Investigación Clínica
ISSN: 0535-5133
Universidad del Zulia

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Investigación Clínica, vol. 59, no. 2, 2018, April-June, pp. 161-178
Universidad del Zulia

DOI: https://doi.org/10.22209/IC.v59n2a06

Invest Clin 59(2): 161 - 178, 2018 https//doi.org/10.22209/IC.v59n2a06

Neuropeptides in the human brainstem.

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Key words: neuropeptides; mesencephalon; pons; *medulla oblongata*; human.

Abstract. The physiological importance of the brainstem has made it one of the most studied structures of the central nervous system of mammals (including human). This structure receives somatic and visceral inputs and its neurons send motor efferences by means of the cranial nerves, which innervate the head, neck and sensory organs, and it mediates in several actions such as movement, pain, cardiovascular, respiratory, salivary, sleep, vigil and sexual mechanisms. Most of these actions are mediated by neuroactive substances denominated neuropeptides, which are short amino acid chains widespread distributed in the nervous system, that play a role in neurotransmission, neuromodulation (paracrine and autocrine actions), and act as neurohormones. Increased study of these substances has taken place since the 1980s to shed light on both their potential role and the way that they mediate in the organism's different activities. Thus, our aim is a detailed review of available morphologic and physiologic data regarding some neuropeptides in the human brainstem. To such end, we will discuss aspects like: 1) the distribution of neuropeptides in the human brainstem; 2) their possible physiological actions in the human brainstem; 3) neuropeptide coexistences in the human brainstem; and 4) future research in neuropeptides in the human brainstem.

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Neuropéptidos en el tronco del encéfalo humano.

Invest Clin 2018; 59 (2): 161 - 178

Palabras clave: neuropéptidos; mesencéfalo; puente; *medulla oblongata*; humano.

Resumen. La importancia fisiológica del tronco del encéfalo la ha convertido en una de las estructuras más estudiadas del sistema nervioso central de mamíferos (incluido el humano). Esta estructura recibe impulsos somáticos y viscerales, además de enviar eferencias motoras mediante los nervios craneales que inervan la cabeza, el cuello y órganos sensoriales, mediando en diferentes acciones tales como movimiento, dolor, mecanismos cardiovasculares, respiratorios, salivación, sueño y vigilia, como también mecanismos sexuales. Muchas de esas acciones son mediadas por sustancia neuroactivas denominadas neuropéptidos, los cuales son cadenas cortas de aminoácidos ampliamente distribuidos en el sistema nervioso que juegan un papel en la neurotransmisión, neuromodulación (acciones paracrina y autocrina)y actúan como neurohormonas. A partir de los años ochenta se incrementó el estudio de estas sustancias que han permitido ampliar el conocimiento sobre papel y la forma en que estas median en las diferentes actividades del organismo. Por lo tanto, nuestro objetivo es realizar una revisión detallada de los datos morfológicos y fisiológicos disponibles respecto a algunos neuropéptidos en el tronco del encéfalo humano. Para tal fin discutiremos aspectos como: 1) la distribución de neuropéptidos en el tronco del encéfalo humano; 2) las posibles acciones fisiológicas en el tronco del encéfalo humano; 3) la coexistencia de neuropeptidos en el tronco del encéfalo humano; y 4) investigaciones futuras de neuropeptidos en el tronco del encéfalo humano.

Recibido: 25-05-2017 Aceptado: 02-03-2018

INTRODUCTION

A wide variety of techniques have been used to study the distribution of substances as neuropeptides, vitamines and D- aminoacids in the nervous system of vertebrates, including humans. These methods include immunocytochemistry, radioimmunoassay, chromatography, *in situ* hybridization and autoradiography, among others (1-23). Our research group has been developing work in neuropeptide mapping in the brainstem of mammals since 2007. Thus, the distribution of substances such as alpha-neo-endorphin and neurokinin B in the human brainstem has been studied, besides the presence of immunolabeled structures containing neurokinin B, neurotensin, somatostatin-28 [1-12], methionin-enkephalin, leucine-enkephalin, beta-endorphin and adrenocorticotropic hormone in non-human primates (*Macaca fascicularis* and *Saimirís ciureus*) brainstem (1, 8).These studies have reported pioneering data about the presence of fiber and/or cell bodies containing these substances.

The term neuropeptides refers to short amino acid chains synthesized in the nervous system, classified as non-classical transmitters and working generally in synergy with other neurotransmitters, a fact that makes it difficult to determine their post-synaptic effects. However, their importance in the modification of the effect of other neurotransmitters on post-synaptic cells has been established (24). Biochemically, neuropeptides synthesize from the mRNA of the pre-

pro-peptide, originating in turn from DNA. This mRNA is translated by the ribosomes and the action of endopeptidases transforms it into a pro-peptide, which, due to post-translational factors mediated by peptidases and other chemical modifications (glycosylation, phosphorylation, and/or sulfonation), finally forms neuropeptides, which are released into the extracellular space by means of exocytosis (Fig. 1) (25). Once released, neuropeptides can perform different actions: 1) a paracrine action (neuromodulator), where the neuropeptide binds its receptors located in the post-synaptic component near to the place where it was released; 2) an autocrine action (neuromodulator), where the neuropeptide binds to receptors placed in the pre-synaptic component, controlling its release; 3) a neurotransmitter action, where the neuropeptide binds to receptors in the postsynaptic component, favoring chemical synapsis; and 4) an action distant from the place where it was released (blood or cerebrospinal fluid) acting as a neurohormone. Moreover, it is know that neuropeptides appear to be the major signal for volume transmission, a concept introduced twenty years ago concerning cellular communication in the central nervous system that operates via the diffusion of chemical signals in the extracellular fluid pathways of the brain to reach affinity membrane receptors (24, 25).

