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X-RAY DIFFRACTION EVIDENCE FOR MYELIN DISORDER IN BRAIN FROM HUMANS WITH ALZHEIMER'S DISEASE

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Wide-angle X-ray diffraction studies revealed that the lipid phase transition temperature of myelin from brain tissue of humans with Alzheimer's disease was about 12°C lower than that of normal age-matched controls, indicating differences in the physical organization of the myelin lipid bilayer. Elevated levels of malondialdehyde and conjugated diene were found in brain tissue from humans with Alzheimer's disease, indicating an increased amount of lipid peroxidation over the controls. An increase in myelin disorder and in lipid peroxidation can both be correlated with aging in human brain, but the changes in myelin from humans with Alzheimer's disease are more pronounced than in normal aging. These changes might represent severe or accelerated aging.

Introduction

Senile dementia of the Alzheimer type (Alzheimer's disease) is a progressive cerebral degeneration that continues without remission until death [1]. Morphologically, the disease is characterized by large numbers of senile plaques and neurofibrillary tangles in the brain [1–3]. Although Alzheimer's disease is the most common organic cause of intellectual and cognitive deterioration [3,4], there is still considerable uncertainty about its pathogenesis. An increasingly complete picture of Alzheimer's disease, involving psychological, genetic, physiological, structural and chemical aspects is beginning to emerge [1,3,5–8], but there is still debate about the extent and selectivity of neuronal loss. Central to our understanding of the disease is the resolution of the controversy that the disease is an accelerated form of normal aging [5,8–10].

The pathological information concerning normal aging and senile dementia has been difficult to interpret. Neuritic plaques and neurofibrillary tangles do occur during normal aging [3], and this has led to confusion concerning the difference between senile dementia and normal aging. There are biochemical differences, however, between the brains of normal aged persons and those with Alzheimer's disease, with specific losses of choline acetyltransferase and somatostatin from the cortex and hippocampus in patients with Alzheimer's disease [3,11–14].

If Alzheimer's disease represents an accelerated form of aging in the brain, age-related changes should be found in most brain structures. At present, little is known about the differences in structure of myelin from normal aged brain and that from brain tissue of humans with Alzheimer's disease. In Alzheimer's disease, there is some breakdown of myelin, although it is not a major feature and may reflect degeneration of tracts [10]. In the present study, myelin preparations from brain tissue of three humans with Alzheimer's

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disease and from normal brain tissue of three age-matched controls were examined by wide-angle X-ray diffraction to determine if there were differences in the physical structure of myelin. This study also provides evidence that increased lipid peroxidation is found in this disease.

Methods and Materials

Myelin isolation

The brain of three patients who died from Alzheimer's disease and of three normal age-matched control humans who died accidentally or from diseases not affecting the nervous system were obtained within 4–8 h of death. Each control brain was examined by a neuropathologist to rule out other central nervous system pathology. A full neuropathological examination was conducted on each of the Alzheimer's diseased brains to confirm the clinical diagnoses. The cortex was removed and the white matter was separated from the grey matter. Myelin was prepared as described earlier [15].

X-ray diffraction of myelin

Myelin samples for X-ray diffraction studies were prepared as described previously [15]. A portion of the packed myelin pellet was mounted between two jaws of a brass holder. The holder was kept in an atmosphere of nitrogen which had been bubbled through distilled water, until the myelin had formed a thin ribbon spanning the jaws. This treatment removes water from between and around the membranes, allowing them to come together to form an ordered array, but it does not dehydrate them [16]. The holder with prepared specimen was then placed in a chamber on a wide-angle camera (Philips, type 1030), and diffraction patterns were recorded for 4 h on film at various temperatures using CuKα radiation from a point-focus X-ray tube (type PW 2103/01). To ensure that the membranes remained hydrated, air, which had been bubbled through distilled water, was continuously passed through the chamber while the diffraction patterns were being recorded.

The lipid phase transition temperature, defined as the highest temperature at which gel phase lipid can be detected, was determined to within 1 deg.C.

Determination of lipid peroxidation products

Malondialdehyde. Levels of malondialdehyde, a breakdown product of unsaturated fatty acid hydroperoxides, were measured in homogenates of white matter using a modified thiobarbituric acid test [17]. Malondialdehyde levels were calculated relative to a standard preparation from the hydrolysis of 1,1,3,3-tetramethoxypropane [18].

