Simultaneous Assay of Immunoreactive β-Lipotropin, γ-Lipotropin, and \( \beta \)-Endorphin in Plasma of Normal Human Subjects, Patients with ACTH/Lipotropin Hypersecretory Syndromes, and Patients undergoing Chronic Hemodialysis

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**ABSTRACT** We have studied the relative concentrations of the human immunoreactive (IR) peptides γ-lipotropin (hyLPH, [1–58]h\( \beta \)LPH), β-lipotropin (h\( \beta \)LPH), and \( \beta \)-endorphin (h\( \beta \)END, [61–91]h\( \beta \)LPH) using gel exclusion chromatography together with a specific radioimmunoassay (RIA) for hyLPH and a RIA that (because h\( \beta \)END is the COOH-terminus of the h\( \beta \)LPH molecule) measures both h\( \beta \)END and h\( \beta \)LPH on an equimolar basis. In normal subjects, basal plasma IR-hyLPH was often undetectable (<12.5 fmol/ml), but ranged up to 21 fmol/ml, and IR-h\( \beta \)END/h\( \beta \)LPH was 10.8±0.7 fmol/ml; previous studies by others suggest that most of the IR-h\( \beta \)END/h\( \beta \)LPH was probably h\( \beta \)LPH. Both IR-hyLPH and IR-h\( \beta \)END/h\( \beta \)LPH were significantly elevated (\( P < 0.001 \)) in patients undergoing chronic hemodialysis (101.5±12.7 and 23.8±2.0 fmol/ml, respectively). Their IR-hyLPH coeluted with standard hyLPH as a single peak, and IR-h\( \beta \)END/h\( \beta \)LPH coeluted with h\( \beta \)LPH; no distinct peak of IR-h\( \beta \)END was observed. In patients with ACTH/LPH hypersecretion due to Addison’s disease, Nelson’s syndrome, or ectopic ACTH syndrome, IR-hyLPH and IR-h\( \beta \)END/h\( \beta \)LPH were both elevated, and IR-h\( \beta \)END/h\( \beta \)LPH eluted as two peaks, one coeluting with h\( \beta \)LPH and the other with h\( \beta \)END. The molar concentrations of all three peptides were significantly correlated with one another. The lower concentrations of endogenous IR-h\( \beta \)END observed may be due in part to its apparent shorter plasma half-life, as estimated in an Addison’s patient given a cortisol infusion. The biologic significance of these three peptides in circulating blood is still unknown. The increased levels of h\( \beta \)LPH and hyLPH in plasma of patients with chronic renal failure suggest that the kidney may be an important organ for their metabolism.

**INTRODUCTION**

The human \( \beta \)-melanocyte-stimulating hormone (h\( \beta \)MSH)\(^1\) immunoreactivity of human tissues and plasma is now generally accepted to be due to two larger molecules, called lipotropins (LPH), both of which contain the sequence of “h\( \beta \)MSH” in their structures (1–13). The LPH have been isolated from putative extracts of several species (14–18) including man (1, 19–21); h\( \beta \)LPH is a single-chain 91-amino acid peptide, and hyLPH is (1–58)h\( \beta \)LPH; “h\( \beta \)MSH” is (37–58)h\( \beta \)LPH. Thus, both h\( \beta \)LPH and hyLPH cross-react in most “h\( \beta \)MSH” radioimmunoassays (RIA) (2–13). Indeed, several investigators have reported that immunoreactive (IR) “h\( \beta \)MSH” in human tissues and plasma is associated with high molecular weight (HMW) substances, which were thought to be h\( \beta \)LPH.

\(^1\)Abbreviations used in this paper: BSA, bovine serum albumin; END, endorphin; h, human; HMW, high molecular weight; IR, immunoreactive; LPH, lipotropin; MSH, melanocyte-stimulating hormone; RIA, radioimmunoassay.
(7–13) and/or possibly hyLPH (11) on the basis of their apparent molecular weights. Studies of extracts of human tissues, plasma, and media in which ACTH/LPH-producing human pituitary tumor cells were cultured, using gel exclusion chromatography and denaturing conditions, indicated the presence of hyLPH alone or both hyLPH and hβLPH (11, 13). However, the relative concentrations of the two LPH in plasma have not been studied in a systematic manner.

