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Structural Characteristic of Nucleolus and Heterochromatin Aggregates of Rat Brain Tanycytes

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Abstract—This work was aimed at studying the structural organization of nucleolus and constitutive heterochromatin in different types of tanycytes during postnatal development and aging of rats. The distribution of nucleolus argentophilic proteins (nucleolin and nucleophosmin) and heterochromatin aggregates in tanycytes at various stages of postnatal development have been described for the first time using immunohistochemical methods and confocal laser microscopy. The heterogeneity of the size and number of nucleoli was demonstrated both in different tanycytes subpopulations and at different ages of an animal. This may indicate different levels of the tanycyte synthetic activity and the ability to proliferate during early postnatal development and aging. During aging, the distribution of heterochromatin aggregates varies among tanycyte subpopulations: α-tanycytes undergo intense heterochromatization, while β-tanycytes are characterized by a stable organization of the studied compartments of the cell nucleus. The data obtained significantly supplement the modern understanding of organization of the structure of the cell nucleus of tanycytes during normal development and aging. This can subsequently serve as a basis for establishing the role of these subnuclear structures in pathological processes.

Keywords: tanycytes, nucleolus, constitutive heterochromatin, development, aging, nucleolin, nucleophosmin, H4K20me3, immunohistochemistry, confocal laser microscopy

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INTRODUCTION

Tanycytes localized at the bottom of the third ventricle are a peculiar population of glial cells. The bodies of these cells line the infundibular recess, while the basal processes penetrate neural tissue and terminate in blood vessels, twisting them with their extended terminals. The processes of tanycytes also twist around the fenestrated capillaries of the hypophyseal portal system, which is localized in the median eminence. Thus, these cells are involved in formation of the blood-brain and blood-CSF barriers. Their role in regulation of the work of the subjacent hypothalamic nuclei: the ventromedial and arcuate nuclei responsible for the energy balance of a body, has been shown previously. In addition, being progeny of radial glial cells, tanycytes maintain not only their morphology and some cytochemical characteristics but also the ability to proliferate and differentiate into neurons and glia. There are 4 types of tanycytes (α1-, α2-, β1-, and β2-tanycytes), which differ in their localization within the infundibular recess, as well as in the structural, cytochemical and functional characteristics [1, 2]. Nevertheless, despite the keen interest of researchers in tanycytes, the important cellular characteristic such as structural and functional organization of the nucleus in these cells has not yet been investigated. In the study of cell nucleus organization, particular attention is focused on the distribution of heterochromatin aggregates and the morphology of the nucleolus, because these subnuclear structures represent the metabolic status of a cell and are sensitive to degenerative changes.

The nucleolus is one of the important markers reflecting the functional state of a cell. Since the main function of this structure is participation in ribosome biogenesis, the level of protein synthesis in the cell correlates with organization of the nucleolus. In addition, it has been established that the nucleolar proteins are involved in the control of the cell cycle, apoptosis and aging, while dislocation of these proteins from the nucleolus (the so-called "nucleolar stress") can mediate the development of neurodegenerative processes [3–5]. One more characteristic representing cellular activity is the presence of heterochromatin aggregates. Histone H4 trimethylated at lysine 20 (H4K20me3) forms constitutive heterochromatin and participates in gene repression in the promoter regions [6, 7]. In addition, H4K20me3 is important for suppression of the transcription of repetitive DNA and transposons [8]. It has been shown that the suppressed expression of this histone leads to the development of malignant neoplasms [9]. The study of potential changes in organiza-

tion of the nucleolus and heterochromatin aggregates during postnatal development and aging will provide new data on the functional state of tanycytes in the course of normal development.

The present research was aimed at studying the structural and functional organization of the nucleolus and constitutive heterochromatin in different types of tanycytes during postnatal development and aging.

MATERIALS AND METHODS

The research materials were fragments of the diencephalon of male Wistar rats from five age groups: day 7, 14 and 30 of postnatal development ($n = 3$ for each period), adult animals (4–5 months) ($n = 6$), and old animals (20–24 months) ($n = 3$). The animals were kept and killed in accordance with the international rules of the Declaration of Helsinki on the care and use of laboratory animals and the Regulations of Work with Experimental Animals (Order no. 755 of August 12, 1977, of the Healthcare Ministry of the USSR). The brain was fixed in zinc-ethanol-formaldehyde [10], dehydrated and paraffin-embedded by the standard technique. Frontal sections 5-μm thick were made at a level from –3.24 to –4.44 mm relative to bregma [11]. After the standard procedure of deparaffinization, the sections were exposed to thermal antigen retrieval in a modified citrate buffer S1700, pH6.1 (Dako, Denmark) for 23 min in a steamer. It was followed by the double immunohistochemical staining with murine monoclonal anti-vimentin antibodies (clone V-9, Dako) diluted 1 : 100, rabbit polyclonal anti-nucleolin/protein C23 antibodies (Abcam, Great Britain) diluted 1 : 200, murine monoclonal anti-nucleophosmin/protein B23 antibodies (clone FC82291, Sigma-Aldrich, USA) diluted 1 : 100, and polyclonal rabbit anti-histone H4K20me3 antibodies (Abcam) diluted 1 : 500. Secondary reagents were the monovalent Fab-fragment of fluorochrome Rhodamine Red-X (RRX)-conjugated donkey anti-rabbit immunoglobulin diluted 1:50 (Jackson ImmunoResearch, USA) and the monovalent Fab-fragment of biotinylated donkey anti-mouse immunoglobulin diluted 1 : 100 (Jackson ImmunoReaserch). Then the sections were treated with fluorochrome Cy2-conjugated streptavidin (Jackson ImmunoResearch) diluted 1 : 200. The reagents were embedded in water-soluble Fluorescence Mounting Medium (Dako). The resultant preparations were analyzed with a LSM 710 confocal laser microscope (Zeiss, Germany). Image processing and 3D reconstruction of objects were performed using LSM Image Browser and ZEISS ZEN lite software (Zeiss). The diameter and number of nucleoli were determined with the ZEISS Zen lite and Fiji software [12], analyzing no less than 10 randomly selected cells of each type of tanycytes for each period. If there was more than one nucleolus in the nucleus, the mean diameter of the nucleoli was calculated for the largest nucleolus. The data are presented as the mean ± standard error of the mean. For the number of nucleoli, the median (M_e) was calculated in addition to the absolute values. Statistical analysis was performed with Graph Pad Prism 8. After non-parametric Kruskal–Wallis test, the groups were compared by Dunn's test. The differences between the groups were considered significant at $p < 0.05$.

