

Morphological changes in the sphenopalatine ganglion on the background of metabolic disorders in the experiment at rats

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SUMMARY

The sphenopalatine ganglion occupies a special place in neuropathology and dental neuropathy, accompanied by such pronounced symptoms as "vegetative storm". The aim of the research was obtaining information on the external structure of the sphenopalatine ganglion, the morphometric characteristics of its neurons in norm and in experimental diabetes. The study was carried out on male Wistar rats weighing 260-300 g: with a stereoscopic biological microscope, using ophthalmic instruments, we removed almost the entire gland that was not accompanied by significant bleeding under general anesthesia. Peculiarities of the external structure of the sphenopalatine ganglion of the white rat were studied by macro-micro preparations under a binocular microscope at 50 objects pervaded with silver nitrate, according to Christensen.

The sphenopalatine ganglion of the rat is located in the orbit along the lower edge of its medial wall, between it and the infraorbital vascular-neural bundle. Externally, it is a thickening of a large petrosal nerve in the form of a "sausage", with a longitudinal diameter of 4-5.6 mm and a transverse diameter of 383 μ m. The external shape of the ganglion, close to the cylinder, makes it possible, without a

large error, to calculate its volume, which is 0.6 mm³. The number of neurons belonging to the medium distribution decreases in size, both in smaller and larger diameters, and the number of small and large neurons increases, as a result of which the arithmetic mean of the values of morphometric indices increase insignificantly, but it is statistically reliable.

Key words: Sphenopalatine ganglion – Experimental diabetes – Rat – Macro-micro preparations – Facial anatomy

INTRODUCTION

Sphenopalatine ganglion occupies a special place in neuropathology and dental neuropathy, because in clinical practice its lesions are often seen in the form of ganglionitis, accompanied by such pronounced symptoms that they are called "vegetative storm". The close relationship with the sympathetic ganglia and cranial nerves allows the sphenopalatine ganglion to react sharply to the processes associated with vascular pathology (Tebloev et al., 1976; Shuster et al., 1988; Grachev, 1999; William et al., 2016). Anatomy of the human node was studied in detail by Russian and foreign researchers by the middle of the 20th century (Szczyrkowski et al., 2002; Gold, 2005; Bathla and Hedge, 2012; Frautschi et al., 2016) and refined in later publications (Kolesnikov et al., 1995; Tsybul'kin and Poloiko, 2000; Tsybul'kin and

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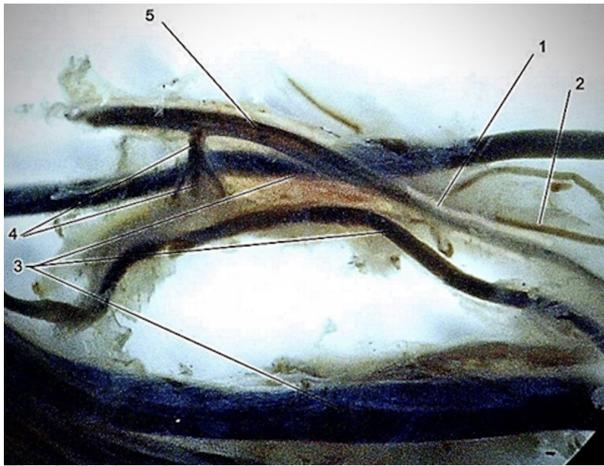


Fig 1. Rat's sphenopalatine ganglion (macro-micro preparation) silver nitrate impregnation according to Christensen, x 10. 1= main petrosal nerve; 2= deep petrosal nerve; 3= infraorbital nerve and its branches; 4= sphenopalatine ganglion branches to the mucosa of the hard palate and nasal cavity; 5= sphenopalatine ganglion.

Kolesnikov, 2001; Ruskell, 2003, 2004; Uryvaev et al., 2007). However, most of the works of recent decades affecting the sphenopalatine ganglion devoted mainly to related clinical problems. Meanwhile, a very small amount of information is known about the sphenopalatine ganglion of the white rat, which refers to laboratory animals, since it is used in various clinical and physiological experiments (Tsybul'kin et al., 1999), and in the manual of P.P. Gambaryan and N.M. Dukelsky does not mention him at all (Hambaryan et al., 1955).

