has a variety of effects on mature macrophage function and protein metabolism (*8*). In addition, purified murine granulocyte CSF and granulocyte-macrophage CSF affect murine neutrophil cytotoxic function (*9*), and semipurified human CSF from placental-conditioned medium enhances the antibody-dependent cell-mediated cytotoxicity of human neutrophils (*10*).

The physiological functions of NIF-T/CSF may be severalfold. In bone marrow this lymphokine can stimulate proliferation and differentiation of effector cells for host defense while, in the periphery, new and existing cells can be activated. In a localized immunological response NIF-T/CSF can retain circulating neutrophils in or away from areas of inflammation. Inappropriate localization or activation of neutrophils may be involved in the pathophysiology of a variety of immune-mediated disorders, such as rheumatoid arthritis. It should now be possible to study the production and function of NIF-T/CSF in normal and pathological environments.

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23 July 1984; accepted 2 October 1984

Elevated Concentrations of CSF Corticotropin-Releasing Factor–Like Immunoreactivity in Depressed Patients

Abstract. *The possibility that hypersecretion of corticotropin-releasing factor (CRF) contributes to the hyperactivity of the hypothalamo-pituitary-adrenal axis observed in patients with major depression was investigated by measuring the concentration of this peptide in cerebrospinal fluid of normal healthy volunteers and in drug-free patients with DSM-III diagnoses of major depression, schizophrenia, or dementia. When compared to the controls and the other diagnostic groups, the patients with major depression showed significantly increased cerebrospinal fluid concentrations of CRF-like immunoreactivity; in 11 of the 23 depressed patients this immunoreactivity was greater than the highest value in the normal controls. These findings are concordant with the hypothesis that CRF hypersecretion is, at least in part, responsible for the hyperactivity of the hypothalamo-pituitary-adrenal axis characteristic of major depression.*

A large proportion of patients with major depression (DSM III) or endogenous depression (Research Diagnostic Criteria) exhibit hyperactivity of the hypothalamo-pituitary-adrenal (HPA) axis as assessed by measurement of plasma concentrations of cortisol (*1*), urinary free cortisol excretion (*2*), and cortisol nonsuppression in response to the synthetic glucocorticoid, dexamethasone (*3*). The site within the HPA axis responsible for this endocrine abnormality is not known. Some evidence for adrenal hyperresponsivity to adrenocorticotropic hormone (ACTH) in depressed patients is available (*4*). Moreover, measurement of plasma ACTH after oral dexamethasone has generally revealed a lack of ACTH suppression to this synthetic glucocorticoid in depressed pa-

