PRESENCE OF IMMUNOASSAYABLE β-LIPOTROPIN IN BOVINE BRAIN AND SPINAL CORD: LACK OF CONCORDANCE WITH ACTH CONCENTRATIONS

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SUMMARY

Discrete areas of freshly obtained adult bovine brain were assayed for their content of immunoreactive β-lipotropin (β-LPH), ACTH and β-endorphin. Highest concentrations (pg/100ug protein) of β-LPH were present in hypothalamus (517 ± 81), hippocampus (218 ± 60), central grey rostral mesencephalic level, pons, striatum, and spinal cord (163-258). Lesser concentrations (49-138) were present in other parts of the limbic system, brain stem, cortex and thalamus. Immunoreactive ACTH concentrations were highest in hypothalamus (1702 ± 487) and hippocampus (210 ± 40), with markedly lesser concentrations (5-24) being present in all the other aforementioned areas. Immunoreactive β-endorphin concentrations in hypothalamus were 1990 ± 510, in hippocampus 280 ± 50.

β-Lipotropin has been demonstrated in the pituitary of several species (1). Endorphin(s) (comprising portions or the whole of the 61-91 sequence of β-LPH) have been reported to be present in both brain and pituitary of several species (2-4). Endorphin-like activity is also present in the brains of hypophysectomized animals (5). Incubation of β-LPH*with brain extracts (6) generates fragments with opioid-like activity suggesting a precursor role for β-LPH. To date, however, there has been no report of the presence of β-LPH in brain. The present study demonstrates the presence of β-LPH in selected areas of the bovine brain. Since ACTH is present in the brains of both normal and hypophysectomized rats (7) and β-LPH and ACTH have been reported to occur within the same cell in the pituitary (8), ACTH concentrations were also determined in all of the areas assayed to determine if the distribution pattern was similar to that of β-LPH.

Abbreviations: *β-LPH, β-lipotropin; ACTH, Adrenocorticotropic hormone; $_{h}$ACTH, human ACTH; MSH, Melanocyte stimulating hormone CNS: Central nervous system

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TABLE I

Distribution of β-LPH, ACTH and β-endorphin in Bovine Brain

Anatomical Area	(n)	β-LPH Content* pg/100ug prot.	ACTH Content* pg/100ug prot.	β-endorphin** Content pg/100ug prot.
Hypothalamus	3	517 ± 81	1702 ± 487	1990 ± 510
Hippocampus	3	218 ± 60	210 ± 40	280 ± 50
Olfactory Bulb	2	138 ± 94	<5	1500+
Amygdala	1	49	120	700+
Septum	3	278 ± 55	11 ± 3	
Striatum	4	163 ± 41	17 ± 2	930+
Thalamus	3	99 ± 22	12 ± 3	
Mesencephalon:				
Central Grey	3	258 ± 15	24 ± 6	
Pons	3	243 ± 43	6 ± 0.5	
Medulla (tegmentum)	3	123 ± 28	9 ± 1	
Spinal Cord	2	200 ±140	6 ± 1	
Cortex ‡				
Frontal‡	4	124 ± 7	24 ± 6	
Cerebellar‡	3	101 ± 21	7 ± 1	
Pituitary (whole)	3	$1.7 \times 10^6 \pm 0.6$	$1.4 \times 10^6 \pm 0.2$	$0.61 \times 10^6 \pm 0.13$

*Immunoreactive content determined on 0.2N HCl extracts.

**Immunoreactive content determined on fractions after G-75 chromatography (see methods). Where concentrations are not noted for a given area, G-75 chromatography was not performed either because β-endorphin concentrations of acid extracts were below the limits of detection of the assay or lack of parallelism of dilutions of tissue extracts with that of the standard curve indicated possible cross-reactivity with β-LPH.

+n=1

‡determined on 500mg sections

MATERIALS AND METHODS

Adult female bovine brains (n=4) and spinal cords (n=2) were obtained seven minutes following exsanguination. Selected areas (Table I; not all areas were obtained from each animal) were dissected grossly, using standard neuroanatomical landmarks. Tissues were immediately frozen on dry ice and then kept at -45°C. All sections save those from cortex, spinal cord and medulla (of which only portions were excised) comprised the entire anatomical extent of a given area.