RESULTS

The immunohistochemical double staining with vimentin (the marker of tanycytes and ependymocytes) and nucleolin (the marker of the nucleolus) demonstrates that the dorsal region of the bottom of the third ventricle is lined with cuboidal cells–ependymocytes, which are then replaced by cells with basal process–tanycytes (Fig. 1).

Among tanycytes, α1-tanycytes have the most dorsal localization and their processes are directed toward the ventromedial nucleus of the hypothalamus (Fig. 1, enlarged fragment 1). In young and adult animals, these cells are characterized by the presence of 1 to 4 nucleoli. For the most part, there were 1–3 nucleoli (97% cases), and only single cells had 4 nucleoli (3% cases). In old animals, the number of nucleoli was usually 1–2, rarely 3. α2-tanycytes are localized more ventrally relative to α1-tanycytes. The processes of these cells are directed toward the arcuate nucleus (Fig. 1, enlarged fragment 2). In 7- and 14-day young rats, the number of nucleoli was 1–3; beginning from the first month of postnatal development, these cells are characterized by the presence of 1–2 nucleoli; in adult animals, there was 1 nucleolus in most cases. The lateral regions of the median eminence are lined with β1-tanycytes, which form a pseudostratified structure. The central region of the median eminence is occupied by β2-tanycytes. It is just β-tanycytes that contact the fenestrated capillaries. These types of tanycytes are characterized by the presence of 1–2 nucleoli (Fig. 1, enlarged fragments 3 and 4, respectively). It should be noted that the nucleoli of tanycytes in most cases are localized in the periphery of the nucleus, often contacting the nuclear envelope.

The measurement of the diameter of the largest nucleolus has shown that the nucleolar diameter changes with aging (Table 1, Fig. 2). All types of tanycytes are characterized by an increase in the size of this subnuclear structure during early postnatal development (statistically significant for day 7 and 14 of postnatal development compared to one-month-old animals for β1-tanycytes and to adult and old animals for all types of tanycytes). The maximum size of the nucleolus is observed in adult animals, while the diameter of the largest nucleolus decreases with aging. In addition, the nucleolar diameter varies between different types of tanycytes within the same age group. For example, in 30-day young rats, the nucleolar diameter in α1- and α2-tanycytes was statistically different when compared with the nucleolar diameter in

