Proc. Natl. Acad. Sci. USA
Vol. 75, No. 6, pp. 2950–2954, June 1978
Medical Sciences

β-Lipotropin is the major opioid-like peptide of human pituitary and rat pars distalis: Lack of significant β-endorphin

(plasma endorphin/corticotropin/opiates/radioimmunoassay)

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Communicated by Hans Popper, March 27, 1978

ABSTRACT **β-Lipotropin is the predominant opioid peptide of the human pituitary and rat pars distalis and is present in concentrations essentially equimolar with corticotropin. When freshly obtained nonfrozen rat anterior pituitaries were homogenized with 0.2 M HCl, approximately 98% of the immunoreactivity detected utilizing an antiserum that crossreacts equally with β-lipotropin and β-endorphin coeluted with ^{125}I-labeled human β-lipotropin upon molecular sieve chromatography. The remainder of the activity eluted with synthetic human β-endorphin. Similar results were obtained for human pituitary. HCl homogenization of thawed tissue or homogenization of fresh tissue with acetic acid yielded substantially greater concentrations of β-endorphin and decreased concentrations of β-lipotropin. In human subjects, acute anterior pituitary stimulation using either insulin-induced hypoglycemia or vasopressin administration was associated with increased plasma β-lipotropin and corticotropin levels. At the time of peak concentrations, no significant levels of β-endorphin were detectable. These data indicate the lack of significant amounts of β-endorphin in human pituitary. Additionally, there appears to be no specific intrapituitary conversion of β-lipotropin to β-endorphin.**

There is little information available regarding the physiological role of β-endorphin in humans. It has been shown that corticotropin (ACTH) and β-lipotropin (β-LPH) are both contained in a common 31,000-dalton glycoprotein precursor molecule (1, 2), that these peptides are both present in pituitary corticotropic cells (3, 4) with suggestive evidence that they may be localized within the same secretory granules (3), and that they are secreted concomitantly in the human after insulin-induced hypoglycemia or vasopressin administration (5). Material with opioid activity (not definitively characterized as β-endorphin) appears after incubation of β-LPH with aqueous rat brain extracts (6) at neutral pH. The possibility therefore exists that similar endorphin generation might occur within the pituitary. Although β-endorphin has been localized immunocytochemically in cells of the anterior and intermediate lobes of the rat pituitary gland, to date there is no evidence that it occurs within the same cells as does β-LPH.

By utilizing a combination of radioimmunoassay and molecular sieve chromatography, it has been found that the major opioid peptide of rat pituitary extracts is β-endorphin (7), with a minor component of β-LPH comprising approximately 10% of the total immunoreactivity. (It was not stated whether these were extracts of total pituitary or of anterior lobe only). An anterior lobe β-endorphin content of approximately 340 pmol was reported with a total pituitary content of 760 pmol (7). It was further demonstrated that, in the rat, ACTH and β-endorphin are secreted concomitantly *in vivo* under acute stress conditions (7, 8) and *in vitro* when monolayer cultures of anterior pituitary cells are incubated with purified corticotropin-releasing factor (7).

In contrast, another study (9) found that rat anterior pituitary contains approximately 100 pmol of β-LPH and only 2 pmol β-endorphin. Lowry and coworkers (10, 11) have proposed that corticotropic cells in both the pars distalis and the pars intermedia (in those species having such a lobe) synthesize ACTH and β-LPH. They further proposed that both peptides are secreted in the intact form from the pars distalis, whereas in the pars intermedia, ACTH and β-LPH serve as precursors for smaller fragments—ACTH being cleaved to α-melanotropin (α-MSH) and corticotropin-like intermediate lobe peptide (CLIP; ACTH$^{18-39}$) and β-LPH, to γ-LPH, β-MSH (β-LPH$^{41-58}$), and β-endorphin.

Chretien *et al.* (12) have isolated both β-LPH and β-endorphin from frozen human pituitary but did not quantify the yield. Li *et al.* (13) isolated 0.73 μmol of β-endorphin from 1000 fresh human pituitaries. Assuming that β-LPH is present in the same molar concentrations as ACTH in anterior pituitary and assuming an average pituitary weight of 500 mg, this would correspond to a concentration of 0.7 nmol of β-endorphin per pituitary as compared to a concentration of approximately 110 nmol of β-LPH.

