

β -Lipotropin: Localization of cells and axons in rat brain by immunocytochemistry

(opiate peptides/endorphin/corticotropin/anatomy)

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ABSTRACT Using specific antisera to human β -lipotropin, we have visualized cells and axons with β -lipotropin-like immunoreactivity in rat brain and pituitary. The β -lipotropin so localized is well delineated and contained in the cytoplasm of cells and in beaded axons. Areas of greatest β -lipotropin content are hypothalamus (with cell bodies in the medial basal hypothalamus and arcuate regions), periventricular nucleus of the thalamus, ansa lenticularis, zona compacta of the substantia nigra, medial amygdaloid nucleus, zona incerta, periaqueductal central gray area, locus ceruleus, and a few fibers in the reticular formation. The question of the exact relationship of β -lipotropin and methionine-enkephalin remains open, because some brain areas contain both substances and some areas contain only one or the other.

β -Lipotropin is a 91 amino acid peptide (1-3) containing several smaller biologically active peptides: β -melanotropin (4), corticotropin (4-10), γ -lipotropin (6), methionine-enkephalin (7), and α - (8), β - (9), and γ - (10) endorphins. This pluripotent peptide has been localized by immunohistochemical techniques to the corticotrophs of the anterior pituitary and to the cells of the intermediate lobe (11). It is stored and released in the same vesicles as corticotropin (12). No specific function has been attributed to the entire peptide, yet several of the derivative peptides are thought to have roles in stress and pain responses (ref. 13; H. Akil, S. J. Watson, and J. Barchas, unpublished data). It has been proposed that β -lipotropin may be a precursor for the endorphins (9, 14) or possibly for methionine-enkephalin (7). Using antiserum raised against human β -lipotropin (15, 16) we investigated the immunohistochemical localization of this substance in cells and axons in rat brain.

MATERIALS AND METHODS

Rabbit antiserum against human β -lipotropin was prepared by four weekly subcutaneous injections of 3 mg of the peptide with Freund's complete adjuvant (16). The resulting antibody showed no crossreactivity with human melanotropin, methionine-enkephalin, or leucine-enkephalin. The COOH-terminal fragment of β -lipotropin (i.e., β -endorphin) was 1/30 as effective in producing 50% inhibition as β -lipotropin itself. Furthermore, there was no detectable change in immunocytochemical anatomy with the addition of 100 nM β -endorphin, whereas 3 nM β -lipotropin could completely block all specific activity.

Sections of rat brain were prepared for immunohistochemistry according to the procedure of Hökfelt *et al.* (17) and Coons (18). Briefly, 200-g male Sprague-Dawley rats were anesthetized with pentobarbital (50 mg/kg) and perfused via the aorta with cold (4°) 4% formaldehyde in 0.1 M phosphate buffer. The

perfusion was maintained for 30 min at 80-120 mm Hg. The brain was removed, blocked, placed in the perfusion solution for 2 hr, and then kept overnight in 5% sucrose/phosphate-buffered saline. The blocks of tissue were then frozen onto cryostat chunks with liquid nitrogen. The blocks were sectioned (10 μ m), mounted on gelatin-coated slides, and kept frozen until used. The sections were incubated overnight at 4° with anti- β -lipotropin antibody diluted 1:500 with 0.3% Triton/phosphate-buffered saline. After incubation, the sections were washed in phosphate-buffered saline for 30 min. They were then incubated with fluorescein isothiocyanate-tagged goat-anti-rabbit antibody (Cappel Labs, Downington, PA.) at 1:80 dilution for 30 min at 37°. The sections were washed, coverslipped with buffered glycerine, and viewed through a Leitz Orthoplan fluorescence microscope.

Control sections were incubated with the specific antiserum and an excess of authentic β -lipotropin (200 nM). Anatomical distribution was correlated with coronal sections from the stereotaxic atlases of König and Klippel (19), Palkovits and Jacobowitz (20), and Jacobowitz and Palkovits (21).

