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MORPHOLOGICAL FEATURES OF NEURONS INNERVATING DIFFERENT PARTS OF THE LARGE INTESTINE

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The purpose of the study was to obtain complex morphological data on neurons innervating different parts of the large intestine. The object of the study was rats (n=17) of the Wistar line. The study used a universal method of impregnation. In addition, a 3D model of neurocytes of the ganglia of the intermuscular plexus of the large intestine was made. The results of the study showed that the large intestine has intra-organ ganglia located in the intermuscular and submucosal plexuses. The intermuscular plexus (Auerbach) of the colon has the form of a network with cells of different shapes and consists of nerve nodes containing Dogel cells of the 1st and 2nd type. In the intramural nodes of the large intestine, there are a large number of sensitive nerve endings. At the same time, a significant number of these endings in the nodes are formed by the processes of type 2 Dogel cells. A study of a three – dimensional image of a type 2 Dogel cell from the intermuscular plexus of the large intestine showed that this cell has an ovoid shape. The cell is flattened transversely and elongated longitudinally. The volume of its pericaryon is 2785.11 μm^3, the volume of the nucleus is 647.7 μm^3.

Key words: large intestine, type 2 Dogel cells, intramural nodes, submucosal plexus, intermuscular plexus.

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МОРФОЛОГІЧНІ ОСОБЛИВОСТІ НЕЙРОНІВ, ЩО ІННЕРВУЮТЬ РІЗНІ ДІЛЯНКИ ТОВСТОЇ КИШКИ

Метою дослідження було отримання комплексних морфологічних даних про нейрони, що інервують різні ділянки товстої кишки. Об'єктом дослідження стали щури (n=17) лінії Вістар. У дослідженні використано універсальний метод імпрегнації. Крім цього, виготовлено 3D модель нейроцитів гангліїв міжм'язового сплетення товстої кишки. Результати дослідження показали, що товста кишка має внутрішньоорганні ганглії, розташовані в міжм'язовому і підслизовому сплетеннях. Міжм'язове сплетіння (Ауербаха) товстої кишки має вигляд мережі з осередками різної форми і складається з нервових вузлів, що містять клітини Догеля 1-го і 2-го типу. В інтрамуральних вузлах товстої кишки є велика кількість чутливих нервових закінчень. При цьому значну кількість цих закінчень у вузлах утворено відростками клітин Догеля 2-го типу. Дослідження тривимірного зображення клітини Догеля 2-го типу з міжм'язового сплетення товстої кишки показало, що ця клітина має овоїдну форму. Клітка сплощена у поперечному і подовжена у поздовжньому напрямку. Обсяг її перікаріона дорівнює 2785,11 мкм^3, обсяг ядра - 647,7 мкм^3.

Ключові слова: товста кишка, клітини Догеля 2-го типу, інтрамуральні вузли, подслизистое сплетіння, міжм'язової сплетіння.

The work is a fragment of the doctoral dissertation: "The study of the structural organization of the autonomic nervous innervation of the large intestine in normal in human and experimentally".

Neural theory is an unshakable physiological dogma. However, some of its provisions should be revised in the light of new information. It turned out that in addition to synapses, there are other structures in the nervous system that can carry out a different form of interneuronal communication [8]. So there are peculiar phenomena of cytoplasmic fusion in the nervous system of a number of invertebrates. It is impossible to ignore the detection of syncytial communication of neurocytes in mollusks, crustaceans, polychaetes and other invertebrates. The discussion about the possibility of syncytial communication in the nervous system arose in the literature after the publication of the first materials on this topic [3, 4]. This is not surprising, since this publication was preceded by more than a century of discussion by supporters of neural theory, led by Santiago Ramon and Cahalem and reticularists (supporters of the syncital organization of the nervous system), led by Camilo Golgi. The previous discussion was based on the results of light microscopy with low resolution and insufficiently informative results of staining of biopsies with methylene blue and Bilnovsky-Gros impregnation. All the evidence of both neuronists and reticularists was based on drawings obtained using drawing machines [3].

Even at the beginning of the twentieth century, A. S. Dogel presented a huge material for the development of neural theory, but he was wary of it, did not accept it blindly, saw numerous exceptions in it, which allowed us to approach it to some extent with a critical assessment [4, 6].

