AGE-ASSOCIATED CHANGES IN THE LEVELS OF OXIDATIVE STRESS MARKERS IN THE BRAIN OF OXYS RATS

*Shcheglova T.V., Kolosova N.G., Sinitsina O.I., Vasunina E.A., Loskutova L.V.*
Institute of Cytology and Genetics SB RAS, 630090, Novosibirsk, Russia, e-mail: scheglov@nibochnsc.ru
*Corresponding author

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Resume

Motivation: Oxygen free radicals have been hypothesized to play an important role in aging and a number of pathologic processes. Free radicals are formed in the brain as part of normal metabolic processes such as mitochondrial respiration. High oxygen uptake and low antioxidant defense increase vulnerability of the brain to oxidative damage. Behavior of the young OXYS rats, characterized by accelerated senescence, is similar to that of old Wistar rats. OXYS rats have lowered response to novel stimuli, higher anxiety, and impaired capability of one-trial learning. Therefore, the OXYS strain could be a good model to study human degenerative diseases and the mechanism of oxidative protein and DNA damage and repair in mammals.

Results: Here we have analyzed for the first time age-dependent and interstrain differences in oxidative stress markers and antioxidant enzymes in the brain of OXYS and Wistar rats. We have shown that the learning and memory impairments usually can be registered in the OXYS rats at 3-4 months of age and that these symptoms significantly increase with age. At the same time, the level of protein carbonyl groups in the OXYS rats was elevated (>1.4-fold), compared with that in Wistar, only for the ages of 12-14 months and older. The level of an antioxidant enzyme superoxide dismutase was 1.8-fold higher in the brain of young OXYS rats. Thus, cognitive impairment in the OXYS rats precedes detectable changes in these oxidative stress markers. Our data are consistent with the view of oxidative stress as a mosaic process, dependent on damage of DNA, proteins and lipids in the brain.

Introduction

Aging is influenced by various environmental factors, of which free radicals are thought to be the most important, as proposed by Harman in 1956. Accumulation of oxidative damage in different components of brain membranes with age leads to their depolarization, changes in neuron sensitivity, deviations from optimal conditions of functioning of receptors, channels, enzymes, and modulators, as well as to change in a number of membrane receptors and their affinity to neuromediators. This ultimately leads to functional brain impairment.

OXYS rats show some chronic aging pathologies such as short lifespan, accelerated degeneration of thymus, liver, myocardium, bone, and development of cataracts. The OXYS strain is argued to be a model for studying human degenerative diseases. One of the potential causes of age-related neuronal damage is reactive oxygen species. The objective of this study was to investigate relationships between brain functioning and changes in the oxidative stress proxies in the brain of OXYS (from 3 to 24 month of age), as compared to control Wistar rats.

Methods

Male white Wistar and OXYS rats from the Institute of Cytology and Genetics of RAS (Novosibirsk, Russia) from 3 to 24 months of age were used in this study. The animals were housed in colonies under standard conditions. The rats were sacrificed by decapitation, and the brains were frozen at -70°C.

Protein carbonyl content was measured after reaction with 2,4-dinitrophenylhydrazide and quantifying the resulting protein hydrazone derivatives spectrophotometrically at 370 nm (extinction coefficient, 22x10³ l·mol⁻¹·cm⁻¹). The concentration of carbonyl groups was expressed as nmol carbonyl per mg protein (Reznick, Packer, 1994). The protein content was determined by Bradford assay, using bovine serum albumin as a standard.

Lipid peroxidation (LPO) was determined using conjugated diene (CD) (Steinbrecher, 1990).

To assay for SOD activity in brain extracts, xanthine/xanthine oxidase-mediated ferricytochrome c reduction assay (Flohe, Otting, 1984) was used. Samples (40 µl) were added to 550 µl of the reaction mixture containing 0.025 µmol of xanthine in 0.1 mM NaOH, and 0.01 µmol of cytochrome c in 50 mM phosphate buffer, pH 7.8, supplemented with 0.1 mM EDTA and 10 mM sodium azide. The reaction was initiated by adding 10 µl of the xanthine oxidase solution (0.5 U/ml in 0.1 mM EDTA). The absorbance change was monitored for 3 min at 25°C. SOD activity was calculated from the absorbance at 550 nm using a concurrently run standard curve and expressed as units of activity per mg protein. One unit of SOD activity is defined as the amount of enzyme that inhibits the rate of cytochrome c reduction under the conditions specified by 50%.
Total levels of reduced glutathione (GSH) in brain extracts were measured spectrophotometrically at 412 nm by method of Tietz (1969) using 2-nitro-5-thiobenzoic acid. The concentration of thiols was expressed as nmol thiol per mg protein.

