Increased Number of Vasopressin- and Oxytocin-Expressing Neurons in the Paraventricular Nucleus of the Hypothalamus in Depression

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Background: Cerebrospinal fluid levels of arginine vasopressin (AVP) and oxytocin (OXT) have been found to change in mood disorders. In the present study, the numbers of AVP-immunoreactive (IR) and OXT-IR neurons were determined in the paraventricular nucleus (PVN) of the human hypothalamus.

Methods: Postmortem brain tissue was fixed in formalin, embedded in paraffin, and stained for AVP and OXT using immunocytochemical techniques. The number of IR neurons in the PVN was estimated by morphometry in eight depressed patients ranging in age from 21 to 85 years and eight age-matched controls ranging in age from 23 to 88 years.

Results: The numbers of AVP-IR and OXT-IR neurons in the PVN of patients with mood disorder were increased by 56% and 23%, respectively. No differences were found in AVP-IR or OXT-IR cell numbers between three patients with major depression and three patients with bipolar depression. The numbers of AVP-IR and OXT-IR neurons in two patients with depression not otherwise specified were within the same range as in the six other patients with a mood disorder.

Conclusions: The AVP and OXT neurons were activated in the PVN in patients with major depression or bipolar disorder. This activation may be associated with activation of the hypothalamic-pituitary-adrenal axis in these patients, since both AVP and OXT are known to potentiate the effects of corticotropin-releasing hormone. Because of their central effects, activation of AVP and OXT neurons may also be related to symptoms of major depression or bipolar disorder.

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THREE HYPOTHALAMIC systems are currently considered to be involved in depression: (1) The suprachiasmatic nucleus (SCN), the clock of the hypothalamus, shows strong annual variations in neuronal activity[1-3] that may be related to annual fluctuations in mood.[4,5] (2) The corticotropin-releasing hormone (CRH) neurons of the paraventricular nucleus (PVN), which regulate the hypothalamic-pituitary-adrenal (HPA) axis, have recently been found to be strongly activated in depression.[6,7] This finding is of particular interest since there are similarities between signs and symptoms of major depression and the behavioral effects of centrally administered CRH in laboratory animals[8] and of transgenic mice with CRH overproduction.[9] (3) The hormone levels of arginine vasopressin (AVP) and oxytocin (OXT) were found to be changed in the cerebrospinal fluid (CSF) in mood disorders.[10,11] These CSF levels are influenced by the synthesizing activity of the AVP and OXT neurons of the PVN.[12]

The present report deals with changes in AVP and OXT neurons in the PVN in patients with depression. Arginine vasopressin– and OXT-producing neurons from the PVN and supraoptic nucleus in the hypothalamus project to the neurohypophysis, where they are released into the general circulation. In the periphery, these peptides are involved in the regulation of diuresis and reproductive processes.[13-15] In addition, nerve fibers containing AVP and OXT innervate a large number of brain areas,[16,17] where AVP and OXT act as neurotransmitters or neuromodulators.[18,19] These extrahypothalamic projections are thought to be implicated in the regulation of body temperature, blood pressure, and osmolality, as well as in cognition, attention, and mood.[10,11,20]

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MATERIALS AND METHODS

Eight patients, four men and four women aged 21 through 85 years (mean±SEM, 61±8 years) with a clinical diagnosis of mood disorder according to *DSM-III-R*,[28] were entered in this study. The patients were selected using the clinical records and, if necessary, an interview with the clinician who treated the patients. Three patients fulfilled *DSM-III-R* criteria for major depression (patients 1, 3, and 5), three had bipolar disorders (patients 2, 4, and 7), one had an organic syndrome with depressed mood following a cardiac arrest and resuscitation (patient 6), and one had an unspecified depressive disorder, ie, not fulfilling *DSM-III-R* criteria for major depression (patient 8). Control brains were obtained from subjects without any evidence of a psychiatric disorder and were matched for age and sex (**Table 1**). The controls consisted of four men and four women aged 23 through 88 years (mean±SEM, 60±9 years).

