Dysregulation of plasma pro-opiomelanocortin-related peptides in neurotic depression

Andrea R. Genazzani, Felice Petraglia¹, Elena Sinforiani², Francesca Brambilla³, Fabio Facchinetti and Giuseppe Nappi²

Department of Obstetrics and Gynaecology, University of Modena, Neurological Institute² C. Mondino, University of Pavia, Psychiatric Hospital³, P. Pini, Milan, Italy

Abstract. In order to assess whether a central hypothalamic impairment could account for the pro-opiomelanocortin (POMC)-related peptide over-secretion in depressive disorders, plasma B-lipotropin (B-LPH), B-endorphin (B-EP) and cortisol concentrations were measured in 9 patients affected by neurotic depression: a) every 4 h over a 24-h period; b) in response to insulin-induced hypoglycaemia (0.1 IU/kg body weight), and c) during dexamethasone (DXM) administration (0.5 mg × 4/day for 2 days). Eight age-matched healthy volunteers (controls) were also studied. B-EP and B-LPH were determined by specific radioimmunoassays after plasma extraction and gel chromatography. Compared with the controls, the patients showed a 3 times higher plasma B-EP, twice the normal B-LPH levels, and a 20% cortisol increase. The neurotic depressed patients showed and evening-related decrease in the levels of the 3 hormones, expressed as mean values, similar to that in the controls, whereas the single cosinor analysis revealed a significant circadian rhythm of B-LPH and B-EP only in 3 and 2 patients, respectively. Insulin-induced hypoglycaemia (ITT) stimulated the release of B-LPH and cortisol in both groups, whereas the B-EP increase was absent in the patients. DXM reduced plasma cortisol and B-LPH levels in controls and patients, but in the latter it failed to reduce the B-EP concentrations. The present data indicate that neurotic depressed patients are characterized by increased activity of the hypothalamic-pituitary-adrenal axis, with maintained circadian rhythmicity. The B-EP unresponsiveness to ITT and DXM contradicts the normal responses of B-LPH and cortisol to the same tests and suggests a dysregulation of plasma B-EP secretion in neurotic depressed patients.

An impaired glucocorticoid sensitivity of hypothalamic neurons secreting corticotropin-releasing factor (CRF), leading to hyperactivity of pituitary corticotropes and the adrenal gland has been demonstrated in depressed patients (Schildkraut 1965; Sachar et al. 1980).

Since the basic papers of Carroll’s group (Carroll 1977; Carroll et al. 1981) defining the early escape of plasma cortisol from dexamethasone (DXM) suppression as a typical phenomenon in melancholia, several investigators have studied the functional activity of the hypothalamic-pituitary-adrenal axis in affective disorders. More recently it has been shown that besides ACTH, other peptides, namely B-lipotropin (B-LPH) and B-endorphin (B-EP), are synthesized in the pituitary corticotropes from the same parent molecule, pro-opiomelanocortin (POMC) (Grossman & Rees 1983).

Few data, however, are available on the circulating POMC-related peptide plasma levels in patients with affective disorders, such as ‘borderline’ depressed patients who also present a high prevalence of neuroendocrine abnormalities (Sternbach et al. 1983).

Increased B-EP and B-LPH concentrations were found to co-exist with normal ACTH levels in a first series of patients of both sexes suffering from ‘secondary’ affective disorders (Brambilla et al. 1981) and these observations were later corroborated by similar findings in women whose 're-
active' depressive symptomatology started before or after the menopause (Genazzani et al. 1982).

The present paper reports the circadian variations of circulating B-EP, B-LPH and cortisol levels as well as their responsiveness to dexamethasone suppression or insulin-induced hypoglycaemia in a group of male patients affected by neurotic depression according to DSM III criteria.

Materials and Methods

Subjects

Nine males, aged 27–60 years, affected by depressive neurosis with a 2- to 5-year history of the disease and 8 healthy volunteers (controls) aged 25–53 years, were studied. All of them were hospitalized at least 5 days before starting the protocol. The diagnosis was assessed according to DSM III depressive sub-classes, and patients fell into the 300.40 diagnostic category: depressive neurosis (dysthymic disorders). Patients and controls were interviewed with relatives. The Hamilton Rating Scale for Depression (Hamilton 1960) was applied, and the patients’ scores ranged from 14 to 21, equivalent to a mild depression (Couch & Hassanein 1979). Only one patient had a family history of neurotic depressive disorders, whereas another presented a family history of alcoholism. The length of the current clinical episode ranged from 2 months to 5 years. Six patients had not had any spontaneous remissions since the onset of the disease, whereas in the remaining 3 patients it was possible to discriminate periods of normal mood, lasting less than one month.

