In both, thalamus and hypothalamus immunoreactive fibers showed a widespread distribution. In the thalamus, a high density of these fibers was mainly found in the midline nuclei, whereas in the hypothalamus a high density was in general observed by the whole structure. In general, immunoreactive fibers were thin, short or medium in length, non-branched and with varicosities. In general, in the four diencephalons used in this study, the distribution of the immunoreactive structures (fibers and cell bodies) and the density of such structures were quite similar.

Immunoreactive cell bodies

A low density of cell bodies containing neurotensin has been observed in the zona incerta (Fig. 1d, e). In the hypothalamus, a moderate density was found above the fornix (Fig. 1b, c) and in the caudal part of the lateral hypothalamic area (Fig. 1d, e), whereas a low density was observed in the anterior (Fig. 1a) and dorsal hypothalamic areas (Fig. 1c, 2b), rostral part of the lateral hypothalamic area (Fig. 1a–c, 2c, d), suprachiasmatic nucleus (Fig. 1a, 2e, f), periventricular region of the hypothalamus (Fig. 1b, c and 3b) and in the ventromedial hypothalamic nucleus (Fig. 1b, c).

Immunoreactive fibers

In the thalamus, a high density of immunoreactive fibers was observed in the central medial (Fig. 1b–e), reuniens (Fig. 1a–d, 3c), rhomboid (Fig. 1b, c, 3d), subparafascicular (Fig. 1e) and dorsal part of the reticular thalamic nuclei (Fig. 1a–d) and a moderate density in the anterodorsal thalamic nucleus (Fig. 1a, b) and in the paraventricular thalamic nucleus (Fig. 1a–e, 3e). Single immunoreactive fibers were visualized in the medial habenular nucleus (Fig. 1d, e), paracentral thalamic nucleus (Fig. 1c, d) and in the medial and lateral parts of the ventroposterior thalamic nucleus (Fig. 1c–e) and a low density in the anteromedial (Fig. 1a, b), anteroventral (Fig. 1a, b), centrolateral (Fig. 1c, d), laterodorsal (Fig. 1c–e), lateroposterior (Fig. 1d, e), ventroanterior (Fig. 1a), ventrolateral (Fig. 1b), ventromedial (Fig. 1b–d), reticular (Fig. 1a–e) and mediodorsal (Fig. 1b–e, 4d) thalamic nuclei and in the lateral habenular nucleus (Fig. 1d, e). A low density of immunoreactive fibers was also observed in the zona incerta (Fig. 1d, e, 4f).

In the hypothalamus, a high density of immunoreactive fibers containing neurotensin was found in the anterior, dorsal and lateral hypothalamic areas (Fig. 1a–e), lateral mammillary nucleus (Fig. 1e), periventricular region (Fig. 1a–e), around the fornix (Fig. 1a–d, 4b, c), posterior hypothalamic nucleus (Fig. 1d, e, 4e), median eminence (Fig. 1c), paraventricular hypothalamic nucleus (Fig. 1a, b, 3f), suprachiasmatic nucleus (Fig. 1a) and in the ventromedial hypothalamic nucleus (Fig. 1b, c). A moderate density was found in the arcuate nucleus (Fig. 1c, d) and single fibers

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Table 1. Density of fibers and cell bodies containing neurotensin in the alpaca diencephalon

