Myelin Proteins in Aging Human Brain

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ABSTRACT

Autopsy material of 13 persons who died between 70 and 89 yrs old and of patients who died between 27 and 44 yrs old was studied. White matter of temporal, parietal, and occipital lobes was investigated using histological and biochemical methods. According to results of neuropathological studies, the material of aged patients was divided into two subgroups: (a) brains with vascular changes only and (b) patients with senile atrophy of Alzheimer type. Chemical changes found in all studied brain lobes included a mild decrease in Wolfgram protein content with reciprocal increase in large basic protein content, together with a marked decrease in myelin yield. The abovementioned chemical changes were almost identical whether they were only vascular changes or whether senile atrophy of Alzheimer type was also present. It seems, therefore, that the degeneration of vessels is the decisive factor in the pathogenetic mechanism of myelin lesions in the aged brain.

Index Entries: White matter; vascular change; myelin proteins; Wolfgram protein; basic protein; aging; Alzheimer disease.

INTRODUCTION

Studies of the senile brain have disclosed some morphometric and ultrastructural abnormalities in the white matter as well as deviations in the chemistry of myelin, mainly of its lipids (Horrocks, 1973; Horrocks et

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al., 1979; Wender et al., 1988). Less known is the problem of myelin proteins in aging, including the senile atrophy of Alzheimer type. Therefore, as a continuation of our studies on aged human myelin, we have evaluated the myelin proteins.

The main question to be answered is how far are the myelin proteins primarily or secondarily involved in the aging process, including Alzheimer disease. The latter one is, from a pathological point of view, predominantly the gray matter disease. However, sporadic findings of concomitant involvement of white matter have also been published (England and Brun, 1986).

In line with our previous studies on myelin lipids in the aging brain, we have divided our material into two subgroups according to principal neuropathological changes. In the first group, the vascular degeneration was dominating in brain pathomorphology, whereas in the second group the alterations in the nervous tissue itself—the senile atrophy of Alzheimer type in form of senile plaques and neurofibrillary tangles in neurones—were also noticed.

MATERIALS AND METHODS

The studies were conducted on autopsy material of 13 persons who died at the age of 70–89 yrs (mean 78.8 yrs) with the defined neurological and psychiatric syndrome of stroke and/or dementia. The results were compared with those established for autopsy material of 6 persons who died at the age of 27–44 yrs (mean 34.3 yrs) who suffered from diseases with no clinical and pathological symptoms of involvement of the central nervous system. Time between death and autopsy varied from 7–13 h. Slices of parietal, temporal, and occipital lobes were submitted for histological and biochemical evaluation.

Isolation of Myelin

The source of the myelin fraction was the white matter, excised from the brain according to macroscopic morphological criteria. Homogenization of the nervous tissue was performed at 0–4°C in a glass homogenizer (clearance of 0.18 mm). The myelin fraction was isolated by the method described by Norton and Poduslo (1973). Brain homogenates were layered on a discontinuous sucrose gradient (0.32M and 0.85M, pH 7.0) and centrifuged in a swing-out rotor at 75,000g for 30 min. Subsequently, the crude myelin fraction was washed three times with distilled water, each wash followed by centrifugation at 12,000g for 10 min. The final sediment was lyophilized and stored at –20°C.

Myelin yield was established according to Horrocks (1967). White matter from particular cerebral lobes was weighed and its myelin fraction was isolated as described by Horrocks (1967). The myelin fraction was

freeze dried, and myelin yield was computed. The results were presented as mg of dry myelin/g of wet tissue.

Extraction of Myelin Proteins

Myelin proteins were extracted by means of 0.4% sodium dodecylsulphate (SDS) in 0.01M tris-HCl buffer of pH 7.0 (2 ml/mg of myelin proteins). The protein extract was separated from the insoluble residue by means of centrifugation at 100,000g for 30 min.

Determination of Proteins

Polyacrylamide gel electrophoresis was performed according to Agrawal (1974) in a 12% polyacrylamide gel prepared immediately before use. 0.1 mL of the protein solution, containing 30–35 mg of protein, was layered on top of the gel. Electrophoresis was started at 0.1 mA per gel. As soon as the marker dye penetrated the gel, the current was increased stepwise till 2 mA per gel was reached. The electrophoretic separation was terminated as soon as the marker dye reached the bottom of the gel.

The separated proteins were then stained for 18–24 h by means of Coomassie Brilliant Blue B-250 followed by a thorough rinsing of the gel in 50% methanol alternating with 10% acetic acid, as described by Agrawal (1974) (see Fig. 1).

