Decreased number of oxytocin neurons in the paraventricular nucleus of the human hypothalamus in AIDS

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SUMMARY

The number of immunocytochemically identified vasopressin (AVP) and oxytocin (OXT) neurons was determined morphometrically in the paraventricular nucleus of the hypothalamus of 20 acquired immunodeficiency syndrome (AIDS) patients and 10 controls. The AIDS group consisted of 14 homosexual males (age range 25–62 years), four of whom had a probable HIV-1 associated dementia complex, and six non-demented heterosexuals (four males and two females, age range 21–73 years). Ten males without a primary neurological or psychiatric disease served as a control group.

The number of OXT-expressing neurons in the paraventricular nucleus of both groups of AIDS patients was ~40% lower than that of the controls. In contrast, the three groups showed no significant differences in the number of AVP-expressing neurons in the paraventricular nucleus. Since there were no significant differences in the number of AVP and OXT cells between the homosexual and heterosexual subjects with AIDS, the morphological difference in the paraventricular nucleus seems to be related to AIDS and not to sexual orientation.

No inflammatory changes were found in the paraventricular nucleus area. The selective changes in the OXT neurons of the paraventricular nucleus may be the basis for part of the neuroendocrine, autonomic dysfunction or vegetative symptoms in AIDS.

INTRODUCTION

Hypothalamic disturbances such as endocrine, autonomic dysfunction or vegetative symptoms have frequently been reported in patients with acquired immunodeficiency syndrome (AIDS). These include adrenal insufficiency, hyperprolactinaemia, central diabetes insipidus and changes in the hypothalamo-pituitary gonadal axis (Croxson *et al.*, 1989; Dluhy, 1990; Merenich *et al.*, 1990) as well as weight loss, fever and fatigue (Hellerstein *et al.*, 1990). However, so far no studies have been performed in relation to the question whether hypothalamic neurons might be affected in AIDS and thus be the basis for at least some of these symptoms. The present study was aimed at a central

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structure for such regulatory functions, namely the paraventricular nucleus of the hypothalamus. We determined the number of two of its major populations, i.e. vasopressin (AVP) and oxytocin (OXT) neurons. These neurons project to the posterior lobe of the pituitary and the median eminence, where they secrete the peptides into the general and portal blood circulation, respectively. Vasopressin acts as an antidiuretic hormone on the kidney and as a vasopressor (for review, *see* Gash and Boer, 1987). Oxytocin is involved in labour and lactation (Swaab and Boer, 1979; Swaab, 1982) and in ejaculation (Murphy *et al.*, 1987). Moreover, paraventricular nucleus fibres containing AVP and projecting to the external zone of the median eminence are involved in the regulation of the adrenal function and of stress response (Vandesande *et al.*, 1977). In addition, animal studies have shown that AVP and OXT cells in the paraventricular nucleus project to the brain areas outside the hypothalamus (Swanson, 1977; Buijs *et al.*, 1978), where the neuropeptides most probably act as neurotransmitters (Buijs, 1987). Animal studies have shown that central AVP and OXT also play a role in the expression of various functions, such as eating, cardiovascular regulation, nociception and thermoregulation (Buijs, 1987; Argiolas and Gessa, 1991; Olson *et al.*, 1991).

MATERIALS AND METHODS

The brains of 20 subjects who had died of AIDS and 10 male controls who had died of other causes were obtained by autopsy. Ten of the AIDS patients were non-demented male homosexuals [age 25–43 years; 36.7 ± 2.1 years (mean $\pm$ SEM)], four were demented male homosexuals (age: 25–62 years; 39.07 ± 2.34 years) and six were non-demented heterosexuals (four males and two females: age 21–73 years; 36.7 ± 7.5 years). Earlier studies had not shown a sex difference in either OXT (Wierda *et al.*, 1991) or AVP cell number of the paraventricular nucleus (P. F. Van der Woude *et al.*, unpublished results). Two of the heterosexual AIDS patients had contracted AIDS through blood transfusion, two through sexual contact and two had been drug addicts. The subjects of the control group (aged 23–88 years; 53.9 ± 7.9 years) had not suffered from primary neurological or psychiatric disorder.

