Simultaneous Assay of Immunoreactive β-Lipotropin, γ-Lipotropin, and β-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 (hγLPH, [1–58]hβLPH), β-lipotropin (hβLPH), and β-endorphin (hβEND, [61–91]hβLPH) using gel exclusion chromatography together with a specific radioimmunoassay (RIA) for hγLPH and a RIA that (because hβEND is the COOH-terminus of the hβLPH molecule) measures both hβEND and hβLPH on an equimolar basis. In normal subjects, basal plasma IR-hγLPH was often undetectable (<12.5 fmol/ml), but ranged up to 21 fmol/ml, and IR-hβEND/hβLPH was 10.8±0.7 fmol/ml; previous studies by others suggest that most of the IR-hβEND/hβLPH was probably hβLPH. Both IR-hγLPH and IR-hβEND/hβLPH 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-hγLPH coeluted with standard hγLPH as a single peak, and IR-hβEND/hβLPH coeluted with hβLPH; no distinct peak of IR-hβEND was observed. In patients with ACTH/LPH hypersecretion due to Addison's disease, Nelson's syndrome, or ectopic ACTH syndrome, IR-hγLPH and IR-hβEND/hβLPH were both elevated, and IR-hβEND/hβLPH eluted as two peaks, one coeluting with hβLPH and the other with hβEND. The molar concentrations of all three peptides were significantly correlated with one another. The lower concentrations of endogenous IR-hβEND 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 hβLPH and hγLPH in plasma of patients with chronic renal failure suggest that the kidney may be an important organ for their metabolism.

INTRODUCTION

The human β-melanocyte-stimulating hormone (hβMSH)[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 "hβMSH" in their structures (1–13). The LPH have been isolated from putuitary extracts of several species (14–18) including man (1, 19–21); hβLPH is a single-chain 91-amino acid peptide, and hγLPH is (1–58)hβLPH; "hβMSH" is (37–58)hγLPH. Thus, both hβLPH and hγLPH cross-react in most "hβMSH" radioimmunoassays (RIA) (2–13). Indeed, several investigators have reported that immunoreactive (IR) "hβMSH" in human tissues and plasma is associated with high molecular weight (HMW) substances, which were thought to be hβLPH

These studies were reported in preliminary form at XIIth Acta Endocrinologica Congress, Munich, Germany, 26–30 June 1979.

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Received for publication 11 February 1980 and in revised form 17 September 1980.

[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 hγLPH (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 hγLPH alone or both hγLPH and hβLPH (11, 13). However, the relative concentrations of the two LPH in plasma have not been studied in a systematic manner.

If hγLPH 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 hγLPH, but not hβLPH (29). In the present study, we have investigated the concentrations and relative plasma distributions of immunoreactive hβLPH, hγLPH, 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

hγLPH RIA. The hγLPH RIA was performed as described (29), using antiserum R1547 raised in a rabbit injected with synthetic (37–58)hγLPH (generously provided by Ciba-Geigy, Ltd., Basel, Switzerland) conjugated to bovine serum albumin (BSA) by the glutaraldehyde reaction (33). Purified hγLPH 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)hγLPH 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 ^{125}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 hβEND, synthetic hACTH (Ciba-Geigy), synthetic αEND (kindly provided by R. Guillemin), and hβLPH and hγLPH (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, hγLPH, 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 (V_0); unlabeled hβLPH, hγLPH, and hβEND (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^2 (Cobe Laboratories, Inc., Lakewood, Colo.; 4 patients); Vivacell 1.5 m^2 (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

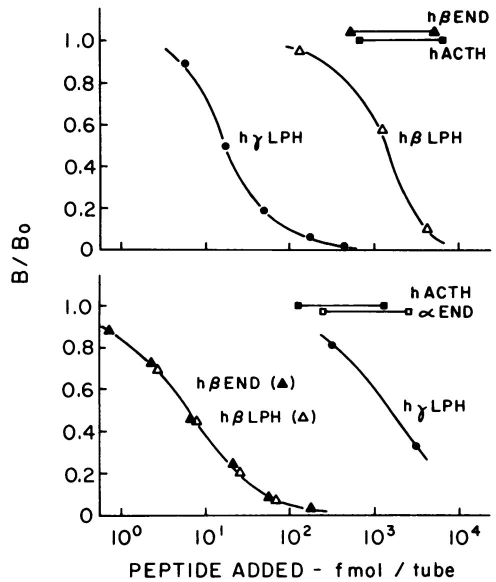

FIGURE 1 Specificities of the hγLPH and hβEND/hβLPH RIA. The competitive binding curves generated by highly purified hγLPH and hβLPH and by synthetic hβEND, hACTH, and αEND are shown.