The obtained gels were stored in 7% acetic acid until they were scanned by means of densitometry at 610 nm, using a microdensitometer (Kipp-Zonen, Holland) with integrating device, and the results were expressed in percentage of total proteins.

Myelin proteins separated in polyacrylamide gels were identified using standard proteins of known molecular mass (Fig. 2). The protein of 55 kDa was identified as Wolfgram protein, of 23 kDa as Folch-Lees proteolipid, of 21 kDa as Agrawal protein (DM-20), of 17 kDa as large component of basic protein, and of 13.8 kDa as a small component of basic protein.

RESULTS

Morphological characteristics of the presented material is summed up in Table 1. The neuropathological data correlated with clinical pattern led to the subdivision of the studied material to the group of patients with vascular changes only and the group with mixed changes (i.e., senile atrophy of the Alzheimer type and generally moderate vascular alterations). The latter alterations comprised in both subgroups arteriosclerotic or amyloid angiopathy and their ischemic sequelae in the brain. The presence of multiple senile plaques and, in more advanced cases, also of neurofibrillary tangles in neurocytes, characteristic for the senile atrophy of Alzheimer type, was documented.

Fig. 1. Picture of protein pattern from densitometer. 1. Small basic protein, 2. Large basic protein, 3. Agrawal protein - DM 20, 4. Folch-Lees proteolipid, 5. Wolfgram protein. (a) control case (parietal lobe) (b) aged brain (parietal lobe) (c) aged brain (occipital lobe).

The white matter was generally well-preserved, the profound demyelination was never seen and only some perivascular pallor as well as small foci of decoloration in myelin staining were visible.

Results found in electrophoretic studies of myelin proteins of particular cerebral lobes are presented in Tables 1-4 and in Fig. 1. The statistically significant changes noticed in all studied brain regions of the aged included a small decrease in Wolfgram protein content, a mild relative elevation in large basic protein content and a marked decrease in myelin

Fig. 2. Estimation of molecular mass of myelin proteins in control material. Log 10 of molecular mass of protein standard presented as R_f function. Every point represent the mean of 10 estimations from myelin of control material: 1. phosphorylase, 2. bovine serum albumin, 3. ovalbumin, 4. carbonic anhydrase, 5. inhibitor of topsoil tripsine, 6. α -lactalbumin, WP = Wolfgram protein, PLP = Folch-Lees proteolipid, AP = Agrawal protein (DM20), LBP = large basic protein, SBP = small basic protein. $\circ$: standard proteins. $\bullet$: myelin proteins of control material.

yield, observed in comparison to the control material. The findings were almost identical in both subgroups of the studied material (cases with vascular and senile changes and cases with vascular changes only).

DISCUSSION

In spite of extensive studies, search for biochemical substrates of dementia in senility remains distant from definite conclusions. In Alzheimer dementia, deficient function of cholinergic neurons and its receptor, especially in the basal forebrain, seems to be of great importance. In some patients, other transmitter systems, mainly the serotonergic and noradrenergic neurons, are severely damaged (Rossor and Iversen, 1986; Mann and Yates, 1986).

Most of the proteins involved in pathological process in Alzheimer disease are insoluble (Buee et al., 1989), including Tau proteins (each variant Tau 64 and 69) characteristic for neuronal neurofibrillary tangles and amyloid, deposited mainly in senile plaques and in cerebral vessels. Wiśniewski (1990) emphasizes, in summary of these studies, that a subset of brain reticulo-endothelial cells (i.e., the microglia, pericytes, macrophages, and fibroblast-like cells) are defective in Alzheimer disease

