PLASMA MEASURES OF B-ENDORPHIN-LIKE IMMUNOREACTIVITY
IN DEPRESSIVES AND OTHER PSYCHIATRIC SUBJECTS

J. Matthews, H. Akil, J. Greden, and S. Watson

Mental Health Research Institute
University of Michigan
Ann Arbor, Michigan 48109

Summary

We report the utilization of a very sensi-
tive radioimmunoassay for plasma B-END, which
has enabled us to examine the suppressibility of
B-END-like material, under dexamethasone chal-
lenge, on a population of depressed patients.
We have found that B-END-like material in plasma
is suppressed by dexamethasone in a psychiatric
control population, whereas suppression is less
likely in endogenously depressed patients.
There also appears to be a dissociation between
cortisol and B-END measures (tested at 4:00 PM)
in the endogenously depressed group.

Several lines of evidence demonstrate an abnormality in the
hypothalmic-pituitary-adrenal axis in patients with endogenous
depression (1,2,3). There are multiple levels where the defect
might be localized. The dexamethasone suppression test (DST)
measures the pituitary-adrenal interface. This paper attempts
to move the study of the endocrinology of depression to the
level of the pituitary and brain. Thus, we are interested in
measuring beta-endorphin since it is part of the same precursor
molecule, pro-opiomelanocortin, as ACTH (4,5). We have used a
dexamethasone challenge to begin to look at the regulation of
the pituitary pro-opiomelanocortin system. Although one study
reports no suppression with dexamethasone in normals, the plasma
radioimmunoassay is difficult and may lead to false negatives
(6). We have therefore addressed here the following two
questions: a) Does dexamethasone suppress B-END in human
plasma? and b) Is the pattern of B-END suppression altered in
endogenous depression?

Methods

Blood collection was carried out via angiocatheter into
chilled EDTA containing vacutainers. The samples were centri-
fuged at 4°C for 10 minutes and were then aliquoted into plastic
storage vials and acidified to a pH of 2.0 with 1 N HCl. The
acidified serum was stored at -70°C until extraction. We used
Sep Pak C_{18} cartridges (Waters) for our extraction procedure
which provided 90% recovery with H^3-B-END spiked human serum
(9).

The radioimmunoassay was carried out in 150 mM Na phosphate buffer with 1% human serum albumin at pH 8.2 under disequilibrium incubation. The antiserum (rabbit Brenda) was used at a final concentration of 1:40,000 and separation was carried out by immunoprecipitation with goat antirabbit IgG. Cross-reactivity studies with our antibody showed 100% cross-reactivity with B-LPH and N-acetyl-B-END, 50% with B-END$_{1-27}$, and 30% with N-acetyl-B-END$_{1-27}$, whereas there is no cross-reactivity with alpha-END$_{1-27}$, gamma-END, B-END$_{2-5}$, B-END$_{2-17}$, methionine enkephalin, or alpha-melanocyte-stimulating hormone. The assay is capable of measuring <1.5 fmoles/assay tube (normal resting levels of B-END-like immunoreactivity are 2-5 fmoles/ml). The validation of the assay and the chromatography of the material being measured have been described (9).

Using this procedure we studied a mixed psychiatric control population (N=5) versus a group of endogenous depressives (N=6). Under dexamethasone challenge serum cortisol and serum B-END levels were measured. An 18 gauge angiocatheter was inserted into an antecubital arm vein at 1:30 PM; 20 ml blood samples were obtained at 20 minute intervals between 3:30 and 4:30 PM. All subjects were then given 1 mg of dexamethasone at 11:30 PM that evening and the catheter study was carried out in an identical fashion the following day. Both cortisol and B-END measures are reported as the means of the four time points between 3:30 and 4:30 PM. Thus: 1) simultaneous cortisol and B-END measures were obtained; 2) subjects were their own controls; and 3) repeated measures enable us to control for stress and pulsatile release of B-END. The 4:00 PM time period was chosen since this is the time when the dexamethasone suppression test is most sensitive in separating endogenously depressed patients from normal individuals.

<center>Results</center>

Table 1 shows the results. The non-endogenous psychiatric control group showed a mean suppression of their B-END levels of 45% + 8 as compared to 18% + 8 suppression for the endogenous depressives. The mean Hamilton rating for depression for the control group was 6 + 2 and 24 + 1 for the endogenous depressives. Further, there is an absence of overlap between pre- and post-dexamethasone B-END levels in the controls, whereas there was considerable overlap in endogenous depressives. The post-dexamethasone B-END values were very closely clustered in the controls in contrast to much greater variability in the endogenous depressives.

Since endogenously depressed patients usually exhibit cortisal values of greater than 5.0 ug/dl after dexamethasone, we have reviewed the B-END data for a similar cutoff. A conservative preliminary post-dexamethasone value of 2.5 fm/ml was above all non-endogenous post-dexamethasone values.