In view of the discrepancies reported by different investigators with regard to rat anterior pituitary β-endorphin content and the limited information available with regard to human pituitary β-endorphin content, human pituitaries were assayed for their ACTH, β-LPH, and β-endorphin contents after determination of the efficacy of three different methods of tissue homogenization to ascertain if a particular method of extraction would result in β-LPH degradation. Rat intermediate and posterior lobe tissue was also assayed for its β-LPH and β-endorphin contents for comparison to anterior lobe results. (In order to maintain the integrity of the tissue, no attempt was made to separate intermediate from posterior lobes.) Additional studies were performed to determine whether acute stress resulted in increases in human plasma β-endorphin concentrations.

MATERIALS AND METHODS

Radioimmunoassay Techniques. ACTH was assayed as described (14). β-Endorphin was quantified by using an antiserum that reacts with both β-endorphin and β-LPH on an equimolar basis. The β-LPH antiserum used contains two distinct binding sites, one site binding both β-LPH and β-endorphin with equal affinity (COOH-terminal determinant) and one site binding only β-LPH. To quantify β-LPH specifically in the presence of β-endorphin, the antiserum is allowed to react

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Abbreviations: ACTH, corticotropin (adrenocorticotropic hormone); LPH, lipotropin; MSH, melanotropin (melanocyte-stimulating hormone).

with an excess of synthetic β_h-endorphin, thus making available only the specific site for binding both the labeled and unlabeled β-LPH (complete characterization of this antiserum will be reported elsewhere). The assay protocol is similar to the one we have described for β_o-LPH antiserum (15). None of the antisera used in this study crossreacted with myelin basic protein, at tested concentrations up to 2.5 μg/ml. Human plasma β-LPH levels were assayed as described (16). All pituitary peptide concentrations are expressed as pg/100 μg of protein. Protein concentrations were determined on an aliquot of unspun homogenate by the method of Lowry *et al.* (17).

Molecular Sieve Chromatography. All tissue extracts were subjected to chromatography on Sephadex G-50 (fine) to separate immunoreactive β-LPH-like activity from that of β-endorphin. The columns were 1 × 50 cm, with a sample volume 1–1.5% of the column volume, and were eluted with 0.1 M HCl at a flow rate of 0.2 ml/min. Sephadex G-50 gives a sharp separation of β-LPH and β-endorphin (as characterized with synthetic standards and tissue extracts), and the combined effect of the HCl elution and slow flow rate slightly retards the β-LPH peak (both marker and extracts), separating it from immunoreactive material eluting in the void volume. The void volume peak, which always comprised less than 5% of the total immunoreactivity detected, is presumably the common precursor of ACTH and β-LPH recently described by Mains and colleagues (1). No further characterization of this material was performed for this study.

Extraction of Pituitary Tissue. Rat anterior pituitaries were obtained by decapitation of male Sprague–Dawley (250–350 g) rats. Depending upon the experiment, the tissue was either homogenized immediately as described below or kept at room temperature (moistened with 0.9% NaCl) for 6 hr prior to homogenization. (Because human tissue is only available several hours post mortem, six rat anterior pituitaries were left at room temperature for 6 hr prior to extraction to check the effect of the extent of autolysis prior to extraction.) One pool of six rat anterior pituitaries was frozen on dry ice, thawed, frozen a second time, and then kept for 6 hr at room temperature, to check the effect of such tissue handling. Three different extraction methods were used to determine if degradation of β-LPH can occur as a consequence of the method of extraction. Twelve freshly obtained rat anterior pituitaries were pooled for each method. Tissue was homogenized in 2 ml of 0.2 M HCl, 2 ml of 0.2 M acetic acid, or 2 ml of 1 M acetic acid preheated to 90°. The homogenates were then centrifuged at 12,000 × *g* for 30 min. The acid extract was frozen at −40° until assayed. An additional pool of fresh rat intermediate plus posterior lobes from 22 rats was homogenized in 0.2 M HCl and the extract was treated as described above. Three whole human pituitaries and one anterior pituitary were obtained at autopsy performed at various times after death (corpses were refrigerated prior to autopsy). This tissue was not frozen but, based on the results of foregoing experiments, was immediately placed in 5 ml of 0.2 M HCl, brought to the laboratory, and homogenized and treated as above.*

Calculation of β-LPH/β-Endorphin Molar Ratios. No specific antiserum to rat β-LPH is yet available. Therefore, such ratios were obtained by respectively summing the activities in the immunoreactive peaks that coeluted with standard ^{125}I-labeled human β-LPH and β-endorphin.† Ratios in human pituitary were similarly calculated. In addition, total immunoreactivity of the extract prior to chromatography was quantified by using the β_h-LPH antiserum, in both the presence and absence of excess β-endorphin. Values obtained with these two methods were in good agreement.

