

## DISCORDANT CHANGES IN PLASMA ACTH AND $\beta$ -LIPOTROPIN/ $\beta$ -ENDORPHIN LEVELS IN CUSHING'S DISEASE PATIENTS WITH DEPRESSION

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### SUMMARY

Cushing's Disease is often associated with a depressive syndrome, with mood, vegetative, and cognitive abnormalities of variable severity. In 11 patients with (pituitary ACTH-dependent) Cushing's disease (10 women, 1 man), we studied the relationship between severity of the depressive syndrome and concordance of changes in ACTH and  $\beta$ -lipotropin/ $\beta$ -endorphin ( $\beta$ -LPH/ $\beta$ -E) levels at baseline and in response to metyrapone and dexamethasone. For each condition, blood samples were drawn at 0800h, 1200h, 1600h, and 2200h. Six patients were categorized as mildly depressed (mean [ $\pm$ SD] depressed mood score =  $0.17 \pm 0.4$ ; modified Hamilton Depression scale score =  $7.6 \pm 4.5$ ) and five as severely depressed (mean depressed mood score =  $2.4 \pm 0.5$ ; modified Hamilton Depression scale score =  $15 \pm 5.6$ ) ( $p < 0.05$ ). ACTH and  $\beta$ -LPH/ $\beta$ -E were measured by radioimmunoassay. For each experimental condition, changes in levels were scored as concordant if the two peptides moved in parallel between sampling points. There was a relationship between greater severity of depression and more frequent discordant changes in ACTH and  $\beta$ -LPH/ $\beta$ -E levels: The six patients with mild depression exhibited 23 concordant and 3 discordant change patterns, while the five patients with severe depression showed 8 concordant and 15 discordant patterns. The mean percentage of concordant patterns per patient differed significantly between the two groups (mildly depressed =  $90.0 \pm 16.7$ ; severely depressed =  $34.6 \pm 8.7$ ) ( $p < 0.001$ ). When each study condition was examined separately, differences in the frequency of concordance between the groups reached significance during the post-metyrapone phase and with 8.0 mg dexamethasone administration. These initial findings, taken together with data in related areas, suggest that greater diversity in regulation and cosecretion of ACTH and  $\beta$ -LPH/ $\beta$ -E may occur than is currently suspected. Such diversity may play a role in the relationship between HPA axis dysregulation and mood disorders.

### INTRODUCTION

PATIENTS WITH CUSHING'S DISEASE (CD) provide a useful model for studying the association of psychiatric abnormalities and dysfunction of the hypothalamo-pituitary-adrenal (HPA) axis. In his original description of CD, Cushing (1932) noted that "emotional disorders" were a prominent feature of the clinical presentation. Since that time, the characteristic phenomenology has

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been defined more precisely as a depressive syndrome, with abnormalities in mood, cognition, and vegetative symptoms (Starkman *et al.*, 1981; Starkman, 1987). Amelioration of depression has been demonstrated after treatment that lowers cortisol levels (Jeffcoate *et al.*, 1979; Cohen, 1980; Kelly *et al.*, 1983; Starkman *et al.*, 1986). ACTH also may play a role; patients with ACTH-independent Cushing's syndrome of adrenal origin have hypercortisolemia but suppressed ACTH levels, and are less likely to have severe depressed mood than patients with CD, who have elevations in both cortisol and ACTH (Carroll, 1977; Starkman *et al.*, 1981).

As with ACTH,  $\beta$ -lipotropin and  $\beta$ -endorphin ( $\beta$ -LPH/ $\beta$ -E) are derived from proopiomelanocortin. There is some evidence that these two peptides have opposing effects: following microinjection into the periaqueductal gray of rats:  $\beta$ -E exerts an inhibitory action and produces sedated immobility, while ACTH produces the opposite, an excitatory syndrome of fearful hyperreactivity (Jacquet, 1982). Initial animal studies *in vivo* and *in vitro* indicated that, under basal and challenge conditions, ACTH and  $\beta$ -LPH/ $\beta$ -E are secreted together not only in concomitant but also in equimolar fashion (Guillemin *et al.*, 1977; Mains & Eipper, 1981). Other studies have suggested that discordant and non-equimolar secretion might occur; e.g., in rats receiving two consecutive exposures to restraint stress separated by a 5-min interval, the plasma  $\beta$ -LPH/ $\beta$ -E response to the second stress was significantly potentiated, while the ACTH response was not (DeSouza & VanLoon, 1985).

