LIPOTROPIN: LOCALIZATION BY RADIOIMMUNOASSAY OF ENDORPHIN PRECURSOR IN PITUITARY AND BRAIN[1]

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SUMMARY

Beta-Lipotropic Hormone (LPH) was estimated in pituitary and brain by a radioimmunoassay specific for the N-terminal, non-opiate portion of the protein, and endorphin activity by an opiate radioreceptor assay. The intermediate pituitary is most concentrated in LPH and endorphin. Gel filtration indicated the presence in the pituitary of intact LPH and N-terminal fragments but only intact LPH in brain. Endorphin activity in pituitary is associated primarily with a component of 3000 daltons and to a lesser extent with one of about 2000 daltons. Brain endorphin activity is mostly accounted for by a 2000 dalton component, enkephalins representing an apparently minor activity. In pituitary and brain LPH and endorphin are entirely associated with a 12,000 g/10 min fraction.

A family of peptides with morphine-like actions has been isolated from brain and pituitary (1-5). These peptides (endorphins) share a common N-terminal pentapeptide sequence and individual members vary in length. The peptide sequence is contained within a 91-amino acid residue protein, beta-Lipotropic Hormone (LPH) identified in the pituitary gland (6-9). The localization of paired basic amino acid residues in the LPH sequence suggests points of cleavage yielding active peptide fragments. A protein structure of this type is found in several other endocrine cells and the major hormone structure in each case is contained within the prohormone (10). Thus, beta-MSH represents one hormonal peptide contained within LPH and the endorphin sequence,

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another. In analogy with other hormones, one would expect LPH to be sequestered within a secretory package consisting of prohormone, cleaving enzyme(s) and active peptide fragments.

In previous work from our laboratory (11,12) endorphin activity was associated with cytoplasmic granules obtained from homogenates of brain and pituitary. Essentially all of the activity in these tissues is contained within the granules. Pituitary endorphin is apparently derived from parenchymal cells of the intermediate lobe of the pituitary gland, whereas the cellular source of granules in brain remains to be determined. We now show that the distribution of immunoreactive LPH parallels that of endorphin; it is most concentrated in the intermediate pituitary and associated with the same granule fraction from pituitary or brain.

EXPERIMENTAL

Antibodies to ovine beta-LPH were developed in rabbits against the hormone conjugated to ovalbumin with carbodiimide. The ratio of LPH to ovalbumin in the conjugate was 10:1. (We are grateful to Dr. P. Schiller for preparation of the conjugate). Three days before immunization animals were injected subcutaneously with 0.5 ml of crude Bordella pertussis vaccine. The first immunization was carried out with 500 ug LPH in complete Freund's adjuvant intradermally, and a booster given at six weeks. A second injection contained 250 ug LPH in incomplete adjuvant. Methionine^5, enkephalin (LPH 61-65) and leucine^5 enkephalin were obtained from Peninsula Laboratories, San Carlos, CA, and alpha-endorphin (LPH 61-76) from Dr. R. Guillemin. Bovine pituitary glands and brains were obtained from freshly killed cattle of unspecified age and sex and transported on ice to the laboratory. About 2 hr elapsed between death of the animal and extraction of tissue. For estimation of LPH, 10% homogenates of tissues in ice cold water were extracted with a final concentration of 2 M acetic acid and serial dilutions of the extracts made in 0.05 M TRIS buffer, pH 7.4. Dilutions of tissue extracts were assayed by solid phase radioimmunoassay, as previously described for other pituitary hormones (13). For gel filtration the 480,000 g-min fraction, containing essentially all the tissue LPH from 0.5 - 1.0 g tissue was extracted in 10 volumes of 2 N acetic acid, freeze-dried, and the residue in 1 ml of 2 N acetic acid was applied to the column. The sample was eluted with 2 N acetic acid, with a flow rate of 0.2 ml/min and 0.5 ml fractions collected. Column dimensions: G-15 and G-50, 1 x 47 cm, and G-25, 1.5 x 66 cm. Endorphin activity was estimated by a radioreceptor assay with ^{3}H-naloxone as previously described (11,12). Centrifugation pellets were suspended in 2 N acetic acid, stirred at room temperature for 30 min, centrifuged, and the supernatant assayed.

