Elevated Plasma Thymopoietin Associated with Therapeutic Nonresponsiveness in Major Depression

Gideon Goldstein, Maurizio Fava, Michael Culler, Alan Fisher, Karl Rickels, R. Bruce Lydiard, and Jerrold Rosenbaum

Background: Stress predisposes to major depression, and hyperactivity of the stress-activated hypothalamic-pituitary-adrenal (HPA) axis occurs in this disease. Thymopoietin, an active fragment of thymopoietin (TP), reduces endocrine and behavioral responses to experimental stress, possibly by lowering plasma TP (pTP) levels.

Methods: Plasma TP and the HPA hormones arginine vasopressin (pAVP), adrenocorticotropic hormone (pACTH), and plasma cortisol (pCORT) were measured in 21 untreated depressed patients and 21 matched control subjects. Clinical responses to antidepressants were evaluated in 17 depressed patients.

Results: Plasma TP was elevated in depression (p < .002), with in 8 out of 21 (38%) depressed patients having significant elevations (p < .03). For 17 patients whose antidepressant responses were evaluated, nonresponsiveness occurred in 6 out of 7 (86%) with elevated pTP (>7.5 pg/mL) versus 3 out of 10 (30%) with normal pTP (p < .05).

Conclusions: The significant association of elevated pTP with nonresponsiveness to antidepressant drugs may signify a distinct pathogenesis for the depression of patients with elevated pTP. Biol Psychiatry 2000;48: 65–69 © 2000 Society of Biological Psychiatry

Key Words: Major depression, plasma thymopoietin, plasma arginine vasopressin, pulsatile secretion, therapeutic response, stress

Introduction

Early adverse life events and chronic stress predispose to major depression (Heim et al 1997), a highly prevalent disease causing extensive morbidity (Kessler et al 1994; Lopez and Murray 1998). The synthetic thymopoietin (TP)-derived peptide thymopentin (Goldstein et al 1979) and its analog IRI-514 attenuate experimental stress responses in rats (Klusa et al 1990; Menzaghi et al 1996) and also appear to lower levels of pTP (unpublished data). Thus, plasma thymopoietin (pTP), which circulates as a 20 kd protein corresponding to the N-terminal 187 amino acids of larger intracellular forms of TP (Berger et al 1996; Harris et al 1994), may be involved in stress responses associated with depression.

Corticotropin releasing factor (CRF) is a critical mediator of behavioral stress responses within the brain (Koob et al 1993), and activation of the hypothalamic-pituitary-adrenal (HPA) axis is a peripheral manifestation of CRF hypersecretion in major depression (Owens and Nemeroff 1993). Although antidepressant drugs are widely used and effective, nonresponse to antidepressant treatment of adequate dose and duration is observed in 19% to 34% of patients (Fava and Davidson 1996; Keller et al 1995). We studied the plasma levels of TP and of the HPA hormones arginine vasopressin (AVP), adrenocorticotropic hormone (ACTH), and cortisol as possible biologic correlates of depression or responsiveness to antidepressant drugs.

Methods and Materials

Study Population

Twenty-one untreated outpatients (11 men, 10 women) fulfilling the DSM-III-R criteria for a current major depressive episode lasting at least 1 month were studied along with 21 age-matched (within 2 years) and gender-matched normal control subjects. Depressed patients were free of antidepressant drugs for 1 month before obtaining the plasma samples. Routine hematology, blood chemistry, and urinalysis tests were performed. A medical history, complete physical exam, and psychiatric evaluation with the structured clinical interview for DSM-III-R (SCID-P) were obtained for all subjects plus the 17-point Hamilton Depression Rating Scale (HDRS). A depression rating greater than 16 was required at entry. The protocol was approved by Institutional Review Boards, and all subjects signed Informed Consent forms. The 17 depressed study subjects from the Depression Clinical and Research Program of the Massachusetts General Hospital were entered into one of two trials involving open treatment for 8 weeks with fluoxetine 20 mg daily or nortriptyline 50–150 mg...
daily to maintain a therapeutic plasma range of 50–150 ng/mL. Response data from these patients was utilized to determine, in a blinded fashion, the clinical responses to drug treatment, using an intent-to-treat analysis. A full response required a decline of the HDRS 50% to 7 and a partial response required a 25% to 50% decline in depression rating (Fava et al 1994).