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

RESULTS

RIA

hγLPH RIA. Antiserum R1547 bound 35% of labeled (37–58)hγLPH 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 hγLPH 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 hγLPH 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 hγLPH ($n = 9$), 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 ^{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 hγLPH RIA and 3 fmol/ml plasma for the hβEND/hβLPH RIA.

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

Basal plasma IR-hγLPH 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-hγLPH 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

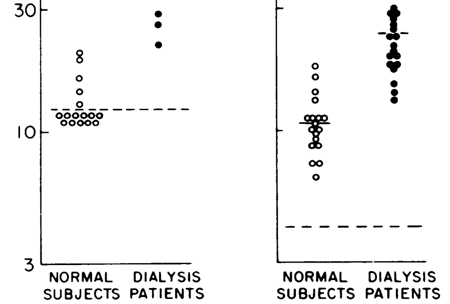

FIGURE 2 Basal morning plasma levels of IR-hγLPH (left) and IR-hβEND/hβLPH (right) in normal subjects and in hemodialysis patients. The broken lines indicate the sensitivity threshold of each assay for IR-peptide in extracted plasma; the solid lines indicate the means.

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

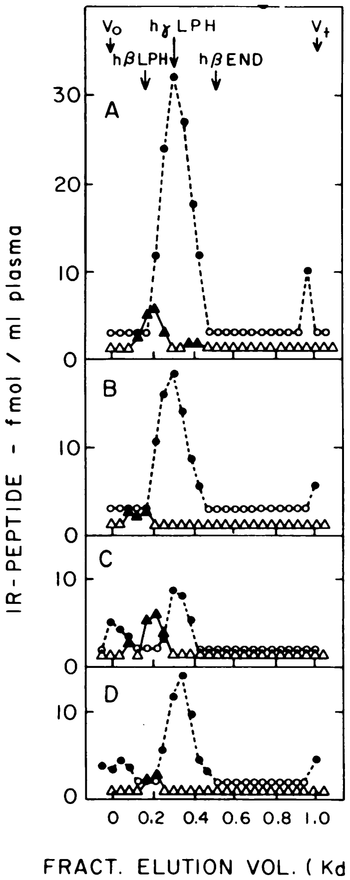

FIGURE 3 Sephadex G-50 Fine gel exclusion chromatography of extracted plasma from hemodialysis patients. Plasmas from a basal blood collection from each of four hemodialysis patients (A–D) were extracted, lyophilized, reconstituted in RIA standard diluent, and subjected to gel exclusion chromatography. Each eluate fraction was assayed in both the hγLPH (●) and the hβEND/hβLPH (▲) RIA. The overall (plasma extraction plus column chromatography) recoveries of IR-hγLPH 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 (V_0), unlabeled highly purified hγLPH and hβLPH or synthetic hβEND, and NaCl(V_t) is indicated. Open symbols indicate nondetectable IR-peptide at the concentration plotted.

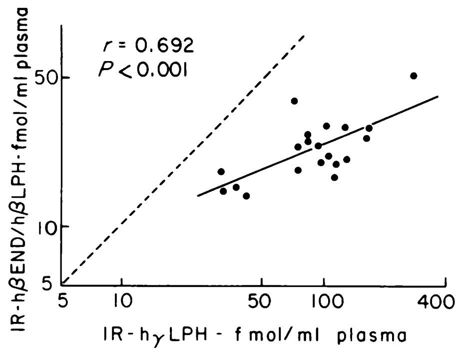

FIGURE 4 Correlations between IR-hβLPH and IR-hβEND/hβLPH determined simultaneously in the plasma of 20 hemodialysis patients. The broken line represents equimolarity.

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-hγLPH and IR-hβEND/hβLPH were similar, both for the extraction procedure and from the Sephadex G-50 column, hγLPH predominated on a molar basis in the plasma of these hemodialysis patients (Fig. 3).

In 20 hemodialysis patients who had simultaneous IR-hγLPH and IR-hβEND/hβLPH determinations, a significant correlation was found between plasma IR-hγLPH 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-hγLPH appeared as one peak coeluting with standard hγLPH, and IR-hβEND/hβLPH appeared as two major peaks coeluting with standard hβLPH and hβEND, respectively (Fig. 5).