Frozen tissue was homogenized for 30-60 seconds in 20 volumes of 0.2N HCl in either (depending on tissue weight) a siliconized Oster blender or a siliconized glass tube with a Teflon pestle. A protein determination (9) was performed on an aliquot of the unspun homogenate. The remaining homogenate was centrifuged at 18,000g for 20 minutes at 4°C and the supernatant frozen in aliquots until subsequent assay. When radioimmunoassay was performed on such specimens, they were first neutralized with 1N NaOH and recentrifuged. (There was no loss of added β-LPH or $_h$ACTH as a result of such neutralization). Speci-

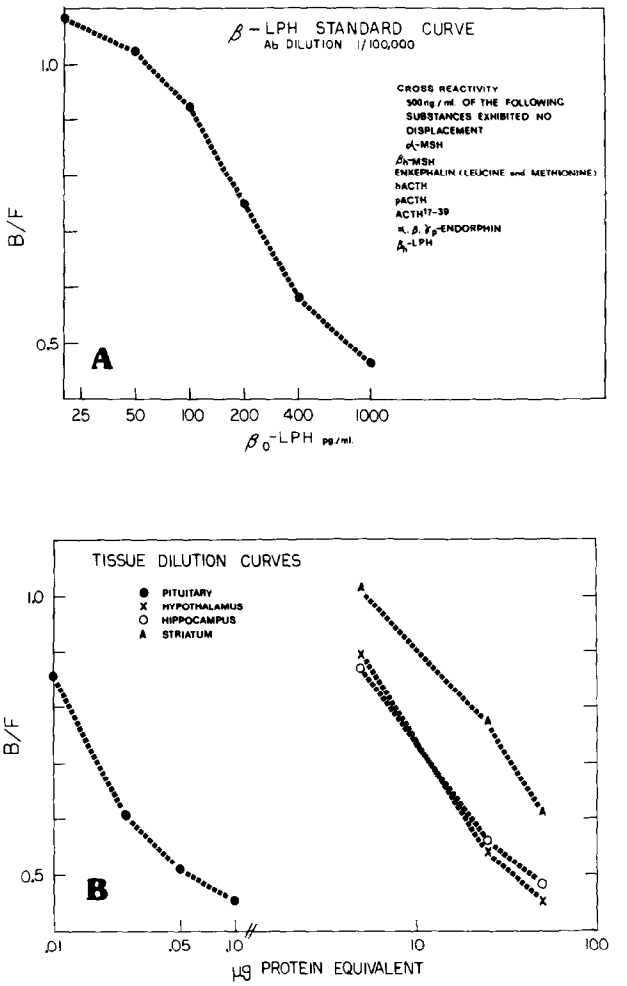

Figure 1. A: Standard curve for β-LPH. The ratio of bound to free labelled hormone is plotted on the ordinate. The abscissa depicts the dose of peptide (pg/tube). Crossreactivity studies with indicated peptides are also depicted. B: Parallelism of displacement curves of indicated tissue extracts with the standard curve obtained for β-LPH, (depicted in Figure 1.A).

mens that were to be subjected to G-75 Sephadex chromatography were first concentrated by ultrafiltration (using a filter with a molecular weight cut off of 1000) prior to neutralization. All immunoassays were performed in duplicate and at three different dilutions.

Immunoassay of β-LPH: This was performed using a rabbit antibody (at a titer of 1/100,000) to synthetic ovine β-LPH, and synthetic ovine β-LPH was used as a standard and for iodination (10). (The iodination procedure was modified, using silicic acid, rather than QUSO for extraction). The calculated specific activity varied from 87-118uCi/ug. Approximately 10pg ^{125}I-β-LPH/ml was added as tracer. Separation of bound and free LPH was achieved with polyethylene glycol. The limit of sensitivity was 25pg β-LPH/ml. The intra-assay

