Anxious–Retarded Depression: Relation with Plasma Vasopressin and Cortisol

Remco FP de Winter[1,2], Albert M van Hemert[1,2], Roel H DeRijk[1,2], Koos H Zwinderman[3], Ank C Frankhuijzen-Sierevogel[4], Victor M Wiegant[4] and Jaap G Goekoop*[1,2]

[1]Rijngeest Groep, Oegstgeest/Leiden, The Netherlands; [2]Department of Psychiatry, Leiden University Medical Center, Leiden, The Netherlands; [3]Department of Medical Statistics, Leiden University Medical Center, Leiden, The Netherlands; [4]Department of Pharmacology and Anatomy, Rudolf Magnus Institute for Neurosciences, University Medical Center Utrecht, The Netherlands

Dysregulation of the hypothalamus–pituitary–adrenal (HPA) axis is related to melancholic or endogenous depression; however, the strength of this relationship depends on the definition of the specific depression subcategory. A two-dimensionally defined subcategory, anxious–retarded depression, is related to melancholic depression. Since arginine vasopressin (AVP) activates the HPA axis, and both major depression and the melancholic subcategory are associated with elevated plasma AVP levels, we investigated whether the plasma AVP level is also elevated in anxious–retarded depression, melancholic depression and anxious–retarded melancholic depression, and whether plasma AVP and cortisol levels are correlated in these subcategories. A total of 66 patients with major depression not using oral contraception were investigated. Patients with anxious–retarded depression had a highly significant AVP–cortisol correlation, while no such correlation was found in patients with nonanxious–retarded depression. Log-transformed mean plasma AVP values were higher in patients with anxious–retarded depression than in patients with nonanxious–retarded depression. Patients with anxious–retarded melancholic depression also had a significantly elevated level of plasma AVP and a highly significant correlation between plasma AVP and cortisol levels. The correlation was low in patients with melancholic depression. Anxious–retarded depression may be a useful refinement of the melancholic subcategory with regard to dysregulation of the HPA axis and plasma AVP release.
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INTRODUCTION

Hyperactivity of the hypothalamus–pituitary–adrenal (HPA) axis is a robust characteristic of major depression (Holsboer, 1999; Scott and Dinan, 1998). Basal plasma levels of cortisol and urinary excretion of cortisol are raised (Carroll *et al*, 1976a, b), and the secretion of corticotrophin-releasing hormone (CRH) is increased (Holsboer, 1999; Scott and Dinan, 1998). Several studies have shown that the HPA axis is dysregulated in depression. For example, the HPA axis is resistant to suppression by dexamethasone in the dexamethasone suppression test (DST) (Carroll *et al*, 1981), the release of adrenocorticotrophic hormone (ACTH) after CRH challenge is diminished (Thalen *et al*, 1993), and CRH-induced release of ACTH and cortisol is increased after dexamethasone in the Dex–CRH test (Heuser *et al*, 1994). In, on average, 30–50% of depressed patients the HPA axis is not suppressed in the DST, with the highest rates of nonsuppression being found in patients with melancholic, endogenous, familial or psychotic subcategories of depression (Nelson and Davis, 1997; Rush *et al*, 1996). The failure to suppress the HPA axis may be because of reduced negative feedback via glucocorticoid receptors, facilitation of CRH-induced ACTH release, or both. It may be partially mediated by increased CRH release. This suggestion is supported by the finding that cerebrospinal fluid (CSF) levels of CRH are higher in patients in whom the HPA axis is not suppressed than in patients in whom the HPA axis is suppressed in the DST (Pitts *et al*, 1995). Reduced CRH-dependent ACTH release has also been associated with DST nonsuppressor status (Thalen *et al*, 1993), and has been attributed to negative feedback by high basal cortisol levels and/or downregulation of pituitary CRH receptors (Ur *et al*, 1992). The Dex–CRH test is more sensitive than the DST for detecting changes in HPA axis regulation. Depending on age and sex, 90% of depressed patients exhibit increased release of ACTH and cortisol (Heuser *et al*, 1994), possibly as a result of increased CRH release and reduced negative feedback by downregulated glucocorticoid receptors (Modell *et al*, 1997).