A number of authors have developed and used in the experiment a method for modeling small rodents of diabetes mellitus, affecting all organs and tissues, including the nervous system (Scow, 1957; Shalyapin, 1962; Baranov and Gasparian, 1983; Heidt et al., 2007; Vasil'ev et al., 2019). In this connection it is interesting to find out what effect diabetes is having on the nerve nodes, in particular, on the rat's sphenopalatine ganglion.

The aim of the research was obtaining information on the external structure of the sphenopalatine ganglion, the morphometric characteristics of its neurons in norm and in experimental diabetes.

MATERIALS AND METHODS

The study was carried out on male Wistar rats weighing 260-300 g. The operation was performed under general anesthesia: we used two-component anesthesia by xylazine 0.1 ml / 100 g animal weight subcutaneously, after 10 minutes intravenous propofol (diprivan) 0.1 ml of official solution per 100 g animal weight. On the midline we opened the abdominal cavity; in the loop of the duodenum was found the pancreas, whose structural features are not significantly different from that of man; and under a stereoscopic biological microscope, using ophthalmic instruments, we re-

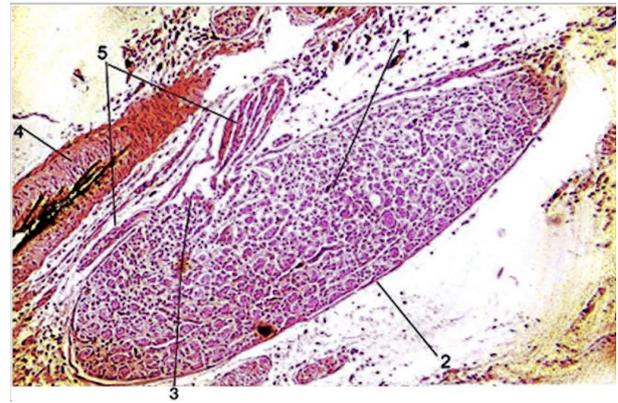


Fig 2. Rat's sphenopalatine ganglion Histogram, hematoxylin-eosin coloration, x 20. 1= sphenopalatine ganglion; 2= capsule of the sphenopalatine ganglion; 3= sphenopalatine ganglion's orifice; 4= infraorbital artery; 5= sphenopalatine ganglion branches. Small, medium and large-caliber neurons are visible.

moved almost the entire gland that was not accompanied by significant bleeding. The location of the gastrointestinal tract on the common mesentery facilitates the location of the pancreas. The outer structure of the sphenopalatine ganglion was examined on 50 objects impregnated with silver nitrate, according to Christensen, using the macro-micro preparation method under a binocular microscope with an increase from 1 x 10 to 4 x 10 with a shameless illuminator. The impregnation method was performed according to the following procedure: the preparations were fixed in acidic formalin for 2 weeks, and then washed in distilled water for 20 minutes. After that, the material was fixed in a 0.5% solution of silver nitrate in a dark dish at 37° C in a thermostat for 1 day. After one day, the preparation was washed 3-4 times in distilled water and placed in 5% formalin solution for 30 minutes, then treated with running water for 15 minutes. The resulting material was placed in a 5% hyposulphite solution for 10 min, then washed for 30 min in running water

In 50 series of histological sections in the frontal (5) and horizontal (5) planes stained with hematoxylin-eosin (even) and in Mallory (odd), the morphological and morphometric characteristics of the neurons were studied normally. Similarly, the same indices were studied in rats with artificially induced diabetes mellitus (42 subjects). The measurements were carried out on standardized photos in Adobe Photoshop XCV Edition, using a table to transfer pixels to micrometers at different values of the objective and the eyepiece of the microscope. Diabetes mellitus was modeled by the V.G. Chaliapin method [19]. For histological examination, animals surviving two weeks after the operation of subtotal removal of the pancreas and having a blood glucose level of at least 13 mmol / l were used, which is characterized as true diabetes.