Conjugated diene. Conjugated dienes, which are formed during the peroxidation of unsaturated fatty acids, exhibit spectra characterized by an intense absorption at 233 nm and were estimated by the method of Buege and Aust [19].

Results

X-ray diffraction

The changes in the lipid phase properties of myelin in Alzheimer's disease tissue were examined by X-ray diffraction. Diffraction patterns were recorded at 46 °C, which is below the transition temperature of normal adult myelin [15]. However, myelin isolated from the white matter from diseased brain was exclusively liquid-crystalline (dis-

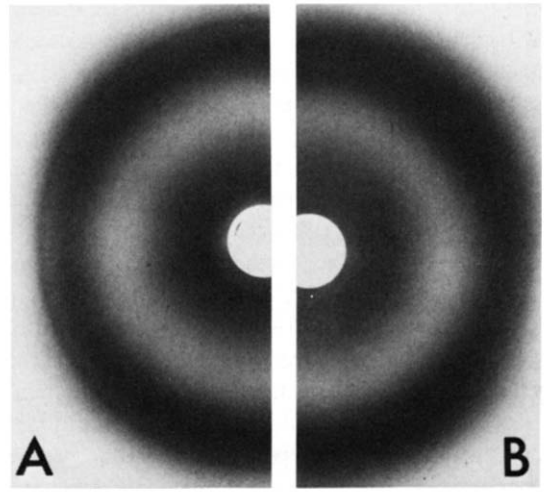

Fig. 1. Wide-angle X-ray diffraction patterns for isolated myelin from human brain. Diffraction patterns were recorded at 46 °C. (A) Pattern from age-matched control myelin featuring (from outside to inside) a sharp band centered at a Bragg spacing of 4.15 Å and two broad diffuse bands centered at Bragg spacings of 4.6 and about 10 Å. (B) Pattern for myelin isolated from white matter of Alzheimer's disease brain featuring (from outside to inside) two broad bands centered at Bragg spacings of 4.6 and 10 Å.

ordered). This was apparent from the fact that the only lipid reflection detectable in diffraction patterns recorded from this myelin was a broad diffuse band centered at a Bragg spacing of 4.6 Å (Fig. 1B), which is known to be derived from the irregular spacings between the fatty-acid side-chains of phospholipid in the liquid-crystalline state [20,21]. The corresponding pattern for normal myelin at the same temperature featured a sharp reflection at a Bragg spacing of 4.15 Å representing gel-phase (ordered) lipid, as well as the diffuse 4.6 Å reflection derived from liquid-crystalline lipid (Fig. 1A). The sharp 4.15 Å reflection in wide-angle diffraction patterns has been well characterized as an indication of a close hexagonal packing of long-chain paraffins [22]. Its appearance in wide-angle patterns from membranes also reflects an ordered crystalline phase of the lipid in which there is a close hexagonal packing of the hydrocarbon chains [23–26]. These observations indicate that control myelin contains a mixture of lipid phases at this temperature verifying results of a previous study [15].

To confirm that detection of the gel phase in adult myelin simply reflected lipid gel phase and was not attributable to dehydration, specimens used for diffraction studies were analyzed gravimetrically to determine their water content. Myelin containing 75–80% moisture with respect to final dry weight still gave rise to the diffraction patterns featuring the sharp reflection at 4.15 Å.

The presence of gel-phase lipid can also be detected by measuring the transition temperature, which is operationally defined as the highest temperature at which gel-phase lipid can be discerned and reflects the composition of phospholipids, in particular fatty acids, contributing to the gel phase [15]. Thus, above the transition temperature, membrane lipid is exclusively liquid-crystalline, whereas below the transition temperature the membranes contain a mixture of liquid-crystalline and gel-phase lipid. The intensity of the 4.15 Å reflection in wide-angle diffraction patterns serves as a relative measure of the amount of gel phase lipid in the membranes. If the temperature is progressively reduced below the transition temperature, the proportion of lipid in the gel phase increases. In all of these experiments, the myelin remained hydrated and the transition temperatures for both Alzheimer's diseased myelin and normal myelin were thermally reversible. The mean transition temperature for control, age-matched myelin was 55 °C, whereas for Alzheimer's diseased myelin it was 37 °C (Table I).

