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Neurohistological alterations in the superior cervical ganglia following ischemic stroke

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Background. The sympathetic nervous system is a primary candidate for neurogenic cerebral blood volume control through the release of norepinephrine and / or neuropeptide Y from perivascular sympathetic fibers. Cerebral vessels are richly supplied by sympathetic fibres whose predominant sources originate in the superior cervical ganglion (SCG).

Methods. We performed neurohistological examination on 8 μm-thick paraffin section stained with cresyl violet and on 1 μm-thick epoxy resin section stained with methylene blue bilateral samples of the SCG obtained from 4 stroke patients, two of whom died on 6th and 9th day after the stroke onset, and the other two, in 3 and 3.5 months.

Results. In all the cases, the ganglia contained individual neurons or neuron clusters whose perikaryon had an increased affinity to basic dyes. Lymphocytic infiltrates were detected at the sites of the concentration of dark-stained neurons. In addition to that, the ganglia of the subjects who died in 3 and 3.5 months following the stroke onset contained neuron clusters some of which had signs of dying nerve cells, whereas the others had typical signs of the regeneration of nerve cells – swelling of the cell body, dispersion of the Nissle substance and alteration of the satellite glial cells. The signs of neurodegenerative alteration were more pronounced in the ganglia of the stroke-affected side.

Conclusion. We associated these histopathological findings with a retrograde reaction of the neuronal cell body to axonal damage, which occurs in the ischemic focus of blood vessels innervated by the SCG. The relationship of these finding with the pathological axotomy is discussed.

Key words: superior cervical ganglion, neurohistology, alteration, ischemic stroke, human, autopsy

INTRODUCTION

Neurohistological studies have shown that human cerebral arteries are supplied by extensive perivascular nerve plexuses (1, 2). In the latter studies, the use of denervation and retrograde tracing in combination with histochemical and immunohistochemical techniques has demonstrated that these perivascular plexuses contain sympathetic fibres storing norepinephrine (NA) and neuropeptide Y (NPY) (3–6). The varicosities of the terminals of these fibres containing NA and NPY are situated at 200–300 nm from the muscular cells of the media (7, 8). It has been demonstrated that the density of the sympathetic terminals on the surface of the media, the amount of varicosities in it, and the distance between varicosities and the muscular cells of the media in the arteries at the base of the brain is greater than that in the pial arteries (7, 8). These neuroanatomical indices correlate with the vasomotor effect of the sympathetic neural control (8, 9).

The predominant source of the sympathetic innervation of cerebral arteries is bilateral SCG (3, 10). These ganglia are important peripheral centres of the autonomic regulation of cerebral blood circulation (11–13). Therefore, we present our findings of a neurohistological study of the SCG obtained from four patients affected by ischemic stroke.

METHODS

The data on age, sex, survival, results of the neuropathological examination of the brain of stroke patients taken from their case histories and autopsy records, as well as histopathological findings in the SCG are presented in the Table.

The SCG were obtained during autopsy and divided into the upper, the middle, and the lower thirds. The specimens for staining with cresyl violet and hematoxylin-eosin were fixed by immersing them into 4% 0.1 M (100 mol/m^3) phosphate buffer (pH 7.4) paraformaldehyde solution at the temperature of 18 °C for 24–28 hours, embedded in paraffin and cut on 8 μm-thick section. Semithin of 1 μm sections was stained with methylene blue

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Table. **Age, sex, survival, pathological findings in the brain of stroke patients and histopathological findings in their superior cervical ganglia**