Hypothalamic nuclei					
Nuclei	**Cell Bodies**	**Fibers**	**Nuclei**	**Cell bodies**	**Fibers**
Anterior hypothalamic area (AHy)	+	+++	Median eminence (ME)	–	+++
Arcuate nucleus (Arc)	–	++	Paraventricular hypothalamic nucleus (PVH)	–	+++
Dorsal hypothalamic area (DA)	+	+++	Perifornical region. Above the Fornix (f)	++	+++
Fornix (f)	–	–	Periventricular area of the hypothalamus	+	+++
Lateral hypothalamic area (LH)	+/++	+++	Posterior hypothalamic nucleus (PH)	–	+++
Lateral mammillary nucleus (LM)	–	+++	Suprachiasmatic nucleus (sch)	+	+++
Mammillothalamic tract (mt)	–	–	Supraoptic hypothalamic nucleus (SO)	–	s
Medial mammillary nucleus (MM)	–	s	Ventromedial hypothalamic nucleus (VMH)	+	+++
Thalamic nuclei					
Nuclei	**Cell bodies**	**Fibers**	**Nuclei**	**Cell bodies**	**Fibers**
Anterodorsal thalamic nucleus (AD)	–	++	Reticular thalamic nucleus (Rt)	–	+/+++
Anteromedial thalamic nucleus (AM)	–	+	Reuniens thalamic nucleus (Re)	–	+++
Anteroventral thalamic nucleus (AV)	–	+	Rhomboid thalamic nucleus (Rh)	–	+++
Central medial thalamic nucleus (CM)	–	+++	Stria medullaris (sm)	–	–
Centrolateral thalamic nucleus (CL)	–	+	Subparafascicular thalamic nucleus (SPF)	–	+++
Lateral geniculate nucleus (LG)	–	–	Subthalamic nucleus (STh)	–	s
Lateral habenular nucleus (LHb)	–	+	Ventroanterior thalamic nucleus (VA)	–	+
Laterodorsal thalamic nucleus (LD)	–	+	Ventrolateral thalamic nucleus (VL)	–	+
Lateroposterior thalamic nucleus (LP)	–	+	Ventromedial thalamic nucleus (VM)	–	+
Medial habenular nucleus (MHb)	–	s	Ventroposterior thalamic nucleus, lateral part (VPL)	–	s
Mediodorsal thalamic nucleus (MD)	–	+	Ventroposterior thalamic nucleus, medial part (VPM)	–	s
Paracentral thalamic nucleus (PC)	–	s	Zona incerta (ZI)	+	+
Paraventricular thalamic nucleus (PVA)	–	++			

Cell bodies (+ — low density; ++ — moderate density); Fibers (s — single fibers; + — low density; ++ — moderate density; +++ — high density). – denotes lack of immunoreactivity.

in the medial mammillary nucleus (Fig. 1e) and the supraoptic hypothalamic nucleus (Fig. 1a, b).

Discussion

This is the first report describing the distribution of immunoreactive structures containing neurotensin in the alpaca diencephalon. The neuroanatomical findings reported here add to our current knowledge about the neurotensinergic system in the camelid central nervous system, since the mapping of neurotensin-immunoreactive fibers and cell bodies in the alpaca brainstem has been carried out previously [12]. The origin of the immunoreactive fibers in the alpaca diencephalon is unknown as well as we have no data indicating whether the neurotensin-immunoreactive cell bodies observed in the alpaca diencephalon are local and/or projecting neurons. However, our data suggest that many neurons could be projecting neurons, since a widespread distribution of immunoreactive fibers containing neurotensin has been observed. It is important to note that this study was carried out in animals not treated with colchicine and it is known that sometimes the visualization of a widespread distribution of peptidergic cell bodies only occurred in colchicine-treated animals [30]. Because the administration of colchicine in alpacas must be ruled out for ethical reasons, other techniques (*e.g.*, *in situ* hybridization) must be used to elucidate the complete distribution of neurons containing neurotensin in the alpaca diencephalon.

The widespread distribution of the neurotensinergic system observed in the alpaca diencephalon suggests that the neuropeptide might be involved in many physiological mechanisms, as it has been reported in other mammals. Thus, neurotensin would be involved

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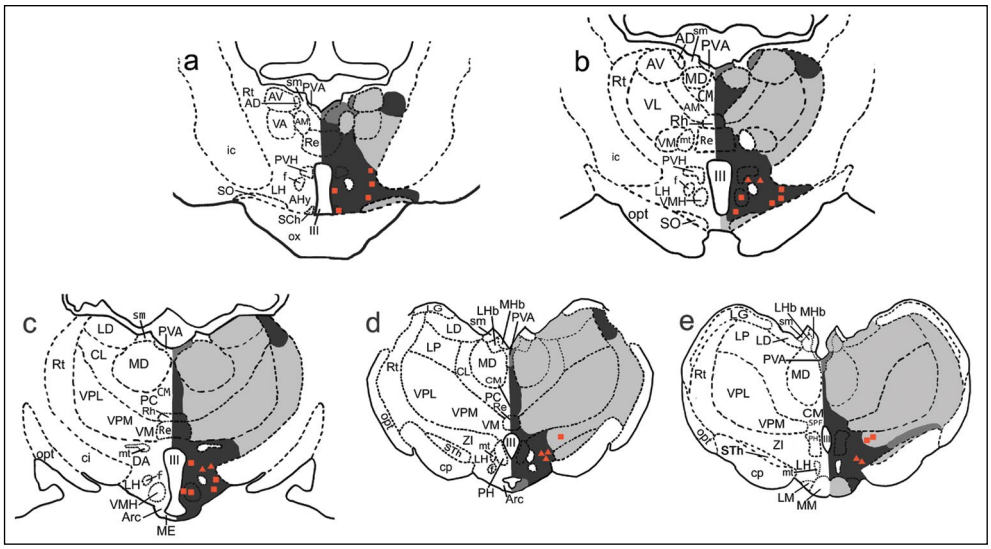

