The Vasopressin and Oxytocin Neurons in the Human Supraoptic and Paraventricular Nucleus; Changes With Aging and in Senile Dementia

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The neuropeptides vasopressin (AVP) and oxytocin (OXT) are supposed to be involved not only in peripheral functions (e.g. diuresis, labour and lactation) but also in central processes that are frequently disturbed during aging and senile dementia (e.g. fluid and electrolyte homeostasis and cognitive functions). A concomitant decrease in activity of the hypothalamo-neurohypophyseal system (HNS) with aging has been postulated in the literature, but has not yet been established.

In order to investigate possible age-related changes in the human HNS, immunocytochemically identified AVP and OXT neurons in the paraventricular and supraoptic nucleus (PVN and SON) were analysed morphometrically in subjects from 10 to 93 years of age, including patients with senile dementia of the Alzheimer type (SDAT). Cell size was used as a parameter for peptide production.

Mean profile area of OXT cells did not show any significant changes with increasing age. Mean profile area of AVP cells, however, showed an initial decrease up to the sixth decade of life, after which a gradual increase was observed. Size of AVP and OXT cell nuclei did not change significantly with aging. Observations in brains from patients with SDAT were within the range for their age group.

The present results do not support degeneration or diminished function of the HNS in senescence or SDAT, as generally presumed in the literature, but suggest an activation of AVP cells after 80 years of age. The activation of AVP cells in senescence is in accordance with previous findings in the aged Wistar rat.

INTRODUCTION

Vasopressin (AVP) and oxytocin (OXT) neurons project from the paraventricular and supraoptic nuclei (PVN and SON) to the neurohypophysis, where AVP and OXT are released into the bloodstream¹. In the periphery, these peptides are involved in the regulation of diuresis, lactation and labour^{35,36}. In addition, AVP and OXT fibers innervate a large number of brain areas^{3,4,38}, where AVP and OXT most probably act as neurotransmitters⁵. These extrahypothalamic projections are thought to be the anatomical basis for the effects of AVP and OXT on the regulation of body temperature, blood pressure and osmolality, and on cognitive functions (e.g. refs. 7, 9 and 11).

In the past decade, changes related to aging in the vasopressinergic and oxytocinergic system have re-

ceived increasing attention, especially since Legros²³ observed a decrease in the blood levels of neurophysins in men between 50 and 60 years of age. Moreover, the finding of improved performance in tests involving attention and memory in men of the same age following administration of vasopressin as a nose spray²⁴, seemed to support the concept of degenerative changes in the vasopressinergic system with advancing age. Since then, vasopressin has been advocated as a potential therapeutic drug in older age and in senile dementia (e.g. ref. 44). Also in the rat, the older literature unanimously proposed a decreased neurosecretory activity in senescence^{17,29,40}, although the parameters studied did not permit such a conclusion. Recently, however, the rat hypothalamo-neurohypophyseal system (HNS) was shown to be activated in senescence^{16,18,28}.

In order to investigate whether the presumed func-

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tional deterioration of the human HNS during aging was reflected by morphological changes in the hypothalamic production sites of AVP and OXT, we measured, in the present study, profile areas of immunocytochemically (ICC) identified AVP and OXT neurons in the PVN and SON from the first to the tenth decade of life. Cell size has been shown to be a reliable parameter for peptide production (see Discussion). The same parameters were determined in brains from patients with senile dementia of the Alzheimer type (SDAT).

MATERIALS AND METHODS

Brains of 32 patients, including 3 who had clinically been diagnosed and pathologically confirmed as cases of senile dementia of the Alzheimer type (SDAT), and one in which Alzheimer's disease had been diagnosed at the age of 59 and who died at the age of 70, were obtained at autopsy (for clinical information see Table I). The brains were fixed in 10% formaldehyde at room temperature. Generally after 1 month the hypothalamic area, containing the PVN and SON was dissected, dehydrated in graded ethanol, and embedded in paraffin via toluene.

