# Presence of Immunoreactive β-Endorphin in Normal Human Plasma

## A CONCOMITANT RELEASE OF β-ENDORPHIN WITH ADRENOCORTICOTROPIN AFTER METYRAPONE ADMINISTRATION

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ABSTRACT To elucidate whether or not β-endorphin exists in plasma of normal subjects, plasma extracts obtained before and after metyrapone administration were subjected to gel exclusion chromatography, and fractions obtained were assayed by a sensitive radioimmunoassay for β-endorphin. The basal plasma level of β-endorphin was 5.8±1.1 pg/ml (mean±SE, $n = 5$), which rose significantly to the level of 48.9 ±3.8 pg/ml after a single oral dose (30 mg/kg of body wt) of metyrapone administration ($P < 0.001$). Plasma ACTH levels also increased from the mean basal level of 73±4 pg/ml to 269±41 pg/ml after metyrapone administration. These results indicate that β-endorphin, distinct from β-lipotropin, exists in normal human plasma and that it is released from the pituitary concomitantly with ACTH.

## INTRODUCTION

Several structurally related peptides with opioid activity have been isolated from the brain and pituitary of several species (1–3). Among these, β-endorphin, which corresponds to 61–91 amino acids of β-lipotropin (β-LPH),[1] is the most potent in analgesic activity (4), opiate receptor binding (5), and behavioral effect (6). However, the physiological role of β-endorphin is still unknown. Recent studies on the cell-free translation product directed by messenger RNA of the pituitary gland (7) and the biosynthetic product of ACTH-producing mouse pituitary tumor cell line (8) have revealed that both ACTH and endorphins come from the common precursor molecule. However it is still unknown whether β-endorphin is present in plasma, especially in that of man. The present study was designed to examine the existence of β-endorphin in normal plasma before and after metyrapone administration.

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[1] *Abbreviation used in this paper*: β-LPH, β-lipotropin.

## METHODS

Five male volunteers with apparently normal endocrine function, aged 26–36 yr, were studied. The basal blood samples were taken at 9:00 a.m. at resting state on the first day. All subjects took a single dose of metyrapone (30 mg/kg of body wt, Ciba-Geigy Ltd. Basel, Switzerland) orally at 12:00 p.m. on the same day. The subsequent blood samples were taken at 9:00 a.m. on the next day. All blood samples were withdrawn into chilled, plastic syringes and transfered to chilled, siliconized disposable glass tubes which contained Trasylol (500 kallikrein inactivator units/ml, Delbay Pharmaceuticals Inc., Div. Schering Corp., Bloomfield, N. J.), and EDTA (1 mg/ml). Plasma was separated by centrifugation in a refrigerated centrifuge. An aliquot of plasma was immediately frozen at −20°C for radioimmunoassay of ACTH and thawed only once at the time of assay. The remaining portion of plasma was immediately processed for extraction of β-endorphin.

*Extraction of β-endorphin.* β-endorphin was extracted according to the method of Donald (9) with slight modifications. In brief, 20 ml of plasma from the basal blood samples and 4 ml of plasma obtained after metyrapone administration were subjected to extraction. 50 mg of silicic acid (100 mesh, Mallinckrodt Inc., St. Louis, Mo.) was added to plasma samples divided into 2-ml samples, and the mixture was agitated in a Vortex mixer (Thermonics, Co. Ltd., Tokyo) for 1 min at 4°C. The mixture was then centrifuged in a refrigerated centrifuge. The precipitates were washed with 3 ml of cold, distilled water and centrifuged in a refrigerated centrifuge. β-endorphin was separated from silicic acid with 2 ml of 40% acetone in 1% acetic acid by vortexing for 1 min at 4°C and then centrifuged in a refrigerated centrifuge. The eluates from the same blood sample were combined and lyophilized after the acetone had been evaporated. The lyophilized eluates were reconstituted in 0.3 ml of 0.05 M phosphate buffer which contained 0.5% human serum albumin (Fraction V, ICN Pharmaceuticals, Inc., Cleveland, Ohio), 500 kallikrein inactivator units/ml of Trasylol and 0.4% 2-mercaptoethanol (Nakarai Chemicals, Ltd., Kyoto, Japan) (standard diluent). The recoveries of the extraction procedures, as monitored by the

addition of β-LPH or β-endorphin in hormone-free plasma, were 35 and 80%, respectively.