The AIDS patients were diagnosed according to the criteria of the Centers for Disease Control (1987). According to the recently published nomenclature and research case definitions for neurological manifestations of human immunodeficiency virus-type 1 (HIV-1) infection (Report of a Working Group of the American Academy of Neurology AIDS Task Force, 1991) four of the AIDS patients were classified as probable HIV-1 associated dementia complex. General pathology and neuropathology (for results *see* Table 1) were performed either at the Free University of Amsterdam (Dr W. Kamphorst) or at the Academic Medical Center of the University of Amsterdam (Dr D. Troost). Brains were weighed and fixed in 10% formaldehyde and kept at room temperature for ~ 1 month. Details of age, post-mortem delay, fixation time and clinical diagnosis of the subjects are given in Swaab and Hofman (1990) and in Table 1. None of the controls or the patients had died from raised intracranial pressure. The hypothalamic area, containing the paraventricular nucleus, was dissected, dehydrated in graded ethanol and embedded in paraffin. Serial 6 μ m frontal sections were cut on a Leitz microtome and mounted on chrome alum-coated object slides. Every fiftieth section was stained with thionine in order to locate the paraventricular nucleus before immunocytochemical staining.

Immunocytochemistry

Two series of sections taken at regular 300 μ m intervals throughout the region in which the paraventricular nucleus could be discerned in the thionine-stained material were stained immunocytochemically for AVP and OXT, respectively.

In order to remove cross-reactivity from the AVP (Truus, 18-9-85) and OXT antisera (O-1-V, 4-4-75), the antisera were pre-adsorbed twice with OXT- or AVP-glutaraldehyde-coupled Sepharose beads, respectively (cf. Pool *et al.*, 1984). The second incubation resulted in a complete removal of the cross-reactivity in the assay. In addition, cross-reactivity had been checked in alternating 6 μ m sections of the

paraventricular nucleus, and revealed no cells staining with both antisera (Wierda *et al.*, 1991). Mounted sections were hydrated and stained according to the following procedure: (i) incubation with purified AVP-antiserum 1:300, or purified OXT-antiserum 1:250 in 0.05 M Tris containing 0.9% NaCl (TBS, pH 7.6) with 0.5% Triton X-100 (all incubations were performed at room temperature for 1 h and subsequently overnight at 4°C in plastic boxes to prevent evaporation); (ii) washing in TBS (2×10 min); (iii) incubation with goat anti-rabbit IgG serum (Betsie) 1:100 in TBS at room temperature for 30 min; (iv) washing in TBS (2×10 min); (v) incubation with peroxidase-anti-peroxidase (PAP) 1:500 in TBS at room temperature for 30 min; (vi) washing in TBS (2×10 min); (vii) rinsing in 0.05 M Tris-HCl (pH 7.6); (viii) incubation with 0.5 mg/ml 3-3'-diaminobenzidine (DAB; Sigma) in 0.05 M Tris-HCl containing 0.01% H₂O₂ at room temperature for 10 min; (ix) rinsing in aquadest followed by dehydration in graded ethanol at room temperature; (x) coverslipping the sections with Entellan.

Morphometry

Cross-sectional areas of the paraventricular nucleus in AVP- and OXT-stained sections were measured with a Calcomp 2000 digitizer connected to an HP 9000/835 computer and with a Zeiss microscope with PLAN 2.5× objective and PLAN 12.5× oculars. When the cross-sectional area of the paraventricular nucleus extended beyond the field of vision in a particular section, this area was measured step-wise using a quadrangular grid on one of the oculars as a reference. All sections containing three or more stained paraventricular nucleus neurons were included in the measurements. The paraventricular nucleus was measured at the right-hand side of the brain except when the nucleus was not completely present within the dissected tissue from that side.

The volume of the AVP and OXT cell populations in the paraventricular nucleus was determined by integrating area measurements from the most rostral to the most caudal sections of each population (Van Eden *et al.*, 1984).

Numerical AVP and OXT cell densities in the paraventricular nucleus were estimated by counting the total number of nuclear profiles in immunoreactive neurons per unit area followed by a discrete 'unfolding' procedure (Weibel, 1979) with the modification proposed by Cruz-Orive (1978) and a correction for section thickness (6 µm). For this purpose, nuclear profile areas were measured with the equipment described above but with a PLAN 40× objective. In order to take local fluctuations in cell density into account, AVP and OXT cell nuclei in the paraventricular nucleus were sampled in a 'random systematic' way (Uylings *et al.*, 1986) by measuring all nuclear profiles in every two hundredth section throughout the paraventricular nucleus (i.e. at 1200 µm intervals). This method is independent of tissue shrinkage.

