Structure and Functional Loading of Cerebral Arterial Smooth Myocyte Population

A. N. Gansburgsky and A. V. Jaltzev

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 147, No. 6, pp. 708-711, June, 2009
Original article submitted October 29, 2008

The cerebral arterial smooth myocytes of dogs with experimental hypertension (n=12), its correction (n=10), and of intact dogs (n=8) were studied. The structure of myocyte population changed under conditions of vascular wall hyperfunction: the size of the cytoplasm and nuclei, DNA content in the nuclei, mitotic activity, and percentage of binuclear forms increased. Thickening of the tunica media in intraorgan arteries was caused by such cytological mechanisms as leiomyocyte hyperplasia and hypertrophy. The cells shrank after elimination of hypertension, while vascular polyploidy persisted.

Key Words: *hypertension; disease correction; smooth myocytes; mitotic activity; polyploidy*

We previously showed that changes in functional load of the coronary arterial wall after correction of hypertension did not lead to disappearance of the vascular myocyte polyploidy, which had developed during hypertension [5]. Study of the cerebral arterial smooth myocytes (SM) during hypertension and after its correction is essential for theoretical and practical medicine [7,9,12], because this structural component largely determines the strength of the vessels [6,14]. Simulation of aortic coarctation on animals, leading to hyperfunction of the cerebral arterial walls [2,13], and its subsequent correction open new vistas in this trend of research.

We studied the structure of the cerebral arterial SM population under conditions of their different functional load.

MATERIALS AND METHODS

Coarctation of the aorta was modeled surgically [8] in 22 dogs aged 3-4 months. After 12 months, the defect was repaired in 10 of them and observation was continued for another 12 months. Intact animals served as controls (n=8). The animals were sacrificed by bleeding (under narcosis). Smooth myocytes from the middle cerebral artery were isolated by alkaline dissociation [1], the preparations were stained with hematoxylin and eosin and after Feulgen (hydrolysis in 5 N HCl at 37°C for 12 min). Linear dimensions of SM and their nuclei were measured by a screw ocular micrometer and their areas and volumes were calculated [10]. The mitotic index and percentage of binuclear forms were evaluated (3000 cells were analyzed). The content of DNA in the nuclei of mononuclear and binuclear myocytes was analyzed on a MIF-K cytophotometer at λ=580 nm. The data were processed by variation statistics methods.

RESULTS

The size of SM and their nuclei in the tunica media of cerebral artery increased in animals with experimental hypertension (Fig. 1, *b*) in comparison with the control (Fig. 1, *a*), which was confirmed by the results of cyto- and karyometry (Table 1). The length of SM increased 1.2 times, transverse section 1.6 times, area and volume 1.9 and 3 times, respectively. The same picture was observed for SM nuclei: the length increased 1.1

Department of Histology, Cytology, and Embryology, Department of Pathology with a Course of Section, Yaroslavl State Medical Academy, the Russian Ministry of Public Health, Russia. ***Address for correspondence:*** Pavlov@hist.yma.ac.ru. A. N. Gansburgsky