An increased release of CRH and reduced glucocorticoid feedback cannot fully explain nonsuppression of the HPA axis in the DST or a positive Dex–CRH test. Numerous

animal data obtained under physiological and pathophysiological conditions support the view that this dysregulation of the HPA axis may involve amplification of the effect of CRH by AVP (Scott and Dinan, 1998). AVP produced by parvocellular and magnocellular hypothalamic neurons synergizes with CRH at the pituitary level to stimulate ACTH release (Antoni, 1993). Chronic psychological stress induces a five-fold increase in the number of AVP-containing CRH neurons (de Goeij et al, 1992). Moreover, highly anxious Wistar rats and old Wistar rats have a positive response in the Dex–CRH test, which appears to depend on an increased synthesis and release of AVP from parvocellular neurons of the hypothalamic paraventricular nucleus (PVN) (Hatzinger et al, 2000; Keck et al, 2002). Pathological conditions such as adrenalectomy or CRH1 receptor deficiency likewise result in an increased number of AVP-containing neurons in the PVN and in the synthesis and release of AVP from the PVN, respectively (Kiss et al, 1984; Muller et al, 2000). Finally, in humans CRH cannot override dexamethasone-induced suppression of the HPA axis, whereas the addition of (lysine)vasopressin leads to nonsuppression of the HPA axis in the DST, like that seen in depressed patients (Von Bardeleben et al, 1985).

Thus AVP has become a peptide of major interest in depression research and may be specifically involved in the hyperactivity of the HPA axis in certain subcategories of major depression. In support of this hypothesis, plasma AVP levels are elevated in patients with major depression and in patients with the melancholic subcategory compared with healthy control subjects (Van Londen et al, 1997). Moreover, plasma AVP and cortisol levels are positively correlated in depressed patients (Inder et al, 1997a). Interestingly, plasma AVP levels are higher in hypercortisolemic depressed patients than in patients with normal cortisol levels and healthy controls (Inder et al, 1997a). The finding that CSF AVP is not significantly elevated in patients in whom the HPA axis is not suppressed in the DST (Pitts et al, 1995) suggests that AVP release may be increased in these individuals. These data suggest that increased AVP release is related to a specific subcategory of depression in which the HPA axis is not suppressed during the DST, rather than to depression in which responses are increased in the Dex–CRH test.

Investigation of DST nonsuppression in subcategories of major depression has shown that the strength of the relationship between nonsuppression and endogenous or melancholic depression depends on the definition used. The nonsuppression rate is lower if the endogenous or melancholic subcategory is defined according to the *Diagnostic and Statistical Manual for Mental Disorders* (DSM)-III (American Psychiatric Association, 1980) or DSM-IV (American Psychiatric Association, 1994) than if melancholic depression is defined according to Research Diagnostic Criteria (RDC) or Newcastle Criteria (Rush and Weissenburger, 1994). In another study, we found that anxious–retarded depression had a significant overlap with melancholic depression according to the DSM-IV (Goekoop et al, 2002 unpublished data). The definition of anxious–retarded depression was based on median scores for two dimensions, anxiety and retardation, and appeared to be associated with a family history of depression (Goekoop et al, 2002 unpublished data).

Since plasma AVP levels are raised in major depression and melancholic depression (Van Londen et al, 1997), the present study primarily investigated whether plasma AVP levels are raised in anxious–retarded depression. Since plasma AVP and cortisol levels are correlated (Inder et al, 1997), we also investigated whether this is the case for anxious–retarded depression. Patients with nonanxious–retarded depression were used as control subjects. We also measured plasma AVP and cortisol levels, and their correlation, in patients with melancholic depression (DSM-IV) and in patients with nonmelancholic depression, hypothesizing that elevated plasma AVP levels and a positive correlation between AVP and cortisol levels would be found in patients with melancholic depression. We further explored whether the relation between plasma AVP level and anxious–retarded depression was due to a relation between plasma AVP level and melancholic depression or *vice versa*. We therefore compared the AVP levels and AVP–cortisol correlation in patients with anxious–retarded–melancholic depression and in the category of all depressed patients.

METHODS

Subjects

A total of 81 patients with major depression recently admitted to the in and outpatient university clinic of the Rijngeest Groep were recruited for a 2-year cross-sectional, prospective follow-up study of the role of stress hormones in the outcome of depression. All patients were referred to the study by the psychiatrist who made the initial diagnosis of major depression. After confirmation of the diagnosis by RFPdeW, using a semistandardized interview, the patient was asked to participate in the study. Written informed consent was obtained for all patients, and the Ethics Committee of Leiden University Medical Center (LUMC) approved the informed consent protocol.