Serial 6 μ m sections were cut transversally on a Leitz microtome, mounted on chrome-alum-coated object slides, and stored at room temperature. Before use, the sections were deparaffinized in xylene and hydrated via graded ethanol. Every 50th section was stained with thionin (0.1% thionin in acetate buffer, pH 4) for 15 min. From the central region of the PVN and SON, one section was selected for double peroxidase staining with AVP- and OXT-antiserum (cf. ref. 41).

Immunocytochemistry

In a preliminary set of experiments (SDAT patients not included) two alternating sections from the central region of the PVN and SON had been selected for staining with AVP-adsorbed OXT-antiserum (O-1-V-AVP, 1:400) and OXT-adsorbed AVP-antiserum (126-OXT, 1:50–1:200) respectively, followed by dehydration and embedding in Entellan (Merck), in order to prevent the possibility of cross-reactivity^{27,33}. The AVP-antiserum (126, 19-3-73) was preadsorbed with OXT (Organon, batch No. TH 644 AK) and the OXT-antiserum (O-1-V, 4-4-75)

with AVP (Organon, batch No. TH 932 AK) on solid phase (CNBr-activated agarose beads; Sepharose 4B, No. GB 19090) until cross-reaction on these beads was eliminated. Specificity tests were performed according to Pool et al.²⁷. However, OXT-adsorbed AVP-antiserum failed to stain AVP cells in 7 brains of older patients (No. 81020, 80286, 81064, 81100, 81033, 81267 and 81251; 64.4 ± 8.9 years, mean and S.E.M., respectively), while AVP-adsorbed OXT-antiserum revealed a good staining. In order to be able to identify AVP and OXT cells in all the patients, a double immunoperoxidase technique was therefore applied, staining both AVP and OXT cells in the same section (cf. ref. 41).

Sections were stained for AVP and OXT (all antisera were diluted in phosphate buffered saline (PBS), pH 7.4), using the following protocol: (a) 10% goat serum containing 0.5% Triton (10 min); (b) AVP-adsorbed OXT-antiserum (O-1-V-AVP) 1:400, containing 0.5% Triton (1 h at room temperature and subsequently overnight at 4 °C; the object slides were covered to prevent evaporation of the antiserum); (c) washing in PBS (2 $\times$ 10 min); (d) goat anti-rabbit IgG serum (Betsy) 1:50 (30 min); (e) washing in PBS (2 $\times$ 10 min); (f) peroxidase-anti-peroxidase (PAP) 1:400–1:1000 (30 min); (g) washing in PBS (2 $\times$ 10 min); (h) rinsing in 0.05 M Tris/HCl (Merck), pH 7.6; (i) 0.5 mg/ml 3-3'-diaminobenzidine (DAB; Sigma) in 0.05 M Tris/HCl, containing 0.01% H₂O₂ (10 min); (j) rinsing in aqua dest; (k) electrophoresis at 20 V/cm in 0.05 M glycine/HCl buffer (Merck) pH 2.2, containing 30% dimethylformamide (Sigma) (120 min); (l) washing in PBS (30 min); (m) 10% goat serum containing 0.5% Triton (10 min); (n) AVP-antiserum (126, 19-3-73) 1:800, containing 0.5% Triton (1 h at room temperature and subsequently overnight at 4 °C; the object slides were covered to prevent evaporation of the antiserum); (o) washing in PBS (2 $\times$ 10 min); (p) goat anti-rabbit IgG serum (Betsy) 1:50 (30 min); (q) washing in PBS (2 $\times$ 10 min); (r) PAP 1:400–1:1000 (30 min); (s) washing in PBS (2 $\times$ 10 min); (t) rinsing in Tris/HCl; (u) filtrate of 5 mg 4-Chloro-1-naphthol (Koch-Light) in 0.5 ml ethanol and 9.5 ml Tris/HCl, containing 0.01% H₂O₂ (10 min); (v) rinsing in aqua dest, followed by embedding in glycerin jelly.

