Hypothalamic vasopressin and oxytocin mRNA expression in relation to depressive state in Alzheimer’s disease
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Gerben Meynen, Unga Unmehopa, Michel Hofman, Dick Swaab, Witte Hoogendijk

Abstract

Objective: Vasopressin (AVP) and oxytocin (OXT), produced in the hypothalamic paraventricular (PVN) and supraoptic nucleus (SON), are considered to be involved in the pathophysiology of major depressive disorder (MDD). The objective of this study was to determine for the first time the relationship between AVP and OXT gene expression and depressive state in both nuclei in Alzheimer’s disease (AD).

Methods: Post mortem brain tissue was obtained from a prospectively studied cohort of 23 AD disease patients, using the Cornell scale for depression in dementia to measure depressive symptoms and DSM-IIIR criteria to diagnose a depressive episode. In addition, brain tissue from six control subjects was obtained. We determined the amount of AVP and OXT mRNA in the PVN and SON by in situ hybridisation.

Results: The group of AD patients did not differ significantly from the 6 control subjects with respect to the amount of AVP or OXT mRNA in the PVN or SON. Also, no significant difference was found between depressed and non-depressed AD patients with respect to the amount of AVP or OXT mRNA in either the PVN or SON. In addition, no significant relationship was found between the Cornell score and AVP or OXT mRNA in both nuclei.

Conclusions: The results of this study indicate that in depression in AD AVP and OXT gene expression at the level of the PVN and SON is unchanged. For AVP, this means a difference with MDD. In addition, we found unaltered OXT mRNA expression in both nuclei in AD.
Chapter 6

Introduction

Depression has a high prevalence in Alzheimer’s disease (AD) (Lee et al., 2003; Lyketsos et al., 2004). Recently, Starkstein et al. found that 26% of the AD patients suffered from minor depression while 26% suffered from major depression, and that both major and minor depression constituted clinically relevant syndromes of depression in Alzheimer’s disease (Starkstein et al., 2005). Also mild levels of depression can produce significant functional impairments, and the severity of psychopathological and neurological impairments increases with increasing severity of depression (Starkstein et al., 2005). In addition, depressive symptoms may seriously increase caregiver burden and are frequently the reason for hospitalization (Steele et al., 1990).

Little is known on the pathophysiology of depression in AD, while the pathophysiology of depressive disorder has been related to hyperactivity of stress-regulating brain systems, such as the hypothalamic-pituitary-adrenal (HPA) axis and aminergic systems, including the noradrenergic system (Antonijevic, 2006). In previous studies we did not find a difference between depressed and non-depressed AD patients in either the total number of noradrenaline producing locus coeruleus neurons (Hoogendijk et al., 1999b) or in the concentration of noradrenaline, serotonin, dopamine or their metabolites in the cerebral cortex, hippocampus, amygdala and locus coeruleus (Hoogendijk et al., 1999a). Also, while in major depressive disorder (MDD) an increased cerebrospinal fluid (CSF) cortisol level is present (Nemeroff et al., 2005; Gerner et al., 1983), in depressed AD patients CSF cortisol levels are not higher than in non-depressed AD patients (Hoogendijk et al., 2006). Recently, however, we found a correlation between CRH neuron number in the PVN and depressive symptoms in AD patients (Meynen et al., 2007b) as measured by the Cornell scale for depression in dementia (Alexopoulos et al., 1988).

Vasopressinergic and oxytocinergic neurons located in the paraventricular (PVN) and supraoptic (SON) nucleus of the hypothalamus 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 populations. First, the PVN contains parvocellular neurons that secrete arginine vasopressin (AVP) together with corticotropin-releasing hormone (CRH) from axon terminals into the portal circulation of the stalk/median eminence. 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 general circulation in response to osmotic and non-osmotic stimuli (Swaab, 2003). Converging evidence points to
Hypothalamic vasopressin and oxytocin mRNA expression

the role of AVP in the pathophysiology of depression. In MDD the number of AVP-immunoreactive neurons in the PVN is increased (Purba et al., 1996), in particular those colocalizing CRH (Raadsheer et al., 1994). Recently, we found AVP mRNA expression to be increased in the SON in depressed subjects, and in both the PVN and SON in patients with a melancholic type of depression (Meynen et al., 2006). In accordance with these findings several studies showed 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). In addition, Van London et al. found increased AVP plasma levels in melancholic depressed patients (Van Londen et al., 1997). Also, a major SNP haplotype of the AVP V1b receptor has been found to protect against recurrent major depression (Van West et al., 2004). Animal studies are supportive of a role of hypothalamic AVP in depression. Nakase et al. found that in a rat model for depression AVP mRNA in the magnocellular neurons of the PVN was increased (Nakase et al., 1998). 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).

