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Immunohistochemical mapping of pro-opiomelanocortin- and pro-dynorphin-derived peptides in the alpaca (*Lama pacos*) diencephalon

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ABSTRACT

Using an indirect immunoperoxidase technique, we studied the distribution of cell bodies and fibres containing non-opioid peptides (adrenocorticotropin hormone (ACTH), alpha-melanocyte-stimulating hormone) and opioid peptides (beta-endorphin (1–27), alpha-neo-endorphin, leucine-enkephalin) in the alpaca diencephalon. No immunoreactive cell bodies containing ACTH were found. Perikarya containing the other four peptides were observed exclusively in the hypothalamus and their distribution was restricted. Perikarya containing alpha-melanocyte-stimulating hormone or alpha-neo-endorphin showed a more widespread distribution than those containing leucine-enkephalin or beta-endorphin (1–27). Cell bodies containing pro-opiomelanocortin-derived peptides were observed in the arcuate nucleus, anterior and lateral hypothalamic areas and in the ventromedial and supraoptic hypothalamic nuclei, whereas perikarya containing alpha-neo-endorphin (a pro-dynorphin-derived peptide) were found in the arcuate nucleus, dorsal and lateral hypothalamic areas, and in the paraventricular, ventromedial and supraoptic hypothalamic nuclei. Immunoreactive cell bodies containing leucine-enkephalin were found in the lateral hypothalamic area and in the paraventricular hypothalamic nucleus. Immunoreactive fibres expressing pro-opiomelanocortin-derived peptides were more numerous than those expressing pro-dynorphin-derived peptides. A close anatomical relationship was observed: in all the diencephalic nuclei in which beta-endorphin (1–27)-immunoreactive fibres were found, fibres containing alpha-melanocyte-stimulating hormone or alpha-neo-endorphin were also observed. Fibres containing beta-endorphin (1–27), alpha-melanocyte-stimulating hormone or alpha-neo-endorphin were widely distributed throughout the diencephalon, but fibres containing ACTH or leucine-enkephalin showed a moderate distribution. The distribution of the five peptides studied here is also compared with that reported previously in other mammalian species. The widespread distribution observed indicates that both the pro-dynorphin and the pro-opiomelanocortin systems are involved in multiple physiological actions (e.g., food intake, thermoregulation, neuroendocrine and reproductive mechanisms) in the alpaca diencephalon.

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; 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; SNC, substantia nigra, pars compacta; SNR, substantia nigra, pars reticulata; SO, supraoptic hypothalamic nucleus; SPF, subparafascicular thalamic nucleus; STh, subthalamic nucleus; sumx, commissura supramamillaris; 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.

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Introduction

Since 2007 knowledge of the chemical neuroanatomy of neuropeptides in South-American camelids has increased considerably. This is because, using immunocytochemical techniques, reports have been made about the distribution of calcitonin gene-related peptide in the alpaca brainstem and diencephalon (Coveñas et al., 2012; de Souza et al., 2008; Marcos et al., 2011, 2013), the presence of somatostatin-28 (1–12) in the alpaca diencephalon (Coveñas et al., 2011) and the distribution of fibres and cell bodies containing neurotensin or leucine-enkephalin in the alpaca brainstem (de Souza et al., 2007, 2014). Moreover, the colocalization of calcitonin gene-related peptide and somatostatin-28 (1–12) with tyrosine hydroxylase has been also reported in the alpaca brainstem and diencephalon respectively (Marcos et al., 2011, 2013). In all cases, the studies were carried out in control animals (alpacas not treated with colchicine). To date, in the alpaca diencephalon, a region of the central nervous system involved in important functional mechanisms, the mapping of only two neuropeptides (calcitonin gene-related peptide and somatostatin-28 (1–12)) has been carried out (Coveñas et al., 2011, 2012; Marcos et al., 2013). Thus, no studies addressing the presence of adrenocorticotropin hormone-, alpha-melanocyte-stimulating hormone- and opioid peptide-immunoreactive structures in the alpaca diencephalon have been performed. Currently, the only opioid peptide mapped in the alpaca is leucine-enkephalin, and then only in the brainstem (de Souza et al., 2007).