Figure 1. Distribution of neurotensin-immunoreactive fibers and cell bodies in frontal planes of the alpaca diencephalon from rostral **(a)** to caudal levels **(e).** Cell bodies are represented by closed squares (low density) and triangles (moderate density). Immunoreactive fibers are represented by slightly dark (low density or single fibers), moderately dark (moderate density) and strongly dark (high density). Abbreviations: III — third ventricle; AD — anterodorsal thalamic nucleus; AHy — anterior hypothalamic area; AM — anteromedial thalamic nucleus; Arc — arcuate nucleus; AV — anteroventral thalamic nucleus; CL — centrolateral thalamic nucleus; CM — central medial thalamic nucleus; cp — cerebral peduncle; DA — dorsal hypothalamic area; f — fornix; ic — capsula interna; LD — laterodorsal thalamic nucleus; LG — lateral geniculate nucleus; LH — lateral hypothalamic area; LHb — lateral habenular nucleus; LM — lateral mammillary nucleus; LP — lateroposterior thalamic nucleus; MD — mediodorsal thalamic nucleus; ME — median eminence; MHb — medial habenular nucleus; MM — medial mammillary nucleus; mt — mammillothalamic tract; opt — optic tract; ox — optic chiasm; PC — paracentral thalamic nucleus; PH — posterior hypothalamic nucleus; PVA — paraventricular thalamic nucleus; PVH — paraventricular hypothalamic nucleus; Re — reuniens thalamic nucleus; Rh — rhomboid thalamic nucleus; Rt — reticular thalamic nucleus; sch — suprachiasmatic nucleus; sm — stria medullaris; SO — supraoptic hypothalamic nucleus; SPF — subparafascicular thalamic nucleus; STh — subthalamic nucleus; VA — ventroanterior thalamic nucleus; VL — ventrolateral thalamic nucleus; VM — ventromedial thalamic nucleus; VMH — ventromedial hypothalamic nucleus; VPL — ventroposterior thalamic nucleus, lateral part; VPM — ventroposterior thalamic nucleus, medial part; ZI — zona incerta.

in feeding, sexual activity, vigilance behavior, homeostasis, thermogenesis and neuroendocrine mechanisms [3, 5, 8, 13]. Future studies should be focused on such possible functions and on the distribution of neurotensin receptors in the alpaca diencephalon.

Neuropeptides in the alpaca diencephalon

In order to compare adequately the distribution of neuropeptides in the alpaca diencephalon, here male alpacas were only used, since in previous works in which the distribution of neuropeptides was carried out; male animals were exclusively used [27–32]. Moreover, all animals, used here and in the previous studies, were always maintained at 0 m on the sea level (from birth to death) and were not treated with colchicine. The distribution of immunoreactive structures containing calcitonin gene-related peptide (CGRP), adrenocorticotropic hormone (ACTH), leucine-enkephalin, beta-endorphin (1-27), alpha-melanocyte-stimulating hormone (αMSH) or alpha-neo-endorphin and somatostatin-28 (1-12) has been previously reported in the alpaca diencephalon [27, 28, 32].

In all the thalamic and hypothalamic nuclei in which we have observed neurotensin-immunoreactive fibers, the presence of fibers containing beta-endorphin (1-27), αMSH, alpha-neo-endorphin or somatostatin-28 (1-12) has been also reported [27, 28, 32]. Moreover, fibers containing CGRP, ACTH, or leucine-enkephalin [28, 32] were observed in all the hypothalamic nuclei in which we have visualized neurotensin-immunoreactive fibers. Thus, there is a close anatomical relationship in the alpaca diencephalon between the above-mentioned neuropeptides. Moreover, the data suggest a possible functional interaction between these neuropeptides and a possible coexistence of them.

