Comparative Analysis of LPO Products in Brain Structures of Wistar and OXYS Rats of Different Age

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We analyzed the content of LPO products in various brain areas of Wistar and OXYS rats characterized by early appearance of changes in the emotional and cognitive spheres typical of aging. Marked differences between brain regions were found in both strains, but were more pronounced in Wistar rats. The content of LPO products in OXYS rats was higher in the midbrain, hippocampus, nuclei accumbens and striatum at the age of 2 months and in the midbrain, hippocampus, and nuclei accumbens at the age of 18 months. At the age of 18 months the content of LPO products was higher than at the age of 2 months in the amygdala and nuclei abbumbens of Wistar rats and in the nuclei accumbens and midbrain of OXYS rats, while in the hippocampus and hypothalamus of both rat strains and in the striatum of OXYS rats the content of LPO products at the age of 18 months was lower than at the age of 2 months. These results indicate that deviations in cognitive and emotional spheres of OXYS rats develop against the background of LPO activation in brain structures critical for training and memory, which indicates their relationship with oxidative stress.

Key Words: *early aging; brain; oxidative stress; OXYS rats*

Oxidative stress (imbalance in the pro- and antioxidant system associated with accumulation of oxidative injuries of macromolecules) plays an important role in age-related changes in training and memory processes and in the pathogenesis of neurodegenerative diseases [7,12]. High sensitivity to oxidative stress determined by a genetic metabolic defect in OXYS rats is the cause of accelerated aging [11] and early manifestation of changes in the emotional and cognitive spheres characteristic of aging humans and animals.

It was previously shown that 3-4-month-old OXYS rats demonstrate high anxiety, low capacity to single training, and impaired concentration to unimportant information [2,3]. These signs precede accumulation of protein and lipid oxidation products in the brain (the content of these products increases with age, but only starting from the age of 12-14 months this parameter in OXYS rats significantly surpassed that in Wistar rats). During the development of neurodegenerative processes some regions of the brain are the first targets of oxidative stress. For example, in Alzheimer's disease activation of free-radical processes results from neuronal degeneration and death in brain regions involved in training and memory processes: cortex, hippocampus, and subcortical nuclei. In Parkinson's disease degeneration of dopaminergic neurons and accumulation of damaged macromolecules occur in the substantia nigra, while in Huntington's disease the targets of oxidative processes are striatal neurons [10]. We proceeded with investigations of the relationship between behavioral deviations in OXYS rats and oxidative stress. Here we compared activity of free-radical processes in different brain structures (midbrain, hippocampus, hypothalamus, amygdala, frontal cortex, nuclei accumbens,

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and striatum), using the contents of LPO products as the markers.

MATERIALS AND METHODS

The study was carried out on 42 male Wistar and OXYS rats aged 2 and 18 months (Institute of Cytology and Genetics). The animals were kept under standard vivarium conditions. The amount of material for investigation of some brain structures was limited, and therefore we measured the content of primary (conjugated dienes) and final (Schiff bases) LPO products using a method requiring ~0.5 mg lipids and not requiring weighing and pretreatment of easily oxidized tissues [1]. The rats were decapitated, brain structures were isolated on the cold, lipids were extracted by the method of Folch with chloroform-methanol 2:1 mixture containing 0.005% butylhydroxytoluene. The extraction was carried out at -8°C for at least 8 h, after which 0.74% KCl was added (1/5 volume). The lower organic phase was transferred into weighing bottles and the solvent was evaporated under vacuum. Dry lipid samples were dissolved in methanol-hexane 5:1 mixture, the content of conjugated dienes was measured by absorption at λ=233 nm, the content of fluorescent LPO products (Schiff bases) by fluorescence at 440 nm (excitation at λ=360 nm). The results were standardized for total lipid content (after repeated drying the samples were dissolved in ethyl ether-ethanol 4:1 mixture, 5% H_2SO_4 was added, and lipid concentration was measured by absorption at λ=650 nm). A mixture of cholesterol and palmitic and oleic fatty acids (1:1:1) served as the reference sample. Fluorometry was carried out on an MPF-4 spectrofluorimeter (Hitachi), spectrophotometry on a Hitachi-556 spectrophotometer. The results were statistically processed using dispersion analysis (ANOVA/MANOVA, Statistica 5) with *post hoc* comparison of the mean group values (Newman-Keuls test, the animal genotype and age and brain structure were considered as independent factors.