We hypothesized that dysregulation in the cosecretion of ACTH and  $\beta$ -LPH/ $\beta$ -E might occur in some patients with CD, and that such heterogeneity in cosecretion might be associated with the severity of the depressive syndrome. Our findings suggest that a higher incidence of discordant changes in plasma peptide levels occurs in patients with CD who have more severe manifestations of the depressive syndrome.

## SUBJECTS AND METHODS

### *Subjects*

Patients with suspected Cushing's syndrome were studied in the University of Michigan General Clinical Research Center (CRC). The diagnosis of CD was established by clinical criteria, including moon facies, truncal obesity, skin and muscle atrophy, and most, if not all, of the following biochemical findings: lack of normal cortisol circadian rhythm; excessive cortisol secretion as indicated by high plasma cortisol and urinary free cortisol concentrations, increased cortisol secretion rate, failure to suppress cortisol normally after 2 mg dexamethasone, and normal or elevated plasma ACTH levels.

Eleven consecutive patients ultimately diagnosed as having pituitary ACTH-dependent Cushing's syndrome (Cushing's disease) were included in this study. Nine were Caucasian and two were black. Ten were female, and one was male, approximating the 80% ratio of women to men usually occurring in this disorder. The age range was 23 to 66 yr (mean = 37 yr; median = 32 yr).

### *Psychiatric evaluation*

The psychiatric evaluation and ratings were performed by one investigator (MNS) during the diagnostic hospitalization prior to the establishment of the medical diagnosis and without knowledge of hormone or peptide profiles. Details of the semistructured psychiatric interview have been reported previously (Starkman *et al.*, 1981). Forty-five items covering affective, vegetative, and cognitive disturbances were studied by symptom report and/or observation during the interview. Items were scored on the basis of the patient's experience of the symptom in the month preceding the evaluation. In particular, depressed mood was scored on a four-point rating scale (absent, mild, moderate, or severe) based on the patient's description of quality, intensity, and frequency of depressed mood.

The Hamilton Rating Scale for Depression (Hamilton, 1967), a composite of the affective, vegetative, and cognitive items which constitute the depressive syndrome, also was used. We modified the standard 17-item Hamilton scale by omitting items 15, 16, and 17, which are inappropriate in patients with Cushing's syndrome

(hypochondriacal preoccupation with bodily symptoms; loss of insight by attributing nervous symptoms to physical illness; and weight loss, since weight gain is frequently a result of glucocorticoid excess). Thus, scores are based on a 14-item Hamilton scale, which is not equivalent to standard Hamilton Depression Scale scores. Our prior studies indicate that depressed mood scores and modified-Hamilton scores are significantly correlated (Starkman *et al.*, 1986). However, the modified-Hamilton and depressed mood scores are not always of the same magnitude: the depressed mood score measures one component of the depressive syndrome, while the Hamilton scale is a composite including vegetative somatic symptoms as well as irritability and anxiety, which may be present in patients with CD in the absence of significant depressed mood.

#### *Experimental protocol*

Patients were studied following their admission to the CRC for a 10-day inpatient protocol. An indwelling venous catheter was inserted 1 hr prior to the initiation of baseline studies and left in place for the duration of the study. The patients were studied under the following conditions: (1) 24-hr baseline, beginning at 0800h; (2) metyrapone administration to block cortisol synthesis and stimulate ACTH and  $\beta$ -LPH/ $\beta$ -E release (750 mg orally every 4 hr for six doses); (3) post-metyrapone phase, when endogenous cortisol synthesis resumed and ACTH and  $\beta$ -LPH/ $\beta$ -E secretion were suppressed; and (4) dexamethasone suppression (0.5 mg every 6 hr for 2 days, followed by 2 mg every 6 hr for 2 days).

