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Abnormal tau species are produced during Alzheimer's disease neurodegenerating process

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Tau proteins were detected in human brain using two polyclonal antibodies: anti-paired helical filaments and anti-human native tau proteins. Both antisera detected identically the normal set of tau proteins in control brains. Moreover they detected two abnormal tau variants of 64 and 69 kDa exclusively in brain areas showing neurofibrillary tangles and senile plaques. Tau 64 and 69 were abnormally phosphorylated as revealed by the decrease in their molecular mass observed after alkaline phosphatase treatment. Therefore, tau 64 and 69 are specific markers of the neurofibrillary degeneration of the Alzheimer type and might be useful tools for studying the first pathological events that lead to neuronal death.

Alzheimer's disease; Protein, tau; Paired helical filament; Phosphorylation

1. INTRODUCTION

Alzheimer's disease is a neurodegenerative brain disorder characterized by a dementia accompanied by a severe loss of large cortical neurons, the accumulation of PHF in the degenerating neurons and extracellular amyloid deposits [1].

The analysis of PHF has been undertaken in recent years in order to obtain clues about the etiopathogenesis of AD. Many converging results have shown that microtubule associated tau proteins are most probably the major structural proteins of PHF [2–8]. An abnormal phosphorylation of tau proteins during AD has been suggested [6,9,10], but this point is still being discussed since there is no direct biochemical evidence for such an abnormality [11].

Up to now, studies using anti-tau or anti-PHF antibodies failed to observe new variants among the set of tau proteins in Alzheimer brains.

We present here a comparison of the tau profile in normal and Alzheimer brain homogenates, using well characterized antisera against PHF that detect exclusively tau proteins and against tau proteins from human origin. These immunological tools reveal the presence of two pathological forms of tau proteins which are abnormally phosphorylated, in brain areas showing neurofibrillary degeneration of the Alzheimer type.

2. MATERIALS AND METHODS

Tau proteins were detected in SDS homogenates from Alzheimer brains (10 brains of definite AD, aged between 55 and 85) and 10 age-matched control brains. The diagnosis of Alzheimer's disease was established according to NINCDS-ADRDA classification [12]. Patients with Alzheimer's disease were 55, 60 and 62 years old and patients with senile dementia of the Alzheimer type were 75–85 years old. Controls were 53–98 years old and died of non-neurological diseases. Different regions were simultaneously studied for the neuropathologic diagnosis and for biochemical analysis, as in [3]. Brain tissue was homogenized in the Laemmli sample buffer [13] 1:10 (w/v), and heat treated. Brain proteins were separated by SDS-PAGE and electrophoretically transferred to nitrocellulose paper for tau immunodetection as in [14]. After

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Abbreviations: AD, Alzheimer's disease; PHF, paired helical filaments; SDS-PAGE, SDS-polyacrylamide gel electrophoresis; DTT, dithiothreitol

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a dialysis against buffer containing 50 mM Tris, pH 8.3, 50 mM NaCl, 1 mM Mg and 0.2 mM DTT, some samples were dephosphorylated using the calf intestine alkaline phosphatase (Boehringer Mannheim) at 100 U/ml [15]. The enzyme action was stopped by heating after addition of an equal volume of the Laemmli sample buffer. The anti-PHF whose properties at the light microscopic, electron microscopic and biochemical levels have already been described [3,14,16], was used at 1/200. The anti-tau antiserum was obtained by immunizing a rabbit with heat-stable human tau proteins purified with a preparative SDS-PAGE, and was used at 1/10000.

3. RESULTS

3.1. *Immunoblot detection of tau proteins in normal brain and Alzheimer's disease brain*

The electrophoretic profile of tau proteins from the normal human brain consists of four bands between 45 and 62 kDa (fig.1). Our anti-PHF and anti-tau detect identically these four proteins.

In Alzheimer brain, in addition to the normal set of tau proteins, two bands at 64 and 69 kDa were stained (lanes 1, 3 and 9). Tau 64 and 69 were always present together in regions with neurofibrillary degeneration while they were absent from regions without neurofibrillary tangles and neuritic plaques. Indeed, they were detected in temporal (lane 1), frontal (lane 3), parietal and occipital cortex, but were never found in caudate nucleus (lane 4), cerebellum, spinal cord and thalamus. Tau 64 and 69 detection was generally strong in the temporal, lower in the frontal and weak in the occipital cortex which often contains less NFT and SP. The staining appeared increased in Alzheimer brains having an early onset. Homogenates of AD brain contained a diffuse smear of immunoreactive material throughout the running gel (lanes 1, 3, and 9). The intensity of this background was proportional to the immunolabelling of the two pathological variants (cf. lanes 1 and 3). We also found tau 64 and 69 in a frontal biopsy (lane 7) of a 58-year-old patient who began to develop an unusual dementia with frontal clinical signs. Their detection was stronger than in all the autopsic pieces studied up to now (cf. lane 7 with lanes 1, 3, 9). The histological study revealed that many NFT and SP were present in this tissue.

Tau 64 and 69 were never found in the different areas of control brains (lanes 2 and 6). Even in the cortical homogenates from the oldest patient, tau 64 and 69 were never detected. Nevertheless, tau 64 and 69 were found in the hippocampus from an 80-year-old control (lane 5). The histological study of this tissue revealed the presence of NFT and SP.

