Original Paper

Support for two increased vasopressinergic activities in depression at large and the differential effect of antidepressant treatment

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Abstract
Animal models of depression support a pathogenetic role for vasopressinergic activation involving increased arginine vasopressin (AVP) release and AVP receptor (V1b) synthesis. Evidence of this has been found particularly in patients with highly anxious-retarded (HAR) and above-normal AVP (ANA) depression. A general pathogenetic theory however predicts vasopressinergic activities to play a role in at least all major depressive disorders, and antidepressant (AD) treatment to be mediated by vasopressinergic reduction. We tested these hypotheses by re-analysing the data of 66 depressed patients; 27 with and 39 without AD treatment. The plasma AVP concentration and the AVP-cortisol correlation were used as presumed parameters of AVP release and pituitary V1b receptor function. A high AVP-cortisol correlation ($r = 0.72$; $p < 0.001$) was found in the non-AD group, and no correlation in the AD treatment group. AD treatment did not relate to plasma AVP concentration. The AVP-cortisol correlation in HAR and ANA depression was not explained by a low rate of AD treatment. These human data support the hypothesis of increased AVP release and receptor function as pathogenetic characteristics of major depression, and show selective normalization of the AVP-cortisol correlation, which is supposed to reflect the receptor function, by AD treatment.

Introduction

Depression is thought to be characterized by dysregulated emotional and behavioural responses to stress, in which increased hypothalamus–pituitary–adrenal (HPA) axis activation, limbic disinhibition and a prefrontal inhibitory deficit may play pathogenetic roles (Goekoop, 2009; Swaab et al., 2000; Wotjak et al., 2002). Accumulating evidence supports a crucial role for AVP in these pathogenetic processes. A switch from corticotroph-releasing factor (CRF) to AVP-driven regulation of the HPA axis has been found in a rat model of chronic stress and depression (Aguilera et al., 2008), and is thought to maintain sufficient HPA axis activation by compensating for chronic stress-induced blunting of the pituitary CRF receptor. AVP is co-expressed with CRF in the parvocellular neurons of the hypothalamic paraventricular nucleus (PVN), and this AVP stimulates anterior pituitary V1b receptors, which are increased in number (Aguilera et al., 2008). These vasopressinergic mechanisms have a synergising effect on the pituitary CRF function, which is reduced at the same time. A more complex variant of stress-induced vasopressinergic co-activation of the HPA axis, which comprises parvocellular and magnocellular AVP synthesis and release, has been found in the genetically inbred high-anxiety behaviour (HAB) rat model (Landgraf, 2006). At rest this male rat model shows genetically increased synthesis of AVP in both the parvo- and the magnocellular divisions of the PVN (Wigger et al., 2004), as well as a, presumably adaptive, reduction of pituitary V1b receptor expression (Salome et al., 2006). After mild stress the HAB rat shows hyper-reactivity of the HPA axis and increased release of AVP from both parvocellular and magnocellular neurons (Engelmann et al., 2004). Increased dendritic release of magnocellular AVP during

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stress has been interpreted to counteract overshooting adrenocorticotropic hormone (ACTH) secretion by locally inhibiting parvocellular neuronal activity in the PVN (Engelmann et al., 2004). In addition it is thought to overstimulate at the same time extra-hypothalamic limbic sites involved in the expression of emotional behaviour, and there to induce a pathophysiological loss of control over emotional responses (Engelmann et al., 2004). Finally, vasopressinergic stimulation has been found to be a necessary step in the production of stress-induced depression-like behaviour mediated by such extra-hypothalamic sites (Mlynarik et al., 2007), while in the HAB rat it has also been found to be involved in insufficient prefrontal inhibitory function (Kalisch et al., 2004).

The role of vasopressin in animal models of depression has further been tested pharmacologically by the use of vasopressin antagonists, especially vasopressin-1b (V1b) receptor antagonists, which were found to produce AD-like effects on depression-like behaviour of stressed animals as well as on their increased HPA axis function (Griebel et al., 2005; Keck et al., 2002; Wigger et al., 2004). Finally, the chronic use of the monoamine reuptake inhibitor paroxetine has been found to reduce the increased vasopressin synthesis in the PVN, increased CRH-induced release of ACTH, and the behavioural responses of anxiety and immobility in the HAB rat model (Keck et al., 2003). Moreover, other monoamine reuptake inhibitors have been found to act on the PVN via the V1b receptor (Stewart et al., 2008). These animal model data support the hypothesis that vasopressinergic mechanisms mediate the AD effects of both the classical AD drugs and vasopressin antagonists independent of the genetic predisposition to affective dysregulation.

