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SPIN LABEL STUDIES ON SYNAPTOSOMAL MEMBRANES OF RAT BRAIN CORTEX DURING AGING

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Summary: Membrane lipids of brain synaptosomes of 2, 12 and 24 months old rats were labeled by stearic acid spin probes with a doxyl group in the C-5 and C-16 position, respectively. We demonstrated that the hydrophobic region of synaptosomal membranes became more rigid during the life span studied, and the region located nearer the surface of membranes displays a significantly decreased fluidity only in the oldest group. Membrane proteins labeled with a nitroxide derivative of maleimide suggest that conformational changes take place during aging.

Functionally one of the most important parameters of biological membranes is the fluidity of the hydrophobic lipid regions. This region plays a regulatory role in a series of biochemical events catalyzed by membrane-bound enzymes. An age-dependent increase in membrane microviscosity of synaptosomes from rat brain cortex has been demonstrated by fluorescence anisotropy measurements (1). These results suggested a need for further studies on the physico-chemical state of the synaptosomal membranes during aging. The present paper describes our results obtained with spin labels of lipids and proteins of synaptosomal membranes.

MATERIALS AND METHODS

N-oxyl-4',4'-dimethyloxazolidine derivatives of stearic acid with the doxyl groups in C-5 and C-16 positions (5NS and 16NS, respectively) were purchased from Syva Co.U.S.A. The protein label, N-(1-oxyl-2,2,6,6,-tetramethyl-4-piperidinyl)-maleimide (MSL) was prepared by Prof.Dr.K.Hideg, Pécs, Hungary.

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Synaptosomes were isolated from the brain cortex of female CFY rats of 2, 12 and 24 months of age, designated as young, adult and old groups, respectively. The brain cortex of 2 rats was pooled together in each experiment. The method of synaptosome preparation was that of Gray and Whittaker (2) as applied by Whittaker and Barker (3). Protein content of the synaptosomal fractions was determined by a modified folin-reaction (4). Ultrastructural studies were performed for checking the purity of the synaptosomal fractions (1).

Incorporation of the labels was carried out as follows: the necessary amount of label was dissolved in ethanol, and the solvent was evaporated from the test tube in vacuum. Known amounts of synaptosomes were added in aqueous suspensions to the tubes, stirred for 2 min rigorously, then kept at 4°C overnight. The necessary label concentration was selected during preliminary experiments carried out in 5 steps between 1 and 16 µg label per mg of synaptosomal protein. The best label to protein ratio was 8 µg/mg. Similar concentrations of label were used also by others (5, 6) for the lipid probes. For the MSL label others have used somewhat lower concentrations (6), nevertheless, in order to remove the excessive label, we washed the synaptosomes with phosphate-buffered saline (PBS, pH 7.4). Since the 3rd washing medium contained insignificant amounts of the label (Fig.3), the measurement was carried out routinely in the 4th dilution of PBS.

ESR spectra of 30 µl lipid probes were recorded at 37.0 $\pm$0.5°C, whereas those of the MSL-labeled fractions at 22.0$\pm$0.5°C by means of a JEOL spectrometer type JES FE 3X.

The polarity-corrected order parameter S was calculated from the spectra according to Hubbell and McConnell (7) for the 5NS-labeled samples. For the 16NS labeled ones, we used the motion parameter τ_0, corresponding to the rotational correlation time, τ_C, as calculated and explained by Eletr and Inesi (8). For evaluation of the results obtained with MSL-label, we used the ratio of the spectral amplitudes of weakly (w) and strongly (s) immobilized label molecules bound to the SH-groups of proteins as described by Butterfield et al. (9). It should be noted that although this method of evaluation was criticized (10), claiming that the w fraction is the free label in solution displaying a line broadening due only to the viscosity of the medium, the fact that the third washing medium did not contain significant amount of free label clearly indicates the validity of this kind of spectral evaluation for membrane preparations. The parameter w/s has been used by other authors in recent years (11-13) for evaluation of ESR spectra of maleimide-derivatives incorporated into biological membranes.

RESULTS AND DISCUSSION

ESR spectra of spin labeled synaptosomal membranes are shown in Figs.1-3 for various age groups.

The results calculated from the respective spectra are summarized in Table 1. It is remarkable that although the experiments were carried out on samples prepared each time in-

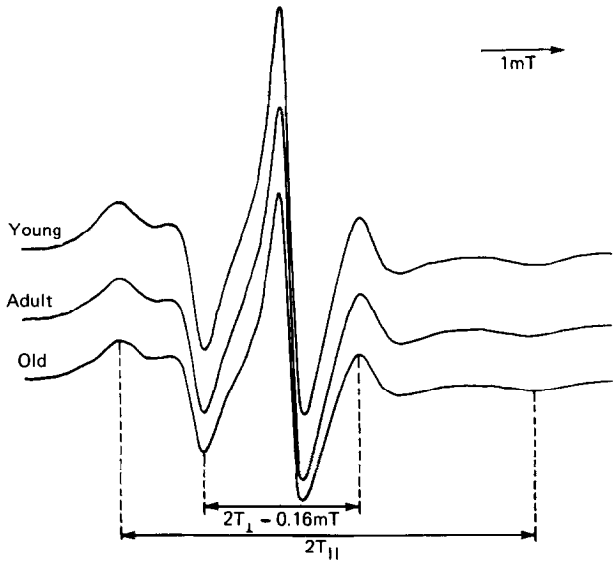

Figure 1. Typical examples of the ESR spectra of 5NS probe in young, adult and old samples of synaptosomes. Instrumental parameters were: 9.127 GHz microwave frequency, 0.2 mT modulation amplitude at 100 kHz modulation frequency, response time 0.3 sec, sweep 10 mT/8 min, microwave power 5 mW, amplitude 1x1000. The arrow in the upper right corner indicates the scale and direction of low to high magnetic field. Two-ended arrows at the bottom show the localization of the measured values in the spectra.