Table 1
Neuropathological Characteristics of the Studied Material

Age	Sex	Time between death and autopsy	Brain wt. g	Diagnosis
<u>Group A</u>				
<u>Cases with arteriosclerosis^a and parenchymatous senile changes^b</u>				
70	M	7	1306	Cerebral arteriosclerosis. Necrosis in basal ganglia. Moderate senile atrophy.
74	F	8	1303	Moderate cerebral arteriosclerosis. Senile atrophy of severe intensity.
78	F	9	1265	Cerebral arteriosclerosis. Senile atrophy of severe intensity.
78	F	12	1192	Cerebral arteriosclerosis. Moderate subarachnoidal hemorrhage. Senile atrophy.
79	F	12	1107	Cerebral arteriosclerosis. Necrosis in the left parietal lobe (size 2 × 3 cm). Senile atrophy.
84	F	10	1206	Cerebral arteriosclerosis. Senile atrophy of severe intensity.
84	F	13	1152	Moderate cerebral arteriosclerosis. Senile atrophy of severe intensity.
<u>Group B</u>				
<u>Cases with arteriosclerosis only</u>				
74	F	10	1104	Cerebral arteriosclerosis. Status lacunaris.
74	M	11	1272	Cerebral arteriosclerosis. Status lacunaris.
77	F	8	1075	Cerebral arteriosclerosis. Necrosis in basal ganglia (size 0.5 × 0.7 cm). Status lacunaris.
78	F	9	1190	Cerebral arteriosclerosis. Necrosis in the right parietal lobe (size 3 × 4 cm).
85	M	10	1092	Cerebral arteriosclerosis. Status lacunaris. Subarachnoidal hemorrhage.
89	M	7	1185	Cerebral arteriosclerosis. Status lacunaris.

^aThe term cerebral arteriosclerosis comprises degenerative vascular changes and its ischemic sequelae in the brain.

^bDiagnosis of senile atrophy is based on the presence of senile plaques and neuron fibrillary tangles.

in such a way that the amyloid precursor protein (APP) is abnormally cleaved to generate the beta-peptide that polymerizes into amyloid fibers. Tau protein and amyloid are not deposited in the myelin sheath and not represented in the myelin fraction.

Deviation in contents of chemical compounds and in their metabolism in structural components of nervous tissue in the aging brain, including cases of Alzheimer senile atrophy, are less known. According to

Table 2
Pattern of Myelin Proteins of the Cerebral White Matter
from the Temporal Lobe of Aged Patients^a

	Control cases <i>n</i> = 6	Total material of aged brain <i>n</i> = 13	Cases with vascular and senile changes <i>n</i> = 7	Cases with vascular changes only <i>n</i> = 6
Small basic protein (SBP)	17.8 ± 0.9	17.6 ± 1.6	18.0 ± 1.7	16.5 ± 3.5
Large basic protein (LBP)	13.3 ± 2.5	16.2* ± 1.6	15.9 ± 1.7	18.0 ± 0.4
Agrawal protein (AP) – DM 20	12.4 ± 1.4	11.5 ± 0.7	10.8 ± 1.0	12.3 ± 1.3
Folch-Lees proteolipid (PLP)	31.0 ± 1.8	34.8 ± 2.0	36.6 ± 2.6	30.9 ± 4.1
Wolfgram protein	25.5 ± 1.3	19.9* ± 1.3	18.7 ± 1.5	22.4 ± 1.1
Yield of myelin (in mg dry myelin per gram of wet tissue)	84.7 ± 1.2	67.1* ± 1.4	67.8* ± 2.0	66.2* ± 2.1

^aResults in percent of total protein. Mean ± SEM. Statistical evaluation was done with Student's *t*-test.

*Differs from control at *p* ≤ 0.05.

Table 3
Pattern of Myelin Proteins of the Cerebral White Matter
from the Parietal Lobe of Aged Patients^a

	Control cases <i>n</i> = 6	Total material of aged brain <i>n</i> = 13	Cases with vascular and senile changes <i>n</i> = 7	Cases with vascular changes only <i>n</i> = 6
Small basic protein (SBP)	16.5 ± 1.9	16.0 ± 1.9	16.0 ± 1.7	15.8 ± 1.9
Large basic protein (LBP)	13.3 ± 2.2	15.8* ± 2.2	16.3 ± 3.7	15.6 ± 3.0
Agrawal protein (AP) – DM 20	12.0 ± 0.8	11.5 ± 0.8	11.5 ± 0.5	11.7 ± 1.3
Folch-Lees proteolipid (PLP)	35.0 ± 2.3	37.0 ± 2.3	36.3 ± 6.1	37.3 ± 1.7
Wolfgram protein	23.2 ± 1.3	19.7 ± 1.3	19.9 ± 6.1	19.5 ± 1.1
Yield of myelin (in mg dry myelin per gram of wet tissue)	83.7 ± 1.2	67.0 ± 1.3	67.8 ± 1.9	66.1 ± 2.0

^aResults in percent of total protein. Mean ± SEM. Statistical evaluation was done with Student's *t*-test.

*Differs from control at *p* ≤ 0.05.

