Abstract  Synapse loss is crucially involved in cognitive decline in Alzheimer’s disease (AD). This study was performed to investigate the distribution and density of chromogranin B-like immunoreactivity in the hippocampus of control compared to AD brain. Chromogranin B is a large precursor molecule found in large dense-core vesicles. For immunocytochemistry we used an antiserum raised against a synthetic peptide (PE-11) present in the chromogranin B molecule. Chromogranin B-like immunoreactivity was concentrated in the terminal field of mossy fibers, the inner molecular layer of the dentate gyrus and in layer II of the entorhinal cortex. In AD, chromogranin B was detected in neuritic plaques. The density of chromogranin B-like immunoreactivity was significantly reduced in the inner molecular layer of the dentate gyrus and in layers II, III and V of the entorhinal cortex in AD brains. The present study demonstrates that chromogranin B is a marker for human hippocampal pathways. It is particularly suitable for studying nerve fibers terminating at the inner molecular layer of the dentate gyrus. It is present in neuritic plaques, and its density is reduced in a layer-specific manner.

Key words  Human brain · Hippocampus · Alzheimer’s disease · Chromogranin B · Synapse loss

Introduction

Progressive impairment in memory and cognition is a clinical key feature of Alzheimer’s disease (AD), but the pathogenesis of the lesions leading to cognitive decline is not well understood. AD is morphologically characterized by the presence of neurofibrillary tangles and senile plaques. Loss of neurons and loss of synapses are of principal importance in intercellular communication in neural circuits in AD [26]. Correlation of synaptic and pathological markers with cognition of the elderly suggests that cytoskeletal alterations are important in AD [7].

Chromogranin B belongs to the chromogranins which include chromogranin A, chromogranin B [15], secretogranin II, and 7B2 [24]. Chromogranins are found in large dense-core vesicles throughout the endocrine and nervous tissue [9]. They are stored together with different peptide hormones and neuropeptides. Neuropeptides are soluble constituents of large dense-core vesicles. They are differentially affected in AD [6, 14]. For example, the concentration of somatostatin is reduced both in the brain tissue and cerebrospinal fluid [34]. In AD there are dramatic reductions in human corticotropin-releasing factor concentration and reciprocal increases in CRF receptor density in the cortex [1]. Other neuropeptides, such as vasoactive intestinal polypeptide, are not changed [12, 37].

The molecular properties and the distribution of secretogranin II and chromogranin A are better characterized than those of chromogranin B. Chromogranin A and B mRNA have been detected in pyramidal neurons of human cerebral cortex by in situ hybridization histochemistry [8]. Several peptides derived from chromogranin B have been demonstrated in various tissues [2]. Recently, we performed a detailed immunohistochemical mapping for chromogranin B in rat brain, and investigated the degree of proteolytic processing [22] using an antibody generated against a synthetic peptide (PE-11) derived from the chromogranin B sequence. Our results demonstrated a significant processing of chromogranin B as well as its widespread and distinct distribution [22]. In human brain,
PE-11-like immunoreactivity (PE-11-LI) is strongly concentrated in several areas including the bed nucleus of the stria terminalis, hippocampus, hypothalamus, and different brain stem nuclei [25].

There were several objectives for the present study. Firstly, we intended to investigate whether chromogranin B-LI is present in the human hippocampal formation, the subiculum and the entorhinal cortex. A detailed immunocytochemical study may reveal which hippocampal pathways contain this peptide. We were particularly interested in whether chromogranin B-LI is found, as in the rat, in a high concentration in the inner molecular layer of the dentate gyrus. In the second part of the present study, the staining pattern of chromogranin B-LI was investigated in AD. A main objective was to determine whether chromogranin B is present in neuritic plaques and if so, how the distribution of chromogranin B-containing plaques correlates with that of chromogranin B-LI. In addition, we studied the density of chromogranin B-LI in the different parts of the hippocampus and entorhinal cortex in AD compared to age-matched controls.

The main goal of the present study was to establish a further neuronal marker for the hippocampal pathways in man, and to provide a neuroanatomical baseline for changes in AD.

Methods and materials

Control subjects

The brains of seven adult humans (three males and four females; average age 76.5 ± 8.4 years), with no known neurological or psychiatric disease, were obtained at routine autopsy at a postmortem interval of 17 ± 3.5 h. Hospital and other medical records confirmed that these subjects had normal intellectual function until the time of their deaths (Table 1). Histologically three brains displayed Braak stage V–VI, which is consistent with the human sequence. The antiserum only reacts with the free C-terminal part of PE-11 since an elongated peptide (chromogranin B 552–574) only reacts in the radioimmunoassay (RIA) when it is first subjected to trypsin digestion [22]. No cross-reactivity was found with peptides derived from chromogranin A and secretogranin II or with the following neuropeptides: galanin, substance P, neuropeptide Y, neurotensin and calcitonin gene-related peptide.

Chromatographic analysis of chromogranin B-LI at different postmortem intervals

Samples (~ 150 mg each) of the hippocampus and entorhinal cortex were dissected. They were cut into coronal slices, about 1 cm thick, and immediately fixed by immersion in cold 4% paraformaldehyde in sodium phosphate buffer (PBS), pH 7.2, for 1 week. One block was dehydrated in graded ethanols, embedded in paraffin and cut serially in 3-μm-thin coronal sections. The remaining slices were rinsed in PBS for 1 day, and were sequentially transferred to 5%, 15% and 20% sucrose in PBS for 1 day each. These slices were frozen in isopentane (–45°C) for 3 min and stored at –70°C. Free-floating sections were prepared using a freezing microtome.

Tissue blocks containing the caudal amygdala, the entire hippocampus and entorhinal cortex were dissected. They were cut into coronal slices, about 1 cm thick, and immediately fixed by immersion in cold 4% paraformaldehyde in sodium phosphate buffer (PBS), pH 7.2, for 1 week. One block was dehydrated in graded ethanols, embedded in paraffin and cut serially in 3-μm-thin coronal sections. The remaining slices were rinsed in PBS for 1 day, and were sequentially transferred to 5%, 15% and 20% sucrose in PBS for 1 day each. These slices were frozen in isopentane (–45°C) for 3 min and stored at –70°C. Free-floating sections were prepared using a freezing microtome.

Table 1: Information about the patients from whom brains were obtained. GDS stage [30]: 1 no cognitive decline, 6 severe cognitive decline, 7 very severe cognitive decline; MME as defined in [10] (GDS Global Deterioration Scale, MME Mini Mental State Examination)

<table>
<thead>
<tr>
<th>Sex</th>
<th>Age (years)</th>
<th>GDS</th>
<th>MME</th>
<th>Braak</th>
<th>Cause of death</th>
<th>Brain weight (g)</th>
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<tbody>
<tr>
<td>M</td>
<td>71</td>
<td>1</td>
<td>28</td>
<td>–</td>
<td>Myocardial infarction</td>
<td>1,250</td>
</tr>
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<td>27</td>
<td>–</td>
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<td>27</td>
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<td>28</td>
<td>–</td>
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<tr>
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<tr>
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<tr>
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<td>12</td>
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<tr>
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<td>VI</td>
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<tr>
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<td>4</td>
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