0007-4888/09/1476-0764

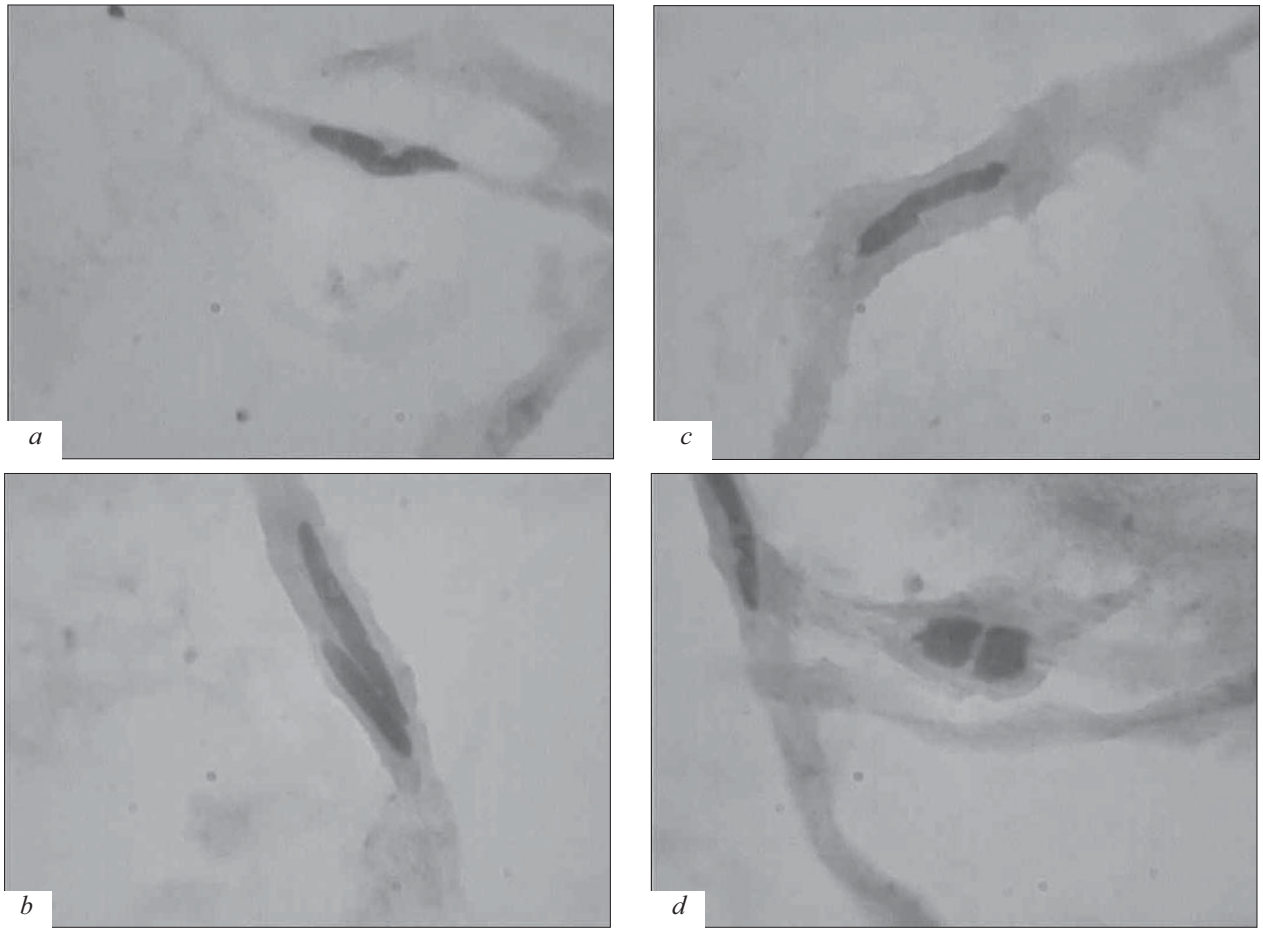

Fig. 1. Isolated SM from the middle cerebral artery in the control (*a*) and in hypertension (period of observation 12 months): *b*) myocyte hypertrophy; *c*) binuclear cell; *d*) mitosis telophase. Alkaline dissociation. Hematoxylin and eosin staining, ×1000.

times, transverse section 1.5 times, area and volume of the nuclei 1.7 and 2.8 times, respectively. Cytophotometry showed a 2-fold increase in DNA content in mononuclear myocytes (Fig. 2). Smooth myocytes divided by mitosis (Fig. 1, *d*). Mitotic activity reached 0.21% (no mitoses were detected in the control). This was paralleled by increment in the percentage of binuclears in the SM population (Fig. 1, *c*): the percent of these cells in the control was just 0.3% *vs.* 2.9% (10-fold more) after defect modeling. These changes reflected hypertrophy, hyperplasia, and polyploidy in the structure of SM population of the cerebral artery tunica media under conditions of long-term hyperfunction caused by hypertension.

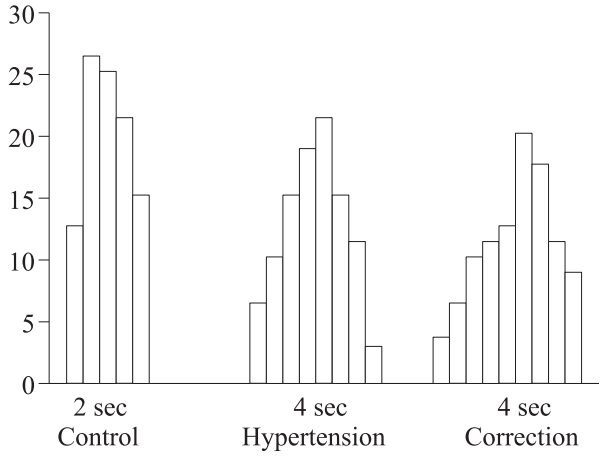

Fig. 2. Content of DNA in the SM nuclei of the dog middle cerebral artery tunica media. Ordinate: percentage of mononuclear myocytes. Bars: classes of DNA-synthesizing nuclei of mononuclear smooth myocytes.

Surgical repair of experimental hypertension led to partial normalization of the morphometric values in the SM population (Table 1): the length and transverse section of cells decreased 1.1 and 1.2 times, the area and volume 1.3 and 1.6 times, respectively. However, the values did not reach the control level and were 1.4 and 1.8 times higher for the area and volume, respectively. This trend was detected in measurements of the myocyte nuclei: the length and transverse section decreased 1.1 and 1.3 times, area and volume 1.3 and 1.7 times, respectively, but still 1.2- and 1.4-fold surpassed the control values. No proliferation of SM

TABLE 1. Cyto- and Caryometry of Smooth Myocytes of the Middle Cerebral Artery in the Control and Experimental Animals (*M±m*)