If hyLPH circulates in blood, then the complementary COOH-terminal (61–91)hβLPH fragment of hβLPH—β-endorphin (hβEND), the potent endogenous opiate peptide—should also be found in the circulation (11). Several authors have recently reported IR-hβEND in human plasma under basal conditions (22–25) and those of ACTH/LPH hypersecretion (25–28).

We have recently developed a RIA that measures hyLPH, but not hβLPH (29). In the present study, we have investigated the concentrations and relative plasma distributions of immunoreactive hβLPH, hyLPH, and hβEND in normal subjects, in patients undergoing hemodialysis because of chronic renal failure, a condition that is associated with high plasma “hβMSH” immunoreactivity (30–32), and in patients with syndromes associated with ACTH/LPH hypersecretion. The plasma disappearance rates of the three endogenous peptides have also been studied in a patient with Addison’s disease.

**METHODS**

**RIA**

**hyLPH RIA.** The hyLPH RIA was performed as described (29), using antiserum R1547 raised in a rabbit injected with synthetic (37–58)hyLPH (generously provided by Ciba-Geigy, Ltd., Basel, Switzerland) conjugated to bovine serum albumin (BSA) by the glutaraldehyde reaction (33). Purified hyLPH prepared in our laboratories from fresh-frozen human pituitary glands (kindly provided by the National Pituitary Agency, National Institute of Arthritis, Metabolism, and Digestive Diseases) by a modification of the method of Chretien and Li (16) was used as a standard, and synthetic (37–58)hyLPH was used for radioiodination. Incubation was carried out for 2 d at 4°C, tracer was added, and the incubation continued for an additional 2 d. Specificity studies were performed with synthetic hβEND, synthetic hACTH (Ciba-Geigy), and hβLPH purified in our laboratories from fresh-frozen human pituitary glands (National Pituitary Agency) by a modification of the method of Li (14).

**hβEND/hβLPH RIA.** The RIA for hβEND/hβLPH was performed using antiserum R2489, which was raised in a rabbit injected intradermally with partially purified hβLPH prepared in our laboratories. Synthetic hβEND (Bachem, Inc., Torrance, Calif.) was used both for radioiodination and as standard. Iodination was performed as previously described (34), and the 125I-labeled tracer was repurified by Sephadex G-50 Fine gel exclusion chromatography before each assay. Incubation was carried out for 3 d at 4°C, and tracer was added at the beginning of incubation. Specificity studies were performed with synthetic hβEND, synthetic hACTH (Ciba-Geigy), synthetic αENK (kindly provided by R. Guillemin), and hβLPH and hyLPH (our preparations).

**Plasma samples.** Blood was collected in cold tubes containing EDTA (15 mg EDTA/10 ml of blood), plasma was prepared, and 2-ml aliquots of plasma were stored at −70°C until they were extracted with silicic acid (34). The extracts were lyophilized and reconstituted in buffer for RIA and/or gel exclusion chromatography. For each RIA, hormone-free plasma specimens (outdated blood bank plasma that had been preextracted with silicic acid) containing known amounts of added hβLPH, hyLPH, and hβEND were similarly extracted and were used to construct the standard curves and correct for losses during extraction. All results were expressed in femtomoles of immunoreactive peptide per milliliter plasma.

**Sephadex G-50 gel exclusion chromatography**

A 0.9 × 60-cm column was packed with Sephadex G-50 Fine gel which was equilibrated and developed at 4°C with RIA standard diluent. Samples of 0.8 ml were applied and eluted at a flow rate of 20 ml/h (descending flow, 50 cm hydrostatic pressure); 1-ml fractions were collected. The column was calibrated with BSA as a void volume marker (V0); unlabeled hβLPH, hyLPH, and hβEND (each measured by RIA); and NaCl as a total volume marker (V1). BSA and NaCl were added to each sample to determine the fractional elution volumes (Kd) of the immunoreactive materials for each run. Fractions eluted from the column were directly analyzed in both RIA.

**Normal subjects and patients**

Normal values were determined in 18 healthy volunteers (10 females, 8 males) whose blood was collected between 0800 and 0900.