The neuropathological diagnosis of depressed patients and controls was performed at the Free University of Amsterdam (Wouter Kamphorst, MD, PhD), the Academic Medical Center of the University of Amsterdam (Dirk Troost, MD, PhD), or the Netherlands Brain Bank, Amsterdam (Frans C. Stam, MD, PhD). For clinicopathological information see Table 1. Brains were weighed, and the hypothalamus was dissected and fixed in 4% formaldehyde for about 1 month. The hypothalamic area containing the PVN was dehydrated in graded ethanol and embedded in paraffin. Serial 6-μm frontal sections were cut on a Leitz microtome (Leica Instruments GmbH, Nussloch, Germany). Every 50th section was mounted on a chrome-alum–coated object slide, deparaffinized, hydrated, and stained with thionine to locate the PVN before immunocytochemical staining and to determine neuropathological alterations in the hypothalamus. Two series of sections taken at regular 300-μm intervals throughout the region in which the PVN could be discerned by thionine staining were stained immunocytochemically for AVP or OXT.

IMMUNOCYTOCHEMICAL PROCEDURES

To remove cross-reactivity from anti-AVP (obtained from rabbit Truus, January 29, 1989) or anti-OXT (obtained from rabbit 02-T, January 29, 1989), the antisera were preadsorbed twice with OXT or AVP glutaraldehyde-coupled sepharose beads.[29] The second incubation resulted in complete removal of cross-reactivity in the assay.[30] In addition, similarly purified antisera had been checked for cross-reactivity in alternating 6-μm sections of the PVN and had revealed no cells staining with both antisera.[30] Mounted sections were hydrated and stained using the following procedure: (1) incubation with purified AVP antiserum (Truus OXT), 1:1000, or purified OXT antiserum (02-T AVP), 1:1000, in 0.05-mol/L TRIS containing 0.9% sodium chloride (TRIS-buffered saline [TBS], pH 7.6) with 0.5% Triton X-100 (All incubations were performed for 1 hour at room temperature and subsequently overnight at 4°C in plastic boxes to prevent evaporation.); (2) washing in TBS (twice for 10 minutes each time); (3) incubation with goat anti–rabbit IgG serum (obtained from goat Betsie), 1:1000, in TBS at room temperature for 30 minutes; (4) washing in TBS (twice for 10 minutes each time); (5) incubation with peroxidase antiperoxidase, 1:500, in TBS at room temperature for 30 minutes; (6) washing in TBS (twice for 10 minutes each time); (7) rinsing in 0.05-mol/L TRIS hydrochloride (pH 7.6); (8) incubation with 0.5-mg/mL 3-3′-diaminobenzidine (Sigma Chemical Co, St Louis, Mo) in 0.05-mol/L TRIS hydrochloride containing 0.01% hydrogen peroxide at room temperature for 10 minutes; (9) rinsing in distilled water followed by dehydration in graded ethanol at room temperature; and (10) coverslipping with Entellan (a rapid-mounting medium for microscopy) (Merck, Darmstadt, Germany).

MORPHOMETRY

Cross-sectional areas of the PVN in AVP- and OXT-stained sections were measured with a digitizer connected to an HP 9000/835 computer (Hewlett-Packard Co, Palo Alto, Calif) using a Zeiss microscope (Carl Zeiss Inc, Thornwood, NY) with a PLAN ×2.5 objective and PLAN ×12.5 eyepieces. If the cross-sectional area of the PVN extended beyond the field of vision in a particular section, this area was measured stepwise. A quadrangular grid in one of the eyepieces was then used as a reference. The sections containing three or more stained neurons were included in the measurements. The PVN was measured on the right side of the brain, except for two cases in which the nucleus was not entirely present within the dissected tissue on that side.