The results obtained by the two procedures were compared with respect to the morphometrical par-

ameters. The highly significant correlation ($r = 0.80$; $P < 0.001$) of the data obtained by the two methods and the absence of neurons stained with both DAB and 4-Chloro-1-naphtol in the double-stained sections, with both procedures resulting in identical localization of OXT and AVP cells in their typical anatomical distribution, made clear that the double-stained sections could be used for further analysis.

Morphometry

In a section through the central part of the PVN and the dorsolateral SON (SONdl), cellular and nuclear profile areas of all stained vasopressin and oxytocin neurons containing a nucleus were measured with a digitizer (MOP AMO2, Kontron Messgeräte or Calcomp), using a Zeiss microscope with a Plan 40 $\times$ objective and 12.5 $\times$ Plan oculars. In addition, the cross-sectional areas of the PVN and SONdl were measured with Plan 1 $\times$ or 2.5 $\times$ objectives in order to allow for the determination of cell density. The mean nuclear diameters and volumetric densities of AVP and OXT neurons were estimated by means of a discrete deconvolution procedure (cf. ref. 43), in which section thickness and the modification proposed by Cruz-Orive⁸ were included. Since only AVP and OXT cell profiles containing a nucleus were measured, the deconvolution procedure could not be applied to the cell profile data. Therefore, mean cellular profile areas are presented.

Statistics

The variation in profile area, nuclear diameter and AVP and OXT cell density over the various age groups and possible effects of post-mortem delay and the duration of fixation were tested by means of analysis of variance (ANOVA; 0.05 level of significance). If P -values were below 0.05, differences between pairs of means were tested with the Student–Newman–Keuls multiple range test (SNK; 0.05 level of significance).

RESULTS

Staining intensity, distribution and ratio of AVP and OXT cells

In the PVN and SON of the patients of the youngest age group (No. 81306, 81043 and 81241; 10, 16 and 19 years old, respectively) both AVP and OXT

containing cells stained darkly, with a homogeneous distribution of the reaction product over the entire cytoplasm.

In AVP cells of subjects of the older age groups an unstained area in the periphery of the cytoplasm was frequently found. This phenomenon was observed from the age of 28 years onwards and did not show any apparent change with advancing age. In OXT cells no pronounced unstained area was found.

Apart from individual variations in shape and size of the PVN and SON, the anatomical distribution of AVP and OXT cells in the hypothalamus was similar in all subjects. In the central part of the SONdl, the great majority of immunoreactive cells ($90.2 \pm 0.9\%$; mean $\pm$ S.E.M., respectively) contained AVP, while a few OXT containing cells were found in the dorsal zone of the nucleus. In the medial part of the SON (SONm), the relative number of OXT cells was larger, and the preferential dorsal position of OXT cells was found to be less pronounced than in the SONdl.

Immunoreactive neurons situated between the PVN and the SON — the accessory supraoptic nucleus — contained mainly OXT.

In the central part of the PVN, $53.0 \pm 1.7\%$ of the magnocellular neurons contained AVP. OXT containing neurons extended more laterally than AVP containing neurons, although OXT cells were also frequently found in the medial zone of the PVN (along the ependym of the third ventricle), thus being scattered over a larger area than AVP cells.

Morphometry

Since none of the morphometrical parameters showed a significant sex difference ($P > 0.50$, Student's t -test), values from male and female patients were pooled.

Both in the SONdl and, to a lesser extent, in the PVN the mean profile area of AVP cells was significantly larger than OXT cells (50.6 and 38.4% respectively, Student's t -test, both $P < 0.001$). The mean profile area of OXT cells in the PVN and SONdl did not differ (Student's t -test, $P > 0.50$). Mean profile area of AVP cells was 12.7% larger in the SONdl than in the PVN (Student's t -test, $P < 0.05$).

Mean profile area of OXT cells was similar in all age groups (ANOVA, $P > 0.50$). AVP and OXT nuclear diameter did not show any significant change

TABLE I

Brain material

Abbreviations used: AD: Alzheimer's disease; f: female; m: male; nd: not determined; SDAT: senile dementia of the Alzheimer type.