Human studies support the involvement of vasopressinergic mechanisms in depression. The findings are: post-mortem increased AVP expression in the hypothalamic PVN (Purba et al., 1996; Raadsheer et al., 1994) in depression, and particularly in melancholic depression (Meynen et al., 2006), genetically increased V1b expression and AVP synthesis in two types of childhood-onset depression (Dempster et al., 2007, 2009), increased ACTH response to the synthetic vasopressin receptor agonist desmopressin in melancholic depression (Dinan et al., 2004), and increased correlation between plasma AVP and cortisol concentrations in patients with depression (Inder et al., 1997) or suicidality (Brunner et al., 2002), as well as in genetically predisposed patients with HAR (De Winter et al., 2003) or ANA depression (Goekoop et al., 2006). Furthermore, an increased plasma AVP concentration has been found in melancholic depression (Van Londen et al., 1997), and above-normal plasma AVP concentrations have been found to be related to increased nocturnal motor activity (Van Londen et al., 1998) and familial depression (Goekoop et al., 2006). Finally, full remission of depression has been found after 5 years to be associated with a decrease of plasma AVP concentration to a mean afternoon value of about 2 pg/mL (Van Londen, 2003).

These vasopressinergic data suggest a high degree of translationability of the affective dysregulations in human and animal subjects. The most conspicuous difference between the rodent models and human subjects is the absence of stress-induced plasma AVP concentrations in the male rats

(Jezova et al., 1995; Williams et al., 1985) that are used in these animal models. This 'anomaly' will be dealt with in the discussion of this paper. The degree to which specific analogies have been found, on the other hand, is very high. The most outspoken example is the analogy between the ANA subcategory of depression and HAB rat model, because both have an increased genetic predisposition for the combination of anxiety and (psychomotor) retardation symptoms (Goekoop et al., 2006), low socializing behaviour (Goekoop et al., 2009a), and a negative relation with plasma norepinephrine (Goekoop et al., 2009b).

Within the realm of human depressive subjects, the long-term general state-dependence of plasma AVP concentrations is interpreted as representing chronic stress-induced AVP release (Van Londen, 2003), and the correlation found between plasma AVP and cortisol concentrations as indirect evidence of increased pituitary V1b expression (Goekoop and Wiegant, 2009). The genetic predisposition (De Winter et al., 2004) and deficient personality development (De Winter et al., 2007) found in the HAR subcategory are held to represent a subgroup of depression with genetically increased vulnerability, presumably corresponding with the early-onset subcategory with a genetic polymorphism of the V1b receptor (Dempster et al., 2007). Patients with this HAR subcategory would therefore be genetically predisposed to depression by an increased V1b receptor function, which in the chronic stress-induced rat model is environmentally induced.

The conclusion from our studies of vasopressinergic mechanisms in subcategories of depression with high association with familial depression is that the HAR subcategory may have genetically increased V1b expression, that ANA depression may have genetically increased AVP synthesis, and that anti-vasopressinergic treatment would be useful for both subcategories (Goekoop and Wiegant, 2009). However, the chronic stress model of depression with its increased AVP synthesis and V1b receptor function in all rats, and the finding that extra-hypothalamic vasopressinergic stimulation is necessary for the production of depression-like symptoms, result in a more general vasopressinergic theory of depression which predicts that increased vasopressinergic activation plays a necessary role in at least all major depressive disorders and not just in genetically predisposed subcategories. This theory also predicts that AD treatment should result in the reduction of this vasopressinergic activation in all patients. These general hypotheses have not yet been investigated.

In the present study we tested these hypotheses by comparing non-AD-treated and AD-treated patients with a major depressive episode. To this end we re-analysed the data from the 66 depressed patients, in which we previously found support for the HAR and ANA subcategories. This sample comprised 27 patients with and 39 patients without AD treatment. At the same time we tested whether the ANA and HAR subcategories were still characterized by increased AVP-cortisol correlations when accounting for the effect of AD treatment. To explore the potential role of the severity of the depressive illness and coping style in the use of AD the severity was assessed by means of the Montgomery Asberg Depression Rating Scale (MADRS) (Montgomery and Asberg, 1979). Reduced coping was assessed by means of the Temperament and Character Inventory (TCI). As far as the

latter is concerned we were particularly interested in the character trait of Self-Directedness (Cloninger et al., 1993), which has been found to show a state-dependent reduction in all depressed patients (De Winter et al., 2007) and could represent a characteristic of reduced prefrontal inhibitory function (Goekoop, 2009).

Methods and materials

Subjects

We re-analysed the data from the same patient sample in which we previously found support for the HAR and ANA subcategories of depression (Goekoop and Wiegant, 2009). All patients fulfilled DSM-IV criteria (American Psychiatric Association, 1994) for major depression and scored >20 on the MADRS (Montgomery and Asberg, 1979). Data from 66 of the 89 initially included patients were used after excluding patients using oral contraception and missing AVP and cortisol data. Mean age was 41 years (SD: 12 years); 39 patients (59%) were female. The melancholic subtype of depression according to DSM-IV criteria was present in 34 (52%) of the 66 patients. Twenty-five (38%) had HAR depression, defined by combined above-median scores for Anxiety and Retardation (De Winter et al., 2004) and 14 patients (21%) had ANA depression, defined by a plasma AVP concentration above 5.6 pg/mL AVP (Goekoop et al., 2006). Nine of the 25 HAR patients (36%) had ANA depression. ANA depression was present in 10 of the 34 (29%) patients with melancholia, and melancholia was present in 74% of patients with ANA depression. HAR was present in 22 of the 34 (65%) with melancholia, and melancholia was present in 88% of patients with HAR depression.