A special merit in obtaining new evidence in favor of the existence of syncytial connections in the nervous system belongs to O. S. Sotnikov. Analysis of the preparations of a number of well – known

authors of the past proves the presence of syncytial connections of neurocytes in them. Thus, schematized images of human spinal neurocyte variants demonstrate the possibility of syncytial fusion of multiple independent branches of the same neurocyte to form a network of branches merging into a single myelin fiber. This method of forming a syncytial connection by branches of a single neurocyte with their fusion into a single myelin fiber is considered as the Dogel principle, in contrast to the Yang principle, which involves the fusion of the processes of many independent neurocytes [3, 4].

Nervous regulation of intestinal functions is one of the least studied problems of the physiology of the gastrointestinal tract. The autonomic nervous system includes many ganglia, nerve plexuses, and single neurons. However, the ganglia of the gastrointestinal tract differ from other intra – and extra – organ ganglia in a number of structural and physiological features, which supports the continuing interest of many researchers [2] and served as the basis for the allocation of the nervous apparatus of the gastrointestinal tract in a special department of the autonomic nervous system – the metasympathetic nervous system [2]. Until recently, the main attention was paid to the study of bulbar mechanisms of regulation of motor activity of the upper gastrointestinal tract, which are implemented with the participation of the vagus nerves [7, 10].

The exact spatial localization and morphological features of neurons innervating different parts of the large intestine remain unclear.

The purpose of the study was to obtain complex morphological data on neurons innervating different parts of the large intestine

Materials and methods. The object of the study was rats (n=17) of the Wistar line, aged 3–4 months, with a weight of 180–320g. Rats were chosen as a biomodel, taking into account their physiological adequacy, resistance to infection, ease of maintenance, and not high cost. The animals were kept and euthanized in accordance with the EC Directive on the Protection of Animals Used for Experimental and Scientific Purposes (86/609 CE).

To achieve this goal, a universal method was developed, based on the classical impregnation methods: intravascular – Ranier-Goyer and immersion – Bilshovsky-Gros. The method has been tested on laboratory animals: cats, dogs, and white rats [1].

Animals under ether anesthesia were conjugated to the abdominal aorta and the portal vein was crossed. Through the abdominal aorta, a 5 % glucose solution was first perfused until a pure perfusate appeared in the portal vein. Then a solution of barium hydroxide was perfused, which is a physiological tracer that passes from the bloodstream through the interstitial space to the lymphatic microvessels. The argifilia of tissue structures is determined by the pH level of silver hydroxide deposition from a solution of its nitric acid salt. Complete precipitation occurs at pH 11–13. Getting into the lumen of the lymphatic microvessels, where the pH rises sharply to 9, the solution of barium hydroxide precipitates on the vascular wall, thereby increasing its argiphilia.

A solution of barium hydroxide is prepared in advance on the basis of a 5 % glucose solution, which is saturated with poorly soluble calcium and magnesium hydroxides. Then, 1.5 g of barium hydroxide is added to 1 liter of the solution. 3 – 5 minutes after perfusion of the solution through the abdominal aorta – portal vein, the test material is taken and fixed in 15 % amethanol formalin, neutralized with sodium tetraborate (0.5 g per 1 liter of solution). The optimal period of fixation of the material is determined empirically by its trial impregnation every 2 days. Further manipulations are carried out according to the classical Bilshovsky-Gros method. Total preparations with a thickness of up to 300 microns are rinsed with distilled water, dried on a filter, and then placed in a solution of silver nitrate (from 1 to 10 %).

The concentration of the first silver and its exposure to the preparation is determined experimentally in each specific case. Thanks to the hydroxides Mg" and Ba', the blood and lymphatic microvessels acquire the same argyrophilia as the autonomic nerve fibers and neurocytes. After the first silver, as required by the classical Bilshovsky-Gros method, the material is transferred to ammonia silver, the concentration and exposure of which on the preparation is also determined experimentally.

In addition, a 3D model of neurocytes of the ganglia of the intermuscular plexus of the large intestine was made. For this purpose, after fixing a fragment of the intestinal wall in 10 % amethanol formalin (5 days), its frontal and horizontal sections with a thickness of 30.0–40.0 microns were made. The sections were encased in canadian balsam and examined under a "Lessa 1000DM" microscope with a digital video system. Morphometric studies of cells were carried out in the Ymadei software package, and the construction of their 3D models was carried out in the AN5Gs Apace claim v 19.2 program.

