

# Simultaneous Assay of Immunoreactive $\beta$ -Lipotropin, $\gamma$ -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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## Research Article

We have studied the relative concentrations of the human immunoreactive (IR) peptides  $\gamma$ -lipotropin (h $\gamma$ LPH, [1-58]h $\beta$ LPH),  $\beta$ -lipotropin (h $\beta$ LPH), and  $\beta$ -endorphin (h $\beta$ END, [61-91]h $\beta$ LPH) using gel exclusion chromatography together with a specific radio-immunoassay (RIA) for h $\gamma$ LPH 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-h $\gamma$ LPH was often undetectable ( $<12.5$  fmol/ml), but ranged up to 21 fmol/ml, and IR-h $\beta$ END/h $\beta$ LPH was  $10.8 \pm 0.7$  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-h $\gamma$ LPH and IR-h $\beta$ END/h $\beta$ LPH were significantly elevated ( $P < 0.001$ ) in patients undergoing chronic hemodialysis ( $101.5 \pm 12.7$  and  $23.8 \pm 2.0$  fmol/ml, respectively). Their IR-h $\gamma$ LPH coeluted with standard h $\gamma$ LPH 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-h $\gamma$ LPH 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 [...]

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# Simultaneous Assay of Immunoreactive $\beta$ -Lipotropin, $\gamma$ -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  $\gamma$ -lipotropin (h $\gamma$ LPH, [1–58]h $\beta$ LPH),  $\beta$ -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 h $\gamma$ LPH 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-h $\gamma$ LPH was often undetectable ( $<12.5$  fmol/ml), but ranged up to 21 fmol/ml, and IR-h $\beta$ END/h $\beta$ LPH was  $10.8 \pm 0.7$  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-h $\gamma$ LPH and IR-h $\beta$ END/h $\beta$ LPH were significantly elevated ( $P < 0.001$ ) in patients undergoing chronic hemodialysis ( $101.5 \pm 12.7$  and  $23.8 \pm 2.0$  fmol/ml, respectively). Their IR-h $\gamma$ LPH coeluted with standard h $\gamma$ LPH 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-h $\gamma$ LPH 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 h $\gamma$ LPH 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)<sup>1</sup> 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 pituitary extracts of several species (14–18) including man (1, 19–21); h $\beta$ LPH is a single-chain 91-amino acid peptide, and h $\gamma$ LPH is (1–58)h $\beta$ LPH; "h $\beta$ MSH" is (37–58)h $\gamma$ LPH. Thus, both h $\beta$ LPH and h $\gamma$ LPH 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

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<sup>1</sup>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 h $\beta$ LPH (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)h $\beta$ LPH fragment of h $\beta$ LPH— $\beta$ -endorphin (h $\beta$ END), the potent endogenous opiate peptide—should also be found in the circulation (11). Several authors have recently reported IR-h $\beta$ END 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 h $\beta$ LPH (29). In the present study, we have investigated the concentrations and relative plasma distributions of immunoreactive h $\beta$ LPH, hyLPH, and h $\beta$ END in normal subjects, in patients undergoing hemodialysis because of chronic renal failure, a condition that is associated with high plasma “h $\beta$ MSH” 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 Chrétien 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 h $\beta$ END, synthetic hACTH (Ciba-Geigy), and h $\beta$ LPH purified in our laboratories from fresh-frozen human pituitary glands (National Pituitary Agency) by a modification of the method of Li (14).

**h $\beta$ END/h $\beta$ LPH RIA.** The RIA for h $\beta$ END/h $\beta$ LPH was performed using antiserum R2489, which was raised in a rabbit injected intradermally with partially purified h $\beta$ LPH prepared in our laboratories. Synthetic h $\beta$ END (Bachem, Inc., Torrance, Calif.) was used both for radioiodination and as standard. Iodination was performed as previously described (34), and the <sup>125</sup>I-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 h $\beta$ END, synthetic