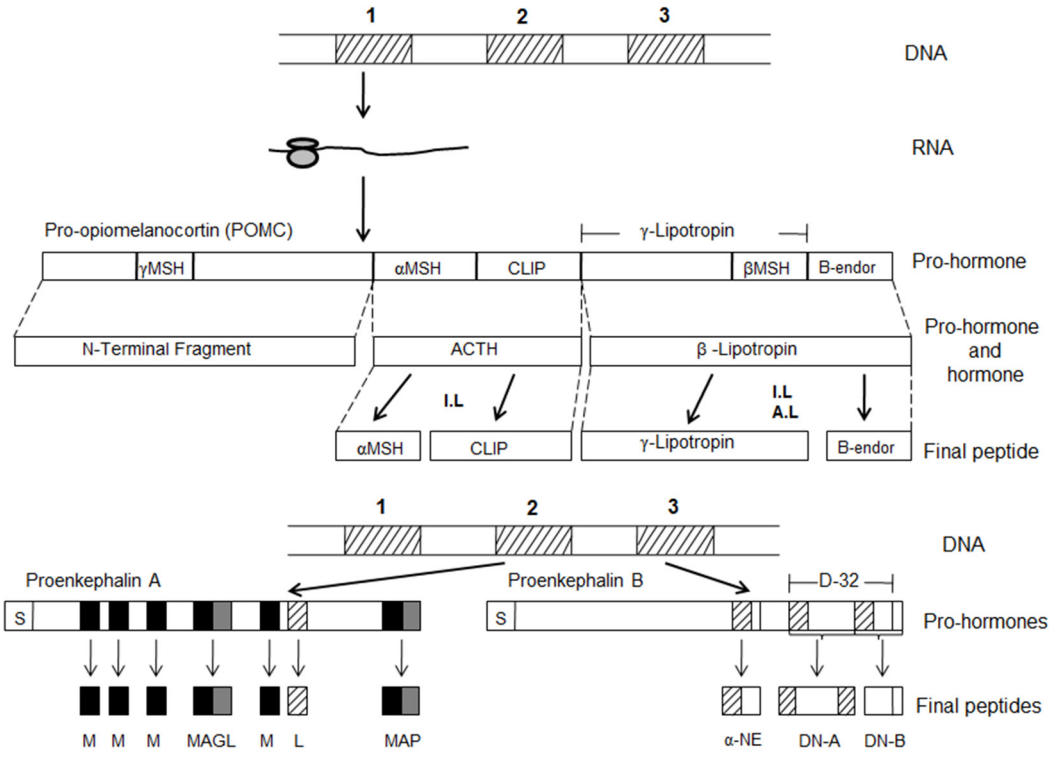


Fig. 1. Pathways for biosynthesis of opioid peptides. These arise from three genes. Gene 1 (above) give rise the precursor molecule termed pro-opiomelanocortin (POMC). Posttranslational processing produces the peptides shown: α-, β- and γ- melanophore stimulating hormone (MSH); β-endorphin, adrenocorticotropic hormone (ACTH) and corticotropin-like intermediate lobe peptide (CLIP). The enkephalins (below), arise from two other genes that code for proenkephalin A and B: M, met-enkephalins(Tyr-Gly-Gly-Phe-Met); L: leu-enkephalin (Tyr-Gly-Gly-Phe-Leu); MAGL (M-Arg-Gly-Leu); MAP: (M-Arg-Phe); α-neo-endorphin (α-NE); dinorphin-A (DN-A); dinorphin-B (DN-B); dinorphin 32 (D-32). I.L., Intermediate lobe of pituitary; A.L., anterior lobe of pituitary (Based on Shepherd, 1989).

Different techniques have been developed to demonstrate the presence of neuropeptides, one of which is the immunocytochemical method, used to detect the presence and distribution of these or other neuroactive substances in fibers and/or cell bodies, an advantage over other methods through which tissue preservation is not possible (1). Additionally, immunocytochemistry can be complemented with other techniques, like *in situ* hybridization, allowing comparison of the location where a substance is synthesized and its site of expression, enabling different immunocytochemical markings in the same region, facilitating establishment of the possible coexistence and/or colocalization of several substances, which could, in turn, yield an explanation of the relation between them. Consequently, the immunocytochemical method allows detection of several neuroactive substances under conditions of experimentation.

In humans, the study of the presence of neuropeptides has been developed in different regions of the brainstem (2-6, 26, 27), one of the most widely studied structures. This structure is anatomically divided in three portions: *medulla oblongata*, pons and mesencephalon (from caudal to rostral region). It has been implicated in sleep, vocalization, eye movement, analgesia and heart rate, as well as in sexual, visual, attentive, auditive and motor mechanisms (6). It also receives somatic and visceral inputs, and its neurons send motor efferences by means of the cranial nerves, which innervate the head, neck and sensory organs (1).

According to the previous, the aim of this work is a detailed description of the distribution of some neuropeptides in the human brainstem, taking into account the different techniques previously described based on a bibliographic search. Also, previous results published by our research group regarding the experience in the distribution of these neuroactive substances in the brainstem of mammals, including that of humans, will be considered.

NEUROPEPTIDES DISTRIBUTION IN THE HUMAN BRAINSTEM

The distribution of some neuropeptides in the human brainstem is shown in Table I and Figs. 2 and 3). Nomenclature of the different nuclei and/or tracts was carried out according to Haines' atlas of the human brain (28), and the same atlas was used for the terminology of the brainstem regions.

Opiate Peptides

There are three families of opioid peptides, classified according to their precursors: pro-opiomelanocortin, proenkephalin and pro-dynorphin (7, 29, 30). Alpha- and gamma-endorphin, beta-endorphin and methionine-enkephalin (metenk) are produced from pro-opiomelanocortin; met-enk, methionine-enkephalin-Arg6-Phe7, methionine-enkephalin-Arg6-Gly7-Leu8 and leucine-enkephalin (leu-enk) are produced from pro-enkephalin; leu-enk, dynorphin A, dynorphin B (rimorphin) and alpha- and beta-neoendorphinare produced from pro-dynorphin (8, 31-34). The non-opiate peptide adrenocorticotropin hormone (ACTH) is exclusively derived from pro-opiomelanocortin, and hence this hormone is a good marker for the pro-opiomelanocortin system (8).

In general, opiate receptors have been found in the central nervous system of mammals, of which three types have been identified: mu, delta, and kappa. While enkephalins are also known to be very active in mu and delta receptors, dynorphin has been determined to be a relatively selective agonist to receptor kappa (31), reinforcing the theory about the importance of opiate peptides and their receptors in the phenomenon of analgesia, given that the presence of these substances in regions mediating pain transmission has been demonstrated (35).

Use of radioimmunoassay and immunocytochemistry techniques has led to detection of two POMC-derived peptides in the human brainstem: β-endorphin and α-MSH 36-39).These studies have not only evidenced

TABLE I
DISTRIBUTION OF NEUROPEPTIDES IN THE BRAINSTEM

Nuclei	NP																											
	β-End		Leu.E		Met-E		DYN		α-neo-E		SP		NK		NKB		NPY		SOM		CRH		ORX		FF2		OXY	
	CB	F	CB	F	CB	F	CB	F	CB	F	CB	F	CB	F	CB	F	CB	F	CB	CF	CB	CF	CB	CF	CB	F	CB	F
CeGy	-	-	-	+	-	+	-	-	+	+	-	-	+	-	+	++	+	+	+++	+	-	-	-	-	-	-	-	-
DLF	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
DCNu	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-
DMNu	-	-	-	+	-	+	-	-	-	+++	-	+	-	-	-	+	+	+	(R)	+	-	-	-	-	-	-	-	-
FacNu	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	(R)	-	(R)
HyNu	-	-	-	-	-	-	-	-	-	+++	-	-	-	-	-	+	-	-	+++	(R)	-	-	-	-	-	-	-	-
LCNu	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
LVN	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	(R)	-	-	-	-	-	-	-	(R)
LRNu	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
MAO	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-
ML	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-
MVN	-	-	-	-	-	-	-	-	-	+	-	+	-	-	-	+	+	+	-	(R)	-	-	-	-	-	-	-	-
NuAm	-	-	-	-	-	-	-	-	-	+++	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
NuCu	-	-	-	+	-	+	-	-	-	+++	+	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	+	+
NuGr	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	(R)	-	-	-	-	-	-	-	(R)
PO	-	-	-	+	-	+	-	-	-	-	-	-	-	-	-	-	+	+	-	+(R)	-	-	-	-	-	-	-	(R)
RaNu	-	-	-	-	-		-	-	-	-	-	-	-	-	-	-	-	+	+		-	-	-	-	-	-	-	-
RetF	-	-	-	+	-	+	-	-	+	+++	+	-	+	-	-	-	+	+	-	+	-	-	-	-	-	-	-	-
SCP	-	-	-	+	-	+	-	-	-	-	+	+	-	-	-	-	-	-	-		-	-	-	-	-	-	-	-
SCPL	-	+	-	+	-	+	-	+	-	-	+	+	-	-	-	-	-	+++	-	+	+	-	-	-	-	-	-	-
SO	-	-	-	+	-	+	-	-	-	-	-	-	-	-	-	-	-	+++	-	-	-	-	-	-	-	-	-	-
SolNu	-	-	-	+	-	+	-	-	-	++	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	(R)	(R)	+
SolTr	-	-	-	+	-	+	-	-	-	-	-	-	-	-	-	-	+	+++	-	(R)	-	-	-	-	-	-	-	-

TABLE I. (*Continuation*)

