Short Communication

Stable vasopressin innervation in the degenerating human locus coeruleus in Alzheimer's disease

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Abstract

The vasopressin (VP) innervation of the human locus coeruleus (LC) was immunocytochemically investigated in Alzheimer's disease (AD) patients and non-demented controls. A dense innervation of VP fibers was present throughout the entire rostro-caudal length of the LC in both, controls and AD-patients. The VP immunoreactivity was confined to fibers; no signs of cell body staining could be found. Comparison of five non-demented control subjects and five AD patients on fifteen different levels throughout the LC revealed that the VP innervation of this nucleus remained intact in AD, even in the rostral part of the LC, which is the most affected region with respect to neuronal loss.

Key words: Human brain; Alzheimer's disease; Locus coeruleus aging; Immunocytochemistry

Vasopressinergic systems have been described throughout the rat, monkey and human brain [3,4,29,30,32] and may functionally be related to circadian rhythms and processes like memory and learning [10,24] that are all affected in Alzheimer's disease (AD). The various vasopressin (VP)-containing nuclei have different patterns of change during aging and in AD [11,15,31,33].

A region in the human brain which is densely innervated by VP-fibers is the LC [12], its noradrenaline (NA)-containing neurons located bilaterally in the dorsal pontine brainstem at the ventrolateral edge of the fourth ventricle and clearly visible in unstained material due to the presence of neuromelanin in its neurons. The LC provides an extensive noradrenergic innervation throughout the brain [18] and is thought to be involved in global functions such as emotion, level of vigilance, sleep–wake cycle [25], cognition and memory [14]. During aging and even more so in AD, an extensive neuronal loss in the LC was found [5,6,28], which appeared to be topographically distributed; the rostral part of the LC being the most, the medial part

less and the caudal part the least affected [6]. In rat brain it has been reported that the antero-dorsal neurons of the LC project mainly to the cortex and hypothalamus [19] (e.g. to the PVN). The preferential loss of rostral neurons in the human LC may consequently be related to cortical and hypothalamic impairment. Pilot experiments carried out at our laboratory did not reveal a change in the VP innervation in the caudal part of the LC (Fliers, personal communication), but since then a preferential loss of rostral LC neurons has been described in AD [6]. This study investigated, therefore, whether the VP-innervation in the LC was affected in the degenerated LC of AD patients.

The region from the colliculus superior to the apertura lateralis of the fourth ventricle, containing the entire LC, was collected at rapid autopsy from five non-demented controls and five clinically and neuropathologically confirmed AD patients (see Table 1 for clinical data). There was no significant difference between controls and AD patients as far as age, post-mortem delay, CSF-pH or brain weight were concerned (Table 1). The tissue blocks were fixed by immersion in 2.5% glutaraldehyde/1% paraformaldehyde in 0.05 M phosphate-buffered saline (PBS), pH 7.4 at 4°C. After 24 h the fixative was renewed and the tissue fixed, for at least 1 week and at most 1 month. The tissue blocks were trimmed, followed by freezing

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in freon-12 at -80°C and subsequently stored in sealed plastic containers at -80°C . The complete LC was cut serially into $40\text{ }\mu\text{m}$ cryostat sections, mounted on uncoated glass slides and stored at -20°C . The uncoated slides made it possible for the sections to be wiped off the glass into a buffer and to be used free-floating in immunocytochemical staining [12]. Every twenty-fifth section was mounted on chrom-alum-coated slides and stained with thionin for morphological orientation. Based on the thionin-stained sections the LC was subdivided into a rostral, medial and caudal part, according to Chan-Palay and Asan [6]. Since in AD-patients neuromelanin-cells had been reported to disappear preferentially in the rostral part of the LC, it was decided to delineate the rostral part in AD patients on the basis of the mean length of the rostral part in control patients. The length of the rostral region of AD patients was estimated by determining the rostral/medial division point of the LC and adding the mean number of rostral sections of control patients to this point (i.e. $n = 75$) in rostral direction.