*Gel exclusion chromatography.* A 0.2-ml aliquot of reconstituted solution was applied after centrifugation on a 0.7 × 49-cm column of Bio-Gel P-60 (Bio-Rad Laboratories, Richmond, Calif.), equilibrated with standard diluent, and eluted with the same diluent at 4°C. The flow rate was 2 ml/h and the fraction volume was 0.74 ml. The β-endorphin content of each fraction was measured by radioimmunoassay. Blue dextran was used as markers for void volume, $^{125}$I-β-LPH for β-LPH, $^{125}$I-β-endorphin for β-endorphin, and $^{125}$I for the salt peak. Recoveries of $^{125}$I-β-LPH and $^{125}$I-β-endorphin applied to the column were 90% in both cases.

*Radioimmunoassays.* Radioimmunoassay for β-endorphin was performed by talcum absorption method as described previously (10). The minimal detectable quantity of β-endorphin was 1 pg. Human β-endorphin and human β-LPH (both donated by C. H. Li) equally displaced $^{125}$I-β-endorphin from the antiserum, when compared on a molar basis; but human ACTH, α-melanocyte-stimulating hormone, human β-melanocyte-stimulating hormone, α-endorphin, γ-endorphin, Leu$^5$-enkephalin, and Met$^5$-enkephalin failed to displace $^{125}$I-β-endorphin from the antiserum, even when quantities as much as 10 ng were added. Radioimmunoassay for ACTH was performed with the CIS ACTH radioimmunoassay kit (11) (Atomic Energy Laboratory of Biomedical Products, Gif-sur-Yvette, France). The minimal detectable quantity of ACTH was 45 pg/ml.

## RESULTS

The elution profiles obtained by gel filtration of plasma extracts from five normal men are shown in Fig. 1. Two major peaks with β-endorphin immunoreactivity were found in most cases; one peak eluted in the position compatible with $^{125}$I-β-LPH (designated as β-LPH hereafter) and the other peak in the elution position of $^{125}$I-β-endorphin (designated as β-endorphin). Immunoreactivity compatible for β-endorphin was distinctly separated from that of β-LPH. A minor peak was observed in, or near, the void volume in two of five cases. Immunoreactive β-endorphin, β-LPH, and ACTH levels in plasma before and after metyrapone administration are summarized in Table I. The mean basal level of β-endorphin in five normal men was 5.8