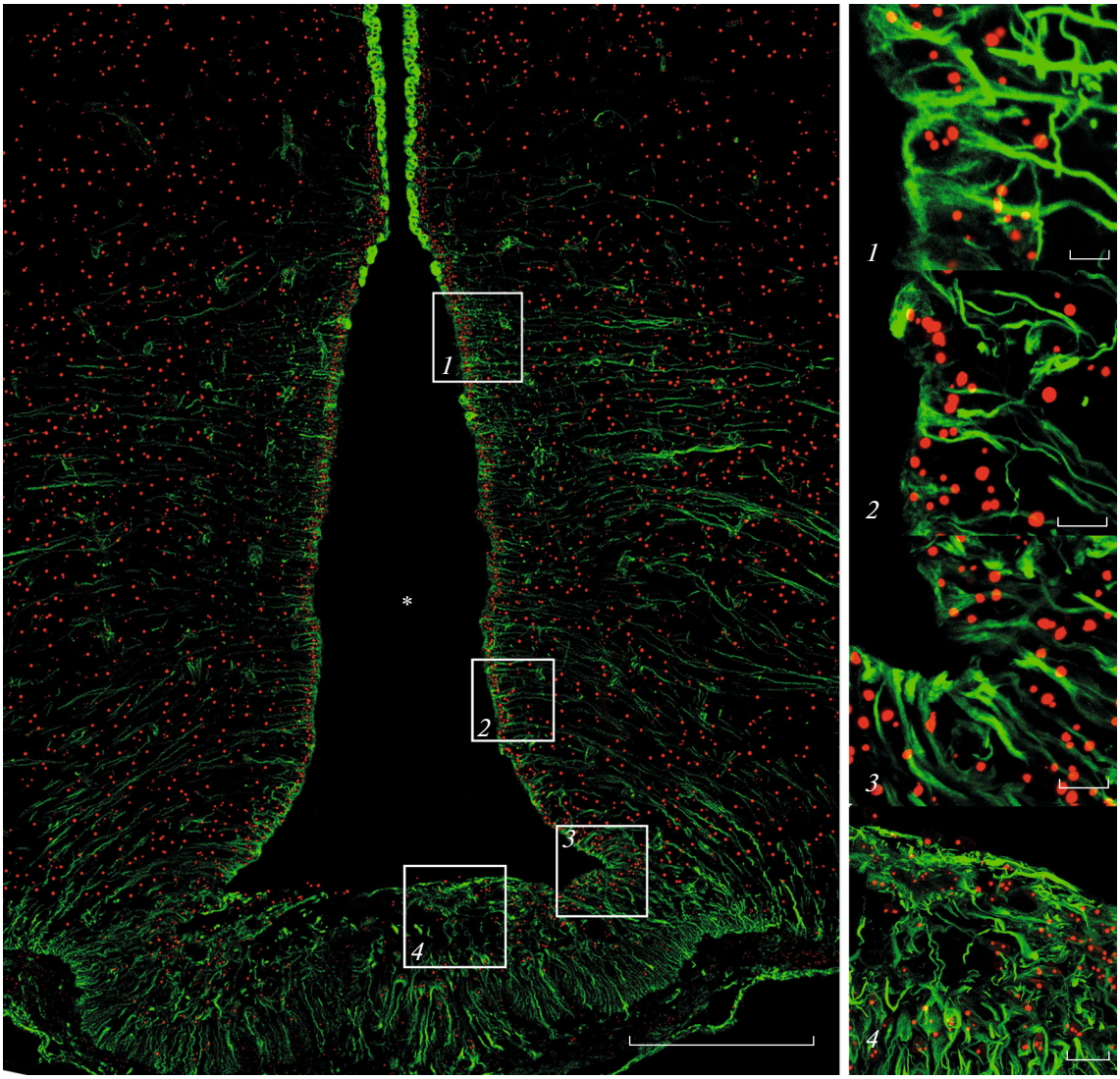

Fig. 1. The bottom of the third ventricle of rat brain. An adult animal. Double immunohistochemical staining with vimentin (*green*) and nucleolin (*red*). A single optical section. Scale bar, 200 μm. Enlarged fragments: (*1*) α1-tanycytes; (*2*) α2-tanycytes; (*3*) β1-tanycytes; (*4*) β2-tanycytes. Projection 16 (fragment *1*), 26 (fragment *2*), 21 (fragment *3*) and 36 (fragment *4*) of optical stacks. Objective: C-Apochromat 63×/1.20 W Korr M27. Water immersion. Scale, bar: 5 μm (fragment *1*), 10 μm (fragments *2* and *3*), and 20 μm (fragment *4*). *Asterisk* (*) shows the cavity of the third ventricle.

β1-tanycytes ($p < 0.0001$ for both types of tanycytes); the sizes of nucleoli in β1- and β2-tanycytes were also different ($p = 0.0006$). Adult and old rats showed the difference between the sizes of nucleoli in α1-tanycytes compared to other types of tanycytes (α2, β1 and β2) ($p < 0.0001$ for all reference groups, except for α1 and α2 in old animals; here, $p = 0.0003$). In 14-day young rats, the nucleoli of α1- and β1-tanycytes were different ($p = 0.0071$). In 7-day young rats, there was no statistically significant difference between the sizes of nucleoli. The data on the number and size of nucleoli at different stages of postnatal ontogenesis are given in Table 1.

The more detailed study of structural and functional organization of the nucleolus included the double immunohistochemical staining with argentophilic proteins of the nucleolus: nucleolin (protein C23) and nucleophosmin (protein B23), which mediate histochemical staining of the nucleolus by silver impregnation (AgNOR technique). It has been shown that the pattern of distribution of these two proteins in the nucleus is different. Nucleolin is usually distributed as a torus or a biconcave disc (Fig. 4a), while the central part of the nucleolus is either not stained at all or stained very weakly, as well as occurs in the form of single aggregates in the nucleoplasm of all types of tanycytes. At the same time, nucleophosmin is localized only in the nucleoli of tanycytes. In addition, the pattern of nucleophosmin distribution in these subnuclear structures varies a lot between the nucleoli. As a

Table 1. Morphological characteristics of nucleoli in different populations of tanycytes during postnatal development and aging

Parameter	Age	Tanycyte subpopulation			
		α1	α2	β1	β2
Number of nucleoli	P7	1–4 $M_e = 2$	1–3 $M_e = 2$	1–3 $M_e = 2$	1–2 $M_e = 2$
	P14	1–3 $M_e = 2$	1–3 $M_e = 2$	1–2 $M_e = 2$	1–3 $M_e = 1$
	P30	1–4 $M_e = 2$	1–2 $M_e = 2$	1–2 $M_e = 1$	1–3 $M_e = 1$
	4–5 months	1–4 $M_e = 2$	1–3 $M_e = 2$	1–2 $M_e = 1$	1–2 $M_e = 1$
	20–23 months	1–3 $M_e = 1$	1 $M_e = 1$	1–2 $M_e = 1$	1–2 $M_e = 1$
Mean diameter of the largest nucleolus ± error of the mean, μm	P7	1.492 ± 0.03	1.473 ± 0.03	1.479 ± 0.04	1.360 ± 0.06
	P14	1.367 ± 0.05	1.489 ± 0.07	1.623 ± 0.05	1.513 ± 0.06
	P30	1.528 ± 0.03	1.516 ± 0.04	1.991 ± 0.06	1.612 ± 0.07
	4–5 months	1.679 ± 0.04	2.087 ± 0.05	2.171 ± 0.05	2.059 ± 0.07
	20–23 months	1.611 ± 0.04	1.948 ± 0.06	2.068 ± 0.06	2.202 ± 0.1