The volume of the AVP and OXT cell groups in the PVN was determined by integrating area measurements from the most rostral to the most caudal sections of each population of cells.[31]

Numerical AVP and OXT cell densities in the PVN were estimated by counting the total number of nuclear profiles of immunoreactive neurons per unit area, followed by a discrete unfolding procedure[32] with the modification proposed by Cruz-Orive[33] and a correction for section thickness. For this purpose, nuclear profile areas of AVP or OXT cells were measured using the equipment described above, with a PLAN ×40 objective. To take fluctuations in cell density into account, AVP and OXT cell nuclei in the PVN were sampled in a random, systematic way[34] by measuring all nuclear profiles in every 200th section throughout the PVN (ie, at 1200-μm intervals).

The total numbers of AVP and OXT cells in the PVN were computed by multiplying the average numerical cell density times the volume of the population.

STATISTICS

Differences among the groups were assessed using the Mann-Whitney *U* two-tailed test. Values are expressed as mean±SEM. The critical level for statistical significance was $P \leq .05$.

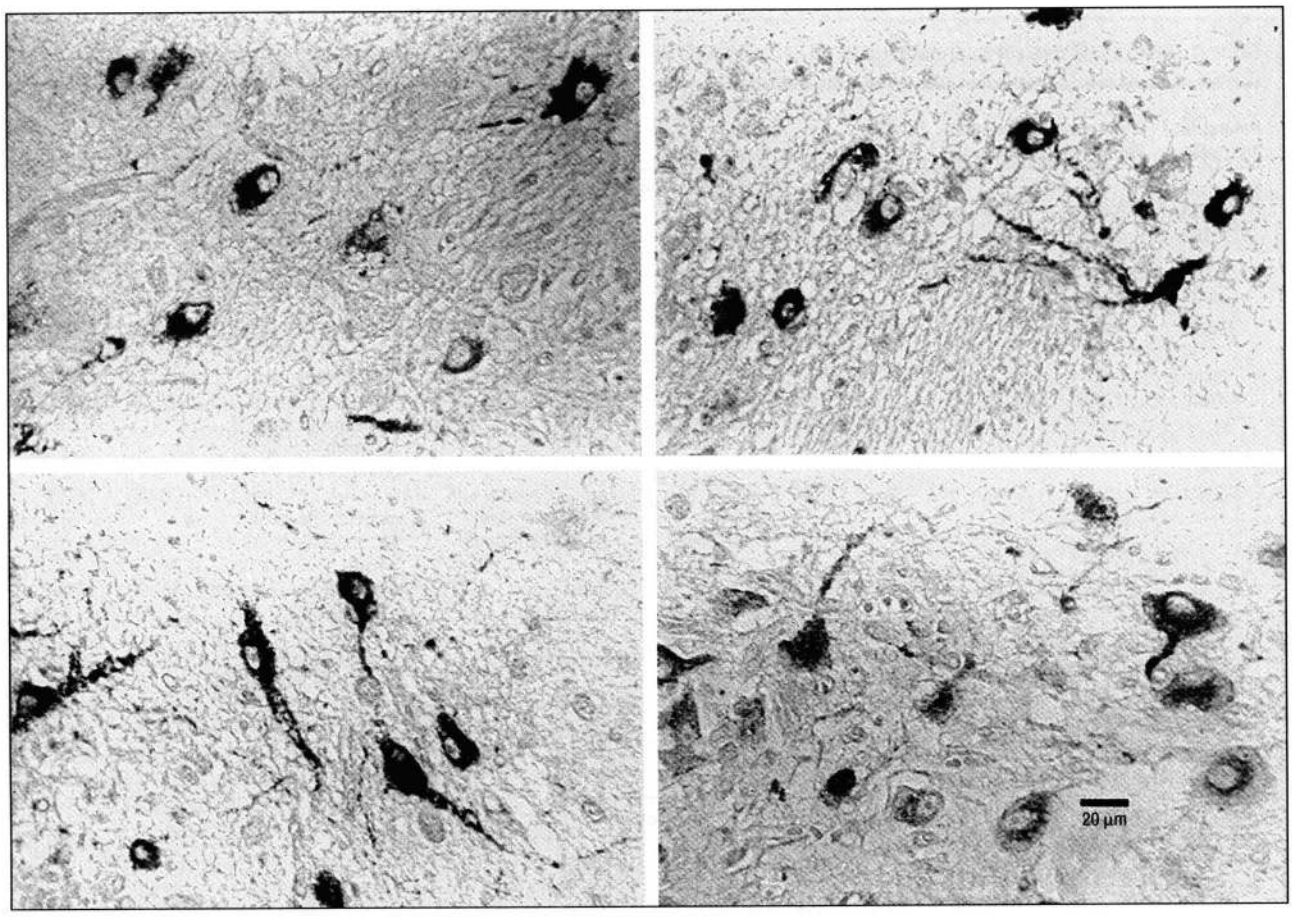
There are at least two opposite lines of evidence for the possible changes in activity of AVP- and OXT-producing neurons in mood disorders. First, patients with depression often manifest hyperactivity of the HPA axis.[6,7,21] In addition, AVP is involved in the modulation of the activity of the HPA axis, eg, by potentiating a CRH-induced corticotropin release from the pituitary.[22,23] Moreover, CRH and AVP are colocalized in an increased number of PVN neurons in depressed patients.[6] On the basis of these two findings one would expect increased activ-

