Increased arginine vasopressin mRNA expression in the human hypothalamus in depression: a preliminary report
Increased arginine vasopressin mRNA expression in the human hypothalamus in depression: a preliminary report

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Abstract

Background: Elevated arginine vasopressin (AVP) plasma levels have been observed in major depressive disorder, particularly in relation to the melancholic subtype. Two hypothalamic structures produce plasma AVP: the supraoptic nucleus (SON) and the paraventricular nucleus (PVN). This study intends to establish which structure is responsible for the increased AVP plasma levels in depression.

Methods: Using in situ hybridisation we determined the amount of AVP mRNA in the PVN and SON in post mortem brain tissue of 9 depressed subjects (6 with the melancholic subtype) and 8 controls.

Results: In the SON a 60% increase of AVP mRNA expression was found in depression compared to controls. In the melancholic subgroup AVP mRNA expression was significantly increased in both the SON and the PVN compared to controls.

Conclusions: We found increased AVP gene expression in the SON in depressed subjects. This may partly explain the observed increased AVP levels in depression.

Introduction

Neurons located in the paraventricular (PVN) and supraoptic (SON) nucleus of the hypothalamus that release arginine vasopressin (AVP) to the pituitary are
considered to be involved in the signs and symptoms of depression (Swaab et al., 2000). The vasopressinergic neurons of the PVN consist of two overlapping populations. First, the PVN contains parvocellular neurons that secrete AVP together with corticotropin releasing hormone (CRH) from axon terminals into the pituitary portal circulation. This AVP potentiates the release of adrenocorticotropic hormone (ACTH) by CRH in the anterior pituitary (Antoni, 1993). Second, magnocellular neurons in the PVN, together with magnocellular neurons from the SON, project to the posterior pituitary, where they release AVP directly into the blood, for instance in response to osmotic stimuli (Swaab, 2003).

In post mortem tissue we previously showed that in major depression (MD) the number of AVP-immunoreactive neurons in the PVN (Purba et al., 1996), and in particular those colocalizing CRH (Raadsheer et al., 1994), is increased. In support of the idea that hypothalamic AVP may be involved in the pathogenesis of depression, Nakase et al. found that in a rat model for depression the expression of AVP mRNA in the magnocellular neurons of the PVN was increased (Nakase et al., 1998). Moreover, Keck et al. showed in an animal model of depression that a reduction of the hypothalamic vasopressinergic hyperdrive following paroxetine treatment contributes to clinically relevant behavioral and neuroendocrine effects (Keck et al., 2003). Interestingly, while in acute stress CRH is the main cause of increased ACTH release, animal models show that in chronic stress there is a switch from CRH to AVP-stimulation of ACTH release (Scott et al., 2002). Since depression is a state that lasts for weeks at a minimum, these findings are in line with the hypothesis that the non-suppression in the combined dexamethasone/CRH test in depressed subjects is due to increased hypothalamic AVP release (Holsboer, 2001).

In accordance with these findings several studies found that depressed subjects have elevated AVP plasma levels (Van Londen et al., 1997; Inder et al., 1997; De Winter et al., 2003), which normalize as patients improve (Van Londen, 2003). Importantly, De Winter et al. observed that patients with an anxious-retarded or ‘melancholic’ type of depression had elevated AVP plasma levels (De Winter et al., 2003). This observation is in line with Van London et al., who found increased AVP plasma levels in melancholic depressed patients (Van Londen et al., 1997). There are also indications that elevated AVP plasma levels are related to riskful symptoms of depression, such as psychomotor agitation (Van Londen et al., 1997) and suicidal behavior (Inder et al., 1997).

To find out 1) whether the observed increase in AVP mRNA in an animal model of depression could also be found in depressed humans and 2) which hypothalamic structure, PVN or SON, might be responsible for the increased
plasma AVP levels in depression, we performed in situ hybridization on AVP mRNA in the PVN and SON of depressed and non-depressed subjects. Because of the indications that AVP release might especially be altered in the melancholic type of depression, we also analyzed our data for the melancholic subgroup of depressed patients.

