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Early Response to Venlafaxine Antidepressant Correlates with Lower ACTH Levels Prior to Pharmacological Treatment

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A link between stressful life events and development or exacerbation of depression has been established via a large body of evidence. An alteration in the regulation of the hypothalamic–pituitary–adrenal (HPA) axis in depression has also been associated with an increase in cortisol secretion. As arginine-vasopressin (AVP) plays an important role in the activation of HPA axis during stress, the present study investigated ACTH and cortisol secretory response induced by an AVP-related peptide desmopressin (ddAVP) in patients with major depression. Prior to antidepressant treatment, endocrinological parameters were evaluated and correlated with the clinical response to venlafaxine treatment, which offers a dual antidepressant action. Depressive patients with no other psychiatric pathology were evaluated with 17-item Hamilton Depression Scale (HAM-D) in order to follow-up the response to venlafaxine. After 1 wk of treatment, 60% of patients reduced their initial HAM-D score to at least 25%; this group was classified as early responders. The other group (40%) started to reduce significantly their HAM-D score after 3 wk of treatment and was classified as late responders. After 6 wk of treatment both groups have reduced HAM-D score to at least 25% of the baseline score. Prior to the pharmacological treatment, both early and late responders showed salivary cortisol rhythm and urinary free cortisol (UFC) in 24-h similar to healthy subjects. However, we did observe differences in basal ACTH secretion, showing that the late responder group had higher basal ACTH than both early responders and controls. The ddAVP challenge promoted a robust secretion of ACTH only in late responders, suggesting a different sensitivity of pituitary vasopressin receptor. The differences in clinical response to venlafaxine among depressive patients seem to be related to endocrinological parameters.

Key Words: Depression; cortisol; ACTH; venlafaxine; ddAVP; AVP; CYP2D6*4.

Introduction

A link between stressful life events and development or exacerbation of depression has been established via a large body of evidence *(1)*. At the cellular level, evidence has emerged indicating neuronal atrophy and cell loss in areas related to the limbic system in response to stress and depression. The neuronal atrophy in parallel with cognitive deficits in both stress and major depression *(2)* are the result of a cortisol level increase by a hyperactivation of the hypothalamic–pituitary–adrenal (HPA) axis *(3)*.

HPA activity is driven by the secretion of corticotrophin-releasing hormone (CRH) from the hypothalamic paraventricular nucleus (PVN), which in turn stimulates the secretion of adrenocorticotrophic hormone (ACTH) from the pituitary gland *(4)*. ACTH stimulates glucocorticoids secretion (cortisol in human) from adrenal cortex. Glucocorticoids interact with their receptor (GR) including those present in the HPA axis where they are responsible for the feedback inhibition of ACTH secretion from pituitary and CRH secretion from hypothalamus *(4)*. Various studies have shown that GR-mediated feedback inhibition is impaired in major depression, allowing CRH hypersecretion and elevation of plasmatic cortisol levels *(5)*. In fact, 50% of depressed patients do not suppress their cortisol secretion following dexamethasone administration, a synthetic corticoid *(5)*; however, antidepressant treatments reverse this condition *(5–7)*.

Although CRH secretion from PVN is the dominant regulator of HPA axis, the role of vasopressin (AVP) as a co-secretagogue has recently been recognized *(8)*. Osmotic stimulation increases the AVP expression in magnocellular neurons of PVN and supraoptic nuclei (SON) while chronic stress paradigms in rodents are associated with AVP neuron activation in the parvicellular division of PVN *(9)*. Thus,

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the CRH effect on ACTH secretion is strongly potentiated by AVP *(10)*. AVP acts on three receptors named V1, V2 and V3 [V(1b)], the latter being predominantly found on the anterior pituitary, providing a mechanism by which AVP facilitates corticotrope responsiveness *(11)*.

In depressive patients, CRH hypersecretion with a concomitant elevation in serum cortisol is associated with hypophyseal downregulation of CRH receptor 1 (CRHR1) *(12)*. CRHR1-deficient mice exhibit a compensatory activation of the AVP system, enabling the animals to maintain normal levels of basal ACTH *(13)*. Thus, the higher levels of cortisol in depressive patients could be induced by the activation of the AVP system. In fact, an increased AVP gene expression has been observed in human hypothalamus from depressed subjects *(14)*. Also, the administration of the AVP analog desmopressin (1-deamino-8-D-arginine vasopressin) to major depressive patients induces an augmented ACTH and cortisol secretion compared with control subject *(15)*. This evidence suggests that the anterior pituitary vasopressin receptors are more sensitive to AVP in depressive patients, which might partly account for the higher ACTH and cortisol secretion in this pathology *(15)*.