Table 1. Clinicopathological Information and OXT-IR and AVP-IR Cell Numbers in the Paraventricular Nucleus*

Patient No./ Age, y/Sex	Brain Weight, g	Postmortem Delay, h	Fixation Time, d	Cause of Death	Clinical Diagnosis (*DSM-III-R*)	Medication in Last Month	No. of Cells	
							OXT-IR	AVP-IR
Patients With Depression								
1/21/M	1492	24	33	Overdose of opiate	Major depression	Amytriptyline	27 190	34 856
2/39/M	1220	48	30	Respiratory insufficiency	Bipolar disorder	Moclobemide, 450 mg/d, clorazepam, 2.5 mg/d, fluphenazine, lithium carbonate	25 470	34 431
3/55/F	1320	7	30	Cardiac failure	Major depression with mood-congruent, psychotic features	Maprotiline, 150 mg/d, oxazepam, 100 mg/d, flupentixol, 5 mg/d	36 794	34 964
4/63/M	1210	24	33	Cardiac failure	Bipolar disorder	Haloperidol, 15 mg/d, temazepam, 20 mg/d, diazepam, 40 mg/d, promethazine, 100 mg/d	37 940	32 003
5/70/M	NA	48	28	Cardiac failure	Major depression with mood-congruent, psychotic features	Fluvoxamine, 250 mg/d, lorazepam, sulpiride, 800 mg/d, lisinopril, 5 mg/d	26 436	26 222
6/73/F	1032	96	28	Cardiac failure	Organic mood syndrome, depressed	Tranylcypramine, 70 mg/d	33 922	38 657
7/80/F	1300	24	69	Bronchopneumonia	Bipolar disorder	Lithium carbonate, haloperidol amantadine	32 052	39 549
8/85/F	1260	72	49	Bronchopneumonia	Depressive disorder not otherwise specified	Trazodone	28 395	29 015
Mean±SEM/ 61±8/...	1262 ±52	43±10	38±5	...	...	...	31 025 ±1711†	33 712 ±1598‡
Controls								
1/23/M	1310	13	11	Acute death, brain-stem encephalitis	...	Antibiotics	20 546	10 461
2/30/F	1330	24	39	Acute death, intramural dissecting hematoma of coronary artery, cardiac failure	...	No medication	27 958	11 481
3/42/F	1370	5	33	Metastatic carcinoma of mamma, multiple cerebral and meningeal metastases, radiation, bronchopneumonia	...	Nicomorphine	20 390	21 683
4/63/M	1420	33	35	Myocardial infarction, cardiac failure	...	No medication	25 632	28 400
5/68/M	1366	7	58	Myocardial infarction, cardiac failure following bypass thrombosis	...	Streptokinase and trinitroglycerine	26 505	20 692
6/83/M	1280	26	42	Perforated diverticulitis of colon, sepsis, myocardial infarction	...	Antibiotics, heparin infusion	25 504	21 645
7/81/F	1210	30	NA	Liver cirrhosis, liver carcinoma, alcohol intoxication, pneumonia	...	Vitamin B (Neurobion), antibiotics	31 116	27 604
8/88/F	1030	11	11	Basal cell carcinoma of ear, cardiac failure, urine infection	...	Digitalis, antibiotics	24 743	30 881
Mean±SEM/ 60±9/...	1290 ±43	19±4	33±6	...	...	...	25 299 ±1264†	21 606 ±2658‡