	Patient 1	Patient 2	Patient 3	Patient 4
Age and sex	68 year, male	77 year, male	69 year, female	74 year, male
Survival after ischemic stroke	8 days	9 days	3 months	4 days (3.5 months after the first stroke)
Time period from death to autopsy	8 hours	5 hours	4 hours	3.5 hours
Pathological findings in the brain	Infarction in the stage of resorption in the right frontoparietal region	Infarction in the stage of resorption in the left frontoparietal region	Cystis and gliosis in the left temporooccipital region	Recent infarction with perivascular hemorrhage in the brainstem. Cystis and gliosis in the right temporooccipital region
Histopathological findings in ganglia Right side ganglion				
Light neurons, m ± SD	10.1 ± 4.8	12.4 ± 4.6	8.3 ± 3.1	16.2 ± 11.6
Dark neurons, m ± SD	27.4 ± 14.2*	13.0 ± 7.6	6.9 ± 5.1	13.0 ± 15.7
Left side ganglion				
Light neurons, m ± SD	10.8 ± 7.7	7.6 ± 5.6	4.4 ± 4.8	21.1 ± 11.1
Dark neurons, m ± SD	8.7 ± 10.5	21.5 ± 10.9*	15.2 ± 8.8*	17.2 ± 11.6
Neurodegenerative signs of the neuron death				
Right side ganglion	Not apparent	Not apparent	Petty areas in middle thirds	Petty areas in middle thirds
Left side ganglion	Not apparent	Not apparent	Petty areas in middle thirds	Not apparent

* $p < 0.001$ comparing mean numbers of the light and dark neurons.

Note. In all the cases, the lumen of the arteries at the base of the brain was narrower by, on the average, 40–50% due to atherosclerotic plaque.

according to Ridgeway. For this purpose specimens were fixed in a mixture containing 2% paraformaldehyde and 2.5% glutaraldehyde in 0.1 M (100 mol/m^3) cacodylate buffer (pH 7.4) for at least 4 hours at room temperature. Afterwards, the samples were post fixed for 2 hours with 1% osmium tetroxide solution in 0.1 M (100 mol/m^3) cacodylate buffer (pH 7.4), then dehydrated and embedded in the mixture of Epon 812 and Araldite.

We have noticed that the affinity of neurons to cresyl violet was not uniform: in some neurons the cell body was stained light with a clearly visible nucleus and the composition of the Nissl substance, while in other nerve cells the staining was intense. The neurons of the first type were thus attributed to the group of light neurons, and the others, to the group of dark neurons. We evaluated the quantitative ratio of these neurons by calculating such neurons in every sixth serial section of the middle third of the ganglion in randomly selected visual fields of the microscope (at the magnification of 250x; Jeneval, Carl Zeiss Germany microscope). Since the distributions of the data obtained were not suitable for a normal distribution test, the evaluation of the difference in the number of light and dark neurons was performed using Wilcoxon Signed Ranks Tests. Statistical analysis was performed using SPSS 12 software package.

RESULTS

The neurohistological examination revealed that in all the cases sympathetic ganglia contained individual neurons or neuron clusters whose cytoplasm had an increased affinity to basic dyes (cresyl violet or methylene blue) (Figs. 1A, 1B, Fig. 2A). The neurons with hyperchromophilic characteristics had slight deformities of the perikaryon. The foci of lymphocytic infiltrates were detected in the clusters of such neurons as well as around the adjacent fine blood vessels (Fig. 1C). In the first three cases, ganglia from the side affected by stroke contained 2.5, 2.8 and 3.5 times more dark neurons, respectively, than the light ones (Table). Meanwhile, there was no significant difference between the amount of dark and light neurons in the ganglia situated on the opposite side from that affected by stroke and in the ganglia of both sides in case No. 4 (Table).