As summarized by Table 2, B-END levels above 2.5 fm/ml identified five out of the six endogenous depressives, whereas post-dexamethasone cortisol levels identified only two of the six endogenous depressives. Note that there is good agreement for the absence of abnormal B-END and cortisol responses in the non-endogenous groups, whereas the agreement for abnormal

Table 1

Non-endogenous Psychiatric Controls

Subject	BE Plasma level (fm/ml) Pre dex	Post dex	Suppression	Hamilton Depression Ratings
1	6.8 + 0.8	1.8 + 0.3	70%	10
2	3.7 + 0.4	1.8 + 0.3	51%	1
3	2.6 + 0.2	2.0 + 0.2	23%	7
4	2.8 + 1.2	1.5 + 0.3	46%	8
5	2.4 + 0.3	1.6 + 0.1	33%	-
X + S.E.	3.5 + 0.7	1.7 + 0.1	45% + 8	6 + 2

Endogenous Depressives

Subject	X BE Plasma level (fm/ml) Pre dex	Post dex	Suppression	Hamilton Depression Ratings
6	3.6 + 0.4	5.2 + 0.8	0%	21
7	3.3 + 0.8	3.2 + 1.7	4%	22
8	10.2 + 1.6	5.8 + 1.8	43%	38
9	1.8 + 0.3	1.2 + 0.2	32%	21
10	5.4 + 0.9	3.8 + 0.5	31%	18
11	3.9 + 1.2	4.0 + 0.9	0%	24
X + S.E.	4.7 + 1.3	3.8 + 0.7	18% + 8	24 + 1

Table 2

		Abnormal BE Suppression >2.5 fm/ml		Abnormal Cortisol Suppression >5.0 ug/dl	
Non-endogenous	#1	(-)		(-)	
	#2	(-)		(-)	
	#3	(-)	$\frac{0}{5}$	(-)	$\frac{0}{5}$
	#4	(-)		(-)	
	#5	(-)		(-)	
Endogenous	#6	(+)		(-)	
	#7	(+)		(-)	
	#8	(+)	$\frac{5}{6}$	(-)	$\frac{2}{6}$
	#9	(-)		(+)	
	#10	(+)		(+)	
	#11	(+)		(-)	

responses is poor in the endogenously depressed group. It should be noted, however, that the combination of B-END and cortisol measures around the 4:00 PM time point identifies all of the endogenously depressed patients.

Discussion

These results suggest that B-END-like material in plasma is suppressed by dexamethasone in a non-endogenous psychiatric population, whereas suppression is less likely in endogenously depressed patients. Of interest is the observation that the combination of both cortisol and B-END-like measures identifies all of the endogenously depressed patients. The dissociation between cortisol and B-END-like values in the endogenously depressed patients warrants further investigation in view of reports suggesting that B-END and ACTH are secreted in equimolar amounts (7). Simultaneous measures of ACTH, B-END, and cortisol may help explain this lack of agreement and possibly help localize the defect. This discrepancy may also be related to the 3:30 to 4:30 PM sampling time since B-END and cortisol are regulated differently and have different half lives (8). There may be a time period at which cortisol and B-END measures are more tightly linked. Thus, the best time point post-dexamethasone B-END measurement needs to be determined. Twenty-four hour circadian rhythm studies are thus imperative. The large differences in the Hamilton ratings in the psychiatric controls versus the endogenous depressives suggests that severity of illnes may account for the lack of suppression in the endogenous depressives; thus, future studies need to control for this variable. Studies on normal controls and sex differences are now being carried out.

Acknowledgements

References

1. J.L. GIBBONS, Arch. Gen. Psychiatry 10 572-575 (1964)
2. B.J. CARROLL and B.M. DAVIES, Brit. Med. J. 1 789-791 (1970)
3. B.J. CARROLL, G.C. CURTIS and J. MENDELS, Arch. Gen. Psych. 33 1051-1058 (1976)
4. B. EIPPER and R. MAINS, J. Supremolecular Structure 8 247-262 (1978)
5. S. NAKANISHI, A. INOUE, T. KITA, M. NAKAMURA, A.C.Y. CHANG, S.N. COHEN and S. NUMA, Nature 278 423-427 (1979)
6. N.H. KAHIN, S.C. RISCH, R.M. COHEN, T. INSEL and D.L. MURPHY, Science 209 827-828 (1980)
7. R. GUILLEMIN, T. VARGO, J. ROSSIER, S. MINICK, N. LING, C. RIVIER, W. VALE and F. BLOOM, Science 197 1357-1360 (1977)
8. M. ENDO, J. ENDO, M. NISHIKUBO, T. YAMAGUCHI and N. HATONANI, Psychoneuroendocrinology Workshop Conf. Int. Soc. Psychoneuroendocrinology, Mieken (Karger, Basel), 22-31 (1974)
9. C. CAHILL, J. MATTHEWS and H. AKIL, (submitted)