Considering the psychological, social, and economic implications of depression, an early clinical response to an antidepressant treatment is a major therapeutic goal. Thus, identifying markers used as early predictors are crucial for antidepressant treatment. We investigated whether treatment response is predicted by HPA axis parameters or by sensitivity of the axis to a vasopressin analog (ddAVP). Venlafaxine, which was used as antidepressant, is a selective serotonin and noradrenaline reuptake inhibitor and offers a dual antidepressant action *(16)*. Dual mechanism of action for venlafaxine provides a significantly greater efficacy to achieve remission *(17)*. Our current pilot study in patients with major depression (i) compared cortisol secretory rhythm, baseline, and desmopressin-induced ACTH, and cortisol secretion with those of control subjects; (ii) correlates endocrinological parameters with the clinical response to venlafaxine antidepressant treatment.

Results

Response to Antidepressant Treatment

The severity of depression was evaluated prior to and during venlafaxine treatment. The mean HAM-D score was 24 ± 1.2 (n = 18) at baseline (d 0). Venlafaxine treatment induced different responses in major depressive patients. After 1 wk of venlafaxine treatment, 60% of patients responded early (early responders) to the antidepressant treatment by reducing their initial HAM-D score (>25%) (Fig. 1). The other patients (40%) were classified as late responders because they reduced significantly their HAM-D score by 25% after 3 wk of treatment (Fig. 1). A repeated-measure analysis of variance (ANOVA) of HAM-D score demonstrated a significant effect of group (early responders versus late responders) to venlafaxine during treatment ($F_{4.68}$ = 6.91; $p < 0.001$). As shown in Fig. 1, the clinical response to venlafaxine was significant different between both groups after 1, 2, and 3 wk of pharmacological treatment.

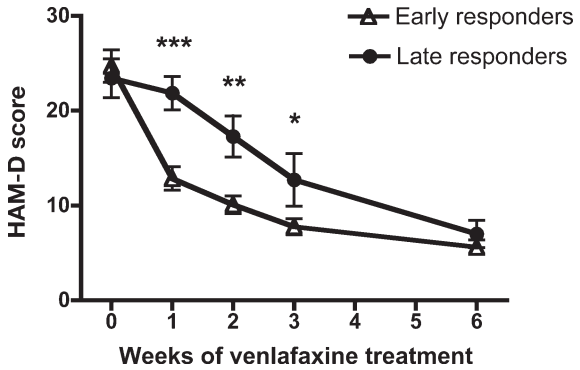

Fig. 1. Effect of venlafaxine treatment on HAM-D score evolution. Early response to venlafaxine was defined as ≥25% reduction HAM-D score from baseline to wk 1 and represented the 60% of patients. ***$p < 0.008$, **$p < 0.002$, *$p = 0.055$ vs early responder.

After a 6-wk treatment, the early responders had reduced to a 23% their initial HAM-D score (Table 1). Also 70% of early responders had remitted (HAM-D score ≤ 7). In addition, after a 6-wk treatment, late responders reduced their initial HAM-D score to 29.5% ($p < 0.001$). However, only 40% of these patients remitted after 6-wk treatment. The differences in the clinical response between these groups of patients could not be explained by differences in the initial HAM-D score, age, or gender (Table 1). Also, both groups of patients had reported previous depression episodes.

Venlafaxine is primarily metabolized by the highly polymorphic cytochrome P450 (CYP) enzyme CYP2D6 to yield a pharmacologically active metabolite, *O*-desmethylvenlafaxine and to a lesser extent by CYP3A4, to yield *N*-desmethylvenlafaxine *(18)*. Polymorphisms related to CYP2D6 (CYP2D6*4 and CYP2D6*3) can produce reduction in the enzymatic activity, generating poor metabolizer phenotypes *(18)*. The difference in the response to pharmacological treatment does not seem to be related to a poor drug metabolism as shown by the absence of CYPD6*4 allele in depressive patients (data not shown).

Neuroendocrine Characterization of Depressive Patients and Controls

Endocrinological parameters related to HPA activity were evaluated prior to venlafaxine treatment. In order to obtain an easier comparison, endocrinological results were separated accordingly to a venlafaxine clinical response. These were compared with a group of 10 age- and weight-matched controls. Thus, early and late responders to venlafaxine presented body mass index (BMI) similar to controls (Table 2). In addition, patients did not exhibit higher levels of 24-h urinary free cortisol (UFC) than healthy controls, displaying values under the normal range (<100 µg/24 h)

Table 1
Characteristics of Early Responders and Late Responders

Parameters	Early responders	Late responders	Statistics
Age, yr[a]	39.4 (31.7–47.0)	47.1 (35.0–59.3)	$t = -1.3369$ $p = 0.1999$
Gender (%)[b]			
Female	71.4 (39.0–94.0)	42.9 (10.0–81.6)	$z = 1.24$ $p = 0.214$
Male	28.6 (6.0–61.0)	57.1 (18.4–90.1)	
HAM-D wk 0[a]	25.1 (21.7–28.5)	23.4 (18.4–28.4)	$t = -0.6597$ $p = 0.5188$
HAM-D wk 6[a]	8.4 (5.5–11.3)	7.0 (3.5–10.5)	$t = -0.6834$ $p = 0.5042$

Early response to venlafaxine was defined as ≥ 25% reduction HAM-D score from baseline to wk 1.