The psychiatrist at the inpatient or outpatient clinic made the diagnosis of major depression (DSM-IV). Patients were included if this diagnosis was confirmed by an independent investigator (RFP) using a semi-standardized interview for these DSM-IV diagnoses. Patients with an organic disorder or a schizophrenic or other primary psychotic disorder, or bipolar depression were excluded. Depressed patients with panic disorder were not included (they participated in a different research project). The Ethical Committee of the Leiden University Medical Centre (LUMC) approved the informed consent protocol. Written informed consent was obtained from all patients. The study was performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

Intensity rating and assessment of personality

The MADRS (Montgomery and Asberg, 1979) was used to assess the severity of the depressive disorder, and the TCI to assess the personality trait of Self-Directedness (Cloninger et al., 1993).

Psychotropic treatment and smoking habit

Thirty-nine of the 66 patients (59%) had been using an AD drug for at least 2 weeks, 9 (14%) used an antipsychotic drug, and 38 patients (58%) a benzodiazepine. To analyse the effect of psychotropic drug dosage as covariate, currently accepted equivalent values of the dosages were computed (Moleman and Birkenhaeger, 1998). Mean antipsychotic, AD and benzodiazepine dosages were 0.4 mg (SD = 1.4) haloperidol equivalents, 94 mg (SD = 98) imipramine equivalents, and 22 mg (SD = 34) chlordiazepoxide equivalents. Smoking habit was quantified by the mean number of cigarettes on a daily basis during the last month; the mean number was 10 (SD = 13).

Plasma AVP and cortisol

Within 7 days of the CPRS interview, blood samples were drawn on a single day under standardized conditions between 09 : 00 and 09 : 30 and between 15 : 30 and 16 : 00. All patients refrained from ingesting alcohol and from undertaking strenuous physical exercise for 12 hours before the study. They sat down 15 minutes before venipuncture. Smoking was not allowed for 30 minutes 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 minutes plasma was separated and stored at –80°C. Plasma AVP-like immunoreactivity, further referred to as AVP, was determined as described previously (Van Londen et al., 1997) by radioimmunoassay (RIA) using an antibody raised in a rabbit in the Rudolf Magnus Institute. The limit of detection (mean blank + 2 × SD as the criterion) was 0.5 pg/mL for plasma (extracted assay), and the intra- and inter-assay coefficients of variation (CV) were 9.9% and 15.9%, respectively. All samples were processed and radioimmunoassayed in duplicate, in one and the same run. The performance of the assay was in the range of the values measured, ED-20, -50 and -80 being 32, 4 and 0.5 pg/mL, respectively. The intra-assay CV was determined using samples taken from a plasma pool with an AVP concentration of around 4 pg/mL, which were processed independently before RIA. This is close to the cut-off point of 5.6 pg/mL for ANA depression (Goekoop et al., 2006).

Total plasma cortisol was measured by high-performance liquid chromatography (HPLC) with UV detection as previously described (Van Londen et al., 1997). The lower limit of detection was 3.62 ng/mL, and the intra- and inter-assay CV were 2.9% and 5.8%, respectively.

For each patient, mean daytime plasma AVP and cortisol levels were computed from the morning and afternoon values to reduce the effect of potential random error in sampling and assessment. Patients with a mean daytime plasma AVP level above 5.6 pg/mL were considered to have above-normal plasma AVP (Goekoop et al., 2006), those with levels below 5.6 pg/mL to have a normal plasma AVP concentration. As plasma AVP was not normally distributed (Kolmogorov–Smirnov $Z = 1.907$; $p = 0.001$), we used log-transformed values (lnAVP) in linear correlation analyses. LnAVP values were normally distributed (Kolmogorov-Smirnov $Z = 0.943$; $p = 0.337$).

Data analysis

Student's *t*-tests were used to investigate demographic and global clinical differences between the AD and non-AD

groups as well as their plasma hormone concentrations. Multivariate analyses of variance (MANOVAs) were used to test whether lnAVP or cortisol concentration depended on any of the three subclasses of AD drugs (selective serotonin reuptake inhibitor (SSRI), serotonergic noradrenergic reuptake inhibitor (SNRI) or tricyclic antidepressant (TCA)), or on a combination of these three drug types with HAR or ANA depression. Analogously multivariate analyses of covariance (MANCOVAs) were used to search for potential relations between lnAVP and plasma cortisol and dosages of these subtypes of treatment, as well as with interactions between ANA or HAR depression and these dosages. Pearson's correlations were used to investigate the correlation between lnAVP and cortisol concentrations in the whole patient group as well as in subcategories. We used analysis of covariance (ANCOVA) to compare the correlations between lnAVP and cortisol in AD and non-AD groups, with plasma cortisol concentration as dependent variable, AD treatment as fixed factor, lnAVP as covariate, and the interaction between lnAVP and AD treatment. As the duration of the illness of the present episode was non-significantly related with the non-AD group, we added this variable as a covariate to the ANCOVA model. To test whether AD subtypes could be differentially associated with the correlation between lnAVP and cortisol ANCOVA with plasma cortisol concentration as dependent variable, AD subtype as the division of treatments into four subcategories (SSNRI, SNRI, TCA and no treatment) as fixed factor, lnAVP as covariate, and the interaction between lnAVP and AD subtype division. To test whether the two new subcategories of depression, HAR and ANA depression, were still characterized by the correlation between lnAVP and plasma cortisol after accounting for the effects of AD treatment, we added these variables as fixed factors to the latter ANCOVA model in subsequent ANCOVAs using the interaction between each of these two subcategories and lnAVP to the model. As in our previous studies, smoking habit, MADRS score and age were used as covariates in analogous analyses; we also added these variables as covariates to the ANCOVA model. The analyses were carried out with the SPSS 12.0.

