BIOGERONTOLOGY

Age-Related Changes in Proteoglycan Composition in Rat Brain

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Brain proteoglycans play an important role in learning and memory processes and in the pathogenesis of neurodegenerative disease. Brain proteoglycans in Wistar rats and early aging OXYS rats at the age of 1, 2, and 14 months were compared. Proteoglycan content in OXYS rats aged 1 and 2 months is 2-fold lower than in Wistar. The content of proteoglycans 7-fold decreases in Wistar and OXYS rats with age at the expense of heparan sulfates and chondroitin sulfates and by the age of 14 months, the difference between the strains is leveled. These results indicate that cognitive and emotional disorders observed in OXYS rats by the age of 3 months develop against the background of significant changes in the content and composition of proteoglycans.

Key Words: *proteoglycans; chondroitin sulfates; heparan sulfates; brain; aging*

Proteoglycans (PG) are a class of glycosylated proteins containing linear chains of sulfated glycosaminoglycans (GAG) constructed from repeating disaccharides. High negative charge of GAG chains causes PG interactions with a wide spectrum of cellular molecules mainly due to sulfates and carbonyl groups. The functions of PG in the CNS are numerous: regulation of cell resistance to damage, modulation of the effects of growth factors and other regulatory molecules essential for axon growth [12].

Heparan sulfate PG stabilize the receptor—ligand interactions [4] and are involved in nerve chain formation at different stages: neuron development, axon growth, and synapse formation [8]. Chondroitin sulfate PG interact with growth factor molecules and play the leading role in the development of the nervous system during the early postembryonic period and formation of the extracellular matrix [5].

The nervous system PG play an important role in learning and memory processes and in the function of the hypothalamic—pituitary system [3,11, 12]. Chondroitin sulfate PG are involved in the pathogenesis of such diseases as epilepsy, sclerosis, Alzheimer's disease [5]. Quantitative changes in different PG classes during the embryonic and early postembryonic development were described [6,9], but the information about the composition of PG and their changes with age is extremely scanty.

The OXYS rats develop early changes in the mental and cognitive spheres, characteristic of aging humans and animals [7]. By the age of 3-5 months, OXYS rats exhibit behavioral features typical of old animals: high anxiety in the elevated plus maze test [2], disorders in associative training, and reduced exploratory activity [10]. Structural and functional changes in the cerebral bloodflow charac-

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teristic of chronic ischemia are detected by the age of 13 months [1]. The use of the rapid aging model seems to be perspective for studies of age-associated cerebral dysfunctions, which can promote understanding of the PG role in molecular mechanisms of aging.

We studied the composition of PG and their changes in Wistar rats and early aging OXYS rats during different periods of life.

MATERIALS AND METHODS

Experiments were carried out on male Wistar and OXYS rats from Laboratory of Experimental Animal Breeding, Institute of Cytology and Genetics. The animals were kept under common vivarium conditions on standard rations with free access to water and food. The animals were decapitated under ether narcosis. The brain was rapidly removed, directly frozen in liquid nitrogen, and stored at -70°C. The tissue was homogenized in TRI Reagent (Ambion; 1:10). Chloroform was added to TRI Reagent (0.2 V per 1 V, respectively) and the mixture was centrifuged for 40 min at 8000*g*. Water phase was precipitated with isopropanol. The precipitate was hydrolyzed with 0.5 N NaOH (1 h, 37°C) in order to remove RNA. The DNA admixtures were precipitated with perchloric acid to a final concentration of 0.5 N with subsequent centrifugation. After removal of nucleic acids, GAG were precipitated from the supernatant with ethanol (3 V; 1 h, -18°C). Glycosaminoglycans from the brain of one animal were dissolved in 100 μl distilled water and identified by horizontal electrophoresis in 1% agarose gel (2 mm thick) on a 5×10-cm plate in 50 mM barium acetate buffer (pH 5.0; 4°C, 1 h, 10 V/cm). After electrophoresis, the gel was stained with 0.1% toluidine blue in 1% acetic acid. Chondroitin sulfates A, B, C and heparan sulfates served as the markers. The type of GAG was identified by the disappearance of the band in the gel after treatment of the samples with chondroitinase AC or nitrous acid specifically destroying heparan sulfate chains. Quantitative evaluation was carried out using Gel-Pro Analyzer software.

The results were statistically processed using post-hoc comparison of the means for groups (Newman—Keuls test). The genotype and age were assumed as the independent factors. Each experimental group consisted of 5-6 animals.