AVP differs only two amino-acids from oxytocin (OXT), a neuropeptide with many effects in social interactions and reproduction (Gimpl et al., 2001). In addition, OXT is a satiety peptide (Gimpl et al., 2001). In the central nervous system OXT is predominantly synthesized in neurosecretory cells located in the paraventricular (PVN) and supraoptic nucleus (SON) of the hypothalamus (Gimpl et al., 2001). Hypothalamic OXT is not only released as a neurohormone via the posterior pituitary into the systemic circulation, but also into the brain, acting on other nerve cells (Ishunina et al., 2002). While AVP potentiates HPA-axis activity, OXT is considered to attenuate the stress-induced activity of the HPA-axis in various species, including humans (Legros, 2001). In addition, OXT inhibits basal HPA-axis activity (Neumann et al., 2000). While finding elevated AVP plasma levels in depressed patients, Van Londen et al. observed normal OXT levels, but described a larger variability in these levels compared to controls (Van Londen et al., 1997). In a post mortem study in depressed subjects the number of OXT expressing neurons in the PVN was found to be increased (Purba et al., 1996). Recently, also in a post mortem study, we reported an increase of OXT gene expression in the PVN in melancholic type compared to non-melancholic type depressed patients (Meynen et al., 2007a).

In AD, AVP mRNA expression in the PVN and SON has been found to be unchanged (Lucassen et al., 1997), while OXT mRNA expression in these nuclei in AD has not yet been determined. Because of the findings of an increased
hypothalamic AVP and OXT in depression and the function of these peptides in HPA-axis regulation, we determined in the present study AVP mRNA and OXT mRNA in the PVN and SON nucleus in the post mortem brains of a cohort of AD patients, prospectively studied with follow-up for depression and depressive symptomatology.

**Methods**

**Subjects**

Demented patients from eight nursing homes were studied at six-month intervals in the framework of a prospective longitudinal study of depression in AD, as described in detail before (Hoogendijk et al., 1999a). Written informed consent for brain autopsy and for the use of brain tissue and clinical records for research purposes was provided by the patients’ next of kin before subjects entered the study. The study was approved by the institutional review board for biomedical research. Patients with major neuropathological co-morbidity were excluded. 23 patients fulfilled the criteria of the National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer’s Disease and Related Diseases Association (NINCDS-ARDA) and criteria of The Consortium to Establish a Registry for Alzheimer’s Disease (CERAD) for probable AD (see table 1).

Diagnosing depressive disorder in AD patients is complicated. Firstly, according to the DSM-IIIR and DSM-IV depression in AD cannot not be classified as major depression or MDD, because it occurs during the course of a neurodegenerative disease. Secondly, diagnosing depression in AD is difficult because of a profound overlap of symptoms between depression and AD. When using only DSM-IIIR criteria to diagnose depression in AD it is unclear whether the observed symptoms are due to AD itself. In order to avoid this pitfall of symptom overlap, Alexopoulos et al. designed the Cornell scale for depression in dementia (Alexopoulos et al., 1988). In the present study the presence of depressive symptoms in AD was established using both DSM-IIIR criteria and the Cornell scale (Alexopoulos et al., 1988).

The last evaluation was 3 months before death on average, except for one patient from which only the Cornell score of 24 months before death was available, the mean Cornell score being 10.4, SEM 1.4. DSM-III-R criteria (except for the absence of an organic factor, i.e. AD) were used to diagnose major depressive episodes in the course of AD and according to the DSM-III-R nine out of the 23 AD patients suffered from a major depression at the last evaluation before they died. These nine patients had higher Cornell scores (>10) than the 14
patients who did not fulfil the criteria for depression (Cornell ≤10) (p<0.0001). Post mortem, a systematic neuropathological evaluation was performed (Van de Nes et al., 1998), with an estimation of the severity of AD changes according to the classification of Braak (Braak et al., 1991; Hoogendijk et al., 1999b). A control group of 6 subjects was obtained from the Netherlands Brain Bank in accordance with the formal protocols for use of human brain material and clinical information for research purposes. Control subjects had never suffered from a depressive episode and post mortem neuropathological assessment did not show features of any neurological disease (for exceptions see table 1), including dementia. Exclusion criteria consisted of psychiatric diseases during lifetime and the use of corticosteroids or alcohol abuse (Harding et al., 1996; Sivukhina et al., 2006). The hypothalami were dissected and fixed in 0.1 M phosphate buffered 4% w/v formaldehyde (pH 7.2) at room temperature and after a period of one month (39.16 days, SEM 4.53) embedded in paraffin.