RESULTS

Antibody directed against β -lipotropin produced a sharp, well-localized demonstration of β -lipotropin-like immunoreactivity (see Figs. 1 and 2). This antiserum showed no cross-reactivity to human α -endorphin, methionine-enkephalin, leucine-enkephalin, corticotropin, human β -melanotropin, or prolactin (H. Akil, J. D. Barchas, and C. H. Li, unpublished data).

In pilot studies, β -lipotropin was located in the anterior and intermediate lobes of rat pituitary (11, 12). This reaction could be blocked by the addition of excess β -lipotropin to the antiserum (Fig. 1B).

β -Lipotropin was located in hypothalamic cells and beaded axons throughout the brainstem and limbic system. No activity was seen in the cerebellum, hippocampus, cerebral cortex, or spinal cord.

Medulla/Pons. A few fibers were seen in the region of the nucleus raphe magnus and the nucleus origin is nervi facialis and between the fibers of the lemniscus medialis. Sparse fibers were also located in the nucleus ambiguus, in the region of both the nucleus olivaris superioris and inferioris, and in the fasciculus longitudinalis medialis. Moderate fiber densities were found in the nucleus parabrachialis dorsalis and in the lateral reticular formation ventral to the nucleus originis nervi trigemini and medial to the nervi trigemini itself. Heavy concentrations of β -lipotropin were found throughout the locus ceruleus.

Mesencephalon. Heavy fiber concentrations were seen in the substantia grisea centralis adjacent to the aqueduct and projecting laterally to the area of the lemniscus lateralis. In the

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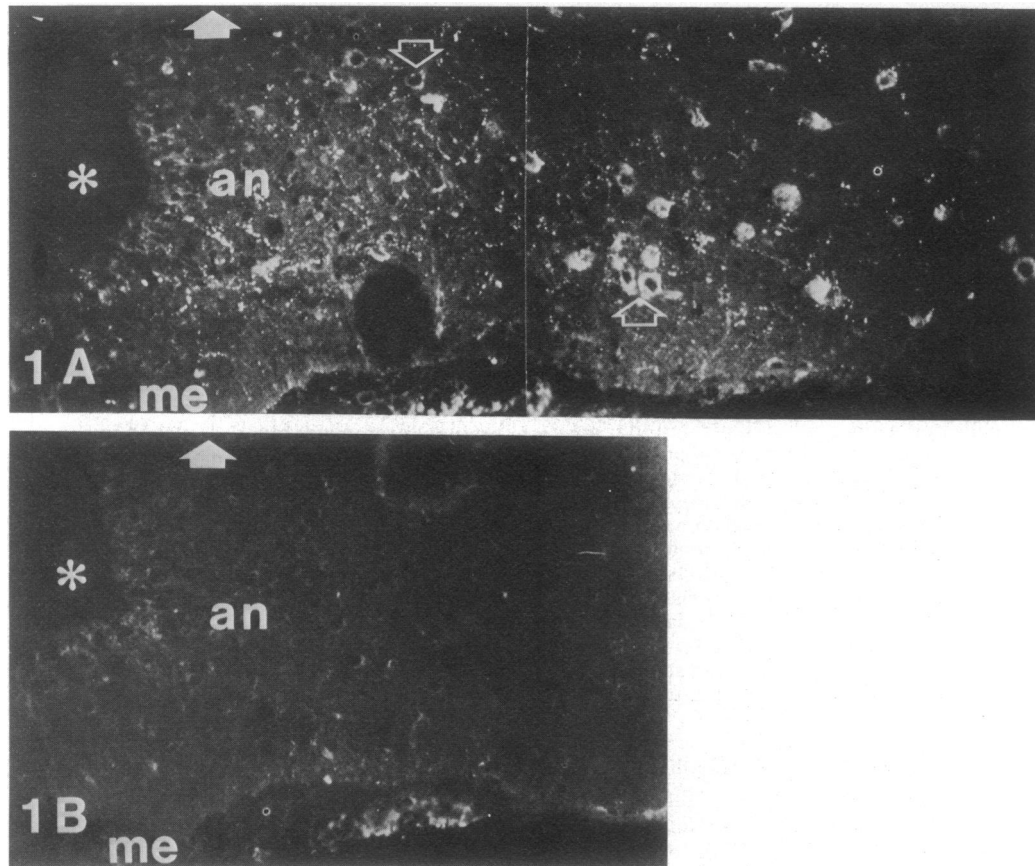