RESULTS

Factor dispersion analysis showed that the content of LPO products in the brain depended on the structure ($F_{6.216}$=28.9, p<0.000003 for conjugated dienes and $F_{6.217}$=8.95, p=0.001 for Schiff bases), age ($F_{1.217}$=14.6, p=0.0002 for conjugated dienes and $F_{1.217}$=10.7, p=0.001 for Schiff bases), and genotype of animals ($F_{1.217}$=3.3, p=0.05). The content of LPO products in brain structures greatly varied and was minimum in the midbrain irrespective of animal age and genotype (Figs. 1, 2). In young rats the content of LPO products was maximum in the frontal cortex irrespective of the genotype, while at the age of 18 months it was high in the cortex and amygdala of Wistar rats and in the nuclei accumbens of OXYS rats.

The relationships between age, genotype, and structure ($F_{6.216}$=4.7, p=0.0002) indicate differences in age-related changes in the content of LPO products in different brain structures of Wistar and OXYS rats. Analysis of the relationships between changes in the sign on genotype and age showed that these factors were negligible only for the frontal cortex. The content of primary LPO products (conjugated dienes) in the midbrain of OXYS rats was higher than in Wistar rats at the age of 2 and 18 months ($F_{1.16}$=20.3, p=0.01 and $F_{1.18}$=5.9, p<0.032, respectively), but this parameter did not depend on age. On the other hand, the content of final LPO products (Schiff bases) in the midbrain depended both on age ($F_{1.31}$=4.52, p<0.041) and genotype ($F_{1.31}$=4.2, p<0.042), their content in OXYS rats

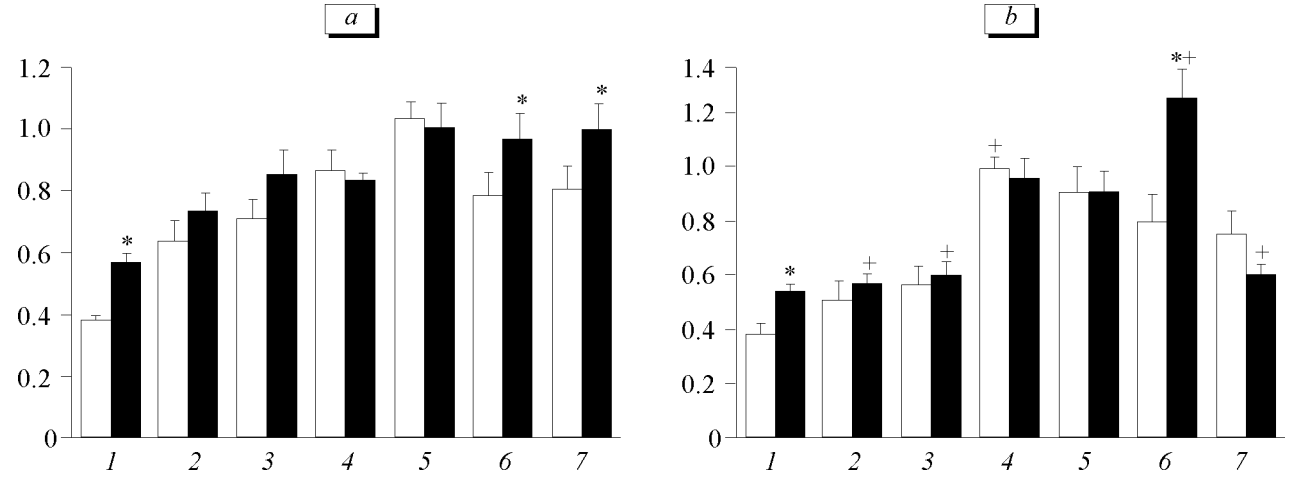

Fig. 1. The content of conjugated dienes in brain structures of 2-month-old (*a*) and 18-month-old (*b*) Wistar and OXYS rats (n=8-12). Light bars: Wistar; dark bars: OXYS rats. *1*) midbrain; *2*) hippocampus; *3*) hypothalamus; *4*) amygdala; *5*) frontal cortex; *6*) nuclei accumbens; *7*) striatum. Ordinates: conjugated dienes, E 233 nm/mg lipids ($M \pm m$). Here and in Fig. 2: p<0.05: *compared to Wistar rats; +between 2- and 18-month-old rats.

