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α-MSH in rat brain: occurrence within and outside of β-endorphin neurons

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After the discovery of the two enkephalins[14,30] and β-endorphin (β-END)[5,6,11,12,18,28], these peptides and related substances were the focus of a large number of immunohistochemical and lesion studies[1–4,7,9,13,21–24,29,31,32,34–38]. These studies indicated the separateness of the enkephalin and β-END systems in rat central nervous system[3,27,34]. The enkephalin systems are composed of many lightly stained cells and fine fibers localized throughout the neuraxis[9,13,29,32,35]. β-END neurons can be visualized only in the arcuate nucleus with some cells spreading laterally out toward the lateral hypothalamus[2,3,23,25,31,34,36–38]. It appears that the single β-END cell group distributes its axons and fibers throughout the central nervous system, with very long projection pathways through hypothalamus, dorsal thalamus, periaqueductal gray finally ending in the region of the locus coeruleus. Immunohistochemical studies using antisera against β-lipotropin (β-LPH) reveal an identical distribution[23,36,38].

In the original immunohistochemical study by Moon et al.[20], β-LPH was localized in pituitary corticotrophs and all the cells of the intermediate lobe. Subsequent visualization studies in pituitary using β-END antibody[4] further confirmed that β-END and β-LPH are located in the same cells as ACTH. Pelletier et al.[25] in elegant electron microscopic studies demonstrated that β-LPH and ACTH were found in the same granules of pituitary corticotrophs and intermediate lobe cells. Since β-LPH and β-END had been shown to exist in rat brain[2,3,17,23,25,31,34,36,38], it seemed reasonable to attempt to localize ACTH-like immunoreactivity in rat brain as well. Immunochemical and immunocytochemical studies using antisera against various portions of ACTH[2,16,21,24,31,34,37] revealed the existence of ACTH-like material in brain, which appeared to survive hypophysectomy. In subsequent studies it was possible to demonstrate that β-END, β-LPH and ACTH immunoreactivity were found within the same hypothalamic neurons[2,21,23,31,34]. Thus, it appears likely that the 31K precursor[8,19,26] for pituitary ACTH, β-LPH and β-END is present in rat hypothalamic neurons as well.

Eipper et al.[8] and Chretien et al.[10] have shown that the pituitary demonstrates two biosynthetic end points arising from the 31K pro-opiocortin precursor. The anterior lobe corticotrophs appear to cleave the precursor to ACTH and β-LPH, whereas intermediate lobe cells appear to carry the synthesis one step further to β-END and α-MSH. Although ACTH-like immunoreactivity had been demonstrated in β-END neurons[2,21,22,34], it was not clear whether the immunohistochemical demonstration was of ACTH 1–39 or of a cross-reacting α-MSH and corticotropin-like intermediate lobe peptide (CLIP) contained within brain neurons. Since some investigators have reported that α-MSH immunoreactivity in rat brain[2,15,22,33] exhibits a similar distribution to the 'β-END/β-LPH/ACTH' system previously described, it appeared reasonable to study the possibility of β-END and α-MSH co-existence in the same neuron. In this study we therefore attempted to visualize α-MSH-like immunoreactivity (α-MSH-LI) in rat central nervous system and to determine whether α-MSH-LI was localized in β-END immunoreactive cells in the arcuate hypothalamus.

Forty-eight hours prior to sacrifice adult Sprague–Dawley rats were injected intracerebroventricularly with 75 μg of colchicine in 75 μl. They were then anaesthetized with 50 mg/kg of pentobarbitol and prepared for immunohistochemistry as reported elsewhere[36]. The animals were perfused via the ascending aorta with 4% cold formaldehyde phosphate buffer for 30 min. The brain was extracted, cut into 4–6 mm slabs, frozen and sectioned on a microtome for immunohistochemistry. Standard immunohistochemical techniques were used to demonstrate α-MSH and β-END. Specifically, 4 α-MSH antisera (kind gifts of Drs. Weber and Voigt, Ulm, G.F.R. and Immunonuclear Corporation, Stillwater, Minn., 373, 378 and 379) were used at 1:300 dilution in 0.3% Triton PBS buffer. Sections were incubated at 37 °C for 1 h and 4 °C for 48 h. They were washed in PBS for 30 min, then exposed to goat antirabbit-FITC (Cappel Labs) for 1 h at 37 °C, washed again for 30 min, coverslipped and viewed. β-END antiserum from 'Melinda' (a kind gift from Drs. Richard Mains and Betty Eipper from the University of Colorado) was used at a dilution of 1:500 under similar conditions. α-MSH-LI demonstration could be blocked with 1 μM authentic α-MSH. This antiserum could not be blocked with 1 μM β-END, ACTH 17–39, ACTH 1–39, or ACTH 1–24. β-END antiserum could also be blocked with 1 μM β-END and was 100% cross-reactive with β-LPH (a kind gift of C. H. Li, University of California), but could not be blocked by α-MSH or ACTH fragments. A large number of 4 μm serial sections were cut on a cryostat and sequentially stained for α-MSH or β-END. Noting common anatomical landmarks, photographs were taken in order to visualize the same cells in several different sections. Since both the β-END and α-MSH immunoreactive cells were approximately 12–15 μm in diameter, it was possible to get 3 or perhaps 4 sections through the same cell.