<i>Patient number</i>	<i>Sex</i>	<i>Age (years)</i>	<i>Brain weight (g)</i>	<i>Postmortem delay (h)</i>	<i>Fixation (days)</i>	<i>Clinical diagnosis</i>	<i>Medicines used</i>
81306	f	10	1270	<16	32	tracheobronchitis; aspiration pneumonia; cardiac failure	antibiotics
81043	m	16	1940	17	49	multiple traumata; cerebral contusion; transtentorial herniation	
81241	m	19	1900	9	34	retroperitoneal chondrosarcoma	
82181	m	27	1560	nd	40	drug addiction; sepsis (St. Aureus)	antibiotics
81058	m	28	1510	23	32	medial cerebral artery aneurysm; vena cava superior syndrome; lung embolism	
81255	f	30	1460	24	39	intramural dissecting haematoma of coronary artery	
81251	m	31	1330	29	30	multiple traumata; small subarachnoidal haemorrhage	
80271	f	35	1200	8	36	acute lymphoblastic leukemia	VM26, ara-c
81012	f	38	1360	3	47	cervix carcinoma	antibiotics
81093	m	42	1510	22	42	metastatic bronchogenic carcinoma; pneumothorax	
81267	m	43	1260	23	53	non-Hodgkin lymphoma; sepsis	corticosteroids cyclofosfamide, hexamethylmelamine, oncovin, prednison, antibiotics
83173*	f	46	1360	nd	33	steroid-producing adrenal carcinoma; virilization; post-operative haemorrhage	antibiotics
81020	f	50	1210	7	40	M. Kahler; renal insufficiency	alkeran, vindesine, prednison, antibiotics, pentazoline-6-7,-benzomorfan
82165	f	52	1370	5	33	metastatic carcinoma of mamma; multiple cerebral and meningeal metastases	5-FU, adriamycine, cyclofosfamide, vincristine, MTX, nicomorphin-hydrochloride
82161	f	57	1220	45	35	polymyalgia rheumatica; endocarditis; mitralis- and aorta stenosis; lung embolism and haemorrhagic infarction	anticoagulants, prednison, digoxine
4724	m	59	1350	4	53	emphysema pulmonum; pneumothorax	
80087*	f	60	1110	nd	198	acute monoblastic leukemia	cytosar, vincristine, daunomycine
4727	m	61	1400	22	51	myocardial infarction; cardiac failure	propanolol
81014	f	64	1090	8	44	haemorrhagic peptic ulcer; hypovolemic shock; renal insufficiency	prednison, cimetidine antacida, diuretics
80286	m	66	1520	13	19	metastatic bronchus carcinoma; bronchopneumonia	
83170	f	70	800	14	34	AD/SDAT	
8560	f	70	1200	30	34	SDAT; lung embolism	
4725	f	72	1200	21	53	endocarditis lenta; glomerulonephritis; renal insufficiency; cerebral infarction right frontal lobe	

TABLE I (continued)

Patient number	Sex	Age (years)	Brain weight (g)	Postmortem delay (h)	Fixation (days)	Clinical diagnosis	Medicines used
81032	m	74	1410	13	48	cardiac failure; bronchopneumonia	digoxine, diuretics, antibiotics, theophylline, alimemazine-tartrate
81064	m	83	1280	22	42	diverticulitis	antibiotics
82175	m	85	1400	16	44	chronic myelocytic leukemia; bronchopneumonia	antibiotics, diuretics allopurinol, hydroxyurea
8484	m	87	1275	42	292	SDAT; pneumonia	
81100	f	88	1030	11	35	basalioma of ear; cardiac failure; amnesic syndrome; anemia	antibiotics
81033	f	90	1110	13	48	femur fracture; bronchopneumonia	antibiotics
84050	f	90	860	2	22	SDAT; anemia	
8538	f	90	1300	6	33	non-toxic goiter	
8533	f	93	1020	nd	32	expressive aphasia	

* Used for morphometrical analysis of the suprachiasmatic nucleus only.

