Parvalbumin-immunoreactive neurons in the human central nervous system are decreased in Alzheimer's disease

J. Satoh1, T. Tabira1, M. Sano2, H. Nakayama3, and J. Tateishi4

1 Division of Demyelinating Disease and Aging, National Institute of Neuroscience, NCNP, 4-1-1 Ogawahigashi, Kodaira, Tokyo 187, Japan
2 Department of Neurology, Faculty of Medicine, Tokyo Medical and Dental University, Tokyo, Japan
3 National Center Hospital for Nervous, Mental, and Muscular Disorders, NCNP, Kodaira, Tokyo, Japan
4 Department of Neuropathology, Faculty of Medicine, Kyushu University, Fukuoka, Japan

Received August 12, 1990/Revised, accepted October 1, 1990

Summary. Immunohistochemical localization of the Ca2+-binding protein parvalbumin (PV) was investigated in the adult human central nervous system (CNS). The antiserum against purified rat skeletal muscle PV specifically recognized certain neuronal populations and their processes. Strongly positive were Purkinje, basket and stellate cells of the cerebellum, cerebral cortical nonpyramidal cells, and neurons in the thalamic reticular and ventrolateral nuclei, subthalamic nucleus, lateral and medial geniculate bodies, vestibular and cochlear nuclei, spinal trigeminal nucleus, cuneate and gracile nuclei, and dorsal nucleus of Clarke. Negative were cortical pyramidal neurons, neurons of the autonomic nerves, and neurons in the caudate nucleus, putamen, dentate nucleus, inferior olive, and substantia gelatinosa. The number and size of PV-immunoreactive neurons were significantly decreased in Alzheimer's disease. However, the decrease was not disease specific.

Key words: Parvalbumin – Ca2+-binding protein – Interneuron – Alzheimer’s disease

Parvalbumin (PV) is a water-soluble, heat-stable, low molecular weight (Mr = 12,000), acidic protein, which has been shown to be a Ca2+-binding protein [17, 18, 27]. It was detected in the fast-contracting muscle fibers in higher vertebrates [11, 19, 32]. Interestingly PV has been shown to be present in non-muscle tissues biochemically [3] and immunohistochemically [13], particularly in the central nervous system (CNS) of the rat [2, 8–10, 14–16, 23, 25, 26], mouse [33, 34], cat [35, 37, 38], zebra finch [5, 6, 40] and monkey [16, 35]. With regard to human CNS, two studies have been reported, one confined to the hippocampus of a patient with temporal lobe epilepsy [4] and the other confined to retina [15].

The first aim of the present investigation was to determine the localization of immunoreactive PV in the whole human CNS. The second aim is to investigate PV-immunoreactive neurons in Alzheimer’s disease (AD). Arai et al. [1] demonstrated that PV-immunoreactive neurons are lost and their size is decreased in AD, while Morrison et al. [30] contradicted this. We conclude that PV-immunoreactive neurons are significantly decreased in AD but that this decrease is not disease specific.

Materials and methods

Antibodies

Antiserum directed to rat skeletal muscle PV was raised in rabbits [36]. Briefly 0.5 mg of PV, purified from Wister rat skeletal muscle, [13], in 0.5 ml phosphate-buffered saline (PBS) was emulsified with an equal volume of complete Freund's adjuvant. One week after the first inoculation, a booster immunization with the same emulsion was given. Serum was obtained 2 months after the initial sensitization and its specificity was tested by double immunodiffusion and immunoblotting. No cross-reaction with either bovine brain calmodulin or S-100 protein was found. Rabbit anti-bovine brain S-100 antibody was purchased from Advance Co., Tokyo.

Materials

Control material was obtained at autopsy from a 45-year-old woman with myasthenia gravis, who committed suicide by hanging; a 35-year-old woman with schizophrenia and bronchial asthma, who died of asphyxia; a 76-year-old man without any disease, who died of acute cardiac failure; and an 82-year-old man with aortic stenosis and mitral valve insufficiency, who died of cardiac failure. Brain weights were 1,430 g, 1,370 g, 1,290 g, and 1,310 g, respectively. All cases showed no significant pathological changes in the CNS. Four AD patients and two disease controls were included in this study. Clinical and pathological profiles of these patients are listed in Table 1.

