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DEVELOPMENT AND DIFFERENTIATION OF THE INTERMUSCULAR AND SUBMUCOSAL NERVE PLEXUS OF COW EMBRYOS LARGE INTESTINE

Vladimir ZDOROVININ, Julia KULIKOVA, Ildar MAVLUDOV, Tatiana DOROFEEVA

Penza State Agriculture University, 30 Botanicheskaya Street, 440014, Penza, Russia

Corresponding author email: zdorovinin.va@pgau.ru

Abstract

The article describes the development and differentiation of the intermuscular and submucosal nerve plexuses of the large intestine of the fetuses of cows from 50 days of age to birth. On days 50-70, the muscular membrane of the intestinal wall is built from bundles of smooth muscle cells, clearly divided into inner (annular) and outer (longitudinal) layers. Ganglia are laid between them, forming the intermuscular nerve plexus. The submucous nerve plexus differentiates later. The development of the nervous apparatus of the rectal wall is ahead of the development of similar structures of the colon and cecum. Enhanced growth and differentiation of nerve cells in fetuses is noted from 95-120 days. The first synapse-like contacts were observed in the intestinal ganglia at the age of 4.5 months of embryonic development. Enhanced differentiation and growth of nerve cells of the submucous nerve plexus is observed in fetuses of a cow at 6-7 months. By the end of the embryonic period and in early postnatal ontogenesis, connective tissue gradually overgrows the developing ganglion of the colon wall. Before the birth of the fetus, the nervous tissue of the colon wall forms a complex intramural nervous system, represented by four plexuses - intermuscular, submucosal, actually mucous and sub-serous. There are a large number of mature synaptic contacts. The study examines the stages of morphological changes in the development of intestinal ganglia from the aggregation of neural cells to the formation of a complex intramural nervous system in the walls of the colon.

Key words: *glia, glial processes, intermuscular nerve plexus, nerve fibers, neuroblasts, neurons, preganglionic fibers, submucosal nerve plexus, synaptic vesicles.*

INTRODUCTION

The study of the patterns of development of the digestive system, including the large intestine, is an important prerequisite for the development of a nutritional system, prevention and diagnosis of various diseases.
A significant number of studies devoted to the digestive system of animals touch upon general questions of the structure and patterns of growth of the gastrointestinal tract in ontogenesis. The morphology and histology of the small intestine has been studied in some detail (Bogolepov, 1994).
Analysis of the available sources shows that the large intestine in fetuses and in newborn calves (especially its immune and nervous systems) has not been sufficiently studied from the histological and cytochemical points of view. Therefore, in the course of the study, tasks were set and solved for the study of 1) the laying, formation and specialization of the nerve plexuses of the colon in fetuses and newborn calves; 2) the development and differentiation of the intermuscular and submucosal nerve plexuses of the large intestine of the fetuses of cows from 50 days of age to birth; 3) consideration of the stages of morphological changes in the development of intestinal ganglia from the aggregation of neural cells to the formation of a complex intramural nervous system in the walls of the colon (Sosunov, 1996).

MATERIALS AND METHODS

The material for the study was fetuses and newborn calves, their colon (its components: blind, colon, rectum). Pieces of tissue were taken from different parts of the colon: cranial, middle, and caudal. The material was fixed by a standard method for histological, histo-physiological and histochemical studies (Teltsov, 2002). Cytometry was performed by measuring the short and long diameters of the nucleus, the height and width of the cell

(Muller, 1992). The area of the nucleus and the cell was calculated and the nuclear-cytoplasmic ratio was derived. For the statistical analysis of all results, such indicators as the arithmetic mean, the arithmetic mean error, the correlation coefficient, and the error probability were used (Furness, 2000).

RESULTS AND DISCUSSIONS

At 3 months of age, the fetuses of cows, the nerve ganglia of the colon wall become more compact, the number of cellular nerve elements and fibers increases. The glial layers in the ganglia are well developed, and on the periphery of the ganglion there are flattened bodies and leaf-like processes of cells, which can be considered glioblasts. It should be noted the presence of desmosome-like contacts between neuroblasts and glial cells. On the periphery of the ganglion, there are small bundles and individual collagen fibers that limit it from the surrounding tissues. At this time, synaptic contacts appear with osmiophilic active zones and a few small light-colored synaptic vesicles. There are also large granular vesicles in the cell bodies and in the nerve processes. In the cytoplasm of neuroblasts, single tubules of the granular endoplasmic reticulum appear. In neuroglial cells, the content of tubules of the endoplasmic reticulum also increases. At this time, one can see how glial cells surround large bundles of nerve fibers.