20 male patients who were undergoing hemodialysis for chronic renal failure were studied after giving informed consent. The etiologies of the renal failure included nephrosclerosis (n = 11), chronic glomerulonephritis (n = 5), polycystic kidney disease (n = 2), and chronic pyelonephritis (n = 2); one patient was anephric. Dialysis was performed three times a week for 4 h, using four different types of dialyzers: Gambro Lundia Major (Gambro, Inc., Newport News, Va.; 14 patients); Cobe PFP 1.6 m² (Cobe Laboratories, Inc., Lakewood, Colo.; 4 patients); Vivacell 1.5 m² (B. D. Drake Willock, Div. of Becton, Dickinson & Co., Portlan, Ore.; 1 patient); and CF 1500 (Travenol Laboratories, Inc., Morton Grove, Ill.; 1 patient). None of the patients had evidence of pituitary or adrenal disease. Two patients had a history of prolonged glucocorticoid treatment, discontinued more than a year previously, for renal transplant and therapy of glomerulonephritis. Blood was withdrawn between 0730 and 0830, just before beginning dialysis.

Basal plasma samples were also obtained from seven patients with primary adrenal insufficiency (Addison’s disease), one patient with Nelson’s syndrome, and two patients with ectopic ACTH/LPH syndrome (pancreatic islet cell carcinoma and oat cell carcinoma).

After giving his informed consent, one patient with Addison’s disease had his daily cortisol maintenance therapy cautiously tapered over several days before his admission to the Vanderbilt Clinical Research Center, where the therapy was discontinued completely. The next day a slow infusion of normal saline into a forearm vein was started at 0700; cortisol hemisuccinate (Solu-Cortef; Upjohn Co., Kalamaazoo, Mich.) was then given as a bolus (1 mg/kg), followed by a continuous 3-h infusion (1 mg/kg/hr). Blood samples for hormone determinations were withdrawn every 20 min via a
purified hyLPH and hACTH, RIA. The competitive binding curves generated by highly purified hyLPH and hβLPH and by synthetic hβEND, hACTH, and αEND are shown.

cannula inserted into a vein of the opposite forearm, starting 20 min before the bolus injection of cortisol.

RESULTS

RIA

hyLPH RIA. Antiserum R1547 bound 35% of labeled (37-58)hyLPH at a final dilution of 1:6,000. Significant displacement of tracer (B/B₀ < 0.90) was usually obtained with 3.5 fmol of added unlabeled hyLPH per tube (Fig. 1). Purified hβLPH showed 1% cross-reaction on a molar basis; no cross-reaction was observed with either hβEND or hACTH (5,000 fmol/tube).

hβEND/hβLPH RIA. Antiserum R2489 bound 30% of labeled hβEND at a final dilution of 1:18,000. Significant displacement of tracer was usually seen with 0.8 fmol added unlabeled hβEND/tube (Fig. 1). Purified hβLPH cross-reacted on an equimolar basis. Purified hyLPH showed 0.5% cross-reactivity, presumably on the basis of minor contamination with hβLPH or hβEND, inasmuch as it shares no common sequence with hβEND, and synthetic αEND and hACTH demonstrated no cross-reactivity (2,000 fmol/tube).

Plasma samples. Recoveries of added standard hormones extracted from hormone-free plasma were similar: 70.7±3.8% (mean±SEM) for hβLPH (n = 6), 68.2±2.7% for hyLPH (n = 9), and 76.3±2.1% for hβEND (n = 8); recovery of each peptide was constant over concentrations ranging from 8 to 2,000 fmol/ml plasma. To avoid any possible variation in extraction recoveries (35), the volume of plasma extracted was kept constant at 2 ml. Plasma samples extracted in this manner caused no damage to 125I-labeled tracers as assessed either by QUSO (QUSO G-32, Philadelphia Quartz Co., Philadelphia, Pa.) or excess first antibody in either RIA (36). Plasma values were calculated as femtomoles IR-peptide per milliliter after correcting for extraction recoveries; the sensitivity was 12.5 fmol/ml plasma for the hyLPH RIA and 3 fmol/ml plasma for the hβEND/hβLPH RIA.

Plasma IR-hyLPH and IR-hβEND/hβLPH in normal subjects and hemodialysis patients

Basal plasma IR-hyLPH in 16 normal volunteers was undetectable (<12.5 fmol/ml) in 11 and ranged up to 21 fmol/ml in 5 others, and basal plasma IR-hβEND/hβLPH was 10.8±0.7 fmol/ml in 18 normal volunteers (Fig. 2).