FIG. 1. (A) β -Lipotropin-positive cell bodies and beaded fibers in the median eminence (me) and arcuate nucleus (an) of an adult male rat (asterisk is third ventricle, and solid arrow points dorsally). Note that the cell bodies (open arrows) show dark unstained nuclei with a ring of positively stained cytoplasm. ($\times 142$.) (B) Serial section incubated as a control with the same antiserum but with an excess of authentic human β -lipotropin. ($\times 142$.)

more rostral central gray region, moderate fiber densities were seen in the ventral midline projecting toward but not penetrating the nucleus interpeduncularis. Light to moderate fiber concentrations were seen throughout the reticular formation, particularly medial to the dorsal aspect of the crus cerebri and in the substantia nigra zona compacta. Small numbers of fibers were detected in the nucleus centralis corporis geniculate medialis and the dorsomedial aspects of the superior and inferior colliculi.

Diencephalon and Telencephalon. Heavy concentrations of β -lipotropin fibers were found immediately surrounding the third ventricle (Fig. 2A), especially in the nucleus arcuatus and the lateral median eminence (Fig. 1 A and B). β -Lipotropin-reactive cell bodies were scattered throughout the nucleus arcuatus and along the ventral-most border of the hypothalamus. The cells of the nucleus periventricularis hypothalami were heavily covered with β -lipotropin-positive terminals. A group of β -lipotropin-reactive cells were found in the nucleus supraopticus. However, unlike all other β -lipotropin-positive structures, these cells could not be blocked with an excess (200 nM) of β -lipotropin. Heavy fiber patterns were seen throughout the remainder of the hypothalamus in the zona incerta (Fig. 2C), ansa lenticularis (Fig. 2D), and stria terminalis. In the amygdala, only the nucleus amygdaloideus medialis was heavily invested with fibers. However, β -lipotropin fibers seemed to abut the medial margin of the nucleus amygdaloideus centralis. A heavy fiber distribution was seen throughout the nucleus periventricularis thalami (Fig. 2B) with a small number of fibers flowing ventrally toward the hypothalamus at its

caudal extreme. Moderate numbers of fibers were seen in the nucleus lateralis septi and tractus diagonalis Broca and were contiguous with the large number of fibers seen in the rostral stria terminalis and in the nucleus accumbens.

A few β -lipotropin fibers were seen in the inner layer of the median eminence and in the middle layer of the infundibular stalk. It seems highly unlikely that pituitary β -lipotropin is responsible for the great number of fibers seen in brain. Parallel immunohistochemical studies carried out on hypophysectomized rats showed no detectable change in brain β -lipotropin distribution.

DISCUSSION

In this paper, we have described evidence of the presence of β -lipotropin in rat brain and its localization in cells and structures, with a beaded axonal appearance. β -Lipotropin-positive fibers are seen throughout the medial brainstem with little activity detected in the cerebellum, hippocampus, or cerebral cortex. This pattern of distribution has been confirmed on a regional basis by radioimmunoassay (H. Akil, J. D. Barchas, and C. H. Li, unpublished data).

The system of β -lipotropin fibers seems to have many points of contact with the catecholamine neuronal networks. Norepinephrine cells of the locus coeruleus appeared to have especially heavy contact with β -lipotropin fibers. Other points in common with norepinephrine fibers include the periaqueductal central gray area, the region of the dorsal catecholamine bundle, the periventricular nucleus of the thalamus, most of the hypothalamus, the lateral septal nucleus, the nucleus accumbens,