Nuclei	NP																											
	β-End		Leu.E		Met-E		DYN		α-neo-E		SP		NK		NKB		NPY		SOM		CRH		ORX		FF2		OXY	
	CB	F	CB	F	CB	F	CB	F	CB	F	CB	F	CB	F	CB	F	CB	F	CB	CF	CB	CF	CB	CF	CB	F	CB	F
SpTNu	-	-	-	+	-	+	-	-	+	+++	+	+	-	++	-	+	++	+	-	+	-	-	-	-	-	-	-	(R)
SpVN	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-
TrapB	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-
VesNu	-	-	-	+	-	+	-	-	-	++	+	-	-	-	-	-	-	++	+	-	-	-	-	-	-	-	-	-
NuPp	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-
LL	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+++	-	+	-	-	-	-	-	-	-	-
LocCer	-	-	-	+	-	+	-	-	-	-	+	+	-	-	-	-	+	+++	+	(R)	+	-	-	-	-	-	-	-
SVN	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
ISNu	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	++	-	-	-	-	-	-	-	-	-	-	-	-
TriMoNu	-	-	-	+	-	+	-	-	-	+	-	-	-	-	-	-	-	+++	-	-	-	-	-	-	-	-	-	-
IC, CNu	-	-	-	+	-	+	-	-	-	-	+	+	+	-	-	+	+	+	-	-	-	-	-	-	-	-	-	-
IC, LZ	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-
IPNu	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	-	-	-	-	-	-	-	-
OcNu	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+++	-	-	-	-	-	-	-	-	-
SC	-	-	-	-	-	-	-	-	+	+++	+	-	-	-	+	-	-	-	+	-	-	-	-	-	-	-	-	-
SCNu	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
SN	-	-	-	(R)	-	(R)	-	-	+	-	-	-	-	++	-	+	+	+	-	+	-	-	-	+	-	-	-	(R)
TroNu	-	-	-	+	-	+	-	-	-	-	-	-	+	++	-	-	+	+	-	+	-	-	-	-	-	-	-	-
VTegA	-	-	-	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	+	-	(R)	-	-

Distribution of β-endorphin (β-End), leucine-enkephalin (Leu-E), metionine-enkephalin (Met-E), dinorphin (DYN), alfa-neo-endorphin (α-neo-E), Substance P (SP), Neurokinin (NK), Neurokinin B (NKB), Neuropeptide Y (NPY), Somatostatin (SOM), Corticotropin Release Hormone (CRH), Orexin (ORX), Neuropeptide FF2 (FF2) y Oxytocin (OXY).

The first column contains the abbreviations of nuclei in the brainstem. **CB** Cell Bodies, **(+)** Presence (+ Low density; ++ moderate density; +++ High density); **(-)** Absence; **(R)** Receptor.

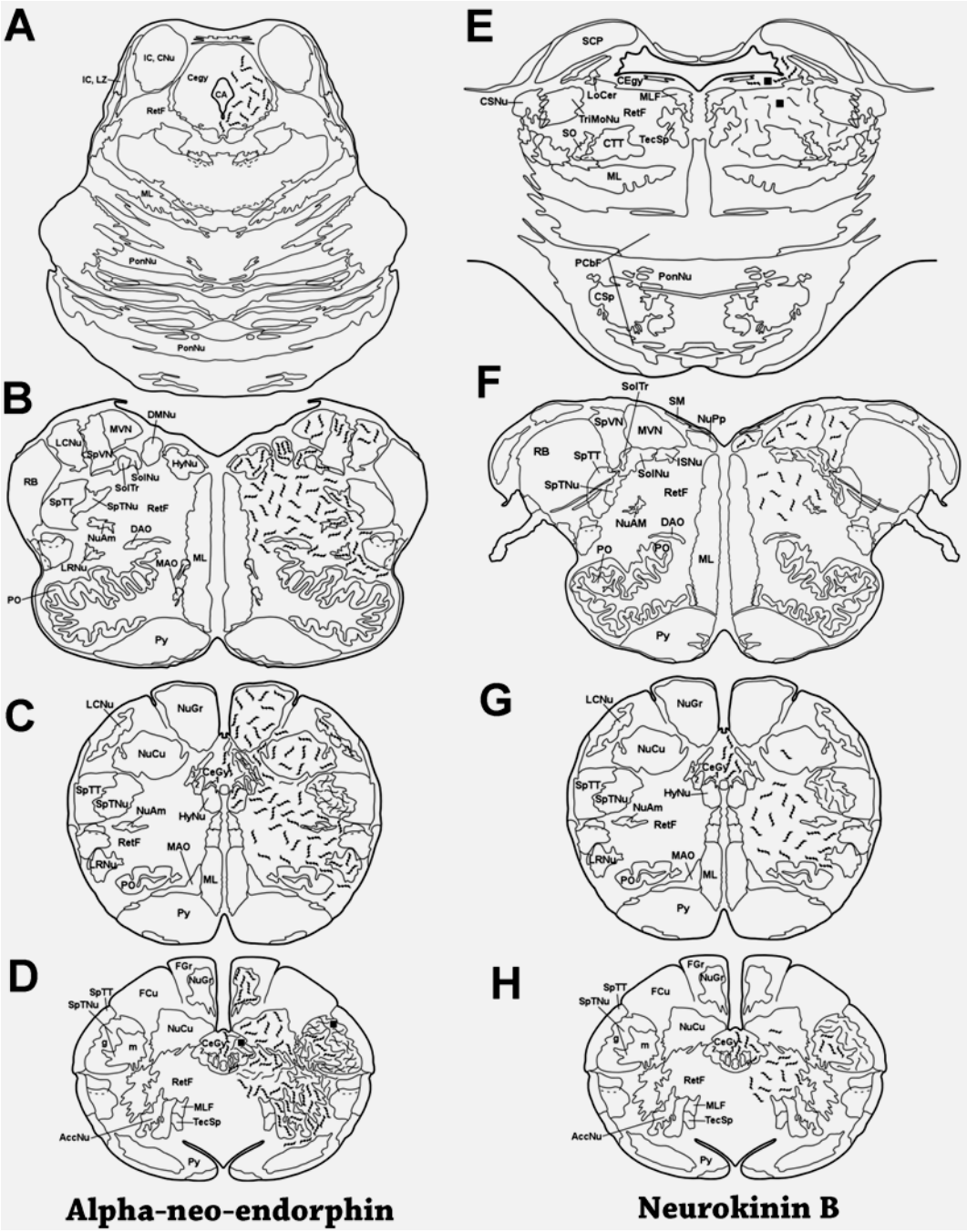


Fig. 2. (A-H). Schematic drawings of coronal section (rostral to caudal region) depicting the distribution of alpha-neo-endorphin (A-D) and neurokinin B (E-H) immunoreactivity in the human brainstem. The low, moderate and high distribution of fibers and cell bodies containing these neuropeptides is represented by symbols (∼) and (■▲●) respectlly.Density of immunoreactive perikarya was considered to be higher when more than 10 cell bodies/section were found; density was moderate when 5-10 cell bodies/section were observed, and low when fewer than 5 cell bodies/section were visualized. Aditionally, immunoreactive fibers were considered short (< 90 mm), medium (90-120 mm) or long in length (> 120 mm) and cell bodies were considered small (diameter below 15 μm), medium-sized (15-25 μm), and large (above 25 μm). This figure is redrawn from the maps of alha-neo-endorphin and neurokinin B published in reference (6), with permission of Springer.