[a]Data represent the mean values and the confidence interval (CI: 95%) is shown in parenthesis. Data were analyzed using two-tail *t*-test.

[b]Data were expressed as percentage of total subject in the group and were analyzed with the probability test. Values of $p < 0.05$ were statistically significant.

Table 2
Clinical and Endocrinological Characteristics of Patients with Major Depression and Control Group

Groups	BMI (kg/m^2)	UFC (μg/24 h)	Salivary cortisol (mg/dL) 8 AM	3 PM	11 PM	DST
Early responder ($n = 11$)	26.2 ± 1.3	65.4 ± 9.2	0.8 ± 0.17	0.40 ± 0.04	0.30 ± 0.08[a]	0.13 ± 0.02[d]
Late responder ($n = 7$)	25.4 ± 1.8	39.9 ± 11.4	0.69 ± 0.15	0.30 ± 0.04	0.10 ± 0.01[b]	0.10 ± 0.0[e]
Control ($n = 10$)	24.7 ± 0.5	56.3 ± 9.2	0.85 ± 0.1	0.32 ± 0.04	0.14 ± 0.02[c]	0.11 ± 0.01[f]

Urinary free cortisol (UFC) was measured in urine collected during 24-h. Cortisol rhythm was measured at 8 AM, 3 PM, and 11 PM as free salivary cortisol. In the dexamethasone suppression test, salivary cortisol was measured at 8 AM following 1 mg dexamethasone at 11 PM the previous night. Data represent the mean values ± SEM and were analyzed using non-parametric Kruskal–Wallis ANOVA test and Dunn's post-test.

[a]$p < 0.001$ vs 8 AM; [b]$p < 0.01$ vs 8 AM; [c]$p < 0.05$ vs 8 AM ; [d]$p < 0.0003$ vs 8 AM; [e]$p < 0.012$ vs 8 AM; [f]$p < 0.05$ vs 8 AM.

(Table 2). However, when compared with early responders and controls, late responders displayed the lowest UFC values, but not statistically significant (Table 2). Also, there were no differences in the 24-h urine collection volumes between depressed subjects and healthy controls (data not shown).

In order to detect a possible alteration in cortisol secretion, we determined salivary cortisol rhythm in depressive patients and control subjects. Table 2 shows that the salivary cortisol was high in the morning (8 AM), decreased in the afternoon (3 PM), and significantly decline at night (11 PM) in depressive and control groups. However, the early responders to venlafaxine had higher cortisol levels at 11 PM than late responders, although this was not statistically different. The 1 mg dexamethasone ingestion at 11 PM suppressed the cortisol secretion (dexamethasone suppression test, DST) measured the next morning in all groups (Table 2). However, it was important to determine if ddAVP, an analog of AVP that promotes ACTH secretion, produces a similar effect on depressive and on control groups. Baseline ACTH prior to ddAVP administration was similar between early responders and controls (11.9 ± 0.8 vs 11.74 ± 0.8 pg/mL). However, in late responders, the baseline ACTH levels were significantly higher (40%) than those in early responders and controls (16.7 ± 1.8 pg/mL, $p < 0.01$). In contrast, basal cortisol secretion was similar among depressive groups and control subjects (early responders: 9.9 ± 1.2 μg/mL, late responders: 11.0 ± 1.4 μg/mL, controls: 11.4 ± 0.75 μg/mL).

During ddAVP test, ACTH-induced secretion was observed in all groups after 5 min of AVP analog injection (Fig. 2A). In order to make an easier comparison, the area from the concentration–time curve (AUC) for ACTH secretion was calculated in each group using the trapezoid method. This analysis revealed that late responders secreted significantly higher levels of ACTH (70–80%) (2889 ± 438 pg/mL/min) when compared to controls (1540 ± 95 pg/mL/min) or early responders (1754 ± 129 pg/mL/min) (Fig. 2B). In agreement with this, δ of ACTH response to ddAVP (maximum peak of the curve minus basal secretion) was higher in late responders than controls (22.85 ± 8.1 pg/dL vs 5.04 ± 1.04 pg/dL) (Fig. 2C). Baseline cortisol levels were similar in depressive groups and control subjects (9.9 ± 1.2 μg/dL; 11 ± 1.4 μg/dL; 11.36 ± 1.4 μg/dL for early responders, late responders, and controls, respectively). However, after 15–30 min of ddAVP injection, a slight increase in cortisol was observed in depressive patients (Fig 3A). The AUC of