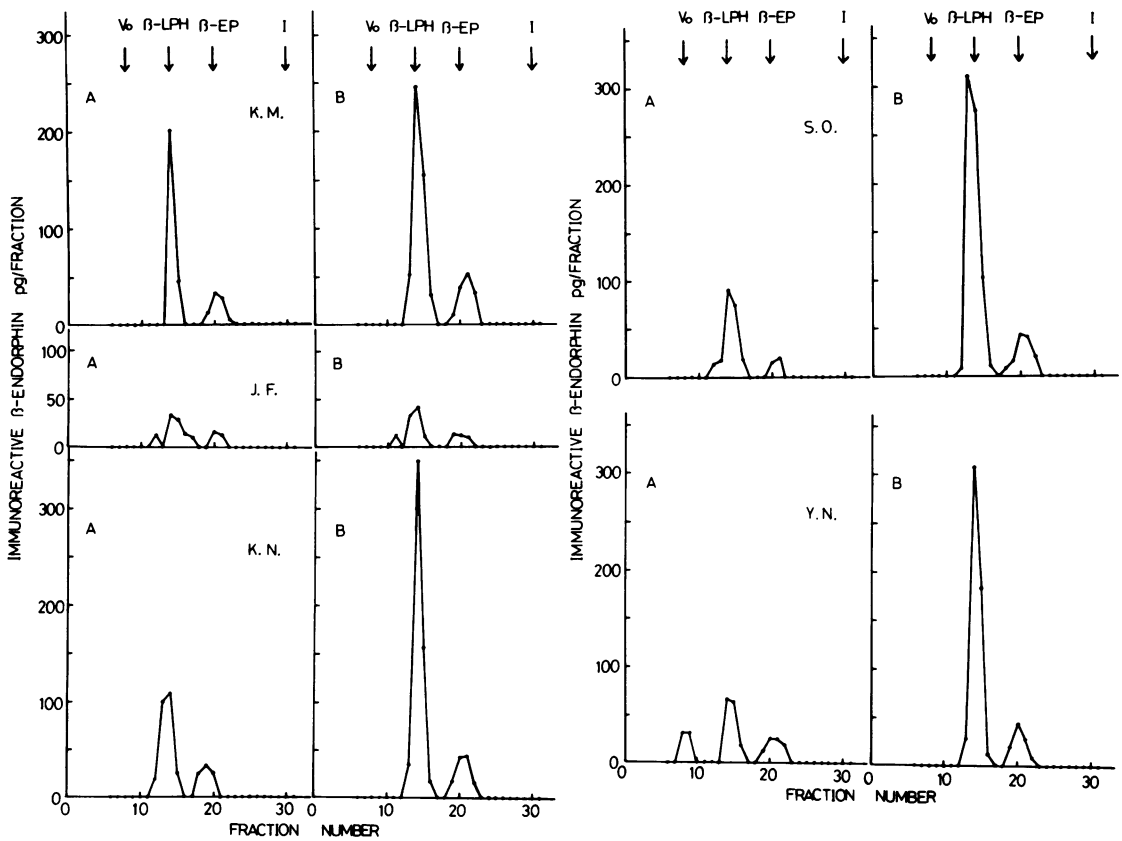


FIGURE 1 Elution profiles obtained by gel filtration (Bio-Gel P-60, 0.7 × 49 cm) of the plasma extracts from five normal men (K.M., J.F., K.N., S.O., and Y.N.). A: Before metyrapone administration, the lyophilized extracts from 20 ml plasma were reconstituted in a 0.3-ml standard diluent. The 0.2 ml of reconstituted solution was applied to the column. B: After metyrapone administration, the lyophilized extracts from 4 ml plasma were reconstituted in a 0.3-ml standard diluent. The 0.2 ml of reconstituted solution was applied to the column. $V_0$, void volume; β-EP, β-endorphin; and I, iodine.

TABLE I

*Plasma β-Endorphin, β-LPH, and ACTH Levels in Five Normal Subjects before and after Metyrapone Administration*

| Case | Age | Before metyrapone | | | After metyrapone | | |
|---|---|---|---|---|---|---|---|
| | | β-Endorphin | β-LPH | ACTH | β-Endorphin | β-LPH | ACTH |
| | *yr* | *pg/ml* | *pg/ml* | *pg/ml* | *pg/ml* | *pg/ml* | *pg/ml* |
| K.M. | 26 | 7.5 | 147.9 | 62 | 64.3 | 1,444.4 | 320 |
| J.F. | 28 | 2.7 | 51.8 | 77 | 17.6 | 255.7 | 110 |
| K.N. | 29 | 7.8 | 149.6 | 75 | 56.0 | 1,663.7 | 300 |
| S.O. | 30 | 3.3 | 126.9 | 65 | 59.4 | 1,823.5 | 284 |
| Y.N. | 36 | 7.5 | 79.8 | 85 | 47.4 | 1,593.0 | 333 |
| Mean | | 5.8 | 111.2 | 73 | 48.9 | 1,356.1 | 269 |
| SE | | 1.1 | 17.4 | 4 | 3.8* | 252.0‡ | 41§ |

* $P < 0.001$.
‡ $P < 0.001$.
§ $P < 0.02$.