The total number of AVP and OXT cells in the paraventricular nucleus was computed by multiplying the average numerical cell density with the volume of the population in question.

Neuropathology

For the neuropathological evaluation of the paraventricular nucleus area. One section from the central part of the nucleus was stained with haematoxylin and eosin (H&E), one with the monoclonal antibody DAKO-M701, one with the monoclonal antibody DAKO-M857 (against HIV-1), one with glial fibrillary acidic protein (GFAP) and one with DAKO-X907 against cytomegalovirus (CMV).

For DAKO-M701 and DAKO-M857. The monoclonal antibody DAKO-M701 reacts with the Leucocyte Common Antigen (LCA), which is expressed in all leucocytes, including B- and T-lymphocytes, macrophages, granulocytes microglia and in some multi-nucleated giant cells (Warnke *et al.*, 1983; Budka, 1986; Johnson *et al.*, 1988; Troost *et al.*, 1989).

The monoclonal antibody DAKO-M857 reacts with the p24 protein in cells infected with HIV type 1, e.g. in lymphocytes, in monocytes and macrophages, Langerhans cells of skin, in follicular dendritic cells and in brain cells of monocyte/macrophage or microglial lineage (Lübke *et al.*, 1988; Daugharty *et al.*, 1990).

Deparaffinized sections were incubated in methanol containing 0.3% H₂O₂ for 20 min in order to block endogenous peroxidase, rinsed in phosphate buffered saline (PBS) pH 7.4 or TBS pH 7.6, incubated with LCA for 1 h (DAKO-M701), diluted 1:100 at room temperature or incubated with DAKO-M857 diluted 1:100 at room temperature for 1 h and subsequently overnight at 4°C in plastic boxes to prevent evaporation, and stained with the avidin-biotin-peroxidase complex (ABC) (Hsu *et al.*, 1981) and with DAB/H₂O₂. The sections were counterstained with Mayer haemalum or haematoxylin, dehydrated and mounted in depex or Entellan.

TABLE 1. PATHOLOGY AND NEUROPATHOLOGY OF SUBJECTS

Patient no.	Sex (F/M)	Age (years)	Brain weight (g)	Post-mortem delay (h)	Fixation (days)	PVN		ME		Clinical diagnosis and neuropathology	Treatment		Cell no.	
						LCA	CMV	LCA	CMV		AZT	AF	OXT	AVP
Homosexuals														
1. 89793	M	25	1530	47	28	-	-	+	ND	AIDS, pneumonia	(-)	(-)	12465	16361
2. 88305	M	30	1480	4	31	+	-	+	-	AIDS, CM	(+)	(+)	14551	24372
3. 87673	M	30	1640	24	26	+	-	+	-	AIDS, PCP	ND	ND	10046	13046
4. 86426	M	32	1440	49	11	+	-	-	-	AIDS, PCP	(-)	(-)	12957	20192
5. 86398D	M	37	1210	ND	ND	++	-	ND	ND	AIDS, CMV, PCP, Kaposi sarcoma	(-)	(+)	23695	20347
6. 87396	M	39	>1330	24	28	++	-	+++	-	AIDS, PML	(+)	(-)	12475	11936
7. 87446D	M	40	1500	ND	ND	-	-	ND	-	AIDS, cerebral toxoplasmosis	(+)	(+)	11493	10911
8. 87159D	M	41	1520	48	43	+	-	ND	ND	AIDS, CMV, cerebral toxoplasmosis	(-)	(-)	21649	10776
9. 88285	M	41	>1240	12	34	+++	-	+++	ND	AIDS, bronchopneumonia, CM and toxoplasmosis	(+)	(+)	15870	18482
10. 86415	M	42	1340	4	35	+++	-	ND	ND	AIDS, disseminated Kaposi sarcoma and generalized MAI	(-)	(+)	12235	8955
11. 88384	M	42	1340	19	30	+	-	+	ND	AIDS, CM, meningoencephalitis	(+)	(+)	15132	33038
12. 86262	M	43	>1260	2	96	+	-	-	-	AIDS, disseminated Kaposi sarcoma and pneumonia	(-)	(-)	22682	20269
13. 87224	M	43	>1340	24	17	+	-	+++	ND	AIDS, PCP, Kaposi sarcoma, CM	(-)	(+)	21548	36874
14. 86381D	M	62	1350	ND	ND	+	-	-	ND	AIDS, Kaposi sarcoma	(-)	(+)	13354	19092
Heterosexuals														
1. 89592	M	21	1500	17	26	+	-	-	-	AIDS, MAI, pneumonia, CVA	(-)	(+)	19425	20455
2. 86436	M	30	1430	8	35	+	-	+	-	AIDS, PCP, lung tuberculosis, toxoplasmosis, heroin addiction	(-)	(+)	9796	14767