Immunoassay Procedures
Two 90-mL blood samples, the first drawn between 8 and 9 AM and the second between 4 and 6 PM, were drawn into prechilled EDTA-vacutainer tubes, centrifuged in the cold, and the plasma aliquoted into chilled polypropylene tubes and stored at 2–70°C to 2–80°C. Commercial radioimmunoassay kits from Diagnostic Systems Laboratories (Webster, TX; AVP) and ICN Biomedicals (Costa Mesa, CA; cortisol) were used in accordance with the manufacturers’ instructions, and ACTH was measured as previously described (Nicholson et al 1984) using reagents from IgG Corp. (Nashville). pTP was measured by ELISA as previously described (Goldstein and Culler 1997). Briefly, plasma was extracted using a C-18 SepPak and assayed with a sandwich ELISA using affinity purified rabbit antibody to synthetic TP1–19 for capture and mouse monoclonal antibody to synthetic TP30–50 (6E10) as the detector antibody. Synthetic TP1–50 was used as a standard. More recently, a direct high-sensitivity plasma TP assay has been developed in collaboration with R&D Systems (Minneapolis).

Statistical Analyses
Statistical analyses were performed on Prism Version 3.0, GraphPad Software (San Diego) unless otherwise specified. The significance of depression and control subjects and AM/PM differences was assessed by repeated measures two-way ANOVA using SAS (SAS Institute, Cary, NC). With no significant differences in AM/PM values, the mean AM/PM values in each subject were used for subsequent comparisons. Between-group differences were assessed with a two-tailed t test with Welch’s correction. Correlation of TP and AVP values in depression was assessed with the Spearman correlation coefficient. pTP and pAVP distributions tested as Gaussian and cutoff values of mean ± 1.645 SD in control subjects were used to define significant elevations (p < .05). Fisher’s exact test, two-tailed, was used to analyze 2 × 2 contingency tables; p > .05 was considered nonsignificant.

Results
Study Subjects
Twenty-one depressed subjects (22 men, 20 women) aged 40 ± 12 (mean ± SD) and 21 matched control subjects with identical age and gender distributions were entered into the study. HDRS scores at entry were 23 ± 4 in depressed patients and 1 ± 1 in control subjects (mean ± SD; Table 1).

Comparisons of Depressed and Control Subjects and AM/PM Differences
Plasma TP (p < .002) and pAVP (p < .002) levels were significantly elevated in depressed subjects but there were no significant between-group differences in pACTH or pCORT levels. None of the hormones showed significant AM/PM differences nor was there a significant group-by-time interaction. The mean AM/PM values for each parameter similarly reflected these differences (Table 1).

Incidence of Elevation of pTP and pAVP Levels in Depression
Using cut-off values of 7.5 pg/mL for pTP and 5.0 pg/mL for pAVP, pTP was elevated in 8 out of 21 (38%) depressed subjects versus 1 out of 21 (5%) control subjects (p < .03; Figure 1), and pAVP was elevated in 6 out of 21 (29%) depressed subjects versus 1 out of 21 (5%) control subjects (ns).

Table 1. HDRS Scores and pTP, pAVP, pACTH, and pCORT Levels in 21 Depressed Subjects and 21 Matched Healthy Control Subjects

<table>
<thead>
<tr>
<th></th>
<th>Control subjects (mean ± SD)</th>
<th>Depressed subjects (mean ± SD)</th>
<th>p value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>HDRS</td>
<td>1 ± 1</td>
<td>23 ± 4</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>pTP (pg/mL)</td>
<td>5.2 ± 1.4</td>
<td>7.7 ± 3.0</td>
<td>.002</td>
</tr>
<tr>
<td>pAVP (pg/mL)</td>
<td>2.9 ± 1.3</td>
<td>4.9 ± 2.4</td>
<td>.002</td>
</tr>
<tr>
<td>pACTH (pg/mL)</td>
<td>17 ± 9</td>
<td>17 ± 9</td>
<td>ns</td>
</tr>
<tr>
<td>pCORT (pg/mL)</td>
<td>14 ± 9</td>
<td>14 ± 6</td>
<td>ns</td>
</tr>
</tbody>
</table>

HDRS, Hamilton Depression Rating Scale; pTP, plasma thymopoietin; pAVP, plasma arginine vasopressin; pACTH, plasma adenocorticotropic hormone; pCORT, plasma cortisol.

* Two-tailed t test with Welch’s correction.
Decreased Clinical Response Rate in Subjects with Elevated pTP

Among depressed subjects, 41% (7 out of 17) of those treated with fluoxetine (13) or nortryptiline (4) had full (5) or partial (3) responses. 86% (6 out of 7) of patients with elevated pTP were nonresponders versus 30% (3 out of 10) nonresponders in patients with normal pTP \((p, .05; \text{Figure 2})\). The response rates for subjects divided on the basis of pAVP levels did not differ statistically.

Relationship between pAVP and pTP in Depressed Subjects

Plasma AVP elevations were found in 3 out of 8 (38%) subjects with elevated pTP versus 3 out of 13 (23%) subjects with normal pTP \((p < .05; \text{Figure 2})\). The response rates for subjects divided on the basis of pAVP levels did not differ statistically.