Insulin Tolerance and Vasopressin Tests. These were performed as described (18).

RESULTS

Effects of Method of Extraction on Immunoreactive β-LPH, β-Endorphin, and ACTH Concentrations in Rat Pars Distalis. When 0.2 M HCl was used to homogenize the tissue, approximately 98% of the immunoreactivity measured with the β-endorphin antiserum coeluted with [^{125}I]iodo-β_h-LPH on Sephadex G-50 chromatography (Fig. 1*A*). The β-LPH to ACTH molar ratios were essentially unity, indicating the equimolar presence of these two peptides. Use of 0.2 M acetic acid (Fig. 1*B*) or 1 M acetic acid at 90° to homogenize the tissue resulted in a large loss of β-LPH activity and a marked increase of β-endorphin activity, as well as a marked decrease in the β-LPH/ACTH molar ratio (Table 1).

β-LPH and β-Endorphin Concentrations in Rat Intermediate and Posterior Lobe. In contrast to the findings in rat pars distalis, with homogenization in 0.2 M HCl the predominant peak of immunoreactivity in intermediate plus posterior lobes (β-endorphin antibody), comprising approximately 83% of the total activity, coeluted with synthetic β_h-endorphin on Sephadex gel chromatography (not shown). Most of the remainder of the activity eluted with [^{125}I]iodo-β_h-LPH. This corresponds to concentrations of about 178 pmol/100 μg of protein for β-endorphin and 37 pmol/100 μg of protein for β_h-LPH.

Effect of Exposure to Room Temperature and of Freezing and Thawing on β-LPH, β-Endorphin, and ACTH Immunoreactivity. When fresh rat anterior pituitaries were homogenized in 0.2 M HCl after being kept at room temperature for 6 hr, there was a modest decrease in β-LPH and an increase

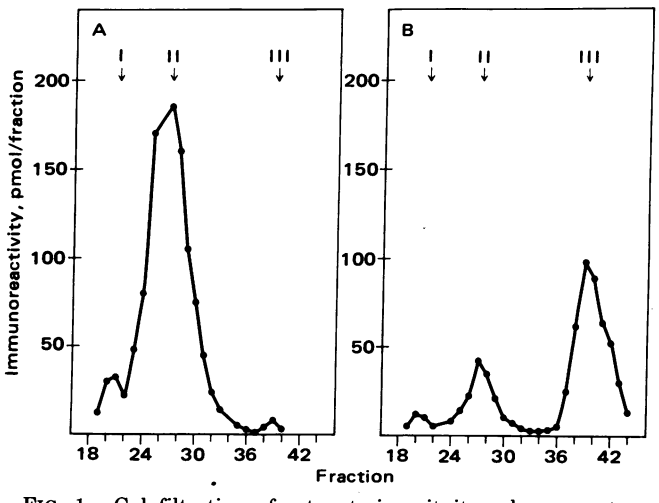

FIG. 1. Gel filtration of rat anterior pituitary homogenates. Freshly removed anterior lobes were pooled and homogenized in either 2.5 ml of 0.2 M HCl (*A*) or 2.5 ml of 0.2 M acetic acid (*B*). An aliquot of the supernatants (12,000 × *g*, 30 min) was applied directly to a Sephadex G-50 column (1 × 50 cm) equilibrated with 0.1 M HCl. Immunoreactivity was quantified by using a β-endorphin antiserum that crossreacts with β-LPH on an equimolar basis. Arrows: I, void volume (blue dextran); II, [^{125}I]iodo-β_h-LPH; III, [^{125}I]iodo-β_h-endorphin (synthetic).

* Similar results were obtained when tissue was placed in 0.2 M HCl, left at room temperature for 30 min, frozen, and then homogenized.