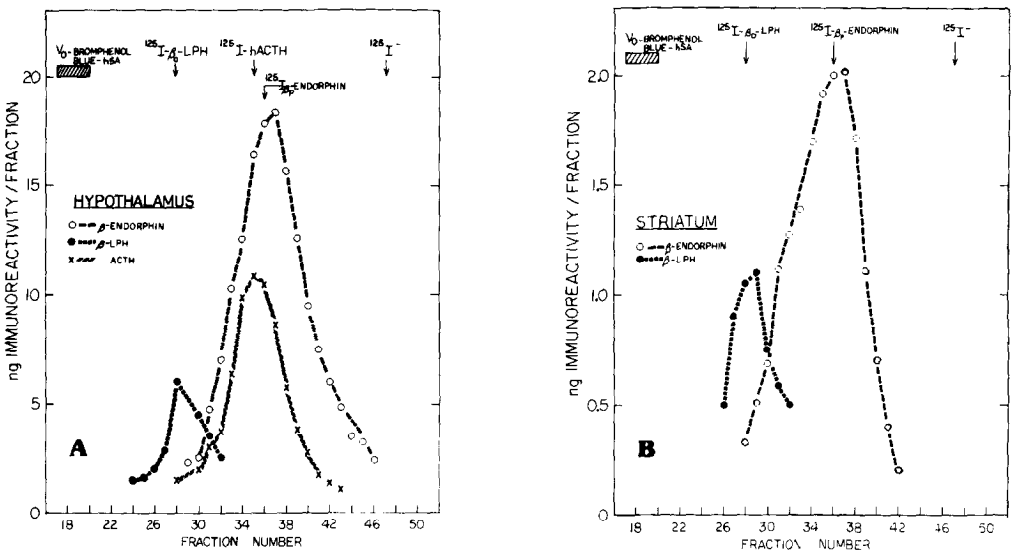

Figure 2. Sephadex G-75 chromatography of A:Hypothalamus B:Striatum. Homogenates of hypothalamus and striatum were concentrated two and six-fold respectively by ultrafiltration prior to neutralization with 1N NaOH. 0.5ml was applied to a 1 x 50cm column and eluted with 0.5% human serum albumin in 0.15M NaCl. ACTH was undetectable in the chromatogram of striatum due to low endogenous levels (see Table I). Arrows indicate the peak of the V_e of the designated labelled markers. Recoveries from the column were: Hypothalamus: ACTH-96%, β-LPH-75%, β-endorphin-82%. Striatum: ACTH-84%, β-LPH-62%, β-endorphin-77%.

coefficient of variation was 5.3%, that of interassay variation 8.1%. Specimens to be assayed were extracted with silicic acid (10) modified to exclude the acid wash (which would elute β-LPH). Recovery of added β-LPH was 80%. Fig. 1A depicts the standard curve and cross-reactivity with other peptides. Fig. 1B indicates representative dilution curves of extracts of three CNS areas and of pituitary, demonstrating parallelism with that of the standard curve.

Immunoassay of ACTH: This was performed as previously described (10). In addition to the reported characterization of the antibody employed (reactivity on an equimolar basis with $ACTH^{1-39}$ and $ACTH^{11-24}$ and lack of reactivity with α-MSH, β-MSH, $ACTH^{1-10}$, $ACTH^{17-39}$) this antibody exhibited no cross-reactivity with β-LPH, α-, β-, or γ-endorphin. Dilution curves of extracts of three CNS areas demonstrated parallelism with that of the standard curve.

Immunoassay of β-endorphin: This was performed using an antibody to synthetic porcine β-endorphin at a titer of 1/10,000 supplied by Dr. R. Guillemin. Partial characterization of this antibody has been described (11). There was also no cross-reactivity with $ACTH^{1-39}$, $ACTH^{25-39}$, α-MSH, β_h-MSH. Synthetic porcine β-endorphin was used as a standard and for iodination. The calculated specific activity was 188uCi/ug. Approximately 40pg ^{125}I-β-endorphin was achieved with polyethylene glycol. The limit of sensitivity at the antibody concentration employed in this study was 200pg/ml. (When antibody is used at a titer of

1/30,000, the minimum sensitivity is 50pg/ml). The intra-assay coefficient of variation was 6.8%, that of interassay variation 9.3%. Dilution curves of extracts of three central nervous system areas (hippocampus, hypothalamus, striatum) and of pituitary demonstrated parallelism with that of the standard curve. Due to the cross-reactivity with β-LPH all assays of tissue for β-endorphin content were performed on Sephadex G-75 eluates comprising the elution volume of β-endorphin. When peaks of B-endorphin and β-LPH overlapped, a "batch" technique was employed in which dry Sephadex G-50 was added to the eluates to further discriminate (taking advantage of the differential partition of β-endorphin and β-LPH in the V_i volume) between the contributions of β-LPH and β-endorphin to immunoreactivity measured with the endorphin antibody.