Sections at this age reveal from 8 to 12 neuroblasts, single differentiating young neurocytes. The latter are polygonal and form one or two processes.

In fetuses of 90-120 days of age, in the nerve ganglia lying in the area of mesentery attachment, along with a large number of neuroblasts (10-20), multi-process young differentiating neurocytes are revealed (Figure 1).

In fetuses of cows 3-5 months with age, the number of mature cells with a large nucleus increases in the ganglia. At this time, neuro- and glioblasts can be clearly differentiated by the shape of the nucleus and body.

Figure 1. Section of the intermuscular nerve plexus of the colon wall of a 4-month-old fetus

Large nerve processes are located both in the center and periphery of the ganglion.
The loops of the intermuscular nerve plexus acquire a varied shape, the bundles of nerve fibers that form them become more powerful, the ganglia are located at the intersections and branching of the bundles, the neurons are at various stages of differentiation.
At 4-4.5 months of age, most of the neurocytes have a well-developed lamellar Golgi complex, with a large number of bordered vesicles near its cisterns. Large granular vesicles and small vesicles are also found in many cells. At the age of 4-4.5 months it is possible to isolate synapses in the intermuscular nerve plexus. In the early stages of their development, synapses look likedelicate buttons, dumbbells, loops that end on the bodies of nerve cells. In this case, near the nucleus, the thinnest branches of the preganglionic fibers. At the point of contact, a small depression is formed on the body of the neuron.
In fetuses of cows at the middle stage of development (from 5 to 7 months), the ganglia become even more dense, due to a decrease in the volume of free intercellular space. Many neuroblasts and young neurons are in contact with each other, forming simple and desmosome-like connections. This is due to the fact that the processes of glial cells, especially in the central parts of the forming ganglia, are still not well developed. The ganglia include type 1 Dogel cells. In close connection with these cells, neurons arise with two to four long, multiply branching processes that extend from different poles of the cell and leave the ganglion. Neurons with such processes in their morphological characteristics belong to type 2 Dogel cells. Dogel type 2 cells are pear-shaped, elongated, body contours are smooth, and processes are long.
The presence of long and short (receptor) dendrites is noted. Long dendrites extend from the poles, penetrating the ganglion and without branching, extend far beyond the ganglion. Short dendrites, shortly after leaving the cell body, repeatedly branching and form receptor endings either on the territory of the ganglion or outside it.
At the late stage of fetal development in cows (from 7 months to birth), the volume of the neuropil increases, and the number of synaptic contacts also increases. The active zones become longer, the number of small synaptic vesicles, characterized by a variety of shapes and sizes, increases significantly (Figure 2). In many differentiating neurons, processes with well-developed cytoplasmic organelles were observed.

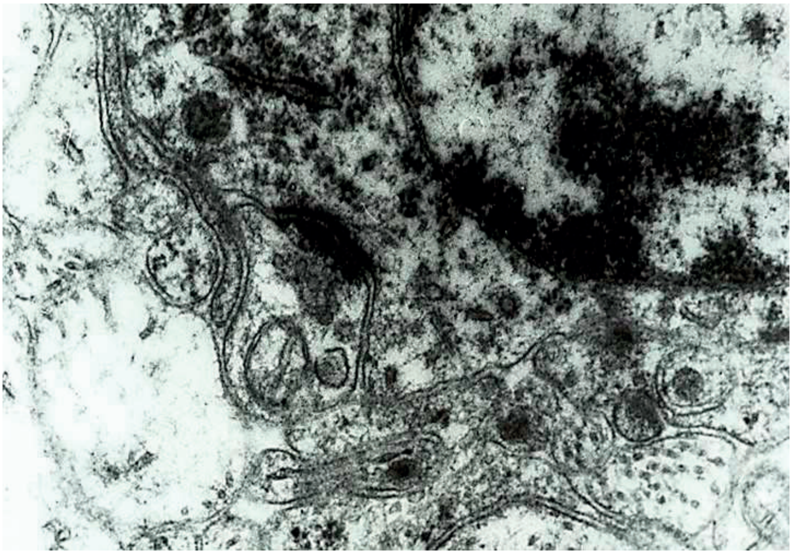

Figure 2. Ultrastructure of specialized interneuronal contact of the intermuscular nerve plexus of the rectal wall of a 4.5-month-old fetus. Uv. 30,000

Sometimes it was possible to observe a close arrangement of bundles of nerve processes directly near the cytolema of a smooth muscle cell. During this period, nerve fibers with varicose veins filled with large granular vesicles, mitochondria and single synaptic vesicles are found in the neuropil of the ganglion.