hACTH (Ciba-Geigy), synthetic  $\alpha$ END (kindly provided by R. Guillemin), and h $\beta$ LPH 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 h $\beta$ LPH, hyLPH, and h $\beta$ END 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 ( $V_0$ ); unlabeled h $\beta$ LPH, hyLPH, and h $\beta$ END (each measured by RIA); and NaCl as a total volume marker ( $V_t$ ). BSA and NaCl were added to each sample to determine the fractional elution volumes ( $K_d$ ) 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 PPD 1.6 m<sup>2</sup> (Cobe Laboratories, Inc., Lakewood, Colo.; 4 patients); Vivacell 1.5 m<sup>2</sup> (B. D. Drake Willock, Div. of Becton, Dickinson & Co., Portland, 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., Kalamazoo, Mich.) was then given as a bolus (1 mg/kg), followed by a continuous 3-h infusion (1 mg/kg per h). Blood samples for hormone determinations were withdrawn every 20 min via a

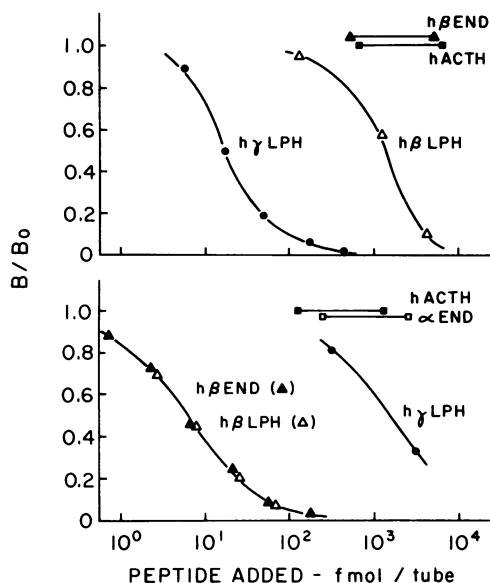


FIGURE 1 Specificities of the  $h\gamma$ LPH and  $h\beta$ END/ $h\beta$ LPH RIA. The competitive binding curves generated by highly purified  $h\gamma$ LPH and  $h\beta$ LPH and by synthetic  $h\beta$ END,  $h$ ACTH, and  $\alpha$ END are shown.

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

## RESULTS

### RIA

**$h\gamma$ LPH RIA.** Antiserum R1547 bound 35% of labeled (37–58) $h\gamma$ LPH at a final dilution of 1:6,000. Significant displacement of tracer ( $B/B_0 < 0.90$ ) was usually obtained with 3.5 fmol of added unlabeled  $h\gamma$ LPH per tube (Fig. 1). Purified  $h\beta$ LPH showed 1% cross-reaction on a molar basis; no cross-reaction was observed with either  $h\beta$ END or  $h$ ACTH (5,000 fmol/tube).

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

**Plasma samples.** Recoveries of added standard hormones extracted from hormone-free plasma were similar:  $70.7 \pm 3.8\%$  (mean  $\pm$  SEM) for  $h\beta$ LPH ( $n = 6$ ),  $68.2 \pm 2.7\%$  for  $h\gamma$ LPH ( $n = 9$ ), and  $76.3 \pm 2.1\%$  for  $h\beta$ END ( $n = 8$ ); 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  $^{125}$ I-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  $h\gamma$ LPH RIA and 3 fmol/ml plasma for the  $h\beta$ END/ $h\beta$ LPH RIA.

### Plasma IR- $h\gamma$ LPH and IR- $h\beta$ END/ $h\beta$ LPH in normal subjects and hemodialysis patients

Basal plasma IR- $h\gamma$ LPH 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- $h\beta$ END/ $h\beta$ LPH was  $10.8 \pm 0.7$  fmol/ml in 18 normal volunteers (Fig. 2).

Plasma IR- $h\gamma$ LPH was markedly increased in hemodialysis patients ( $101.5 \pm 12.7$  fmol/ml) with almost no overlap with normal subjects (Fig. 2). Plasma IR- $h\beta$ END/ $h\beta$ LPH ( $23.8 \pm 2.0$  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- $h\beta$ END/ $h\beta$ LPH 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