rule, protein B23 is distributed as a circle along the periphery of the nucleolus (Fig. 3a). More rarely, this protein can be localized within the nucleolus as a biconcave disc (Fig. 3b), a horseshoe (Fig. 3c), or separate globules within the nucleolus (Fig. 3d), as well as distributed as a rounded structure with a surface groove (Figs. 3e, 3f). The distribution pattern does not change with aging; however, the central part of the nucleolus, where nucleolin and nucleophosmin do not occur, becomes more marked (Fig. 4a).

The study of the distribution of constitutive heterochromatin in tanycytes and its spatial relationships with the nucleolus included the double immunohistochemical staining with H4K20me3 and nucleophosmin. In tanycytes, constitutive heterochromatin occurs as single rounded aggregates. They are localized mainly along the periphery of the nucleus, though there are also diffusively distributed small-sized lumps in the central part of the nucleus. It has been shown that the typical feature of all types of tanycytes is the increasing size of heterochromatin globules during early development. With aging, the content of heterochromatin in α-tanycytes considerably increases both in the periphery of nuclear cells and in the central parts of the nucleus, while in α1-tanycytes it can form a continuous peripheral heterochromatin layer (Fig. 5). In β-tanycytes, the content of heterochromatin aggregates undergoes insignificant changes with aging. The analysis of the spatial arrangement of heterochromatin aggregates relative to the nucleoli has shown that in α1- tanycytes, in the first week of development and in adult animals, almost all nucleoli are submerged in heterochromatin and surrounded by the latter from all sides (Fig. 5). Tanycytes α2 and β in the first week of development, as well as all types of tanycytes at the later stages of development, were shown to contain perinucleolar heterochromatin, which adjoins the nucleolus on one side as a small globule (Fig. 6). The nucleoli completely submerged in heterochromatin were not found at these stages of development. However, in most nucleoli there was a zone of overlap (colocalization) of nucleophosmin and H4K20me3 formed in the region of heterochromatin adjoining. As a rule, this zone is small and located at the interface between these two proteins (Fig. 4b). In singe cases, there is perinucleolar heterochromatin that forms nucleolar invagination (Fig. 6).

DISCUSSION

The study of nuclear architecture is now one of the major trends of modern cell biology. Neurobiological studies provide a lot of data on organization of the nucleus and its components in neurons of different cerebral structures [13–15] but unfairly little attention is paid to the analysis of glial cells and tanycytes in particular [16]. In addition, organization of the tanycyte nucleus during postnatal development and aging has never been studied previously. Hence, the data presented in this work are relevant both for neurobiology and for cell biology in general.

In the present work we have studied the nucleoli of different types of tanycytes during early postnatal development and aging. It has been established that the number of nucleoli does not change during early development but decreases with aging. In addition, tanycytes are characterized by age-related increase in the size of this subnuclear structure. Our results agree