Our study shows for the first time in the alpaca diencephalon the presence of peptidergic cell bodies in the suprachiasmatic nucleus and zona incerta.

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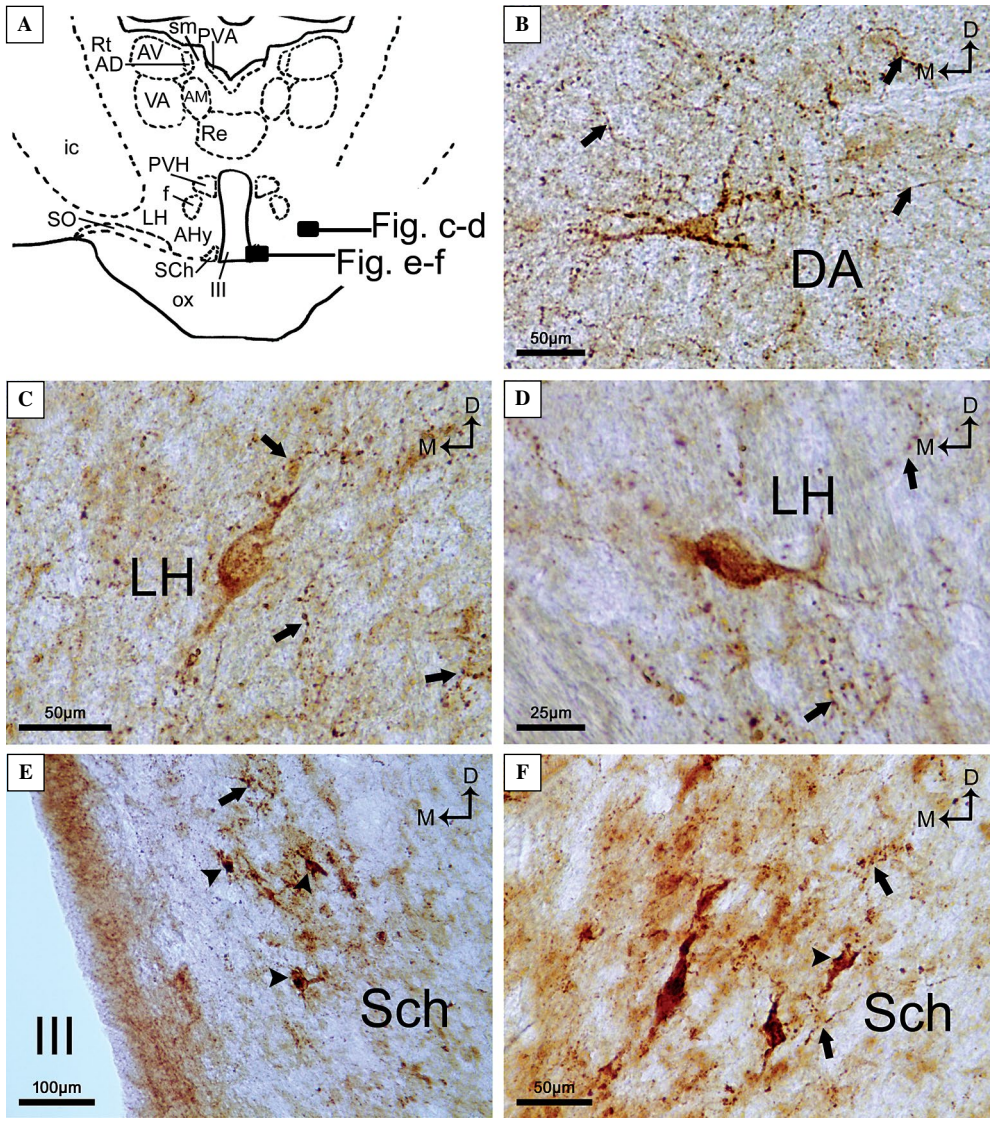

Figure 2. Neurotensin-immunoreactive cell bodies (arrowheads) in the alpaca hypothalamus. **(A)** Frontal section of the diencephalon at the level of the optic chiasm (ox). For nomenclature of the nuclei and tracts, see list of abbreviations in Figure 1. The photographs shown in **C–F** were respectively taken from the regions delimited by the rectangles in **A** (indicated as Fig. c–d and Fig. e–f). Cell bodies containing the peptide located in **(B)** the dorsal hypothalamic area (DA) (see Fig. 1c), **(C, D)** the lateral hypothalamic area (LH) and **(E, F)** in the suprachiasmatic nucleus (Sch). Arrows: immunoreactive fibers.