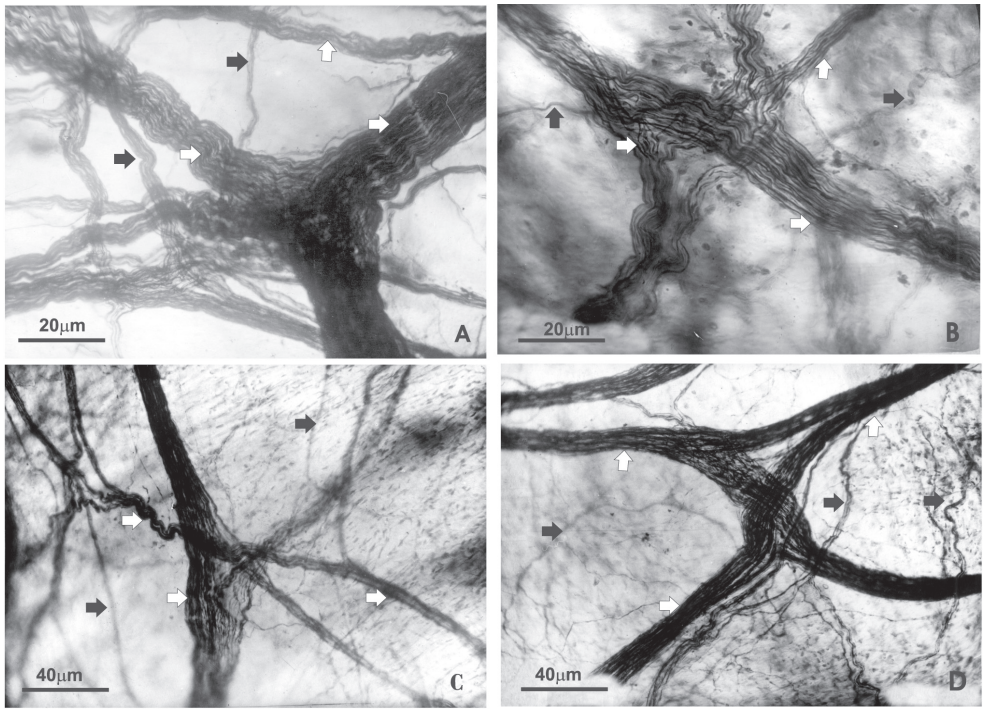

Fig. 1. Perivascular nerve plexuses of the human arteries at the base of the brain. The superficial layer (white arrow) is composed of nerves of various thicknesses, extending to the pial and intraparenchymal arteries. The nerve fibres of deep layer (black arrow) are situated at the adventitial-medial border. A: basilar, B: posterior communicating, C: middle cerebral, D: anterior cerebral arteries. Bielschewsky's silver impregnation method (Preparations supplied from the doctoral dissertations of Stropus R and Tamasauskas K from the Institute of Anatomy, Kaunas University of Medicine)

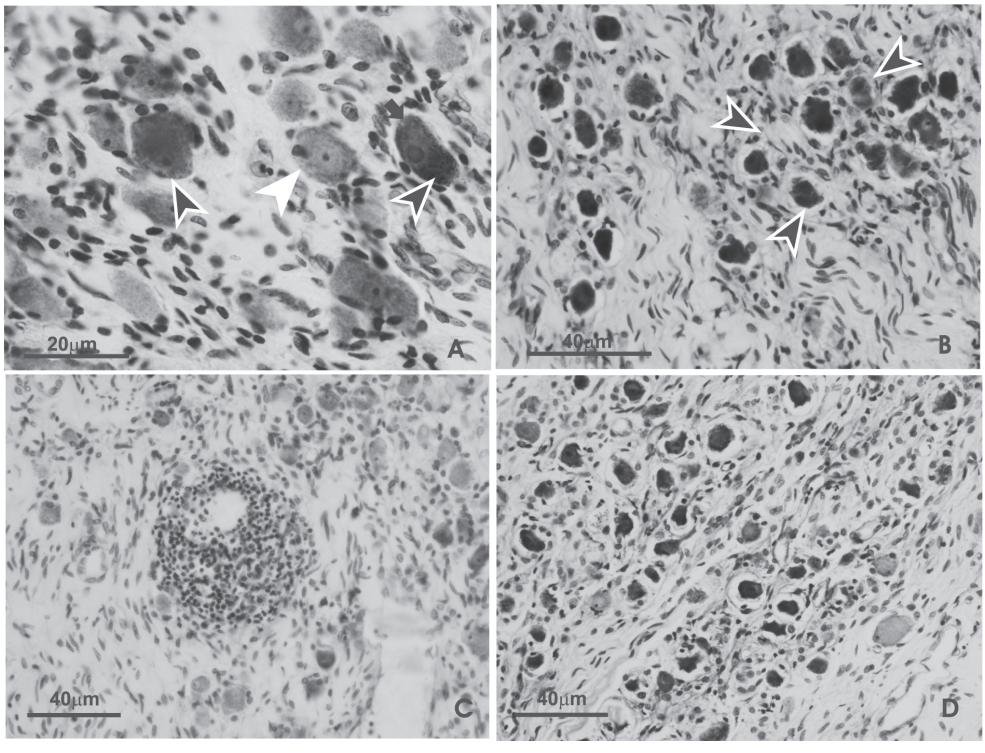

Fig. 2. Signs of neuropathological alteration detected by staining paraffin section with cresyl violet in the ganglia of the stroke-affected side. A: individual dark-stained (black arrowhead) and light-stained (white arrowhead) neurons. B: neuron clusters (arrowheads) whose cytoplasm had increased affinity to cresyl violet and lymphocytic infiltrates in such clusters. C: lymphocytic infiltrates around the adjacent fine blood vessels. D: petty focus of neurodegenerative signs. A, B: patient 2. C: patient 3 and D: patient 4.