**OXT-IR indicates oxytocin-immunoreactive; AVP-IR, arginine vasopressin–immunoreactive; and NA, not available.*
†P=.03.
‡P=.003.

ity, especially of parvocellular AVP neurons, in depression. Concerning blood and CSF levels of AVP, OXT, and their neurophysins, changes in different directions have been reported that may depend, at least partly, on the type of depression. Increased CSF levels of neurophysins were found in patients who had bipolar affective disorder compared with patients who had unipolar depression and controls.[24] However, the basal levels of AVP neurophysin in plasma and CSF were found to be lower in a group of patients with major depression.[10,25-27]



Frontal section (6 µm) of paraventricular nucleus of human hypothalamus. Top left, Control subject (patient 6) (antioxytocin). Top right, Patient with mood disorder (patient 2) (antioxytocin). Bottom left, Control subject (patient 6) (antivasopressin). Bottom right, Patient with mood disorder (patient 2) (antivasopressin).

To obtain direct information on possible changes in PVN neurons in mood disorders, in the present study, numbers of AVP-IR and OXT-IR neurons in the PVN were determined in depressed patients.

RESULTS

Age, brain weight, postmortem delay, and fixation time did not differ between patients with a mood disorder and controls (P=.87, P=.45, P=.11, and P=.82, respectively). Although there was no significant difference in postmortem time between controls and patients with a mood disorder and earlier studies had shown that postmortem interval does not affect AVP and OXT staining,[30,35,36] the potential effect of postmortem interval on the number of peptidergic neurons was investigated by applying linear regression analysis. No significant relationship was found between postmortem interval and the number of OXT-IR neurons (r=.63, P>.1) or AVP-IR neurons (r=.20, P>.6) in the PVN.

The intensity of the OXT staining was found to be similar in controls and patients with a mood disorder, but the intensity of the AVP staining was only slightly diminished in the latter group (**Figure**).

The number of OXT-IR neurons in the PVN was 23% higher in patients with a mood disorder than in controls (P=.03), and the mean nuclear diameter of the OXT-IR neurons was greater in patients with a mood disorder than in controls (P=.03) (**Table 2**).

The number of AVP-IR neurons in the PVN was 56% higher in patients with a mood disorder than in controls (P=.003), whereas the nuclear diameter of the AVP-IR neurons did not differ significantly between the two groups (Table 2). There were no differences between the three patients with major depression and the three patients with bipolar disorder in either OXT or AVP cell numbers (P=.82 for each). The AVP-IR and OXT-IR neuron numbers of the two patients with a depressive disorder not otherwise specified (Table 1, patients 6 and 8) were within the same range as in the six patients with major depression or bipolar disorder.

COMMENT

Mood disorders are characterized by persistent disturbances of mood, cognitive, psychomotor, and biological functions.[37] The symptoms of depression in older patients are basically not different from those in younger patients: depressed mood, apathy and anhedonia, insomnia, weight loss, feelings of worthlessness or inappropriate guilt, psychomotor agitation and retardation, concentration problems, loss of memory, and suicidal ideation or thoughts of death.[38]

Table 2. OXT-IR and AVP-IR Cell Populations in the Paraventricular Nucleus*

	OXT-IR Neurons			AVP-IR Neurons		
	Volume, mm^3	Neuron No., $\times 10^3$	Cell Nuclear Diameter, μm	Volume, mm^3	Neuron No., $\times 10^3$	Cell Nuclear Diameter, μm
Controls (n=8)	3.89±0.49	25.30±1.26	8.83±0.20	2.74±0.38	21.60±2.66	9.35±0.45
Depressed patients (n=8)	3.57±0.27	31.02±1.71	9.64±0.35	3.29±0.35	33.71±1.59	10.18±0.41
P†	.67	.03	.03	.29	.003	.17

*Values are mean± SEM. OXT-IR indicates oxytocin-immunoreactive; AVP-IR, arginine vasopressin–immunoreactive.
†Differences were assessed using the two-tailed Mann-Whitney U test.