In our work, we were guided by the rules of treat-

Table 1. Morphometric characteristics of neurons sphenopalatine ganglion in normal and in the experiment

Indicator		Norm	Diabetes	t
Smaller diameter	min	19	19	3,01
	Max	36	36	
	M	25,7	27,3	
	σ	2,5	4,7	
	m	0,2	0,33	
Indicator		Norm	Diabetes	t
Smaller diameter	min	25	25	5,3
	Max	45	45	
	M	31,7	35,4	
	σ	3,0	6,0	
	m	0,2	0,42	
Indicator		Norm	Diabetes	t
Neuron Volume	min	5900,0	5900,0	3,35
	Max	37179,0	37179,0	
	M	15059,9	18936,5	
	σ	4698,1	9257,7	
	m	332,2	654,6	
Indicator		Norm	Diabetes	t
Surface area of the neuron in 1 mm ³	min	1303	1303	3,2
	Max	4503	4503	
	M	2411,2	2759,8	
	σ	480,2	922,3	
	m	34,0	65,2	
Indicator		Norm	Diabetes	t
Volume of neurons in 1 mm ³	min	0,319	0,158	9,9
	Max	0,456	0,78	
	M	0,404	0,509	
	σ	0,07	0,08	
	m	0,005	0,006	
Indicator		Norm	Diabetes	t
Surface area of neurons in 1 mm ³	min	51,1	35,0	9,972
	Max	73,0	121,0	
	M	64,8	74,1	
	σ	7,13	11,4	
	m	0,5	0,8	

ment of laboratory animals in accordance with the "Helsinki Declaration" of the World Medical Association of 2000, the basic moral and ethical principles of conducting biomedical experiments on animals, formulated by the stands for Council for International Organizations of Medical Sciences (CIOMS), to conduct biomedical research using animals of the Ethical Code of 1985, the European Convention for the Protection of Vertebrates animals used in the experiment and for other scienti-

fic purposes, including the main provisions of the CIOMS Code of Ethics, the Council Directive on the approximation of laws, regulations and administrative provisions of EU states on the protection of animals used for experiments and other scientific purposes.

RESULTS AND DISCUSSION

The sphenopalatine ganglion of the rat is located

Table 2. Distribution of neurons sphenopalatine ganglion "small", "medium", "large" units of the rat

Option		M	C	K	M	σ	m	t
		Number of observations						
Minorities Signatory diameter	Norm	56	135	9	25,7	2,5	0,2	3,01
	Experiment	72	65	63	27,3	4,7	0,33	
Larger diameter	Norm	94	103	3	31,7	3,0	0,2	5,3
	Experiment	72	55	73	35,4	6,3	0,42	
Volume of neurons	Norm	194,0	3,0	3,0	15059,9	4698,1	332,2	3,55
	Experiment	107,0	42,0	51,0	18936,5	4699,0	654,6	
Surface area of neurons	Norm	94,0	103,0	3,0	2411,2	480,2	34,0	3,2
	Experiment	72,0	65,0	63,0	2759,8	975,5	65,2	
Volume of neurons in 1 mm ³	Norm	194	3	3	0,404	0,07	0,005	9,9
	Experiment	107	42	51	0,509	0,08	0,006	
Surface area of neurons in 1 mm ³	Norm	94	103	3	64,8	6,13	0,51	9,07
	Experiment	72	65	63	74,1	6,64	0,8	

in the orbit along the lower edge of its medial wall, between it and the infraorbital vascular-neural bundle (Figs. 1, 2). Externally, it is a thickening of a large petrosal nerve in the form of a "sausage". Analysis of micro preparation allows us to state that the deep petrosal nerve has nothing to do with the sphenopalatine ganglion, and its branches extend to the mucous membrane of the nasal cavity and the palate, like the branches of the infraorbital nerve. The external shape of the ganglion, close to the cylinder, makes it possible, without a large error, to calculate its volume, which is 0.6 mm³. The sphenopalatine ganglion of the rat is covered with a thin capsule consisting of one layer of connective tissue cells.