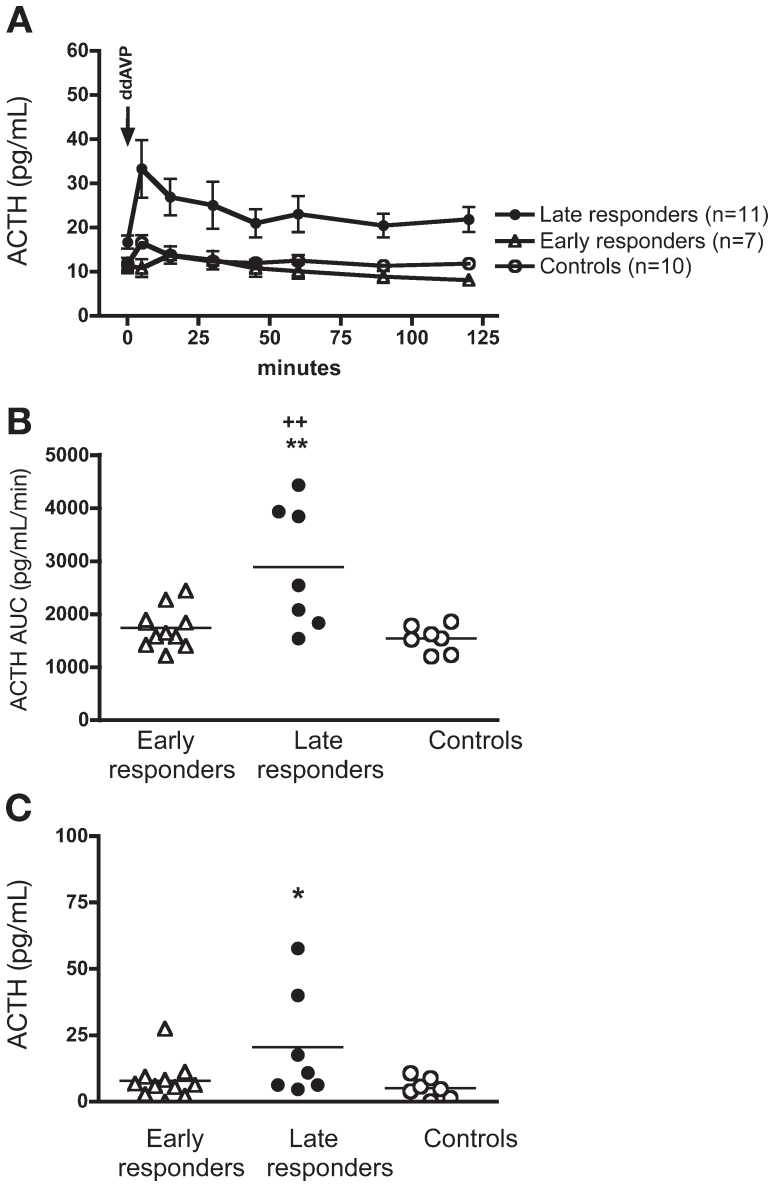

Fig. 2. ACTH response to an injection of ddAVP (8 μg) in depressive patients and healthy subjects. (**A**) Time course of ACTH secretion. (**B**) Area under curve (AUC) of ACTH secretion. (**C**) Delta ACTH (δ-ACTH) was considered as the maximum hormone level following the ddAVP injection minus baseline. Data represent the mean values ± SEM and were analyzed using non-parametric Kruskal–Wallis ANOVA test and Dunn's post-test. Mean value ± SEM of control group: basal ACTH 11.7± 0.8 pg/mL; δ-ACTH 5.0 ± 1.04 pg/mL; basal cortisol 11.4 ± 1.4 μg/dL; δ-cortisol 0.2 ± 0.2 μg/dL. $^{**}p < 0.01$ vs early responder; $^{++}p < 0.01$ vs control.

cortisol was similar in all the three groups (Fig. 3B). However, δ cortisol secretion (the maximum peak of the minus basal secretion) was higher in early (2.73 ± 0.9 μg/dL) and late responders (3.3 ± 0.8 μg/dL) when compared to controls (0.15 ± 0.15 μg/dL) (Fig. 3C).

Discussion

Venlafaxine is a mixed serotonin and noradrenaline reuptake inhibitor used as a first-line treatment of depressive disorders *(17)*. In the current pilot study we demonstrated for the first time that patients with high ACTH response during the challenge with ddAVP behave as late responders to venlafaxine treatment. This difference in the response to pharmacological treatment does not seem to be related to poor drug metabolism as shown by the absence of CYP2D6*4 allele, although we did not study other CYP polymorphisms.

After 1-wk treatment, 60% of patients responded early to venlafaxine treatment by reducing baseline HAM-D score to at least 25%. The other group, which represents 40% of patients, started to reduce significantly their HAM-D score after 3-wk treatment. All patients included in this study had previous depressive episodes and the difference in the clinical response to venlafaxine had not been related to baseline HAM-D score. Also, the difference in clinical response between early and late responders might not be related to poor metabolization of venlafaxine, a proposal based mainly