TABLE I

COMPARISON OF THE LIQUID-CRYSTALLINE TO GEL-PHASE TRANSITION TEMPERATURES OF MYELIN ISOLATED FROM NORMAL AGE-MATCHED BRAINS AND ALZHEIMER'S DISEASE BRAIN

Means and standard errors of the means for separate experiments are shown in parentheses; $n = 3$.

	Age (years)	Transition temperature (°C)
Alzheimer's disease	74	38
	66	45
	67	32
		(38.3 ± 3.7)
Age-matched controls	74	50
	64	55
	66	52
		(52.3 ± 1.5)

Malondialdehyde and conjugated diene

The level of lipid peroxidation was higher in the white matter from the Alzheimer's diseased brains than in white matter of normal brains. The extent

TABLE II

COMPARISON OF THE LEVELS OF MALONDIALDEHYDE AND CONJUGATED DIENE IN WHITE MATTER HOMOGENATES OF ALZHEIMER'S DISEASE WHITE MATTER AND AGE-MATCHED CONTROL WHITE MATTER

Means and standard errors of the means for separate experiments are shown; $n = 3$.

	Age (years)	Lipid peroxidation products	
		Malondialdehyde (nmol/mg dry wt.)	Conjugated diene (absorption/mg dry wt.)
Alzheimer's disease	74	0.26 ± 0.03	0.49 ± 0.04
	67	0.24 ± 0.02	0.48 ± 0.01
	66	0.24 ± 0.02	0.45 ± 0.04
Age-matched controls	74	0.13 ± 0.01	0.33 ± 0.01
	64	0.13 ± 0.02	0.31 ± 0.01
	66	0.18 ± 0.01	0.32 ± 0.01

of lipid peroxidation was determined by measuring the accumulation of conjugated diene, a product of lipid peroxidation, in the homogenates of brain white matter. The level of conjugated diene from the white matter of Alzheimer's diseased brain was 1.5-times higher than in white matter of control material (Table II). The level of malondialdehyde, another product of lipid peroxidation, was also higher (2-times) in homogenates of white matter from Alzheimer's diseased brain than in corresponding homogenates from control brains (Table II).

Discussion

Wide-angle X-ray diffraction has provided evidence for significant differences in the physical organization of the lipid bilayer in Alzheimer's disease myelin compared to normal myelin isolated from patients of similar ages. The absence of gel-phase lipid in wide-angle X-ray diffraction patterns of Alzheimer's disease myelin at 46 °C and its presence in corresponding patterns for normal myelin indicate that the lipid bilayer of the diseased myelin was more disordered than that in normal myelin.

The increase in disorder in the myelin lipid bilayer from the Alzheimer's disease brain was associated with an increase in lipid peroxidation in the brain white matter in comparison with control brains of similar age. The degree of lipid peroxidation was reflected in the levels of malondialdehyde which were 2-fold higher in Alzheimer's disease white matter homogenates than in controls. Increased levels of conjugated diene, another product of lipid peroxidation, substantiated further the conclusion that lipid peroxidation was more extensive in Alzheimer's disease.

We have recently demonstrated that lipid peroxidation is intimately involved in the process of normal aging of human myelin, and that there is also a progressive decline with age in the lipid phase transition temperature of myelin reflecting decreased stability of the bilayer [15]. This age-related decrease in transition temperature of normal myelin was also evident in the present study. However, superimposed on the age-related trend was a very much larger decrease in transition temperature of myelin from Alzheimer's diseased brain tissue. Indeed, in one of the three age-matched samples, the transition temperature for myelin from diseased brain was lower than the corresponding control by 18 °C.

The higher levels of malondialdehyde and conjugated dienes in white matter from diseased brain suggest that free-radical-mediated lipid peroxidation may lead to increased myelin disorder. That lipid peroxidation can decrease markedly the transition temperature of isolated myelin was demonstrated in a recent study in which potassium superoxide was used to generate free radicals [27]. The accumulation of free radicals in myelin represents a mechanism by which lipid peroxides can be formed.

One characteristic of Alzheimer's disease is an altered capillary permeability that possibly restricts local access of water and metabolites to the brain or prevents the removal of metabolic waste products with potential cytotoxic effects [28,29]. Although the relationship between Alzheimer's disease and normal aging is not clearly understood at present, a restricted circulation that enhances the accumulation of cytotoxic products could promote the production of free radicals and thus initiate lipid peroxidation.

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