Group	Cell length, μ	Transverse section of cell, μ	Nucleus length, μ	Transverse section of nucleus, μ	Cell area, μ^2	Cell volume, μ^3	Nucleus area, μ^2	Nucleus volume, μ^3
Control	46.4±1.4	13.9±0.3	15.7±0.2	6.4±0.1	506±10.0	4710±64.0	79.0±2.5	336±7.0
Arterial hypertension	55.0±1.7*	22.0±0.5*	18.0±0.4*	9.2±0.2*	950±14.0*	13 920±94.0*	127±4.0*	763±10.0*
One year after elimination of arterial hypertension	50.0±1.8*++	18.0±0.7*+++	16.5±0.5*+	7.3±0.4*++	707±12.0*+++	8470±81.0*+++	95.0±4.4*++	460±8.0*+++

Note. *$p<0.01$, **$p<0.02$ compared to the control; +$p<0.05$, ++$p<0.02$, +++$p<0.01$ compared to arterial hypertension.

was noted. Hence, reduction of the functional load of vascular wall after correction of long-standing cerebral hypertension promoted reversibility of the cerebral arterial SM hypertrophy. On the other hand, DNA content in the nuclei (Fig. 2) and content of binuclear elements (3%) virtually did not change in myocyte population after correction of the defect.

The study showed that experimental hypertension causes changes in the structure of SM population in the middle cerebral arteries tunica media: development of hypertrophy, proliferation, and polyploidy processes. This phenomenon was also detected in SM of coronary arteries [5]. Hence, previously detected thickening [10] of the tunica media of intraorgan arteries under conditions of hyperfunctioning is caused by cytological mechanisms: SM hyperplasia and hypertrophy.

When discussing the mechanism of polyploid transformation of smooth muscle tissue it was emphasized that the increase of tangent tension in the vessels is paralleled by activation of DNA synthesis in myocyte nuclei. The present study revealed an increase in the count of binuclear SM and of mitotic activity in the cerebral arteries under conditions of hypertension. Various destructive factors lead to an increase in the content of binuclear forms in cell populations of the vascular wall [3], which is regarded as a variant of polyploidy [11]. We found that SM population was most reactive after cryodestruction of the rat aorta: the index of labeled nuclei increased more than 50-fold in comparison with the control [4]. Hence, hyperfunction of the vessels (hypertension) leads to the formation of polyploid SM as a result of polyploidizing mitosis, a variant of incomplete mitosis [11]. Our findings also indicate mitotic origin of binuclear elements: the proportion between DNA content in the nuclei of the same binuclear vascular myocyte is close to 1. Replication of DNA provides cell hyperplasia or polyploidy, underlying the intracellular regeneration processes [7].

Alleviation of the functional load of the cerebral arterial wall leads to changes in the SM population structure, similar to those developing after correction of hypertension in the coronary arteries. The size of vascular myocytes and their mitotic activity change depending on the functional loading. SM polyploidy (increase in DNA content and of percentage of binuclear cells), resultant from hyperfunction, persists after reduction of the loading. Irreversibility of polyploidy in cell populations is confirmed in published reports [11].

REFERENCES

1. V. Ya. Belov, M. E. Kogan, and T. A. Leontyeva, *Tsitologiya*, **117**, No. 11, 1332-1338 (1975).

2. N. A. Belokon' and V. P. Podzolkov, *Congenital Heart Diseases* [in Russian], Moscow (1991).
3. A. N. Gansburgsky and A. V. Pavlov, *Ontogenez*, **24**, No. 6, 79-82 (1993).
4. A. N. Gansburgsky and A. V. Pavlov, *Morfologiya*, **113**, No. 2, 66-70 (1998).
5. A. N. Gansburgsky and A. V. Jaltzev, *Byull. Eksp. Biol. Med.*, **138**, No. 11, 589-591 (2004).
6. A. L. Zashikhin and Ya. Selin, *Visceral Smooth Muscle Tissue* [in Russian], Archangelsk-Umeo (2001).
7. D. S. Sarkisov, M. A. Pal'tsev, and N. K. Khitrov, *Common Human Pathology* [in Russian], Moscow (1997).
8. S. V. Shormanov and A. V. Jaltzev, *Byull. Eksp. Biol. Med.*, **123**, No. 5, 591-593 (1997).
9. S. V. Shormanov A. V. Jaltzev, I. S. Shormanov, and S. V. Kulikov, *Morfologiya*, **131**, No. 1, 44-49 (2007).
10. A. V. Jaltzev, *Ibid.*, **122**, No. 6, 34-37 (2002).
11. V. Ya. Brodsky and I. V. Uryvaeva, *Genome Multiplication in Growth and Development*, Cambridge (1985).
12. H. T. Patel, A. Madani, and Y. M. Paris, *Pediatr. Cardiol.*, **22**, No. 1, 53-57 (2001).
13. P. S. Rao, *Semil. Nephrol.*, **15**, No. 2, 87-105 (1995).
14. H. Veno, P. Kanellaris, and A. Agrotis, *Hypertension*, **36**, No. 1, 89-96 (2000).