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

By integrating the amount of IR-hγLPH 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-

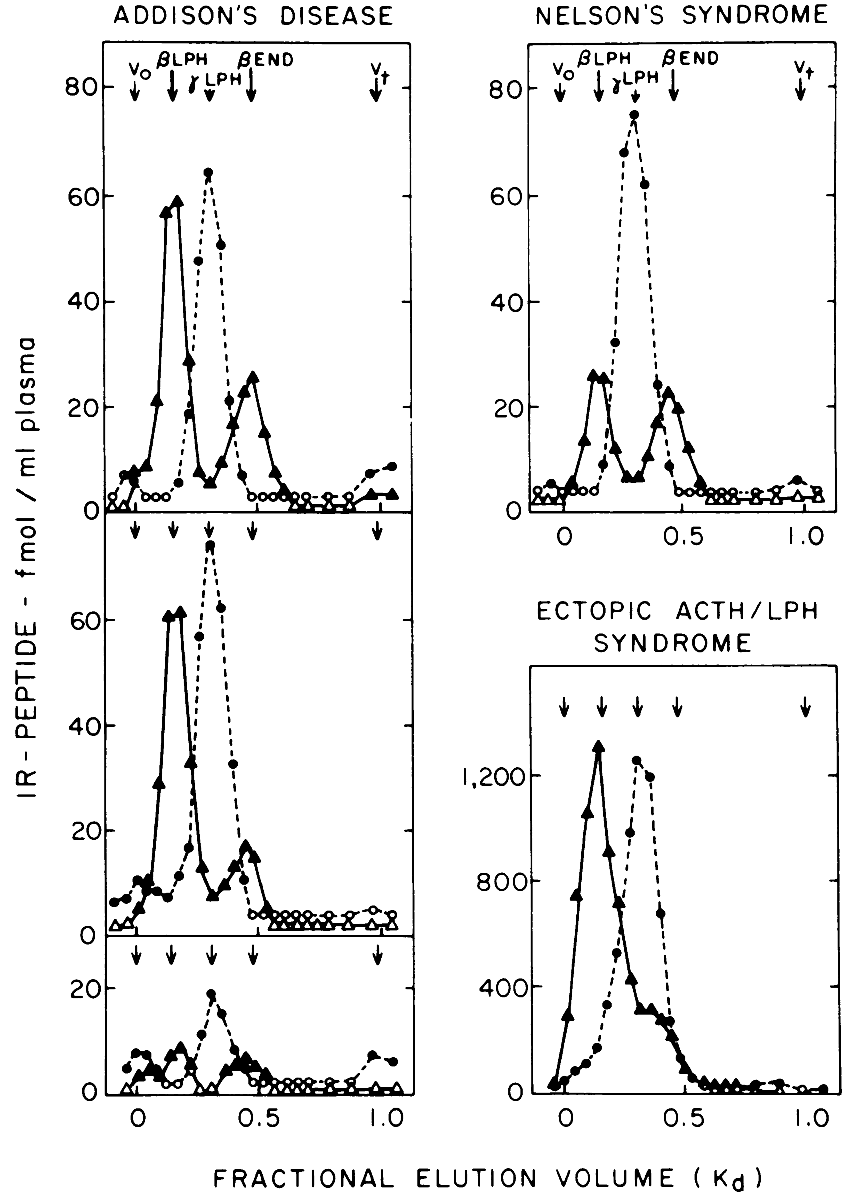

FIGURE 5 Sephadex G-50 Fine gel exclusion chromatography of extracted and unextracted plasma from patients with ACTH/LPH hypersecretion. Extracted plasma samples from three patients with Addison's disease and one with Nelson's syndrome and unextracted plasma from a patient with the ectopic ACTH/LPH syndrome were subjected to gel exclusion chromatography. Each eluate fraction was assayed in both the hγLPH RIA (●) and the hβEND/hβLPH RIA (▲). The overall (plasma extraction plus column chromatography) recoveries of IR-hγLPH 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, hγLPH, and hβEND in plasma obtained from patients with ACTH/LPH hypersecretion from various causes. The molar concentrations of plasma hβLPH and hγLPH were significantly correlated, and hγLPH 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 hγLPH on a molar basis (Fig. 6B). It follows that plasma hβEND concentration