±1.1 pg/ml (mean±SE), which rose significantly to 48.9±3.8 pg/ml after oral administration of metyrapone ($P < 0.001$, by Student's $t$ test). Plasma β-LPH levels increased from 111.2±17.4 pg/ml to 1,356.1±252.0 pg/ml, which were estimated according to the cross-reaction with β-endorphin ($P < 0.001$). Plasma ACTH levels also increased from the mean basal level of 73 ±4 pg/ml to 269±41 pg/ml after metyrapone administration ($P < 0.02$). Immunoreactive β-endorphin, β-LPH, and ACTH increased concomitantly after metyrapone administration. To exclude the possibility of the conversion of β-LPH into β-endorphin in the process of the extraction and gel filtration, 7.5 ng of β-LPH was added to fresh, hormone-free plasma, extracted, applied to the same column, eluted, and assayed in the same way. No conversion of β-LPH into β-endorphin was observed, as shown in Fig. 2.

## DISCUSSION

The present study demonstrates the existence of β-endorphin immunoreactivity in normal human plasma with elution position corresponding to the standard β-endorphin on gel exclusion chromatography. We have previously observed β-endorphin immunoreactivity in plasma from patients with Nelson's syndrome and Addison's disease, which behaved like the standard β-endorphin on gel exclusion chromatography.[2] This fraction showed a parallel dilution curve with that of standard β-endorphin in radioimmunoassay and, therefore, seemed to be β-endorphin. The existence of β-endorphin in human plasma has been suggested by the observation that γ-lipotropin, an N-terminal 1-58 fragment of β-LPH, is present in human plasma and the pituitary (12), because β-endorphin corresponds to the remaining amino acid residues (61–91) of β-LPH molecule. Guillemin et al.

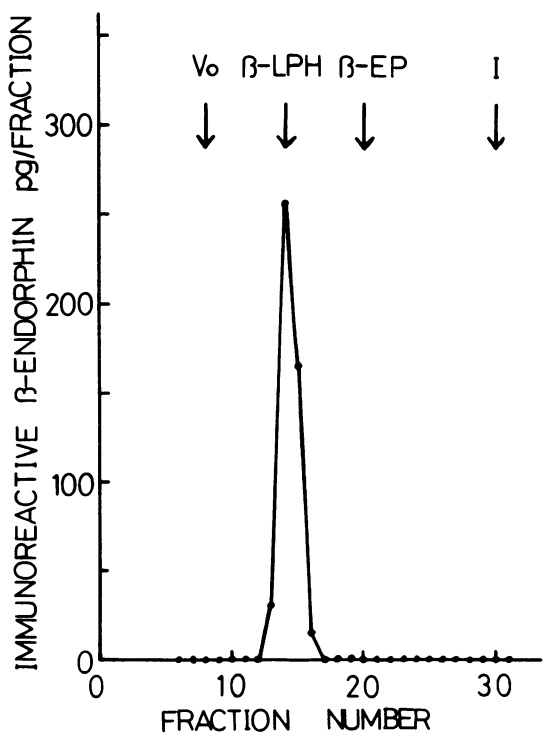


FIGURE 2 An elution profile obtained by gel filtration (Bio-Gel P-60, 0.7 × 49 cm) of the extract from fresh hormone-free plasma to which 7.5 ng of β-LPH was added. The lyophilized extract was reconstituted in a 0.3-ml standard diluent. The 0.2 ml of reconstituted solution was applied to the column. $V_0$, void volume; β-EP, β-endorphin; and I, iodine.

[2] Nakai, Y., K. Nakao, S. Oki, H. Imura, and C. H. Li. Submitted for publication.

(13) reported the presence of immunoreactive β-endorphin in rat plasma with radioimmunoassay for β-endorphin. However, the antiserum which they used, like ours, cross-reacted with β-LPH (14) and could not differentiate between the molecules. Therefore, gel exclusion chromatography as well as sensitive radioimmunoassay are required to demonstrate β-endorphin in plasma. The possibility that β-endorphin is produced by the degradation of β-LPH during extraction and gel filtration procedures seems unlikely because authentic β-LPH added to hormone-free plasma was recovered without noticeable change after extraction and gel exclusion chromatography. Another important finding in the present experiment is that β-endorphin changes parallelly with ACTH and β-LPH after the administration of metyrapone. This suggests that β-endorphin is secreted from the pituitary gland concomitantly with ACTH and β-LPH in normal subjects. This is also consistent with recent discoveries of the common precursor molecule which produces both ACTH and β-endorphin (7, 8). The physiological significance of β-endorphin secreted into the blood must await further clarification.

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