Plasma IR-hyLPH was markedly increased in hemodialysis patients (101.5±12.7 fmol/ml) with almost no overlap with normal subjects (Fig. 2). Plasma IR-hβEND/hβLPH (23.8±2.0 fmol/ml) was also significantly increased (P < 0.001) in these patients when compared with that of normal subjects; however, 6 of the 20 dialysis patients had plasma IR-hβEND/hβLPH values that fell within the range of our normal subjects. No significant difference in either IR hormone was found according to the type of dialysis membrane used. A significant correlation (r = 0.592, P < 0.01) existed.
between plasma IR-hyLPH and the duration of chronic hemodialysis, but no such correlation was found for IR-hβEND/hβLPH.

Sephadex G-50 gel exclusion chromatography of extracted plasma from four hemodialysis patients

Most of the IR-hyLPH in the plasma extracts of four hemodialysis patients appeared in a single large peak that coeluted with standard hyLPH (Fig. 3). In two patients (Fig. 3C, D) some IR-hyLPH was observed in the void volume; the significance of this apparent HMW IR-hyLPH remains to be determined.

The IR-hβEND/hβLPH in the plasma extracts of the same patients appeared in a single small peak coeluting with standard hβLPH; no distinct peak eluting at the position of hβEND was observed (Fig. 3). Since the recoveries of IR-hyLPH and IR-hβEND/hβLPH were similar, both for the extraction procedure and from the Sephadex G-50 column, hyLPH predominated on a molar basis in the plasma of these hemodialysis patients (Fig. 3).

In 20 hemodialysis patients who had simultaneous IR-hyLPH and IR-hβEND/hβLPH determinations, a significant correlation was found between plasma IR-hyLPH and IR-hβEND/hβLPH (Fig. 4).

Sephadex G-50 gel exclusion chromatography of plasma from patients with ACTH/LPH hypersecretion

Plasma extracts from three patients with Addison's disease and one patient with Nelson's syndrome, and unextracted plasma from one patient with the ectopic ACTH/LPH syndrome, were subjected to gel exclusion chromatography, and each eluate fraction was subjected to both RIA. In each case, IR-hyLPH appeared as one peak coeluting with standard hyLPH, and IR-hβEND/hβLPH appeared as two major peaks coeluting with standard hβLPH and hβEND, respectively (Fig. 5).

Correlations between plasma hβLPH, hyLPH, and hβEND in conditions of ACTH/LPH hypersecretion

By integrating the amount of IR-hyLPH and IR-hβEND/hβLPH under each gel chromatography peak in Fig. 5 and in chromatograms from four additional patients with Addison's disease and one with ectopic ACTH syndrome (data not shown), using the same molar scale, correcting for recovery of each, and know-

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**FIGURE 3** Sephadex G-50 Fine gel exclusion chromatography of extracted plasma from hemodialysis patients. Plasmas from a basal blood collection from each of four hemodialysis patients (A–D) were extracted, lyophilized, reconstituted in RIA standard diluent, and subjected to gel exclusion chromatography. Each eluate fraction was assayed in both the hyLPH (•) and the hβEND/hβLPH (▲) RIA. The overall (plasma extraction plus column chromatography) recoveries of IR-hyLPH and IR-hβEND/hβLPH from the plasma of the four patients were similar, in the range of 48–60% and 36–56%, respectively. Calibration of the column with BSA (V₀), unlabeled highly purified hyLPH and hβLPH or synthetic hβEND, and NaCl(V₆) is indicated. Open symbols indicate nondetectable IR-peptide at the concentration plotted.

**FIGURE 4** Correlations between IR-hβLPH and IR-hβEND/hβLPH determined simultaneously in the plasma of 20 hemodialysis patients. The broken line represents equimolarity.
ADDISON'S DISEASE

NELSON'S SYNDROME

ECTOPIC ACTH/LPH SYNDROME

FRACTIONAL ELUTION VOLUME (Kd)

FIGURE 5 Sephadex G-50 Fine gel exclusion chromatography of extracted and unextracted plasma from patients with ACTH/LPH hypersecretion. Extracted plasma samples from three patients with Addison's disease and one with Nelson's syndrome and unextracted plasma from a patient with the ectopic ACTH/LPH syndrome were subjected to gel exclusion chromatography. Each eluate fraction was assayed in both the hyLPH RIA (●) and the hβEND/hβLPH RIA (▲). The overall (plasma extraction plus column chromatography) recoveries of IR-hyLPH and IR-hβEND/hβLPH ranged from 58 to 76% and 57 to 100%, respectively. Open symbols indicate nondetectable IR-peptide at the concentration plotted.