Immunohistochemical staining

All tissues were immersed in 10% formalin, dissected out, and embedded in paraffin. Sections 5 μm were deparaffinized and fixed
<table>
<thead>
<tr>
<th>Case</th>
<th>Diagnosis</th>
<th>Age, Sex</th>
<th>Duration (years)</th>
<th>Brain weight (g)</th>
<th>Senile plaques</th>
<th>Neurofibrillary tangles</th>
<th>Amyloid angiopathy</th>
<th>Arteriosclerosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>AD</td>
<td>68, F</td>
<td>19</td>
<td>970</td>
<td>+++</td>
<td>+++</td>
<td>+ + + +</td>
<td>+++ + + +</td>
</tr>
<tr>
<td>2</td>
<td>AD</td>
<td>72, F</td>
<td>18</td>
<td>790</td>
<td>+++</td>
<td>+++</td>
<td>+ + + +</td>
<td>+++ + + +</td>
</tr>
<tr>
<td>3</td>
<td>AD</td>
<td>64, F</td>
<td>11</td>
<td>930</td>
<td>+++</td>
<td>+++</td>
<td>+ + + +</td>
<td>+++ + + +</td>
</tr>
<tr>
<td>4</td>
<td>AD</td>
<td>59, M</td>
<td>12</td>
<td>1020</td>
<td>+++</td>
<td>+++</td>
<td>+ + + +</td>
<td>+++ + + +</td>
</tr>
<tr>
<td>5</td>
<td>PSP</td>
<td>65, F</td>
<td>8</td>
<td>910</td>
<td>+++</td>
<td>+++</td>
<td>+ + + +</td>
<td>+++ + + +</td>
</tr>
<tr>
<td>6</td>
<td>Pick</td>
<td>59, M</td>
<td>8</td>
<td>1205</td>
<td>+++</td>
<td>+++</td>
<td>+ + + +</td>
<td>+++ + + +</td>
</tr>
</tbody>
</table>

AD: Alzheimer's disease; PSP: progressive supranuclear palsy

Again in the Bouin's fixative overnight. They were treated with: (1) methanol containing 0.3% H2O2 at 4°C for 30 min; (2) normal sheep serum (Cappel) diluted 1:20 in PBS at 4°C for 30 min; (3) rabbit anti-rat PV serum diluted 1:300 in 1% bovine serum albumin (BSA, Sigma) in PBS at 27°C for 90 min; (4) peroxidase-coupled goat anti-rabbit IgG (Cappel) diluted 1:50 in 1% BSA-PBS at 37°C for 45 min; and (5) diaminobenzidine tetrahydrochloride (DAB), with thorough intervening washes with PBS. Three types of controls were performed to test the specificity of the immunohistochemical reaction: (1) the antiserum was absorbed with excess of PV; (2) rabbit anti-bovine brain S-100 antibody was diluted 1:500 in 1% BSA-PBS; and (3) using normal rabbit serum. For the absorbed antiserum, 400 μg of PV was added to 1 ml of 1:300 diluted anti-PV serum and incubated for 48 h at 4°C.

**Morphometry**

For morphometric analysis, pictures were taken at the magnification of 150 x from similar regions, and all PV-immunoreactive cells

---

**Fig. 1a-d.** The immunolabeling of parvalbumin (PV) in the cerebellum. a Purkinje cells and their dendritic arborizations and axons are markedly immunoreactive to PV. Small neurons of the molecular layer are also positive but granule cells are negative. b Staining with the antiserum absorbed with an excess of PV. c Staining with anti-bovine brain S-100 antibody shows positive immunoreactivity in Bergman’s glia and their fibers. d Anti-PV staining of the dentate nucleus shows negative or only weakly positive neurons and positive axons of Purkinje cells. a, b, d ×165, c ×330