RESULTS AND DISCUSSION

A schematic of the LPH molecule is shown in Figure 1, with the sequences between paired basic amino acid residues indicated. The LPH anti-

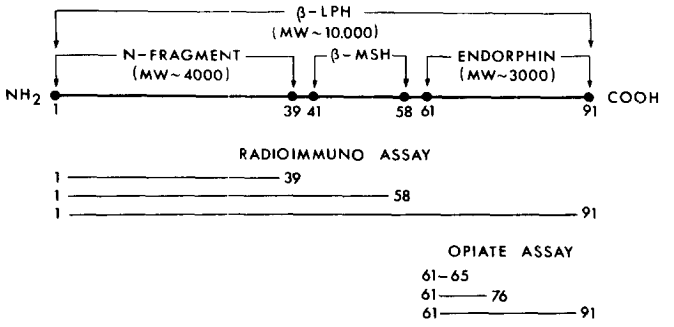

FIGURE 1. Schematic of ovine LPH showing location of known peptide products and the fragments determined by radioimmunoassay and opiate radioreceptor assay.

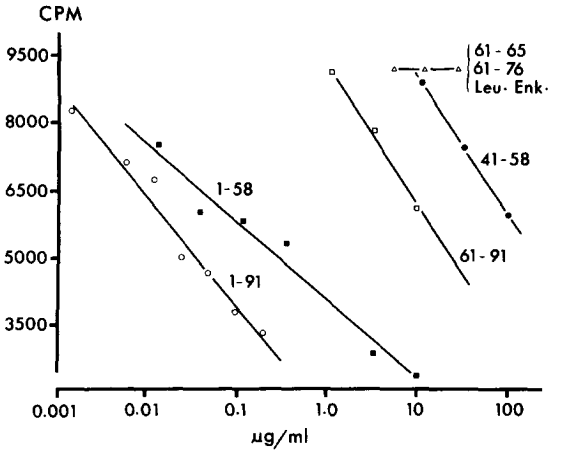

FIGURE 2. Activity of LPH (1-91) and peptide fragments in the solid-phase radioimmunoassay.

serum appears to be directed primarily against the N-terminal portion of the polypeptide (Figure 2). Sequence 1-58 (gamma-LPH) reacts almost as well as does the intact LPH molecule. Beta-MSH (residues 41-58) shows very low cross-reactivity, indicating antigenic determinants to reside within the sequence 1-40. The opiate-like C-terminal fragment (residues 61-91) shows only about 0.1% cross-reactivity. Methionine enkephalin (residues 61-65), leucine enkephalin and alpha-endorphin (residues 61-76) are completely inactive in the assay. Several purified bovine anterior pituitary hormones