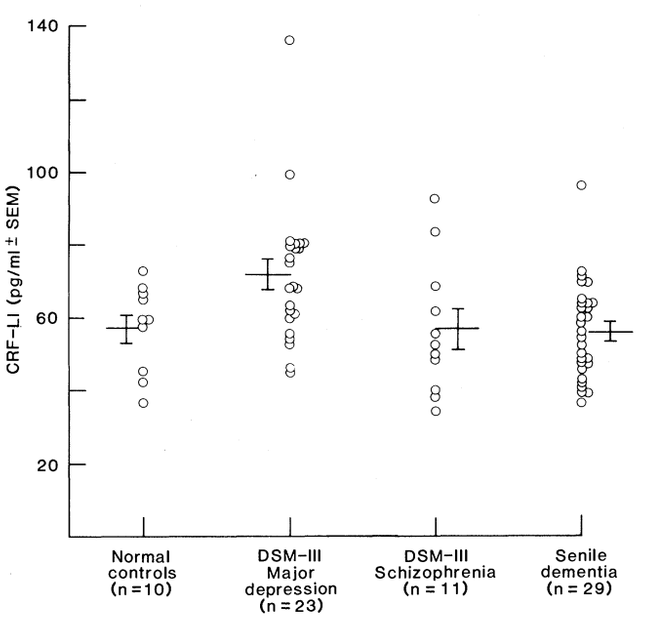

Fig. 1. Concentration of CRF-LI in CSF of normal controls (5 males and 5 females), DSM-III major depression (13 males and 10 females), DSM-III schizophrenia (8 females and 3 males), and senile dementia (DSM-III primary degenerative dementia or multi-infarct dementia, or both, 20 females and 9 males). Because the data distribution in the depressed patients was slightly skewed, the data were analyzed by both parametric (ANOVA and Student-Newman-Keuls test) and nonparametric (Mann-Whitney *U* test) methods. By both methods the CSF CRF-LI concentrations were significantly elevated in the depressed patients when compared to the other diagnostic groups and the normal controls ($P < 0.05$, ANOVA and Student-Newman-Keuls test; $P < 0.025$, Mann-Whitney *U* test).

tients (*5, 6*). This latter finding suggests that abnormal HPA activity in depressed patients arises in pituitary or suprapituitary (that is, central nervous system) sites.

In 1981, Vale and his colleagues (*7*) elucidated the sequence of ovine corticotropin-releasing factor (CRF). This 41-amino-acid peptide, isolated from hypothalamus, is normally released into the hypothalamo-hypophysial portal system where it is transported to the adenohypophysis to stimulate the release of ACTH, β-endorphin and other proopiomelanocortin products. Rat and human CRF have now also been discovered and have identical sequences (*8*). Recently, Gold and his colleagues (*9*) comprehensively reviewed their data from a series of studies concerning both ACTH and cortisol responses of depressed patients to intravenously administered ovine CRF. They concluded that the data were most compatible with the hypothesis that HPA hyperactivity in depressed patients results from hypersecretion of CRF. We now report that patients with major depression have elevated concentrations of CRF-like immunoreactivity (CRF-LI) in cerebrospinal fluid (CSF) when compared to normal healthy volunteers and patients with schizophrenia or dementia.

Samples of CSF were obtained by lumbar puncture at 9:00 a.m. from normal controls [number of subjects (n = 10), mean age ± standard error of the mean ($\overline{X}$ = 34.2 ± 3.2 years); age range (21 to 55 years)] and patients who were drug-free for at least 2 weeks and met one of the following Diagnostic and Statistical Manual of Mental Disorders (DSM-III)(*10*) diagnoses: major depression (n = 23, $\overline{X}$ = 47.7 ± 3.0 years; 25 to 70 years), schizophrenia (n = 11, $\overline{X}$ = 46.6 ± 3.7 years; 27 to 66 years), or senile dementia (primary degenerative dementia or multi-infarct dementia, or both, n = 29, $\overline{X}$ = 81.0 ± 1.0 years; 73 to 92 years) as previously described (*11*). The CSF samples were frozen and stored at −80°C, coded, and assayed by members of the research team ignorant of the diagnostic identity of the samples. Duplicate 400-μl CSF samples were lyophilized and reconstituted in radioimmunoassay buffer. The CRF concentration was measured by a specific radioimmunoassay as described by Vale *et al.* (*12*) with the use of an antiserum $_0$C30 raised in rabbits against ovine CRF and a tracer of ^{125}I-Tyr^0-rat CRF prepared with chloramine-T and purified by high-performance liquid chromatography (HPLC). Optimal binding of the iodinated CRF tracer to the CRF antiserum was determined and that dilution (1:72,000) was used in the assay. The only modification in the CRF radioimmunoassay previously described (*12*) was our use of goat antiserum to rabbit immunoglobulin G instead of Pansorbin to precipitate the CRF-antiserum complex. Sample radioactivity was counted on a LKB Rackgamma II gamma counter with a counting efficiency of 66 percent for ^{125}I. The sensitivity of the CRF radioimmunoassay was 2.5 pg per tube. No other known endogenous mammalian peptides cross-react with this CRF antiserum (*13*).