† This is justified because recoveries of added β-endorphin and β-LPH were similar (92–96%; n = 9).

Table 1. Effect of extraction medium on immunoreactive β-LPH, β-endorphin (EP), and ACTH concentrations in rat pars distalis

Extraction method	n	pmol/100 μg protein β-LPH	β-EP	ACTH	Molar ratios LPH/EP	LPH/ACTH
0.2 M HCl	12	17.3	0.3	18.1	58.0	0.96
1 M acetic acid at 90°	12	6.5	5.9	13.2	1.1	0.49
0.2 M acetic acid	12	3.5	8.6	10.9	0.4	0.32

Thirty-six freshly removed rat anterior pituitaries were arbitrarily divided into three pools of 12 each. A given pool was homogenized in either 0.2 M HCl, 0.2 M acetic acid, or 1 M acetic acid preheated to and maintained at 90° for 5 min after homogenization. Total immunoreactive β-endorphin and ACTH-like activity was determined on the unchromatographed extracts, and an aliquot of the extracts was also subjected to gel filtration.

in β-endorphin concentrations (Table 2). Under these conditions, approximately 88% of the total β-LPH and β-endorphin activities still eluted with [^{125}I]iodo-β_h-LPH on Sephadex G-50 chromatography. When the tissue was immediately frozen on dry ice and then allowed to thaw completely prior to homogenization, approximately 80% of the activity eluted with [^{125}I]iodo-β_h-endorphin (Fig. 2). When the thawed tissue was maintained at room temperature for 6 hr prior to homogenization, virtually all of the β-LPH activity disappeared and a small peak of β-endorphin activity remained (representing less than 10% of the β-LPH activity of the control pool).

Human Pituitary Immunoreactive β-LPH, β-Endorphin, and ACTH. The human pituitary, like the rat anterior lobe, contains much more β-LPH than β-endorphin. There was an increase in the β-endorphin concentration as a function of the time the tissue was homogenized post mortem, with a resultant decrease in the β-LPH to β-endorphin molar ratio (Table 3). In the freshest pituitary, the β-LPH activity comprised approximately 95% of the total immunoreactivity measured by the β-endorphin antiserum. This activity coeluted with [^{125}I]-iodo-β_h-LPH on Sephadex G-50 chromatography (Fig. 3). The Sephadex G-50 β-LPH peak was also quantified with the β-LPH antiserum and yielded a value nearly identical (within the error of the assay system) to the molar concentrations obtained with the β-endorphin antiserum. This is an indication that this peak contained little γ-LPH, because the β-endorphin antiserum used does not crossreact with γ-LPH, whereas the β-LPH antiserum does. If there had been a significant concentration of immunoreactive NH_2-terminal fragments in the Sephadex G-50 LPH peak, the value obtained with the β_h-LPH antiserum would be greater than that obtained with the β-endorphin antiserum.

Table 2. Effect of temperature and freeze-thawing on immunoreactive β-LPH, β-endorphin (EP), and ACTH concentrations in rat pars distalis

Handling prior to homogenization	n	pmol/100 μg protein β-LPH	β-EP	Molar ratios LPH/EP
None	6	15.9	0.3	53.00
6 hr at room temp.	6	12.1	1.6	7.60
Freeze, thaw	6	2.9	11.5	0.25
Freeze, thaw, 6 hr at room temp.	6	<0.05	1.3	—

Twenty-four freshly removed rat anterior pituitaries were arbitrarily divided into four pools of 6 each. Each pool was submitted to one of the following treatments: immediately homogenized; maintained at room temperature for 6 hr and then homogenized; frozen on dry ice and completely thawed prior to homogenization; and frozen and thawed and then left at room temperature for 6 hr prior to homogenization. All tissue pools in this experiment were homogenized in 0.2 M HCl.

Even 48 hr post mortem, approximately 80% of the molar activity measured by the β-endorphin antiserum coeluted with standard β_h-LPH on Sephadex G-50 chromatography. This immunoreactive peak was a little broader than that obtained with the 3-hr sample, and the molar concentration of this peak as quantified with the β_h-LPH antiserum was approximately 14% greater than the value obtained with the β-endorphin antiserum. This increased activity probably does correspond to NH_2-terminal fragments generated by degradation of β-LPH.