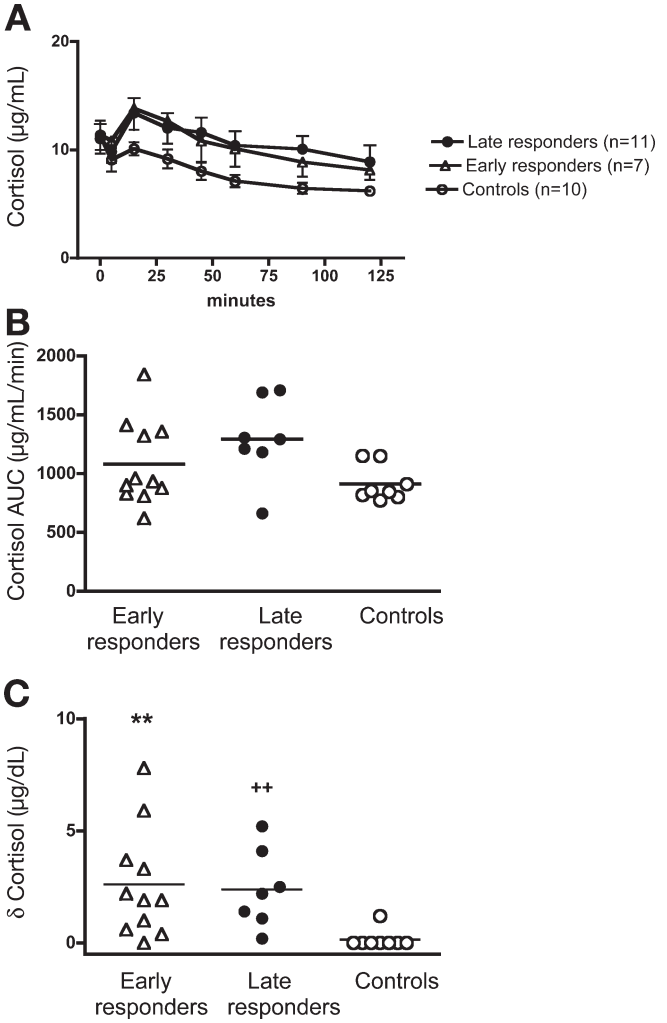

Fig. 3. Cortisol response to an injection of ddAVP (8 µg) in depressive patients and healthy matched subjects. (**A**) Time course of cortisol secretion. (**B**) Area under curve (AUC) of cortisol secretion. (**C**) Delta cortisol (δ-cortisol) was measured as the maximum hormone level following the infusion minus baseline. Data represent the mean values ± SEM and were analyzed using nonparametric Kruskal–Wallis ANOVA test and Dunn's post-test. $^{**}p < 0.01$ vs control; $^{++}p < 0.01$ vs control.

by the absence of CYP2D6*4 allele *(19)*. Zurich metaanalyses showed that the early onset of improvement was highly predictive of later outcome; on average, 70% of patients showing improvement within the first 14 d became responders *(20)*. Also, differences between active treatments and placebo emerged within the first 5 d and reached a point of maximum distinction around d 14 *(20)*. Then, the early response to antidepressants observed in our study may be related to the venlafaxine action based on the response and on the remission of the patients under study.

The lag phase to observe significant therapeutic effects of antidepressants is probably related to changes in neural plasticity that should occur to induce an appropriate clinical response *(21,22)*. Thus, biological changes necessary to promote response are likely to occur by the end of the second week of treatment in responder patients *(20)*. An emerging hypothesis to explain depression suggests that the inability of neuronal systems to exhibit appropriate, adaptive plasticity could contribute to the pathogenesis of depression *(21)* and that antidepressant treatment may exert their therapeutic effects by stimulating appropriate adaptive changes in neuronal systems through the activation of signal transduction pathways and changes in neural gene expression *(21)*. Recent studies have demonstrated that chronic antidepressant administration enhances the brain derived neurotrophic factor (BDNF) expression within hippocampal and cortical neurons and can prevent the stress-induced decrease in BDNF expression *(21)*. Moreover, stress induces neuronal atrophy/death and decreases neurogenesis of hippocampal neurons and these effects may be observed under chronic glucocorticoid administration *(23)*. In line with these, several studies have shown that neuronal atrophy and cell loss in areas related to the limbic system are observed in response to stress and in depression *(3,24–26)*. In this context, the loss of the HPA axis regulation in patients with major depression is identified by increased plasma levels of cortisol (especially at the circadian nadir) *(27)* and is the most consistently demonstrated neuroendocrine abnormality in major depression *(28)*.

The increased activity of the HPA axis is thought to be related to some degree to an altered feedback inhibition of glucocorticoids. Through binding to their receptor in HPA axis tissues, endogenous glucocorticoids act as a potent negative regulator of the HPA axis activity, controlling the synthesis and release of CRH in the PVN *(29)*. This feedback regulation of the HPA axis occurs through two different subtypes of receptors referred as type I or mineralocorticoid receptor (MR), and the type II or glucocorticoid receptor (GR) *(29)*. MR has a high affinity for endogenous corticosteroid and is believed to play a role in the regulation of circadian fluctuations in these hormones *(29)*. In contrast to MR, GR has a high affinity for dexamethasone and a lower affinity for endogenous corticosteroids *(29)*. GR is believed to be important in the regulation of the stress response, i.e., when endogenous levels of glucocorticoids are high *(29)*. Because patients with major depression exhibit elevated circulating cortisol levels, several studies have shown the possibility that the number or function of corticoid receptors are reduced in depressive patients. However, a recent study reports that MR-mediated negative feedback remains intact in depressive patients *(30)*. In contrast, several approaches have found a lack of response of GR in depressed patients, suggesting a glucocorticoid resistance *(5)*. The data supporting the notion that the GR action is impaired in major depression comes from different studies demonstrating a non-suppression of cortisol secretion following administration of the synthetic glucocorticoid, dexamethasone (dexamethasone suppression test, DST) *(4)* and also showing lack of inhibition of ACTH response to CRH following dexamethasone pretreatment (dexamethasone/CRH test) *(5)*.

