VARIABILITY IN INTRAMURAL GANGLIA AND EFFERENT NEURONS ALONG THE DIFFERENT ROUTES OF THE BILE

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Abstract. *The gallbladder ganglia are sites of complex modulatory interactions that ultimately affect the function of muscle and epithelial cells in the organ. This study aimed to identify the morphological features of intramural ganglia and efferent neurons in the gallbladder and extrahepatic biliary tract. Neurohistological methods, including silver nitrate impregnation according to the Bilshovsky-Gros method and Campos, were used to morphometrically study the intramural nervous apparatus of the gallbladder wall, common bile duct, and hepatic ducts in 12 dogs, 6 of which underwent right-sided cervical vagotomy.*

The results showed that the largest nerve ganglia are located in the wall of the gallbladder, particularly in the neck of the bladder, and the common bile duct. In contrast, the walls of the hepatic ducts contain small microganglia and single neurocytes. The intramural nervous apparatus of the gallbladder and extrahepatic bile ducts demonstrated pronounced polymorphism in the shape of the bodies of efferent neurons.

Key words: *biliary system, gallbladder, extrahepatic bile ducts, intramural ganglia, efferent neurocytes.*

Digestion is controlled through the enteric system, central nervous system, and integrative centers in the sympathetic ganglia. The degree to which the enteric and central nervous systems control digestion differs significantly along the digestive tract [10,15]. The enteric nervous system is recognized as a complex neural network that controls various cell populations, including smooth muscle cells, mucosal secretory cells, endocrine cells, microvasculature, immune and inflammatory cells. This network is organized in several plexuses, each of which provides a completely autonomous control of the functions of the gastrointestinal tract [8,10,13]. Although the intramural ganglia of the gallbladder originate from the same neural crest cells that inhabit the intestine, they exhibit structural, neurochemical, and physiological characteristics that differ from the intermuscular and submucosal plexuses of the enteric nervous system. Many results indicate that gallbladder ganglia are not simple relay stations, but rather sites of complex modulatory interactions that ultimately affect the functions of muscle and epithelial cells in the organ [5,12]. The study of the morphology of the neurons of the digestive tract is devoted to classical [1,2], as well as studies of the 21st century. Immunohistochemical determination of the presence or absence of neuronal matter (i.e., chemical coding for enteric neurons) has become an effective and easily applicable tool for distinguishing between types of enteric neurons in the guinea pig and later in other species. However, the morphological correlates of this chemical and functional heterogeneity have not yet been fully studied. An attempt was made to combine the results of morphological and immunohistochemical features of human intestinal neurons [6]. Currently, 3 types of efferent intestinal neurons have been identified. The first type includes short neurons, which, due to the shape of their "short" processes, demonstrated by immunohistochemistry for neurofilaments, apparently correspond to the descriptions of type I neurons given by Dogel. They have short or lamellar dendrites. The second type is spiny neurons, due to their short processes they also correspond to Dogel type I neurons. Sometimes the dendrites have dilated, lamellar endings or branching points, but their basic appearance is spiny and the entire cell looks like a hedgehog. Type I hairy neurons are considered the third type; these are uniaxial, short-dendritic neurons that project from the myenteric plexus to the mucous membrane; they were not included in Dogel's classification. These cells were first described by Stach in 2000 in pigs and guinea pigs as type IV neurons. No chemicals specific to these three types of ENS efferent neurons have been found. Common to them is the content of choline acetyltransferase, and it is also not found in all types of spiny neurons. The morphological and chemical heterogeneity of ENS neurons may be associated with the presence of their regional features, which has been established by a number of researchers. In this regard, research on neurons in various areas of the gastrointestinal tract should be continued to understand physiological functions, pathological processes, and, ultimately, options for therapeutic interventions. This can be facilitated by all available methodological approaches, both “classical” and “modern” [6]. Thus, the widespread use of immunohistochemistry to study the innervation of the organs of the biliary system [7,11,14] does not detract from the effectiveness of traditional neurohistological methods, since until now the classification of ENS neurons is based on their morphological features. In this regard, such studies can be continued to study different parts of the digestive tract, in particular, the biliary system [16] in the comparative aspect of its constituent parts.

The aim of this study was to identify the features of the morphology of intramural ganglia and efferent neurons of the gallbladder, extrahepatic biliary tract using silver nitrate impregnation.

Material and methods. The intramural nervous apparatus of the gallbladder wall, common bile duct and hepatic ducts was studied in 12 dogs. Of these, 6 animals were subjected to right-sided cervical vagotomy in order to identify synaptic endings on the neurons of the intramural ganglia of the biliary system. All experiments, keeping and slaughter of animals were carried out in accordance with the permission and strictly according to the requirements of the ethical committee of the Republic of Uzbekistan. Vagotomy was performed under etaminal sodium anesthesia; for this, a 5% solution of the substance was administered intraperitoneally to dogs. The studies of these animals were carried out 3 days after the operation. Animals were euthanized under etaminal sodium anesthesia followed by transection of the abdominal aorta. The gallbladder and extrahepatic bile ducts were fixed in 12% neutral formalin in a stretched form with wooden needles on a paraffin plate. Formalin was neutralized with a saturated solution of sodium tetraborate. The reaction of formalin was periodically checked with a universal indicator PKC, the processing of the material was started at the first shifts of the reaction to the acid side. Sections 75–100 μm thick were obtained using an MK-25 microtome cryostat. The sections were impregnated with silver nitrate according to the Bilshovsky-Gros method and according to Campos; in some cases, the sections were additionally stained with carmine. The average number of efferent neurons was determined by counting them in the intramural ganglia in different parts of the biliary system with a microscope magnification of about 20, approx. For mathematical data processing, the Student's method was applied with the determination of the arithmetic mean M, the average error of relative values and the coefficient of reliability of the difference. Differences were established as significant at $P<0.05$. Histological preparations were studied and photographed using a LeicaGME microscope (Leica, India) coupled with a LeicaEC3 digital camera (Leica, Singapore) and a Pentium IV computer. Photo processing was carried out using Windows Professional application programs.