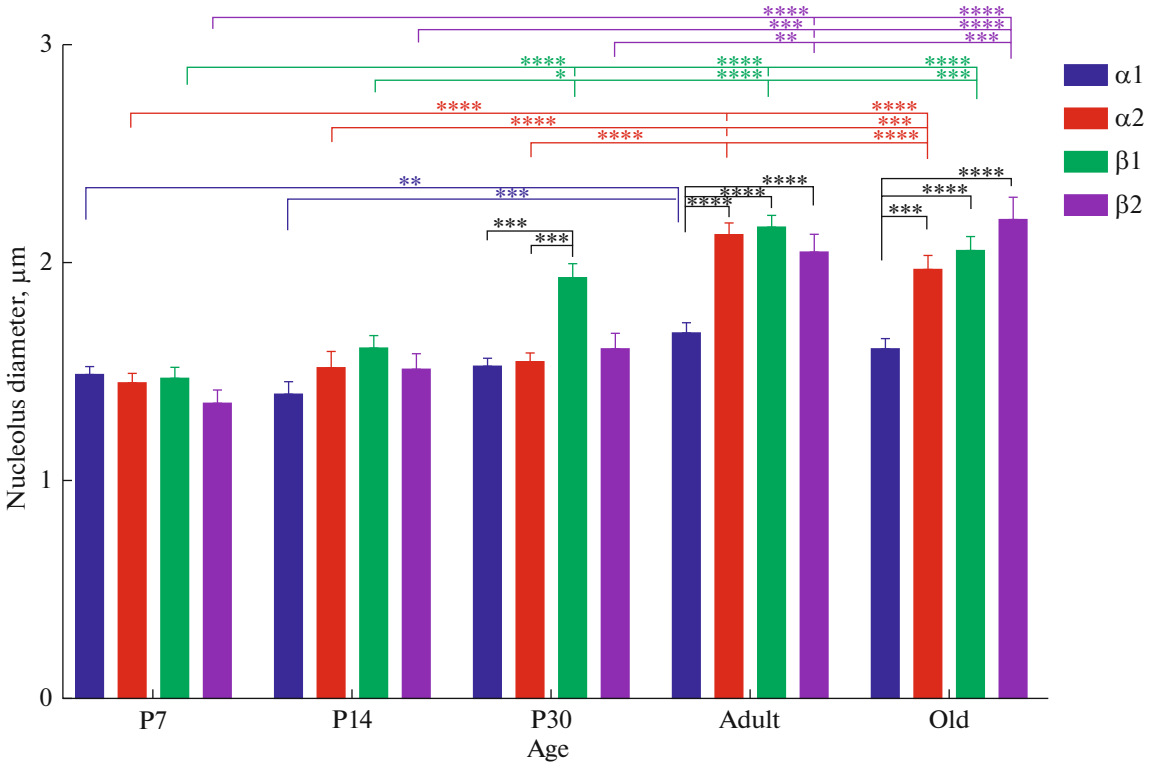

Fig. 2. The age-related dynamics in the size of the nucleolus in different subpopulations of tanycytes. Black line shows the statistically significant difference between various types of tanycytes within the same age. Colored lines show the statistically significant difference in the diameter of nucleoli in each of the tanycyte populations (according to the legend) between different age groups. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$.

with the published data. It has been shown that nucleolar organizer regions (NORs), the number of which determines the maximum possible number of nucleoli in a cell, tend to merge with age and to form larger nucleoli [17]. In addition, we have demonstrated the dependence between the size of nucleoli and the synthetic activity of cells, as well as the number of nucleoli and the degree of cell differentiation [18, 19]. This fact may indicate that the number of nucleoli in tanycytes decreases with age as a result of fusion of nucleoli into larger ones, as well as with the regular increase in synthetic activity during cell formation in the course of early postnatal development.

We have also noted the differences in organization of nucleoli between different types of tanycytes, which, despite being a single population of cells, vary considerably in their functional and cytochemical properties. This is due to the fact that they interact with various structures in the mediobasal hypothalamus and mediate their functions. Beginning from day 30 of postnatal development, the largest nucleolus is present in β1-tanycytes, while the sizes of nucleoli of other types of cells under study did not show any statistically significant differences from each other. In adult and old animals, large nucleoli occur in tanycytes α2, β1, and β2. This fact may indicate that β1-tanycytes have a high synthetic activity even in early postnatal development, while α2- and β2-tanycytes have such activity in the later periods of development. In addition, the findings allow us to distinguish the population of α1-tanycytes characterized by the presence of small nucleoli, but their number is greater compared to other types of analyzed cells (except for α2-tanycytes) throughout postnatal development. It is consistent with the view that tanycytes of this type perform only transporting but not secretory function. In addition, since the number of nucleoli correlates with the degree of cell differentiation, the presence of a great number of nucleoli may be indicative of different proliferation abilities of tanycytes. According to the published data, all types of tanycytes during early postnatal development are characterized by the ability to proliferate and differentiate into neurons and glial cells, while in mature animals this property is maintained only in α-tanycytes, while β-tanycytes are committed neural progenitor cells [1].

In the present work we have studied two main argentophilic proteins of the nucleolus: nucleolin (protein C23) and nucleophosmin (protein B23/NPM1). It has been shown that both nucleolin and nucleophosmin are characterized by distribution as a ring or a biconcave disc. However, distribution patterns of these proteins in the nucleoli of tanycytes are slightly different. For example, the peaks of fluo-

