Monoamine Oxidase Activity, Lipid Peroxidation, and Morphological Changes in Human Hypothalamus during Aging

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 131, No. 6, pp. 694-696, June, 2001
Original article submitted February 5, 2001

Progressive gliosis of the hypothalamus during aging in humans is accompanied by activation of monoamine oxidase B activity, intensification of lipid peroxidation, and inhibition of SDH. Density of capillaries decreases. The role of monoamine oxidase B in the induction of lipid peroxidation is discussed.

Key Words: *monoamine oxidase; lipid peroxidation; gliosis; capillary; hypothalamus*

Many aspects of aging, in particular, aging of the brain are still open to discussion [2]. Morphobiochemical parallels of involutive processes in the hypothalamus, the structure determining aging of the whole organism, are of considerable interest. It was hypothesized that age-related disturbances of the homeostasis are associated with changes in threshold sensitivity of the hypothalamus to regulatory factors resulting from activation of monoamine oxidase (MAO) and intensification of lipid peroxidation (LPO) [7]. Here we studied the relationship between morphological reconstructions in the neuron-glia-capillary system and ontogenetic changes in oxidative and reduction enzymes and LPO.

MATERIALS AND METHODS

We performed postmortem examination of 72 hypothalamic specimens from humans (21-87 years), whose death was not directly related to CNS and cardiovascular pathologies. The samples were taken within 12 h after death. There were 4 age groups: 21-35-year-old women and 22-35-year-old men (middle age 1), 36-55-year-old women and 36-60-year-old men (middle age 2), 56-74-year-old women and 61-74-year-old men (elderly), and 75 years or above (senescent).

Deparaffinized slices (10 μ) were fixed in 10% neutral formalin and stained with hematoxylin and eosin by the Nissl's method. Alkaline phosphatase activity in the endothelium of capillaries was measured in nonfixed slices by the method of Berston. Succinate dehydrogenase (SDH) activity in the nervous tissue was estimated by the method of Nachlas. The number of nerve and glial cells and glial index were determined as described previously [1]. Standard set of morphometric parameters of the capillary bed was evaluated [10]. SDH activity was measured by direct photometry at 515 nm and expressed in arbitrary optical density units.

MAO-B activity was determined spectrophotometrically by the rate of semicarbazones formation [3,4].

The content of lipid peroxides (LP) was estimated by spectrophotometry with separate measurements of LPO product content in heptane and isopropanol phases of the lipid extract [3,4]. LPO products reacting with 2-thiobarbituric acid (TBA) were determined colorimetrically. To evaluate the resistance of lipids to free radical oxidation, we studied accumulation of TBA-reactive substances (TBARS) in 2.0-2.5% brain homogenates incubated *in vitro* on air at 37°C for 1 h. *In vitro* LPO intensity (oxidation index) was evaluated by accumulation of TBARS and expressed in percents [3].

The results were analyzed by Statistica 5.0 for Windows software. Significance of differences was evaluated using Student's *t* test for independent samples. Correlation analysis was performed.

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0007-4888/01/1316-0586$25.00

RESULTS

During aging, the count of hypothalamic neurons decreased (per volume unit of nervous tissue), which was accompanied by substitutive gliosis and an increase in the glial index (Table 1).

The age-related proliferation of glial cells is probably responsible for the increase in hypothalamic MAO-B activity. Enzyme activity in senescent individuals (75 years and elder) 2.5-fold surpassed that in middle-age individuals. Previous studies showed that glial cells possess the highest MAO-B activity compared to other CNS structures [8]. The coefficient of correlation between these parameters attested to their functional relationship (r=0.99, p<0.05).

The dynamics of LPO and MAO activity in human hypothalamus during ontogeny were similar. The contents of heptane-extracted LP and primary or end isopropanol-soluble LPO products were maximum in senescent individuals and 2.0-5.8-fold surpassed that in middle-age 1 group. This agrees with previous data on the important role of MAO-dependent generation of free radicals in involution of the nervous tissue [8]. H_2O_2, a product of MAO-catalyzed reaction irrespective on the nature of oxidized substrates, is a potent inductor of LPO [3,6]. It should be emphasized that the age-related activation of MAO was accompanied by an increase in the sensitivity of hypothalamic lipids to *in vitro* free radical attack. The oxidation index in elderly and senescent individuals far surpassed that in the middle-age 1 group. The increase in hypothalamic lipid sensitivity to LPO-inducing factors probably contributes to LP accumulation in this brain structure in elderly and senescent individuals.