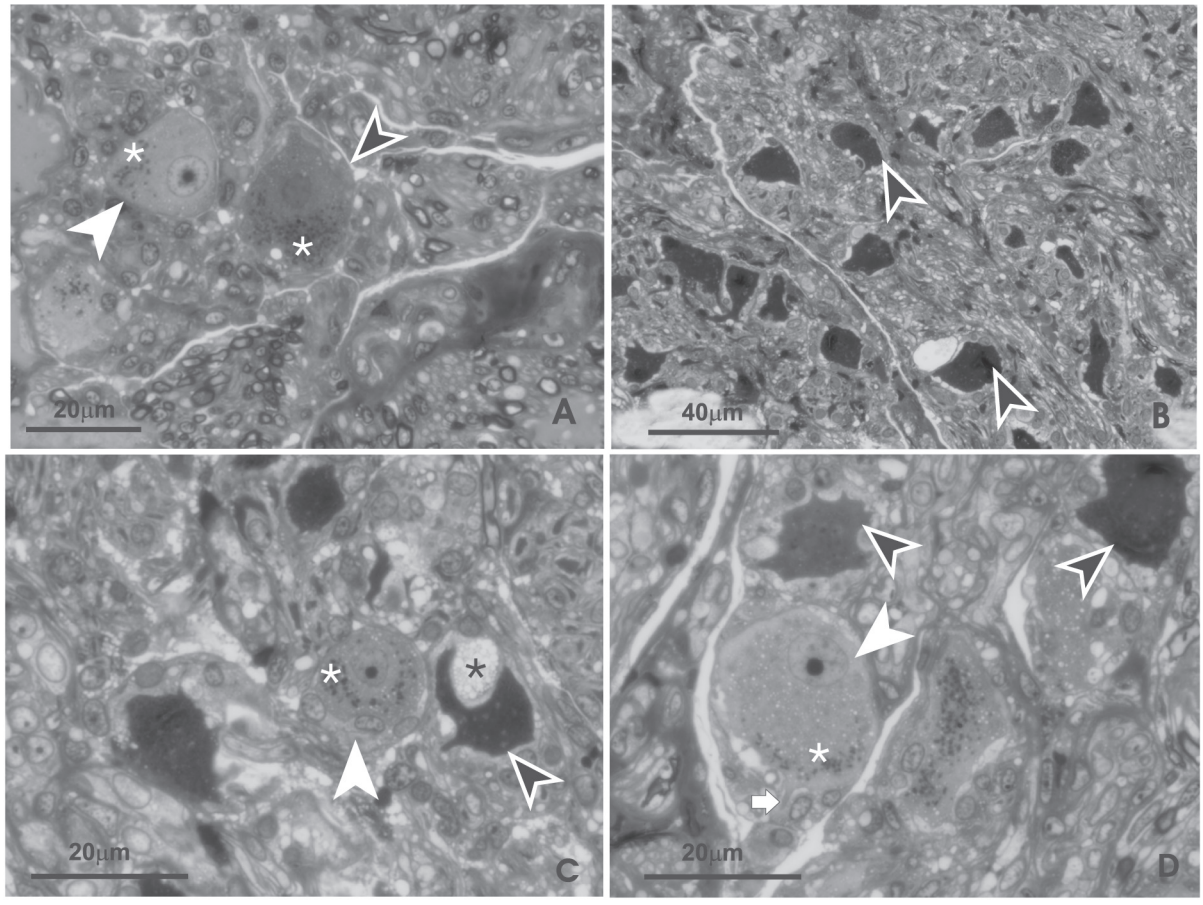

Fig. 3. Signs of neurodegenerative alteration detected by staining epoxy resin 1 μm section with methylene blue in the ganglia of the stroke-affected side. A: dark stained (black arrowhead) and light stained (white arrowhead) neurons. B: petty focus neurodegenerative signs of neuron death. C: neuron with signs of death – homogenously stained and deformed cell body (black arrowhead), large vacuoles outside its margin (black asterisk); there is an unaffected neuron beside (white arrowhead). D: neuron with signs of hypertrophy (white arrowhead) – swollen cell body, chromatolytic changes and vacuoles in cytoplasm, proliferations of satellite cells (white arrow); beside neurons with signs of death (black arrowheads). A: patient 1, B, C: patient 3 and D: patient 4.