Table 4
Pattern of Myelin Proteins of the Cerebral White Matter
from the Occipital Lobe of Aged Patients^a

	Control cases <i>n</i> = 6	Total material of aged brain <i>n</i> = 13	Cases with vascular and senile changes <i>n</i> = 7	Cases with vascular changes only <i>n</i> = 6
Small basic protein (SBP)	18.6 ± 1.8	14.7 ± 1.4	15.8 ± 2.2	13.7 ± 1.5
Large basic protein (LBP)	15.6 ± 1.6	16.6 ± 1.4	16.5 ± 1.3	16.7 ± 3.5
Agrawal protein (AP) – DM 20	9.6 ± 1.1	11.7 ± 0.7	10.1 ± 0.5	13.7 ± 1.1
Folch-Lees proteolipid (PLP)	33.1 ± 1.8	38.1 ± 1.3	36.5 ± 0.8	40.1 ± 2.7
Wolfgram protein	23.1 ± 2.1	18.9* ± 1.1	21.4 ± 1.2	15.8 ± 1.4*
Yield of myelin (in mg dry myelin per gram of wet tissue)	85.0 ± 1.1	67.3* ± 1.6	67.8* ± 2.1	66.7* ± 2.4

^aResults in percent of total protein. Mean ± SEM. Statistical evaluation was done with Student's *t*-test.

*Differs from control at *p* ≤ 0.05.

Horrocks (1978), the abnormalities in plasmalogen content, in plasmalogenase activity, and changes in polyphosphoinositides of the gray matter may be at least partially responsible for the abovementioned impaired function of neurotransmitter receptors in Alzheimer dementia.

The problem of myelin lipids in aging human brain was thoroughly discussed in our previous paper (Wender et al., 1988). We have concluded that the marked decrease in myelin yield, as well as some minor changes in pattern of myelin lipids, seem to be signs of aging that are not connected directly with atrophy of the Alzheimer type.

In our material, the aging human brain has been shown to exhibit small changes in myelin proteins and only a mild decrease in Wolfgram protein content. The increase in large basic protein content observed in some cerebral lobes seems negligible, resulting only from a concomitant relative shift, with no greater significance for the pathogenetic mechanism of aging process.

In their monographic contributions to the field of myelin protein analysis, Lees and Brostoff (1984) as well as Norton and Cammer (1984) have discussed the problem concerning some discrepancies in the term of Wolfgram protein. The designation of Wolfgram fraction is generally used to describe a mixture of high mol wt components, whereas the term referred to as the Wolfgram protein is a doublet or triplet band observed on SDS gels at a position corresponding to a mol wt of 45–55 kDa. We

have used the designation Wolfgram protein in the latter meaning. According to studies of Drummond and Dean (1980) as well as those of Sprinkle et al. (1980) the so understood Wolfgram protein is characterized by the 2'3'-cyclic nucleotide 3'phosphohydrolase (CNP) activity or is even identical with this specific myelin protein.

In our studies we have noticed rather high values of Wolfgram protein and low content of proteolipid. In this respect, Lees and Brostoff (1984) noted that the resolution of different gel systems differs significantly and, consequently, differences result in the studies conducted by various investigators. However, it is almost generally accepted that proteolipid and myelin basic protein contribute in 60 to 80% in total myelin proteins. In our studies this contribution has amounted to almost 65%.

The subsequent question that arises is whether the decrease of Wolfgram protein, established in aging brain, represents a proof of true alteration in the molecular composition of myelin or rather is a result of relative changes due to selective vulnerability to postmortem autolysis. Some arguments speak in favor of the first hypothesis. First of all, the time between death and removal of the brain from the body has been similar in the control group and in the aged individuals and, then, the results of chemical studies have not shown any correlation with this time.

Great similarity was noted between the two subgroups of our material, and the aged people with vascular changes only have lost even more Wolfgram protein than those with vascular and senile changes. These latter findings are only apparently paradoxical ones. First of all, it should be added that in cases with Alzheimer disease the vascular changes have been generally milder than in arteriosclerotic brains. Almost identical similarity has been noticed in our lipid studies of aging brains, which has led us to the conclusion that the degeneration of vessels is one of the decisive factors in the pathogenetic mechanism of myelin lesion in the aged brain. It seems, therefore, that the degenerative process of the nervous tissue itself, known under the term of senile atrophy of Alzheimer type, contributes only insignificantly to the chemical changes in structural components of the myelin.

Comparing the changes associated with aging in the two main components of myelin bilayer (i.e., in proteins and lipids) it should be stressed that the lipid fraction is more abnormal, demonstrating an increase in lysophosphatidylcholine content and a marked decrease in myelin yield. In contrast to this, the myelin proteins seem to be more stable in the course of aging.

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