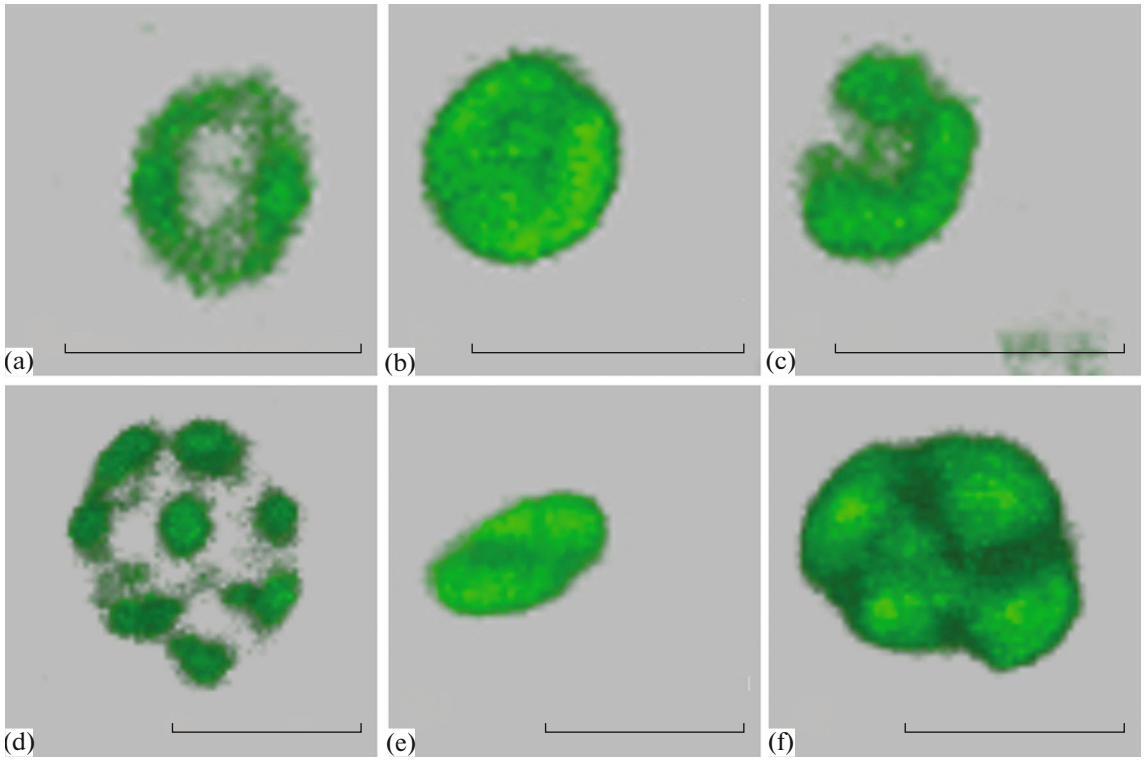

Fig. 3. Distribution pattern of nucleophosmin (protein B23) in the nucleoli of tanycytes. Confocal laser microscopy. 3D reconstruction. (a, b, c) α1-tanycytes, old animals; (d, f) β1- tanycytes, old animals; (e) α1-tanycytes, day 7 of postnatal development. Scale bar, 2 μm.

rescence intensity of these proteins do not coincide: the peak of fluorescence intensity of nucleolin is closer to the center compared to that of nucleophosmin, which is drawn toward the periphery of the nucleolus (Fig. 4a). Such distribution is probably due to the fact that, though both proteins participate in ribosome biogenesis, they are also involved in different stages of the assembly of proribosomal particles. For example, nucleolin has a high affinity to single-stranded rDNA in the nontranscribed spacer region of rDNA localized upstream of the transcription start site and, consequently, regulates rRNA transcription; this protein is also necessary for rRNA maturation and correct assembly of ribosomal particles, i.e., is involved in the early stages of maturation of ribosomal subunits [20, 22]. Nucleophosmin regulates the late stages of maturation of ribosomal particles: it regulates rRNA reprocessing, acting as an endoribonuclease, is responsible for the release of 28S rRNA through binding to the second internal transcribed spacer (ITS2) of pre-RNA and involved in the export of ribosomal subunits to the cytoplasm [23, 24]. The absence of response or weak response to proteins under study in the central regions of the nucleolus may be due to the fact that the fibrillar center (FC) is often localized here, which usually lacks the proteins under study, as can be seen most clearly in large neurons where the giant FC has been described [25].

It is known that C23 is a multifunctional protein detected in the nucleolus, the nucleoplasm, the cytoplasm, and on the plasma membrane of different cells. Various posttranslational modifications and shuttling of nucleolin mediate its multiple functions. It has been shown that this protein, in addition to participation in ribosome biogenesis, is involved in chromatin organization and stability, DNA and RNA metabolism, cytokinesis, cell proliferation and survival, angiogenesis, apoptotic regulation, stress response and microRNA processing, participation in the intercellular signaling, as well as some pathological processes [20, 25, 26]. The present study has shown that nucleolin in tanycytes is localized, in addition to the nucleolus, in the nucleoplasm as diffusely distributed lumps, but not in the cytoplasm nor on the cell plasma membrane. Nonuniform distribution of nucleolin within the nucleoplasm is probably due to the fact that this protein can act as a histone chaperone and locally regulate transcriptional activities of different genes. At the same time, the absence of this protein in the cytoplasm and on the plasma membrane can be associated either with insufficient sensitivity of the immunohistochemical method for detecting low levels of the protein in these cellular compartments, or with the fact that in tanycytes it is involved mainly in the nucleolar and nuclear processes.