Measurement of AVP and OXT mRNA in PVN and SON
For in situ hybridization histochemistry (ISH) the method of Liu et al (2000) was used for AVP and of Guldenaar et al. for OXT (Guldenaar et al., 1995). All steps were performed under RNAse-free conditions. In short, 6 µm sections were taken at a distance of 600 µm throughout the PVN and SON and mounted on amino-alkyl-silane (AAS) coated object slides. After deparaffinization in xylene and rehydration through graded ethanols, they were deproteinated in 0.2N HCl for 20 minutes (min), and treated with proteinase K (10 µg/ml at 37 ºC) for 15 min. The reaction was stopped in glycine buffer for 30 seconds. 0.1% Triton X-100 in phosphate buffered saline (PBS) was used for delipidation. Washing steps took place in 1x PBS. For AVP mRNA ISH we used an oligonucleotide that was complementary to bp 411-458 of the human prepro-AVP precursor (Lucassen et al., 1997; Shibahara et al., 1983). For OXT mRNA ISH we used a 45 bp oligonucleotide probe complementary to bases 1166-1210 of the human prepro-OXT-neurophysin-gene (GB M11186). The oligonucleotides were end-labelled with 35S-dATP, using terminal transferase and purified by ethanol precipitation. The hybridization buffer consisted of 0.5 M NaCl, 1x Denhardt’s solution, 10 mM Tris-HCl, 1 mM EDTA, 10% dextran sulphate, 0.5 mg/ml yeast tRNA, 50% formamide and 200 mM dithiothreitol (DTT). Hybridization took place overnight at 42 ºC for AVP and 33 ºC for OXT. In order to prevent evaporation, sections were covered with sterile coverslips. After hybridization, coverslips were carefully removed in 2x standard saline citrate (SSC) and subsequently the slides were washed in 1x SSC at 50 ºC for AVP and 45 ºC for OXT for 30 min, 0.1x SCC for 30 min, and at room temperature (RT) in 0.1x SCC for another 30 min.
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Table 1. Clinico-pathological data of the subjects
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Clinical and pathological data of the subjects. Abbreviations: BS=Braak score, CS=last Cornell score (mean 10.43, SEM 1.41), D=depression, DM=diabetes mellitus, Dyst=dysthymia, Fix=fixation time (in formaldehyde, in days: mean 39.16 days, SEM 4.53), G=gender, inf=infarction, MD=major depression, NBB= Netherlands Brain Bank number, No=period of time with no affective disorder, PA=pathological anatomy, PMD=post mortem delay (time between death and fixation of the brain material, in hours, mean 4.62, SEM 0.378). Mean age=82.00, SEM 1.31.
Kodak Biomax MR-1 films were firmly pressed on the slides and exposed at RT for 3 days (see figure 1 for microphotographs of the signal). Standards consisted of labelled probe in hybridization mix, spotted on filter membrane in a graded series and were apposed to the films together with the tissue sections. Grey values of these standards were measured to determine the relationship between grey value and radioactive concentrations.

Quantitative analysis of AVP and OXT mRNA
Within the Image Pro image analysis package (Media Cybernetics Inc., Silverspring, Maryland, version 4.5) macros were developed for densitometric analysis of film autoradiographs. The procedures have been described in detail before (Lucassen et al., 1995). In brief, a standard curve was constructed describing the relationship between grey value and radioactive density. In order to quantify the ISH signal on film, images of the autoradiographs (1 pixel = 13x13 micrometer) were digitized with a Sony black and white CCD measuring camera. In these images the PVN and SON were outlined. With the standard curve every pixel within these outlines was translated into radioactive concentrations and the mean density, the area of the outline and an integrated density value (mean density x area) per outline were calculated. These integrated density values for the PVN and SON were separately plotted for all sections taken into account their mutual distance. The total area under this curve is an estimate for the total amount of radioactive label and thus for the total amount of AVP mRNA present in the region of interest.