In fact, many of the beneficial effects of antidepressants are exerted through their action on the HPA system in depressed patients. Indeed, the antidepressant treatment is associated with a reduction of activity in basal and stress-induced hyperactive HPA axis and probably is required to observe clinical response or remission *(31–33)*. A number of studies in humans have also shown that manipulating GR function with both agonists *(34)* and antagonists *(35)* has an antidepressant effect. These results seem to be controversial probably because the effects have been observed in non-responder patients to traditional treatment *(34)* or in patients with atypical depression *(36)*. However, the therapeutic effects of the GR antagonist could also be explained as glucocorticoid resistance in the depressive disorder, where the hypercortisolemia is seen as a compensatory mechanism in the presence of reduced brain sensitivity to glucocorticoids *(37)*. Ridder et al. *(38)* elegantly demonstrated in animals the sufficiency of a reduction in GR for the manifestation of depression-like behaviors and HPA axis dysfunction following a severe stressor, adding further evidence that links HPA axis alteration with depression; however, the GR reduction requirements for the manifestation of depression-like behaviors has not been proven so far. Nonetheless, clinical and experimental studies have shown that antidepressants increase GR function, leading to resolution of glucocorticoid resistance *(5)*. In line with this, recent reports support the notion that variants of GR gene *(39,40)* and of components of the GR heterocomplex *(41)* might play a role in the etiology of major depression and contribute to the variability of antidepressant response.

In the current study, early and late responders to venlafaxine showed similar cortisol rhythms measured as free salivary cortisol. However, differences in circadian cortisol secretion should be more evident in a 24-h plasma sampling period *(27)*. A significant percentage of depressed patients has shown a hypersecretion of cortisol, as manifested by increased 24-h urinary free cortisol and elevated plasma concentration of cortisol *(30)*. The increase in cortisol could be due, at least partly, to an altered enzyme 11-β-hydroxysteroid dehydrogenase (11β-HSD) that regulates glucocorticoid levels by converting cortisol into cortisone and vice versa; however, some studies have reported a lowered activity of 11β-HSD *(42,43)* in depressive patients, while others have reported no alteration in the enzyme activity *(44)*. In the present study, we did not detect differences in FUC between depressive groups and controls. This finding may be explained by evidence showing that cortisol hypersecretion is not always present during the clinical manifestation of depression *(5)* and even when present, the variations do not occur at all times during the episode *(45)*.

We also observed that, after dexamethasone administration, free salivary cortisol concentration was suppressed in early and late responders and in controls, considering a cortisol value for suppression of <5 μg/dL *(46)*. We obtained similar results in DST by evaluating the cortisol level in plasma (data not shown). Only three patients showed cortisol levels above 0.1 μg/dL following DST. The lack of differences in post-DST salivary cortisol between depressive patients and healthy subjects is consistent with a metaanalysis study that showed that high DST non-suppression rates were primarily related to a subsample of depressed patients who met the criteria for psychotic depression (64%) compared to those with non-psychotic or non-melancholic depression (41%) *(47)*. The same study showed that the rate of non-supression was low (12%) in non-psychotic outpatients with major depression *(47)*. Also, the non-suppression observed in the DST has been related to depression severity *(48)* and could identify depressed patients with a higher risk of suicidal behavior *(49,50)*. A more recent report has described that non-suppression to DST is observed in depressive patients with high level of stress *(50)*. Thus, our study shows a low prevalence of non-suppressors that could be explained in terms of a non-severe depression and/or a low risk of suicidal behavior in our population of depressive patients.

Although we did not observe differences in DST and free urinary cortisol between depressive patients, we found differences in both basal cortisol and ACTH between early and late responders to venlafaxine prior to pharmacological treatment. These findings indicate that both groups differ in HPA-axis sensitivity that could be explained by changes in the regulation of ACTH secretion. In recent years, the role of AVP has been increasingly recognized as ACTH secretagogue *(8)*. The neurons located in the PVN and SON nuclei of the hypothalamus that release vasopressin to the pituitary are involved in the signs and symptoms of depression *(10)*. The AVP neurons of the PVN consist of two overlapping populations. First, PVN contains parvocellular neurons that secrete AVP together with CRH from axon terminals into the pituitary portal circulation. This AVP potentiates ACTH release by CRH in the anterior pituitary *(51)*. Second, magnocellular neurons in PVN together with magnocellular neurons from SON, project their axons to the posterior pituitary, where they release AVP directly into the blood, as for instance, in response to osmotic stimuli *(10)*.