Patients were included if they fulfilled DSM-IV criteria for a major depressive episode (American Psychiatric Association, 1994) and scored at least 21 on the Montgomery Åsberg Depression Rating Scale (MADRS) (Montgomery and Åsberg, 1979). Exclusion criteria were bipolar disorder; treatment with lithium, carbamazepine or valproate; first episode of major depression at age 60 years or older; alcohol or drug abuse or dependence; pregnancy; or clinical evidence of a condition associated with abnormal plasma AVP release, such as the syndrome of Inappropriate Secretion of Anti Diuretic Hormone.

Because short-term drug withdrawal may influence the regulation of the HPA axis (Kraus and Grof, 1985), and because we considered long-term withdrawal to be not feasible as it may lead to a high drop-out rate among patients with severe depression, patients continued to take their prescribed medications during the investigation. In exploratory analyses we confirmed that oral contraceptives decrease plasma vasopressin levels (Ekstrom et al, 1992; Kostoglou-Athanassiou et al, 1998) and increase plasma cortisol levels (Amin et al, 1980). Therefore, 15 patients taking oral contraceptives were excluded, as were patients with depression in relation to panic disorder.

Demographic, Clinical and Treatment Characteristics

Of the 66 depressed patients with a mean age of 41 years (SD = 11.7), 59% were female, 53% had a positive family history, and 56% were outpatients ($n = 37$). The mean number of previous depressive episodes was 1.59 (SD = 1.95). A total of 29 patients experienced their first episode of major depression. The average duration of the current episode was 6.8 (SD = 7.0) months. A total of 31 patients smoked more than one cigarette a day. Altogether, 45 patients did not use alcohol, 20 patients consumed one to three alcoholic beverages daily, and one patient consumed maximally four alcoholic beverages daily in the month before the investigation. Alcohol consumption was thus lower than that associated with an increased risk of depression (five consumptions; Wang and Patten, 2001). All patients refrained from using alcohol for 12 h before the investigation.

Of the 66 patients, nine used a neuroleptic drug, 39 an antidepressant drug, 16 a selective serotonin reuptake inhibitor (SSRI), 15 a serotonergic and noradrenergic reuptake inhibitor (SNRI), eight a tricyclic drug (TCA), and 38 a benzodiazepine. For correlational studies, currently accepted equivalent values of the dosages were computed (Moleman and Birkenhaeger, 1998). Of the patients on antidepressant treatment, five additionally used a neuroleptic plus a benzodiazepine, two a neuroleptic, and 20 a benzodiazepine. Two psychotically depressed patients used only an antipsychotic drug and 13 patients used only a benzodiazepine.

Assessments

Psychopathology RFPdeW performed the psychopathological assessment using a semistandardized interview. This interview encompassed the DSM-IV criteria for depressive disorder, subcategorization of DSM-IV melancholic depression, and the Comprehensive Psychopathological Rating Scale (CPRS) (Åsberg et al, 1978; Goekoop et al, 1992). The CPRS is a widely used scale for the assessment of psychopathological signs and symptoms. The inter-rater reliability is comparable with that of the Present State Examination (Goekoop et al, 1991), and factor analysis in a heterogeneous patient sample has shown that its 65 items can be reduced to five global factors of psychopathology, one of which is a bipolar component (Goekoop et al, 1992). Three of these five components represent nonpsychotic psychopathology. They are called emotional dysregulation, motivational dysregulation (the bipolar component comprising two dimensions of inhibited and disinhibited motivational dysregulation, respectively), and autonomic dysregulation. The two psychotic dimensions are called perceptual disintegration and behavioral disintegration (Goekoop et al, 1992). All six dimensions conform to the Rasch model (Goekoop and Zwinderman, 1994).