Fig. 1. Mean cellular profile area and mean nuclear diameter of vasopressin and oxytocin cells (AVP and OXT cells) in the paraventricular and dorsolateral supraoptic nucleus (PVN and SON) as a function of age. Bars indicate mean values per two decades, vertical lines indicate standard errors of the mean (S.E.M.), *n* representing the number of brains examined in each age group. Open circles represent values from SDAT patients. a: AVP cells in the PVN. There is a significant effect of age on mean cellular profile area (ANOVA, $P < 0.02$), values in the 80–100 year old group (marked by *) being higher than in the 20–80 year old groups (Student–Newman–Keuls, $P < 0.05$). No significant effect of age on mean nuclear diameter is found (ANOVA, $P > 0.15$). b: OXT cells in the PVN. No significant effects of age on mean cellular profile area (ANOVA, $P > 0.60$) or on mean nuclear diameter (ANOVA, $P > 0.90$) are observed. c: AVP cells in the SON. There is a significant effect of age on mean cellular profile area (ANOVA, $P < 0.02$), values in the 80–100 year old group (marked by *) being higher than in the 40–60 year old group (Student–Newman–Keuls, $P < 0.05$). No significant effect of age on mean nuclear diameter is present (ANOVA, $P > 0.40$). d: OXT cells in the SON. No significant effects of age on mean cellular profile area (ANOVA, $P > 0.50$) or on mean nuclear diameter (ANOVA, $P > 0.60$) are found.

over the various age groups (ANOVA, $P > 0.15$ and $P > 0.60$, respectively). However, mean profile area of AVP cells, both in the PVN and in the SONdl, showed an initial decrease until the sixth decade, after which a gradual, statistically significant (both $P < 0.02$) increase was observed (Fig. 1).

Neither AVP nor OXT cell density showed a statistically significant change with increasing age (ANOVA, $P > 0.10$).

AVP and OXT cellular profile area and nuclear diameter in SDAT patients fell within the normal range for their age group (Fig. 1).

No significant effects of post-mortem delay or duration of fixation were found on nuclear diameter, cellular profile area and AVP or OXT cell density (ANOVA, $P > 0.10$).

DISCUSSION

The present study was undertaken in order to investigate whether the presumed deterioration of HNS function in man (cf. ref. 23) was reflected by changes in AVP or OXT cell size, being a parameter for peptide production. The use of autopsy material implicated a considerable individual variation in post-mortem delay until fixation and duration of fixation (Table I). However, no significant effects of these variables on the parameters studied were found.

The shape of the PVN and SON and the anatomical distribution of AVP and OXT cells were generally in accordance with the description of Dierickx and Vandesande¹². In both the PVN and the SONdl we found AVP cell profiles to be larger than OXT cell profiles. Feremutsch¹⁵ already distinguished large, often vacuolated cells with irregular Nissl-substance around the nucleus and smaller, more darkly and homogeneously Nissl-stained cells in the PVN, but a distinction between the peptides produced by the two cell types became possible only by means of immunocytochemistry. Dierickx and Vandesande¹³ found AVP cells to be larger and less homogeneously stained than OXT cells in both the PVN and the SON, while in the monkey a similar difference in size was reported²¹. In the SONdl we found a predominance of AVP cells ($90.2 \pm 0.9\%$), comparable to the figure that Dierickx and Vandesande¹² reported (more than 95% AVP cells). However, in the central

part of the PVN $53.0 \pm 1.7\%$ of the magnocellular neurons were found to be AVP cells, while Dierickx and Vandesande¹² reported 70–80% AVP cells in the PVN. The reason for this discrepancy might be the site in the PVN where the measurements were performed. Our data were calculated from observations in one section per patient from the central part of the PVN. In patient No. 83170 (female, 71 years), however, AVP/OXT cell ratios in the PVN and SONdl were followed in 6 sections over a total distance of 1.5 mm. While in the SONdl the AVP/OXT cell ratio was relatively constant over this distance, in the PVN the relative number of OXT cells was larger in the rostral and caudal areas as compared with the central region, similar to earlier observations in the rat PVN³⁴.