All analyses were performed using statistical software (SPSS Inc, Chicago). Differences among groups were statistically evaluated using Kruskal-Wallis test. The Spearman test was used for correlations. All tests of significance were two-tailed. Statistical significance was set at p<0.05.

Results
The group of 23 AD patients did not differ significantly from the 6 control subjects concerning AVP mRNA both in the PVN (p=0.3) and SON (p=0.3) and concerning OXT mRNA both in the PVN (p=0.5) and SON (p=0.7). No statistically significant differences we found when comparing the groups of 6 control subjects, the 9 depressed AD patients and the 14 non-depressed AD patients: for AVP mRNA in the SON p=0.4, for AVP mRNA in the PVN p=0.6, for OXT mRNA in the SON p=0.14, for OXT mRNA in the PVN p=0.8. In addition, in the group of 23 AD patients no significant relationship was found between the Cornell score for depression in dementia and AVP mRNA in the PVN (rho=0.1, p=0.5) or SON (rho=0.4, p=0.08) and OXT mRNA in the
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PVN \((\rho=0.05, p=0.8)\) or SON \((\rho=0.3, p=0.2)\). Furthermore, no significant relationship was found between the Braak score and AVP mRNA in both PVN \((\rho=0, p=1)\) and SON \((\rho=0.09, p=0.7)\) or OXT mRNA in the PVN \((\rho=-0.1, p=0.5)\) or SON \((\rho=-0.14, p=0.5)\).

**Discussion**
To our knowledge, this is the first report in which both AVP mRNA and OXT mRNA have been quantified in the PVN and SON of AD patients that were prospectively followed-up for depression and depressive symptoms. We found that the group of AD patients did not differ significantly from controls concerning AVP mRNA expression in both nuclei, which confirms earlier findings of unaltered AVP mRNA expression (Lucassen et al., 1997). We also found that OXT mRNA expression is unchanged in both PVN and SON in AD. This is in accordance with earlier findings of Wierda et al. concerning OXT neuron number in the PVN in AD (Wierda et al., 1991). In addition, we did not find a correlation between the Braak score and the amount of OXT and AVP mRNA in either PVN or SON, indicating that the stage of the AD process as such is not of influence on the expression in these nuclei. We, however, do have to take into account that this

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**Figure 1. Photomicrograph**
A) Vasopressin mRNA and B) oxytocin mRNA signal on film of the supraoptic nucleus (SON) and paraventricular nucleus (PVN) of representative subjects (III, third ventricle). Bar = 1 mm.
was a population with end-stage AD (Braak scores 4-6) and, therefore, it could be that in earlier stages the gene expression of AVP or OXT is influenced.

AD patients that died while suffering from a depression, i.e. fulfilling the criteria of depression on the last evaluation before dying, did not differ significantly from the non-depressed AD group and the controls. In addition, the amount of AVP and OXT mRNA in the PVN and SON was not significantly related to the Cornell score. These results indicate that increased AVP gene expression in the PVN and SON is not involved in depression in AD, while in depressive disorder AVP mRNA is increased in the SON, and in melancholic-type depressed patients in both the PVN and SON (Meynen et al., 2006). This is in contrast to the number of CRH neurons in the PVN that we previously found to be increased in both depression (Raadsheer et al., 1994) and depression in AD (Meynen et al., 2007b). The present results points to a different pathophysiology of depression in AD compared to MDD at the level of hypothalamic AVP expression. This finding could indicate that AVP antagonists that are currently developed as treatment strategy for depression (Scott et al., 2002) would not be likely to be of beneficial effect in depression in AD.

In conclusion, the results of this study indicate that in depression in AD AVP and OXT gene expression at the level of the PVN and SON is unchanged. For vasopressin, this means a difference with MDD. In addition, for the first time we report unaltered OXT mRNA expression in both nuclei in AD compared to controls.

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Reference List
Antonijevic IA. Depressive disorders -- is it time to endorse different pathophysiologies? Psychoneuroendocrinology 2006; 31: 1-15.
Braak H, Braak E. Neuropathological staging of Alzheimer-related changes. Acta Neuropathol
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Chapter 6


Purba JS, Hoogendijk WJ, Hofman MA, Swaab DF. Increased number of vasopressin- and oxytocin-expressing neurons in the paraventricular nucleus of the hypothalamus in depression. Arch Gen Psychiatry 1996; 53: 137-143.