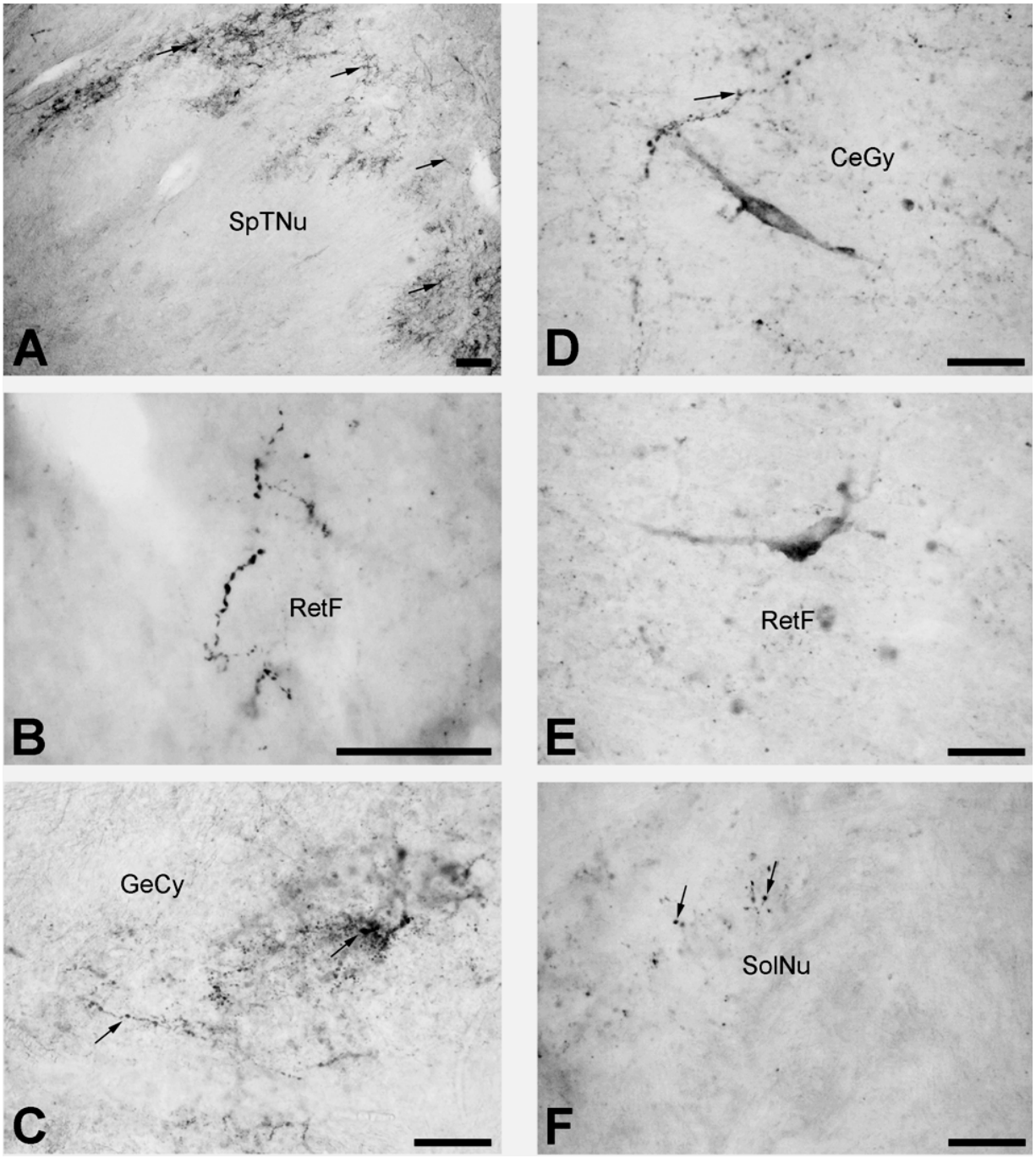


Fig. 3. (A-F). Neuropeptides immunoreactivity in the human brainstem. (A-C) Alpha-neo-endorphin fibers in the spinal trigeminal nucleus, reticular formnation and periaqueductal gray matter. These nuclei contain low (B), moderate (C) and high (A) density of fibers (arrows). (D-F) shown cell bodies and fibers (arrows) containing neurokinin B in the periaqueductal gray matter, reticular formation and solitary nucleus. Scale bar= 100 μm for (A, C, D, E, F); 50 μm for (B). Panels A, C and F are provided from Ewing Duque-Díaz. Panels B, D and E are from Duque E, Mangas A, Salinas P, Díaz-Cabiale Z, Narváez JA, Coveñas R. Mapping of alpha-neo-endorphin-and neurokinin B-immunoreactivity in the human brainstem. *Brain StructFunct*. 2013; 218(1): 131-149 with permission of Springer.

the presence of high levels of β-endorphin in the middle regions of the midbrain, and of moderate levels of same in the regions of pons and medulla oblongata, but also identified β-endorphin-labeled cell bodies in the lateral and middle cuneate nucleus, as well as fibers in the lateral parabrachial nucleus, jointly labeled with β-endorphin and α-MSH.

Additionally, the presence in the human mesencephalon of fibers labeled with derivates of Pro-enkephalins such as met-enk, met-8 andleu-enk has been demonstrated (5, 9, 11,12), located in the substantia nigra, ventral tegmental area, between the brachium conjuntivum and the lemniscus medialis, lateral parabrachial nucleus adjacent to the trochlear nerve root, periaqueductal gray matter pontine region, locus coeruleus, reticular formation, and inferior olivary nucleus, suggesting modulatory mediation in different actions like respiratory and motor mechanisms. In addition, methionine-enkephalin and leucine-enkephalin receptors have been reported in the substantia nigra of the human brainstem and diencephalon during aging (12); however, the results did not show significant changes in the levels of these neuropeptides in this region with respect to zones like the pallidum and the caudate. These findings suggest that during the phenomenon of aging, enkephalins in these regions can modulate the action of dopaminergic neurons, given the close relation between enkephalins and these pathways, especially the pathways affecting receptors D1 and D2, resulting in motor effects.

Coveñas *et al.* (5) demonstrated the presence of Arg^6-Phe^7-Leu^8 (Met-enk-8)-methionine-enkephalin-labeled cell bodies and/or fibers in the whole human brainstem. This study evidenced different densities of immunolabeled structures (high, moderate and low density) in regions such as the reticular formation, (medulla oblongata, pons and mesencephalon), medial vestibular nucleus, solitary nucleus, dorsal motor nucleus of vagus, periaqueductal gray matter, spinal trigeminal nucleus, ambiguus nucleus, solitary tract, cuneate nucleus, superior olivary nucleus, inferior colliculus, among others (Table I).

On the other hand, Fordor *et al.* (38), evidenced the presence of B-dynorphin-labeled fibers in the rostral region of the lateral parabrachial nucleus. Additionally, a recent immunocytochemical study demonstrated the presence of immunoreactive structures by another Pro-dynorphin derivate, alfa-neoendorphin, in several nuclei and regions of the human brainstem (6) with a low density of cell bodies in the medullar periaqueductal gray matter and the spinal trigeminal nucleus (gelatinous pars), as well as high, moderate and low densities in zones like the gelatinous and magnocellular portion of the spinal trigeminal nucleus, principal sensorial nucleus, dorsal motor nucleus of vagus, hypoglossal nucleus, superior colliculus, medial vestibular nucleus, nucleus ambiguus, cuneate nucleus and reticular formation (Table I).

a) Tachykinins

Substance P (SP) is one of the best known members of this family, and one of the first to be isolated. This peptide can be obtained from a pro-hormone or in association with substance K (also denominated neurokinin A) of a precursor derived from the gen of per-pro-tachykinin A (30, 31, 40, 41); among its best studied actions is its mediation in nociceptive events, including pain. On the other hand, neurokinin B derives from the gen of pro-tachykinin B (30). Other members of the tachykinin family exist, like: kassinin, eledoisin and fisalaemin. Bombesin was isolated in amphibians, and its homologue in mammals is gastrin-releasing peptide (GRP), in which both molecules have the same carboxi-terminal heptapeptide (30).

Studies have detected immunoreactive structures for various tachykinin-derived peptides in the human brainstem by means of different immunocytochemical methods, demonstrating the presence of SP in regions

of the brainstem (13, 14, 27, 39). Cell bodies immunostained with SP were detected in superior colliculus, reticular formation (medulla oblongata, pons and mesencephalon) and solitary tract nucleus, as well as immunoreactive fibers containing SP in the inferior colliculus, braquium conjuntivum, parabrachial nucleus, locus coeruleus, spinal trigeminal nucleus, dorsal motor nucleus of vagus, medial vestibular nucleus and cuneate nucleus. Also, and by means of radioimmunoassay and autoradiography, high levels of SP were observed in the mesencephalon, pons and medulla oblongata (42, 43).