All patients were drug-free for at least 10 days prior to the study. They had previously been treated with tricyclic antidepressants and benzodiazepines. Most patients presented minor sleep disturbances such as difficulty in falling asleep and/or frequent awakenings during the night.

Experimental protocol

In order to study the circadian variability of the various hormones in all subjects, blood samples were taken every 4 h for 24 h starting at 08.00 h. On the third day they were submitted to insulin-tolerance test (ITT) (0.1 IU/kg body weight) and blood samples were collected prior to, and 15, 30, 45, 60, 90 and 120 min after iv insulin injection. On the fourth day at 08.00 h, both groups were given 0.5 mg of dexamethasone treatment and in the subsequent 24 h. To avoid the stress related to venous puncture, blood samples for both the circadian rhythm measurements and the insulin-tolerance test were collected by an iv cannula inserted in a forearm vein connected to a 3-way stopcock, and main-

Hormone assays

B-EP and B-LPH plasma levels were evaluated by two specific RIAIs after silicic acid extraction and Sephadex G-75 column (45 × 1.5 cm) chromatography, essentially as described previously (Brambilla et al. 1981; Faccinetti et al. 1983). The recovery of 200 pg of standard B-LPH and B-EP added to 15 samples of pooled plasma from healthy volunteers was 70.4 ± 5.7% and 76.1 ± 7.6%, respectively. Plasma B-EP and B-LPH concentrations were expressed as fmol/ml and corrected for the recovery estimates.

Highly purified human B-LPH, anti-B-LPH (N-terminal) and anti-B-EP (C-terminal) sera were kindly supplied by Prof. C. H. Li (San Francisco, CA). Synthetic B-EP was provided by Organon (Oss, NL).

Anti-B-LPH serum cross-reacted 100% with γ-LPH and 16% with B-EP. Anti-B-EP serum fully cross-reacts with B-LPH, but does not recognize 1–16, 1–17, 1–26 and 1–27 endorphines.

The peptides were labelled by the chloramine T method (Greenwood et al. 1965). The sensitivity of both RIAs was 1 fmol. Inter- and intra-assay coefficients of variation were 9.5 ± 1.5% (B-LPH) and 9.0 ± 1.0% (B-EP) and 6.1 ± 0.3% (B-LPH) and 5.5 ± 1.0% (B-EP), respectively.

Cortisol plasma levels were assayed by RIA utilizing the commercially available materials supplied by RADIM (Roma, Italy); the assay sensitivity was 2 ng, and inter- and intra-assay coefficients of variation were 8.5 ± 1.1% and 5.0 ± 0.3%, respectively. Plasma cortisol levels were expressed in ng/ml.

Statistical analysis

The results were evaluated using the Student’s t-test for paired and unpaired data and ANOVA. The study of circadian variations was also performed by means of single cosinor analysis (Halberg 1969).

Results

For each subject, the ‘basal’ values were calculated, as the average value of the single levels measured in the 4 blood samples collected at 08.00 h during the circadian rhythm study, before ITT and DXM administration. In the patients, all basal hormone values were significantly higher than in the
Morning basal concentrations (m ± sd) of B-endorphin (B-EP), B-lipotropin (B-LPH), and cortisol in controls and neurotic depressed patients (closed bars). In each patient the values originate from the mean of 4 samples taken on different days. One asterisk: $P < 0.05$. Two asterisks: $P < 0.01$.

Healthy controls (Fig. 1). In particular, the B-EP values were 3 times and the B-LPH twice those of the normal volunteers. Cortisol levels exceeded the control values by about 20%.

In the healthy controls, the 24-h secretory pattern of the 3 hormones confirmed a significant reduction of B-EP, B-LPH and cortisol at 20.00 and 24.00 h in comparison with the morning values. Depressed patients showed a concomitant significant reduction of the 3 hormones at the same times (Fig. 2). It should be pointed out that cortisol nadir values were similar in both groups despite the significant difference observed in the morning samples.

The evaluation of the same data using a single cosinor analysis confirmed a significant secretory rhythm of each hormone in all the control subjects, whereas in the 9 patients a significant circadian rhythmicity of cortisol was found only in 6, of B-LPH in 3, and of B-EP in 2.

Insulin administration was accompanied by a similar fall of glucose and rise of cortisol and B-LPH in both groups (Fig. 3). In the controls, B-EP increased progressively to reach the highest concentration after 60 min. On the other hand, no significant changes in the B-EP plasma levels were observed in the depressed patients (Fig. 3).