In two patients, one of whom died in 3 months following the stroke and the other one on the 4th day after the recurrent stroke but having survived for 3.5 months after the first stroke (case No. 4), the middle thirds of the ganglia on the side affected by stroke contained clusters of neurons with typical signs of nerve cell death: intensely and homogenously stained and deformed cell bodies, and vacuoles in the perikaryon or outside its margins (Fig. 1D, Figs. 2B, 2C). Besides, there were individual cells with signs of hypertrophy: swollen cell body, containing microvacuoles, and light-stained cytoplasm (Fig. 2D). Proliferation of the satellite glial cells was detected in the environment of these cells. A focus of similarly changed neurons, only smaller in size, was detected in the middle portion of the ganglion taken from the side opposite to that affected by stroke in patient 3.

DISCUSSION

In all the SCG taken from the stroke patients who survived for various periods of time after the stroke onset, the present study revealed the dual signs of neurodegenerative alteration. Some signs, such as the increased affinity of the cytoplasm to basic dyes in individual neurons and neuron clusters, and lymphocytic infiltrates in the environment of such neurons, were detected in all the ganglia, whereas the others, such as the signs of neuron death and regeneration, were detected only in the ganglia of the patients who survived for 3 months after the stroke.

In the first three cases the detected signs of the neuropathological alteration of the ganglia were more pronounced in the ganglia located on the side affected by stroke. We associate such asymmetry of the manifestations of changes with the specific features of the sympathetic innervation of cerebral arteries. It has been demonstrated that the majority of neural fibres from the SCG of one side extend to the arteries of cerebral hemispheres ipsilaterally while few do contralaterally (3, 10). These findings could explain why similar distribution of light and dark neurons was detected in the SCG of both sides in patient No. 4. In this case, the focus of 2nd ischemic stroke that resulted in the patient's death was located in the brainstem, i.e. in the pool of the basilar artery whose perivascular plexus contains fibres of the SCG of both sides, reaching the artery through analogous plexuses of vertebral arteries (1).

We failed to find any reports on the investigation of the associations of the signs of neurological alteration in sympathetic ganglia with ischemic stroke. However, there are a number of

reports (15–18) indicating that with age and in the presence of certain diseases structural changes of the parenchyma and stroma develop in human autonomic ganglia, including SCG. Schmidt (17) emphasized that neurodegenerative alteration is most pronounced not in the nerve cell body itself, but rather in the terminal part of the sympathetic axons located inside the ganglion.

We did not find any reports describing the changes in the tinctorial properties of the cytoplasm of the neurons of human SCG in the presence of pathology. However, it was noticed that in rats, during the course of postnatal development of the SCG, the affinity of the cytoplasm of degenerating nerve cells to basic dyes increases, and therefore these cells become pyknotic (19). In clinical and experimental neuropathology, an increased affinity of the cytoplasm of the central nervous system neurons to several histological stains is known very well. These nerve cells were called dark, collapsed, argyrophilic or eosinophilic neurons (20–23). This study revealed that dark neurons are generated *in vivo* as acute or delayed consequence of several pathological situations. By now only one theory (21) explains their appearance: an initial and localized damage is extending to all the neuronal structures producing a marked disorganization with similar morphological characteristics.