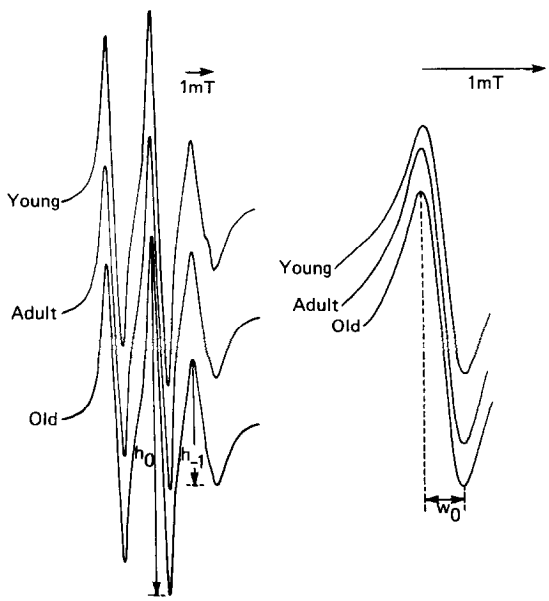

Figure 2. ESR spectra of the 16NS probe. Instrumental parameters were the same as indicated at Fig.1 except that W_0 was calculated from the central line of spectra recorded at an expanded magnetic field sweep (±2.5 mT), in order to assure more precise reading, as shown in the right side of the figure.

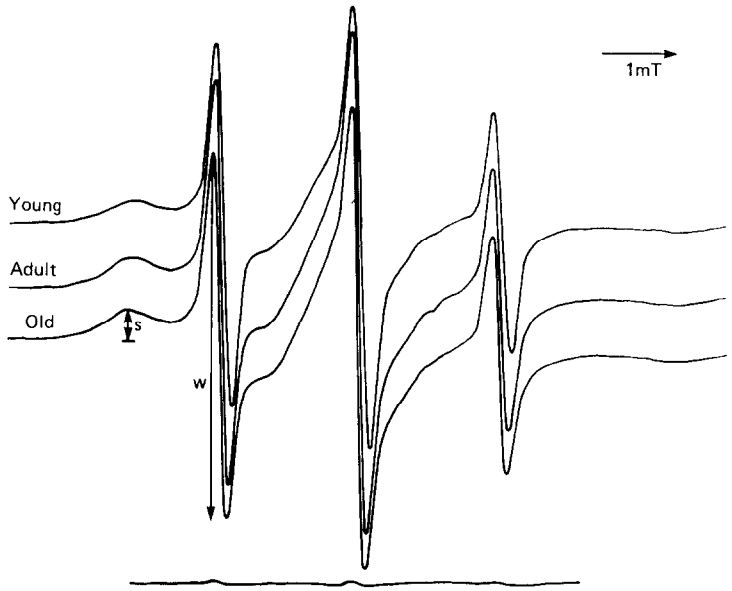

Figure 3. ESR spectra of the MSL label. Instrumental parameters were the same as indicated for Fig.1. The lowest curve corresponds to the 3rd washing medium.

dividually, the results regarding the lipid labels fell in a rather narrow range for each age group. With the MSL label we noticed a slight change with incubation time. Therefore, we ran parallel young, adult and old samples each time with identical incubation times, and expressed the adult and old values as a percentage of the young one. For technical reasons, we could not always keep the same incubation time in different experiments, therefore, the relative values are more reliable statistically.

The results demonstrate that all three parameters displayed age dependence. Only the 5NS label showed an insignificant difference between young and adult rats, although there was an increasing tendency. One can conclude from these data that the lipid layer of the synaptosomal membranes became more rigid during aging, however, this is expressed more in the more hydrophobic region than nearer to the hydrophilic face of the membrane.

Table 1.

Order parameter (S), motion parameter (τ_0) and w/s ratio in rat brain synaptosomal membranes of 2, 12 and 24 months of age (mean ± S.D.)

Age group	n	S	n	τ_0 (ns)	n	w/s (%)
YOUNG	6	0.5537 ±0.0031	10	1.210 ±0.025	9	100.00 ± 3.70
p (young-adult)	<	N.S.		0.01		0.01
ADULT	7	0.5563 ±0.0025	16	1.261 ±0.019	9	88.51 ± 3.29
p (adult-old)	<	0.01		0.01		0.01
OLD	7	0.5636 ±0.0038	21	1.303 ±0.017	9	79.12 ± 4.82
p (young-old)	<	0.01		0.01		0.01

Note: n indicates the number of measurements; p indicates the significance level as calculated using Student's "t" test. The absolute value of the young w/s ratio was an average of 17.

The state of membrane protein SH-groups is a sensitive reflection of protein conformation (9, 14, 15).Since the MSL label is bound to SH-groups yielding the w and s fractions of the bound label, the decrease in the w/s ratio resulting from both an increase of s and a decrease of w, can be interpreted as a sign of conformational changes of the proteins in the synaptosomal membranes.

The findings are consistent with the membrane hypothesis of aging (16, 17). This hypothesis predicts that free radical damage of the membrane components may result in an increased cross-linking of components, and this is the basis for a decreased ion and water permeability of the nerve cell membrane in old animals. As a consequence, the cells accumulate potassium and loose water to the intracellular space. This is the physicochemical basis for an increased condensation of the

chromatin and a reduced rate of RNA synthesis (18). Various experimental approaches support this assumption (1, 19, 20-22). Both the decreased fluidity of the lipid layer and the altered conformation of the membrane proteins in the old rats may be a manifestation of free-readical induced damage to the membrane, resulting in serious impairment of membrane functions.

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