Statistical analysis. The results were presented as mean ± Er of at least 3 different experiments for each brain extract sample of each rat. The results are an average of brain extracts from at least 5-8 different animals. All data assessed by the one-way or two-factor analysis of variance (ANOVA).

Results and Discussion

Impairments of cognitive functions (lowered response to novelty, higher anxiety, and impairment ability to one-trial learning) are usually the earliest symptoms of worsening of memory in senescents humans and animals (Loskutova, Kolosova, 2000).

Learning and memory impairments can usually be noticed in OXYS rats at 3-4-month of age; these symptoms become progressively worse with age. In order to find a possible biochemical explanation of this phenomenon, we have analyzed relationships between several physiological indices of brain impairment, changes in the levels of oxidative damage of proteins and lipids, and others oxidative stress proxies in the brain of OXYS (from 3 to 24 month of age) in comparison with the same parameters of control Wistar rats.

First, we have examined whether there was age-dependent oxidative damage to proteins and lipids in brain tissues of OXYS and Wistar rats. We have determined protein groups and lipid peroxides content in OXYS and Wistar rats at 3, 10, and 14 and 24 months of age. Fig. 1 demonstrates that the level of lipid peroxides and protein carbonyl content increased with age in the brain of rats from both strains (Fig. 1). Interestingly, a significant difference between OXYS and Wistar rats can be revealed only for 12-14-months old or older rats; ~1.4-fold (statistically significant) higher level of these parameters in brain of OXYS as compared with Wistar rats was revealed. The relative amount of protein carbonyl groups for OXYS and Wistar rats at 24 month of age, in comparison 4-month old rats, was increased by a factor of 3.3 and 2.3, respectively, while the content of lipid peroxides increased 2.7- and 1.8-fold.

![Fig. 1. Comparison of a relative content of protein carbonyl groups (a) and lipid peroxides (b) in the brain of OXYS and Wistar rats of different age.](image)

Under normal circumstances, nuclear and mitochondrial oxygen radicals may be detoxified by superoxide dismutase (SOD), decreasing the levels of protein, lipid, and DNA modifications. In order to estimate possible differences in such detoxification in OXYS and Wistar rats we have compared SOD activities in the brain of rats as well as age-related changes in SOD and non-enzymatic ROS scavengers’ vitamin E and glutathione. The activity of SOD in brain of OXYS rat at 24 months of age was ~1.7-fold (statistically significant) lower than in Wistar rats of the same age.

However, there was no significant difference in protein carbonyl groups in the brain of 3-4-month-old OXYS and Wistar rats, even though young OXYS rats already demonstrate pronounced learning and memory impairment. At the same time, we have observed a significant difference in the level of superoxide dismutase between young OXYS and Wistar rats; SOD activity was higher 1.8-fold (Fig. 2).
At the same time, the level of reduced glutathione in OXYS was 1.3-fold lower than in Wistar rats. Vitamin E is a natural antioxidant protector of mammalian organisms. However, there was no detectable difference in the level of vitamin E for two analyzed rat strains. Since SOD is inducible, increased levels of ROS in OXYS can lead to elevated SOD activity in response to increased oxidative damage.

Thus, our results suggest that accumulation of detectable levels of oxidative stress markers (oxidized proteins and lipids) with age occurs slower than manifestation of cognitive impairment in the OXYS rats. In order to uncover a possible reason for this, we have analyzed relative levels of LPO in different regions of the 4-month-old OXYS and Wistar rat brain (cortex, hippocampus, midbrain and cerebellum). No pronounced interstrain difference was found. At the same time, two-way ANOVA analysis revealed statistically significant differences in the levels of LPO for specific regions of rat brains; both OXYS and Wistar rats demonstrated interstructural differences according to Fisher indices (F) (F_{3,33} = 5.8, p = 0.003 for Wistar and F_{3,26} = 4.8, p = 0.008 for OXYS rats). The LPO levels were higher in the cortex of Wistar and in the hippocampus of OXYS rats. In addition, according to our preliminary data, DNA damage with formation of etheno derivatives of adenine and cytosine is more extensive in OXYS than in Wistar rats. Interestingly, etheno DNA lesions are not distributed uniformly in the brain, but there exist specific brain areas accumulating more lesions, leading to a mosaic picture of brain damage. Thus, our results and related studies indicate that the changes in brain function may be related to progressive oxidation of critical brain proteins and lipids. An intriguing possibility is that damage of some critical regions in the brain, leading to cognitive impairment in OXYS rats, may occur in earlier age than more extensive damage of the brain as a whole, which can be registered using analysis of oxidative stress markers.

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**References**