Both α-MSH-LI and β-END-LI were visualized in the same region of the arcuate nucleus. When 4 μm serial sections were analyzed (Fig. 1) it was clear that the same arcuate cells were immunoreactive for α-MSH and β-END. Although some cells were positive for α-MSH or β-END that were seen in serial sections, when a series of 3 sections were studied, all cells in the middle section (α-MSH-LI Fig. 1B) were accounted for in the 2 surrounding sections (β-END-LI Fig. 1A and C).

Fig. 1. A, B and C: taken from the same region of 3 sequential 4 μm sections through rat arcuate nucleus. A and C are from β-END stained sections. B is taken from the second of the 3 sections; it was stained for α-MSH. Cells in B (α-MSH-LI) were numbered 1–8. These same cells were identified with the same number either in A or C (β-END-LI). It was therefore possible to demonstrate that all α-MSH-LI cells in the arcuate nucleus contained β-END-LI. In other photographs a similar analysis also showed that all β-END-LI cells in the arcuate region contained α-MSH-LI. (× 206).

A second set of α-MSH positive cells was detected in several hypothalamic nuclei. This cell group arched over the top of the third ventricle (Fig. 2A), out laterally into the zona incerta, between the fornix and the mammillothalamic tract and countinued down toward the lateral hypothalamic sulcus. The heaviest distribution of these non-arcuate cells was in the region of the dorsal third ventricle and near the lateral sulcus. The visualization of these cells and the α-MSH-LI cells of the arcuate nucleus was completely blocked with the addition of 1 μm α-MSH peptide. It was often possible to visualize both the arcuate and the more dorsal α-MSH positive cells in the same section. In serial sections, using β-END antibody, only the arcuate cells could be visualized (Figs. 1A and C and 2B). In studies of 20 sequential stained sections per rat across 3 rats, every α-MSH stained section contained both cell groups, whereas every β-END stained section contained only the arcuate cells (Fig. 2).

The common cell of origin for α-MSH-LI and β-END-LI in rat brain arcuate nucleus tends to support the hypothesis that β-END cells of rat arcuate nucleus and intermediate lobe of pituitary have similar biosynthetic end points, especially when contrasted with the corticotrophs of the anterior lobe[8,10]. Although all 3 sets of cells contain the 31K pro-opiocortin precursor system[19,26], the anterior lobe mainly produces ACTH and β-LPH, whereas brain and the intermediate lobe seem to produce β-END and α-MSH[8,10]. In the brain, since α-MSH and β-END are contained within the same arcuate nucleus cells and fibers, it is possible that both peptides are simultaneously released during normal neuronal activity. The possible co-release of α-MSH, its function with the CNS and its interactions with β-END merit further study.