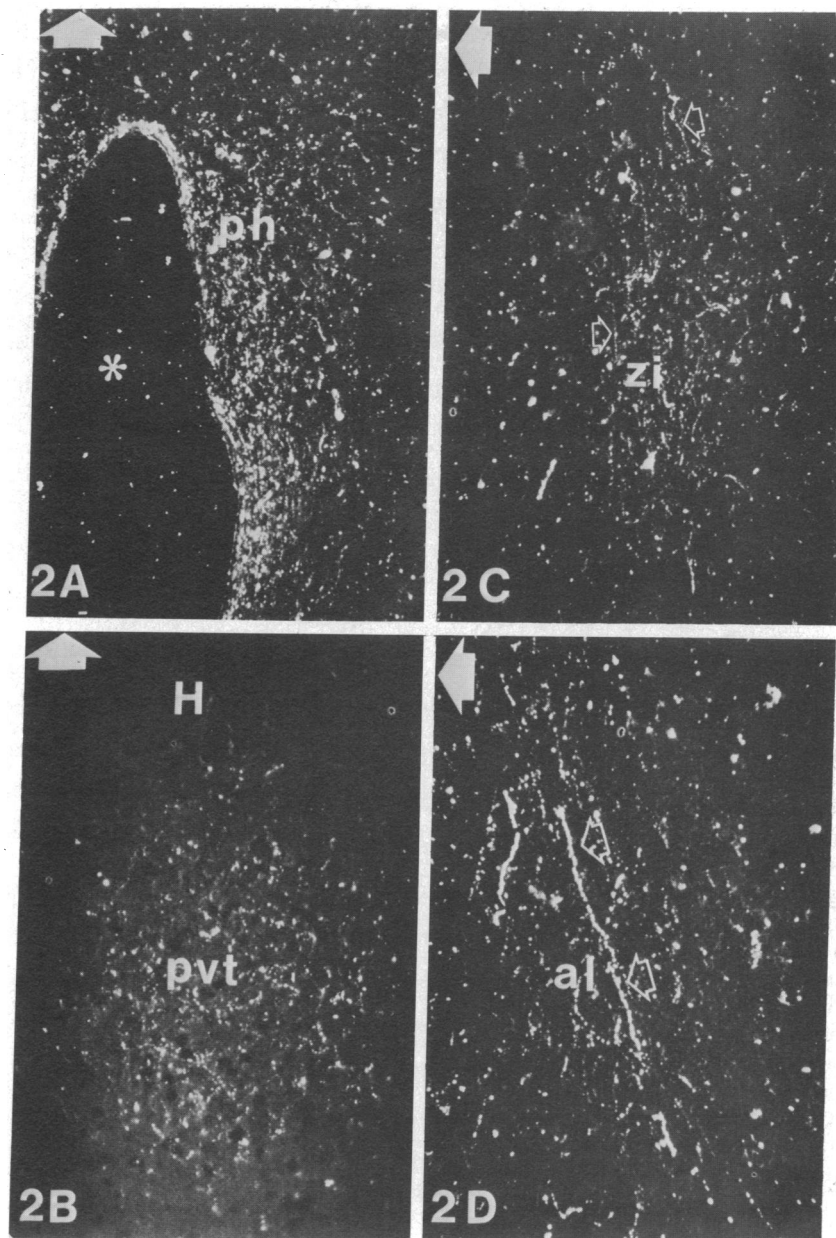


FIG. 2. β -Lipotropin-positive fibers. (A) In the medial periventricular nucleus of the hypothalamus (ph). This area was dorsal to that shown in Fig. 1A. Asterisk is in the third ventricle; solid arrow points dorsally. ($\times 95$.) (B) In the periventricular nucleus of the thalamus (pvt). H, medial habenula; solid arrow points dorsally. ($\times 145$.) (C) In the zona incerta (zi). Note the fine, closely spaced beads (open arrows). Solid arrow points dorsally. ($\times 95$.) (D) In the region of the ansa lenticularis (al). The open arrows indicate a long, beaded, axon-like fiber. Solid arrow points dorsally. ($\times 150$.)

bens, the diagonal band of Broca, the stria terminalis, and the medial amygdoloid nucleus.