The method used in calculating the volume of the cell was to separate its cross-section into separate sites and calculate the area of each of them, followed by adding the results obtained. To determine the area of the shaded area (Fig.4) a number of formulas were applied:

$$A^{(i)} = \frac{(x_{i+1}+x_i)}{2}(y_{i+1} - y_i); \quad (1.1)$$

$$S^{(i)} = A^{(i)}\frac{(y_{i+1}+y_i)}{2} = \frac{(x_{i+1}+x_i)}{4}(y^2_{i+1}-y^2); \quad (1.2)$$

$$J^{(i)} = A^{(i)}\frac{(y_{i+1}+y_i)^2}{4} = \frac{(x_{i+1}+x_i)}{8}(y_{i+1}-y);\ (y_{i+1} + y_i)^2; \quad (1.3)$$

$$J^{(i)} = A^{(i)}\frac{(x_{i+1}+x_i)}{4}\frac{(y_{i+1}+y_i)}{8} = \frac{(x_{i+1}+x_i)^2}{16}(y_{i+1} - y). \quad (1.4)$$

The summation of A and B allowed us to determine the features of the ALBCD figure.

$$A = \sum_{i-1}^{n} A^{(i)} = \sum_{i-1}^{n}\frac{(x_{i+1}+x_i)}{2}(y_{i+1}-y_i); \quad (1.5)$$

$$S_x = \sum_{i-1}^{n} S^{(i)} = \sum_{i-1}^{n}\frac{(x_{i+1}+x_i)}{4}(y_{i+1}-y_i); \quad (1.6)$$

$$J_x = \sum_{i-1}^{n} J_x^{(i)} = \sum_{i-1}^{n}\frac{(x_{i+1}+x_i)}{8}(y_{i+1}-y)\ (y_{i+1}+y_i)^2; \quad (1.7)$$

$$J_{xy} = \sum_{i-1}^{n} J_{xy}^{(i)} = \sum_{i-1}^{n}\frac{(x_{i+1}+x_i)}{16}(y_{i+1}{}^2-y^2); \quad (1.8)$$

$$S_x = \sum_{i-1}^{n} S_y^{(i)} = \sum_{i-1}^{n}\frac{(y_{i+1}+y_i)}{4}(x_{i+1}{}^2 - x^2); \quad (1.9)$$

$$J_y = \sum_{i-1}^{n} J_y^{(i)} = \sum_{i-1}^{n}\frac{(y_{i+1}+y_i)}{8}(x_{i+1}-x)\ (x_{i+1}+ x_i)^2. \quad (1.10)$$

Results of the study and their discussion. As a result of the study, morphological evidence was obtained that there are direct syncytial connections between neurons in the submucosal and muscular plexuses of the large intestine. All elements of the intermuscular and submucosal vegetative plexuses of the rat intestine were identified by the universal method of impregnation.

Studies have shown that syncytial connections of neurons in the autonomic ganglia of the intestine were constantly detected. These were syncytial connections of the processes and bodies of two neurocytes.

According to our data, the large intestine has intra-organ ganglia located in the intermuscular and submucosal plexuses. The intermuscular plexus (Auerbach) of the large intestine has the form of a network with cells of different shapes and consists of nerve nodes containing Dogel cells of type I and II, but the latter numerically significantly predominate (20–25 cells or more).

In the intramural nodes of the large intestine, there are a large number of sensitive nerve endings. At the same time, a significant number of these endings in the nodes are formed by the processes of the Dogel type 2 cells. Most of the processes of these neurons are very long, which go beyond the node; pass as part of the internode strands.

Along the periphery of the node, these neurons have a unipolar, pseudonipolar or bipolar shape, while in the center there are also multipolar cells. The number of processes in these cells ranges from 2 to 6.

The results of the study showed that a connective capsule was formed in each ganglion of the intermuscular and submucosal nerve plexuses of the large intestine.

The structural organization of the microvascular bed of the nervous plexuses of the large intestine is based on the modular principle. Three of their different specializations have been identified: ganglion, interganglionic, and stem. In the ganglia, a microvascular bed is formed, the module of which is built according to the type of network with short but wide arterioles and venules.

To estimate the volume of the pericaryon and the nucleus of isolated type 2 Dogel cells located in the frontal and horizontal planes, we made color photographs of them (fig. 1).