Various centrally active neuropeptides have been suggested to play a role in the pathophysiologic processes of affective disorders.[39] In this respect, it should be noted that in humans the SCN shows very strong annual fluctuations in neuronal activity. The number of AVP-expressing neurons in the SCN is about twice as high in the autumn as in the summer.[1-3] October and November, when the number of AVP-expressing neurons in the SCN is highest, is also when the frequency of depression is highest.[4,40] Since, in addition, light therapy is an effective treatment of seasonal depression and directly stimulates the SCN,[4,40-42] the annual fluctuation in the SCN might be causally related to this type of depression. As a seasonal fluctuation does not occur in the number of AVP neurons in the PVN,[1] annual changes should not have influenced the present data.

A second hypothalamic peptidergic system that has been shown to be related to depression is the HPA axis. This system is known to be hyperactive in depressed patients on the basis of hormone assays.[8] Recently, our group has shown that CRH neurons were strongly activated both in the three patients with bipolar disorder and in the three patients with major depression; ie, the number of CRH neurons had increased threefold to fourfold,[6] and the amount of CRH messenger RNA had doubled[7]; in the patients with major depression, the number of AVP-coexpressing CRH neurons had increased as well.[6] It was remarkable that in the two patients with a depressive disorder not otherwise specified, the CRH neurons were not activated,[6] whereas the OXT and AVP neurons were found to be activated in these two patients in the present report. Whether this means that different types of depression go together with different types of neuropeptidergic responses needs to be studied in more detail.

A third hypothalamic peptidergic system that might be involved in depression is formed by the AVP and OXT neurons of the supraoptic nucleus and PVN.[10] We found an increased number of neurons expressing AVP or OXT associated with depression. This is an indication of increased neuropeptide production and release, as shown, for example, for AVP in the course of aging, where a gradual increase in the number of neurons expressing AVP[36] goes together with other signs of increased peptide production, such as increased nucleolar size[43] and Golgi apparatus[44] and increased plasma levels of AVP.[45] With respect to the fact that we observed activation of AVP, OXT (in the present report), and CRH neurons[6,7] in depression, it is of interest to note that both AVP and OXT potentiate the CRH-mediated corticotropin release.[46-51] The AVP nerve terminals found in the external zone of the rat median eminence originate from the hypothalamic PVN.[52] The PVN also contains the CRH neurons in the human hypothalamus that project to the median eminence.[53] The CRH neurons in the PVN coexpress AVP in increasing numbers during the course of aging.[53] In the rat, OXT neurons that also contain CRH are present in both magnocellular and parvocellular subnuclei of the PVN,[54,55] but in the human PVN this coexistence has not yet been studied.

Increased corticosterone levels have been found in animals treated with OXT,[56] indicating that not only AVP but also OXT may potentiate the effects of CRH release. Our results showed an increase of 56% in AVP-IR neuron numbers and an increase of 23% in OXT-IR neuron numbers in depressed patients. Animal experiments have shown that CRH and AVP also act in a synergistic manner centrally, ie, on behavior.[57] Thus, the increased corticotropin release caused by increased activity of CRH-IR neurons, as observed recently in depressed patients,[6] might be potentiated by increased release of AVP and OXT. Our results show changes that might contribute to the hyperactivity of the HPA axis in depression,[8] confirming the postulation of Bardeleben and Holsboer[58] that the action of CRH in depression is enhanced by AVP. It is also interesting to note that OXT had an effect similar to that of antidepressants in two animal experimental tests that are considered relevant as animal models of depression.[59]