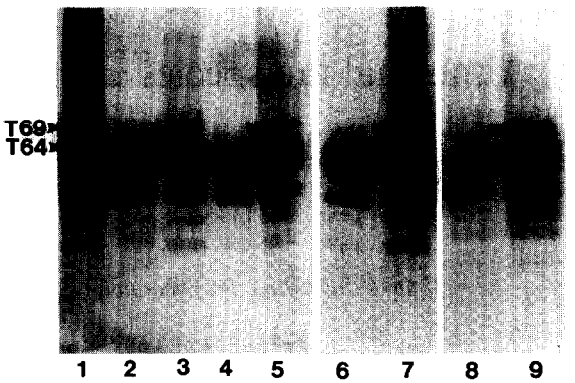

Fig.1. Immunoblot detection of tau proteins with antihuman tau on total brain protein from controls and patients with Alzheimer's disease. 25 μl of SDS homogenates (1:10, w/v) were loaded in each well. Once separated by SDS-PAGE, brain proteins were electrophoretically transferred on nitrocellulose sheets. Then, the anti-tau was added at 1/10000. Lanes: 1, temporal cortex from a 55-year-old Alzheimer patient; 2, temporal cortex from a 53-year-old control; 3, frontal cortex from a 77-year-old Alzheimer patient; 4, caudate nucleus from a 78-year-old Alzheimer patient; 5, hippocampus from an 80-year-old control; 6, frontal cortex from a 62-year-old control; 7, frontal biopsy from a 58-year-old Alzheimer patient (note that there is much more immunoreactive tau 64 and 69 than in the other Alzheimer homogenates); 8, temporal cortex from a 75-year-old Alzheimer patient after a 5 h incubation with the calf intestine alkaline phosphatase (100 U/ml at 37°C). Tau 64 and 69 disappear and the tau profile becomes similar to a control; 9, the same sample prior to dephosphorylation. Tau 64 and 69 have a high molecular mass which is due to their abnormal phosphorylation. They are only detected in brain areas showing NFT and SP and are therefore reliable markers of the neurofibrillary degeneration of the Alzheimer type.

3.2. *Effect of phosphatase treatment*

In order to determine if the slow electrophoretic mobility of tau 64 and 69 was due to a modification of their molecular mass after an abnormal phosphorylation, we have dephosphorylated a cortical brain extract from an Alzheimer patient. After 5 h of incubation with the alkaline phosphatase at 37°C, the two pathological variants disappeared (lane 8). As a control, the same sample was incubated during 5 h with the same amount of phosphatase inactivated by heating (100°C, 15 min): tau 64 and 69 were still detected (lane 8).

4. DISCUSSION

Antisera against PHF and against human tau proteins have already been extensively used for neuropathologic studies at the light and electron microscopic levels [3,14,16] and they are very sensitive tools for the immunohistochemical detection of NFT and SP. Besides, they give identical tau profiles and are therefore suitable for a tau immunodetection.

Our study with these antisera led us to find two abnormal variants of tau proteins that are only detected in brain areas with NFT and SP. They were always present in cortical areas from the 10 Alzheimer patients studied. The staining intensity of tau 64 and 69 was stronger in the regions which are mostly affected during the disease (temporal cortex). The tau 64 and 69 detection was proportional to the smears which probably correspond to the partial dissociation of tau aggregates in PHF [3,5]. Tau 64 and 69 were generally more strongly detected in brain homogenates from Alzheimer patients having an early onset. Their detection was also stronger in a biopsy (mean stage of the disease) than in all the autopsic pieces (ultimate stage of the disease).

In contrast, they were never found in the 10 control brains even if very old, except in the hippocampus of an 80-year-old patient, which contained NFT and SP.

Therefore tau 64 and 69 are reliable markers of the neurofibrillary degeneration of the Alzheimer type.

We analyzed the state of phosphorylation of these proteins, since an abnormal phosphorylation of tau during AD has been suggested [6,9,10]. The incubation of tau proteins from an Alzheimer brain with alkaline phosphatase during 5 h dramatically changed the tau staining pattern: the two pathological variants disappeared. Therefore, since the apparent molecular mass of tau proteins depends upon their phosphorylation state [9,10,15], we conclude that the abnormal molecular mass of tau 64 and 69 is due to a dysfunction in the regulation of tau phosphorylation.

Our results show that during AD there is a pathological process which results in the formation of abnormal tau species easily detectable and quantifiable. Their strong detection in the biopsy is in favour of their early presence during the neurodegeneration process. Moreover, their solubilization with the normal set of tau proteins using mild SDS extractions leads us to think that they are not yet incorporated in PHF which are SDS-insoluble [17]. Thus, the abnormal phosphorylation might be the trigger that precedes and induces their incorporation in the PHF structure. Consequently, their further characterization and the search for experimental conditions able to produce such abnormal tau proteins might be useful to establish an in vitro model of nerve cell degeneration. For example, they might be produced in nerve cell cultures after the addition of aluminium salts [18], or in cells transfected with the Cu,Zn-superoxide dismutase gene [19]. This might be very suitable for the study of the early events that lead to the neurofibrillary degeneration and might help in the search for the primum movens of Alzheimer's disease.

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