Opioid peptides can be classified in three families according to their precursors: (1) beta-endorphin, alpha- and beta-melanocyte-stimulating hormone, adrenocorticotropin hormone, methionine-enkephalin, and alpha-, and gamma-endorphin are produced from pro-opiomelanocortin; (2) leucine-enkephalin, methionine-enkephalin, methionine-enkephalin-Arg-Gly-Leu and methionine-enkephalin-Arg-Phe from pro-enkephalin; and (3) alpha-neo-endorphin, leucine-enkephalin, dynorphin A and dynorphin B from pro-dynorphin. Our main aim in this work was to study and compare for the first time the distribution of the pro-opiomelanocortin and pro-dynorphin systems in the alpaca diencephalon. Thus, using an immunocytochemical technique we studied the distribution of fibres and cell bodies containing beta-endorphin (1–27), alpha-melanocyte-stimulating hormone and adrenocorticotropin hormone (exclusively produced from pro-opiomelanocortin), as well as the distribution of immunoreactive structures containing alpha-neo-endorphin (exclusively produced from pro-dynorphin). In addition, the distribution of leucine-enkephalin (derived from pro-enkephalin and from pro-dynorphin) was also studied, as the mapping of this opioid peptide has been reported previously in the alpaca brainstem (de Souza et al., 2007), but not in the alpaca diencephalon. A further aim was to compare the distribution of adrenocorticotropin hormone/alpha-melanocyte-

Table 1
Density of ACTH-, alpha-melanocyte-stimulating hormone-, and opioid-immunoreactive fibres and cell bodies in the alpaca diencephalon.

Nucleus/tract	ACTH		β-end (1–27)		α-MSH		α-NE		Leu-Enk	
	CB	F	CB	F	CB	F	CB	F	CB	F
AD	–	–	–	+/++	–	+	–	+	–	–
AHy	–	+++	–	+++	+	+++	–	+	–	+++
AM	–	–	–	+/++	–	+/++	–	+	–	–
Around fornix	–	+++	–	+++	–	++/+++	–	+	–	+++
Arc	–	+++	+	+++	+	+	+++	+	–	+++
AV	–	–	–	+/++	–	+	–	+	–	–
CL	–	–	–	+/++	–	+	–	+	–	–
CM	–	+/++/+++	–	+/++	–	++	–	+	–	+/++
DA	–	+++	–	+++	–	+++	+++	+	–	+++
iC	–	–	–	+/++	–	+	–	++	–	–
LD	–	–	–	+/++	–	+/++	–	+	–	–
LG	–	–	–	+	–	+	–	+	–	–
LH	–	++	–	+++	+++	++/+++	+	+/++	+	++
LHb	–	+	–	+	–	+	–	+	–	++/+++
LM	–	+	–	++	–	++	–	++	–	+
LP	–	–	–	+/++	–	+	–	+	–	–
MD	–	+	–	+/++	–	+/++	–	+	–	+
ME	–	+	–	+	–	++	–	+	–	+++
MHb	–	–	–	+	–	+	–	+	–	+++
MM	–	+	–	+++	–	++	–	+++	–	+
mt	–	++	–	+/++/+++	–	+/++	–	+	–	–
PC	–	–	–	+	–	+/++	–	+	–	–
PH	–	+++	–	++/+++	–	+++	–	+	–	++
PVA	–	+/++	–	+/++/+++	–	+/+++	–	+	–	++
PVH	–	+++	–	++/+++	–	++/+++	+++	+	+++	+++
Re	–	+/++	–	+/++	–	++/++	–	+	–	+
Rh	–	+	–	+	–	++/+++	–	+	–	+
Rt	–	–	–	+	–	+	–	+	–	–
sch	–	+++	–	+++	–	+++	–	+	–	+++
sm	–	–	–	+	–	+	–	+	–	–
SO	–	++	–	+++	+	++	+++	+	–	+++
SPF	–	++	–	++	–	++	–	+	–	++
STh	–	+	–	++	–	+	–	+	–	+
sumx	–	–	–	++	–	+++	–	+++	–	+++
VA	–	–	–	+	–	++	–	+	–	–
VL	–	–	–	+	–	+	–	+	–	–
VM	–	–	–	+	–	+	–	+	–	–
VMH	–	+++	–	+++	+	++	+	+	–	+++
VPL	–	–	–	+	–	+	–	+	–	–
VPM	–	–	–	+	–	+	–	+	–	–
ZI	–	+	–	++	–	+++	–	++	–	++

CB, cell bodies (+++, high density; +, low density). F, fibres (+++, high density; ++, moderate density; +, low density). For nomenclature of the nuclei, see list of abbreviations.