We did not find any difference in AVP-IR or OXT-IR cell numbers between patients with major depression and patients with bipolar disorder. This is in agreement with the similar increase in CRH activity found in both groups.[6,7] We do not know which of the two types of OXT and AVP neurons are activated: (1) neuroendocrine neurons that release their peptides into the capillary loops of the pituitary portal system or the neurohypophysis[13-15] or (2) neurons that transport their peptides to other parts of the brain, where they act as neurotransmitters or neuromodulators.[18,35] Currently, we cannot distinguish between these two cell types in the human PVN. In the human PVN, in contrast to that of the rat, these different types of neurons are not localized in particularly well-developed subnuclei.[60] In addition, suitable tracing procedures to solve this problem are not yet available for the postmortem brain.

There is little information in the literature on the possible effects of antidepressant drugs on the AVP or OXT system in the PVN. Although the clinical use of the serotonin transporter inhibitor fluoxetine was recently found to be associated with the occurrence of an inappropriate

antidiuretic hormone syndrome in some patients, no evidence was found that this compound had an effect on osmoregulated AVP release or on the ability of a normal person to excrete a water load.[61] The diabetes insipidus of manic-depressive patients treated with lithium salts seems to be caused by activation of the collecting ducts of the kidney.[62] Although such an effect might lead to a compensating increase in AVP production by the PVN, the similar AVP and OXT changes in major depression and bipolar disorder, the strongly variable amount and kind of medication the patients had received, and the lack of effect of fluoxetine on AVP release indicate that it is more likely that the observed activation of OXT and AVP neurons is related to depression than that it is caused by the drug treatment. The idea that activation of OXT and AVP neurons is related to depression is reinforced by the observation of De Bellis et al,[63] who found significant decreases in CSF CRH and AVP levels and in Hamilton Depression Scale ratings following fluoxetine treatment. The overdose of opiates taken by patient 1 did not influence our conclusions, since opiates inhibit AVP and OXT secretion.[64]

As described above, increased AVP and OXT activity might contribute to the activation of the neuroendocrine HPA axis. Furthermore, increased AVP and OXT activity might affect other parts of the brain as neurotransmitters or neuromodulators. At present, one can only speculate about the possible central effects of OXT and AVP activation in depressed patients. Increased AVP and OXT cell activity might contribute to mood changes and cognitive impairment,[10] eg, by also potentiating the central CRH effects. Indeed, together with a decrease in Hamilton Depression Scale ratings, CSF AVP levels decrease following fluoxetine treatment of depressed patients.[63] Moreover, hypomanic episodes have been observed following administration of AVP.[10] One can also speculate about possible AVP- or OXT-potentiated central CRH effects on stress-related phenomena such as anxiety or sleep disturbances. These phenomena may in turn contribute to the diagnosis of major depression. Another interesting speculation is that the possible amnesiac effect of OXT might have an adaptive value in minimizing the emotional trauma of certain forms of stress, including depression.[65] The activation of OXT neurons may also play a role in the metabolic changes that are disturbed in depression. Various studies have shown that OXT is an important inhibitory factor in the modulation of food intake[66,67] and that it modifies fat and glucose metabolism.[14,67] One might therefore speculate that the weight loss, inhibition of food intake, and decreased motivation to eat seen in depressed patients are based not only on increased CRH activity[21,68,69] but also on the activation of OXT neurons, as observed in the present study.

One future research strategy might be to measure the individual symptoms of depression quantitatively, preferably prospectively. In this way, stronger and more specific correlations between particular symptoms and changes in hypothalamic systems might be detected, eg, between weight loss and the activation of OXT-IR cells in the PVN.

In conclusion, we found increased activity in the OXT and AVP neurons in the PVN of patients with mood disorder, regardless of the type of depression. Confirmation of these findings using quantitative in situ hybridization[70] and determining the exact central and peripheral consequences of this activation require further study.

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