Since we were specifically interested in the main dimensions of unipolar depressive disorders, we used three nonpsychotic dimensions: emotional dysregulation, motivational inhibition and autonomic dysregulation, which correlate highly with the MADRS (Goekoop et al, 1994), Salpetrière Retardation Rating Scale (Weme et al, 1996), and Brief Scale for Anxiety (Goekoop et al, 1994). Emotional dysregulation comprises *general neurotic* signs and symptoms of inner tension, concentration difficulties, reported sadness, pessimistic thoughts, reduced sexual interest, inability to feel, reduced sleep, indecision, apparent sadness, fatigability, failing memory, lassitude, reported muscular tension, reduced appetite, phobias, suicidal thoughts, worrying over trifles, compulsive thoughts, depersonalization and derealization. Motivational inhibition comprises the signs and symptoms related to psychomotor *retardation*, namely apparent sadness, inability to feel (particularly anhedonia), slowness of movement, reduced speech and inappropriate emotional expression (including flattening of affect). Autonomic dysregulation comprises signs and symptoms of predominantly somatic *anxiety*, such as inner tension, reported autonomic symptoms, observed muscle tension, reduced sleep, aches and pains, and observed autonomic symptoms. These three global CPRS dimensions correspond to three dimensions detected in anxious–depressive disorders, called physiological hyperarousal, general distress, and anhedonia (Clark and Watson, 1991), and the correlations between the three CPRS dimensions show similar convergent and divergent relations within samples of patients with major depression: emotional dysregulation correlates moderately with autonomic dysregulation as well as with motivational inhibition, while the latter two correlate only poorly (Goekoop et al, 2002 unpublished data). This suggests that emotional dysregulation is a central dimension of depression and that the other two dimensions are involved in distinctions within major depression, such as have been found separately for anxiety (Maes et al, 1994) and psychomotor retardation (Parker et al, 2000). Hierarchic cluster analysis based on dichotomous ratings for the three CPRS dimensions has shown that unipolar depressive disorders can indeed be distinguished in terms of autonomic dysregulation ('anxiety') and motivational inhibition ('retardation') (Goekoop et al, 2002 unpublished data). We therefore used these two dimensions for the present study.

Four subcategories of depression were constructed based on combinations of scores higher or lower than the median for the dimensions autonomic dysregulation ('anxiety') and motivational inhibition ('retardation'). These subcategories were called anxious–retarded, (nonretarded) anxious, retarded (nonanxious), and undifferentiated depression. The anxious–retarded subcategory was selected for the present study because of its high association with the melancholic subcategory (Goekoop et al, 2002 unpublished data, see also Results).

Biochemical assay procedures Within 7 days of the CPRS interview, blood samples were drawn on a single day under standardized rest conditions between 09.00 and 09.30 and between 15.30 and 16.00. All patients refrained from alcohol and abnormal motor activity (sports) during the 12-h period preceding the study. They sat down 15 min before venipuncture; smoking was not allowed for 30 min before venipuncture. Eating and drinking were allowed *ad libitum*.

Blood was collected in 10-ml Vacutainer tubes and immediately stored at 4°C. Within 30 min, plasma was separated and stored at −80°C. Plasma AVP was determined by radioimmunoassay (RIA); the detection limit was 0.5 pg/ml for plasma (extracted assay) and the intra- and interassay coefficients of variation were 9.9 and 15.9%,

respectively. Total plasma cortisol was measured by high-performance liquid chromatography (HPLC) with UV detection as previously described (Van Londen *et al*, 1997); the detection limit was 0.01 mg/l and the intra- and interassay coefficients of variation were 2.9 and 5.8%, respectively. Plasma osmolality was not measured because it is not associated with plasma AVP levels in patients with depression (Van Londen *et al*, 1997).

Statistical Analyses

The association between anxious–retarded depression and melancholic depression was quantified by Cohen's kappa. Plasma AVP and cortisol levels were calculated as the average of the morning and afternoon values. Plasma AVP values were not normally distributed. Therefore, differences between subcategories were analyzed with Mann–Whitney U tests, and correlations with plasma cortisol level and psychotropic drug dosage were analyzed with Spearman's rank correlations. For multivariate analysis, plasma AVP values were log-transformed into ln(AVP). Differences in ln(AVP) and cortisol between patients with anxious–retarded or nonanxious–retarded depression, melancholic or nonmelancholic depression, and anxious–retarded melancholic depression or the category of all other patients with depression were analyzed with Students' t-tests. Pearson's correlations were used for the correlations between ln(AVP) and cortisol. The effects of medication, age (dichotomized as older or younger than the median of 42 years, as well as older or younger than the menopausal age of 50 years) and sex on ln(AVP) were investigated by using an analysis of variance (ANOVA). The effects of these variables on the correlation between ln(AVP) and cortisol were analyzed by ANOVA using ln(AVP) as the dependent variable and cortisol as the covariate. The association between ln(AVP) and anxious–retarded depression or melancholic depression was also investigated by ANOVA. All calculations were carried out using SPSS 9.0 (SPSS inc. Chicago).