The results (see Fig. 1) were statistically analyzed by both parametric [analysis of variance (ANOVA) and Student-Newman-Keuls test] and nonparametric (Mann-Whitney U test) methods (*13*). The CSF concentration of CRF-LI was significantly increased (by both methods of statistical analysis) in patients with major depression compared to either the normal controls or the patients with schizophrenia or senile dementia. Of the 23 patients with major depression, 11 had concentrations of CRF-LI greater than the highest value obtained in the normal control group. Dexamethasone suppression tests (1 mg administered orally at 10 p.m. with the serum cortisol measured at baseline and at 4 p.m. and 10 p.m. the following day) were conducted in the depressed patients as well as in the normal controls. No significant correlations were observed between CSF CRF-LI concentrations and either baseline or post-dexamethasone plasma cortisol concentrations. No significant correlations were observed between CSF CRF-LI and either age or sex of the patient.

These results support the hypothesis that the HPA hyperactivity observed in patients with major depression is due, at least in part, to hypersecretion of CRF. However, it is important to recognize that the relation between CSF CRF-LI and functional activity of central CRF-containing neural systems is unclear. It is also of interest to note that CRF is localized in CNS regions (for example, hypothalamus, amygdala, and other limbic areas) (*14*) that are believed to be pathophysiologically altered in depression. Moreover, intracerebroventricularly administered CRF produces increased emotionality (*15*), increased sympathetic nervous system activity (*16*), reduced sexual behavior (*17*), and reduced appetitive behavior (*18*). Many of these functional effects of CRF are characteristic signs of human depression (*19*). Whether the elevated CSF concentrations of CRF-LI are associated with the "state" of being depressed [as has been reported for the dexamethasone suppression test (*20*)] or whether it is conversely associated with the "trait" of vulnerability to depression is an important issue that remains to be elucidated.

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21. We thank G. Daughtry for aid in statistical analysis and M. Lassiter for typing the manuscript. This work was supported by grants MH-39415 and MH-36157 from the National Institute of Mental Health.

8 June 1984; accepted 6 September 1984

Amino-Terminal Amino Acid Sequence of the Silkworm Prothoracicotropic Hormone: Homology with Insulin

Abstract. *Three molecular forms of prothoracicotropic hormone were isolated from the head of the adult silkworm,* Bombyx mori, *and the amino acid sequence of 19 amino acid residues in the amino terminus of these prothoracicotropic hormones was determined. These residues exhibit significant homology with insulin and insulin-like growth factors.*

Kopec (*1*) first described a factor from the brain of the gypsy moth, *Lymantria dispar,* that causes ecdysis and metamorphosis. Subsequent studies have shown that the brain secretes prothoracicotropic hormone (PTTH), which acts on the prothoracic glands to stimulate release of ecdysone (*2*). The head of the adult silkworm, *Bombyx mori,* contains two kinds of PTTH's with different molecular weights, previously named PTTH-S and PTTH-B according to species specificity (*3*) and now called 4K PTTH (molecular weight ~4400) and 22K PTTH (molecular weight ~22,000). The 4K PTTH is further composed of heterogeneous molecular species, one of which, designated 4K PTTH-I, has been isolated and partially characterized (*4*). We report here the isolation of two new 4K PTTH's, designated 4K PTTH-II and -III, and the NH_2-terminal amino acid sequences of 4K PTTH-I, -II, and -III.

The 4K PTTH-I (50 μg) was isolated from 648,000 heads of male adult *Bombyx* (4.86 kg) by a 15-step purification procedure involving heat-treatment, fractional precipitations, gel filtrations, ion-exchange chromatographies, and high-performance liquid chromatography (HPLC) (*4, 5*). The 4K PTTH's contain one or more disulfide bonds as the biological activity of the crude preparation (involving 4K PTTH-I, -II, and -III) was lost upon treatment with mercaptoethanol or dithiothreitol (*4, 6, 7*). Amino acid composition (molar ratios) of 4K PTTH-I is Asx(4), Thr(3), Ser(1), Glx(4), Pro(1), Gly(4), Ala(3), Cys(4), Val(4-5), Leu(5-6), Phe(2), Tyr(1-2), His(1), and Arg(3). Glycine was determined to be its NH_2-terminal residue by identification of the dansyl derivative (*4*). Injection of 0.1 ng of 4K PTTH-I induced adult development in a pupa of *Samia cynthia ricini* from which the brain had been removed (*8*). At a concentration of $1 \times 10^{-11} M$ (*4*), it could also activate a *Samia* prothoracic gland cultured in vitro.

