ULTRACYTOCHEMICAL ANALYSIS OF E-PTA-POSITIVE SYNAPTIC JUNCTION —THREE CASES OF DOWN'S SYNDROME TOGETHER WITH ALZHEIMER-TYPE DEMENTIA—

KENICHIROU INOMATA¹, HIROAKI IGARASHI¹, FUMIO NASU¹,
MICHIYA SUGAHARA² AND YUKIO TAKASHIMA³

*Department of Anatomy¹ and Psychiatry², Toho University School of Medicine, Tokyo 143
and National Institute of Neuroscience³, NCNP, Kodaira, Tokyo 187*

Received for publication October 23, 1991

The synaptic junction from three autopsy cases of adults with Down's syndrome and Alzheimer-type dementia was examined ultracytochemically, using the ethanolic phosphotungstic acid (E-PTA) method. A noteworthy finding was the presence of variable shaped vesicles which were not observed in the ordinary E-PTA-treated preparations. Many of these vesicles displayed a wide variety of abnormal shapes between the presynaptic dense projections. Such a change has escaped detection in the E-PTA-treated preparation so far. The presynaptic terminal was considered to be one of the central regions for observing pathological changes, associated with Alzheimer's disease. In addition, the possibility is raised that the qualitative analysis of synaptic junctions, using the E-PTA method, facilitates the acquisition of further information concerning synaptic changes.

Down's syndrome is characterized by physical, mental, and functional abnormalities that result from trisomy 21, the presence in the genome of three rather than the normal two chromosomes 21. Most patients with Down's syndrome undergo a premature cognitive decline with aging, and eventually develop the neuropathologic changes of Alzheimer's disease, including amyloid-containing neuritic plaques, and the formation of neurofibrillary tangles (25). Amyloid fibrils consist of hollow rods that are composed of filaments arranged in a tightly coiled helix, with each turn comprising five globular subunits. Miyakawa and Kuramoto suggest that the capillary degeneration that occurs with the formation of amyloid fibrils may constitute the primary change concerning the genesis of neurite plaques. Furthermore, degenerative changes in the microvessels may also be responsible for the loss of neurons in the brain of subjects with Alzheimer's disease (24).

A reaction within the nervous system occurs when an injury is induced around the perinatal stage, and thus provides a central source of knowledge for measuring plasticity. One of the sites in which such plastic changes occur is the synapse located in the neural circuit (4). According to the degenerative study of

synapses by Inomata and Taxi (18), the altered synapses usually underwent osmiophilic degeneration, while finding the persisting synaptic junctions. Ethanolic-phospho-tungstic acid (E-PTA) is the specific reagent for a synaptic junction. Furthermore, the E-PTA method is applied to the pathological observations gathered from postmortem materials. The purpose of the present study is to perform an ultracytochemical observation using the E-PTA method, on three adults with both Down's syndrome and Alzheimer-type dementia.

MATERIALS AND METHODS

The synaptic junction from the three autopsy cases was examined ultracytochemically. The three adults had clinical histories of Down's syndrome coupled with Alzheimer-type dementia, which was confirmed during the autopsy. These brains histologically contained cortical atrophy, neuritic plaques, neurofibrillary tangles, granulovascular degeneration, Hirano bodies and amyloid angiography. For each tissue, there was no significant difference in the clinical histories and neuropathological findings. The majority of observations in this study were obtained from

the frontal cortex area. The materials were rinsed with a cold 0.1 M phosphate buffer, pH 7.3, containing 8% sucrose, then sectioned using a Microslicer (Dosaka EM), into a thickness of 100 μ m. The Microslicer sections were dehydrated with a graded series of ethanol and then left for 1 hr in 1% E-PTA in absolute alcohol, at room temperature. Following dehydration and staining, all sections were treated with propylene oxide and embedded in Poly-Bed 812 (Polysciences). Ultrathin sections were prepared using a Reichert-Jung Ultracut E into a thickness of 50 nm, poststained with uranyl acetate only and examined under a 200 EX electron microscope (JEOL).

