Short communication

Increase in vasopressin binding sites in the human choroid plexus in Alzheimer's disease

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Abstract

Vasopressin binding sites were determined in the choroid plexus of five Alzheimer's disease patients and five non-demented controls using the ¹²⁵I-labelled linear V_{1a}-antagonist. The Alzheimer's disease patients showed a twofold increase in the density of vasopressin binding sites, whereas the increase in the affinity constant K_d did not reach significance.

Keywords: Human brain; Vasopressin binding sites; Linear V_{1a}-antagonist choroid plexus; Alzheimer's disease; Cerebrospinal fluid

Vasopressin (VP) is not only produced as a neurohormone by the supraoptic and paraventricular nucleus (SON and PVN respectively), but also by the suprachiasmatic nucleus, the clock of the brain [26], and by several extra-hypothalamic cell groups as described in rat, monkey and man [3,4,9,27]. As a neurohormone, VP is involved in the regulation of diuresis and blood pressure [5]. Vasopressin containing pathways innervate regions throughout the brain [2–4,6] including the fissures of the choroid plexus [1]. Vasopressin binding sites of the V₁ subtype have been described in many brain regions, including brain microvessels and the choroid plexus [10,28,30–32].

The choroid plexus produces cerebrospinal fluid (CSF) and its epithelium contains receptors for several neuroactive substances, e.g. for serotonin, atrial natriuretic peptide and VP. Vasopressin is thought to play a role in choroid plexus ion and water transport, blood flow and CSF production [19]. Activation of VP receptors decreases the blood flow through the choroid plexus and reduces the CSF production, an effect that can be blocked by a V₁-antagonist [7,8,12].

Aging is accompanied by atrophy of the human brain and enlargement of the CSF spaces [17,33], a change that is even more pronounced in Alzheimer's disease (AD) patients [18]. A decrease in CSF production in aging has been reported [14], but to our knowledge no information is

available concerning CSF production or VP binding sites of the choroid plexus in AD. The present study was designed to determine whether VP binding sites in the choroid plexus were changed in AD patients.

Choroid plexus material was obtained by autopsy and stored at -70°C (Table 1). Neuropathology was performed by Prof. F.C. Stam (Netherlands Brain Bank) or Dr. W. Kamphorst (Free University, Amsterdam). Before use the choroid plexus was weighed and subsequently thawed to room temperature (2–3 min) for homogenization (about 250 mg/10 ml) in 1 mM Tris-HCl (pH 7.5) and 2 mM dithiothreitol (DTT) using a Polytron homogenizer (2×4 s). The homogenate was centrifuged for 20 min at $30,000 \times g$ (Beckman L-80 Ultracentrifuge, SW 25.2, 4°C) and the pellet was resuspended in 40 ml 50 mM Tris-HCl (pH 7.5), 0.25 M sucrose and 0.1 mM DTT, vortexed and centrifuged again. The resulting pellet was resuspended in the same buffer (250 mg original tissue/ml), hand-homogenized using a Potter-Elvehjem homogenator (5 strokes) and a sample was taken for the modified Lowry protein assay procedure [13]. The resulting crude membrane fraction was finally brought to the acquired protein concentration using incubation buffer of the VP receptor assay (50 mM Tris-HCl, 10 mM MgCl₂ and 1 mg/ml BSA, pH 7.5). The procedure was such that tissue from a control and an AD patient were processed simultaneously, both for homogenisation and the VP receptor assay.

As radioligand the VP receptor assay used the linear VP antagonist Phaa-D-Tyr(Me)-Phe-Gln-Asn-Arg-Pro-Arg-

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Tyr-NH₂ (Phaa = phenylacetyl), which was monoiodinated at the phenyl moiety of the tyrosylamide residue at position 9 using Na¹²⁵I and the chloramine-T method [24]. The linear VP antagonist used in this study was found to be a highly potent and specific ligand for V_{1a}-receptors in control studies using rat liver crude membrane preparations (average K_d = 0.05 nM, unpublished results; G.J. Boer, P. te Riele, C. Korting and J.J. van Heerikhuizen). It has a low affinity for oxytocin receptors, if compared with other VP antagonists, and a low affinity for VP receptors of the V_{1b}- and V₂-type [10,24]. Saturation analysis with the crude membrane fraction of the human choroid plexus (10–12 points) was performed with triplicate samples in the following way. Two hundred μ l of the sample (approximately 50–80 μ g of tissue protein per incubation tube) was incubated for 2 h at 25°C with 25 μ l radioligand solution (4.5–300 pM final concentration), either in the presence of 25 μ l displacer solution (1 μ M VP final concentration) or 25 μ l incubation buffer. The reaction was terminated with the rapid addition of 2 ml of incubation buffer using a Skatron cell harvester followed by rapid filtration over a coated filter (Whatman GF/B coated for 2 h in 0.03% polyethyleneimine) and a wash. The radioactivity bound on the crude membranes collected on the filter were counted for 10 min (Cobra 5005, Packard; efficiency 80%). The computer program 'Ligand' (Elsevier-Biosoft) was used to perform the Scatchard analysis to calculate the affinity constant K_d and density B_{max} . In two cases, one control and one AD patient, no Scatchard plot could be calculated [23], despite the fact that binding and subsequent displacement were present. These cases were therefore omitted from the statistical analysis of the K_d and B_{max} .