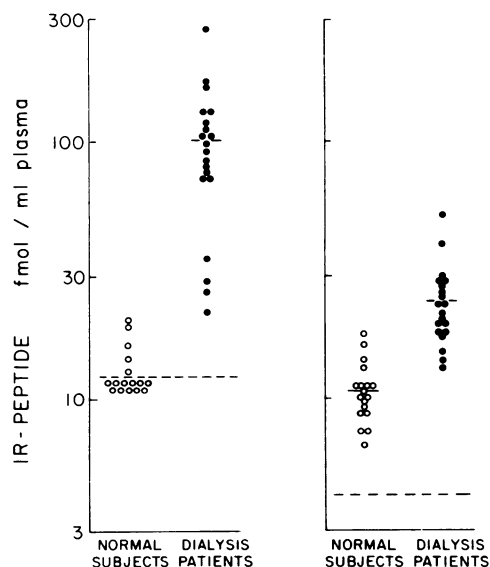
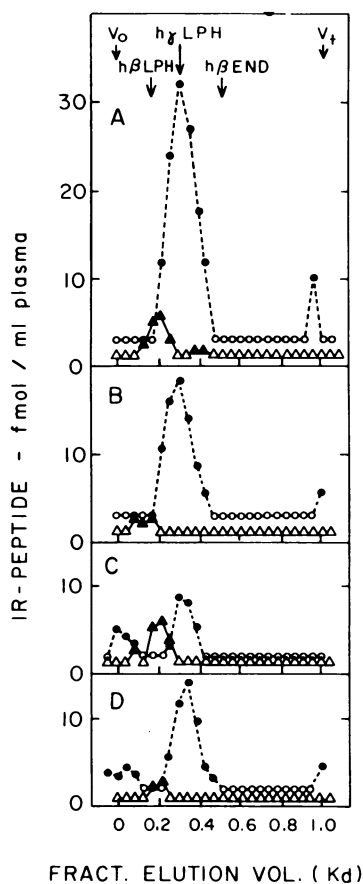


FIGURE 2 Basal morning plasma levels of IR- $h\gamma$ LPH (left) and IR- $h\beta$ END/ $h\beta$ LPH (right) in normal subjects and in hemodialysis patients. The broken lines indicate the sensitivity threshold of each assay for IR-peptide in extracted plasma; the solid lines indicate the means.

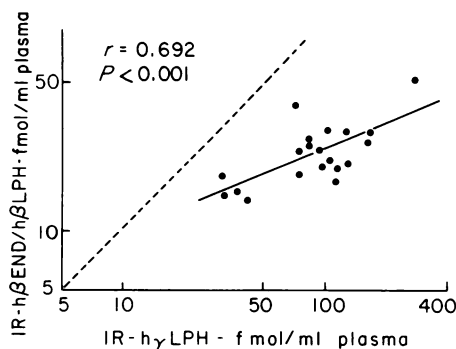
between plasma IR-hyLPH and the duration of chronic hemodialysis, but no such correlation was found for IR-h $\beta$ END/h $\beta$ LPH.

#### *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



**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 h $\beta$ END/h $\beta$ LPH (▲) RIA. The overall (plasma extraction plus column chromatography) recoveries of IR-hyLPH and IR-h $\beta$ END/h $\beta$ LPH 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 ( $V_0$ ), unlabeled highly purified hyLPH and h $\beta$ LPH or synthetic h $\beta$ END, and NaCl ( $V_t$ ) is indicated. Open symbols indicate nondetectable IR-peptide at the concentration plotted.



**FIGURE 4** Correlations between IR-h $\beta$ LPH and IR-h $\beta$ END/h $\beta$ LPH determined simultaneously in the plasma of 20 hemodialysis patients. The broken line represents equimolarity.

in the void volume; the significance of this apparent HMW IR-hyLPH remains to be determined.