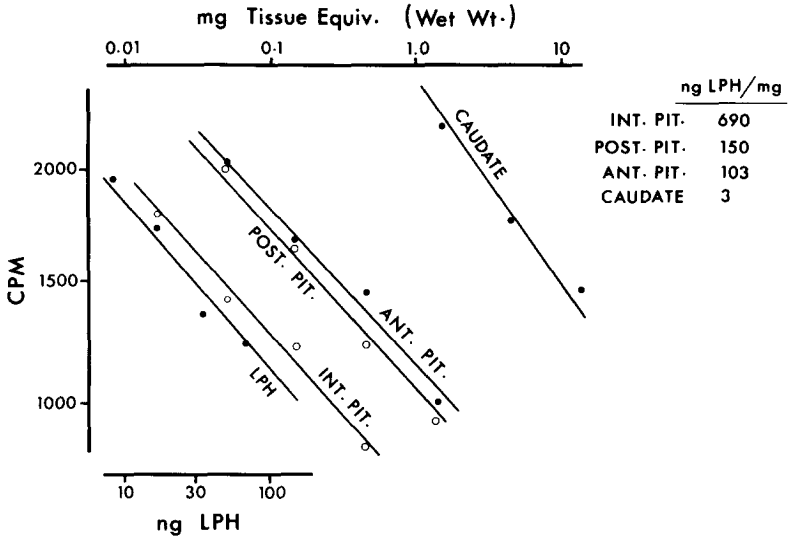

FIGURE 3. Dose-response curves of extracts of bovine pituitary lobes and brain in the LPH radioimmunoassay.

(NIAMD-USPHS) cross-react to less than 0.1%. Thus, the radioimmunoassay (RIA) for LPH estimates the intact protein and the N-fragment. The opiate assay, on the other hand, is selective for the C-terminal region of LPH, specifically for sequences beginning with residue 61.

Extracts of each of the three lobes of the bovine pituitary gland and of brain tissue show parallel dose-response curves in the assay for LPH (Figure 3). As shown previously for endorphin (11,12) the intermediate lobe is most and the anterior lobe least concentrated in LPH. Brain is much less concentrated than pituitary, as represented by the caudate nucleus known to be very rich in endorphin. Regional brain differences in LPH levels are observed and these findings will be presented elsewhere.

Gel filtration, in conjunction with the RIA and opiate radioreceptor assay, permits assessment of the types and sizes of LPH fragments in a given tissue. It can be seen (Figure 4) that the profile of LPH and its cleavage products differs between brain and pituitary. In the pituitary, intact LPH

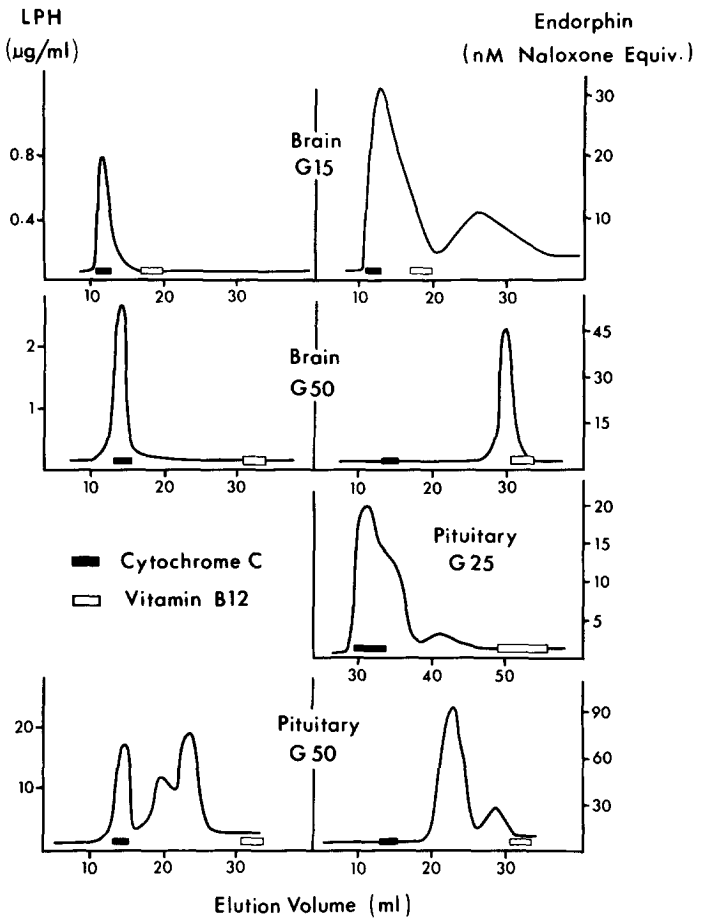

FIGURE 4. Gel filtration of extracts of bovine neuro-intermediate pituitary and of brain. LPH in column fractions is shown in the left and endorphin activity in the right panels.