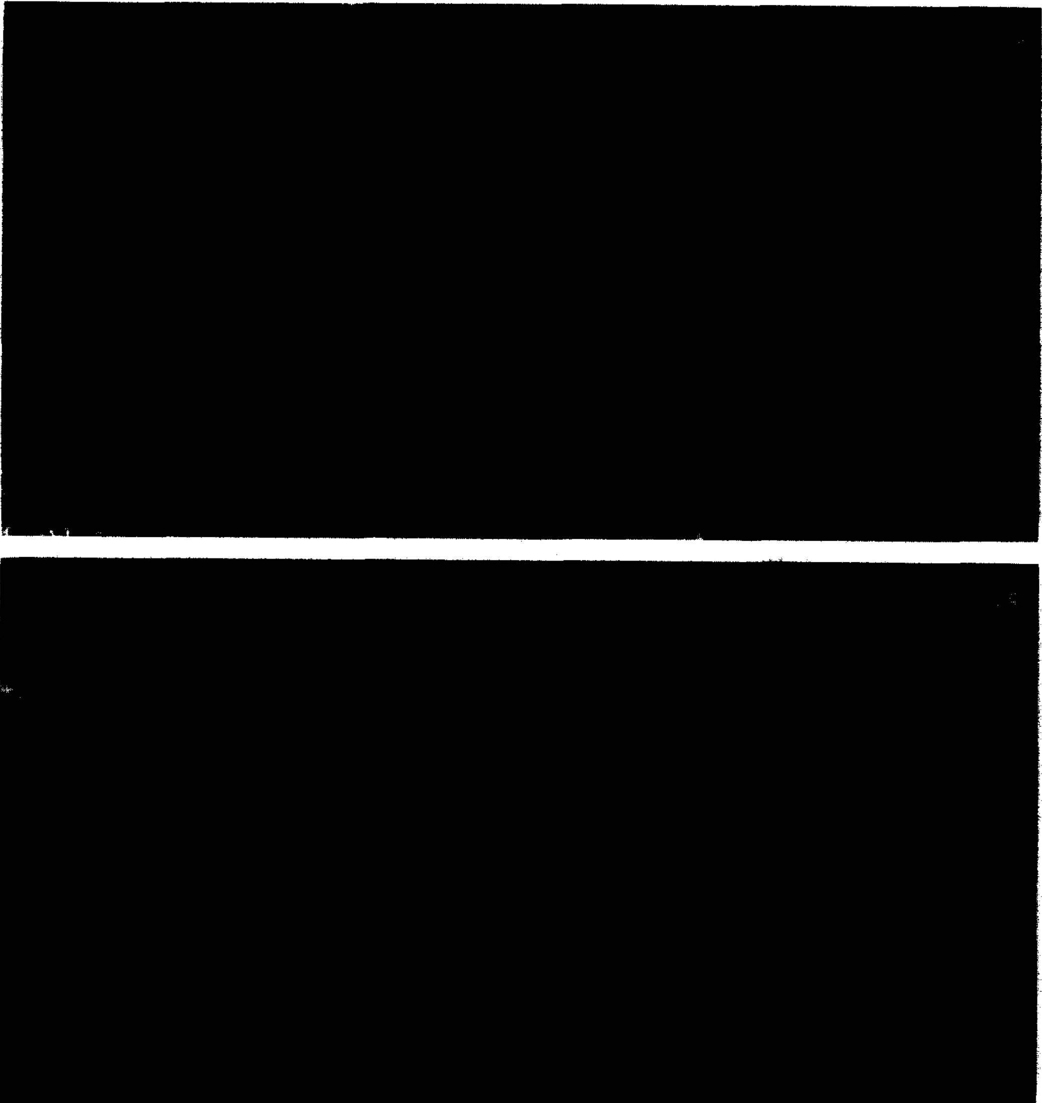

Fig. 2. A and B: montages taken just dorsal and lateral to the top of the third ventricle (note star). A: α-MSH stained cells. B: taken in the analogous region of the serial 4 μm section (NB, starred vessel) demonstrated the absence of β-END stained cells. β-END-LI cells could only be seen in the arcuate region. (× 170).

In contrast to the arcuate system described above, the dorsal-lateral set of α-MSH positive cell bodies seems not to be related to the 31K system, as evidenced by a lack of β-END immunoreactivity. In studies by several laboratories[2,3,25,34,36–38], using several antisera against the 31K precursor, β-LPH, β-END and the 16K fragment (a kind gift of Drs. R. Mains and B. Eipper, University of Colorado), there are no descriptions of cells, other than those in the arcuate nucleus. Furthermore, in previous studies of α-MSH-LI distribution in brain[2,15,22,33], there have been no

published reports of this dorsal α-MSH positive cell group. Our observation is likely to be due to the higher dose of colchicine used in this study (75 μg per animal) and the initial fortuitous use of a highly sensitive antibody (Weber and Voigt). It should be noted that these non-arcuate α-MSH-positive cells were visualized using 4 α-MSH antisera, which were raised using different methods of coupling α-MSH and harvesting antibodies.

It is unclear what the biosynthetic route is for the non-arcuate cells with α-MSH-LI. This study appears to suggest a synthetic route other than the 31K pro-opiocortin precursor. Alternatively the 31K system could be the biosynthetic source, but with rapid turnover of other 31K products (i.e. β-END, β-LPH, 16K etc.) or with different modes of storage.

It is critical to note here that while these cells exhibit interactions with 4 different antisera, we cannot determine that we are indeed studying α-MSH. It is conceivable that a slightly altered form of the peptide has evolved through a different synthetic route, though it retains many structural and immunological properties. Final identification must await further chromatographic and analytical studies.

It would therefore appear that studies attempting to examine ACTH-like immunoreactivity in brain must taken into account at least two potential sources of confusion, the first being that the ACTH-like immunoreactivity in brain may be in the form of α-MSH and CLIP; and second that there may actually be two distinct anatomical and biochemical origins of α-MSH immunoreactivity in brain. While some α-MSH immunoreactivity could derive from pro-opiocortin, a common precursor for ACTH, β-LPH, α-MSH, β-END, the remainder arises from an unknown biosynthetic mechanism. The multiplicity of the systems also carries important implications for pharmacological and behavioral studies attempting to elucidate the function of ACTH, α-MSH and related peptides in brain.

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