The IR-h $\beta$ END/h $\beta$ LPH in the plasma extracts of the same patients appeared in a single small peak coeluting with standard h $\beta$ LPH; no distinct peak eluting at the position of h $\beta$ END was observed (Fig. 3). Since the recoveries of IR-hyLPH and IR-h $\beta$ END/h $\beta$ LPH 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-h $\beta$ END/h $\beta$ LPH determinations, a significant correlation was found between plasma IR-hyLPH and IR-h $\beta$ END/h $\beta$ LPH (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-h $\beta$ END/h $\beta$ LPH appeared as two major peaks coeluting with standard h $\beta$ LPH and h $\beta$ END, 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-h $\beta$ END/h $\beta$ LPH 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-

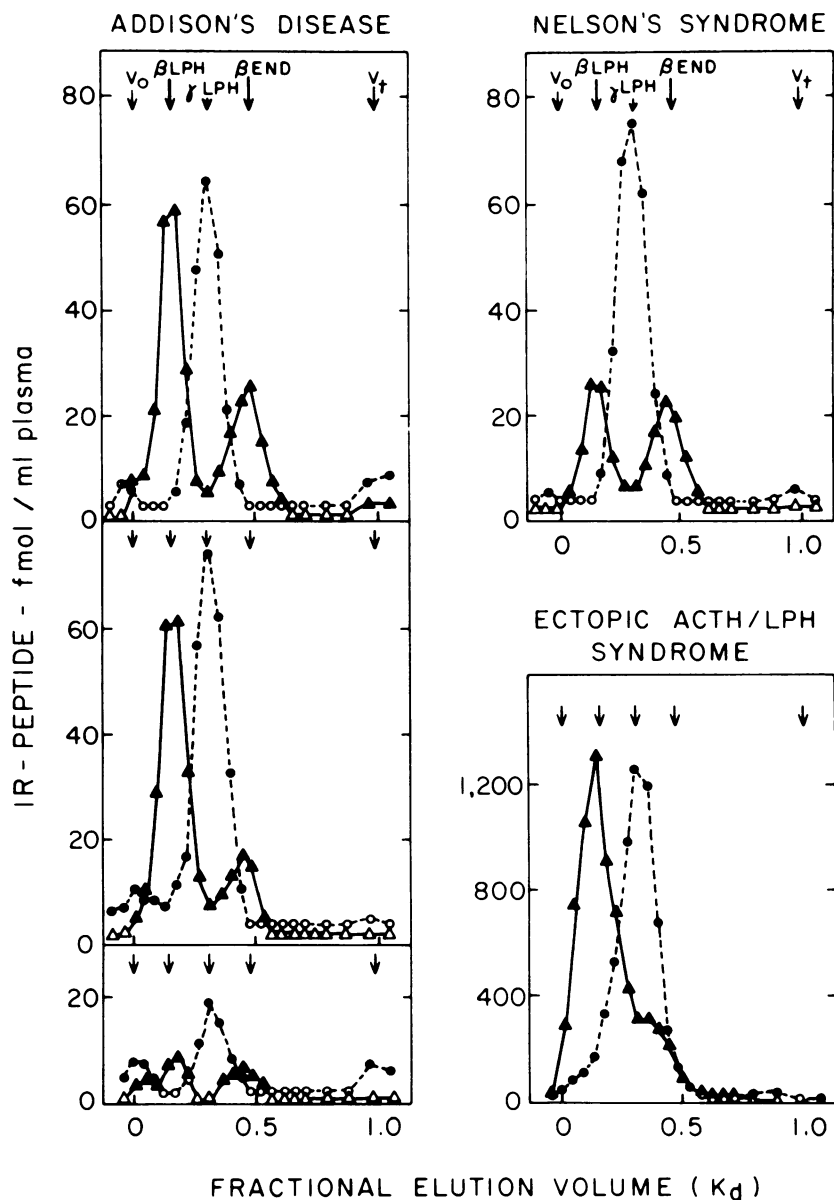


FIGURE 5 Sephadex G-50 Fine gel exclusion chromatography of extracted and unextracted plasma from patients with ACTH/LPH hypersecretion. Extracted plasma samples from three patients with Addison's disease and one with Nelson's syndrome and unextracted plasma from a patient with the ectopic ACTH/LPH syndrome were subjected to gel exclusion chromatography. Each eluate fraction was assayed in both the  $h\gamma$ LPH RIA (●) and the  $h\beta$ END/ $h\beta$ LPH RIA (▲). The overall (plasma extraction plus column chromatography) recoveries of IR- $h\gamma$ LPH and IR- $h\beta$ END/ $h\beta$ LPH 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  $h\beta$ LPH,  $h\gamma$ LPH, and  $h\beta$ END in plasma obtained from patients with ACTH/LPH hypersecretion from various causes. The molar concentrations of plasma  $h\beta$ LPH and  $h\gamma$ LPH were

significantly correlated, and  $h\gamma$ LPH concentrations equaled or exceeded those of  $h\beta$ LPH in all specimens (Fig. 6A). Furthermore, the plasma concentration of  $h\beta$ END correlated significantly with, but was approximately one-third that of  $h\gamma$ LPH on a molar basis (Fig. 6B). It follows that plasma  $h\beta$ END concentration