Human Plasma β-LPH and ACTH Levels after Insulin and Vasopressin Stimulation. After insulin-induced hypoglycemia and vasopressin stimulation in six normal subjects, plasma β-LPH-like immunoreactivity at the time of peak concentrations

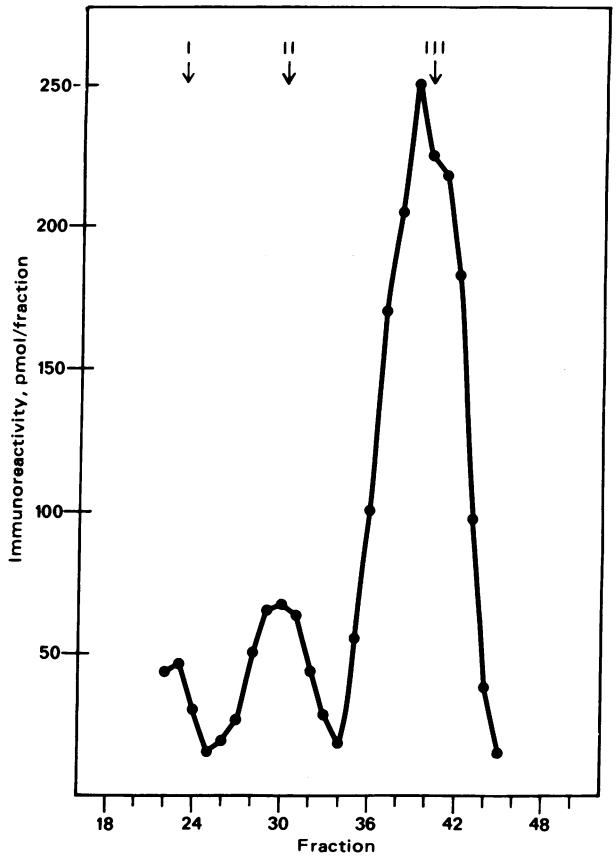

FIG. 2. Gel filtration (Sephadex G-50) of rat anterior pituitary tissue frozen and thawed prior to homogenization. Thawed tissue was homogenized in 0.2 M HCl and an aliquot of the 12,000 × *g* supernatant was applied directly to the column. Arrows: I, void volume; II, [^{125}I]iodo-β_h-LPH; III, [^{125}I]iodo-β_h-endorphin (synthetic).

Table 3. Immunoreactive β-LPH, β-endorphin (EP), and ACTH in human pituitary

Time after death, hr	pmol/100 μg protein LPH	EP	ACTH	Molar ratios LPH/EP	LPH/ACTH
3	117.0	4.9	127.0	23.7	0.92
7	106.0	13.2	96.5	8.1	1.10
20	103.0	14.4	127.0	7.2	0.81
48*	57.7	13.4	76.9	4.3	0.75

Human pituitary tissue was obtained at autopsy at the indicated time post mortem. All tissue was homogenized in 0.2 M HCl.
* Anterior lobe only.

was assayed in the presence and absence of excess synthetic β_h-endorphin. Because the β-LPH antiserum exhibits cross-reactivity with β-endorphin, if β-endorphin were secreted in response to such stimulation, "β-LPH" values obtained with the β-LPH antiserum in the absence of excess β_h-endorphin would be expected to be higher than those obtained with the β-LPH antiserum preabsorbed with β-endorphin. There was close agreement in β-LPH concentrations obtained by both methods (Table 4), suggesting that, under conditions of acute stimulation, β-endorphin is not secreted by the human anterior pituitary. The molar ACTH concentrations of such samples were always higher than those of β-LPH.