Results and discussion. Both impregnation techniques showed identical results. Intramural ganglions with different numbers of neurocytes were found in the wall of the gallbladder and extrahepatic bile ducts of dogs. In the wall of the gallbladder (especially in the neck of the bladder) and the common bile duct are the largest nerve ganglia containing many neurons. Nerve nodes are located at the intersection of bundles of nerve fibers. In the wall of the hepatic ducts there are small microganglia with a small number of nerve cells, as well as single neurocytes. In the lamina propria of the mucous membrane in all parts of the biliary system, single neurons are found. They are located at the intersection of bundles of nerve fibers and very rarely as part of the bundles themselves.

All types of vegetative nerve cells (three types of Dogel cells) and giant nerve cells are found in the wall of the organs of the biliary system. Type I Dogel cells (long-axon neurocytes) have a large polymorphism of localization and shape. Characteristic of these neurons is the presence of a thick and long axon, exceeding the dendrites in these parameters. Long-axon neurocytes are impregnated more intensively than other cells.

These neurons are more often found in the localization of the sphincters of the biliary system. The hyperimpregnated axon of these cells can be traced on preparations at a considerable distance and often enters the bundle of nerve fibers. In the composition of the bundle, they are also distinguished by intense impregnation from the rest of the nerve fibers of the bundle up to a certain distance. The body shape of efferent neurons is usually irregular. In the common bile duct, there are also neurocytes with a cone-shaped body. In the area of confluence of the hepatic ducts, the cystic duct with the common bile duct, there are other (oval, fusiform) forms of neurocyte bodies.

Quite often, individual hyperimpregnated nerve fibers approach efferent neurons, which form a pericellular formation with transient contacts around its body. Transient contacts are formed in the places where the nerve fiber is closest to the body of the neurocyte. We also found nerve cells similar in shape to Type I Dogel cells with neurofibrillary plates (dendritic lamellae) and binucleated.

The study of the comparative density of distribution of long-axon neurocytes in the departments of the biliary system showed that their lowest density is noted in the area of the bottom and body of the gallbladder (1.9 ± 0.09). Significantly more, compared with the body and the bottom, they are located in the neck of the gallbladder (3.4 ± 0.12, $P<0.05$). The highest density of neurons is observed in the common bile duct (4.7 ± 0.14, $P<0.05$).

After right-sided cervical vagotomy, hypertrophied and hyperimpregnated synaptic endings are found on the efferent neurons of the biliary system. Most of them end on the body of neurocytes, forming axosomatic synapses.

The study showed that in the intramural nervous apparatus of the gallbladder and extrahepatic bile ducts, nerve nodes and efferent neurons have a pronounced polymorphism. It should be noted that changes in the number of neurocytes in the intramural ganglia were also found in different parts of the small intestine. The number of neurons in the ganglia gradually decreases in the direction from the duodenum to the end sections of the jejunum of guinea pigs from 48 to 4 [3]. The hyperimpregnated and hypertrophied nerve endings found by us after vagotomy on long-axon neurocytes confirm their belonging to efferent parasympathetic neurons. It is assumed that all gallbladder neurons are cholinergic, since they all express immunoreactivity to choline acetyltransferase. Most of these neurons also express substance P, neuropeptide Y, and somatostatin, and a small population of neurons express vasoactive intestinal peptide (VIP), immunoreactivity, and NADPH-diaphorase enzymatic activity. NADPH-diaphorase activity, nitric oxide synthase immunoreactivity, and VIP immunoreactivity were found to be expressed by the same neurons in the gallbladder of guinea pigs [12]. The content of various substances may be associated with different functional significance of efferent neurons and is reflected in their morphology, causing polymorphism. The giant and binuclear efferent neurocytes found by us, transient contacts on their surface, as well as the formation of dendritic lamellae morphologically characterize the high functional activity of some neurons. Similar structures on the dendrites of efferent neurocytes were also described by B.I. Lavrentiev in his study of the cytoarchitectonics of the intermuscular ganglia of the stomach and esophagus. In this way, the dendrites of type I cells and the lamellae formed by them increase the surface of the cell body and serve to perceive irritation coming through the pericellular apparatus [2]. Different functional orientation of neurocytes is also shown in the study of their development. The established asynchrony in the development of neurons is presented as a manifestation of adaptive evolution. Since ganglion neurons are associated with muscle, connective tissues, glands and other tissue structures, it is possible that they have morphofunctional features. Neurons, apparently, also experience the influence of specific biochemical conditions of the environment in which they are located [4].

Thus, neurohistological impregnation methods revealed polymorphism of intramural ganglions and efferent neurons of the biliary system of the dog. Relatively large nerve nodes are located in the neck of the gallbladder and in the wall of the common bile duct. The intramural nervous apparatus of the biliary system contains a large number of efferent neurocytes with bodies of various shapes. For the first time, as a result of a combination of experimental and impregnation methods, a connection was established between the efferent neurons of the gallbladder and biliary tract and the vagus nerve.

Literature

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