RESULTS

The results of electrophoresis indicate that the brain of Wistar and OXYS rats aged 1, 2, and 14 months contains mainly heparan sulfates and chondroitin sulfates. Bifactorial analysis of dispersions showed the genotype impact for the content of GAG in the rat brain ($F_{1.31}$=4.6; p<0.001). A similar trend of age-specific changes in GAG in Wistar and OXYS rats (Fig. 1) was seen from the relationship between the genotype and age factors ($F_{1.17}$=9.13; p<0.01). The content of summary GAG in Wistar rats aged 1 month was 2-fold higher than in OXYS rats ($F_{1.10}$=21.6, p<0.001). Analysis of individual GAG classes in the brain of 1-month-old animals showed that this difference was due to low content of heparan sulfates, their content in OXYS being 2.7 times lower than in Wistar rats ($F_{1.10}$=18.1, p<0.002). The content of chondroitin sulfates tended to decrease (by 20%; p=0.06). By the age of 2 months, the content of summary GAG in Wistar rats decreased

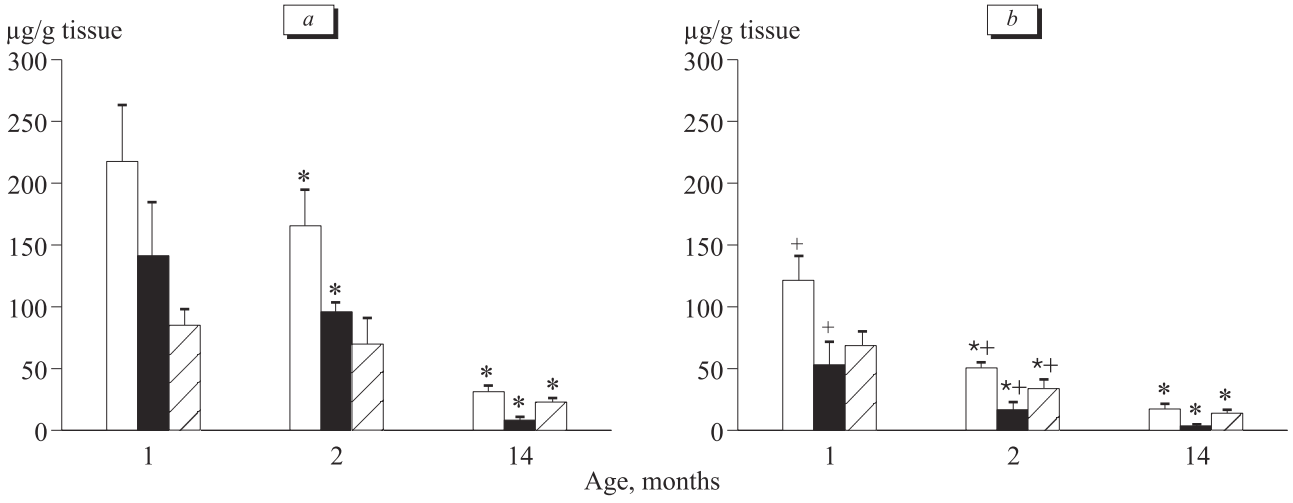

Fig. 1. The composition of GAG in the brain of Wistar (*a*) and OXYS (*b*) rats of different age. Light bars: GAG sum; dark bars: heparan sulfates; cross-hatched bars: chondroitin sulfates. *p<0.05 compared to 1-month-old animals; ^{+}p<0.001 compared to Wistar rats.

by 24% (p=0.019), while in OXYS rats it dropped more than 2-fold (p<0.0004). Post-hoc comparisons revealed differences in the cerebral heparan and chondroitin sulfate levels at the age of 2 months: their content was 5.8 and 2.1 times lower, respectively, in OXYS than Wistar rats. Unifactorial analysis of dispersions carried out separately for each genotype showed that GAG content in the brain at the age of 14 months decreased 7-fold ($F_{1.16}$=7.4; p<0.0001 for Wistar and $F_{1.34}$=11.23; p<0.0001 for OXYS rats). In comparison with the age of 1 month, the most pronounced changes were observed for heparan sulfates: their content decreased 17-fold in Wistar (p<0.001) and 15-fold in OXYS rats (p<0.001). The content of chondroitin sulfates in 14-month-old rats was lower than at the age of 1 month: 3.7 times lower in Wistar (p<0.0001) and 4.9 times in OXYS rats (p<0.0001). The content of heparan sulfates was lower than of chondroitin sulfates in OXYS rats of all age groups, while in Wistar rats this difference was noted only at the age of 14 months.

Hence, PG content in the brain decreased with age in early aging OXYS rats and in Wistar rats with normal aging rate. It is noteworthy that their content in the brain was significantly lower at the age of 2 months than at 1 month, being significantly lower in young OXYS compared to Wistar rats. The proportion of GAG of different classes was also different in the two strains. The differences in the quantitative and qualitative PG composition in the two strains leveled by the age of 14 months (Fig. 1).

Decrease in exploratory activity, increased anxiety, and disorders in learning capacity are regarded as manifestations of early aging of OXYS rats. These signs manifest in this rat strain by the age of 3 months in the presence of other manifestations of early aging [2]. Our findings indicate that these signs are forming against the background of reduced (*vs.* Wistar rats) PG level. Exploratory activity of Wistar rats decreased by the age of 14 months, but their learning capacity at this age can be retained at the level of 3-month-old animals [10]. We therefore do not regard the reduction of PG content in the brain as a sign of aging. In order to understand the physiological role of PG, studies of fine structural changes in PG molecules are needed. These changes can be caused by disorders in the GAG chain biosynthesis linked with elongation enzymes (glycosyl transferases), modification enzymes (sulfotransferases and epimerases), GAG chain hydrolysing enzymes (heparanases, chondroitin sulfatases) or can be due to disorders in the expression of genes encoding the protein part of the molecule.

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