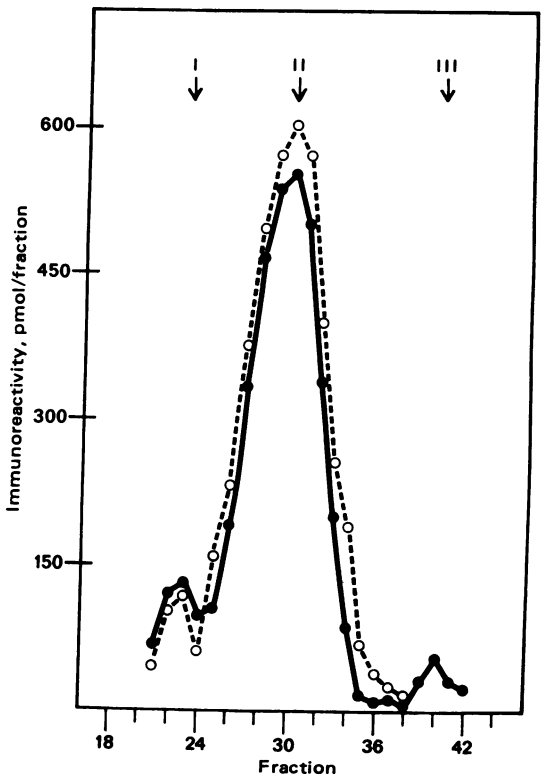

FIG. 3. Gel filtration (Sephadex G-50) of whole human pituitary homogenates. The pituitary (from a male) was obtained 3 hr post mortem and homogenized in 5 ml of 0.2 M HCl. Immunoreactivity was quantified by using a specific β-LPH antiserum preabsorbed with excess β_h-endorphin (O - - O) and with the β-endorphin antiserum (●—●) that crossreacts with β-LPH on an equimolar basis. Arrows: I, void volume; II, [^{125}I]β_h-LPH; III, [^{125}I]β_h-endorphin (synthetic).

Table 4. Peak immunoreactive plasma β-LPH, β-LPH + β-endorphin, and ACTH concentrations after administration of insulin or vasopressin

Subject	fmol/ml "β-LPH"	"β-LPH + β-endorphin"	ACTH
	Insulin		
1	33.0	30.4	59.3
2	43.8	46.7	74.0
3	60.1	64.0	109.0
	Vasopressin		
1	50.0	50.4	57.2
2	18.3	20.1	33.6
3	24.7	29.0	37.5

β-LPH and "β-LPH + β-endorphin" immunoreactive-like activity was determined as follows: Plasma was extracted as described in *Methods*. The extracts were assayed in the presence and absence of excess exogenously added β-endorphin. The β-LPH antiserum employed contains two distinct binding sites; see *Methods* for description. In the absence of added β-endorphin the β-LPH antiserum quantifies both β-LPH and β-endorphin. In the presence of added β-endorphin only β-LPH will be quantified. It is apparent that there is no significant difference between the values obtained with either method, indicating the lack of significant endorphin secretion at the time measured.

DISCUSSION

The present finding that rat and human anterior pituitaries contain intact β-LPH with only a minor component of β-endorphin is in agreement with the report of Rubinstein *et al.* (9), who found a 50:1 ratio of these peptides in rat anterior pituitary tissue by using acetone/HCl extraction. The equimolar concentrations of β-LPH and ACTH found in both of these tissues in the present study (extraction with 0.2 M HCl) lends further support to Lowry and coworkers' (10, 11) proposal that pars distalis corticotropic cells contain and release ACTH and β-LPH simultaneously and is consistent with the demonstration of a common precursor molecule in the pituitary containing both ACTH and β-LPH. Furthermore, the finding of high levels of β-endorphin-like activity in the intermediate plus posterior lobes of the rat pituitary is also predicted by the Lowry and Scott hypothesis (10). [The concentrations found are in general agreement with those reported by Rossier *et al.* (19). All the detected β-endorphin may be confined to the intermediate lobe because Bloom *et al.* (20) demonstrated immunocytochemically that the posterior lobe is devoid of such activity.]

There have been reports of detection of endorphin activity in anterior, posterior, and intermediate lobes of several mammalian species, and in mouse pituitary tumor cells, using an opiate-receptor assay (21–25), radioimmunoassay (7, 15, 19), or immunocytochemical techniques (20). The highest concentrations have been localized to the intermediate lobe (20, 22, 19). With regard to anterior lobe concentrations, studies in which endorphin activity was quantified by opiate receptor assay cannot be compared to the present study because such assays may detect opioid-like peptides other than β-endorphin. Interpretation of immunocytochemical data is difficult because the antiserum used in this study crossreacted with both β-LPH and β-endorphin (20). In other studies (7, 22, 24) in which frozen and thawed tissue or neutral pH or acetic acid extraction was used, which in view of the present findings would be expected to generate β-endorphin, when the molar values obtained were summed and divided by those of ACTH, the ratio was essentially unity (ACTH appears to be relatively stable when acetic acid extraction is used). A similar decrease in

β-LPH and an increase in β-endorphin concentration in rat tissue after 1 M acetic acid extraction has been alluded to by Rubinstein *et al.* (9).