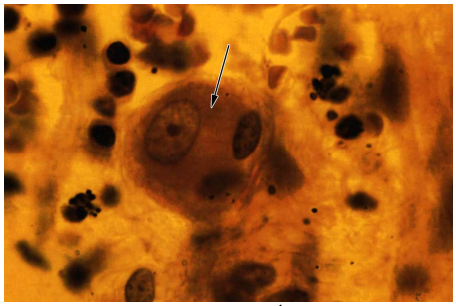

Fig. 1. Dogel cell of the 2nd type in the horizontal plane. Universal impregnation method. Uv. X 900

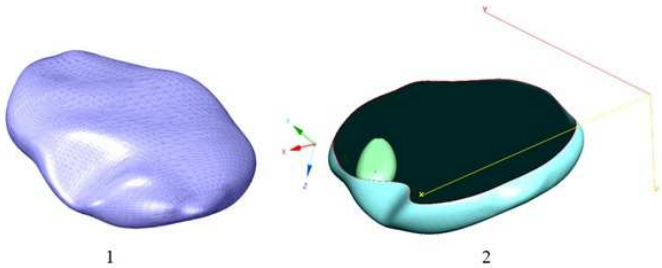

Fig. 2. Three-dimensional model of the nucleus of a type 2 Dogel cell: 1) the shape of the cell nucleus; 2) the location of the nucleus in the cell.

The photos were then converted to the VMR bitmap format (*3DtooL*^{R} software environment). Sections of neurocyte bodies located in the frontal and horizontal sections were cut separately from these images. The resulting images were first combined in a Cartesian coordinate system, and then, after combining the planes and transforming them into an Stl-file, into a solid-state model (in the AutodeskR software environment). The resulting 3D model of the cell and nucleus was reduced by 900 times, in order to obtain a three-dimensional cell and nucleus with absolute dimensions with a ratio of 1:1 to their true dimensions (fig. 2).

A number of assumptions were made in the calculations: 1) the cross section of the cell in the horizontal plane is approximated to a figure of the correct shape; 2) the distance between the sections is 0.1 % of the cell volume; 3) the material of the volume under study is isotropic.

The object of the study, the body of cell H, the overall characteristics of which had to be calculated, is located between two arbitrary planes a and b (fig. 3).

The coordinate system is constructed so that the axis Ox is perpendicular to the planes a and b. The letters a and b indicate the abscissae of the points of intersection of the axis Ox with these planes (a<b). We will assume that the body of the cell H is such that its section F (x) is a plane passing through the point with the abscissa (x) and perpendicular to the axis (Ox). Thus, they are an ellipse (fig. 4).

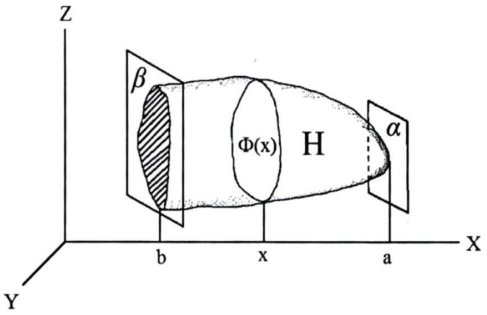

Fig. 3. Cross-section diagram of the Dogel cell type II in the vertical plane

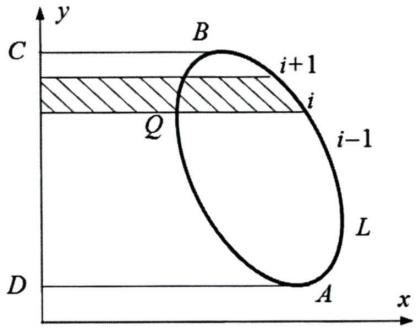

Fig. 4. Scheme for determining the area of the plane of a type II Dogel cell using the numerical method.

The following results were obtained: A type 2 Dogel cell from the intermuscular plexus of the large intestine has a three-dimensional structure of an ovoid shape. The cell is flattened transversely and elongated longitudinally. The volume of its pericaryon is 2785.11 mm3, the volume of the nucleus is 647.7 mm3.

According to our data, syncytial connections of neurons in the autonomic ganglia of the intestine were constantly detected. These were syncytial connections of the processes and bodies of two neurocytes. Therefore, to the question posed in the work of O. S. Sotnikov: “is it possible to present the absolute evidence of the presence of interneuronal syncytium with the help of light microscopy” [3], it is necessary to answer: yes, it is possible. There is no doubt that syncytial interneuronal connections, along with chemical and electrical synapses, make the structural organization of the nervous system more reliable. Obviously, instead of the current representation of “either – or” or synapses, or ephaps, the representation of "and – and" is needed, i.e., both synapses and ephaps. Based on this, we can hope that the discussion about the principle of the organization of the nervous system will end with the unification of the neuronal and syncytial theory into a single neuroscytial theory, as it happened with the unification of the cellular and humoral theories into a single cellular-humoral theory of immunity.