RESULTS

The E-PTA method revealed only synaptic junctions with a high electron density on a pale background (Figs. 1, 2). The reaction products were precipitated on dense projections, cleft materials and postsynaptic thickening was present at the synaptic junctions. The electron-opaqueness of cleft materials was minor as compared with that of dense projections and postsynaptic thickening. Dense projections and postsynaptic thickening were evident at a synaptic junction, although cleft material could hardly be discerned. Highly electron-opaque dot-like presynaptic dense projections were arranged in either a hexagonal or triangular pattern in front of or on the opposite side of the post-synaptic thickening, forming presynaptic grids. However, in some presynaptic

grids presynaptic dense projections were irregularly arranged into the form of an array of discrete low hills, giving the appearance of an immature dense projection. There were no presynaptic dense projections in the perforated areas (Fig. 3). A noteworthy finding was the presence of vesicles of variable shapes not observed in the ordinary E-PTA-treated preparations. Many of them displayed a wide variety of abnormal shapes between presynaptic dense projections. E-PTA-staining was observed on the membrane of distended vesicles (Fig. 4).

DISCUSSION

The present study documented the ultracytochemical changes in the presynapse, in particular the presence of partially E-PTA-positive vesicles along the presynaptic junction. Such a change has escaped detection in the E-PTA-treated preparation so far. Conventional electron microscopy, Maslich *et al.* (23) found that, regardless of amyloid deposits, some presynaptic terminals were distended while containing swollen vesicles and dense bodies. These altered synaptic elements were similar to those found in dystrophic neurites.

According to Takashima *et al.* (30), the findings from a Golgi study of the visual cortex in patients with the Down's syndrome were compared with those from neurologically normal, age-matched control subjects. The dendritic atrophy seen in childhood continued into adulthood, with a marked decrease in dendritic

FIG. 1. E-PTA-positive synaptic junction in the asymmetrical synapse. The reaction products were precipitated on the dense projection (arrow), cleft material and postsynaptic thickening (arrowhead) seen at the synaptic junction. Ultrathin sections were stained with uranyl acetate only.

FIG. 2. E-PTA-positive synaptic junction in the symmetrical synapse. The reaction products were precipitated on the pre- and post-synaptic junction. Ultrathin sections were stained with uranyl acetate only.

FIG. 3. E-PTA-positive synaptic junction. The perforated appearance (arrowhead) was observed on the synaptic junction. Ultrathin sections were stained with uranyl acetate only.

FIG. 4. E-PTA-positive synaptic junction. The vesicles (arrows) displayed a wide variety of abnormal shapes between pre-synaptic dense projections (arrowhead). E-PTA-staining (double arrowhead) was observed on the membrane of the distended vesicles. Ultrathin sections were stained with uranyl acetate only.

branching, dendritic length, and spine frequency in elderly adults with the Down's syndrome. It was suggested that these dendritic abnormalities may be related to mental retardation in children and early dementia in older adults who have Down's syndrome (13, 20, 29). In addition, a significant reduction in the number of dendritic spine was found in the apical and basilar dendritic arbors of CA₁ and CA₂₋₃ pyramidal neurons in patients with Down's syndrome and no Alzheimer's disease when compared to age-matched controls. An additional decrease of Down's syndrome in every segment occurred in Down's syndrome in every segment occurred in Down's patients with Alzheimer's disease, when compared to age-matched controls and Down's patients with no Alzheimer's disease (9). As suggested by Takashima *et al.* (30), the genetic and extrinsic factors may be im-

portant determinants of the Alzheimer-type dementia that is associated with Down's syndrome. Likewise, the pathological nature of the three present cases also share similarities with Alzheimer's disease.