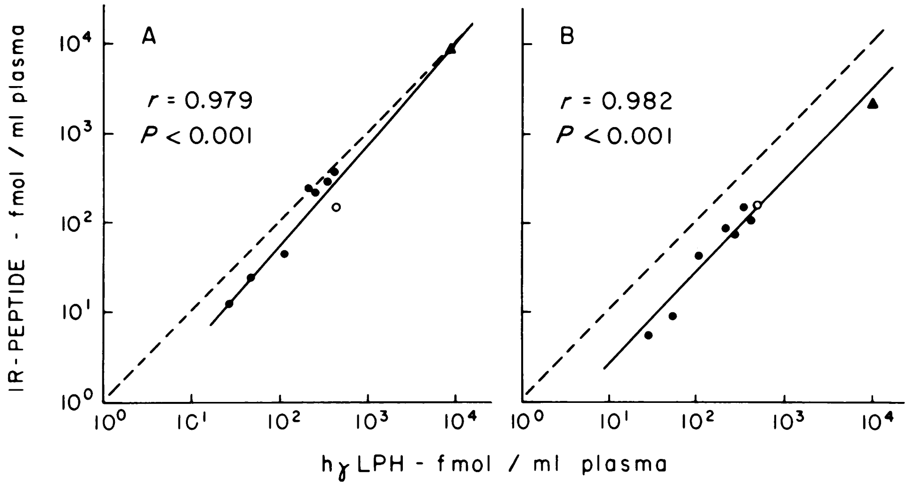

FIGURE 6 Correlations between plasma IR-hγLPH and IR-hβLPH (A) or IR-hβEND (B) in patients with ACTH/LPH hypersecretion. Plasma samples were obtained from patients with Addison's disease (●), Nelson's syndrome (○), and the ectopic ACTH/LPH syndrome (▲), and subjected to Sephadex G-50 Fine gel exclusion chromatography. The actual amounts of hβLPH, hγLPH, 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-hγLPH (A) and IR-hβEND and IR-hγLPH (B) are plotted on identical logarithmic scales. The broken lines represent equimolarity.

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, hγLPH, 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-hγLPH and IR-hβEND/hβLPH, with similar biphasic disappearance curves (Fig. 7). The initial and subsequent half-lives were 80 and 170 min for IR-hγLPH 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, hγLPH, and hβEND in the three samples were calculated in the manner just described. The plasma disappearance rates of hβLPH and hγLPH were similar, whereas hβEND disappeared much more rapidly (Fig. 8).

DISCUSSION

It is now generally accepted that hβLPH and/or hγLPH 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 hγLPH 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 hγLPH 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 hγLPH 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

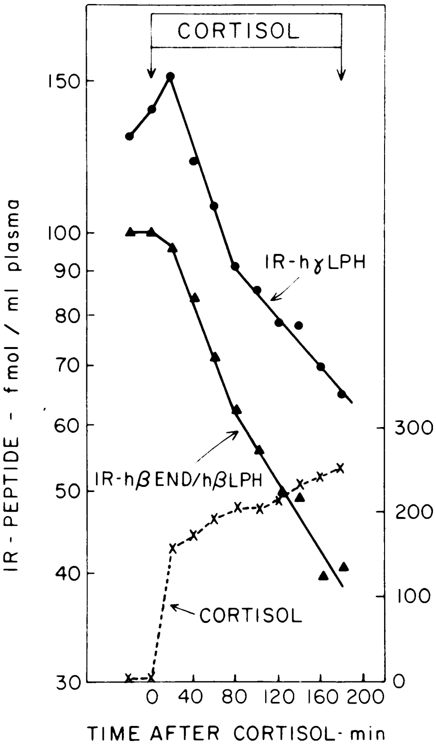

FIGURE 7 Plasma disappearance of IR-hγLPH and IR-hβEND/hβLPH in a patient with Addison's disease who received cortisol hemisuccinate as an intravenous bolus (1 mg/kg) followed by a continuous intravenous infusion (1 mg/kg per h) for 3 h. The patient's IR-hγLPH and IR-hβEND/hβLPH plasma levels had been 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$_2$-terminal hβEND RIA that may measure Met^5-enkephalin, α-endorphin, γ-endorphin, or other metabolites in plasma or tissue (40). The term "IR-hβEND/hβLPH" has been used in this study, recognizing the fact that either hβEND, hβLPH, or both may contribute to the total immunoreactivity in any one sample.

In normal controls, plasma IR-hγLPH was undetectable in 11 of 16 volunteers and was 21 fmol/ml or less in 5 others. Although direct comparison of hγLPH levels to hβEND/hβLPH 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)hγLPH and hγLPH, but only 3% as well with hβLPH, reported plasma IR-(37–58)hγLPH in normal controls in the range of 25 pg/ml. Since the NZ antiserum presumably was measuring only hγLPH, there being no (37–58)hγLPH ("hβMSH") in normal human plasma, this would correspond to about 10 fmol/ml of IR-hγLPH, a value compatible with our findings.