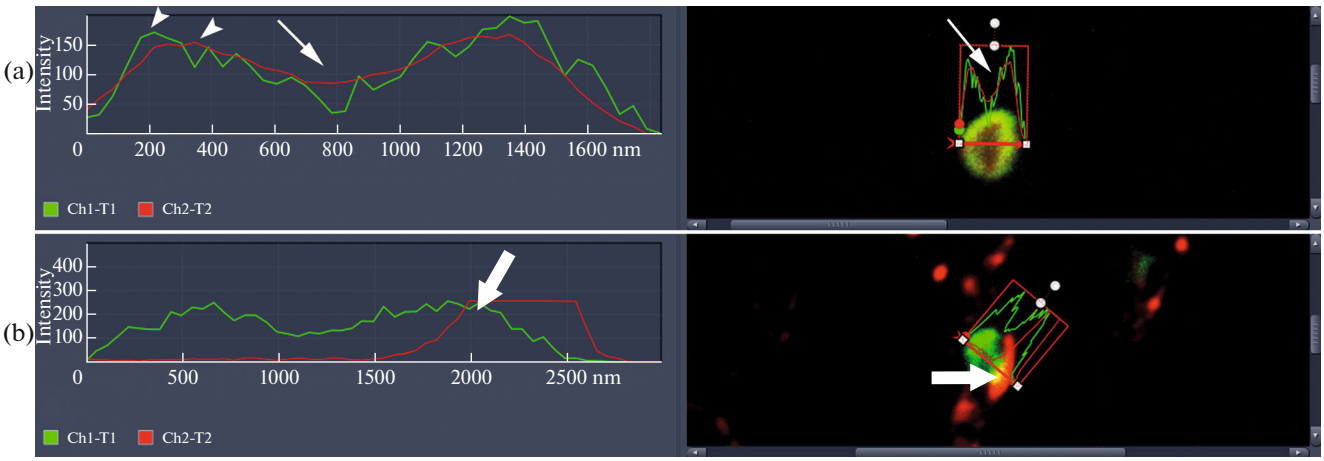

Fig. 4. The fluorescence intensity profile of proteins under study in the nucleolus of tanycytes. Confocal laser microscopy. A single optical section. (a) Distribution of argentophilic proteins nucleolin (*red*) and nucleophosmin (*green*) in the nucleolus of tanycytes in old animals (20–24 months). *Arrow* shows the region of the minimum fluorescence of both proteins. *Arrowhead* points to the fluorescence peaks of the green and red channels. (b) The zone of H4K20me3 (*red*) and nucleophosmin (*green*) co-localization in the nucleolus of tanycytes in old animals (20–24 months). The area of co-localization (*yellow*) is denoted with a *thick arrow*.

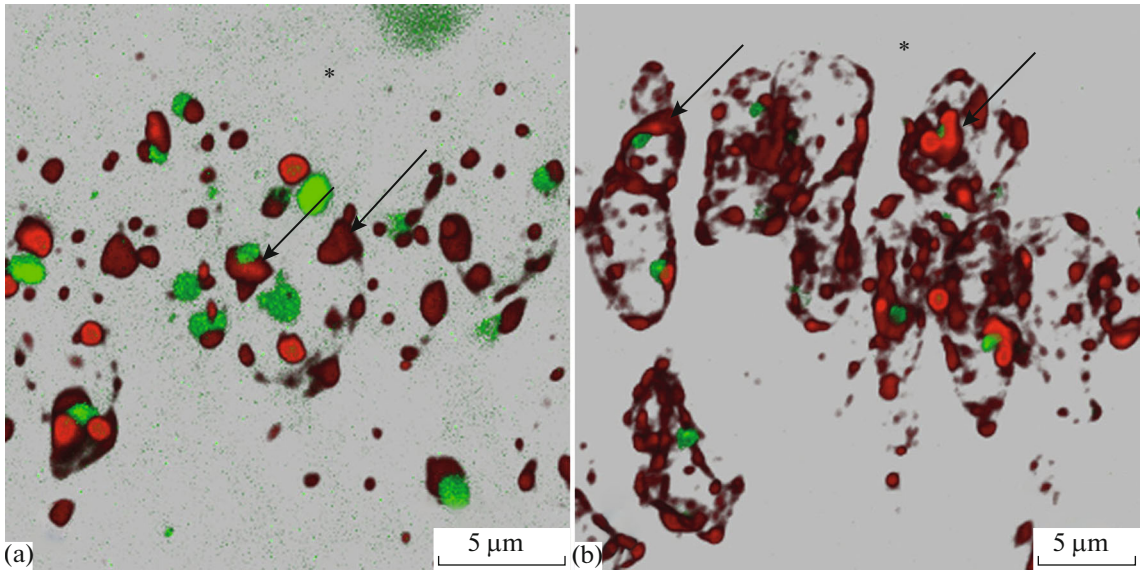

Fig. 5. The distribution of heterochromatin aggregates in α1-tanycytes. Double immunohistochemical staining with H4K20me3 (*red*) and nucleophosmin (*green*). 3D reconstruction of 28 (a) and 29 (b) optical sections (transparent mode). Objective: alpha Plan-Apochromat 100×/1.46 Oil DIC M27 (oil immersion). (a) Day 7 of postnatal development; (b) old animal (20–24 months). *Arrows* point to heterochromatin aggregates. *Asterisk* is the ventricular cavity.

In turn, nucleophosmin is detected only within the nucleolus of cells under study. According to the literary sources, nucleophosmin is localized in the nucleolus (80% of total protein content) and in the nucleoplasm (20% of total protein content), as well as can shuttle between the nucleolus, the cytoplasm and the plasma membrane transporting various proteins. There are two isoforms of nucleophosmin: B23.1 and B23.2. The N-terminal and central domains in these isoforms are identical, while the C-terminal domain (containing the nucleolar localization signal, NoLS) is present only in isoform B23.1. This fact determines preferential localization of isoforms B23.1 and B23.2 in the nucleolus and the nucleoplasm, respectively [27]. The antibodies used in the present study allowed us to detect the C-terminal region of protein B23, i.e., isoform B23.1, which accounts for its identification only within the nucleolus of tanycytes.