3. 89116	M	30	1340	8	26	+++	-	+++	-	AIDS, disseminated non-Hodgkin lymphoma infections, drugs abuse	(+)	(+)	16215	19804
4. 56032	M	32	1340	11	131	+++	+	+++	++	AIDS, CM	(+)	(+)	15387	10666
5. 88309	F	34	1400	12	24	+	+	-	-	AIDS, disseminated histioplasmosis	(-)	(+)	18088	19688
6. 89224	F	73	> 1090	48	38	+	+	+	-	AIDS, pneumonia, epilepsy	ND	ND	13094	16818
Controls														
1. 87110	M	23	1310	13	11	+	-	++	-	Brainstem encephalitis	ND	ND	20546	10461
2. 82181	M	27	1560	24	40	+	-	+++	-	Drug addiction, sepsis (<i>Staphylococcus aureus</i>)	ND	ND	28625	17457
3. 81251	M	31	1330	29	30	-	-	ND	ND	Multiple traumatic, small subarachnoidal haemorrhage	ND	ND	34658	17593
4. 84248	M	37	1370	39	35	++	-	+++	-	Bronchopneumonia, intoxication with alcohol	ND	ND	28521	27473
5. 81267	M	43	1260	23	53	+	-	ND	ND	combined with benzodiazepines	ND	ND	31537	20790
6. 4724	M	59	1350	4	53	+	-	ND	ND	Non-Hodgkin lymphoma, sepsis	ND	ND	25157	19447
7. 84049	M	63	1420	32	35	+	-	ND	ND	Emphysema pulmonum, pneumothorax	ND	ND	26828	29714
8. 81064	M	83	1280	22	42	+	-	++	ND	Myocardial infarction cardiac failure	ND	ND	24370	21856
9. 82175	M	85	1400	16	44	++	-	++	ND	Diverticulitis, myocardial infarction	ND	ND	29126	31631
10. 81097	M	88	1370	47	60	-	-	+	-	Chronic myelocytic leukaemia, bronchopneumonia	ND	ND	16723	23976

AF = antifungicide; AVP = vasopressin; AZT = zidovudine; CM = cytomegalic infection; CMV = cytomegalovirus; CVA = cerebrovascular accident; D = AIDS dementia complex; LCA = leucocyte common antigen; MAI = mycobacterium avium infection; ME = median eminence; ND = not determined; OXT = oxytocin; PCP = *Pneumocystis carinii* pneumonia; PVN = paraventricular nucleus; PML = progressive multifocal encephalopathy; - = no positive cell; + = <20 positive cells; ++ = 20-40 positive cells; +++ = >40 positive cells per PVN or ME in section; (+) = treated; (-) = untreated.

For cytomegalovirus staining. Deparaffinized sections were treated with 0.25% pepsine (Sigma p.7000), in 0.01 M HCl for 15 min, incubated in methanol containing 0.3% H₂O₂ for 20 min in order to block endogenous peroxidase, rinsed in PBS, incubated in 10% normal goat serum in PBS (DAKO-X907) for 15 min, incubated with the CMV antibody Biosoft 207-83 [Clone E 13 (IEA)] for 60 min, diluted 1:100 at room temperature, washed in PBS 3×2 min, incubated in anti-mouse Ig [Fab fragment, biotinylated (DAKO E 413)], diluted in PBS 1:200 containing 10% human AB serum for 30 min, washed in PBS, incubated in the streptavidin ABC complex horseradish peroxidase (DAKO K377), 1:200 in PBS with 10% human AB serum for 30 min, stained with DAB/H₂O₂, counterstained with haematoxylin, dehydrated and mounted in depex.