On the other hand, the presence of neurokinin and neurokinin B-labeled cell bodies and/or fibers in the human brainstem has been demonstrated (4, 6). Although these studies evidenced ample distribution of these tachykinins, the density of immunoreactive structures shows some differences. Thus, Coveñas *et al.* (4) described a high density of cell bodies labeled for neurokinin in regions such as periaqueductal gray matter, reticular formation, dorsal motor nucleus of vagus, dorsal longitudinal fascicle, and inferior colliculus, and Duque *et al.* (6) observed a low density of neurokinin B cell bodies labeled in the periaqueductal gray matter, superior colliculus and pons reticular formation. Both studies determined a similar pattern in the density of fibers containing neurokinin-and-neurokinin B, where immunoreactive fibers were described in similar nuclei: in the spinal trigeminal nucleus, substantia nigra, reticular formation, and solitary tract nucleus. Such differences might arise from the antibody specificity, considering the similarity of methodology applied.

b) Neuropeptide Y

Neuropeptide Y (NPY) is a molecule of 36 amino acids derived from a pre-pro-NPY and belonging into a family that includes polypancreatic peptide (PP) and peptide YY (PYY). This peptide is highly conserved in mammals and it is catalogued as a neuroprotector, additionally to its role in the response to stress and food intake (44), the reason why its study focuses on detection of this substance – receptors and cellular structures– in regions of the cortex and the diencephalon (15-17, 45-48). The presence of cell bodies and/or fibers containing NPY has been described in the human brainstem (49, 50) from the utmost rostral to the utmost caudal region, in nuclei like: periaqueductal gray matter, reticular formation, substantia nigra (pars lateralis), inferior colliculus, locus coeruleus, tegmental dorsal tegmental nucleus of Gudden, trigeminal spinal nucleus, lateral reticular nucleus, medial and inferior vestibular nucleus, dorsal motor nucleus of vagus, and inferior olivary nucleus, interpeduncular nucleus and nucleus laterodorsalistegmenti.

c) Somatostatin

Somatostatin is a cyclic tetradecapeptide that originally isolated of the ovine hypothalamus on the basis to inhibit the release of growth hormone from anterior pituitary cells (25). Several molecular forms of this peptide exist, such as somatostatin-14, somatostatin-28 and a fragment of this latter, somatostatin-28 [1-12] (51). Studies have been conducted in the human brainstem of the distribution of receptors for somatostatin, allowing observation of a relative density (low, moderate, high) in the different regions (medulla oblongata, pons, mesencephalon), and locating high levels of somatostatin receptors in the gracilis nucleus, cuneate nucleus, inferior olivary nucleus, hypoglossal nucleus, dorsal motor nucleus of vagus, and solitary tract nucleus. In contrast, lower levels of somatostatin receptors were observed in the lateral vestibular nucleus and the medial vestibular nucleus (11). By using the immunocytochemistry technique, Chigr *et al.* (27) observed different densities of immunoreactive structures containing somatostatinin the human brainstem. In this study, cell bodies labeled with this neuropeptide were observed in the superior colliculus, supratrochlear nucleus,

dorsal raphe nucleus, medial lemniscus, interpeduncular nucleus, periaqueductal gray matter (pons), medial vestibular nucleus, and reticular nucleus. Additionally, fibers containing somatostatin were found in interpeduncular nucleus, periaqueductal gray matter, cuneate nucleus, substantia nigra, superior central nucleus, locus coeruleus, parabrachial nucleus, dorsal motor nucleus of vagus, trigeminal spinal nucleus, ambiguus nucleus, reticular formation (medulla oblongata), inferior olivary nucleus and accessory olivary nucleus. Also, Bouras *et al.* (52) identified immunoreactive structures (cell bodies and fibers) containing somatostatin-14 and somatostatin-28 (1-12) in the human brainstem. This work allowed observation of not only a high density of cell bodies in the periaqueductal gray matter, oculomotor nucleus, hypoglossal nucleus, trochlear nucleus, but also moderate and low density of cell bodies in nuclei like the ventral tegmental area, locus coeruleus, superior and lateral vestibular nucleus, lateral lemniscus and facial nucleus.

d) Other neuropeptides and neuroactive substances

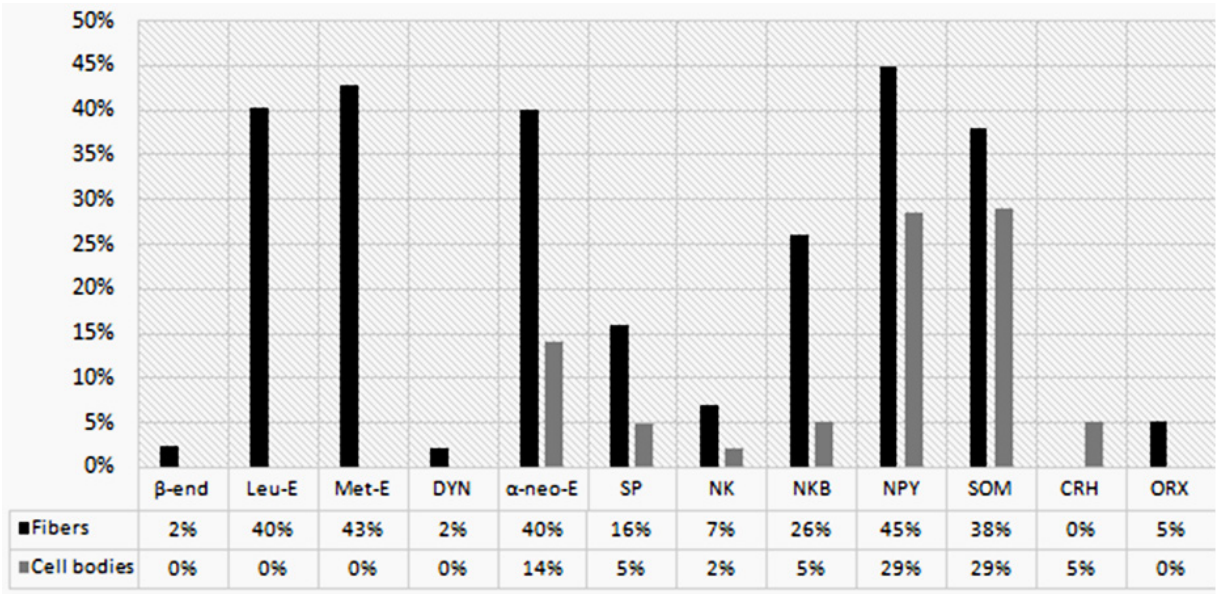
By using the radioimmunoassay technique, increased level of corticotrophin-releasing hormone (CRH) in different pontine nuclei of the brainstem of depression-diagnosed suicide men was observed (53). This work evidenced 30 and 45% CRH increase with respect to raphe nucleus controls in its caudal and dorsal nuclei portions, as well as locus coeruleus. Hasegawa *et al.* (54) demonstrated the presence of fibers containing oxerin B associated with dopaminergic neurons in the ventral tegmental area and the substantia nigra of the post-mortem human brainstem. These findings suggest that the orexinergic mechanism may play an important modulating role in the reward process.

Although neuropeptide FF2 is an 8-amino-acid peptide regularly located in the ventromedial and dorsomedial hypothalamus, it has also been observed in solitary tract nucleus neurons, with fibers projecting towards the medulla, where they develop nociceptive functions (24). A study conducted by Goncharuk and Jhamandas (55) detected the presence of structures (cell bodies and/or fibers) immunolabeled for receptor of FF2 in different structures of the encephalon, among them regions of the brainstem such as the rostral part of the dorsal motor nucleus of vagus, solitary tract nucleus and ventral tegmental area. It must be highlighted that along this work, the highest receptor density was observed in the hypothalamus, suggesting a mediating role of this substance in neuroendocrine regulation.

The use of *in vitro* light microscopic autoradiography allowed to demonstrate the presence of oxytocin receptors in the pars compact of the substantia nigra, the gelatinous layer of the spinal trigeminal nucleus, medio-dorsal region of the solitary tract nucleus and hypoglossal nucleus (56). Additionally, Boccia *et al.* (57) demonstrated through immunohistochemistry the presence of oxytocin receptors in solitary tract nucleus, ambiguus nucleus and hypoglossal nucleus cell bodies and fibers, but not in the inferior olivary.