Results

Antidepressant treatment and demographical and clinical data

The mean age of the non-AD group (42.7; SD 12.7 years) did not differ from that of the AD group (40.2 years; SD 11.0 years); neither did the percentage of female patients (22/39 = 56% versus 17/27 = 63%), nor the severity of the depression according to the MADRS (30; SD 6.5 versus 32; SD 6.1) or the number of previous episodes (1.7; SD 2.4 versus 1.5; SD 1.6). There was a non-significant trend for a longer duration of the illness of the present episode, i.e. the one immediately preceding the start of the study in the non-AD group (8.7 months; SD 9.6 versus 5.5 months; SD 3.7; $t = 1.877$; $p = 0.065$). The Self-Directedness was significantly lower in the AD group (22.1; SD = 6.3) than in the non-AD group (25.8; SD = 7.6; $p = 0.045$).

Table 1. Plasma AVP and cortisol concentrations (SD) in patients on antidepressant treatment (AD) and patients not on antidepressant treatment (non-AD)

	n	AVP (pg/mL)	Cortisol (ng/mL)
AD	39	4.3 (4.2)	142.28 (38.51)
Non-AD	27	4.7 (5.7)	149.44 (45.18)

Within the AD group 22 patients (56%) had melancholia according to DSM-IV, 17 patients (44%) had HAR depression, and 8 (21%) had ANA depression, while in the non-AD group 12 (44%) had melancholia, 8 (30%) HAR depression and 6 (22%) ANA depression. Within the AD group 7 patients (18%) had antipsychotic treatment and 25 (64%) a benzodiazepine, while in the non-AD group 2 (7%) had an antipsychotic drug and 13 (48%) a benzodiazepine. None of these differences were statistically significant. Within the AD group there were 16 patients on SSRI, 15 on SNRI and 8 on TCA treatment. Seventeen of the 25 HAR patients (68%) and 8 of the 14 ANA patients (57%) had AD treatment. The percentages of patients on SSRI, SNRI and TCA in HAR depression were 28%, 20% and 20%, and in ANA depression 29%, 21% and 7%.

Antidepressant treatment and hormone concentrations

Table 1 shows the concentrations of plasma AVP and cortisol in the AD and non-AD groups. Separate Student's *t*-tests did not reveal any statistically significant difference between these two groups for these hormone concentrations ($t = 0.333$; $p = 0.740$, and $t = 0.692$; $p = 0.492$, respectively). MANOVA (*F*- and *p*-values not shown) demonstrated that neither lnAVP nor plasma cortisol concentration depended significantly on any of the three classes of ADs, SSRI ($n = 16$), SNRI ($n = 15$) or TCA ($n = 8$), or on their combination with HAR or ANA depression. An effect of the interaction between AD-subtyping and ANA depression on plasma cortisol ($F = 2.749$; $p = 0.051$) just lacked statistical significance. MANCOVAs with separate SSRI, SNRI and TCA dosages as covariates showed no effect on lnAVP or plasma cortisol in depression in general or specifically in interaction with ANA or HAR depression.

Antidepressant treatment and correlations between hormone concentrations

As published before (Goekoop et al., 2006), Pearson's correlation showed that plasma AVP correlated positively with plasma cortisol in the whole patient sample ($r = 0.37$; $n = 66$; $p = 0.002$). ANCOVA with plasma cortisol concentration as dependent variable and lnAVP as covariate showed that this correlation had an *F*-value of 10.023 (Intercept 121.462; Beta: 20.100; $p = 0.002$; see Figure 1). Within the group on AD treatment the Pearson's correlation appeared to be 0.08 ($p = 0.646$) and in those not on AD treatment 0.72 ($p < 0.001$). The same ANCOVA, using plasma cortisol concentration as dependent variable and lnAVP as covariate, but with AD treatment as fixed factor combined with the
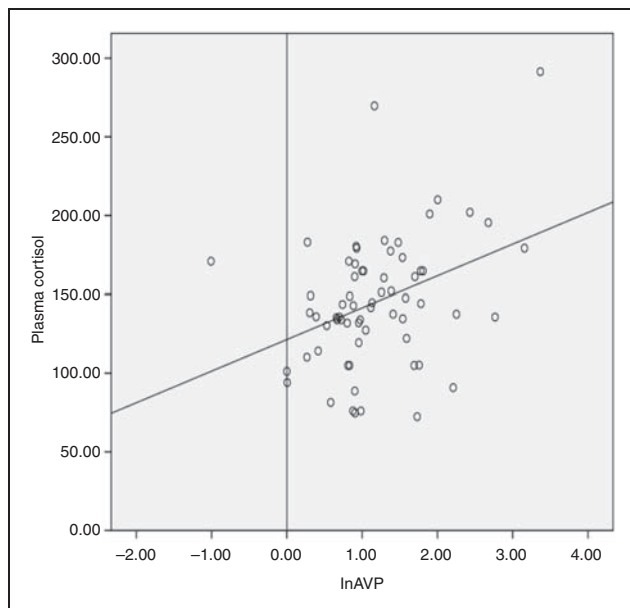
Figure 1. Distribution of 66 depressed patients based on plasma cortisol concentration (ng/mL) and ln-transformed AVP concentration (pg/mL) values. The oblique line represents the regression line of the correlation between lnAVP and plasma cortisol. The vertical line is a reference line representing the Y (plasma cortisol) axis for X (lnAVP) = 0.