For glial fibrillary acidic protein staining. Glial fibrillary acidic protein is a marker for astrocytes (Bignami and Dahl, 1973; Ghandour *et al.*, 1980).

Deparaffinized sections were incubated with anti-GFAP (donated by Dr Bernard Delpuch, Rouen, France) 1:200 in 0.05 M Tris containing 0.9% NaCl (TBS, pH 7.6) with 0.5% Triton X-100 at room temperature for 60 min; rinsed in TBS 2×15 min; incubation with goat anti-rabbit IgG serum (Betsie) 1:50 in TBS at room temperature for 60 min; rinsing in TBS for 2×10 min; incubation with PAP 1:1000 in TBS at room temperature for 30 min; rinsing in Tris-HCl 0.05 M pH 7.6 for 5 min; incubation with 0.5 mg/ml DAB (Sigma) in 0.05 M Tris-HCl containing 0.01% H₂O₂ at room temperature for 10 min; rinsing in aquadest for 10 min; counterstaining with haematoxylin for 15 s; rinsing in tap water 5 min; rehydrating in a graded series of alcohols and xylol; mounting in Entellan.

Statistics

Differences among the groups were tested two-tailed using the Kruskal-Wallis multiple comparison test statistics. Throughout this study values are expressed as mean $\pm$ SEM. The critical level for statistical significance was taken to be 5%.

RESULTS

The quality of the immunocytochemistry staining for OXT and AVP in the paraventricular nucleus was not altered in any drastic way in the AIDS patients (Fig. 1A-D). The intensity of the OXT staining was similar in controls and AIDS patients, and the AVP staining was only slightly diminished in AIDS-affected subjects. An average of 12.4 anti-AVP and 15.3 anti-OXT stained sections, and an average of 230 ± 82 anti-AVP and 210 ± 71 anti-OXT-stained profiles was measured per subject.

However, the OXT cell number in the paraventricular nucleus of homosexual males was >40% lower than that of the (male) reference group ($P = 0.0005$; Table 2). An increase in the mean OXT cell-nuclear diameter was found in homosexual males ($P = 0.01$). The AVP cell number and the volumes of the AVP and OXT cell populations in the paraventricular nucleus did not show any significant differences among the three groups (Table 2; Fig. 2). In addition, AVP cell-nuclear diameter showed a trend towards an increase in the brains of patients who had died of AIDS, particularly in homosexual men ($P = 0.09$; Table 2). Since the number of AVP and OXT cells was not found to be different between the various AIDS groups (Table 2), the diminished OXT cell number as observed in the paraventricular nucleus of these subjects seems to be related to AIDS and not to sexual orientation or dementia.

Neuropathology of the paraventricular nucleus

The H&E staining did not show any signs of local inflammation in the paraventricular nucleus area or median eminence of the 20 subjects, i.e. we found no multi-nucleated giant cells, perivascular macrophages, vacuolization or gliosis. Leucocyte common

FIG. 1. Frontal sections ($6\ \mu\text{m}$) of the paraventricular nucleus (A–D). Some positive oxytocin (OXT) cells or anti-vasopressin (AVP) cells are indicated by arrows, e.g. A, control subject (no. 84248), stained with anti-OXT; B, AIDS patient (no. 87396), stained with anti-OXT; C, control subject (no. 84248), stained with AVP; D, AIDS patient (no. 87396), stained with anti-AVP. Bar = $10\ \mu\text{m}$.

TABLE 2. VOLUME, NEURON NUMBER AND MEAN DIAMETER OF CELL-NUCLEI OF THE OXYTOCIN AND VASOPRESSIN-CONTAINING CELL POPULATIONS IN HUMAN PARAVENTRICULAR NUCLEUS