**Methods and Materials**

**Subjects**

Hypothalami of 9 depressed subjects and a group of 8 control subjects matched for age and sex (see table) were obtained from the Netherlands Brain Bank in accordance with the formal protocols for use of human brain material and clinical information for research purposes. The MD patients were diagnosed during their life and the diagnosis was confirmed by a board-certified psychiatrist (WJGH) retrospectively using the medical record, according to DSM-IV criteria, paying special attention to the presence of melancholic features, also according to DSM-IV criteria. One patient, who used an antidepressant and eventually committed suicide, was diagnosed having depressive disorder not otherwise specified (DDNOS), because his medical record did not yield sufficient information to diagnose MD. Six of the patients fulfilled the criteria for melancholic type of depression. The matched group of controls did not suffer from a primary neurological or psychiatric disease. Exclusion criteria consisted of alcohol abuse, because of the reported loss of AVP neurons in the PVN and SON (Harding et al., 1996), and the use of corticosteroids (Erkut et al., 1998; Kim et al., 2001), except for one patient who used prednisone and was matched with one control who also used prednisone.

*Measurement of AVP mRNA in PVN and SON*

The method of Liu et al. (2000) was used for in situ hybridization histochemistry. For quantitative analysis of AVP mRNA, within the Image Pro Plus version 4.5 image analysis package (Media Cybernetics Inc., Silver Spring, Maryland), macros were developed for densitometric analysis of film autoradiographs. The procedures were described before (Lucassen et al., 1995).

*Statistical analysis*

Differences among groups were evaluated by the non-parametric Mann-Whitney U test. Analysis of covariance was used to correct for possible confounding factors.
### Table 1. Clinico-pathological data of the subjects