Out of each part, i.e. rostral, medial and caudal, five evenly spaced sections were chosen, which were processed for immunocytochemistry and stained using a purified [22] antibody against VP. The immunocytochemical PAP-procedure was adapted from Fliers [12], with a modification concerning the removal of pseudo-peroxidase activity. Before the first antibody (Truus 29/1/86, 1:1000), the sections were incubated for 30' in 10% (v/v) methanol containing 3% (v/v) H_2O_2 in Tris buffer pH 7.6. The ICC procedure resulted in

fifteen sections (five rostral, five medial and five caudal sections) per patient. The sections of all patients were ranked per level according to their VP fiber density in the LC region, using a blind procedure in which the patient numbers were not known. Differences in rank numbers of VP fiber density between control and AD patients were tested by means of the Mann-Whitney *U*-test (two-tailed, corrected for ties, 0.05 level of significance). Differences between control and AD patients in other parameters were tested by means of the Student's *t*-test (two-tailed, 0.05 level of significance).

Immunocytochemical stainings revealed a dense VP innervation throughout the entire rostro-caudal length of the LC of both non-demented controls and AD-patients. It was evident that when compared to controls, AD patients showed a strong reduction in number of neuromelanin-containing neurons, which was most pronounced in the rostral part of the LC, as reported in literature [6,14]. In addition, extracellular deposits of neuromelanin were observed in this region in both groups of patients, but were more evident in AD patients.

In rostral regions of the LC of AD patients, even when only a few neuromelanin containing neurons were present, a dense VP innervation was still found (see Fig. 1). Statistical analysis of the two patient groups per level did not show a significant difference in density of VP-innervation in any of the levels. No obvious sex-difference was found in the density of the VP-innervation of the LC. In both controls and AD patients VP-staining was only present in fibers. No VP

Table 1
Clinical and pathological information on controls and Alzheimer patients

Patient number	Age (yrs)	Sex (m/f)	Postmortem delay (h.min)	pH of CSF at autopsy	Brain weight (g)	Clinicopathological diagnosis	Severity of dementia (*)
<i>Controls</i>							
91-204	71	f	7.25	8.10	1135	Heart failure	
91-182	73	f	4.25	5.87	975	posterolateral myocardial infarct	
92-050	78	f	6.35	7.00	1084	myocardial infarct, pneumothorax	
89-082	84	m	4.05	6.77	1080	Heart failure	
90-206	90	f	4.30	6.70	1040	Lung emboli	
mean	79		5.24	6.88	1063		
S.E.M.	3.5		0.65	0.36	27		
<i>Alzheimer's disease patients</i>							
89-279	67	f	3.50	6.56	890	AD, Cachexia	severe (7)
90-078	83	f	3.20	6.57	1060	AD, Dehydration	severe (7)
90-064	84	f	4.00	6.47	990	AD, CVA	advanced (6)
91-327	87	m	3.30	6.85	1475	AD, Uremia due to dehydration	advanced (6)
88-042	89	f	4.10	6.62	904	AD, Heart failure	severe (7)
mean	82		3.62	6.61	1063		
S.E.M.	3.9		0.18	0.06	108		
<i>P</i>	0.61		0.07	0.49	0.99		

yrs: years; m/f: male/female; h.min: hours.minutes; g: grams; S.E.M.: standard error of means; *P*: value of *P* in Student's *t*-test; AD: Alzheimer's disease; CVA: cerebro-vascular accident; CSF: cerebro-spinal fluid.

* According to Reisberg et al. 1989 [23].

Fig. 1. Photomicrographs of neuromelanin-containing neurons and VP-innervation in the LC of a 78-year-old non-demented control patient (92-050; A,C,E) and a 83-year-old AD patient (90-078; B,D,F) at different levels of the LC: (A,B) rostral, (C,D) medial, and (E,F) caudal according to Chan-Palay and Asan, 1989. Note the presence of the VP-innervation in the LC of the AD-patient in spite of the loss of neuromelanin containing neurons. Bar = 500 μ m.

immunoreactivity was present in cell bodies of the LC-region.