Correlation analysis revealed a direct relationship between the age and hypothalamic LP content (r=0.5-0.84, p<0.05). The only exception was isopropanol-soluble secondary LPO products, whose content considerable decreased during aging and dropped to a minimum in people aging 75 years or more. TBARS content underwent similar changes, but was minimum in elderly people. This can be explained by intensive lipofuscin formation accompanied by consumption of carbonyl LP. Secondary LPO products (carbonyls) interact with amino groups of phospholipid nitrous bases and lysin ε-amino groups with the formation of lipofuscin [9]. Lipofuscin is a derivative of Schiff bases (end LPO products), whose content in human hypothalamus significantly increases during aging (Table 1).

TABLE 1. Age-Related Changes in LPO Intensity, MAO Activity, and Qualitative Parameters of the Neuron—Glia—Capillary System in Human Brain ($M\pm m$)

Parameters	Age groups			
	middle age 1	middle age 2	elderly	senescent
LPO product content, oxidation index				
H1	0.350±0.037	0.420±0.049	0.530±0.052*	0.670±0.022*+o
H2	0.140±0.032	0.160±0.017	0.210±0.018	0.260±0.036
HE	0.055±0.008	0.070±0.017	0.082±0.012	0.110±0.022*
I1	0.370±0.009	0.34±0.02	0.530±0.056	0.710±0.074*+
I2	0.110±0.011	0.220±0.055	0.100±0.012	0.062±0.013*+
IE	0.017±0.005	0.016±0.006	0.069±0.011*+	0.100±0.007*+
TBARS, E_{532}/mg tissue×10^{-3}	1.800±0.154	1.210±0.196	0.920±0.201*	1.040±0.421
LO, %	57.9±10.3	91.00±34.15	145.20±25.19*	200.20±53.68*
MAO activity, nM benzaldehyde/mg tissue/min	0.274±0.069	0.312±0.062	0.428±0.050	0.697±0.082*+o
Capillary length/mm^3, mm	236.50±9.31	281.10±8.03*	261.10±8.69	226.80±6.41+o
Capillary diameter, μ	6.380±0.101	6.030±0.121	5.940±0.109	6.80±0.14*+o
Neuron count	176.30±5.88	280.00±11.12	153.70±5.52+	187.5±8.6+
Glial cell count	686.80±25.67	1318.70±71.79*	920.00±18.19	1157.50±42.84*
Glial index	3.93±0.13	4.80±0.27	6.09±0.19*+	6.28±0.19*+
SDG activity, arb. optical density units	8.46±0.18	6.68±0.16	7.68±0.27	5.85±0.35*+o

Note. H1 and I1: heptane- and isopropanol-soluble conjugated dienes (primary LPO products), respectively; G2 and I2: heptane- and isopropanol-soluble ketodienes and conjugated trienes (secondary LPO products), respectively; GE and IE: end LPO products (Schiff bases) in heptane and isopropanol phases of the lipid extract, respectively; TBARS: content of substances reacting with 2-TBA; and LO: lipid oxidizability. p<0.05: *compared to middle-age 1 group; +compared to middle-age 2 group; °compared to elderly people.

These morphobiochemical changes were accompanied by a considerable decrease in the density of functionally active (alkaline phosphatase-positive) capillaries. Their total length in senescent individuals decreased by 20% compared to that in middle-age 2 individuals ($p<0.05$), which attested to chronic circulatory hypoxia of the nervous tissue. This assumption is confirmed by the age-related increase in capillary diameter. This reaction compensates reduction of microvessels [10] and reflects chronic disturbances in oxygen metabolism [11].

The age-related decrease in SDH activity (key enzyme involved in tissue respiration) in hypothalamic neurons indicates that the circulatory hypoxia is complicated by tissue hypoxia. In light of this, the age-related activation of cerebral MAO-B not only contributes to LPO intensification, but also reflects oxygen demands of the nervous tissue [4,6].

Our results indicate that hypothalamic LPO is activated during aging, which is probably related to chronic hypoxia induced by partial reduction of capillaries and decrease in SDH activity in nerve cells. The data suggest that LP-induced damages to cell membranes and accumulation of ballast substances (Schiff bases) cause nerve cell death. Substitutive diffuse gliosis leads to an increase in cerebral MAO activity and LPO intensification.

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