<table>
<thead>
<tr>
<th>NBB</th>
<th>Sex</th>
<th>Age</th>
<th>PMD</th>
<th>Psychiatric diagnosis</th>
<th>History of depression/suicide attempts</th>
<th>cause of death, clinical diagnosis</th>
<th>medication</th>
<th>psychiatric medication, last month</th>
</tr>
</thead>
<tbody>
<tr>
<td>92003</td>
<td>f</td>
<td>55</td>
<td>7</td>
<td>MD (Me)</td>
<td>Depression lasted year before death. Yes</td>
<td>Cardiac failure, urosepsis, DM II, suspicion of Parkinson</td>
<td>Dextemide, bromocriptine, AB</td>
<td>Fluoxetine, imipramine</td>
</tr>
<tr>
<td>94112</td>
<td>m</td>
<td>61</td>
<td>&lt; 61</td>
<td>MD</td>
<td>12 years before death MD, delusions, mood congruent. No</td>
<td>Pneumonia, infarction of the right hemisphere, DM II</td>
<td>Biperiden 2 mg/day, glibenclamide, AB</td>
<td>Trifluoperazine</td>
</tr>
<tr>
<td>94032</td>
<td>m</td>
<td>71</td>
<td>16</td>
<td>MD (Me)</td>
<td>Several PH admissions due to therapy resistant MD. Yes</td>
<td>Cerebral ischemia after suicide attempt by strangulation</td>
<td>Aminodarine, digoxin, phenprocoumon, D</td>
<td>None</td>
</tr>
<tr>
<td>94094</td>
<td>m</td>
<td>71</td>
<td>14</td>
<td>MD (Me)</td>
<td>Two PH admissions due to MD in years before death. No</td>
<td>Acute respiratory distress syndrome</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>94017</td>
<td>f</td>
<td>72</td>
<td>&lt; 22</td>
<td>MD (Me)</td>
<td>Last 20 years recurrent MD, PH admissions. No</td>
<td>Bronchopneumonia, metastasized mesothelioma</td>
<td>Fluoxetine, metoprolol, D</td>
<td>Lithium, nitrazepam, pance, levomepromazine</td>
</tr>
<tr>
<td>95036</td>
<td>m</td>
<td>74</td>
<td>63</td>
<td>MD (Me)</td>
<td>Admission PH, MD with psychiatric features just before suicide. Yes</td>
<td>Suicide by strangulation. chronic lymphatic leukemia</td>
<td>None</td>
<td>Zuclopentixol, paroxetine, nitrazepam</td>
</tr>
<tr>
<td>93115</td>
<td>m</td>
<td>79</td>
<td>21</td>
<td>DDNOS</td>
<td>Suicide (jumped)</td>
<td>Dexamethasone, D</td>
<td>Zuclopentixol, paroxetine, nitrazepam</td>
<td></td>
</tr>
<tr>
<td>90122</td>
<td>f</td>
<td>85</td>
<td>71</td>
<td>MD (Me)</td>
<td>Last year before death MD, No</td>
<td>Sudden death while in PH, DM II</td>
<td>Inulin</td>
<td>Trazolan</td>
</tr>
<tr>
<td>94055</td>
<td>f</td>
<td>72</td>
<td>28</td>
<td>MD</td>
<td>From 1975 several PH admissions due to MD. No</td>
<td>Heart failure, septic shock, pyelonephritis</td>
<td>Inulin</td>
<td>Brotizolam</td>
</tr>
<tr>
<td>99033</td>
<td>f</td>
<td>61</td>
<td>18</td>
<td>control</td>
<td>MI, gastrointestinal tract bleeding</td>
<td>Unknown</td>
<td>Unknown</td>
<td></td>
</tr>
<tr>
<td>92042</td>
<td>m</td>
<td>61</td>
<td>14</td>
<td>control</td>
<td>Esophagus carcinoma</td>
<td>Unknown</td>
<td>Unknown</td>
<td></td>
</tr>
<tr>
<td>95106</td>
<td>m</td>
<td>74</td>
<td>8</td>
<td>control</td>
<td>Aneurysma aorta, MI</td>
<td>Sympathomimetics</td>
<td>Unknown</td>
<td></td>
</tr>
<tr>
<td>93061</td>
<td>m</td>
<td>70</td>
<td>9</td>
<td>control</td>
<td>Pneumonia, renal failure</td>
<td>Unknown</td>
<td>Unknown</td>
<td></td>
</tr>
<tr>
<td>93139</td>
<td>f</td>
<td>78</td>
<td>6</td>
<td>control</td>
<td>Lung carcinoma</td>
<td>Ranitidine, AB, D</td>
<td>Temazepam</td>
<td></td>
</tr>
<tr>
<td>95093</td>
<td>m</td>
<td>78</td>
<td>7</td>
<td>control</td>
<td>Heart failure, lung embolism, prostate carcinoma</td>
<td>Estramustine 560mg/day</td>
<td>Unknown</td>
<td></td>
</tr>
<tr>
<td>97130</td>
<td>m</td>
<td>58</td>
<td>17</td>
<td>control</td>
<td>Aorta dissection, cardiac tamponade</td>
<td>Unknown</td>
<td>Unknown</td>
<td></td>
</tr>
<tr>
<td>94074</td>
<td>f</td>
<td>85</td>
<td>5</td>
<td>control</td>
<td>Pneumonia</td>
<td>Prednisone, zantac</td>
<td>Unknown</td>
<td></td>
</tr>
</tbody>
</table>

AB, antibiotics; D, diuretics; DDNOS, depressive disorder not otherwise specified; DM, diabetes mellitus; f, female; m, male; MD, major depression; Me, melancholic type (retrospectively); MI, myocardial infarction; NBB, Netherlands Brain Bank number; PH, psychiatric hospital; PMD, post mortem delay.
**Results**