ing the simultaneous concentrations of the IR peptides in the original plasma sample, it was possible to calculate the molar concentrations of hβLPH, hyLPH, and hβEND in plasma obtained from patients with ACTH/LPH hypersecretion from various causes. The molar concentrations of plasma hβLPH and hyLPH were significantly correlated, and hyLPH concentrations equaled or exceeded those of hβLPH in all specimens (Fig. 6A). Furthermore, the plasma concentration of hβEND correlated significantly with, but was approximately one-third that of hyLPH on a molar basis (Fig. 6B). It follows that plasma hβEND concentration
was also correlated significantly with that of hβLPH ($r = 0.998, P < 0.001$), but was about one-third as high (data not plotted).

**Plasma disappearance rates of hβLPH, hyLPH, and hβEND in a patient with Addison’s disease**

The acute rise of plasma cortisol from 2 to 200 µg/dl during cortisol infusion in this untreated Addison’s patient induced a sudden, rapid fall of both IR-hyLPH and IR-hβEND/hβLPH, with similar biphasic disappearance curves (Fig. 7). The initial and subsequent half-lives were 80 and 170 min for IR-hyLPH and 100 and 180 min for IR-hβEND/hβLPH, respectively. To evaluate the relative plasma disappearance rates of all three peptides, three plasma samples obtained at zero time and after 100 and 180 min of cortisol infusion were subjected to Sephadex G-50 gel exclusion chromatography, and the concentrations of hβLPH, hyLPH, and hβEND in the three samples were calculated in the manner just described. The plasma disappearance rates of hβLPH and hyLPH were similar, whereas hβEND disappeared much more rapidly (Fig. 8).

**DISCUSSION**

It is now generally accepted that hβLPH and/or hyLPH circulate in human blood under normal and abnormal conditions and are responsible for overall plasma “hβMSH” immunoreactivity (15–21). Recent results with hLPH RIA that use antisera that do not cross-react with “hβMSH” (35, 37–39) have confirmed observations previously made with “hβMSH” RIA, but have shown that the “hβMSH” was actually the hLPH, corroborating the concept of Scott and Lowry (1). However, considerable ambiguity persists concerning which of the two lipotropins these hLPH RIA are actually measuring; some authors do not address the question (39), others acknowledge the complete cross-reactivity of hyLPH in their “hβLPH” RIA, but do not attempt to differentiate the two hormones (38), and still others describe a “specific radioimmunoassay for human β-lipotropin,” when there is equimolar cross-reactivity with hyLPH in the most sensitive portion of the assay standard curve, and 10–60% cross-reactivity in the remainder (35). We have demonstrated that antisera used in previous “hβMSH” RIA have variable cross-reactivity with the two LPH (40). Tanaka et al. (11) concluded that both hβLPH and hyLPH were present in human plasma and tissue extracts by hLPH RIA of gel exclusion chromatography eluate fractions. Thus, it was both important and feasible to explore the question of the relative concentrations of the two LPH in human plasma and the possible correlation with hβEND concentrations.

The hβEND/hβLPH RIA uses an antiserum that cross-reacts on an equimolar basis with both hβEND and hβLPH. Thus, this RIA is actually a COOH-terminal hβLPH RIA, as is probably the case with most other “hβEND” RIA thus far described. RIA for hβEND that do not cross-react with hβLPH are probably

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**Figure 6** Correlations between plasma IR-hyLPH and IR-hβLPH (A) or IR-hβEND (B) in patients with ACTH/LPH hypersecretion. Plasma samples were obtained from patients with Addison’s disease (●), Nelson’s syndrome (○), and the ectopic ACTH/LPH syndrome (▲), and subjected to Sephadex G-50. Fine gel exclusion chromatography. The actual amounts of hβLPH, hyLPH, and hβEND were calculated by integrating the area under each peak of IR-peptide and correcting for overall recovery of immunoreactivity. Concentrations of plasma IR-hβLPH and IR-hyLPH (A) and IR-hβEND and IR-hyLPH (B) are plotted on identical logarithmic scales. The broken lines represent equimolarity.
NH$_2$-terminal h$\beta$END RIA that may measure Met$^k$-enkephalin, $\alpha$-endorphin, $\gamma$-endorphin, or other metabolites in plasma or tissue (40). The term "IR-h$\beta$END/h$\beta$LPH" has been used in this study, recognizing the fact that either h$\beta$END, h$\beta$LPH, or both may contribute to the total immunoreactivity in any one sample.