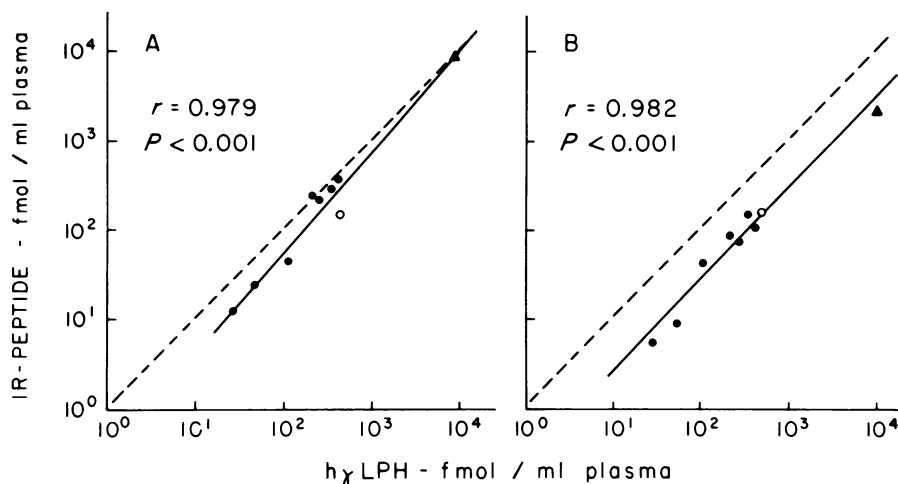


FIGURE 6 Correlations between plasma IR-h $\gamma$ LPH and IR-h $\beta$ LPH (A) or IR-h $\beta$ END (B) in patients with ACTH/LPH hypersecretion. Plasma samples were obtained from patients with Addison's disease (●), Nelson's syndrome (○), and the ectopic ACTH/LPH syndrome (▲), and subjected to Sephadex G-50 Fine gel exclusion chromatography. The actual amounts of h $\beta$ LPH, h $\gamma$ LPH, and h $\beta$ END were calculated by integrating the area under each peak of IR-peptide and correcting for overall recovery of immunoreactivity. Concentrations of plasma IR-h $\beta$ LPH and IR-h $\gamma$ LPH (A) and IR-h $\beta$ END and IR-h $\gamma$ LPH (B) are plotted on identical logarithmic scales. The broken lines represent equimolarity.

was also correlated significantly with that of h $\beta$ LPH ( $r = 0.998$ ,  $P < 0.001$ ), but was about one-third as high (data not plotted).

#### *Plasma disappearance rates of h $\beta$ LPH, h $\gamma$ LPH, and h $\beta$ END in a patient with Addison's disease*

The acute rise of plasma cortisol from 2 to 200  $\mu$ g/dl during cortisol infusion in this untreated Addison's patient induced a sudden, rapid fall of both IR-h $\gamma$ LPH and IR-h $\beta$ END/h $\beta$ LPH, with similar biphasic disappearance curves (Fig. 7). The initial and subsequent half-lives were 80 and 170 min for IR-h $\gamma$ LPH and 100 and 180 min for IR-h $\beta$ END/h $\beta$ LPH, 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 h $\beta$ LPH, h $\gamma$ LPH, and h $\beta$ END in the three samples were calculated in the manner just described. The plasma disappearance rates of h $\beta$ LPH and h $\gamma$ LPH were similar, whereas h $\beta$ END disappeared much more rapidly (Fig. 8).