AVP mRNA signal in the SON of depressed subjects was 60% higher than in controls ($Z=-2.3, p=0.021$, Figure 1). In the PVN the signal in the depressed group was 15% higher than in controls, but this difference was not significant ($Z=-1.3, p=0.21$; Figure 2). Leaving out two patients who suffered from a cerebrovascular accident (CVA), leaving out the patient and control who used prednisone, and leaving out the DDNOS patient had no effect on the outcome. We did not find a significant difference in the amount of AVP mRNA in the SON or PVN between those patients who did or did not use antidepressants. Post mortem delay (PMD) in patients was longer than in controls, but correcting for PMD only increased the significance of the difference we found. In melancholic patients in both SON ($Z=-2.6, p=0.01$) and PVN ($Z=-2.2, p=0.028$) the AVP mRNA signal was significantly increased compared to controls.

**Discussion**

This is the first report in which AVP mRNA has been quantified in the SON and the PVN in depressed subjects and controls. We found a significant (60%) increase in the amount of AVP mRNA signal in the SON of depressed subjects (Figure 1). We also expected to find an increase in AVP expression in the PVN, which would have been in line with our earlier finding of an increased number of AVP-immunoreactive neurons in the PVN in depression (Purba et al., 1996), but the rise we observed in the PVN was not significant, probably due to the
small sample. In the melancholic ‘subgroup’, however, we did find a significant
difference in both the SON and the PVN. This indicates that the increase in AVP
mRNA may be even more pronounced in melancholic type depression, as was
found in earlier studies (De Winter et al., 2003; Van Londen et al., 1997).

An important question raised by our finding is whether increased AVP expression
in the SON could also, at least partly, be responsible for the HPA-axis hyperdrive
in depression. Two points should be considered in this respect. First, animal
studies show that the release of magnocellular AVP, originating in PVN or SON,
may take place in the median eminence, and thus in the portal system, where it
may potentiate ACTH release (Antoni, 1993; Wotjak et al., 1996). Second, it is
possible that an increased AVP release from the SON into the systemic circulation
contributes to the increased ACTH release from the pituitary. A clear indication
that such a mechanism might exist is provided by Gispen-De Wied et al., who

Figure 2.
Signal on film of the supraoptic nucleus (SON) and paraventricular nucleus (PVN) of a representa-
tive patient. Note the intense signal in both nuclei (III, third ventricle).
found that intravenous administration of AVP results in increased levels of both ACTH and cortisol in both controls and depressed subjects (Gispen-de Wied et al., 1992). Another indication is the finding that plasma AVP levels are positively correlated with cortisol levels in depression (Inder et al., 1997; De Winter et al., 2003; Brunner et al., 2002). In addition, there may be a feedback mechanism between the HPA-axis and the SON and PVN: glucocorticoid receptors are present in both the PVN and SON in rats (Morimoto et al., 1996) as well as in rhesus monkeys (Sanchez et al., 2000), and in our group Erkut et al. found that in humans not only AVP in the PVN but also in the SON responds to glucocorticoid plasma levels (Erkut et al., 1998).

In some cases, antidepressants can cause the syndrome of inappropriate antidiuretic hormone secretion (SIADH), suggesting that they influence hypothalamic AVP release (Spigset et al., 1995). We do not consider our results to be due to the use of antidepressants, based on three findings. First, Van Londen found elevated AVP levels in depressed subjects who did not use antidepressant medication at all (Van Londen et al., 1997). Second, in our group no significant difference was found in the amount of AVP mRNA in PVN or SON between patients using antidepressants and those who did not. Third, in an experiment especially designed to determine the influence of antidepressants on the occurrence of SIADH, no increase in either plasma AVP levels, or AVP mRNA levels in PVN and SON was found (Marar et al., 1998).

In conclusion, our results point to the SON being the main source of the observed elevated AVP plasma levels in depression. This finding indicates that besides the PVN, the SON is a factor in the HPA-axis hyperdrive.

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Increased arginine vasopressin mRNA expression