In the lateral hypothalamic area and in the ventromedial hypothalamic nucleus, cell bodies containing neurotensin, αMSH, alpha-neo-endorphin, somatostatin-28 (1-12) or CGRP have been reported [27, 28, 32]. According to the morphological characteristics (*e.g.*, size, shape) of the peptidergic neurons located in both hypothalamic nuclei [27, 28, 32], it seems that a possible coexistence of these neuropeptides could occur. The administration of CGRP into the ventromedial hypothalamic nucleus increases the temperature of the interscapular brown adipose tissue, heart rate, oxygen consumption and colonic temperature [36]. In the anterior and dorsal hypothalamic areas, cell bodies containing neurotensin or CGRP have been also located [28]. In future studies, the coexistence of both neuropeptides should be addressed in the anterior and dorsal hypothalamic areas.

Neurotensin in the mammalian diencephalon

The distribution of fibers and cell bodies containing neurotensin has been carried out studied in rat, cat and human diencephalons [3, 5, 8, 11]. It should be noted that, here, we have used the same anti-neurotensin antiserum and applied the same immunohistochemical method that in the study carried out in the feline [3]. In general, in the hypothalamus, the neurotensinergic

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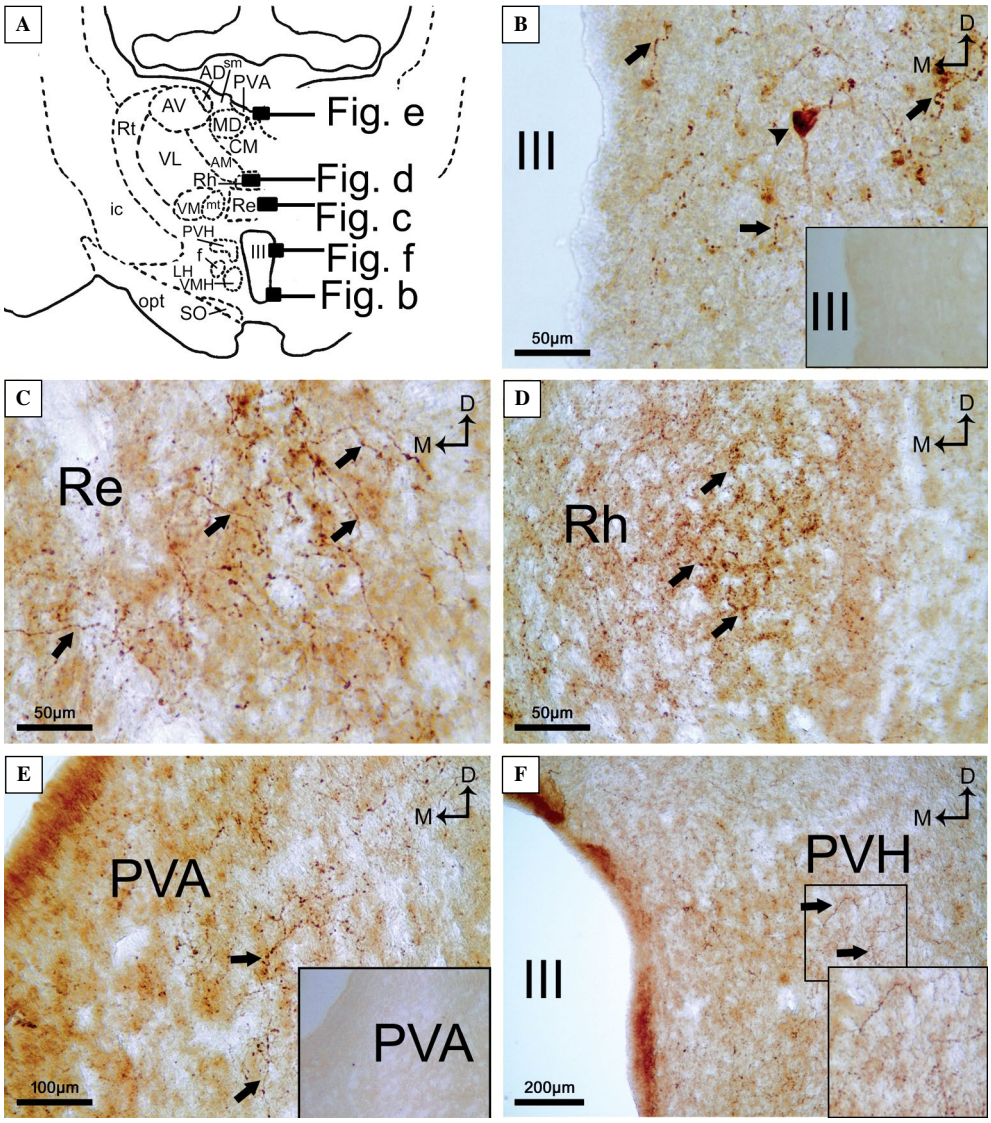