The 4K PTTH-II and -III were separated from 4K PTTH-I in the 13th step of the procedure by ion-exchange chromatography on DEAE-Sepharose CL-6B (Pharmacia) followed by elution with a gradient of 0.1 to 0.5*M* sodium chloride (*4, 5*). The 4K PTTH-I, -II, and -III were eluted from this column at 0.28, 0.30, and 0.33*M* sodium chloride, respectively. As in the case of 4K PTTH-I, reversed-phase HPLC on Develosil ODS-5 (Nomura-Kagaku) led to the isolation of 4K PTTH-II (36 μg) and -III (63 μg), which showed activity in a *Samia* pupa from which the brain had been removed at doses of 0.4 and 0.1 ng, respectively (Table 1).

The NH_2-terminal amino acid sequences of 4K PTTH-I, -II, and -III were determined by Edman degradation with the use of an Applied Biosystem model 470A gas phase sequencer. The phenylthiohydantoin (PTH) derivatives of the amino acids obtained at each cycle of the Edman degradation were identified by reversed-phase HPLC on an Ultrasphere ODS (Altex) column with a gradient of acetonitrile (10 to 50 percent) in 10 m*M* sodium acetate buffer (*p*H 4.5). Analyses were performed with 2.0 nmol each of 4K PTTH-I, -II, and -III. PTH–amino acids from cycles 1 to 19 were unambiguously determined by this method, although PTH–amino acids were not distinctly identified at cycles 6, 7, and 11 for 4K PTTH-I and -II, and at cycles 6, 7,

Table 1. Isolation of 4K PTTH-I, -II, and -III.

Purification step	Weight (mg)	Total activity* (10^3 *Samia* units)	Specific activity (nanograms per *Samia* unit)
Saline extract	$1{,}108 \times 10^3$	6,480	171,000
Crude 4K PTTH	19,400	6,480	3,000
Highly purified 4K PTTH HPLC	37.6	6,480	5.8
4K PTTH-I	0.050	520	0.1
4K PTTH-II	0.036	90	0.4
4K PTTH-III	0.063	630	0.1

*Bioassay. The samples [dissolved in 0.1*M* tris-HCl (*p*H 7.8) or 0.2*M* ammonium acetate] were injected routinely into five pupae of the saturniid moth *Samia cynthia ricini* that had aged 3 to 8 months after removal of the brain. When the sample possessed PTTH activity, the injected pupae underwent wing apolysis in 4 to 7 days. The activity is expressed in terms of the *Samia* unit, which is the minimum amount causing adult development in an assay pupa (*8*).

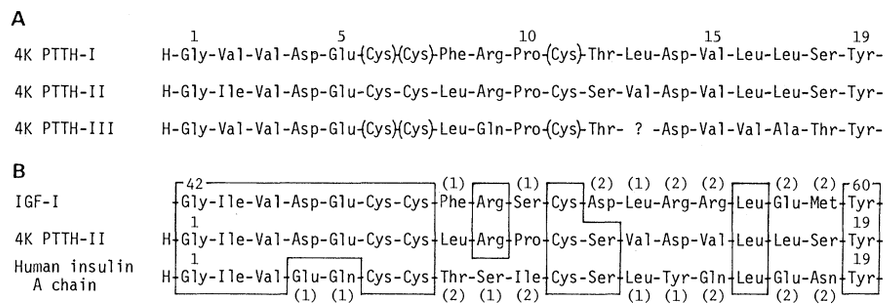

Fig. 1. (A) Amino-terminal amino acid sequence of 4K PTTH-I, -II, and -III. Cysteine residues at positions 6, 7, and 11 in 4K PTTH-I and -III have not yet been identified fully. (B) Homology between 4K PTTH-II and insulin-like growth factor I (IGF-I) and human insulin A chain. The boxed sections indicate identical amino acids. Numbers in parentheses are the minimum number of nucleotide base changes required to generate the amino acid substitution from that in 4K PTTH-II.