There was no significant difference between control and AD patients as far as age, postmortem delay (PMD) or pH of the CSF were concerned (Table 1). Brain weight was significantly reduced in the AD patients group.

Scatchard analysis of the binding data of choroid plexus crude membranes reached no statistically significant differences for the K_d of the linear VP antagonist (Table 1), although the average K_d was 2.7 times increased in the Alzheimer group. The twofold increase of the B_{max} in the AD group was however significant, from which it can be concluded that the number of V_{1a}-receptors on the choroid plexus is increased in the Alzheimer group. Since the PMD almost reached statistical significance ($P = 0.06$), an additional Pearson correlation test was performed between the PMD, K_d and B_{max} , but no significant correlation was found (PMD vs. K_d : $P = 0.42$; PMD vs. B_{max} : $P = 0.9$, showing that differences in PMD had not affected our results. The clinical data gave no indication for an influence of medication on our data.

Changes in VP receptor density might have their origin in changes in the level of circulating VP concentrations. In AD patients the plasma level of VP was found to be decreased [25]. With respect to the VP concentration in the CSF, generally a reduction of VP levels has been reported [15,20,21]. Decreased VP plasma and/or CSF levels might thus have led to upregulation of the V_{1a}-receptor in the choroid plexus, although it is not certain whether the V_{1a}-receptors of the choroid plexus are activated from the luminal (blood) or CSF side. However, it should be noted that one other study reported an increase in CSF-VP levels [29] and another found no differences, not even in severely demented patients [11]. A point of concern is that the studies on plasma and CSF were based on samples col-

Table 1

Clinico-pathological data of control and Alzheimer's disease patients and the affinity constant, K_d , and density, B_{max} , of binding sites of the linear VP-antagonist on crude membrane fractions of choroid plexus

	Age (years)	Sex	Postmortem delay (h · min)	CSF pH	Brain weight (g)	Clinico-pathological data	K_d (pM)	B_{max} (fmol/mg protein)
<i>Controls</i>								
93-105	80	m	07.00	6.75	1405	hypertension, heart failure	33	13.1
93-133	64	m	08.10	6.90	1448	chronic myeloid leukemia	28	21.9
93-143	68	f	10.20	6.45	1310	septic shock	59	24.1
94-051	79	m	03.45	N.D.	1250	myocard infarct	95	23.3
94-053	83	m	08.50	6.70	1120	decompensatio cordis	no fit	no fit
mean $\pm$ S.E.M.	75 $\pm$ 4		7.37 $\pm$ 1.06	6.7 $\pm$ 0.1	1307 $\pm$ 58		54 $\pm$ 15	20.6 $\pm$ 2.5
<i>Alzheimer's disease</i>								
92-039	87	f	03.00	7.04	1094	AD (5) [#] , cachexia	no fit	no fit
92-050	67	f	05.00	6.37	940	AD (7), dehydration	153	36.6
92-052	90	f	05.00	6.40	1023	AD (7), toxic megacolon	186	50.0
93-003	76	m	03.45	6.61	1250	AD (7), Lewy bodies in SN and GC, cachexia	28	32.9
94-002	80	f	06.40	6.16	1128	AD (6), cachexia	99	25.7
mean $\pm$ S.E.M.	80 $\pm$ 4		4.41 $\pm$ 0.38	6.5 $\pm$ 0.2	1087 $\pm$ 52		117 $\pm$ 35	36.3 $\pm$ 5.1
Statistics	$t = 0.94$		$t = 2.31$	$t = 0.09$	$t = 2.81$		$t = 1.65$	$t = 2.76$
(t -test)	$P = 0.37$		$P = 0.06$	$P = 0.94$	$P = 0.02$ *		$P = 0.11$	$P = 0.02$ *

Abbreviations: h = hours; min = minutes; CSF = cerebrospinal fluid; g = grams; f = female; m = male; N.D. = not determined; no fit = no Scatchard plot possible; SEM = standard error of mean; SN = substantia nigra; GC = gyrus cinguli. Statistical significance: * $P < 0.05$; [#] = dementia scale according to Reisberg [22].

lected from patients clinically diagnosed as probable AD [16], but were postmortem not neuropathologically confirmed as AD patients.

The higher number of VP receptors in the choroid plexus of neuropathologically confirmed AD patients as found in the present study point to an increased potency of VP for inhibition of CSF production in this neurodegenerative disorder.

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