RESULTS

Relation Between Anxious–Retarded Depression and Melancholic Depression

In all, 25 patients had anxious–retarded depression and 34 had melancholic depression. Totally, 22 patients (88% of the patients with anxious–retarded depression and 65% of the

patients with melancholic depression) fulfilled criteria for both subcategories. The correspondence between the anxious–retarded and melancholic subcategories was 0.549 (Cohen's kappa, $p < 0.001$).

Plasma AVP and Cortisol in Major Depression

Mean values and effects of drug treatment, age and sex Table 1 shows plasma AVP and cortisol levels and AVP–cortisol correlations in patients with different subcategories of major depression. The mean plasma AVP concentration was 4.5 pg/ml (SD = 4.87). In subgroups of patients on different medications, Spearman's correlations between drug dosage and plasma AVP were 0.267 for the SSRI subgroup ($n = 16$, $p = 0.317$), -0.231 for the SNRI subgroup ($n = 15$, $p = 0.408$), -0.771 for the TCA subgroup ($n = 8$, $p = 0.025$), 0.124 for the antipsychotic subgroup ($n = 9$, $p = 0.750$) and -0.162 for the benzodiazepine subgroup ($n = 38$, $p = 0.333$). ANOVA showed that in the whole group, treatment with SSRI, SNRI, TCA, antipsychotic drug or benzodiazepine was not related to ln(AVP). F values (p values between brackets) related to these drug treatments were 0.134 ($p = 0.715$), 0.007 ($p = 0.935$), 0.818 ($p = 0.369$), 0.069 ($p = 0.794$) and 0.105 ($p = 0.747$), respectively. Likewise, ln(AVP) did not depend on the dosage of these psychotropic drugs. F values (p values between brackets) were 0.682 ($p = 0.412$), 0.330 ($p = 0.568$), 0.532 ($p = 0.468$), 2.263 ($p = 0,138$) and 0.171 ($p = 0.680$), respectively. Finally, there was no interaction between antidepressants and antipsychotics, antidepressants and benzodiazepines, and antipsychotics and benzodiazepines. F values were 0.554 ($p = 0.460$), 0.468 ($p = 0.497$) and 0.445 ($p = 0.507$), respectively. Neither age ($<$ or $\geqslant$ 42 years) nor sex, nor their interaction, was related to ln(AVP). F values were 0.011 ($p = 0.918$), 0.348 ($p = 0.557$), and 0.238 ($p = 0.628$), respectively. If a menopausal age criterion of 50 years was used, then the F values were 2.349 ($p = 0.130$), 0.165 ($p = 0.686$), and 0.003 ($p = 0.957$), respectively.

The mean plasma cortisol concentration was 145.4 mg/ml (SD = 41.2). In subgroups of patients on different medications, Spearman's correlations between drug dosage and plasma cortisol were 0.302 for the SSRI subgroup ($n = 16$, $p = 0.255$), -0.011 for the SNRI subgroup ($n = 15$, $p = 0.969$), 0.072 for the TCA subgroup ($n = 8$, $p = 0.691$), 0.080 for the antipsychotic subgroup ($n = 9$, $p = 0.838$), and -0.126 for the benzodiazepine subgroup ($n = 38$, $p = 0.451$). Treatment with SSRI, SNRI, TCA, antipsychotic drug or

Table I Plasma AVP and Cortisol with SD and Spearman Correlations between Plasma AVP and Cortisol for Patients, with Different Subcategories of Depression

(Sub)categories	n	AVP (pg/ml) Mean (SD)	Cortisol (mg/ml) Mean (SD)	AVP–Cortisol Correlation	p
Major depression	66	4.50 (4.87)	145.4 (41.2)	0.35	0.005
Anxious–retarded	25	6.25[a] (7.06)	148.6 (44.6)	0.56	0.004
Nonanxious–retarded	41	3.44[a] (2.38)	143.4 (39.5)	0.24	0.126
Melancholic	34	5.50 (6.22)	148.5 (41.4)	0.39	0.024
Nonmelancholic	32	3.44 (2.53)	142.1 (42.4)	0.27	0.133
Anxious–retarded melancholic	22	6.75[b] (7.39)	148.6 (47.3)	0.59	0.004
All other patients	44	3.38[b] (2.31)	143.8 (38.4)	0.25	0.098