## DISCUSSION

It is now generally accepted that h $\beta$ LPH and/or h $\gamma$ LPH circulate in human blood under normal and abnormal conditions and are responsible for overall plasma "h $\beta$ MSH" immunoreactivity (15–21). Recent results

with hLPH RIA that use antisera that do not cross-react with "h $\beta$ MSH" (35, 37–39) have confirmed observations previously made with "h $\beta$ MSH" RIA, but have shown that the "h $\beta$ MSH" 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 h $\gamma$ LPH in their "h $\beta$ LPH" RIA, but do not attempt to differentiate the two hormones (38), and still others describe a "specific radioimmunoassay for human  $\beta$ -lipotropin," when there is equimolar cross-reactivity with h $\gamma$ LPH 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 "h $\beta$ MSH" RIA have variable cross-reactivity with the two LPH (40). Tanaka et al. (11) concluded that both h $\beta$ LPH and h $\gamma$ LPH 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 h $\beta$ END concentrations.

The h $\beta$ END/h $\beta$ LPH RIA uses an antiserum that cross-reacts on an equimolar basis with both h $\beta$ END and h $\beta$ LPH. Thus, this RIA is actually a COOH-terminal h $\beta$ LPH RIA, as is probably the case with most other "h $\beta$ END" RIA thus far described. RIA for h $\beta$ END that do not cross-react with h $\beta$ LPH are probably

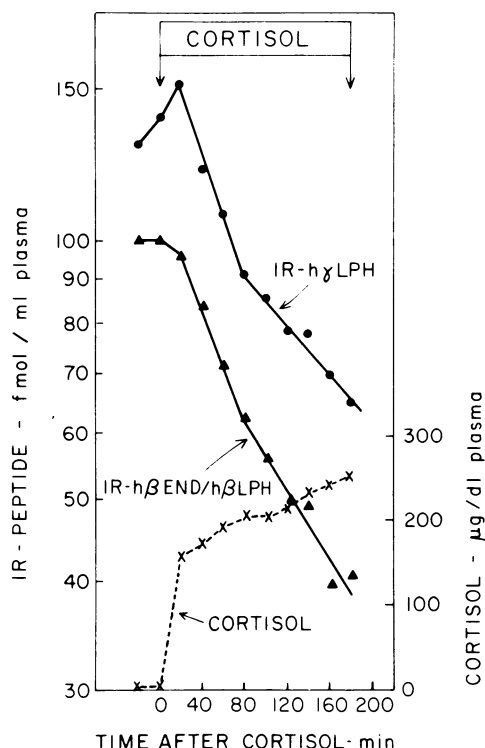


FIGURE 7 Plasma disappearance of IR-hyLPH and IR-h $\beta$ END/h $\beta$ LPH in a patient with Addison's disease who received cortisol hemisuccinate as an intravenous bolus (1 mg/kg) followed by a continuous intravenous infusion (1 mg/kg per h) for 3 h. The patient's IR-hyLPH and IR-h $\beta$ END/h $\beta$ LPH plasma levels had been elevated by careful prior withdrawal of glucocorticoid replacement therapy. Plasma IR-peptide concentrations are plotted on a logarithmic scale; plasma cortisol concentration is plotted on an arithmetic scale.

NH<sub>2</sub>-terminal h $\beta$ END RIA that may measure Met<sup>5</sup>-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 $\beta$ END 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-

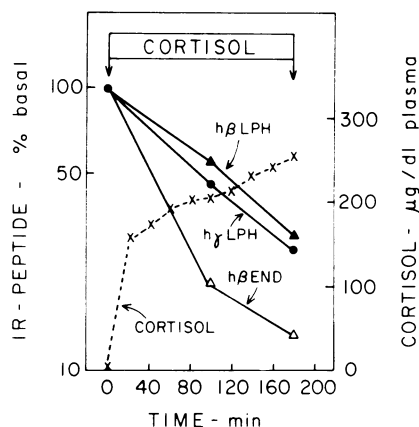


FIGURE 8 Plasma disappearance of endogenous h $\beta$ LPH, hyLPH, and h $\beta$ END in the patient with Addison's disease. Plasma samples were obtained before and after 100 and 180 min of cortisol infusion (Fig. 7) and subjected to Sephadex G-50 Fine gel exclusion chromatography. The actual amounts of h $\beta$ LPH, hyLPH, and h $\beta$ END were calculated by integrating the area under each peak of IR-peptide and correcting for overall recovery of immunoreactivity. Percent basal IR-peptide concentration is plotted on a logarithmic scale; plasma cortisol concentration is plotted on an arithmetic scale.