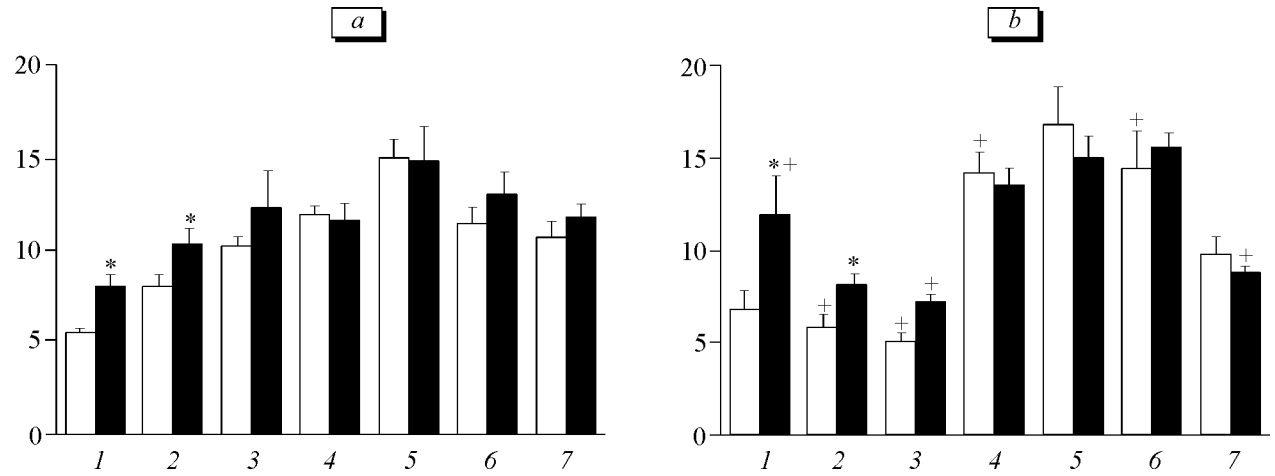

Fig. 2. The content of fluorescent LPO products (Schiff bases) in brain structures of 2-month-old (*a*) and 18-month-old (*b*) Wistar and OXYS rats (*n*=8-12). *1*) midbrain; *2*) hippocampus; *3*) hypothalamus; *4*) amygdala; *5*) frontal cortex; *6*) nuclei accumbens; *7*) striatum. Ordinates: Schiff bases, arb. units/mg lipids.

was higher than in Wistar (1.5 times at the age of 2 months and 1.7 times at the age of 18 months). It is noteworthy that significant (1.5 times) increase in the content of Schiff bases with age was detected only in the midbrain of OXYS rats.

The content of LPO products in the hippocampus depended on the genotype: the content of Schiff bases in young OXYS rats was 28% higher than in Wistar rats ($F_{1.31}$=8.14, p=0.007). This parameter also depended on age ($F_{1.31}$=7.79, p=0.009): the content of LPO products in Wistar and OXYS rats aged 18 months was lower than in young animals, but the age-related changes were significant only for conjugated dienes in OXYS rats and for Schiff bases in Wistar rats (Figs. 1 and 2).

The content of LPO products in the hypothalamus did not depend on the genotype, but it changed significantly with age ($F_{1.31}$=11.74, p=0.002 for conjugated dienes and $F_{1.31}$=18.34, p=0.0002 for Schiff bases). Unexpectedly, the content of LPO products in this structure, like in the hippocampus, was lower in 18-month-old rats of both strains in comparison with young animals: the content of conjugated dienes was 20% lower in Wistar rats and 23% lower in OXYS rats. However according to single-factor analysis for each strain individually, this difference was significant only for OXYS rats ($F_{1.17}$=10.2, p=0.005). The age-related differences in the content of Schiff bases were more significant: their content was 2-fold lower in Wistar rats compared ($F_{1.15}$=38.5, p=0.00002) and 1.7 times lower in 18-month-old OXYS rats compared to 2-month-old animals ($F_{1.15}$=3.97, p=0.05).