According to our data, like the rest of the gastrointestinal tract, the large intestine has its own nervous apparatus, which is represented by neurons of the intra-organ ganglia located in the nodes of the intermuscular plexus of Auerbach and the submucosal plexus of Meissner. According to the literature, the submucosal plexus is two closely related plexuses, the so-called surface plexus between the circular muscle layer and the submucosal layer, and the deep one – in the thickness of the submucosal layer. The superficial plexus has a broad-leaved character. In the ganglion of the intermuscular and submucosal nerve plexuses of the intestine, there is a well-defined connective capsule [6, 9].

It should be noted that, in general, the neurons of the intramural ganglia are characterized by significant variability in the shape and number of processes, which is well revealed in studies performed on tissue culture and in observations on a live neuron [9].

According to the data obtained, the sensitive nerve endings in the intramural nodes of the large intestine are formed by the processes of the second type of Dogel cells. It is believed that Dogel cells of the second type perform a sensory function. It is also possible that there are special functional connections between the neurocytes of the intermuscular and submucosal plexuses [7].

It is believed that the neurocytes in the metasympathetic ganglia are flat in their structure [10]. But the results of our study showed that the Dogel cell of the second type from the intermuscular plexus of the intestine has a three – dimensional structure of an ovoid shape. The cell is flattened transversely and elongated longitudinally.

Conclusion

The large intestine has intra-organ ganglia located in the intermuscular and submucosal plexuses. In the intermuscular plexus of the large intestine, there are nerve nodes containing cells of the Dogel’s 2nd type of ovoid shape.

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THE EFFECT OF CARBON MONOXIDE'S DONOR CORM-2 ON ERYTHROCYTE AQUAPORINS

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Carbon monoxide is part of group of gas transmitters, which are produced by almost all cells and involved in many mechanisms of internal and external communication between cells. Carbon monoxide-releasing molecules are promising for therapeutic use. Such donors include tricarbonyldichlororuthenium (II) dimer. For the research, the aquaporins 1 and 3 were blocked in erythrocyte membranes with the use of mercury chloride and silver nitrate, and then they were added to hypo–, iso– and hyperosmolar solutions. However, tricarbonyldichlororuthenium (II) dimer does not bind to aquaporins 1, but this effect is due to the effect on aquaporins 3. This effect is dose-dependent: the most active concentration was 6–50 μM. It is arguable that tricarbonyldichlororuthenium (II) dimer increases the resistance of erythrocytes to hypertensive stress, stabilizes their volume in a hyper- and hypo-tonic environment by activating aquaporins 3.

Key words: AQP1, AQP3, gas transmitter, erythrocyte membrane.

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ВПЛИВ ДОНОРА МОНООКСИДУ КАРБОНУ CORM-2 НА АКВАПОРИНИ ЕРИТРОЦИТІВ

Монооксид карбону, який належить до групи газотрансміттерів, продукується практично всіма клітинами організму і бере участь у багатьох механізмах міжклітинної сигналізації. Сполуки-донори монооксиду карбону є перспективними для терапевтичного застосування. До таких донорів належить димер трикарбонілдихлororутенію (II). Для дослідження реакції аквапорінів 1 і 3 проводили їхнє блокування в мембранах еритроцитів з використанням хлориду ртуті та аргентум нітрату й отриману суспензію поміщали у гіпо–, ізо– і гіперосмолярні розчини. Було встановлено, що димер трикарбонілдихлororутенію (II) впливає на транспорт води в еритроцитах шляхом дії на аквапорини 3. Цей ефект дозо-залежний: найбільш активна концентрація становила 6–50 μМ. Отже, димер трикарбонілдихлororутенію (II) підвищує стійкість еритроцитів до гіпертонічного стресу, стабілізує їх об'єм в гіпер- і гіпотонічному середовищі впливаючи на аквапорини 3.

Ключові слова: AQP1, AQP3, газотрансміттер, мембрана еритроциту.

This work is a fragment of the research project "The effect of certain vasoactive substances on the central and peripheral lymphoid organs of white mice", state registration No 0117U001764.

Since 1993, carbon monoxide (CO) has been regarded not only as a toxic gas. It is also being studied as a substance that plays an important physiological role in the body [7]. Prior to that, it was found that CO is constantly produced in the human body and significantly increases under conditions accompanied by abnormal decomposition of red blood cells (RBC) [4]. Over time it was found that CO is freely diffusible and traverses all membranes, bypassing transporters and thus can rapidly mediate