bined extraction/chromatography system was ~4 fmol/ml plasma, a level higher than most basal plasma values reported for h $\beta$ END in normal subjects (22–26). Thus, our data do not exclude the possibility that h $\beta$ END is present in the plasma of hemodialysis patients, perhaps even at levels slightly higher than those in most normal subjects. It has been reported that parathyroid hormone fragments accumulate in the blood of patients with chronic renal failure (41). By analogy, one might speculate that hyLPH, or very closely related peptides that are biosynthetic or peripheral metabolic products of h $\beta$ LPH, tend to accumulate in the plasma of patients with chronic renal failure because of relatively slow clearance rates, hence their relative predominance. However, whether the kidneys participate in the peripheral metabolism of the lipotropins is not yet known.

Patients with ACTH hypersecretion of various causes (e.g., Addison's disease, Nelson's syndrome, or ectopic ACTH syndrome) have high plasma levels of IR-hLPH (11, 37, 39) and IR-h $\beta$ END (24–26, 42). To establish further the relative distribution of h $\beta$ LPH, hyLPH, and h $\beta$ END in the plasma of such patients, we subjected plasma specimens to gel exclusion chromatography. The h $\beta$ END/h $\beta$ LPH RIA recognizes h $\beta$ LPH and h $\beta$ END equally on a molar basis, permitting a direct comparison of concentrations of the peptides. The concentrations of h $\beta$ END correlated well with those of hyLPH, but were approximately one-third as high. If these two hormones are produced concomitantly in man, the lower levels of immunoreactive h $\beta$ END must be due to a faster plasma disappearance rate, as would be expected from its faster disappearance during cortisol infusion in a patient with Addison's disease. The levels of h $\beta$ LPH and hyLPH were almost equal in the plasma of these patients. The gel exclusion chromatography data further validated the specificity of the hyLPH RIA for, in these plasma specimens containing high levels of both h $\beta$ LPH and h $\beta$ END, only a single peak of IR-hyLPH was detected, and that co-eluted with standard hyLPH.

$\beta$ LPH was demonstrated to be the biosynthetic precursor of  $\beta$ END in the rat pituitary (43) and the mouse pituitary tumor cell line AtT-20/D-16v (44). Since a similar biosynthetic pathway presumably exists in man, one would anticipate that enzymatic processing of the  $\beta$ LPH precursor would result in concomitant release of  $\beta$ END and  $\gamma$ LPH, just as equimolar quantities of insulin and C-peptide are released as the result of intracellular processing of proinsulin (45). However, the faster plasma disappearance rate of insulin results in a C-peptide-to-insulin ratio > 1.0 in peripheral venous blood (45). To examine whether a similar phenomenon could explain the observed hyLPH-to-h $\beta$ END ratio in plasma, the disappearance rate of each peptide was determined in a patient with Addison's disease.

The observed disappearance rates of endogenous h $\beta$ LPH 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 h $\beta$ LPH and endogenous hLPH by authors (37, 48) using an NH<sub>2</sub>-terminal hLPH 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-h $\beta$ END was apparently faster than that of either lipotropin, at least potentially explaining its relatively lower molar concentration.

Both hyLPH (1) and h $\beta$ END (50–52) are found in the human pituitary, but the h $\beta$ END/hLPH molar ratio has been reported to be very low (53). Human  $\beta$ LPH is not degraded to hyLPH or "h $\beta$ MSH" during the process of blood collection, plasma separation, silicic acid extraction, and gel chromatography (11), and h $\beta$ LPH injected into normal human subjects is not converted to h $\beta$ END (26). However, the possibility that hyLPH and/or h $\beta$ END are produced by peripheral metabolism of h $\beta$ LPH after it is secreted into the circulating blood has not been explored in the present study. Thus, the origin of hyLPH and h $\beta$ END 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 h $\beta$ END.

This study demonstrates that h $\beta$ LPH, hyLPH, and h $\beta$ END 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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