No age-related changes in the content of LPO products in the amygdala were detected in both strains; age-specific changes (accumulation of LPO products) were observed only in Wistar rats. The intensity of LPO in the nuclei accumbens depended on both genotype and age: at the age of 2 months the content of conjugated dienes in OXYS rats was 1.3 times higher than in Wistar rats ($F_{1.15}$=4.6, p=0.041). This parameter increased with age ($F_{1.17}$=4.6, p=0.041) and by the age of 18 months the content of conjugated dienes in OXYS rats was 1.6 times higher than in Wistar rats ($F_{1.16}$=5.8, p=0.0035). On the other hand, no differences in the content of Schiff bases between two rat strains were detected. This parameter increased with age, but only in Wistar rats it was significantly higher at 18 months than at 2 months ($F_{1.17}$=5.26, p=0.024).

The content of LPO products in the striatum did not depend on the genotype, but changed with age: $F_{1.31}$=10.4, p=0.003 for conjugated dienes and $F_{1.30}$=3.5, p=0.060 for Schiff bases. The interactions between the age and genotype factors ($F_{1.31}$=5.9, p=0.021) indicates differences in age-related changes of LPO activity in this brain structure in different rat strains. Indeed, the differences were significant only in OXYS rats: at the age of 18 months the contents of conjugated dienes and Schiff bases were lower than at the age of 2 months by 39% ($F_{1.16}$=19.51, p=0.0004) and 23% ($F_{1.15}$=8.92, p=0.009), respectively.

Hence, various brain regions considerably differed by the intensity of LPO processes in both Wistar and OXYS rats. The differences were more pronounced in Wistar rats irrespective of age and reached 270% between polar structures in young and 240% in 18-month-old animals, in OXYS rats the respective values were 177 and 230%. Our findings confirm previous data on the absence of generalized activation of LPO processes involving all brain regions in OXYS rats. At the same time, in young OXYS rats the content of LPO products is higher in four brain structures involved in training and memory processes (midbrain, hippocampus, nuclei accumbens, and striatum) in comparison with Wistar rats. In 18-month-old rats the

content of LPO products increased (compared to the corresponding control) only in the midbrain, hippocampus, and nuclei accumbens. It is noteworthy that in many brain structures the content of LPO products at the age of 18 months was lower than at the age of 2 months: in the hippocampus and hypothalamus of Wistar and OXYS rats and in the striatum of OXYS rats. Higher level of LPO products (in comparison with young animals) was observed in the amygdala and nuclei accumbens of Wistar rats and in the nuclei accumbens and midbrain of OXYS rats. Presumably, opposite changes are responsible for our previous failure to detect the differences between the two rat strains in the levels of LPO products in brain homogenates [6]. Differences in accumulation of oxidative injuries in brain structures of OXYS rats are confirmed by immunohistochemical studies of the formation of premutagenic etheno-DNA-adducts (1,N^6-ethenodeoxyadenosine) — products of reaction between oxidized lipids and DNA bases [6].

Some reports present direct and indirect proofs that activity of free-radical processes differs in different brain regions and varies with age [5,6]. On the other hand, accumulation of LPO products in the brain with age is paralleled by a drop of LPO capacity [4]. Age-related changes in LPO activity in different brain regions are oppositely directed and less pronounced than differences between brain structures [9], which is in line with our findings.

Different levels of free-radical oxidation products in brain structures are usually explained by insufficiency of antioxidant mechanisms and are regarded as a marker of injuries. On the other hand, a direct relationship was found between the intensity of generation of reactive oxygen species in different regions of the brain, on the one hand, and energy metabolism, electrophysiological activity, on the other [8], and between typological characteristics of animal behavior and oxidative metabolism in the brain (the balance between oxygen delivery and utilization and activity of oxidative enzymes) [4]. The mechanisms underlying the differences in the levels of LPO products in brain structures of Wistar and OXYS rats remain unclear, but it is obvious that deviations in the cognitive and emotional spheres develop in OXYS rats against the background of LPO activation in brain compartments critical for training and memory and are presumably associated with oxidative stress.

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