Group ¹		Oxytocin neurons			Vasopressin neurons		
		PVN volume (mm^3)	Neuron no. ($\times 10^{-3}$)	Cell-nuclear diameter (μm)	PVN volume (mm^3)	Neuron no. ($\times 10^{-3}$)	Cell-nuclear diameter (μm)
Reference group	($n = 10$)	4.53 ± 0.25	26.6 ± 1.6	8.51 ± 0.17	2.73 ± 0.17	22.0 ± 2.0	9.22 ± 0.33
Homosexuals (AIDS)	($n = 10$)	4.23 ± 0.42	14.9 ± 1.3	$9.13 \pm 0.15^*$	3.42 ± 0.58	20.9 ± 2.7	10.18 ± 0.27
Heterosexuals (AIDS)	($n = 6$)	3.88 ± 0.46	15.3 ± 1.4	$8.72 \pm 0.16^*$	2.67 ± 0.16	17.0 ± 1.6	9.82 ± 0.17
AIDS-dementia complex	($n = 4$)	3.63 ± 0.71	17.5 ± 3.3	9.39 ± 0.18	3.34 ± 1.22	15.3 ± 2.6	9.32 ± 0.48
Statistics ²		NS	$P = 0.001$	$P = 0.01$	NS	NS	$P = 0.09$

¹Values are given as mean $\pm$ SEM; ²differences among groups were tested two-tailed using the Kruskal–Wallis multiple comparisons test; NS = not significant; PVN = paraventricular nucleus; * = not significant.

FIG. 2. Oxytocin and vasopressin cell number in the human paraventricular nucleus and vasopressin cell number in the supra-chiasmatic nucleus of three groups of subjects: (i) a male reference group ($n = 10$); (ii) male homosexuals who had died of AIDS ($n = 10$); (iii) heterosexuals who had died of AIDS ($n = 6$; four males and two females). Values represent means $\pm$ SEM. Note that the number of oxytocin cells in the paraventricular nucleus of AIDS patients is only ~60% of that found in the reference group (Kruskal-Wallis multiple comparison test, $*P < 0.05$; $**P < 0.01$; $***P < 0.001$). No significant differences are found in the vasopressin cell numbers of the paraventricular nucleus. The vasopressin cell numbers of the supra-chiasmatic nucleus are significantly larger in homosexual men than in the reference group and in heterosexuals.

antigen (LCA) (DAKO-M701) staining revealed a few positive lymphocytes, some endothelial cells and microglia-like cells in the paraventricular nucleus and median eminence of some subjects of each of the three groups (Table 1; Fig. 3A–D). The number of LCA-positive cells varied greatly within the groups but not between the groups (Table 1). The control subjects also had some LCA-positive cells in the paraventricular nucleus and median eminence (Table 1; Fig. 3A, c). Subjects with relatively strong LCA staining in the paraventricular nucleus (*see* Table 1), patients no. I: 10; II: 3; III: 4, 9, did not have lower OXT or AVP cell counts than the rest (I: 10, OXT = 12 235 neurons, AVP = 8955; II: 3, OXT = 16 215, AVP = 19 804; III: 4, OXT = 28 512, AVP = 27 473; III: 9, OXT = 29 126, AVP = 31 631).

Human immunodeficiency virus staining (DAKO-M857) did not reveal any sign of HIV-1 infected cells in the paraventricular nucleus area of the AIDS patients.

Cytomegalovirus staining (DAKO-X907) was generally negative in the paraventricular nucleus area, except for one heterosexual AIDS patient (no. 56032; Table 1). In addition, intense CMV positivity was observed periventricularly and in the area of the arcuate nucleus in one probable HIV-1 associated dementia complex patient (no. 86381).

Glial fibrillary acidic protein positive subependymal astroglial cells and dense network of fibres were found along the third ventricle. In the region of the paraventricular nucleus astroglial cells and a less dense network of the GFAP positive fibres were observed. Around blood vessels the staining was denser. There was no difference between the controls and AIDS patients in the density of GFAP positive cells or fibres. The four patients suffering from probable HIV-1-associated dementia complex had more intense GFAP in the paraventricular nucleus, but a relationship to OXT cell numbers did not exist. Individual differences were considerable in all groups.

FIG. 3. Frontal sections ($6\ \mu\text{m}$) of the paraventricular nucleus (PVN) (A, B) and the median eminence (ME) (C, D) stained with anti-leucocyte common antigen. A few positive lymphocytes are indicated by arrows, e.g. A, control subject (no. 82181); B, AIDS patient (no. 86398); C, control subject (no. 82181); D, AIDS patient (no. 87396). Bar = $25\ \mu\text{m}$.