interaction between lnAVP and AD treatment, showed a significant effect of AD treatment on this correlation ($F = 9.632$; Beta = -37.465; $p = 0.003$), compared with a Beta of 41.395; $p < 0.001$ in the non-AD group. The AD effect therefore eventually resulted in a beta of this regression line in the AD group of 41.395 – 37.465 = 3.930, which corresponds with the nearly horizontal regression line of the correlation (see Figure 2). The duration of the present episode as covariate in the ANCOVA model was not related to the cortisol concentration and did not change these relations. The same was found for smoking habit, MADRS score and age as covariates. In the next ANCOVAs, with cortisol concentration as dependent variable and lnAVP as covariate, these covariates were therefore no longer added to the ANCOVA model.

Correlations between hormone concentrations in HAR and ANA depression

Figure 3 shows scattergrams of 25 patients with HAR depression and 14 with ANA depression (5 patients having both ANA and HAR depression) based on lnAVP values and plasma cortisol concentrations. ANCOVA using plasma cortisol concentration as dependent variable and lnAVP as covariate, and further the HAR and ANA subcategories as fixed factors combined with the interaction between the HAR subcategory and lnAVP, resulted in an effect of the interaction between lnAVP and HAR depression that did not reach two-tailed significance ($F = 3.666$; Beta = $14.83 + 25.89 = 40.72$; $p = 0.06$). An analogous ANCOVA using the interaction between lnAVP and ANA depression showed a significant
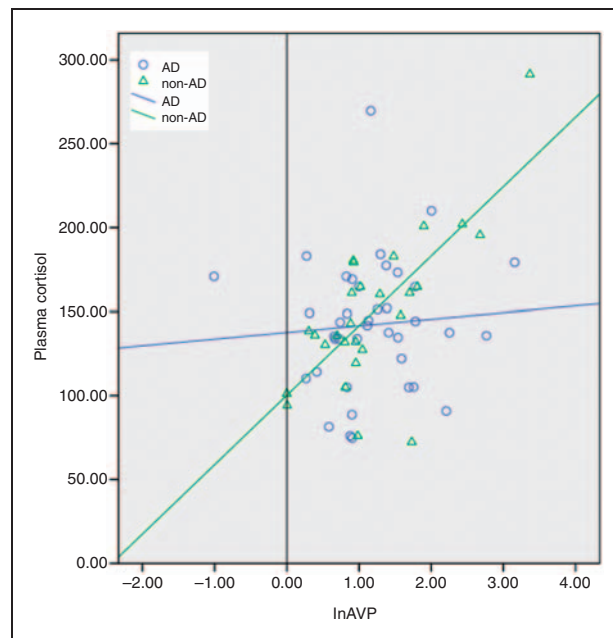

Figure 2. Distribution of 39 depressed patients with antidepressant (AD) treatment and 27 without AD treatment (non-AD) in a scattergram defined by plasma cortisol concentration (ng/mL) and ln-transformed AVP concentration (pg/mL) values. Oblique lines represent regression lines of the correlation between lnAVP and cortisol in the two groups. The vertical line is a reference line representing the Y (plasma cortisol) axis for X (lnAVP) = 0.

effect of the interaction between lnAVP and ANA depression ($F = 4.252$; Beta = $13.01 + 45.77 = 58.78$; $p = 0.043$).

The conclusion of this is that, corresponding with our earlier report based on partial correlations (Goekoop et al., 2006), the slopes (Betas) of the regression lines of the AVP-cortisol correlations in both the ANA and HAR subcategories appeared to be higher than those in the complementary patient groups of non-ANA and non-HAR patients. In contrast to the outcome of the partial correlation, the difference between the correlations in the HAR and non-HAR subcategories just lacked statistical significance in the presently used ANCOVA.