Differences between means (Mann–Whitney): [a]$p = 0.09$; [b]$p = 0.046$.

benzodiazepine was not related to plasma cortisol concentration. F values (p values between brackets) related to these drug treatments were 0.222 ($p = 0.639$), 0.347 ($p = 0.558$), 1.129 ($p = 0.292$), 1.133 ($p = 0.291$) and 0.108 ($p = 0.743$), respectively. Similarly, plasma cortisol concentration was not associated with psychotropic drug dosage. F values (p values between brackets) were 0.119 ($p = 0.732$), 0.022 ($p = 0.881$), 0.442 ($p = 0.508$), 1.493 ($p = 0.227$) and 0.149 ($p = 0.701$), respectively. Interaction effects were analyzed for antidepressants and antipsychotics, antidepressants and benzodiazepines, and antipsychotics and benzodiazepines. F values were 0.596 ($p = 0.443$), 1.439 ($p = 0.235$), and 0.956 ($p = 0.332$), respectively. Neither age ($<$ or $\geqslant$ 42 years) nor sex, nor their interaction, was related to cortisol level. F values were 0.013 ($p = 0.908$), 0.020 ($p = 0.888$) and 0.013 ($p = 0.909$), respectively. If the menopausal age criterion of 50 years was used, then the F values were 1.799 ($p = 0.185$), 0.040 ($p = 0.841$), and 0.062 ($p = 0.804$), respectively.

Correlations between plasma AVP and cortisol levels, and effects of drug treatment age and sex A statistically significant positive correlation (Spearman's $r = 0.35$, $p = 0.005$) was found between plasma AVP and cortisol levels for all 66 patients with major depression. After logarithmic transformation of AVP concentrations, the Pearson's correlation was 0.37 ($p = 0.002$). ANOVA further showed that neither SSRI, SNRI, TCA, antipsychotic drug or benzodiazepine nor the above-mentioned interactions of drug treatments were related to the correlation between ln(AVP) and plasma cortisol, the significance of the association being only slightly reduced ($p = 0.004$) after correction for these factors.

ANOVA of age ($<$ or $\geqslant$ 42 years), sex and their interaction, and cortisol as covariate of ln(AVP) showed that the significance of the covariation between cortisol and ln(AVP) in depression was only slightly reduced ($p = 0.003$). If the menopausal age criterion of 50 years was used, then the significance of the covariation was again slightly reduced ($p = 0.005$).

Plasma AVP and Cortisol in Patients with Anxious–Retarded Depression, Melancholic Depression or Anxious–Retarded Melancholic Depression

Differences between mean values and effects of drug treatment, age and sex Patients with anxious–retarded depression had a higher plasma AVP concentration than patients with nonanxious–retarded depression (Mann–Whitney U test, $p = 0.09$, two-tailed). After logarithmic transformation of AVP concentrations, the difference was statistically significant (t-test, $p = 0.031$, two-tailed). The difference in plasma AVP or ln(AVP) between patients with melancholic or nonmelancholic depression was not significant ($p = 0.090$, Mann–Whitney, and $p = 0.130$, t-test, respectively). Patients with anxious–retarded melancholic depression had a significantly higher AVP and ln(AVP) concentration than all other patients ($p = 0.046$ and 0.013, respectively).

ANOVA showed that dosage of SSRI, SNRI, TCA, antipsychotic or benzodiazepine did not have a confounding effect on the relation between ln(AVP) and anxious–retarded depression. F values (p-values between brackets) were 0.787 ($p = 0.386$), 1.778 ($p = 0.198$), 0.680 ($p = 0.420$), 1.855 ($p = 0.189$), and 0.026 ($p = 0.874$), respectively. Similarly, dosage of SSRI, SNRI, TCA, antipsychotic or benzodiazepine did not affect the relation between plasma cortisol level and anxious–retarded depression. F values (p-values between brackets) were 0.005 ($p = 0.945$), 1.279 ($p = 0.272$), 1.136 ($p = 0.300$), 0.252 ($p = 0.621$) and 0.738 ($p = 0.401$), respectively.

