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Original contribution

Increased expression of the Nogo receptor in the hippocampus and its relation to the neuropathology in Alzheimer's disease☆

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Summary Alzheimer's disease (AD) is the most prevalent cause of dementia in human beings. Its best-known pathologic feature is the presence of senile plaques and neurofibrillary tangles in the brain. Nogo-66 receptor (NgR) is believed to contribute to the inhibitory activities of axon regeneration after injury. This study investigated the expression of NgR in the hippocampus and its relation to the pathologic changes of AD using immunohistochemistry and double-labeling immunofluorescence methods. The results showed that NgR immunoreactivity was present in more than 50% of the pyramidal layer cells of the CA1 to CA4 subfields of the hippocampus. No significant difference was observed in the number of NgR immunopositive cells in the CA1 to CA4 subfields between patients with AD and control subjects, whereas the ratio of NgR immunopositive cells to the total number of pyramidal layer cells was revealed to be significantly higher in the CA1 and CA2 subfields of the hippocampus of patients with AD than that in the same region of the control subjects. Moreover, high numbers of AT-8 immunopositive cells were found to be double-labeled with NgR in the CA1 subfields of patients with AD, whereas only few NgR deposits were observed in the senile plaques of the hippocampus in these patients. These results suggest that NgR may be related to the formation of tangles in AD.

1. Introduction

Alzheimer's disease (AD) is characterized by progressive cognitive, behavioral, and functional declines [1]. The neuropathologic hallmarks of AD include the early loss of neocortical synapses, the formation of neurofibrillary tangles (NFTs), and the presence of neuritic plaques in the hippocampus and temporal cortex [2].

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Nogo-66 receptor (NgR) was primarily found to play an important role in axon regeneration after central nervous system injury [3]. Recently, it was suggested that the normal function of some axon inhibition proteins may be correlated more to the preservation of the wiring of central nervous system than to the suppression of axonal regeneration [4]. NgR and its ligand Nogo-A have been proposed to participate in synaptic plasticity, learning, and memory [5,6]. Several studies have demonstrated that NgR and Nogo-A were involved in the pathology of neurologic diseases such as temporal lobe epilepsy and multiple sclerosis [7,8]. Most recently, NgR was reported to be related to the production of amyloid-β peptide (Aβ), implicating the potential function of NgR in the AD process [9].

Because NgR is identified as a glycosylphosphatidylinositol-linked protein and lack of an intracellular signaling domain, it must rely on additional transmembrane co-receptors to transduce the inhibitory signal [10]. The low-affinity neurotrophic factor $p75^{NTR}$ and another membrane protein known as LINGO-1, both of which have been identified as the co-receptors of NgR, form a transmembrane receptor complex with NgR [11-13]. $p75^{NTR}$ was proposed to play a role in the process of AD and believed to correlate with cell death or neuron apoptosis in AD [14-16]. The previous study from our group showed that $p75^{NTR}$ may be involved in the formation of tangles in AD [17], which raises an indication that NgR may also be involved in the progression of tau hyperphosphorylation.

NgR messenger RNA has been reported to be expressed in the neocortex, hippocampus, and amygdala in the human brain by in situ hybridization [18]; in mouse brain, NgR protein was detected in axons and at synaptic terminals, either presynaptically or postsynaptically [19]. However, NgR protein expression in the human hippocampus has not been reported in detail until now. The current study aimed to observe the expression of NgR in the human hippocampus and its possible relation to the neuropathologic changes in AD.

2. Materials and methods

2.1. Subjects

Hippocampal samples of 10 female patients with AD and 10 nondemented female control subjects matched for age and postmortem delay were dissected at autopsy. Detailed data on the sample are shown in Table 1. The brain material was obtained within the framework of the rapid autopsy program of the Netherlands Brain Bank. Permission was obtained for brain autopsy and for the use of the tissue and clinical information for research purposes. The clinical diagnosis of probable AD was verified neuropathologically. The general pathology and neuropathology of the AD and control brains were performed at the Free University of Amsterdam (Dr W. Kamphorst). Patients with AD showed extensive hippocampal and neocortical senile plaques and tangles. The Braak stages of patients with AD were between V and VI, and those of the control subjects were between 0 and II.

Table 1 Clinicopathologic information on the control subjects and the patients with AD

NBB no.	Age (y)	Brain weight (g)	Postmortem delay (h)	Cerebrospinal fluid (pH)
Patients with AD				
90075	79	1155	4:00	6.53
99002	77	1130	6:05	6.22
90038	84	1185	2:30	6.59
96063	82	1385	9:00	7.45
97091	85	1100	2:09	7.25
96090	84	1116	6:05	6.1
90026	84	990	4:00	6.47
96089	74	934	6:05	7.36
98139	72	935	9:50	6.4
93008	69	1032	4:00	6.03
Control subjects				
96057	74	982	4:00	6.03
98036	69	1264	6:15	6.59
96051	71	1256	4:50	6.65
94063	74	982	5:35	7.04
95078	80	1087	6:15	6.96
97088	78	1351	4:15	6.2
89093	80	1210	8:00	6.07
96084	78	1330	7:30	6.6
91120	73	975	4:25	5.87
90054	70	1116	7:26	6.65

Abbreviation: NBB, The Netherlands Brain Bank.

2.2. Tissue preparation

Brains were removed from the skulls and weighed, followed by 4 weeks of fixation in 4% formaldehyde at room temperature. The main body of the hippocampus was dissected, dehydrated, and embedded in paraffin. Serial 6-μm coronal sections were cut on a Leica microtome (RM 2135; Leica, Deerfield, IL). The first series of sections was stained with 0.5% thionin. The second series was processed for NgR immunohistochemistry. The third series was processed for NgR double-labeling immunohistochemistry with AT-8. The fourth series was processed for NgR immunofluorescent double-labeling with $A\beta_{17\text{-}24}$. The fifth series was processed for NgR immunofluorescent double-labeling with glial fibrillary acidic protein (GFAP).

2.3. Antibody specificity

The monoclonal antibodies AT-8 (MN-1020-B; Endogen, Woburn, MA), $A\beta_{17\text{-}24}$ (MAB1561; Chemicon, Temecula, CA), and GFAP (M0761; DAKO, Glostrup, Denmark) were used to identify hyperphosphorylated tau, senile plaques, and astrocytes, respectively. The specificity of these antibodies had been confirmed previously in our laboratory and in many other research efforts [17,20,21].