DISCUSSION

In general, the human paraventricular nucleus and supraoptic nucleus seem to be rather stable cell groups that are not affected in ageing and dementia, as judged from cell counts

(Hofman *et al.*, 1990; Goudsmit *et al.*, 1990; Wierda *et al.*, 1991), whereas another AVP-containing area situated nearby, i.e. the suprachiasmatic nucleus, shows a pronounced cell loss in these conditions (Swaab *et al.*, 1985, 1987). The number of OXT neurons in the paraventricular nucleus of controls found in the present study ($26.6 \pm 1.6 \times 10^3$) is similar to that measured in males by other investigators in our group ($26.1 \pm 1.8 \times 10^3$) (Wierda *et al.*, 1991). We found an AVP cell number of $22 \pm 2 \times 10^3$, which is comparable with the observation of P. F. Van der Woude (unpublished) ($19.5 \pm 2.5 \times 10^3$) in the same reference group. This illustrates the reproducibility of the measurements.

The main finding of the present paper is that the number of OXT-expressing neurons in the paraventricular nucleus was greatly decreased (40%) in the brain of patients who had died of AIDS. Vasopressin neurons showed only a slight, non-significant decrease of 7%. The strong decrease in OXT neuron number is also interesting because most authors favour the idea that the human immunodeficiency virus cannot be readily demonstrated in neurons and is mainly present in monocyte-derived cells (Sharer, 1992). However, products of the virus infection may have toxic effects on neurons without directly infecting them (Sharer, 1992). Indeed, some reports claim severe cortical nerve cell loss in both adults and children with the HIV infection (Giangaspero *et al.*, 1989; Ketzler *et al.*, 1990; Everall *et al.*, 1991; Gray *et al.*, 1991; Wiley *et al.*, 1991; Sharer, 1992). However, these data concern cell density, whereas total cell counts are only possible in structures that can be delineated, such as the hypothalamic nuclei (Swaab and Uylings, 1987; this paper). The large decrease in OXT neurons in the paraventricular nucleus is even more remarkable since the stainability of this group of neurons was not obviously decreased. The lower OXT neuron number is consequently not simply due to an overall decrease in the amount of OXT in the paraventricular nucleus neurons of AIDS patients. It rather appears that a selective group of paraventricular nucleus cells has stopped producing OXT or is even dying. The lack of change in paraventricular nucleus volume and of cellular reaction in the paraventricular nucleus argues in favour of the first possibility. The decrease in the number of OXT neurons in the paraventricular nucleus seems to be independent of sexual orientation or medication. Although most of the AIDS patients had been treated with a variety of medicines, e.g. AZT (zidovudine and/or antifungal), no connection was found in this group of patients between the kind of medication and the number of OXT neurons in the paraventricular nucleus (Table 1). These findings suggest that the loss of OXT neurons in the paraventricular nucleus of the hypothalamus is a consequence of HIV infection. Swaab and Hofman (1990) and LeVay (1991), on the other hand, have reported that homosexuality, rather than AIDS, may have an effect on the size and neuronal content of some other hypothalamic cell groups. This indicates that the different hypothalamic nuclei show selective differences in relation to sexual orientation or AIDS. In addition, since AIDS-affected subjects were found to have a reduced number of AVP-expressing neurons in the suprachiasmatic nucleus (Swaab and Hofman, 1990), the disease process also affects the hypothalamic nuclei in a selective way, even if the same peptide (i.e. AVP) is involved.

The paraventricular nucleus of the AIDS patients was exempt from signs of cells infected with HIV type 1 and no difference was found in the amount and GFAP-like intensity of the astrocytes. It is, therefore, not clear why the OXT cells in the

paraventricular nucleus are preferentially affected by AIDS. Possible explanations include involvement of the vasoactive intestinal polypeptide (VIP) receptor, corticotropin-releasing factor or local inflammatory processes. Concerning the first possibility, intracerebroventricular administration of VIP to the rat induces a dose-dependent rise of AVP and OXT plasma levels from the paraventricular nucleus (Bardrum *et al.*, 1988), indicating the potential importance of VIP for paraventricular nucleus function. There is considerable structural homology between part of the envelope of HIV (i.e. peptide T) and VIP. It has therefore been proposed that the VIP receptor is the naturally occurring protein by which the virus enters the cell (Ruff *et al.*, 1987; Brenneman *et al.*, 1988). Although peptide T and the VIP receptor have not been found to interact in intestinal cells, which argues against this hypothesis (Nguyen, 1988), this possibility has not yet been tested in the brain. Such an interaction might well be a specific characteristic of the nervous system, because VIP receptors have a different structure in various organs (Said, 1991). However, VIP innervation from the suprachiasmatic nucleus terminates preferentially in the subparaventricular area (Moore, 1992) and not within the paraventricular nucleus itself. Although a few VIP fibres may terminate in the paraventricular nucleus, there is at present no reason to assume that the receptor is preferentially located on the OXT neurons of the human paraventricular nucleus, so that the structural relationship between VIP and peptide T remains a very hypothetical explanation for the selective OXT cell number decrease in AIDS.