Discussion

Early morning and late afternoon plasma levels of the HPA hormones ACTH and cortisol did not prove useful diagnostic measures in major depression. AVP secretion is pulsatile \((\text{Redekopp et al 1986; Wood et al 1994})\) and HPA hyperactivity in depression is related to a greater frequency of episodic hormone release \((\text{Deuschle et al 1997})\). This can be gauged by frequent sampling over 24 hours \((\text{Deuschle et al 1997})\) or by measuring 24-hour urinary cortisol secretion \((\text{Carroll et al 1976})\). These methods have not found widespread acceptance in clinical practice, however. Using only two samples, we found an increase of 48% in mean pAVP level in depression, a 29% incidence of patients with elevated pAVP, and no detectable diurnal rhythm, findings similar to those of van Londen et al \((1997)\), with a mean pAVP increase of 68%, an incidence of elevated pAVP of 31% and no detectable diurnal rhythm. In contrast to our findings of no depression related changes in pCORT, however, van Londen et al \((1997)\) found a 20% elevation of mean plasma cortisol in depression and a statistically detectable diurnal rhythm in their study of 52 depressed patients and 37 control subjects. Depression-related or diurnal changes in pCORT appear small in relation to individual variation and apparently require large numbers of subjects for statistical detection. pAVP elevations in depression probably reflect the increased statistical probability of sampling higher levels during longer and more frequent secretion pulses of AVP, and patients with elevated pAVP do not appear to represent a biologically distinct group. Accordingly, there was no significant correlation of pAVP elevation with the clinical response status of patients treated with antidepressant drugs.

In contrast, pTP was elevated in 7 out of 17 depressed subjects whose clinical responses to antidepressant drugs were evaluated, and most of the subjects with elevated pTP failed to respond to fluoxetine or nortryptyline. Nonresponse to antidepressant drugs is found in 19% to 34% of depressed subjects \((\text{Fava and Davidson 1996; Keller et al 1995})\), and it was interesting that pTP elevations were present in 6 out of 9 (67%) nonresponders but only 1 out of 8 (13%) full or partial responders. Fluoxetine and nortriptyline modify CRF and AVP release by regulating serotonin pathways, catecholamine pathways, or both \((\text{De Bellis et al 1993; Veith et al 1993})\). Nonetheless, CRF and AVP release can be triggered through alternative pathways \((\text{Plotsky et al 1988})\) and, if TP were such a serotonin- and catecholamine-independent stress mediator, it could explain our finding of poor therapeutic responses in depressed patients with elevated pTP. Animal data provide some additional clues. Thymopentin pretreatment in mice and rats inhibited stress-induced behavioral changes, alterations in hippocampal GABA, and benzodiazepine receptor densities and elevations of plasma corticosterone levels, but it did not affect these parameters when administered alone \((\text{Klusa et al 1990})\). IRI-514, an analog of thymopentin, was similarly stress-protective \((\text{Menzaghi et al 1996})\). IRI-514 dose-dependently reversed the behavior induced by social stress if administered at least 1 day earlier, and the effect lasted for several days. Because small peptides are eliminated rapidly \((\text{Adsumali et al 1996})\), direct action of the peptide could not account for these long-lasting modulatory effects on behavior. pTP was measured in normal human volunteers during a phase I study of an orally active
thymopentin analog, IRI-695 (Adsumali et al 1996), and statistically significant decreases in pTP were found starting 12 hours after a single oral dose and persisting through 2 days, the last time point measured (GG, unpublished observations). Thus the behavioral effects of thymopentin and its analogs may be mediated via reduction of pTP levels, and pTP may be a mediator or regulator of stress-induced behavioral changes.

There is a growing literature on intranuclear proteins encoded by the thymopoietin gene, which are involved in cell cycle regulation of nuclear organization (Alsheimer et al 1998; Berger et al 1996; Harris et al 1995; Weber et al 1999). Nonetheless, the immunoassay described in this report is novel and the physiological role of pTP awaits elucidation. The lack of correlation of pTP and pAVP in depression suggests that pTP is not a component of the HPA system although the relation of pTP to stress remains to be elucidated. The levels of pTP in the psychiatrically evaluated control subjects in our study were similar to those of a randomly selected sample of 40 healthy volunteers, and there were no detectable age- or gender-related effects on pTP levels (GG, unpublished observations). There are presently no systematic studies of pTP levels in other disorders.

Although the high incidence of nonresponders in subjects with elevated pTP was statistically significant, the finding is not robust because the small sample size enhances the possibility of a Type I statistical error. If the present findings prove reproducible, pTP assay could be useful in the study of depression. Drugs related to thymopentin may offer new therapeutic possibilities for patients with elevated pTP who are nonresponsive to standard antidepressant drugs.

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References