Figure 3. Neurotensin-immunoreactive cell bodies (arrowhead) and fibers (arrows) in the alpaca diencephalon. **(A)** Frontal section of the diencephalon at the level of the optic tract (opt). For nomenclature of the nuclei and tracts, see list of abbreviations in Figure 1. The photographs shown in **B–F** were respectively taken from the regions delimited by the rectangles in a (indicated as Fig. **B–F**). **(B)** Immunoreactive cell body located in the periventricular region of the hypothalamus. Inset: absence of immunoreactivity in the periventricular region of the hypothalamus when the preabsorption of the anti-neurotensin antibody was carried out with neurotensin. Immunoreactive fibers in the reuniens thalamic nucleus (Re) **(C)**, rhomboid thalamic nucleus **(D)**, paraventricular thalamic nucleus (PVA) **(E)** (inset in (e): absence of immunoreactivity in the paraventricular thalamic nucleus (PVA) when the anti-neurotensin antibody was omitted) and in the paraventricular hypothalamic nucleus **(F)** (inset at bottom right: a higher magnification of the upper rectangle).

innervation in rat, cat, alpaca and humans is quite similar since in these four species neurotensin-immunoreactive fibers have been found through the whole hypothalamus [3, 5, 8, 11]. Moreover, in the four species neurotensin-immunoreactive fibers have been observed in the thalamus along the midline [3, 5, 8, 11]. However, in rat, cat and humans, neurotensin-immunoreactive fibers were totally absent in the lateral region of the thalamus (*e.g.*, reticular thalamic nucleus) [3, 5, 8, 11], but in the alpaca we observed immunoreactive fibers containing neurotensin in the lateral thalamic nuclei. This means that, among the mammalian thalamus, the alpaca shows the most widespread distribution of neurotensin-immunoreactive fibers. It seems that this discrepancy is due to species differences and suggests that neurotensin could be involved in special and unique regulatory mechanisms in the lateral thalamic nuclei of the alpaca.