(about 10,000 daltons) is indicated by the presence of a component excluded from the G-25 gel. Smaller components detected by the N-terminal directed antiserum may represent fragments 1-58 and 1-39, respectively. The major endorphin component, about 3,000 daltons, probably corresponds to the 61-91 fragment. A minor endorphin of about 2,000 daltons can be identified, but no components approaching the size of enkephalin are detectable in pituitary. The position of synthetic enkephalin was determined on each of the columns. In brain, only intact LPH (1-91) is detectable by RIA, indicating that N-

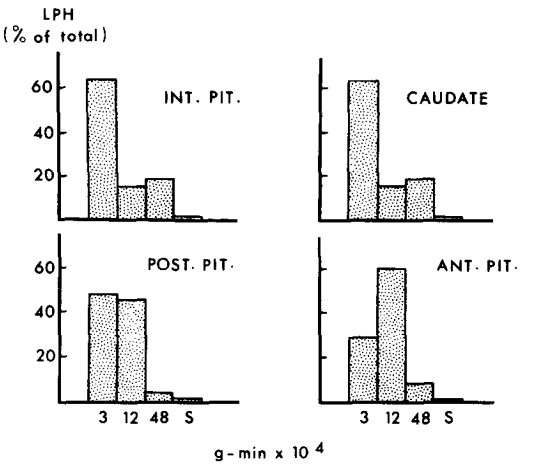

FIGURE 5. Distribution of LPH among subcellular fractions from brain and pituitary lobes.

terminal fragments are degraded or removed soon after their generation. C-terminal opiate fragments are represented by a major component of about 2,000 daltons. Enkephalin activity is present apparently in a relatively small proportion (Figure 4).

Immunohistochemical studies show LPH to be most concentrated in cells of the intermediate pituitary (14-16). We showed that endorphin, presumably derived from LPH, is also most concentrated in the intermediate lobe (11,12). Furthermore, in pituitary and brain the putative prohormone and its peptide products are apparently stored within the same secretory granule. The sedimentation pattern of LPH (Figure 5) is identical to that of endorphin (11,12). Essentially all of the LPH activity is found in particulate components, and the great proportion of LPH-containing granules is sedimented at forces of 12,000 g or less. The paired amino acid residues in LPH and the recognition of beta-MSH and endorphin structures within the protein sequence suggest secretion product packaging analogous to that in several other glands elaborating peptide hormones (10). Although LPH has been shown to possess lipolytic activity (17,18) this biological property is not impressive and,

until recently, the physiological significance of the protein has been enigmatic. Chretien and his colleagues (9) were the first to propose that beta-LPH is synthesized in bovine pituitaries and gives rise to fragment 1-58 (19), thus presumably releasing the C-terminus fragment. However, these last experiments were done before the discovery of endorphin and the release of 61-91 was not verified until recently (unpublished).

In the present studies the major endorphin in the pituitary gland appears to be the 61-91 fragment, smaller components apparently representing artifacts of extensively processed tissue (4,5). In fresh bovine pituitary glands we find the 61-91 component to predominate, although a minor component of about 2,000 daltons is consistently present, presumably alpha-endorphin (residues 61-76). In extracts of fresh beef brain endorphin activity is accounted for chiefly by the component of about 2,000 daltons; another component (about 500 daltons), presumably the pentapeptide identified in brain by Hughes (1) and Simantov and Snyder (20), is only a minor component. The LPH-endorphin complex in pituitary presumably represents a systemic endocrine secretion. Intact LPH, as well as each of the major peptide products, i.e. N-fragment (residues 1-39), beta-MSH (residues 41-58), and beta-endorphin (residues 61-91), are apparently present within the storage granule in the pituitary. In contrast, extracts of brain apparently yield intact LPH only, and two endorphin components, both smaller than the major opiate component (residues 61-91) found in pituitary. LPH in brain may be mobilized at synapses upon demand, to yield products distinct from those in pituitary.

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