For each condition, blood samples were drawn at 0800h, 1200h, 1600h, and 2200h. For the dexamethasone suppression condition, blood was drawn on the second day of each dosage level. Samples were spun immediately and the plasma chilled and frozen at  $-70^{\circ}\text{C}$ .

#### *Peptide assays*

Plasma ACTH was measured by radioimmunoassay (Vague *et al.*, 1971). The antiserum (generously provided by Vague) was used at a dilution of 1:800,000; it has a low cross-reactivity with other peptides and no cross reactivity with  $\beta$ -E. It can detect ACTH in plasma in concentrations as low as 6 pg/ml. Separation of free from bound hormone was carried out with 1.5% charcoal suspension. The tracer was prepared with human ACTH<sub>1-39</sub> (provided by C. H. Li) and iodinated with  $^{125}\text{I}$  by the chloramine-T method (Hunter & Greenwood, 1962). Each sample was run at four dilutions. Quality control studies were carried out with each assay using standard sera and carryover samples from previous assays. The same antiserum and assay method(s) were used for all samples. The inter-assay coefficient of variation was  $4.2 \pm 1.2\%$  at 20% binding and  $8.4 \pm 2.4\%$  at 80% binding. Intra-assay variability ranged from 3.6–10%.

Plasma  $\beta$ -LPH/ $\beta$ -E also was measured by radioimmunoassay. Five milliliters plasma were extracted by a solid phase technique with Sep-Pak extraction filters. Recovery was determined for each sample with  $^{125}\text{I}$ -labeled  $\beta$ -E and was 60–80%. An aliquot of the methanol extract was dried under nitrogen, reconstituted in assay buffer (0.01 M phosphate buffer containing 1% trasyolol, 0.2% mercaptoethanol, and 0.3 HSA; pH 7.8) and assayed. The antiserum (generously provided by Huda Akil) was obtained by injecting New Zealand white rabbits with gluteraldehyde-mediated  $\beta$ -E-thyroglobulin conjugate and used at a dilution of 1:20,000. In order to maximize sensitivity, the assay was run under disequilibrium conditions for the 2-day incubation period. Free from bound hormone was separated with charcoal. Assay sensitivity was 1 fmol/tube, with an  $\text{IC}_{50}$  of 45 fmol. The crossreactivity of the antibody was characterized with camel  $\beta$ -E<sub>1-31</sub>, N-acetyl- $\beta$ -E<sub>1-31</sub>,  $\beta$ -E<sub>1-27</sub>, and N-acetyl- $\beta$ -E<sub>1-27</sub>.  $\beta$ -LPH showed almost full cross-reactivity with this antiserum. Therefore, the primary antigenic determinant appeared to be in the midportion of  $\beta$ -E<sub>1-31</sub>, between positions 2 and 27. No cross-reactivity was observed with ACTH. The inter-assay coefficient of variation was 7.8% for 20% binding and 15.4% for 80% binding. The intra-assay variability ranged from 4–15%.

#### *Rating of discordant changes in peptide levels*

Ratings of concordance of changes in ACTH and  $\beta$ -LPH/ $\beta$ -E levels were performed by one investigator (DES) who was blind to the depression severity status of the patients. Levels of both peptides obtained for each experimental condition (baseline, metyrapone stimulation, post-metyrapone endogenous suppression, and dexamethasone suppression) were graphed. Consecutive levels were considered to be a change if the value differed from the preceding one by more than twice the SD of the assay. For each experimental condition, changes in levels were scored concordant if the two peptides moved in parallel between sampling points and discordant if there were dissociations in the direction of change for at least two of the three sampling intervals.

*Statistical analysis*

Student's *t* test (two-tailed) was used to compare group means. Fisher's exact test or  $\chi^2$  was used to compare differences in the frequency of change patterns between two groups classified according to depressed mood scores: "mild" (none to mild) and "severe" (moderate to severe).