With respect to changes with aging, OXT-adsorbed AVP-antiserum failed to stain AVP cells in a group of 7 older patients. Decreased ICC stainability might indicate a functional change but as such does not indicate the direction in which the change has taken place, since it may be due to a decreased peptide content as a result of either decreased synthesis or increased transport of the compound from the cell body.

The size of AVP cell profiles in the SONdl and the PVN showed an initial decrease until the sixth decade, after which an increase was observed. The changes in cellular profile areas may be interpreted as changes in size of the cell somata, also since the mean nuclear diameters did not show any significant changes over the age groups. Changes in the size of the AVP or OXT producing cell body have been shown to be a reliable parameter for the production of these peptides under a number of different experimental conditions, viz., during osmotic stress^{14,20}, following castration⁴⁵ and during lactation²⁰. The changes in size of the AVP cell profiles therefore suggest a drop in AVP production until 60 years of age, after which an activation of the peptide production takes place. A similar increase in senescence was recently found in the nucleolar size of AVP cells, but not OXT cells, in the PVN and the SONdl in the same brain material¹⁹. This finding reinforces our conclusion of an enhanced synthetic activity in the AVP cells in the oldest age-group. Theoretically, it cannot be ruled out on the basis of the present results that AVP cells are activated in senescence as a compensa-

tory mechanism for cell loss. Although AVP cell density was found not to change significantly during aging, there may be a decrease of total AVP cell number with a concomitant decrease of volume of the PVN and SON. On the other hand, the changes in hormone levels make this possibility very unlikely. Although Legros²³ observed decreased blood levels of immunoreactive neurophysins between 50 and 60 years of age, a secondary increase was subsequently shown after the age of 70²⁵. Recently also other investigators reported an age-related increase of the blood levels of AVP^{18,22,30}. In addition, a similar activation of AVP cells was recently found in the aged rat¹⁶.

The activation of the HNS observed in senescence and senile dementia may be secondary to changes in kidney function. In aged Fisher 344 rats, impaired urinary concentrating ability and decreased AVP-dependent cAMP generation were reported², while in senescent human patients elevated neurophysin levels were found only in patients with impaired kidney function (Legros, personal communication). In addition, an age-related decrease of immunocytochemically stained AVP binding sites in the kidney was recently found in the Wistar rat³⁷. An alternative or additional explanation for the age-related changes in the HNS may involve changes in afferent innervation patterns of the PVN and SON during aging. An age-related decline of noradrenergic innervation of magnocellular SON neurons in the aging rat has been reported³¹, with 50% less catecholaminergic (CA) varicosities in apposition to AVP neurons, some target neurons being hyperinnervated by CA varicosities³².

In brains from patients with SDAT, all morphometrical parameters fell within the range for their age group, which is in contrast with the data of Mann et al.²⁶, who reported decreased nuclear and nucleolar volume of unidentified cells in the PVN and SON of demented patients as compared with age-matched controls. However, no lifespan measurements of these parameters were performed in their study. Therefore, the demented group may have been less

activated, rather than 'degenerated', as compared with the age-matched controls.

The meaning of the hypothesized activation of the HNS in senescence and SDAT for central functions is not clear. Centrally projecting PVN neurons are probably for the majority different from those projecting to the neurohypophysis. Additional sources of extrahypothalamic AVP fibers were recently found in the rat^{6,42}. It was evident from recent lesion and tracing studies in the rat that the AVP fibers of a number of brain areas probably originate from the bed nucleus of the stria terminalis, and not from the 'classical' magnocellular neurosecretory nuclei¹⁰. On the other hand, central and peripheral actions of a number of peptidergic systems may be coupled under certain circumstances (for a review see ref. 36). In this respect it is of interest that Tsuji et al.³⁹ reported higher concentrations of AVP in the cerebrospinal fluid (CSF) of patients with senile dementia as compared with controls. Since the demented patients in their study were older than the control patients, these data are in line with our observations and seem to point to a combined increase of both peripheral and central release of AVP.

Currently, the vasopressinergic and oxytocinergic innervation of extrahypothalamic brain areas is under investigation, in order to see if particular innervation sites show changes during aging and dementia.

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