More pronounced signs of the neurodegenerative alteration revealed in the ganglia of the side affected by stroke allow hypothesizing that this process may be associated with the retrograde reaction of nerve cell bodies to hypoxic damage of their axons that innervate the arteries in the focus of ischemia. Studies on the sympathetic innervation of an experimental myocardial infarction (24) and radionuclide imaging of the hearts of patients who had an infarction (25) indicate that the sensitivity of the sympathetic nerve fibres to ischemia is greater than that of the myocardium, which results in the ischemic zone becoming desympathized. This hypothesis is also supported by the similarity of the alteration signs of neurons following experimental axotomy to those detected in the SCG of stroke patients. It has been found that during the first two weeks after axotomy, changes in somal ribonucleoprotein and protein synthesis of neurons result in alterations in the affinity of the cytoplasm of nerve cells to basic dyes, the formation of microvacuoles in the mitochondria, and the enlargement of nerve cell body due to swelling (26–28). Reaction of the neurons affected by axotomy is accompanied by proliferation of satellite cells and lymphocytic infiltrates (29, 30). Neurons maintain such a condition until their axons reach the target organ again (29). In cases when the axon reinnervation process is disturbed, the structural changes of the early reaction of the nerve cell to axotomy progress to the death of neurons (29, 30).

CONCLUSION

Our findings suggest that ischemic stroke is related to neurodegenerative alteration of neuronal and non-neuronal cells of the SCG, and that this process is not limited to the acute period of the disease, since signs of death and regeneration of neurons were detected after 3 month following the onset of the disease. We advance a hypothesis that these alterations are related to pathological axotomy. This has to be taken into consideration while evaluating the mechanisms of neural regulation of cerebral blood circulation in stroke patients.

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VIRŠUTINIO KAKLINIO SIMPATINIO MAZGO NEUROHISTOLOGINIAI POKYČIAI PO SMEGENŲ INSULTO

S a n t r a u k a

Įžanga. Eksperimentiniais ir klinikiniais tyrimais įrodyta, kad simpatinė nervų sistema yra labai svarbi kraujotakos reguliavimui. Ši funkcija vykdoma per smegenų arterijų išorinio sluoksnio nervinio rezginio gausias simpatines skaidulas, kurių daugiausiai ateina iš viršutinio kaklinio simpatinio mazgo (VSM). Mūsų tikslas – neurohistologiškai ištirti VSM ligonių, patyrusių skirtingos lokalizacijos smegenų insultą bei išgyvenusių skirtingą laiką nuo jo pradžios, ir nustatyti neuronų bei stromos reakcijos požymius į šią patologiją.

Metodai. Ištyrėme abiejų pusių VSM keturių ligonių, iš kurių du mirė 6-ąją ir 9-ąją dieną, o kiti du – po 3 ir 3,5 mėnesio nuo insulto pradžios. Atlikome 8 μm parafininių pjūvių, dažytų krezilo violetu bei eozinu-hematoksilinu, ir 1 μm epoksidinės dervos pjūvių, dažytų metileno mėlynuoju, neurohistologinę analizę.

Rezultatai. Aptikti dviejų tipų neurohistologinės alteracijos požymiai. Pirmajam mes priskyrėme dalies neuronų padidėjusį afinitetą baziniams dažams ir limfocitų infiltraciją šių neuronų ir smulkių kraujagyslių aplinkoje. Pavienių ar įvairaus dydžio židinių, intensyviai nusidažiusių neuronų aptikome visais atvejais ir abiejų pusių VSM, tačiau statistiškai patikimai daugiau jų buvo insulto židinio pusės simpatiniame mazge. Antrajam mes priskyrėme klasterius neuronų, tarp kurių vieni turėjo destrukcijos, o kiti – hipertrofijos požymių: gerokai padidėjusį ląstelės kūną, Nisslio medžiagos granulių dispersiją ir satelitinių – glijos – ląstelių proliferaciją. Šio pobūdžio pakitimai aptikti VSM ligonių, mirusių praėjus trims mėnesiams nuo insulto pradžios.

Išvados. Pirmą kartą pastebėtus VSM neurohistologinės alteracijos požymius po insulto, vadovaudamiesi keliolikos autorių tyrimais apie simpatinių neuronų reakciją į eksperimento metu pakenktus aksonus, mes siejame su retrogradine neuronų aksonų, dirginančių insulto židinio arterijas, reakcija į ischemiją ir jos sukeltos smegenų parenchimos bei stromos destrukcija.

Raktažodžiai: viršutinis kaklinis simpatinis mazgas, neurohistologija, alteracija, smegenų insultas, autopsija