## RESULTS

Of the 11 patients studied, 6 were classified as "mildly" depressed and 5 as "severely" depressed. Mean ( $\pm$ SD) depressed mood score in the mildly depressed group was  $0.17 \pm 0.4$  vs.  $2.4 \pm 0.5$  in the severely depressed group ( $p < 0.001$ ), and mean 14-item Hamilton Depression Scale Score was  $7.6 \pm 4.5$  vs.  $15 \pm 5.6$  ( $p < 0.05$ ).

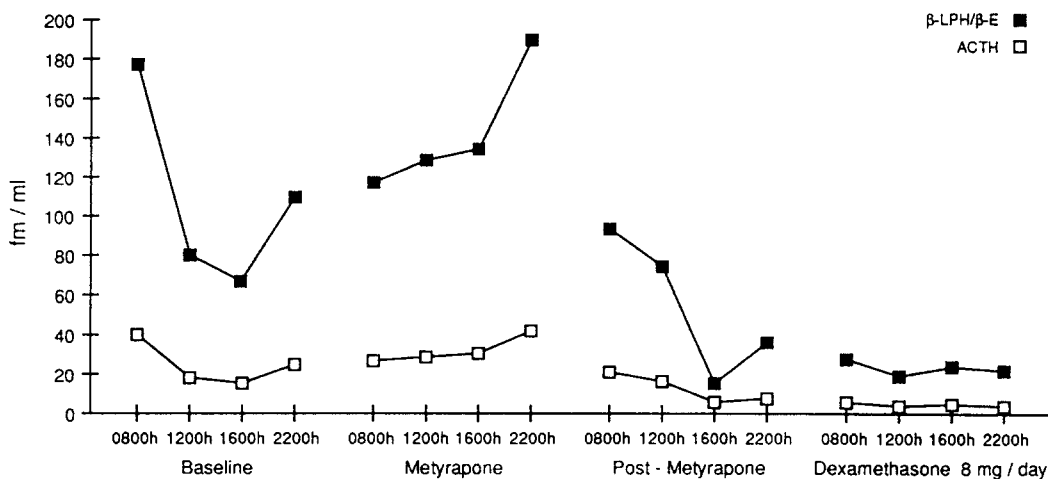


FIG. 1: Concordant changes in ACTH and  $\beta$ -LPH/ $\beta$ -E levels in a patient with mild depression.

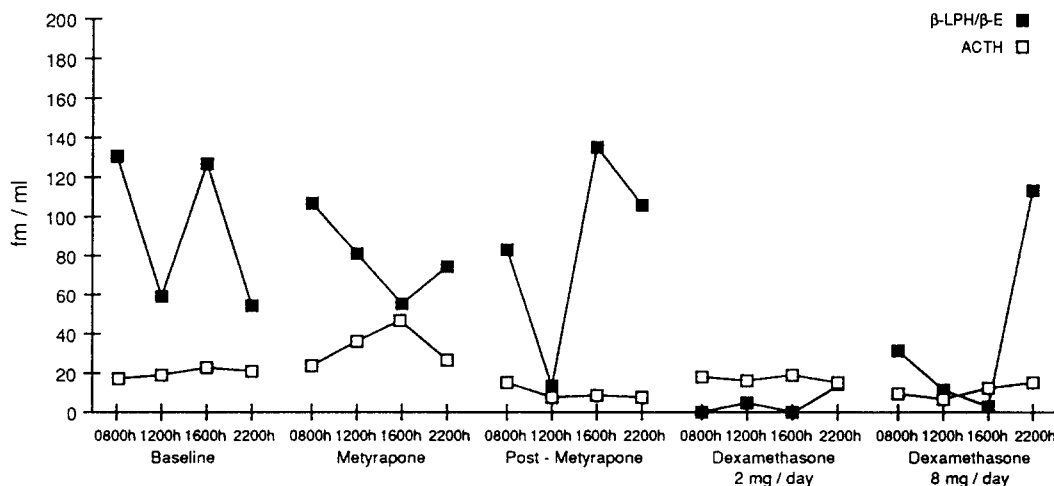


FIG. 2: Discordant changes in ACTH and  $\beta$ -LPH/ $\beta$ -E levels in a patient with severe depression.