The difficulty of delineating the rostral part of the LC in AD-patients, which lost the majority of the neuromelanin-containing neurons in this region, prompted us to define the rostral region in the AD group on the basis of the mean length of this region in control subjects. Our method might have led to a slight overestimation of the length of the rostral region in

AD patients, since the rostro-caudal length of the LC decreases in this disorder [6]. However, the first section studied both in controls and AD patients was 400 μ m caudal from the rostral tip. Furthermore, since a similar dense VP innervation was present both in controls and AD patients at this rostral point, we were confident that the procedure to outline this region in AD subjects was appropriate. The loss of cells in the rostral region of the LC in AD and also in other neurodegen-

erative disorders like Parkinson's disease and Down's syndrome, may be related to the demential symptoms of these disorders [14]. In AD, the main symptoms are thought to be attributed to an impaired function of temporal cortex and hippocampus [20]. Both regions would be innervated by the antero-dorsal region of the LC, based upon data obtained in rat [19]. Since in multi-infarct dementia the loss of NA-containing nerve terminals in the cerebral cortex did not coincide with a degeneration of cortical-projecting, rostral LC cells [14], cortical dysfunction of the noradrenergic system in dementia, does not necessarily seem to affect the LC. The strong degeneration of the LC in AD may consequently be a primary event in the disease process, rather than a result of cortical alterations in AD.

It is known that VP can act as a neuromodulator on noradrenergic cells in the LC of rat [21]. One would expect that, if the neuromelanin-containing neurons are the main targets for the VP fibers, this innervation would be affected when the number of neuromelanin neurons decreases strongly in AD. One of the possible sources for the VP-innervation of the LC is the PVN. Anatomical contacts between the dorsal cap of the rat PVN and the LC have been found [1], whereas a noradrenergic innervation from the LC to the hypothalamic PVN is also described [26,27]. The fact that the number of VP-neurons in the PVN of the human brain does not decrease in AD [31] may thus be related to the observation that the VP innervation of the LC also remains stable, whereas the LC shows a substantial cell loss. The finding of a decreased VP-innervation in the LC of the rat following castration [7] or during aging [11] and the subsequent restoration of the innervation by testosterone substitution in aged rats [15] suggests that an alternative source for the VP-innervation of the LC is a VP nucleus that is sensitive to gonadal hormones, like the BST [9,32]. In the human brain there are extra-hypothalamic VP cells in the BST [12], but their number is so limited that one may wonder whether this could indeed be the source of the extensive VP innervation of the LC. The density of the VP innervation in the human LC makes the larger PVN a much more likely source. Major sex differences have not been observed in our study of the VP-innervation of the human LC, but only one male patient was present in each patient group. So, at present, there is no indication of a sex hormone dependent source of VP fibers in the human LC. Alternatively, the VP innervation of the LC may be derived from the LC itself. We have, however, so far not found any evidence for the latter possibility, since VP cell body staining was not observed in the LC. Since VP-immunoreactive cells were found in the LC of rat and monkey [2,4], the absence of VP-immunoreactive cell bodies may be another example of the major differences in VP systems between species.

One may presume that VP-innervation remains stable in AD because the VP-fibers do not terminate on the large neuromelanin neurons but rather on small, non-pigmented neurons. However, this population of LC neurons is also strongly diminished in AD [16]. Another explanation for the stable VP-innervation of the degenerating LC, is that a major part of the VP-fibers is merely passing through the LC to other regions in the brainstem, e.g. the parabrachial nucleus, the nucleus of the solitary tract or the dorsal motor nucleus of the vagus, all regions where VP has been found in the human brain [13,17]. Subsequently, the number of fibers ending in the LC region may be too small for detection of effects of AD to be possible. The possibility of non-synaptic release of VP in the LC may also explain the stable VP-innervation in AD, since in that case retrograde loss of innervating fibers is not expected. Since neuropeptides and monoamines perform neurophysiological actions which are attributed to non-synaptic release [8,34], this may also explain our current results.

The present study shows that the VP-innervation in the LC was not affected in AD, not even in the rostral regions where in AD hardly any neuromelanin-containing neurons were left.

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