Besides neuropeptides, the presence of neuroactive substances (classic neurotransmitters) has been evidenced by means of diverse immunolabeling techniques in different regions of the human brainstem. Presence was detected of cell bodies and fibers labeled for glycine receptors in the dorsal motor nucleus of vagus, hypoglossal nucleus, solitary tract nucleus, geniculated nucleus, trigeminal spinal nucleus, cuneate nucleus, locus coeruleus, raphe dorsal nucleus, olivary complex, inferior olivary and lateral reticular nucleus (58, 59).

Finally, Fig. 4 shows the percentage of nuclei with immunoreactive structures (cell bodies and fibers) for the different neuropeptides. It evidences that the highest percentage of nuclei with labeled cell bodies corresponds to NPY and somatostatin, with 29%, while the highest number of nuclei



	β-end	Leu-E	Met-E	DYN	α-neo-E	SP	NK	NKB	NPY	SOM	CRH	ORX
Fibers	2%	40%	43%	2%	40%	16%	7%	26%	45%	38%	0%	5%
Cell bodies	0%	0%	0%	0%	14%	5%	2%	5%	29%	29%	5%	0%

Fig. 4. Percentage of nuclei with immunolabeled structures (cell bodies and / or fibers) with the different neuropeptides studied in the human brainstem.

with immunolabeled fibers corresponds to NPY, with 45%. Nuclei with immunolabeled cell bodies suggest these regions may have a role as regulating centers in the release of these substances and are capable of projecting axons towards other areas of the brainstem, while those nuclei containing fibers labeled for the different neuropeptides are part of the projections of diverse origins.

PHYSIOLOGICAL ACTIONS OF NEUROPEPTIDES IN THE HUMAN BRAINSTEM

In general, the neuroactive substances specified in this review show an ample distribution; they are located all along the whole structure of the human brainstem, suggesting that diverse neuropeptides may be involved in a wide range of physiological actions. One example of such is the presence of fibers immunolabeled with Pro-enkephalin-derived opiates, like methionine-enkephalin-8 and pro-dynorphin, like alfa-neo-endorphin in regions like the trigeminal spinal nucleus, periaqueductal gray matter, and solitary tract nucleus, suggesting that these neuropeptides neuromodulate nociceptive mechanisms (pain and temperature), as well as visceral sensitivity (5, 6). On the other hand, the presence of immunoreactive structures containing alfa-neo-endorphin in the superior colliculus suggests this substance can have a modulating action in visual motor coordination (6).

Evidence has been established of the important role of some peptides derived from pro-tachykinins, like NKA, NKB and substance P, whose biological interaction is mediated by three receptors: NK-1, NK-2, and NK-3. Besides, tachykinins are known to be involved in different actions as salivation, smooth muscle contraction regulation, depolarization of potential action in central neurons, hyperactivity, activation of behavioral mechanisms, nociception, etc. (4, 41). Such data are in accordance with previous results where cell bodies and fibers containing neurokinin-and-neurokinin B were observed in regions like trigeminal spinal nucleus, periaqueductal gray matter and interpeduncular nucleus, regions involved in nociceptive and antinociceptive mechanisms, and in the solitary tract nucleus and

parabrachial nucleus, involved in respiratory and cardiovascular mechanisms. Their presence in the dorsal motor nucleus of vagus has been related to smooth muscle contraction mechanisms, substantia nigra, associated with motor mechanisms, and finally the inferior colliculus, involved in auditory mechanisms (4, 6, 60).

NPY, in principle synthesized in the arcuate nucleus, projects itself to many other nuclei where it shows an effect on multiple systems, thus indicating a possible neuromodulating action on the central nervous system (48). As previously mentioned, most studies on the distribution of NPY in humans have involved mostly hypothalamic regions; however, two studies of the human brainstem (49, 50) evidenced the presence of this neuropeptide in caudal regions of the brainstem (medulla oblongata), suggesting that this region receives projections with NPY from the hypothalamus, which allows it posterior and diverse autonomic and neuroendocrine control functions.

Presence of NPY has been associated with an inhibitory role in the hypothalamic hormonal synthesis of somatostatin (27). However, other studies of the human brainstem suggest other possible actions. Presence of somatostatin-immunolabeled structures in the periaqueductal gray matter and trigeminal spinal nucleus suggests a modulating effect on nociceptive activities. On the other hand, their presence in the locus coeruleus can influence the noradrenergic system, given that this nucleus is involved in stress, major depression, and paradoxical sleep response mechanisms (52, 61). The presence of structures immunolabeled for somatostatin in the dorsal motor nucleus of vagus, as well as solitary tract nucleus may suggest that this neuropeptide participates in regulation of some autonomous functions, based on results from previous studies in rats, where the intraventricular administering of somatostatin stimulated intestinal motility and gastric acid secretion (2, 3).

Orexins (hypocretins) are widely studied neuropeptides in the central nervous system of mammals, including that of humans. These substances have been reported to be involved in mechanisms like sleep and alertness reward system, alimentary disorders and energetic balance (54, 62, 63).

Finally, it is important to highlight that the ample distribution of neuropeptides in the brainstem suggests their participation in various physiological actions controlled by this structure.

COEXISTENCE OF NEUROPEPTIDES IN THE HUMAN BRAINSTEM

Presence of different neuropeptides in nuclei and/or tracts of the human brainstem (Table I) suggests possible interaction among these substances, and an elaborate modulating action in physiological functions. The location of neuropeptides in various similar nuclei hints of their coexistence in the same neuron, which is why some studies evidence a possible coexistence of methionine-enkephaline-8 and substance P in the solitary tract nucleus and in the locus coeruleus (9, 11, 12).

Taking into account the distribution and peptidergic characteristics of immunolabeledcell bodies, a possible coexistence can be described between different neuroactive substances summarized below:

- Neurokinin B and somatostatinin the reticular formation of the pons region (2, 3, 6).
- Somatostatin and substance P in the spinal trigeminal nucleus (2, 3, 13, 14, 27, 52).
- Neurokinin B and alfa-neo-endorphin in the periaqueductal gray matter (6).
- β-endorphin, dynorphin A, dynorphin B, enkephalins, SP, NPY, CCK in the parabrachial nucleus and the locus coeruleus (39).

Given that, the coexistences herein proposed are described taking into account cell bodies labeling, double-labeling immunocy-

tochemical studies should be conducted to demonstrate them.

FUTURE RESEARCH IN NEUROPEPTIDES IN THE HUMAN BRAINSTEM

The previous suggests involvement of neuropeptides in many nervous central system actions. Given that data available in the field of the human brainstem are occasionally incomplete, the need arises for in-depth studies of aspects that will shed light on the detailed distribution of these substances and their physiological implication.

Use of diverse methodologies can provide more detailed information on the synthesis of these neuroactive substances. For example, hybridization *in situ* would allow comparison between mRNA distribution and the final molecule of a neuropeptide, evidencing if a given substance is produced in the same region where it acts, or, on the contrary, it is produced in another region and transported to its site of action. Such studies can be supplemented by conducting tracking of not only the receptor of a neuropeptide, but also its activity.

On the other hand, the distribution of peptidases would also allow supplementary study of the action pathways of a neuropeptide. In studying the cellular communication pathway where neuropeptides mediate, it is important to develop electronic microscopy and immunocytochemistry techniques (simple and/or double labeling) to demonstrate whether a neuropeptide is released alone or together with other neuroactive substances.

Given the knowledge of the possible coexistence of neuropeptides, double-or-triple-labeling immunofluorescence studies must be implemented, aided by studies by means of confocal microscopy in order to analyze if co-localization exists between neuropeptides.

The combination of methods such as immunocytochemistry and trac-tracing would allow knowledge of afferent and efferent projections of peptidergic systems in a structure like the brainstem. This would provide wider, more detailed knowledge of the connections that can be established in the different areas of the human brainstem, as well as the level of influence that a region can have on another.

Thus, the results deriving from combining the different techniques mentioned can contribute to in-depth comprehension of the functions of the different neuroactive substances –among them neuropeptides– in the human brainstem.

ACKNOWLEDGEMENTS

This work has been supported by the Research Commission and the School of Medicine of the Universidad de Santander UDES.