Effects of antidepressant treatment on the correlation between lnAVP and cortisol in HAR and ANA depression

Figure 4 shows distributions of HAR and ANA patients with or without AD treatment in scattergrams based on lnAVP values and plasma cortisol concentrations, as well as the regression lines of the lnAVP-cortisol correlations. The addition of AD treatment to the list of fixed factors of the ANCOVA models that were developed above slightly reduced the effects of the interaction between HAR and lnAVP ($F = 3.296$; $p = 0.074$) as well as the interaction between ANA and lnAVP ($F = 4.026$; $p = 0.049$). If instead of AD treatment the AD subtyping into SSRI, SNRI or TCA was used as a fixed factor, then the effect of the interaction between HAR and lnAVP still just lacked statistical
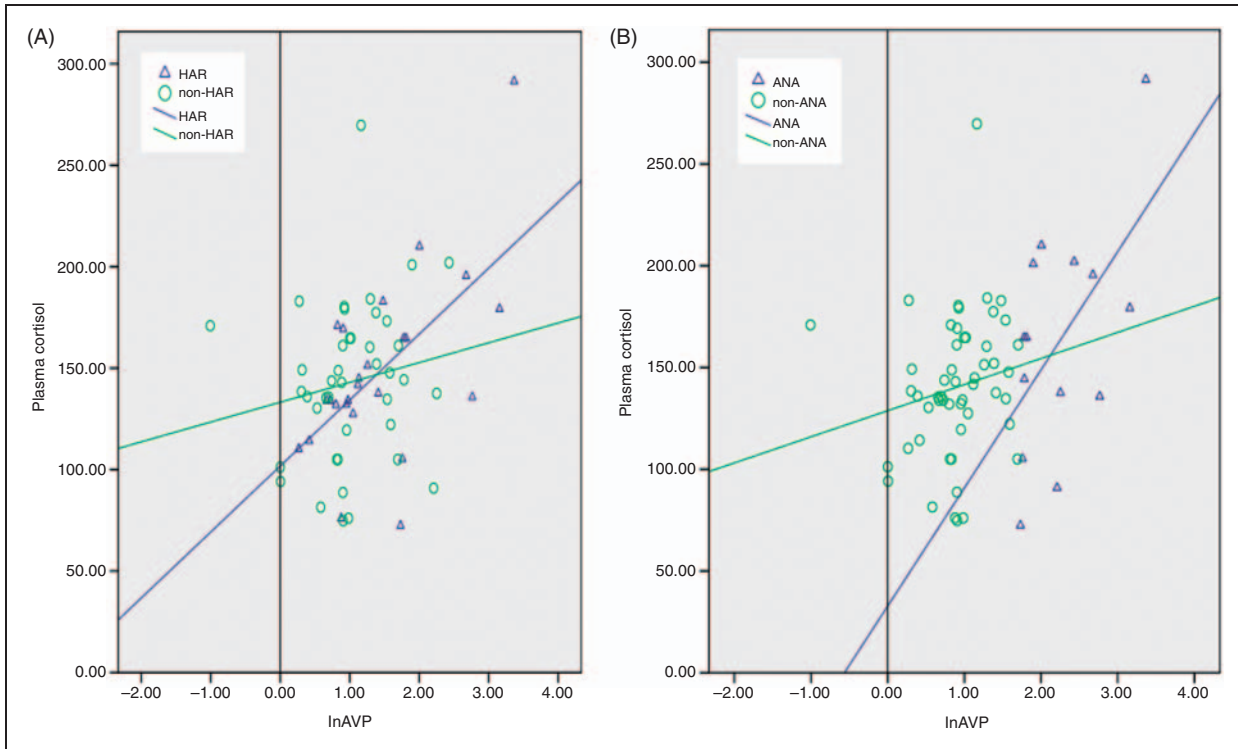
Figure 3. Distribution of (A) 25 patients with highly anxious-retarded depression (HAR) and 41 without highly anxious-retarded depression (non-HAR), and (B) 14 patients with ANA depression (ANA) and 52 without ANA depression (non-ANA), in scattergrams defined by plasma cortisol concentration (ng/mL) and ln-transformed AVP concentration (pg/mL) values. For the meaning of the vertical and oblique lines see Figure 2.