ANOVA showed that age ($<$ or $\geqslant$ 42 years), sex and their interaction did not have a confounding effect on the relation between ln(AVP) and anxious–retarded depression, melancholic depression or anxious–retarded melancholic depression. F values (p-values between brackets) related to age, sex and their interaction were 0.707 ($p = 0.410$), 0.011 ($p = 0.916$) and 1.265 ($p = 0.273$) for the anxious–retarded subcategory, 0.109 ($p = 0.743$), 0.562 ($p = 0.459$) and 0.555 ($p = 0.462$) for the melancholic subcategory, and 0.001 ($p = 0.975$), 0.357 ($p = 0.558$) and 2.554 ($p = 0.127$) for the anxious–retarded melancholic category.

ANOVA with anxious–retarded depression and melancholic depression as independent variables showed that ln(AVP) was only associated with the anxious–retarded subcategory of depression ($p = 0.031$). There was no association between cortisol level and any subcategory of depression.

Correlations between plasma AVP and cortisol and effects of age and sex In patients with anxious–retarded or nonanxious–retarded depression, Spearman's correlations between plasma AVP and cortisol were 0.56 ($n = 25$; $p = 0.004$) and 0.24 ($n = 41$; n.s.), respectively. In patients with melancholic or nonmelancholic depression, these correlations were 0.39 ($n = 34$; $p = 0.024$) and 0.27 ($n = 32$; n.s.), respectively. The correlation between plasma AVP and cortisol levels in the 22 patients with anxious–retarded melancholic depression was 0.59 ($p = 0.004$), while in the 44 other patients it was 0.253 ($p = 0.098$). After logarithmic transformation of plasma AVP concentrations, the Pearson's correlations between ln(AVP) and plasma cortisol were 0.61 ($p = 0.001$) and 0.17 ($p = 0.302$) for patients with anxious–retarded or nonanxious–retarded depression, 0.43 ($p = 0.011$) and 0.27 ($p = 0.141$) for patients with melancholic or nonmelancholic depression, and 0.63 ($p = 0.002$) and 0.16 ($p = 0.289$) for patients with anxious–retarded melancholic depression and all other patients.

After correction for age ($<$ or $\geqslant$ 42 years), sex and their interaction, ANOVA with cortisol concentration as covariate revealed a slightly lower association between cortisol concentration and ln(AVP) in patients with anxious–retarded depression ($p = 0.005$), melancholic depression ($p = 0.017$) or anxious–retarded melancholic depression ($p = 0.004$).

DISCUSSION

We replicated the finding that major depression is associated with a positive correlation between plasma AVP and cortisol levels (Inder et al, 1997). This correlation was due to a highly significant correlation between plasma AVP and cortisol levels in patients with anxious–retarded depression. We also showed that this anxious–retarded subcategory of depression was associated with a higher

plasma AVP level than the complementary nonanxious–retarded category, but this difference was only significant when logarithm-transformed AVP values were used.

In patients with melancholic depression (65% of the patients with anxious–retarded depression), there was a low correlation between plasma AVP and cortisol levels. These patients had a nonsignificantly higher plasma level of AVP than the patients with nonmelancholic depression. Like the patients with anxious–retarded depression, the patients with anxious–retarded melancholic depression (88% of the patients with anxious–retarded depression) had a significantly higher plasma AVP level and a highly significant AVP–cortisol correlation. This suggests that anxious–retarded depression may be a two-dimensional refinement of the melancholic subcategory and a more useful clinical phenotype than the melancholic subcategory as far as external validity involving AVP-related HPA-axis dysregulation is concerned.

The increased plasma AVP level combined with the correlation between plasma AVP and cortisol levels in the patients with anxious–retarded depression is the third report supporting the hypothesis that AVP is involved in the dysregulation of the HPA axis in depression. As already mentioned, one study demonstrated a correlation between plasma AVP and cortisol levels in major depression (Inder et al, 1997), and another study showed that the number of CRH neurons coexpressing AVP in the PVN of the hypothalamus was almost three times higher than in a control group (Raadsheer et al, 1994). However, in an earlier study (Van Londen et al, 1997), we found no correlation between plasma AVP and cortisol levels. The reason for this difference is unclear. Whether drug withdrawal may have played a role will have to be investigated. The previously reported statistically significant increase in plasma AVP level in melancholic depression (Van Londen et al, 1997) was not reproduced in the present study. This may be because this relationship was only found for plasma AVP levels at 23.00, a time not assessed in the present study. Another reason is that DSM-III-R criteria for melancholic depression were used in our previous study.