Both concordant and discordant changes in ACTH and  $\beta$ -LPH/ $\beta$ -E levels were observed. Figure 1 depicts concordant changes under baseline conditions and during metyrapone, post-metyrapone, and during dexamethasone administration in a patient with mild depression (mood score = 0, modified-Hamilton score = 8). Figure 2 illustrates discordant changes in the peptides in a patient with severe depression (mood score = 3, modified-Hamilton score = 23).

As shown in Table I, patients with "mild" depressed mood had predominantly concordant changes in ACTH and  $\beta$ -LPH/ $\beta$ -E levels at baseline and during metyrapone and dexamethasone testing; only one had a discordant pattern at baseline, while another had a discordant pattern during metyrapone stimulation of peptide secretion. In contrast, discordant changes were frequently observed in patients with "severe" depressed mood. The mean percentage ( $\pm$ SD) of concordant patterns per patient differed significantly between the two subgroups: mildly depressed =  $90.0 \pm 16.7$ , and severely depressed =  $34.6 \pm 8.7$  ( $t = 6.7$ ,  $p < 0.001$ ).

Table II indicates the frequency of concordant and discordant changes in the two groups of patients for each of the study conditions. The differences were significant during the post-metyrapone phase and with 8.0 mg dexamethasone administration. When a composite of all scored conditions was made, the six patients with mild depression exhibited 23 concordant and 3 discordant change patterns, while the five patients with severe depression showed 8 concordant and 15 discordant patterns. The difference for this composite of scores also was statistically significant (although such composites pool observations that are not statistically independent, thus violating the assumption of random, independent sampling).

#### DISCUSSION

Discordant changes in ACTH and  $\beta$ -LPH/ $\beta$ -E levels were observed in a subgroup of patients with Cushing's Disease. This contrasts with earlier reports that ACTH and  $\beta$ -LPH/ $\beta$ -E are

TABLE I. CONCORDANT ( $\checkmark$ ) AND DISCORDANT ( $\times$ ) CHANGES IN ACTH AND  $\beta$ -LPH/ $\beta$ -E LEVELS IN 11 PATIENTS RANKED BY DEPRESSED MOOD SCORE

	Baseline	Metyrapone	Post-Metyrapone	DEX 2 $\mu$ g/qd	DEX 8 $\mu$ g/qd
<u>NONE/MILD</u>					
(0,3)*	$\checkmark$	$\checkmark$	$\checkmark$	$\checkmark$	$\checkmark$
(0,4)	$\checkmark$	$\checkmark$	$\checkmark$	$\checkmark$	$\checkmark$
(0,6)		$\checkmark$	$\checkmark$		
(0,8)	$\checkmark$	$\checkmark$	$\checkmark$	$\checkmark$	
(0,9)	$\checkmark$	$\times$	$\times$	$\checkmark$	$\checkmark$
(1,16)	$\times$	$\checkmark$	$\checkmark$	$\checkmark$	$\checkmark$
<u>SEVERE</u>					
(2,7)	$\times$	$\checkmark$	$\times$	$\checkmark$	$\times$
(2,14)	$\times$	$\checkmark$	$\times$	$\times$	$\times$
(2,16)	$\checkmark$	$\times$	$\times$	$\checkmark$	$\times$
(3,15)	$\checkmark$	$\times$	$\times$		
(3,23)	$\times$	$\times$	$\times$	$\checkmark$	$\checkmark$