Sephadex G-75 chromatography: At a sample volume of 1.25% of column volume, similar elution patterns were obtained using either 1 x 50cm or 2.5 x 25cm columns. Columns were equilibrated and eluted with 0.5% human serum albumin (heat-inactivated) in 0.15M saline at 10°C; one ml fractions were collected. Markers were bromphenol blue-human serum albumin for V_o, ^{125}I-labelled ovine β-LPH, ^{125}I-labelled $_h$ACTH$^{1-39}$, ^{125}I-labelled porcine β-endorphin and ^{125}I- for the salt peak. Column eluates were assayed directly. Dilution curves of such eluates exhibited parallelism with their respective standard curves.

RESULTS

β-LPH and ACTH concentrations in 0.2N HCl tissue extracts (Table I): Highest concentrations of β-LPH were present in hypothalamus, septum, hippocampus, the central grey of the mesencephalon, pons, striatum and spinal cord, with lesser concentrations in other noted areas. β-LPH concentrations determined on 500mg aliquots from a given region and compared to those present in the area as a whole, revealed rather uniform concentrations in the hypothalamus, but lack of uniformity in other areas, e.g., hippocampus and striatum. Highest concentrations of ACTH were present in hypothalamus, these were 8 to 15 fold higher than those seen in the hippocampus and amygdala. ACTH concentrations tended to be more uniform throughout a given area. Concentrations in whole pituitary are noted for comparative purposes.

β-endorphin concentrations in Sephadex G-75 eluates (Table I): Marked variability of β-endorphin concentrations was noted within different portions of the limbic system, and there was no correlation between the pattern of distribution of β-endorphin concentrations and those of β-LPH.

β-LPH, ACTH and β-endorphin concentrations determined on Sephadex G-75 fractions of extracts of selected brain areas: Such studies were performed on hypothalamus, hippocampus, striatum and amygdala, choosing areas in which different proportions of β-LPH and ACTH were present on direct assay of acid extracts. Two representative chromatograms are shown in Fig. 2 (A:hypothalamus B:striatum). LPH, ACTH and endorphin activity eluted with the same V_e as their

respective labelled standards. This elution pattern for ACTH differs from our previously reported (7) observation on rat brain ACTH, whose elution pattern was slightly retarded compared to that of the labelled standard.

DISCUSSION

These data provide the first demonstration of immunoreactive β-LPH in discrete nervous system areas. The presence of β-LPH in brain could represent detection either of bound β-LPH, synthesized in the pituitary, or β-LPH synthesized within the brain. In view of the presence of a blood brain barrier for ACTH (12), it would be expected that this would also be present for β-LPH. Pituitary β-LPH, however, might reach the brain by the route of reverse flow in the pituitary portal system (13). Studies in hypophysectomized animals, similar to those which we have reported with regard to brain ACTH concentrations (7), would help to clarify the source of such brain β-LPH.

The data presented with regard to parallelism of brain extracts with that of the standard curve for β-LPH, and the patterns obtained on Sephadex gel filtration indicate similarity of the β-LPH detected in the present assay system with authentic β-LPH. Despite such similarities it still remains to be proven that such immunoreactive β-LPH possesses similar biological lipolytic activity to that of authentic β-LPH. Such studies are in progress.

The detection of β-LPH in brain would support the possibility that it may serve as a precursor of fragments with "endorphin-like" activity found in brains of both intact and hypophysectomized animals (5). In the present study the higher concentrations of endorphin than of β-LPH noted in the various areas studied may reflect enzymatic degradation of β-LPH, such degradation being apparent within 30 minutes (the first time point checked in the reported study (6)).

In the cow, as in the rat, highest ACTH concentrations are present in the hypothalamus and limbic system. ACTH concentrations in bovine hypothalamus are higher than those reported in the rat. Concentrations in hippocampus are similar in both species, while those in amygdala and septum appear to be lower in bovine brain.

In contrast to ACTH, there appear to be significant amounts of β-LPH present within the brain stem and spinal cord. The differences in the distribution pattern of β-LPH and of ACTH raises the question of whether these peptides may be synthesized independently of each other in a given area, unlike in the pituitary, or whether such differences could be secondary to differential distribution of degradative enzymes or uptake sites for these two peptides. Immunocytochemical localization may help to distinguish between these possibilities.

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