The Alzheimer's disease neocortical neuropil is characterized by extensive synaptic pathology (5, 15, 21, 23, 28), focal amyloid deposition, dystrophic neurite formation, glial cell hypertrophy in plaque (7), dendritic atrophy (30) and massive accumulation of abnormal cytoskeletal filaments in dendrites (1). However, very little information is available about the sequence of these neuro-pathological events. Ultrastructural studies of postmortem and biopsied cortical tissues with mild to severe Alzheimer's disease, have shown that the number of synapses was significantly decreased when compared with the number in the age-matched control brains (5, 11, 15).

In this regard, the relationship between preamyloid deposits and neuritic changes was studied using the brains from Alzheimer's and Down's patients. The presynaptic terminal swellings, immunolabeled by anti-synaptophysin and anti-ubiquitin antibodies, supports the view that the deposition of amyloid fibril precursors in the neuropil is closely related to presynaptic terminals (2). The immunoelectron microscopic study by Masliah *et al.* (23) favored the possibility that dystrophic neurites are actually distorted presynaptic terminals. In Alzheimer and in other degenerative diseases, pathological and biochemical lesions in the cholinergic neuron are localized mainly to the presynaptic region (10). A decrease in the number of cholinergic receptors in the cortex, particularly presynaptic nicotinic receptors, enzyme activities related to acetylcholine (ACh) synthesis, hydrolysis and release of ACh in an Alzheimer-type dementia spinal cord remained, caused ACh synthesis to be reduced, while ACh hydrosis unaltered. It was speculated that decreased cord ACh synthesis may be related to electrophysiological abnormalities, which have been reported in the motor nerves of patients with Alzheimer's disease (31). The alternative was also reported: Glutamatergic hypothesis sufficiently explains the dementia of Alzheimer's disease. This is based largely on postmortem evidence indicating reduced binding and uptake of D[³H] aspartate, as well as the loss of numerous other putative markers, such as phosphate-activated glutaminase activity, glutamate concentration, and the number of pyramidal cell perikarya, with this latter change correlating with the severity of dementia (27). The aspartic and glutamic acids cross the blood brain barrier, and are deposited in the brain. Within the brain, in the precise form of aluminum complex, L-glutamic acid is consequently unable to detoxify ammonia from neurons and produce L-glutamine. Accumulation of ammonia is subsequently responsible for the neuronal death, affecting each and every neurotransmitter system (6). Immunologically, calcitonin and calcitonin gene-related peptides (CGRP) were not affected in Alzheimer-type dementia, either as a consequence of a direct effect on peptidergic neurons or secondary to the loss of choline acetyltransferase activity (12). Immunohistochemical studies have shown that many dystrophic terminals within plaques, around neuronal perikarya, or those scattered in the neuropil show abnormal synaptophysin immunoreactivity (21, 22). In the E-PTA-treated preparation, it is impossible to detect whether the E-PTA-positive synaptic junction belongs to the cholinergic synapse or to the glutamatergic synapse, because the mechanism that

determines the relation of a specific stain to synaptic junction remains to be demonstrated. However, the E-PTA method has the advantage of not depending upon the postmortem changes, as compared to the other ultracytochemical stains to synapses. Consequently, it is almost certain that the E-PTA-treated preparations obtained from postmortem brains may increase the available information concerning the pathological changes occurring within synapses.

Secondarily, the perforation of the synaptic junction was noted in the present cases. Qualitatively, the larger synaptic junctions may undergo perforation and subsequent fragmentation and slitting, in order to adapt to changing experimental and environmental situations (3, 8, 14, 26). Jones and Colangelo (19) suggested that these perforations are an important change related to impaired synaptic junctions, determined by using the E-PTA method. However, Inomata *et al.* (16, 17) noted synaptic perforations in both the alcohol-treated and in the control rats, which were determined by using the experimental model for fetal alcohol syndrome. It is difficult to consider these perforations as completely representing the changes of the immature form in an immature-mature continuum.

In conclusion, by using the method to assess the number of synaptic junctions, considerably more information was provided about the synaptic changes which occur during the development of the neurological picture. From the present ultracytochemical analysis, the results suggest that the qualitative analysis of synaptic junctions, obtained through employing method, will increase the pool of available knowledge concerning such synaptic changes.

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