In normal controls, plasma IR-hyLPH was undetectable in 11 of 16 volunteers and was 21 fmol/ml or less in 5 others. Although direct comparison of hyLPH levels to h$\beta$END/h$\beta$LPH in normal subjects is not possible from these data, it is clear that the ranges of their concentrations overlap (Fig. 2), and it is probable that their mean concentrations are, therefore, similar. Gilkes et al. (8), using antiserum NZ, which cross-reacts equally with (37–58)hyLPH and hyLPH, but only 3% as well with h$\beta$LPH, reported plasma IR-(37–58)hyLPH in normal controls in the range of 25 pg/ml. Since the NZ antiserum presumably was measuring only hyLPH, there being no (37–58)hyLPH ("h$\beta$MSH") in normal human plasma, this would correspond to about 10 fmol/ml of IR-hyLPH, a value compatible with our findings.

We also detected IR-h$\beta$END/h$\beta$LPH in the plasma of normal subjects; others (22–24, 26) have found that h$\beta$LPH represents 69–94% of total "IR-h$\beta$END" in the plasma of normal subjects under basal conditions. Therefore, the mean IR-h$\beta$END/h$\beta$LPH value of 11 fmol/ml in our normal subjects probably represents mainly h$\beta$LPH and yields a calculated h$\beta$LPH concentration (0.7–3.4 fmol/ml) that is consistent with those reported by others (22–26).

Although several investigators have reported increased plasma "IR-h$\beta$MSH" in hemodialysis patients (30–32) that behaves as HMW material (32) and reacts in a partially specific "h$\beta$LPH" RIA (35), the relative contributions of hyLPH and h$\beta$LPH to total "IR-h$\beta$MSH" in the plasma of these patients have remained unknown. Our data demonstrate that IR-hyLPH is greatly increased in their plasma and coelutes with standard hyLPH. IR-h$\beta$END/h$\beta$LPH is also increased significantly, and a significant correlation exists between IR-h$\beta$END/h$\beta$LPH and IR-hyLPH in the plasma of hemodialysis patients, with hyLPH clearly predominant. The lack of a detectable h$\beta$END peak after gel chromatography indicates that h$\beta$LPH itself contributes the vast majority of total plasma IR-h$\beta$END/h$\beta$LPH in these patients. The fact that we could not detect h$\beta$END in the plasma of hemodialysis patients does not necessarily mean that it was not present, because the limit of detection in our com-
The observed disappearance rates of endogenous hβLPH and hyLPH were similar to those of hLPH previously reported by several other investigators (32, 46, 47) using different RIA, but somewhat longer than that reported for both exogenous hβLPH and endogenous hLPH by authors (37, 48) using an NH₂-terminal lLPH antiserum. It should be noted that apparent disappearance half-times of polypeptides are strictly dependent upon the sequence of amino acids with which the particular RIA antiserum reacts (49), a subject we have discussed previously (34); the disappearance rate of bioactive LPH from plasma has not yet been measured. The observed plasma disappearance rate of endogenous IR-hβEND was apparently faster than that of either lipotropin, at least potentially explaining its relatively lower molar concentration.

Both hyLPH (1) and hβEND (50–52) are found in the human pituitary, but the hβEND/hLPH molar ratio has been reported to be very low (53). Human βLPH is not degraded to hyLPH or "hβMSH" during the process of blood collection, plasma separation, silicic acid extraction, and gel chromatography (11), and hβLPH injected into normal human subjects is not converted to hβEND (26). However, the possibility that hyLPH and/or hβEND are produced by peripheral metabolism of hβLPH after it is secreted into the circulating blood has not been explored in the present study. Thus, the origin of hyLPH and hβEND found in the peripheral blood of normal subjects remains unknown. In patients with ACTH hypersecretion from a variety of causes, there also exists the possibility that the processing of the common precursor is altered, with relatively increased secretion of hyLPH and/or hβEND.

This study demonstrates that hβLPH, hyLPH, and hβEND are all present simultaneously in the circulating blood, and suggests that their relative ratios in various conditions may depend upon either their relative rates of secretion by the pituitary, their peripheral metabolism and disposition, or both. In light of rapidly increasing information about pituitary biosynthesis and secretion and plasma concentrations of these three hormones, it is interesting to note that virtually nothing is known about their physiologic roles in circulating blood.

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