Dopaminergic systems also have extensive areas of contact with β -lipotropin-containing structures. The dopamine cell areas in midbrain (zona compacta, the medial A 10 group, and the dopamine cells of the periaqueductal central gray area) have moderate contact with β -lipotropin fibers. As noted above, the hypothalamus, nucleus accumbens, stria terminalis, lateral septum, and diagonal band of Broca are potential points of interaction. Of particular interest is the arcuate-median eminence area where both dopamine and β -lipotropin fibers and cells are seen. There were very few β -lipotropin fibers associated with the serotonin-rich raphe dorsalis, magnus, or medianus.

It has been suggested that β -lipotropin might be enzymatically cleaved between amino acids 60 and 61 to produce β -

endorphin [β -lipotropin (61-91)] and perhaps methionine-enkephalin [β -lipotropin (61-65)] as well (7, 9, 14). If β -lipotropin is a precursor for these opiate peptides, then it should be contained in the same neurons. Unfortunately, only partial information is available about the brain distribution and pathways of the specific enkephalins (22, 23) and endorphins. The distribution of methionine-enkephalin cells and fibers has been studied in detail by use of specific antiserum (S. Watson, H. Akil, S. Sullivan, and J. D. Barchas, unpublished data) that does not crossreact with leucine-enkephalin, β -endorphin, β -lipotropin, or any of several methionine-enkephalin fragments, and that exhibits minimal crossreactivity with α -endorphin (24). When the anatomical distributions of methionine-enkephalin and β -lipotropin are compared, no clear answer is found to the question of whether or not they exist in the same neurons. There

are many areas of overlap in the distribution of these two peptides—hypothalamus, periaqueductal central gray area, lateral septal nucleus, lateral reticular formation, area of the ansa lenticularis, zona incerta, zona compacta of the substantia nigra, and periventricular nucleus of the thalamus. There are, however, several areas of striking difference in distribution. The globus pallidus is very heavy in methionine-enkephalin but has no visible β -lipotropin. On the other hand, the median eminence-arcuate area contains β -lipotropin fibers and cells but very little methionine-enkephalin. In the amygdala the central nucleus contains a dense network of methionine-enkephalin fibers but no β -lipotropin. At the medial border of that nucleus there is a moderately heavy accumulation of β -lipotropin fibers. If β -lipotropin is the precursor of methionine-enkephalin, it might be contained in the early axonal segments with methionine-enkephalin in the finer axons and terminals in the nucleus itself.

There is modest evidence against β -lipotropin as a direct precursor to methionine-enkephalin. Aside from the differences noted above, methionine-enkephalin cell bodies are seen in the dorsal part of the rostral and midhypothalamus (anterior and dorsomedial nuclei) as well as the lateral septal nucleus. No β -lipotropin cells are seen in these areas, but they are seen in the pituitary, arcuate nucleus, and basal hypothalamus, where no methionine-enkephalin cells have been reported. The relationship between these peptides must await more detailed anatomical mapping and perhaps biochemical studies involving methionine-enkephalin synthesis.

It has been proposed that β -endorphin may be a normally occurring opiate peptide in mammalian brain (25). There have been no unequivocal immunohistochemical demonstrations of its intraneuronal distribution in the central nervous system. The neuron-like distribution of β -lipotropin presented in this paper may bear on the question of storage of β -endorphin in brain. Because β -lipotropin is only one cleavage step removed from β -endorphin, it may be the immediate precursor and major storage form for that opioid.

Pelletier *et al.* (12) have found that β -lipotropin and corticotropin are located in the same cells in the anterior and intermediate lobes of the pituitary (using noncrossreacting antisera). They further stated that β -lipotropin and corticotropin are both found in the granules of these cells and may well be released together during granule extrusion. It has also been shown in human studies that plasma levels of lipotropin and corticotropin parallel each other under several conditions (26, 27). The relationships among β -lipotropin, the opiate peptides, and corticotropin are very complex and may prove to be a highly productive field of study. It may be that the interface among stress, pain, and mood control is in part mediated by the intimate anatomical and biochemical relationships among these classes of neuroregulators.

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