Senile plaques: staining for acetylcholinesterase and A4 protein: a comparative study in the hippocampus and entorhinal cortex

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Summary. In 20 unselected autopsy cases tissue blocks from the hippocampus with adjacent entorhinal cortex and neocortex were stained for acetylcholinesterase (AChE). From five brains shown to have large numbers of senile plaques tissue, adjacent to that taken for AChE tissue blocks, was embedded in paraffin and sections were immunostained for the A4 protein. The morphological aspects were compared. Equivalent types of plaques and plaque-like structures were observed in the A4- and AChE-stained sections. On selected tissue blocks from patients with many senile plaques two immediately adjacent cryostat sections were stained, one for AChE and one for A4 protein. The same individual plaques could be identified on the two sections. These findings suggest that high AChE activity is intimately associated with the process of A4 protein formation and accumulation in plaques and that this association already occurs at a very early stage of plaque formation.

Key words: Alzheimer's disease — A4 protein — Acetylcholinesterase — Senile plaque — Neuropathology

A decrease in the synthesis of acetylcholine is consistently found in brains of patients with Alzheimer's disease [4, 5, 7]. The relationship between this biochemical alteration on the one hand and the morphological hallmarks of the disease, i.e., of plaques and tangles [1], on the other is still unclear, although there is some evidence for disturbed cholinergic innervation being of pathogenetic relevance for the formation of plaques in the allo- and neocortex [2, 16, 17].

In recent years the enzyme-histochemical staining of acetylcholinesterase (AChE)-containing structures in cerebral tissues has been successfully applied to Alzheimer dementias [16, 23]. A major finding of these investigations consisted in an apparent shift of AChE staining from cortical axons to the senile plaques [16]. However, we do not know whether AChE activity is restricted to certain types of plaques and there has been no systematic investigation aimed at comparing plaques stained for AChE with other techniques, especially with immunohistochemistry. We, therefore, decided to compare the morphological appearances of senile plaques using the Tago-Mesulam technique and by immunohistochemistry with an anti-A4 protein serum.

Material and methods

The right hippocampus including the adjacent entorhinal cortex from patients autopsied less than 12 h after death was prepared and fixed in 4% phosphate-buffered paraformaldehyde (pH 7.4). After the cryo-protection procedure [10] frozen sections of these hippocampi were stained for AChE in accordance to the published protocol [14, 16, 23]. As a control, on selected sections the substrate (acetylcholine iodide) was not added to the incubation medium.

The other parts of the brains were fixed in phosphate-buffered formalin (pH 7.4). After 10—15 days fixation, they were dissected according to our routine neuropathological procedure.

In the five cases which showed the most senile plaques, tissue sections from the hippocampus were also immunostained with an antiserum to the A4 protein diluted 1:500. These sections were from parts of the hippocampus close but not immediately adjacent to the frozen sections on which the histochemistry was performed (average distance from each other 5—10 mm). For immunohistology of A4 protein, paraffin sections were deparaffinized in xylene and alcohol and rinsed in phosphate-buffered saline. After removing peroxidase with 0.3% (v/v) H2O2 in methanol the sections were incubated overnight at 4°C with antisynthetic A4 serum (diluted by 1:500) pretreated with 90% formic acid for 5 min to expose the A4 epitopes. The immunoreactive sites were then visualized using the avidin-biotin-peroxidase method [9]. Pairs of adjacent cryostat sections were taken from two patients with many plaques and one section was stained for AChE and immunostained for A4 protein.

The antiserum against a synthetic peptide homologous to the amino-acid sequence 1—42 of the A4 protein was kindly provided by Prof. Collin Masters, Melbourne, Australia.
Fig. 1 A—D. Various plaques and preplaques from the entorhinal cortex in immunostains of the A4 protein, all photographed with Nomarski optics without counterstain. A Classical plaque. Note intensity of the immunostain in the core as compared to weaker reactivity of the peripheral rim. F: Fully formed plaque. B Deposits of A4 protein of punctate (P) and cotton wool (C) type. Larger deposits probably correspond to early neuritic type (same case as in A). C Preplaques of diffuse (D) punctate, subpial (S) and cotton wool type. D Diffuse and punctate deposits in Layer I of the cortex and subpial deposits (same case as in A and B) Bars = 50 μm

Results

Characteristics of plaques

The immunostain for A4 and the AChE reaction (see Figs. 1, 2) allowed the distinction of many plaques of various morphological types. The same morphological spectrum of plaques was observed with both techniques. In the sections processed as for AChE but incubated without acetylcholine iodide neither axons nor senile plaques were visible.

Two basic types of plaques were observed both with the AChE reaction and the immunostain for the A4 protein: Well delimited, fully formed plaques some of which showed a dense amyloid core in the A4 stain, (Figs. 1A, 2B; designated F); and structures of a different morphology, which we name here preplaques. These were of different types: (a) small, cotton wool like (less than 40 μm in diameter), which were sometimes in the vicinity of vessels or neurons (designated C on the Figs. 1B, 2C—E); (b) large (up to 300 μm diameter) diffuse badly delimited cotton wool like (designated D on the Figs. 1C, 2D); (c) punctate (designated P on the Fig. 1B—D, 2C, E); and (d) subpial surface of the entorhinal cortex (designated S on Fig. 1D, 2E). All these preplaques sometimes formed clusters (Figs. 1D, 2E).

The same individual plaques could be seen in the pairs of adjacent sections (Fig. 3 A, B). Stains for AChE made at pH 7.0 or 8.0 did not differ, which is a slight discrepancy compared with other observations [16]. The high density of plaques was associated with a marked change in the staining pattern: while brains without plaques exhibited a fine network of AChE-positive fibers within the hippocampus and adjacent cortex (Fig. 2A), this network was severely attenuated in the hippocampi most afflicted with plaques (Fig. 2B). Also in the entorhinal cortex with only few senile plaques, the number of AChE-reactive axons was slightly reduced. The plaques themselves appeared as amorphous deposits of reaction product in which no AChE-reactive fibers could be observed. Some neurofibrillary tangles also presented strong AChE activity; a finding which we will not discuss further in this communication (cf. Fig. 2B; N).