A possible alternative endocrinological explanation of the selective changes in OXT neurons is based upon the change in the activity of the hypothalamo-pituitary adrenal axis in AIDS. It has been noted that corticotropin-releasing hormone selectively stimulates OXT, but not AVP, when injected intracerebroventricularly (Demitrack and Gold, 1988). Corticotropin-releasing hormone neurons terminate on OXT neurons in the paraventricular nucleus (Hisano *et al.*, 1992). The diminished activity of the hypothalamo-pituitary adrenal axis in AIDS patients (for references, *see* Introduction) may thus lead to a decrease in the number of OXT-expressing neurons rather than that of AVP neurons, just as we observed in the present study.

A third possible explanation for the loss of OXT-expressing neurons is the involvement of local inflammatory processes. However, neuropathologically there were no inflammatory changes related to AIDS encephalopathy (cf. Gray *et al.*, 1988; Budka, 1989) in the area of the paraventricular nucleus or in the median eminence. In this respect there is a clear difference with HIV encephalitis in the cortex of children, in which a marked inflammatory perivascular infiltrate with abundant multinucleated cells has been observed (Giangaspero *et al.*, 1989).

Animal experiments (Argiolas and Gessa, 1991) suggest that oxytocinergic paraventricular nucleus neurons affected in AIDS contribute to the various symptoms seen in this disease, such as disturbances in metabolism, cardiovascular regulation and thermoregulation (Hellerstein *et al.*, 1990; Cunningham and Sawchenko, 1991). Oxytocin modifies fat and glucose metabolism and stimulates the release of insulin and glucagon (Lederis *et al.*, 1985; Stock *et al.*, 1990; Cunningham *et al.*, 1991). One might conceive, on the basis of the severe weight loss in AIDS (Hellerstein *et al.*, 1990), that this function of the OXT neurons is lost. However, in Alzheimer's disease, a condition which is also characterized by cachexia (Sandman *et al.*, 1987; Singh *et al.*, 1988; Burns *et al.*, 1989; Franklin and Karkeck, 1989), the number of OXT-expressing neurons is not

diminished (Wierda *et al.*, 1991). Cachexia alone is consequently not a sufficient explanation for the greatly decreased number of OXT neurons in AIDS.

The exact functional consequences of the probable decrease in OXT secretion in patients suffering from AIDS are not yet clear. It has been suggested that OXT has an amnesic effect (De Wied, 1983). However, a major role of OXT in the occurrence of dementia does not fit the observation that in senile dementia decreased OXT levels are present in the cerebrospinal fluid (Unger *et al.*, 1971). Moreover, a low number of OXT neurons was observed in all AIDS patients regardless of the presence or absence of dementia (Table 2), so that there seems to be no relationship between the decrease in the number of OXT-expressing neurons and memory disturbance in AIDS. This corresponds with our observation that the number of OXT-expressing neurons also remained the same in the paraventricular nucleus of Alzheimer patients (Wierda *et al.*, 1991).

The increased nuclear size of OXT neurons may be due to a loss of smaller OXT neurons. Alternatively the changed nuclear size indicates an altered activity of the remaining neurons (Edström and Eichner, 1958; Eneström, 1967; Palkovits and Fischer, 1968). It would be worthwhile investigating, by means of *in situ* hybridization, whether the decreased number of OXT-expressing neurons in the paraventricular nucleus in AIDS is reflected by a diminished synthesis of OXT-mRNA, and by decreased OXT blood levels. If so, OXT blood levels may be a good parameter to monitor the degree to which the paraventricular nucleus is affected in AIDS. In that case, OXT concentration in the blood may also give a reliable indication of how the brain would react to therapeutical interventions.

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