Precedents exist for the suggestion that degradation of β-LPH can occur either prior to or as the result of extraction. There is now general acceptance that β_h-MSH (β_h-LPH$^{37-58}$) is an extraction artifact and is not elaborated by the human pituitary. Scott and Lowry (26) and Bloomfield *et al.* (27) demonstrated that prolonged extraction of human pituitary tissue in 1 M acetic acid resulted in a progressive increase in immunoreactive material coeluting with synthetic β_h-MSH and a parallel loss of activity eluting in the region of β-LPH. They postulated this "β_h-MSH" to be an artifact resulting from enzymatic degradation of β-LPH during extraction.

These and the present findings may be related to the weakly acidic properties of acetic acid ($pK_a = 4.75$). Homogenization of tissue in 10 vol of 0.2 M acetic acid results in a homogenate pH of 3.5–3.9. This may be insufficient to inactivate completely lysosomal proteases that have activity optima at acidic pH, whereas the use of 0.2 M HCl lowers the pH to less than 1, inactivating such enzymes. The lower concentrations of β-endorphin seen when tissue is maintained at room temperature (Table 2) or in the cold for several hours (Table 3) (as compared to those in frozen-thawed tissue) may be secondary to compartmentalization of proteases within cells, preventing their access to the secretory granules of the corticotropic cells. When this integrity is lost, either by freezing and thawing or homogenization in a weak acid, proteolysis can occur.

Li and Chung (28) were unable to demonstrate β-LPH in an extract of 1000 camel pituitaries, but they did isolate 14 mg of β-endorphin. They noted that this yield was very close to that expected if the β-LPH concentration of the camel pituitary was the same as in sheep and if all the β-LPH had been degraded to yield β-endorphin. They speculated that degradation had occurred prior to receipt of glands from the slaughterhouse. It is therefore possible that a considerable portion of the β-endorphin detected in the present study in fresh rat pars distalis and in human pituitary obtained 3 hr post mortem (HCl extraction) may also be secondary to proteolysis.

The lack of any significant secretion of β-endorphin into peripheral blood after insulin-induced hypoglycemia or vasopressin administration strengthens this possibility. The present data and the molar ACTH/LPH ratios obtained are in agreement with our previously reported data (5, 16) of simultaneous secretion of β-LPH and ACTH in the human after such stresses. Whether the presence of ACTH/LPH ratios greater than unity reflects different immunoreactive half-lives of these peptides, peripheral LPH degradation, or preferential secretion remains to be determined, as does the physiological role of such secreted β-LPH.

The finding of elevated plasma endorphin levels in rats after acute stress (7, 8) cannot be immediately compared to the results of the present study. The rat has a distinct intermediate lobe which does contain high immunoassayable and bioassayable endorphin concentrations. Crine *et al.* (29) have demonstrated *in vitro* biosynthesis of β-endorphin by bovine intermediate lobe tissue. Under stressful situations, therefore, this lobe may release β-endorphin. On a molar basis, the plasma β-endorphin levels found by Guillemin *et al.* (7) after acute stress are approximately 2.5 times greater than those of ACTH. Even if it were assumed that the anterior lobe contained only β-endorphin and no β-LPH, upon its stimulation one would expect to find essentially equimolar plasma concentrations of β-endorphin and of ACTH (assuming no large difference in immunoreactive half-lives). Accepting this, to attain the molar ratios reported in the above study (7), approximately 60% of the plasma endorphin concentrations would have to be of intermediate lobe origin, derived as postulated by Lowry and coworkers (10, 11).

We thank Dr. R. Guillemin for a generous supply of β-endorphin antiserum. Dr. C. H. Li kindly provided the β_h-LPH antiserum and β_h-LPH and synthetic β_h-endorphin. The ACTH antibody used (West) was a gift of the hormone distribution program, National Pituitary Agency, National Institute of Arthritis, Metabolism, and Digestive Diseases. This investigation was supported in part by National Institutes of Health Grant NS-02893 and The Lita Annenberg Hazen Charitable Fund.

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