Using postmortem tissue of subjects with major depression has demonstrated an increase in the number of AVP-immunoreactive neurons in PVN *(52)*, particularly colocalizing with CRH *(53)*. Also, an increased AVP gene expression in the SON in depressed subjects has been observed *(14)*. This evidence supports the idea that hypothalamic AVP might be involved in the pathogenesis of depression. Interestingly, while CRH is the main cause of increased ACTH release under acute stress, in animal models of chronic stress there is a switch from CRH to AVP stimulation of ACTH release *(54)*. Unlike of CRH, the effect of AVP on ACTH secretion is resistant to glucocorticoid feedback *(55)*. In line with this, post-dexamethasone AVP levels were signifi-

cantly elevated in patients with chronic depression compared with controls *(56)*. These findings are in agreement with the hypothesis that the nonsuppression ACTH secretion in the combined dexamethasone/CRH test is due to increased hypothalamic AVP release in depressed subjects *(54)*. AVP exerts major physiological effects through three different G coupled–receptor isoforms named V(1a), V(1b), and V2 *(11)*. Among these three subtypes, the vasopressin V(1b) receptors are coupled to phospholipase C (PLC) *(57)* and are specifically expressed in pituitary corticotrophs mediating the stimulatory effect of vasopressin on ACTH release *(11)*, not only under stress but also under basal conditions *(58)*.

All this evidence suggests that AVP system could be altered in depressive disorder, enhancing both the ACTH and cortisol secretion. Recently, it has been observed in patients with melancholic depression that ddAVP challenge induced higher levels of ACTH and cortisol secretion than control subjects *(15)*. In the present study, we found prior to antidepressant treatment that early responders to venlafaxine showed basal cortisol and basal ACTH similar to controls. Moreover, the ddAVP challenge in early responders caused an AUC of ACTH and cortisol secretion similar to controls; indicating that this group of depressive patients has the same sensitivity to ddAVP in comparison to healthy subjects. However, ddAVP induced a higher δ cortisol than controls, probably due to a higher adrenal response to ACTH. In contrast, late responders to venlafaxine showed an increased basal ACTH level prior to antidepressant treatment. The difference in basal ACTH suggests that HPA-axis regulation could be altered in patients who display a late response to the antidepressant. In agreement, the ddAVP promoted a robust secretion of ACTH measured as AUC or as δ ACTH, only in late responders to venlafaxine. These findings were also correlated with a higher δ cortisol secretion when compared to controls, although cortisol was as high as to those observed in early responders. The absence of correlation between ACTH increased secretion and cortisol levels in late responders could be explained by the resistance of adrenal cortex to ACTH stimulation probably through to a downregulation of ACTH receptors and/or alteration in the signal transduction pathway associated to this receptor.

The effect of ddAVP among depressive patients could reflect different sensitization or density of AVP receptor. In agreement, it has been reported that ddAVP administration normalized the blunted CRH-mediated ACTH release in depressive subjects, suggesting pituitary V(1b) receptor upregulation *(59)*. Recently, studies in mice lacking the V(1b) receptor gene [V(1b)R(–/–) mice] have shown that the V(1b) receptor regulates stress-induced catecholamine release *(60)*, also suggesting that AVP system participates in stress response. Also, data suggest that AVP regulation of the HPA axis is critical for sustaining corticotroph responsiveness in the presence of high circulating glucocorticoid levels during chronic stress *(61)*. Thus, the prolonged exposure to glucocorticoids although decreases the number of V(1b) receptors, increases the V(1b) coupling efficiency due to an increase in guanyl nucleotide binding protein Gq *(62)*.

We concluded that late responders to venlafaxine treatment displayed a higher sensitivity to ddAVP challenge, suggesting a higher sensitivity of pituitary V(1b) receptors. Probably, these patients could present higher levels of AVP than early responders and controls. In accordance with this, several studies report that depressed subjects with no antidepressant medication have elevated AVP plasma levels *(63,64)*. A highly significant AVP–cortisol correlation has been observed in patients with anxious–retarded depression, while no such correlation has been found in patients with nonanxious–retarded depression *(63)*.

The most relevant finding in the present study is the probable relationship between the endocrinological parameters and the clinical response to venlafaxine in depressed patients.

An association was found between the delayed response to venlafaxine and the high basal ACTH levels. Therefore, the effect of ddAVP in late responders can be explained by an increase in the population of V(1b) receptors and/or by an increase in the sensitivity of the receptors V(1b) receptors. Also, the late response to venlafaxine could be associated with a normalization in the sensitivity of central V(1b) receptor and/or AVP secretion. This study should hopefully be repeated in wider groups of patients and also with other type of antidepressants. Evaluation of changes in basal ACTH, AVP, and ddAVP response in nonresponder patients and in remitter patients would also be useful to have a greater insight of the processes involved and its applicability as response markers.

Our study suggests that the sensitivity of pituitary V(1b) receptor among depressive patients seems to be related to the clinical response to venlafaxine. A systematical investigation of well-defined treatment regimens in a larger number of patients and in a longer period of time becomes necessary to explore whether the ddAVP test could be classified both as a state marker and as a predictor of treatment response in major depression.