Figure 4. Distribution of (A) 25 patients with highly anxious-retarded (HAR) depression either on or not on antidepressant treatment (HAR-AD and HAR-non-AD), and (B) 14 patients with ANA depression either on or not on antidepressant treatment (ANA-AD and ANA-non-AD), in a scattergram defined by plasma cortisol concentration (ng/mL) and ln-transformed AVP concentration (pg/mL) values. For the meaning of the vertical and oblique lines see Figure 2.
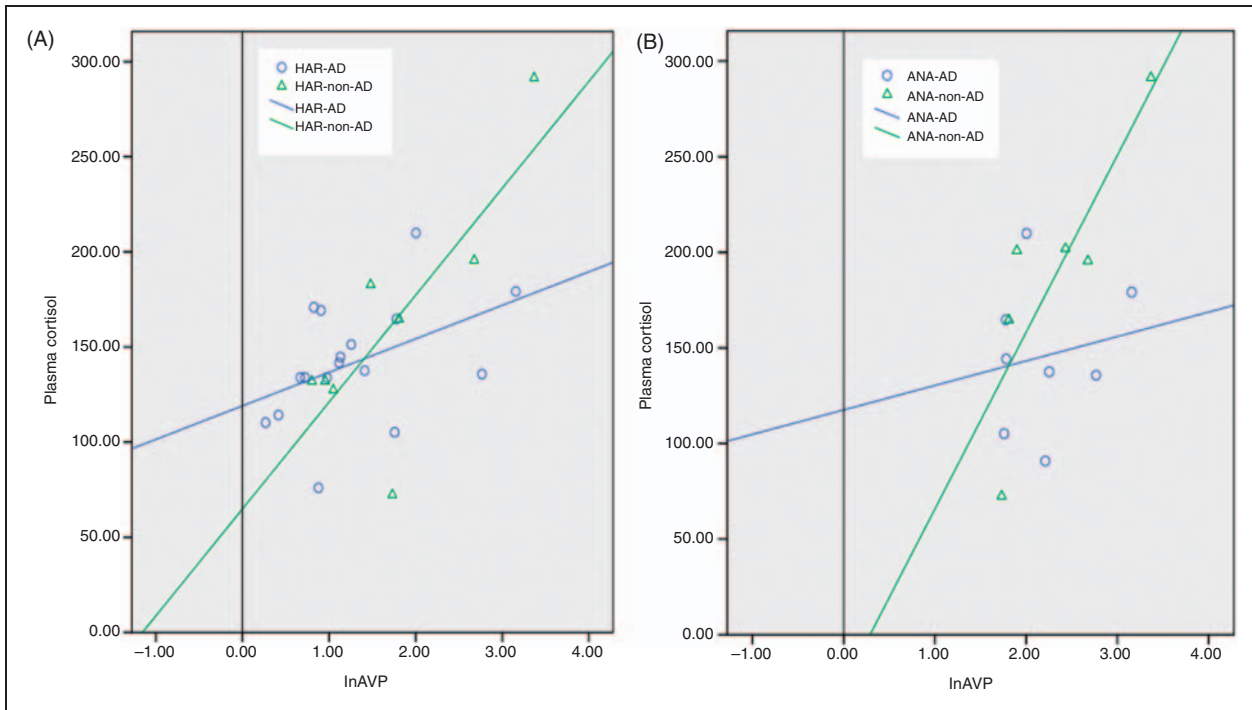
significance ($F = 3.889$; $p = 0.053$), while the effect of the interaction between ANA and lnAVP became just non-significant ($F = 3.980$; $p = 0.051$). This suggests that the type of drug may have a small but relevant effect on the correlation between AVP and cortisol, particularly in HAR depression. If instead of AD subtyping into three AD subtypes the dichotomy between SNRI and the other ADs was used as fixed factor, then this resulted in just significant effects of the interactions between lnAVP and HAR depression ($F = 4.000$; $p = 0.050$), and lnAVP and ANA depression ($F = 4.094$; $p = 0.048$). Summarizing, the correlation between lnAVP and cortisol in the ANA and HAR subcategories was not caused by a potentially low rate of AD treatments in these subcategories. In contrast, both subcategories showed relatively increased slopes compared with all other patients, and this was not caused by the absence of AD treatment. The HAR subcategory could be specifically insensitive for SSRI and TCA treatment.

Discussion

The present study strongly suggests that the correlation of 0.72 between plasma AVP and cortisol concentrations is a general characteristic of non-AD-treated depressive patients, and that the full decrease or normalization of that correlation is a general effect of AD treatment. As no decrease of plasma AVP concentration was found in the AD group compared with the non-AD group, this study also suggests a differential effect of AD treatment on the two components of vasopressinergic activation of the HPA axis in depression. We suppose that the increased AVP-cortisol correlation in untreated patients is due to increased AVP release and increased pituitary V1b receptor function. As far as we know this is the first study to support this peripheral measure as a general characteristic of depression, and to suggest that only the V1b receptor part is most directly reduced by AD treatment.

The data further show that the correlations between plasma AVP and cortisol that have previously been found in ANA and HAR depression (Goekoop and Wiegant, 2009) are not explained by an effect of AD treatment. In fact these two subcategories in particular showed steep slopes of their regression lines of the AVP-cortisol correlation. This suggests a high V1b receptor function specifically in these subcategories, while the relatively weak effect of AD treatment particularly in the HAR subcategory corresponds with a relative resistance to the currently used reuptake inhibitors, with SNRI treatment a potential exception. The genetic factor involved in the increased V1b receptor function which we suppose to be present in this subcategory, and which could correspond with the genetic polymorphism found in early-onset depression (Dempster et al., 2007), could be the cause of this AD resistance and eventually also of the long time to full remission found in this HAR subcategory (De Winter et al., 2006).

The results of this study throw new light on the previous finding of a correlation of 0.55 between plasma AVP and cortisol in 19 depressed patients with several DSM diagnoses not on AD treatment (Brunner et al., 2002). The relatively low correlation could be due to several patients having an adjustment disorder or dysthymic disorder. The other previously reported correlation of 0.49 in 45 unipolar depressed patients could be due to only 67% instead of 100% of these patients having no AD treatment for at least 2 weeks (Inder et al., 1997).