In the control, a DXM load induced a concomitant significant inhibition of B-EP, B-LPH and cortisol, which remained suppressed till the end of the observation period (Fig. 4). In the depressed patients, DXM administration was followed by a prompt and significant inhibition of B-LPH and cortisol levels, which remained suppressed, the former till the 6th h and the latter until 18th h after the last DXM tablet. At that time a significant rise ($P < 0.05$) of B-LPH levels was observed, but the concentrations still remained significantly lower than in the basal condition ($P < 0.05$). In all patients the B-EP levels were unaffected by DXM administration or withdrawal (Fig. 4).

**Discussion**

The present data indicate that patients affected by depressive neurosis are characterized by B-EP, B-LPH and cortisol baseline values significantly higher than in healthy controls. Moreover, it is
Responses of the various hormones to insulin-induced hypoglycaemia (0.1 IU/kg body weight) in controls (C, dotted line) and neurotic depressed patients (D, solid line). Results are expressed as M ± SD. The asterisk indicates significant changes in comparison with baseline values (P < 0.01).

Fig. 3.

Interesting that whereas the B-LPH levels in depressed patients are twice those of controls, the B-EP levels reach values 3 to 4 times as high.

Therefore, in neurotic depression, a hypersecretion of POMC-derived pituitary hormones may co-exist with changes in the enzymatic breakdown of POMC in the anterior pituitary or with further sources contributing to the pool of circulating B-EP. In fact, the synthesis of B-EP has been established also in organs other than the anterior pituitary and the brain, such as the pancreas, (Bruni et al. 1979), testes (Tsong et al. 1982), ovarian follicle (Petraglia et al. 1984), and the vestigial cell layer of the pituitary-intermediate lobe (Shibasaki et al. 1981). The contribution of these organs to the peripheral B-EP levels is not known. The finding that the cortisol increase in our patients is less pronounced than that of B-LPH and B-EP may be attributed to the adaptive phenomena occurring in the chronically hyperstimulated adrenal gland.

The observation of an evening reduction of the mean circulating levels of the 3 hormones in depressed patients sustains a normal function of the central chronobiological regulation of POMC secretion. However, the single cosinor evaluation showed that the circadian rhythm of B-EP and
B-LPH was altered in several patients. This suggests that when single neurotic depressed patients are evaluated through systems ad hoc developed, to assess biological rhythms, a dysregulation of their hypothalamic-pituitary-adrenal (HPA) axis may be revealed. Disturbances in the circadian rhythmicity have been recently reported also in major depressive disorder patients in whom cortisol nadir occurred 3 h earlier than in controls (Linkoski et al. 1985).

The insulin-induced hypoglycaemia, despite similar glucose, cortisol and B-LPH responses in depressed and control subjects, was characterized in the former group by the lack of any significant B-EP increase.

Only one patient showed a concomitant B-EP and B-LPH rise after ITT. This corroborates the hypothesis of specific disorders in the pituitary secretion of the 2 POMC-related peptides in neurotic depression.

The fact that DXM administration promptly inhibited circulating B-EP, B-LPH and cortisol levels in the controls and did not change the B-EP levels in the depressed patients suggests that part of the B-EP is released through a CRF-independent mechanism. This finding goes against the general knowledge that in healthy subjects the basal B-EP and B-LPH levels circulate in equimolar amounts with circadian rhythmicity (Petraglia et al. 1983) from prepubertal (Genazzani et al. 1983) to old age (Facchinetti et al. 1983) and that stressful stimuli (Smith et al. 1981) and exogenously administered CRF (Grossman et al. 1982; Mc Loughlin et al. 1984) induce a concomitant release of the two peptides.

Similarly, in endocrine disease with corticotrophes hyperactivity, such as Nelson's syndrome and Cushing's disease (Ratter et al. 1983) and in endogenous depression (Brambilla et al. 1981) B-EP and B-LPH circulate in equimolar amounts, even though at higher levels than in healthy subjects. The recent finding that adolescent obese children are hyperendorphinaemic and loose the B-EP rhythmicity, while maintaining that of B-LPH and cortisol (Genazzani et al. 1986), corroborates the hypothesis of the existence of pathological conditions characterized by circulating B-EP levels apparently independent of CRF control.

The discrepancy in basal B-EP and B-LPH levels and their different trends after ITT and under dexamethasone offer a biochemical difference between neurotic and endogenous depression where general hyperactivity of the HPA axis and reduced sensitivity to glucocorticoid feedback have been demonstrated (Carroll 1977; Brambilla et al. 1981). The cause of the B-EP hypersecretion and its glucocorticoid non-suppressibility in neurotic depressed patients remains to be elucidated.

References


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