The specific phenotypic characteristic of patients with high plasma AVP levels and a high AVP–cortisol correlation appears to be the combination of both high anxiety and high retardation. This differentiates these patients from highly anxious or highly retarded patients, as well as from highly anxious, low-retarded patients and highly retarded low-anxious patients. The combination of high anxiety, high retardation and high plasma AVP levels with a positive AVP–cortisol correlation suggests a common pathogenetic pathway that involves disinhibition of the HPA axis and disinhibition of the coping systems for fight/flight and behavioral inhibition (Bohus and Koolhaas, 1993). Since these two systems involve CRH and AVP neurotransmission, respectively, reduced negative feedback or increased release of both CRH and AVP may occur in the anxious–retarded subcategory of depression. This could be due to reduced hippocampal and/or hypothalamic glucocorticoid feedback (Sapolsky and Plotsky, 1990; Kovacs et al, 2000) and/or to enhanced noradrenergic activation (Scott and Dinan, 1998). Although it is generally accepted that peripheral plasma AVP levels reflect osmotic regulation and activation of the magnocellular neurosecretory system,

the elevated plasma AVP levels in the patients with anxious–retarded depression could be because of a disinhibited response to psychological stress. In this case, the response could originate in the parvocellular neurons in the hypothalamic PVN and reach the pituitary via the portal circulation, as indicated by the results of animal studies of 'psychological' stress (Keck et al, 2002; Scott and Dinan, 1998). The higher correlation between plasma AVP and cortisol levels may be because of synergy between AVP derived from the parvocellular PVN and CRH at the level of the pituitary. However, increased plasma AVP levels may also directly stimulate adrenocortical glucocorticoid secretion (Guillon et al, 1995), although we have no evidence for this from this study. Increased plasma AVP levels could also originate from the magnocellular system. The lack of a correlation between plasma AVP levels and plasma osmolality in our earlier study (Van Londen et al, 1997) makes the latter explanation unlikely.

Psychotropic agents may have confounded the association between plasma AVP levels or the correlation between plasma AVP and plasma cortisol levels and anxious–retarded depression, because the SSRI fluoxetine has been shown to reduce hypothalamic AVP release in vitro (Altemus et al, 1992), and in some studies antipsychotic drugs have been shown to influence plasma cortisol and AVP levels (Gattaz et al, 1985; Raskind et al, 1987). In these 66 patients with major depression, we only found a negative correlation between TCA dosage and plasma AVP level in the whole group of depressed patients. This finding was no longer significant after correction for multiple assessments. To detect the effects of treatment on plasma AVP levels, it may be better to measure plasma levels of those drugs whose antidepressant effect is known to be related to their plasma concentration. The finding that neither age (<42 or $\geqslant 42$ years; <50 or $\geqslant 50$ years), sex nor their interaction explained the elevated plasma AVP levels in patients with anxious–retarded depression, and that no difference was found between young men and women, suggests that postmenopausal or perimenstrual effects did not confound the data.

We found that the mean plasma AVP level was 4.5 pg/ml, which may seem rather high compared to data reported by others. Our healthy control group of 17 subjects, however, had a mean plasma AVP level of 3.17 pg/ml (range 1.20–9.11 pg/ml; $SD = 1.97$), which is similar to previously reported concentrations (ranging from 1.2 ± 0.6 to 3.5 ± 0.6 pg/ml) measured by RIA after plasma extraction (Glanzer et al, 1984; Viinamaki et al, 1986). This suggests that it is unlikely that the RIA method used resulted in systematically higher plasma AVP values, and that the high mean plasma AVP level in this depressed patient group was due to a subgroup with extraordinarily high levels (the anxious–retarded depression subgroup).

In conclusion, anxious–retarded depression, which has been related to family history, appeared to be a phenotypic subcategory showing a correlation with high plasma AVP levels and a high AVP–cortisol correlation. Anxious–retarded depression was significantly associated with melancholia, and patients with anxious–retarded melancholic depression also had high plasma AVP levels and a high correlation between plasma AVP and cortisol levels. These findings suggest that this two-dimensional

refinement of the melancholic subcategory of depression may be useful for further investigations of plasma AVP-related dysregulation of the HPA axis in familial depression.

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