ABBREVIATION

CeGy: Periaqueductal Grey Matter
CSNu: Central Superior Nucleus
DCNu: Dorsal Cochlear Nucleus
DLF: Dorsal Longitudinal Fasciculus
DMNu: Dorsal Motor Nucleus of Vagus
FacNu: Facial Nucleus
HyNu: Hypoglossal Nucleus
IC, CNu: Inferior Colliculus, Central Nucleus
IC, LZ: Inferior Colliculus, Lateral Zone
IPNu: Interpeduncular Nucles
ISNu: Inferior Salivatory Nucleus
LCNu: Lateral Cuneate Nucleus
LL: Lateral Lemniscus
LocCer: Locus Coeruleus
LRNu: Lateral reticular Nucleus
LVN: Lateral Vestibular Nucleus
MAO: Medial AccesoryOlivary Nucleus
ML: Medial Lemniscus
MVN: Medial Vstibular Nucleus
NuAm: Nucleus Ambiguu
NuCu: Cuneate Nucleus
NuGr: Gracile Nucleus
NuPp: Nucleus Prepositus
OcNu: Oculomotor Nucleus
PO: Inferior Olivary Nucleus
RaNu: Raphe Nucleus

RetF: Reticular Formation
SC: Superior Colliculus
SCP: Brachium conjuntivum
SCPL: LateralParabrachial Nucleus
SN: Substantia Nigra
SO: Superior Olivary Nucleus
SolNu: Solitary Nucleus
SolTr: Solitary Tract Nucleus
SpTNu: Spinal Trigeminal Nucleus
SpVN: Spinal (or inferior) Vestibular Nucleus
SVN: Superior Vestibular Nucleus
TrapB: Trapezoid Body
TroNu: TrochlearNucleus
VesNu: Vestibular Nucleus
VTegA: Tegmental Ventral Area

REFERENCES

1. **Coveñas R, Duque E, Mangas A, Marcos P, Narváez JA**. Neuropeptides in the monkey (*Macaca fascicularis*) brainstem. In: Brain Molecules: from Vitamins to Molecules for Axonal Guidance. Mangas, A., Coveñas, R., Geffard, M, eds. Transworld Research Network, Trivandrum; 2008; p 131-156.
2. **Carpentier V, Vaudry H, Laquerrière A, Tayot J, Leroux P**. Distribution of somatostatin receptors in the adult human brainstem. Brain Res 1996; 734: 135-148.
3. **Carpentier V, Vaudry H, Mallet E, Laquerrière A, Tayot J, Leroux P**. Anatomical distribution of somatostatin receptor in the brainstem of the human fetus. Neuroscience 1996; 73: 865-879.
4. **Coveñas R, Martín F, Belda M, Smith V, Salinas P, Rivada E**. Mapping of neurokinin-like immunoreactivity in the human brainstem. BMC Neurosc 2003; 4:(3) 1-13.
5. **Coveñas R, Martin F, Salinas P, Rivada E, Smith V, Aguilar LA.** An immunocytochemical mapping of methionine-enkephalin-arg-^{6}gly^{7}-leu^{8} in the human brainstem. Neuroscience 2004; 128(4): 843-859.
6. **Duque E, Mangas A, Salinas P, Díaz-Cabiale Z, Narváez JA, Coveñas R**. Mapping of alpha-neo-endorphin-and neurokinin B-immunoreactivity in the human brainstem. Brain Struct Funct2013; 218(1): 131-149.
7. **Duque E, Mangas A, Díaz-Cabiale Z, Narváez JA, Coveñas R**. Neuropeptides in the monkey brainstem. In: Monkeys: Biology, behavior and disorders, Rachel M. Williams, editors. Nova Science Publisher 2011; p 151-166.
8. **Duque-Díaz E, Díaz-Cabiale Z, Narvaéz JA, Coveñas R.** Mapping of enkephalins and adrenocorticotropic hormone in the squirrel monkey brainstem. Anat Sci Int2017; 92: 275-292.
9. **Bouras C, Taban CH, Constantinidis J.** Mapping of enkephalins in human brain: an immunohistofluorescence study on brains from patients with senile and presenile dementia. Neuroscience 1984; 12(1): 179-190.
10. **Belda M, Coveñas R, Narváez JA, Aguirre JA, Tramu G.** An immunocytochemical mapping of methionine-enkephalin-Arg6-Gly^{7}-Leu^{8} in the cat brainstem. Anat Embryol. 2003; 206(5): 399-408.
11. **Rinne JO, Lönnberg P, Marjamäki P, Mölsä P, Säkö E, Paljärvi L**. Brain methionine-and leucine-enkephalin receptors in patients with dementia. Neurosci Lett 1993; 161(1): 77-80.
12. **Rinne JO**. Human brain methionine-and leucine-enkephalins and their receptors during ageing. Brain Res 1993; 624(1): 131-136.
13. **Del Fiacco M, Dessi ML, Atzori MG, Levanti MC**. Substance P in the human brainstem. Preliminary results of its immunohistochemical localization. Brain Res 1983; 264(1): 142-147.
14. **Del Fiacco M, Dessi ML, Levanti LC**. Topographical localization of substance P in the human pot-morten brainstem. An inmunohistochemical study in the newborn and adult tissue. Neuroscience 1984; 12: 591-611.
15. **Dawbarn D, Hunt SP, Emson PC**. Neuropeptide Y: regional distribution chromatographic charatacterization and immunohistochemicaldemostration in post-morten human brain. Brain Res 1984; 296: 168-173.
16. **Widdwson PS**. Quantitative receptor autoradiography demostrates a differential distribution of neuropeptide-Y Y_1 and Y_2 receptor subtypes in the human and rat brain. Brain Res 1993; 631: 27-38.
17. **Jacques D, Dumont Y, Fornier A, Quirion R.** Characterization of neuropeptide Y re-

ceptor subtipe in the normal human brain, including the hypothalamus. Neuroscience 1997; 79 (1): 129-148.