We also detected IR-hβEND/hβLPH in the plasma of normal subjects; others (22–24, 26) have found that hβLPH represents 69–94% of total "IR-hβEND" in the plasma of normal subjects under basal conditions. Therefore, the mean IR-hβEND/hβLPH value of 11 fmol/ml in our normal subjects probably represents mainly hβLPH and yields a calculated hβEND 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-hβMSH" in hemodialysis patients (30–32) that behaves as HMW material (32) and reacts in a partially specific "hβLPH" RIA (35), the relative contributions of hγLPH and hβLPH to total "IR-hβMSH" in the plasma of these patients have remained unknown. Our data demonstrate that IR-hγLPH is greatly increased in their plasma and coelutes with standard hγLPH. IR-hβEND/hβLPH is also increased significantly, and a significant correlation exists between IR-hβEND/hβLPH and IR-hγLPH in the plasma of hemodialysis patients, with hγLPH clearly predominant. The lack of a detectable hβEND peak after gel chromatography indicates that hβLPH itself contributes the vast majority of total plasma IR-hβEND/hβLPH in these patients. The fact that we could not detect hβEND in the plasma of hemodialysis patients does not necessarily mean that it was not present, because the limit of detection in our com-

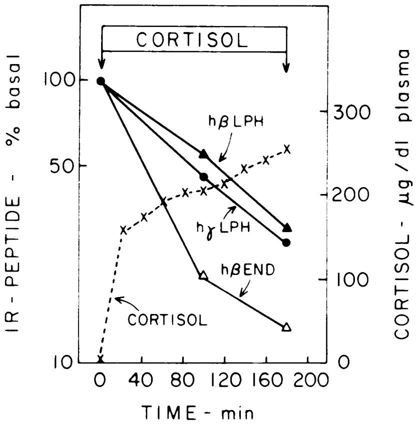

FIGURE 8 Plasma disappearance of endogenous hβLPH, hγLPH, and hβEND in the patient with Addison's disease. Plasma samples were obtained before and after 100 and 180 min of cortisol infusion (Fig. 7) and subjected to Sephadex G-50 Fine gel exclusion chromatoraphy. The actual amounts of hβLPH, hγLPH, and hβEND 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 hβEND in normal subjects (22–26). Thus, our data do not exclude the possibility that hβEND 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 hγLPH, or very closely related peptides that are biosynthetic or peripheral metabolic products of hβLPH, 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-hβEND (24–26, 42). To establish further the relative distribution of hβLPH, hγLPH, and hβEND in the plasma of such patients, we subjected plasma specimens to gel exclusion chromatography. The hβEND/hβLPH RIA recognizes hβLPH and hβEND equally on a molar basis, permitting a direct comparison of concentrations of the peptides. The concentrations of hβEND correlated well with those of hγLPH, but were approximately one-third as high. If these two hormones are produced concomitantly in man, the lower levels of immunoreactive hβEND 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 hβLPH and hγLPH were almost equal in the plasma of these patients. The gel exclusion chromatography data further validated the specificity of the hγLPH RIA for, in these plasma specimens containing high levels of both hβLPH and hβEND, only a single peak of IR-hγLPH was detected, and that coeluted with standard hγLPH.

βLPH was demonstrated to be the biosynthetic precursor of βEND 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 βLPH precursor would result in concomitant release of βEND and γLPH, 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 hγLPH-to-hβEND ratio in plasma, the disappearance rate of each peptide was determined in a patient with Addison's disease. The observed disappearance rates of endogenous hβLPH and hγLPH 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 NH_2-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-hβEND was apparently faster than that of either lipotropin, at least potentially explaining its relatively lower molar concentration.

Both hγLPH (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 hγLPH 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 hγLPH 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 hγLPH 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 hγLPH and/or hβEND.

This study demonstrates that hβLPH, hγLPH, 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.

ACKNOWLEDGMENTS

We gratefully acknowledge the generosity of Dr. R. Guillemin for the gift of synthetic αEND; Ciba-Geigy, Ltd., for providing synthetic hACTH and (37–58)hγLPH; and the National Pituitary Agency, National Institute for Arthritis, Metabolism, and Digestive Diseases, for providing us with 1,000 fresh-frozen human pituitary glands (Lot No. HP72-17). We thank Ms. Yvonne S. Brown, Ms. Barbara J. Sherrell, and Ms. Margaret G. Wilson for their excellent technical assistance and Ms. Sue Warrington for transcribing the manuscript.

These studies were supported in part by National Cancer Institute research grants 5-R01-CA11685 and 5-R25-CA19429 and grant-in-aid 5-M01-RR00095 from the National Institutes of Health. Dr. Bertagna was the recipient of Individual Research Fellowship grant 1-F32-AM05974 from the National Institutes of Health during the course of these studies.

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