\*depressed mood score, 14-item Hamilton Depression Scale score

TABLE II. SUMMARY OF CONCORDANCE OF PEPTIDE LEVEL CHANGES IN 11 PATIENTS

	DEPRESSED MOOD		<i>p</i>
	Mild n = 6	Severe n = 5	
<u>BASAL DAY</u>			
Concordant	4	2	
Discordant	1	3	n.s.
<u>METYRAPONE</u>			
Concordant	5	2	
Discordant	1	3	n.s.
<u>POST-METYRAPONE</u>			
Concordant	5	0	
Discordant	1	5	0.02
<u>DEXAMETHASONE, 2 mg</u>			
Concordant	4	3	
Discordant	0	1	n.s.
<u>DEXAMETHASONE, 8 mg</u>			
Concordant	5	1	
Discordant	0	3	0.05
<u>COMPOSITE OF SCORED CONDITIONS</u>			
Concordant	23	8	
Discordant	3	15	0.001

secreted in a concordant and equimolar fashion (Guillemin *et al.*, 1977; Mains & Eipper, 1981). However, studies in a variety of species do suggest that non-equimolar and discordant secretion of ACTH and  $\beta$ -LPH/ $\beta$ -E also can occur. As previously noted, the plasma  $\beta$ -LPH/ $\beta$ -E response in rats to a second consecutive restraint stress was significantly potentiated, while the ACTH response was not (DeSouza & VanLoon, 1985). In sheep, the anterior pituitary was found to secrete POMC peptides in both synchronous and asynchronous patterns: While the most frequent pattern of ultradian secretion involved concordant release of ACTH and  $\beta$ -LPH/ $\beta$ -E, the second most frequent pattern consisted of ACTH release alone, with no concomitant secretion of  $\beta$ -LPH/ $\beta$ -E (Engler *et al.*, 1989). In a patient with Cushing's syndrome due to an ectopic ACTH-producing malignant thymoma, a 2-hr infusion of somatostatin suppressed both ACTH and  $\beta$ -LPH/ $\beta$ -E in concordant fashion; yet, 1 hr later, ACTH remained suppressed, while  $\beta$ -LPH/ $\beta$ -E had returned to its preinfusion level (Nakashima *et al.*, 1985). Similar dissociations between ACTH and  $\beta$ -LPH/ $\beta$ -E have been observed in cultures of anterior pituitary cells under basal and stimulated conditions, and several explanations for such results have been suggested, such as differences in radioimmunoassay reactivity of precursor forms of the two peptides, differential rates of degradation, or selective secretion of the peptides (Ham & Smyth, 1985).

Both ACTH and  $\beta$ -E have important functions in neurobiology. Even though they are derived from a common precursor and are stored together in secretory granules, the "language" of the neuroendocrine system — pulsatility of secretions and relative differences in concentration of hormones and neurotransmitters — would seem to require mechanisms that would make

possible finely tuned, independent secretion of each of these peptides. Evidence that such mechanisms exist is beginning to accumulate. For example, in normal men, deconvolution techniques to study the secretory behavior of the pituitary gland have revealed that the 24-hr rhythm of ACTH is regulated by secretory burst amplitude alone, while  $\beta$ -E is controlled by secretory burst frequency as well as amplitude (Veldhuis *et al.*, 1990).

We have reported herein a relationship between greater severity of depression and more frequent discordant changes in ACTH and  $\beta$ -LPH/ $\beta$ -E levels, which was most apparent with challenge testing, particularly post-metyrapone, when endogenous levels of cortisol were rising, as well as after several days of suppression by dexamethasone. There are several reports in the literature suggesting dissociation of POMC products in patients with primary depressive disorder. For example, while hCRH administered to healthy control subjects induced a marked release of both ACTH and  $\beta$ -E, in patients with primary depressive disorder it induced an attenuated net ACTH release, whereas  $\beta$ -E was not concomitantly blunted (Rupperecht *et al.*, 1989). In another study, a subgroup of patients with primary depressive disorder showed escape from dexamethasone suppression of either  $\beta$ -LPH/ $\beta$ -E or cortisol (an indirect measure of ACTH), but not necessarily both (Matthews *et al.*, 1986).

Both our sample size and number of sampling points per study day were small. We are currently extending these studies, using more frequent sampling points in a larger group of CD patients. However, our findings to date, taken together with those of other investigators noted above, suggest that greater diversity in regulation and cosecretion of ACTH and  $\beta$ -LPH/ $\beta$ -E may occur than is currently suspected. Such diversity may play a role in the relationship between HPA axis dysregulation and mood disorders.

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