18. **Coveñas R, Gonzalez-Fuentes J, Rivas-Infante E, Lagartes-Donato MJ, Mangas A, Geffard M, Arroyo-Jimenez MM, Cebada-Sanchez S, Insausti R, Marcos P.** Developmental study vitamin C distribution in children's brainstem by immnunohistochemistry. Ann Anat 2015; 201: 65-78.
19. **Mangas A, Coveñas R, Geffard K, Geffard M, Marcos P, Insauti R, Dabadie MP**. Folic acid in the monkey brain: An immunocytochemical study. Neurosc Lett 2004; 362: 258-261.
20. **Mangas A, Coveñas R, Geffard K, Geffard M, Marcos P, Insauti R, Dabadie MP**. Thiamine-like in the monkey brain: An immunocytochemical study. Life Sciences 2006; 79: 1121-1128.
21. **Mangas A, Coveñas R, Bodet D, Geffard M, Aguilar LA, Yajeya J**. Immunocytochemical visualization of D-Glutamate in the rat brain. Neuroscience 2007; 144: 654-666.
22. **Mangas A, Coveñas R, Bodet D, Deleu S, Marcos P, Geffard M**. Vitamins in the monkey brain: an immunocytochemical study. J Chem Neuroanat 2009; 38: 1-8.
23. **Mangas A, Yajeya J, Gonzalez N, Husson M, Geffard M, Coveñas R.** Detection of pantothenic acid-immunoreactive neurons in the rat lateral septal nucleus by a newly developed antibody**.** Folia Histochem Cytobiol 2016; 54: 186-192.
24. **Striedter GF**. Neurobiology: A functional approach. New York (NY): Oxford University Press. 2016; p 58-61.
25. **Coveñas R, Mangas A, Narváez JA**. Introduction to neuropeptide. En: Focus on neuropeptide research, Coveñas R, Mangas A, Narváez J, eds. Transworld Research Network Trivandrum; 2007; p 1-26.
26. **Pammer CS, Fodor M, Palkovits M.** Localization of corticotropin-releasing factor, somatostatin, and vasoactive intestinal polypeptide in the parabrachial nuclei of the human Brain. J Neurosc Res 1988; 20:109-114.
27. **Chigr F, Najimi M, Leduque P, Charnay Y, Jordan D, Chayvialle JA.** Anatomical distribution of somatostatin immunoreactivity in the infant brainstem. Neuroscience 1989; 29(3): 615-628.
28. **Haines DE**. Neuroanatomy. An Atlas of Structure, Section, and Systems. Urban and Schwarzenberg, Baltimore-Munich 1987.
29. **Shepperd G**. Neurobiology, Oxford University press, 3th ed. New York, USA. 1989.
30. **Kandel ER, Schwartz JH, Jessell TM**. Principles of neural science. New York: McGraw-Hill. 2001; 4: p 1227-1246.
31. **Raffa RB**. The action of FMRFamide (Phe-Met-Arg-Phe-NH2) and related peptides on mammals. Peptides 1988; 9: 915-922.
32. **Schwarzer C.** 30 years of dynorphins new insights on their functions in neuropsychiatric diseases. Pharmacol Ther 2009; 123: 353-370.
33. **Manso B, Sanche ML, Medina LE, Aguilar LA, Díaz-Cabiale Z, Narváez JA, Coveñas R.** Immunohistochemical mapping of pro-opiomelanocortin- and pro-dynorphin-derived peptides in the alpaca (*Lama pacos*) diencephalon. J Chem Neuroanat 2014; 59-60: 36-50.
34. **Kowarika, M, Einhäusera J, Jochima B, Büttnerb A, Töllea TR, Riemenschneiderc M, Platzera S, Berthelea A.** Impact of the COMT $Val1^{08/158}$ Met polymorphism on the mu-opioid receptor system in the human brain: mu-opioid receptor, met-enkephalin and beta-endorphin expression. Neurosc Lett 2012; 506: 214-219.
35. **Hollander N**. β-endorphin in the brainstem, pituitary, and spinal fluid of infants at autopsy: relation to sudden infant death syndrome. Forens Scien Inter 1988; 38: 67-74.
36. **Pasi A, Mehraein A, Jehle A, Traub M, Morniroli G, Bär W**. β-Endorphin: regional level profile in the brain of the human infant. Neurochem Int 1992; 20(1): 93-95.
37. **Coquerel, A, Buser M, Tayot J, Pfaff F, Matray F, Proust B**. Beta-endorphin and neurotensin in brainstem and cerebrospinal fluid in the sudden infant death syndrome. Neurochem Int 1992; 20(1): 97-102.
38. **Fodor M, Görcs TJ, Palkovits M.** Immunohistochemical study on the distribution of neuropeptides within the pontine tegmentum—particularly the parabrachial nuclei and the locus coeruleus of the human brain. Neuroscience 1992; 46(4): 891-908.

39. **Mai JK, Estephens TH, Hopf A, Cuello AC**. Substance P in the human brain. Neuroscience 1986; 3: 109-139.
40. **Marcos P, Coveñas R, De León M, Narváez JA, Tramú G, Aguirre JA**. Neurokinin A-like immunoreactivity in the cat brainstem. Neuropeptides 1993; 25: 105-114.
41. **Muñoz M, Coveñas R.** Involvement of substance P and the NK-1 receptor in human pathology. Amino Acids 2014; 46(7): 1727-1750.
42. **Bergström L, Lagercrantz H, Terenius, L**. Post-mortem analyses of neuropeptides in brains from sudden infant death victims. Brain Res 1984; 323(2): 279-285.
43. **Kleiman JE, Hong J, Laradola M, Govoni E, Guilin CJ**. Neuropeptides in the human brain-postmorten studies. Prog Neuropsych Biol Psysich 1985; 9: 91-95.
44. **Enman NM, Sabban EL, Mcgonigle P, Van Bockstaele EJ.** Targeting the neuropeptide Y system in stress-related psychiatric disorder. Neurobiol Stress 2015; 1: 33-43.
45. **Brené S, Lindefors N, Kopp J, Sedvall G, Persson H.** Regional distribution of neuropeptide Y mRNA in postmortem human brain. Molec Brain Res 1989; 6(4): 241-249.
46. **Walter A, Mai JK, Jiménez-Härtel W.** Mapping of neuropeptide Y-like immunoreactivity in the human forebrain. Brain Res Bull 1990; 24(3): 297-311.
47. **Yu MC, Luo CB, Lomg L, Yew DT**. An immunohistochemical study of neuropeptide Y positive site in the developing human hippocampal formation. Develop Brain Res 1993; 72: 277-281.
48. **Ko L, Rotoli G, Grignol G, Hu W, Merchanthaler I, Dudas B**. A putative morphological substrate of the catecholamine-influenced neuropeptide Y (NPY) release in the human hypothalamus. Neuropeptides 2011; 45: 197-203.
49. **Yamazoe M, Shiosaka S, Emson C, TohyamaM**. Distribution of neuropeptide Y in the lower brainstem: an immunohistochemical analysis. Brain Res 1985; 355: 109-120.
50. **Halliday GM, Li YW, Oliver JR, Joh TH, Cotton RGM, Howe PRC, Geffen LB, Blessing WW**. The distribution of neuropeptide Y-like inmunoreactive neurons in the human medulla oblongata. Neuroscience 1988; 26 (1): 179-191.
51. **Marcos P, Arroyo-Jiménez MM, Lozano G, González-Fuentes J, Lagartos-Donate MJ, Aguilar LA, Coveñas R**. Mapping of tyrosine hydroxylase in the diencephalon of alpaca (*Lama Pacos*) and co-distribution with somatostatin-28 (1-12). J Chem Neuroanat 2013; 50-51: 66-74.
52. **Bouras C, Magistretti PJ, Morrison JH, Constantinidis J.** An immunohistochemical study of pro-somatostatin-derived peptides in the human brain. Neuroscience 1987; 22(3): 781-800.
53. **Austin MC, Janosky JE, Murphy HA**. Increased conrticotropin-releasing hormone immunoreactivity in monoamine-containing pontine nuclei of depressed suicide men. Molec Psych 2003; 8: 324-332.
54. **Hasegawa E, Yanagisawa M, Sakurai T, Mieda M**. Orexin neuron ssuppress narcolepsy via 2 distinct efferent pathways. J Clin Invest 2014; 124(2):604-616.
55. **Goncharuk V, Jhamandas JH.** Neuropeptide FF2 receptor distribution in the human brain: an immunohistochemical study. Peptides 2008; 25: 1544-1553.
56. **Loup F, Tribollet E, Dubois-Dauphin M, Dreifuss JJ.** Localization of high-affinity binding sites for oxytocin and vasopressin in the human brain. An autoradiographic study. Brain Res 1991; 555: 220-232.
57. **Boccia ML, Petrusz P, Suzuki K, Marson L, Pedersen CA**. Inmunohistochemical localitation of oxytocin receptors in human brain. Neuroscience 2013; 253: 155-164.
58. **Baer K, Waldvogel HJ, During J, Snell RG, Faull RLM, Rees MI**. Association of gephyrin and glycine receptors in the human brainstem and spinal cord: an immunohistochemical analysis. Neuroscience 2003; 122: 773-784.
59. **Baer K, Waldvogel HJ, Faull RLM, Rees MI**. Localization of glycine receptors in the human forebrain, Brainstem, and cervical spinal cord: an immunohistochemical review. Front Molec Neurosc 2009; 2 (25): 1-11.
60. **Mazzone SB, Geraghty DP.** Respiratory actions of tachykinins in the nucleus of the solitary tract: characterization of receptors using selective agonists and antagonists. Br J Pharmacol 2000; 129: 1121-1131.
61. **Caberlotto L, Fuxe K, Overstreet DH, Gerrard P, Hurd YL**. Alterations in neuropep-

tide Y and Y1 receptor mRNA expression in brains from an animal model of depression: region specific adaptation after fluoxetine treatment. Molec Brain Res 1998; 59(1): 58-65.

62. Kuwaki T. Orexin links emotional stress to autonomic function. Autonom Neurosc Basic Clin 2011; 161: 20-27.

63. Sinto C. Orexin/hypocretin plays a role in the response to physiological disequilibrium. Sleep Med Rev 2011; 15:197-207.