Methods

Subjects

Ten healthy volunteers (four male and six female) were recruited for this study. These subjects did not present current or previous history of psychiatric illness, physical illness, alcohol or drug abuse, and use of any medication or oral contraceptives.

The group of major depressive patients included 18 outpatients (8 male and 10 female) diagnosed by a psychiatrist, following DSM-IV criteria, as having a current episode of major depression. For inclusion, subjects had to be

between 18 and 60 yr old and they had a baseline score at least 15 in the 17-item on the Hamilton Rating Scale for Depression 17 (HAM-D) *(65)*. Using the structured Mini-International Neuropsychiatry Interview (MINI) other psychopathologies were discarded *(66)*. Exclusion criteria were as follows: (i) physical/neurological disease, (ii) abnormal thyroid function, (iii) patients with hypertension, (iv) pregnant women or nursing women, (v) patients with antidepressant medication. All patients and controls had to be free of symptoms of acute or chronic infection, autoimmune disease, and medications that might influence the immune function. Women using oral contraception were not included. The study was approved by the Ethics Committee of the Clinical Hospital, University of Chile. All subjects signed written informed consent.

Hormonal Evaluation Prior to Antidepressant Treatment

Desmopressin Stimulation Test

All patients underwent desmopressin testing prior to venlafaxine treatment. An indwelling catheter was inserted at 0800 h with the subject remaining supine during the whole study period. At 0830 h AM (0-min) 8 μg ddAVP was given as an iv bolus injection. Blood samples for ACTH and cortisol measurements were obtained at –15, 0, 5, 15, 30, 45, 60, 90, and 120 min. Blood pressure and heart rate were recorded during the study period. No side effects were reported during the study period or the day. The total and integrated ACTH and cortisol responses to ddAVP were calculated by the trapezoid method and expressed as the area under the concentration–time curve (AUC) from 0 to 60 min, using the Origin 7.0 software (Originlab Corporation).

Salivary Cortisol Circadian Rhythm and Dexamethasone Suppression Test (DST)

Salivary cortisol measurement is an excellent indicator of free cortisol and was used for cortisol circadian rhythm determination. Salivary samples were taken at 8 AM, 3 PM, and 11 PM, 5 d following ddAVP stimulation test. Samples were centrifuged at 1000*g* during 2 min and the free cortisol was measured in the supernatant. For DST, 1 mg of dexamethasone was given at 11 PM and blood and salivary samples were drawn next day at 8 AM for cortisol determination. Subjects with postdexamethasone cortisol levels >5 μg/dL were considered as non-suppressors according to Vythilingam et al. *(46)*.

Measurement of Urinary Free Cortisol (UFC) Excretion

One 24-h sample of urine was collected from each subject. Subjects were asked to empty their bladder at 8 AM on the first day and start urine collection immediately after. Subjects were asked to void at 8 AM the next day and include this final sample in the 24-h collection. The total urine volume was measured and an aliquot was kept at –20°C until assayed for cortisol. The urinary free cortisol (UFC) excretion (μg/24 h) was calculated as product of urine volume and urinary cortisol concentration.

Hormones Measurements

Cortisol and ACTH were determined by commercial chemiluminescent enzyme immunometric assays (Immulite, Diagnostic Products Corporation, Los Angeles, CA). For cortisol the sensitivity was 1.0 μg/dL and the inter- and intra-assay coefficients of variation were 7.3% and 6.7%, respectively. For ACTH, the sensitivity was 10 pg/mL and the inter- and intraassay coefficients of variation were 5.1% and 4.9%, respectively. Salivary cortisol was measured by Active® Cortisol EIA (Diagnostic System Laboratories, Inc, Texas, USA) with a sensitivity of 0.01 μg/dL.

Pharmacological Treatment with Venlafaxine and Follow-up

The venlafaxine treatment was started after endocrinological evaluation. Depressive patients were treated with a dose of 37.5 mg/d during 1 wk and then maintained at 75 mg/d during 6 mo. The follow-up assessments were done at 1st, 2nd, and 6th wk through HAM-D scale by a trained clinician to rate severity of depression. The early response to the antidepressant was considered as a reduction of at least a 25% from baseline HAM-D score after 1 wk of venlafaxine treatment. Treatment response was defined as ≥50% reduction in HAM-D from baseline to wk 6.

Statistical Analysis

All results are expressed as mean ± SEM. Data were analyzed by non-parametric Kruskal-Wallis ANOVA tests with a Dunns post-hoc test using GraphPad Prism 4.0 (GraphPad Software, San Diego, CA). In order to assess change over time of the variable between groups of patients during venlafaxine treatment, repeated measurement analysis of variance (ANOVA) was used (Satistica 4.5 for Windows). In order to evaluate gender effect on venlafaxine response, data were analyzed with the probability test using the response as dependent variable (STATA 7.0, StataCorp LP). Significance between early and late responders was analyzed using two-tail *t*-test. The level of significance was set at $p \leq 0.05$.

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