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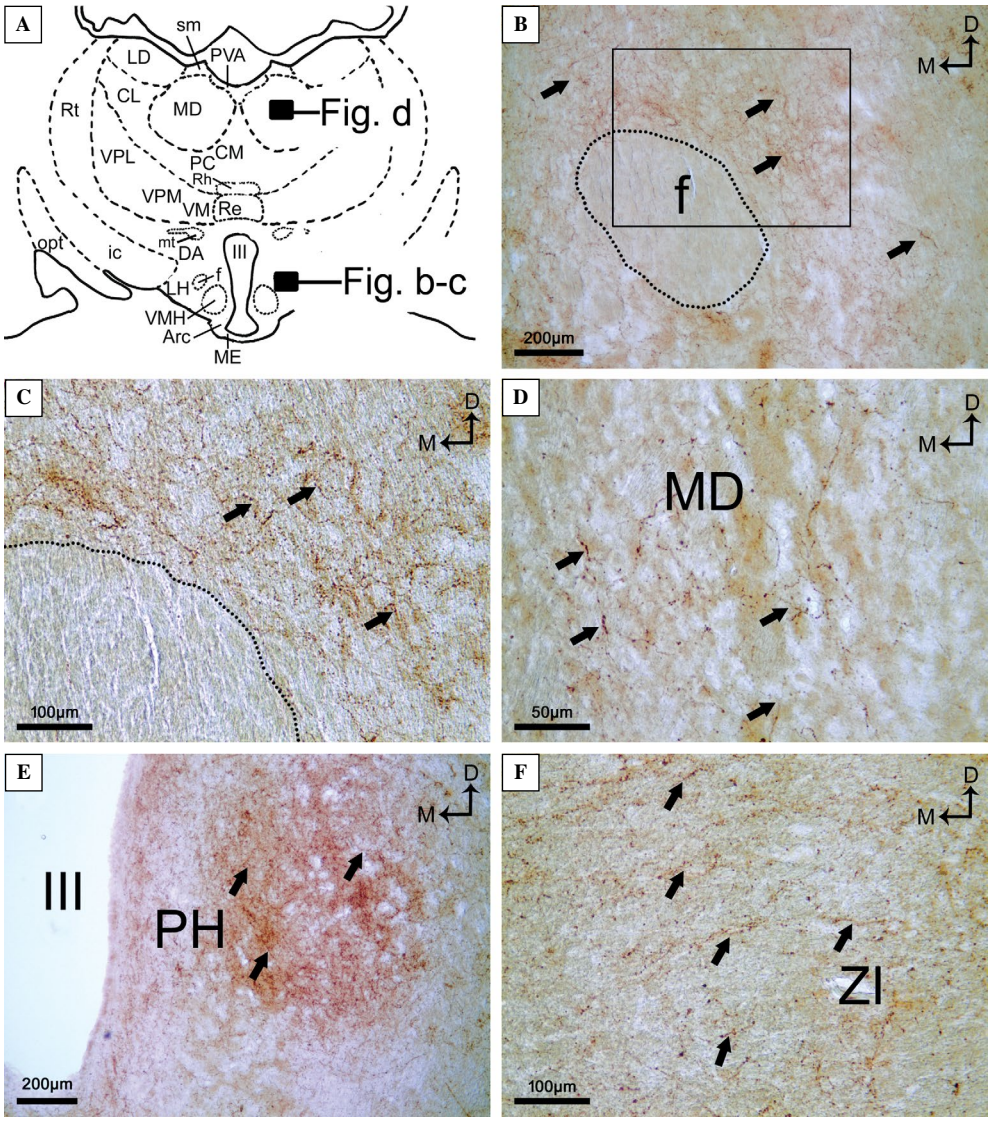

Figure 4. Neurotensin-immunoreactive fibers (arrows) in the alpaca diencephalon. **(A)** Frontal section of the diencephalon at the level of the median eminence (ME). For nomenclature of the nuclei and tracts, see list of abbreviations in Figure 1. The photographs shown in **B–D** were respectively taken from the regions delimited by the rectangles in a (indicated as Fig. b–c and Fig. d). **(B)** Immunoreactive fibers around the fornix (f). **(C)** A higher magnification of the region delimited by the rectangle in **B.** Fibers containing the peptide located in the **(D)** mediodorsal thalamic nucleus (MD), **(E)** posterior hypothalamic nucleus (PH) (see Fig. 1d, e) and **(F)** in the zona incerta (ZI) (see Fig. 1d, e).

Regarding the distribution of thalamic neurotensin-immunoreactive cell bodies, they are more widely distributed in cat than in rat, alpaca and human [3, 5, 8, 11]. In the two latter species, neurotensin-immunoreactive cell bodies were not observed [8]. This discrepancy could be due to technical considerations, since in both rats and cats intraventricular injections of colchicine were carried out [5, 11]. Regarding the hypothalamus, rats, cats and humans [3, 5, 8, 11] showed a more widespread distribution of neurotensin-immunoreactive cell bodies than that observed in the alpaca. In these four species, cell bodies containing neurotensin were found in the lateral hypothalamic area, ventromedial hypothalamic nucleus and in the suprachiasmatic nucleus. In the arcuate nucleus, neurotensin-immunoreactive cell bodies have been visualized in rat, cat and humans, but not in alpacas. The discrepancy in the distribution of cell bodies containing neurotensin in the mammalian hypothalamus could be due to technical considerations (*e.g.*, primary antisera used, administration of colchicine) and/or species differences.

In summary, this study increases our knowledge of the chemical neuroanatomy of neurotensin in

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camelids, since a previous study described the distribution of immunoreactive structures (cell bodies and fibers) containing neurotensin in the alpaca brainstem [12]. The mapping of neurotensin in the alpaca diencephalon may help to better understand the involvement of this neuropeptide in its multiple physiological actions.

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