Distribution of heterochromatin aggregates is also an important characteristic of the cell nucleus. Heterochromatin is a component of several nuclear subcom-

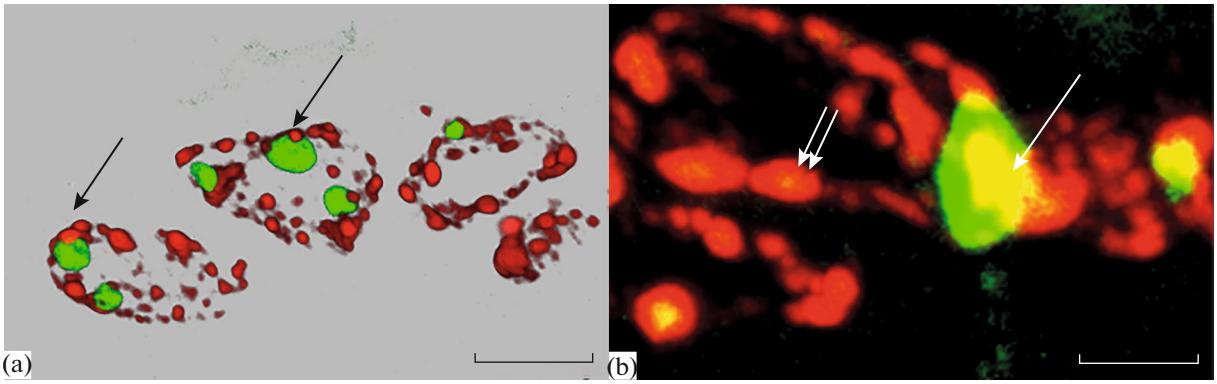

Fig. 6. The perinucleolar heterochromatin in β2 tanycytes. Double immunohistochemical staining with H4K20me3 (*red*) and nucleophosmin (*green*). 3D reconstruction of 24 optical sections: (a) transparent mode; (b) maximum mode. (a) *Arrows* point to perinucleolar heterochromatin. Scale bar, 5 μm. (b) *Arrow* denotes perinucleolar heterochromatin forming invagination into the nucleolus. *Double arrow* denotes heterochromatin aggregates in the periphery of the nucleus of tanycytes. Scale bar, 2 μm.

partments such as lamina-associated domains, telomeric and centromeric regions, perinucleolar heterochromatin, etc. The present study has shown an age-related increase in the sizes of heterochromatin aggregates in the nuclei of α1-tanycytes, though the level of heterochromatin in β-tanycytes varies insignificantly. The literature data on age-related changes in the level of constitutive heterochromatin are contradictory. According to some data, there is a considerable age-related decrease in heterochromatization in different types of cells, the so-called heterochromatin loss model of aging [28, 29]. However, Lezhava [30] has shown intensive heterochromatinization of chromosomes in human lymphocytes with aging. Supposedly, in this case the age-related (after 70 years) accumulation of chromosomal aberrations and aneuploidia occurs as a result of active heterochromatization of DNA and physically impossible access of preparative enzymes to these regions. Progressive age-dependent heterochromatinization contributes to inactivation of some of the previously functioning "active genes". It leads to the block of certain stages of metabolic processes in cellular systems, which occur under normal conditions; as a result, the deficiency of many specific enzymes sooner or later leads to age-related pathologies [30]. Probably, α-tanycytes are also the type of cells undergoing heterochromatinization with aging, which may affect their functions. In this respect, β-tanycytes with aging are characterized by stable organization of the studied cell nucleus compartments, probably due to their unique localization in the area of the median eminence, where fenestrated capillaries (i.e., those lacking the blood-brain barrier) are localized. It seems to be necessary not only for the normal function of β-tanycytes, but also for the adequate work of the hypothalamic-pituitary axis in general.

The study of spatial relationships between nucleoli and heterochromatin has shown that the nucleoli of all types of tanycytes in all periods under study are closely associated with perinucleolar constitutive heterochromatin. The co-localization zone formed in the area of their contact can be evidence of structural and functional interactions between these two compartments. It is known that nucleophosmin contains binding sites for H3-H4 and H2A-H2B histone tetramers, as well as H1 linker histone. When binding to histone, protein B23 acts as a histone chaperone and participates in the formation of a nucleosomal particle [31]. Previously, Lafarga et al. [32] have shown that perinucleolar heterochromatin in some cases forms a chromatine pedicle, which passes through DFC and touches the fibrillar center of the nucleolus. We have also found out that single cells contain perinucleolar heterochromatin, which is invaginated deep into the nucleolus.

Thus, the present study is the first comparative ontogenetic analysis of nucleoli and constitutive heterochromatin in different types of tanycytes during postnatal development and aging. This work has shown heterogeneity in the size and number of nucleoli not only between different subpopulations of tanycytes, but also in the age-related aspect, which can be evidence of different functional status of these cells. The age-related increase in the size and decrease in the quantity of nucleoli have been shown, suggesting the natural increase in synthetic activity in the course of cell formation during early postnatal development. Distribution of the major argentophilic proteins of the nucleolus, nucleolin and nucleophosmin, as well as constitutive heterochromatin, in tanycytes at different stages of development has been described for the first time. It has been demonstrated that nucleolin and nucleophosmin have different spatial localizations within the nucleolus. The distribution of heterochromatin aggregates varies between tanycyte subpopulations with aging: α-tanycytes undergo intensive heterochromatinization, while β-tanycytes are characterized by stable organization of compartments of the cell nucleus under study. The findings significantly complement the modern concept of organization of the nucleolar apparatus and heterochromatin in tanycytes

during normal development and aging, which provides conditions for determining the role of these subnuclear structures in pathological processes under damaging effects of different kinds.

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COMPLIANCE WITH ETHICAL STANDARDS

The authors declare that they have no conflict of interest.

All procedures were performed in accordance with the European Communities Council Directive (November 24, 1986; 86/609/EEC) and the Declaration on humane treatment of animals. The protocol of experiments was approved by the Commission on Bioethics of the Institute of Experimental Medicine.

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