As far as the translation of the AVP-cortisol correlation to animal models is concerned, direct evidence of increased V1b receptor synthesis has been found in a rat model of chronic stress (Aguilera et al., 2008). As this increased synthesis of the V1b receptor coincides with increased synthesis of AVP in parvocellular neurons of the PVN as well as with increased release of ACTH and cortisol (Aguilera et al., 2008), AVP from these neurons is assumed to play the central role in the stimulation of these highly expressed V1b receptors. However, because the amount of AVP released by these neurons into the bloodstream is probably too small to be detected in peripheral plasma, and no stress-related increase of plasma AVP or AVP-cortisol correlation has been found in the male rat model of chronic stress and depression (Aguilera et al., 2008), nor in the stressed male HAB rat (Landgraf et al., 1999), these male rat models are not useful to test the hypothesis of increased V1b receptor function in depression as the cause of the correlating peripheral AVP and cortisol concentrations in human depression. As this correlation is not due to osmotic stimulation of AVP release, because plasma AVP in depression has previously been found to have no correlation with plasma osmolality (Brunner et al., 2002; Van Londen et al., 1997), the most appropriate animal model of depression should show a non-osmotic stress-related AVP response, as has been found in acutely stressed healthy human subjects (Zimmermann et al., 2004) and in chronically depressed patients (Van Londen, 2003). We therefore suggest that tests of the hypothesis of increased AVP release and V1b receptor function in depression could better be carried out in female rat models, which in contrast to male rats do have a non-osmotic stress-induced plasma AVP response (Williams et al., 1985).

While up to now the increased pituitary V1b receptor function was thought to apply particularly to melancholic depression (Dinan et al., 2004), and the involvement of increased AVP synthesis and V1b receptor function could apply particularly to suicidal (Brunner et al., 2002; Inder et al., 1997), anxious-retarded (De Winter et al., 2003; Goekoop et al., 2006) or early-onset (Dempster et al., 2007, 2009) subcategories of depression, the present findings support the hypothesis that the combined increase of AVP release and V1b receptor function is characteristic for depression at large, and suggest that AD treatment induces a down-regulation of the pituitary V1b receptor function. This is comparable with the up-regulating effect on the expression of the pituitary glucocorticoid receptor (Nikisch, 2009).

As far as the increased AVP release in depression in general is concerned, this appears not to be directly reduced by AD treatment. This AVP increase has previously been found to normalize gradually over months to years as AD-treated patients improve, and only in those patients who show full remission (Van Londen, 2003). These results suggest that normalization of AVP release more closely parallels full remission and recovery than the normalization of the pituitary V1b receptor function. The combined results suggest that AD treatment by means of the current monoaminergic drugs

reduces part of the increased HPA axis activation in depression, but does not sufficiently reduce the limbic disinhibition and/or prefrontal deficit that may play additional pathogenetic roles in depression (Goekoop, 2009; Swaab et al., 2000; Wotjak et al., 2002). This differential effect of current AD treatment suggests that drugs with direct anti-vasopressinergic effects could be more effective. The data also suggest that monitoring tests of the effectiveness of AD drugs should differentiate between the two components of AVP release and pituitary V1b receptor function.

Limitations of the present study are the relatively low number of patients, which may have resulted in the low statistical significance of the lnAVP-cortisol correlation within the HAR subcategory; the naturalistic cross-sectional design not controlling for all potential characteristics related to the absence of pharmacological treatment of depressed patients; and the absence of osmolality data that could reconfirm the role of non-osmotic stress in the increased plasma AVP concentration in depression. A further limitation concerns the general measures of AVP and cortisol concentration, based on the mean of morning and afternoon values. Although up to now no statistically significant difference has been found between morning and afternoon AVP concentrations in depression at large (Goekoop et al., 2009b; Van Londen et al., 1997), circadian variability of AVP could still be present in a subcategory of depression, and this could affect the sensitivity and specificity of correlations between AVP and cortisol concentrations for that subcategory. Analogously, while plasma cortisol normally shows strong circadian variability, a reduced variability has been found in depression. As the afternoon concentration has most specifically been found to be related to the increased amount of 24-hour cortisol secretion in depression (Halbreich et al., 1985) this afternoon value could be optimally related to a subcategory of depression, and this could affect the sensitivity and specificity of correlations between AVP and cortisol concentrations for that subcategory. These characteristics and associated effects of AD treatment deserve further study.

As far as potential factors of the absence of AD treatment are concerned, which might confound the interpretation of the correlation between AVP and cortisol as inherent to all depressed patients, this absence could be due to a particularly high severity of depression and low level of Self-Directedness, which would have resulted in a form of self-neglect. However, this possibility is refuted, as in fact the AD treatment group had a non-significantly higher MADRS score and significantly lower Self-Directedness than the non-AD group. This suggests that the acceptance of AD treatment was related to the depression-related loss of Self-Directedness in patients (De Winter et al., 2007), and that the correlation of plasma AVP and cortisol in the non-AD group was not limited to patients with particularly high depression severity.

The present results may imply a further development of the vasopressinergic theory of depression, as they integrate the knowledge of general pathogenetic pathways and AD working mechanisms in animals and humans, and generate predictions for animal models of depression. Major questions that remain to be answered are whether the increased AVP-cortisol correlation, which is thought to represent V1b receptor expression at the pituitary level, is also representative of extra-hypothalamic limbic or prefrontal vasopressinergic mechanisms involved in the dysregulation of the affective and behavioural responses in depressed human subjects, and how well the plasma AVP concentration in depression represents intracerebral vasopressinergic effects. Other remaining questions are to what extent in depressed and manic patients plasma AVP changes are involved in the changes in osmotic behaviour of red cells (Goekoop et al., 1990; Hoeksema et al., 1996) and in the rapid weight changes that have been ascribed to shifts in body water (Kerry et al., 1968).

Acknowledgements

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