The neurons of the sphenopalatine ganglion in the control group rats, which have a nucleolus in this section, are often characterized by an oval shape of the profile, smaller than 19-36 μm in diameter (on average $25.7 \pm 2.5 \mu\text{m}$) and larger diameter (up to 70% of neurons) μm (on average $31.7 \pm 3.0 \mu\text{m}$), less often – a round shape with a diameter of 19-36 μm . The nuclei of neurons, ranging in size from 8 to 15 microns, contain one nucleolus and a bubbling karyoplasm, in the overwhelming majority of cases (more than 90%) light, slightly darker than the granular cytoplasm. Only in a few cells is the nucleus dark. There are also neurons, the cytoplasm of which is only a narrow rim around a large nucleus. Each pericarion is surrounded by a connective tissue capsule that has a dense homogeneous substance on stained hematoxylin-eosin sections, which decisively distinguishes the rat assembly from such a human node. The thickness of this capsule is 3-4 microns. There are 8-10 satellite cells around each neuron. The bodies of the neurons of the node are almost everywhere located evenly and densely, so that neighboring cells are separated either only by connective tissue capsules, often common to them, or by an interval not exceeding 10 μm . In the section of sec-

tion with the area of 9441 mkm² (0.009441 mm²) there are 7 to 10 neurons (on average - 8.875 ± 0.8), which contain a nucleolus in this section. Consequently, 940.05 ± 87.3 neurons can be located on the cut-off area equal to 1 mm², and the sum of the neuron profiles per unit area of the cut (specific density of neurons) is estimated on average at $1237 \times 708 = 876053 \mu\text{m}^2$, which is 87.6 % of the unit area of the cut. In other words, for 1 mm³ the weight of the node should be from 21184.20 to 30263.20 cells, on average - 26858.5 ± 2494.4 . The volume of one neuron is determined in the range from 5900.0 to 37179.0 μm^3 , on the average - $15059.9 \pm 4698.1 \mu\text{m}^3$, and the total volume of neurons contained in 1 mm³ of the node mass ranges from 0.319 to 0.456 mm³, 0.404 mm³, or 40.4% of the volume of the node. The surface area of one pericarion is 1303-4503 μm^2 , on average - $2411.2 \pm 480.2 \mu\text{m}^2$, so that their total area per 1 mm³ of the mass of the node is 51.1-73 mm², on the average 64.8 mm².

In the rats of the experimental group, the neurons are characterized by the same smaller diameter as in the norm (19-36 μm) and the same large diameter (25-35 μm), but the average values of these dimensions are somewhat larger than in the norm - $27.2 \pm 4,7$ and $35.3 \pm 6.0 \mu\text{m}$, respectively (Table 1). It can be also seen from the table that the volume of the neurone of the rat focal nodule under this experimental pathology varies within the same limits as in the norm – from 5900.0 to 37179.0 μm^3 , but the average value increased to $18936.5 \pm 9255.7 \mu\text{m}^3$. The aggregate the volume of neurons contained in 1 mm³ of the substance of the node in this group is estimated at 0.158-0.78 mm³, on average - 0.51 mm³, which is 0.1 mm³ larger than in the control group. The change in the surface area of the neuron of the sphenopalatine ganglion under the experimental conditions is similar to the change in volume: the boundaries of variability remain the same, and the average value

increases to $2759.8 \pm 922.3 \mu\text{m}^2$. The cumulative surface area of neurons contained in 1 mm^3 of the substance of the node in the experimental group is estimated at $35.0\text{-}121.0 \text{ mm}^2$, on the average 74.1 mm^2 , which is 9.3 mm^2 larger than in the control.

Conclusions

Due to the similar structure, it is possible to extrapolate the results of experimental studies from animals to humans. Under the conditions of experimental diabetes mellitus, various sizes of neurons occur in the sphenopalatine ganglion: the number of neurons belonging to the medium distribution decreases in size, both in smaller and larger diameters, and the number of small and large neurons increases (Table 2), as a result of which the arithmetic mean the values of morphometric indices increase insignificantly, but it is statistically reliable. Convincing evidence of a direct relationship between diabetes and pathology of the sphenopalatine ganglion has been obtained.

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