On drugs at this age, the number of differentiating neurocytes increases. The number of nucleoli in them decreases, but as a rule, the neurocyte still contains 2 or more nucleoli. At the late stage of fetal development, the nerve fibers and ganglia of the intermuscular nerve plexus form a large-looped network. There are significantly more differentiating nerve cells in the ganglia of the intermuscular nerve plexus than in the ganglia of the submucosal nerve plexus. The caudocranial gradient in the development of nerve tissue remains at this age. It can be traced when comparing the level of differentiation and the degree of innervation of the walls of the blind, colon and rectum. The most differentiated ganglia are the nerve plexuses of the rectum, less - the colon and blind. Before birth, already well-formed nerve plexuses are presented as mature cells, and neuroblasts and glioblasts, as well as a large number of nerve fibers. Nerve cells differ in the area of the perikaryon and especially clearly in the organization of nuclear chromatin. In the nerve ganglion, the walls of the rectum, cells and nerve fibers are located compactly, there is practically no free intercellular space. Poorly differentiated cells also lie in compact groups. Nerve and glial cells differ well both in chromatin density and in the organization of the cytoplasm. In these terms, the organization of the ganglionic neuropil becomes much more complicated. An increase in the number of their own processes in nerve cells and a weak development of glial membranes leads to the formation in the nerve ganglion, the walls of the rectum, cells and nerve fibers are located compactly is practically no free intercellular space. Poorly differentiated cells also lie in compact groups. Nerve and glial cells differ well both in chromatin density and in the organization of the cytoplasm. In these terms, the organization of the ganglionic neuropil becomes much more complicated. An increase in the number of their own processes in nerve cells and a weak development of glial membranes leads to the formation in the nerve ganglion, the walls of the rectum, cells and nerve fibers are located compactly, there is practically no free intercellular space. Poorly differentiated cells also lie in compact groups. Nerve and glial cells differ well both in chromatin density and in the organization of the cytoplasm. In these terms, the organization of the ganglionic neuropil becomes much more complicated. An increase in the number of their own processes in nerve cells and a weak development of glial membranes leads to the formation in these terms, the organization of the ganglionic neuropil becomes much more complicated. An increase in the number of their own processes in nerve cells and a weak development of glial membranes leads to the formation in these terms, the organization of the ganglionic neuropil becomes much more complicated. An increase in the number of their own processes in nerve cells and a weak development of glial membranes leads to the formation numerous simple contacts between the former. There are a large number of synaptic contacts, and a significant part of them do not differ in structure from mature contacts. In some cases, one terminal is observed to form several contacts with two or more cells, or by the process of one cell of several contacts with two terminals. Apparently, already at this time, the formation of communication relationships in the neuropil of the ganglion was taking place. Neurons and nerve fibers in the nerve nodes are closely adjacent to smooth muscle cells, and due to the frequent absence of glial layers, they come into contact with muscle cells. Nerve fibers running through the muscle membrane can also form simple contacts with smooth muscle cells.

On preparations in the serous membrane, small single nodules are revealed along the nerve fibers. In the nerve ganglia of the colon, dividing neuroblasts are rare. By 8 months at the age of the fetus, the number of neurocytes is maintained due to the differentiation of neuroblasts into neurocytes, and not by mitotic division. Before the birth of the fetus, the nervous tissue of the colon wall forms a complex intramural nervous system, represented by four large plexuses - intermuscular, submucosal, and actually mucous. Each of them has a superficial and deep arrangement of ganglia.

By this time, the glial environment of neurons and nerve processes becomes sufficiently developed. A well-developed neuropil of the intestinal ganglia is characterized by the presence of compactly located numerous nerve fibers and profiles filled with various vesicles (Figure 3).

In fetuses at the mid-stage of development of the PNS ganglion, the walls of the colon are located at different levels. Larger ganglia are localized on the annular layer of the muscle membrane, smaller ones - near the muscle plate of the mucous membrane.

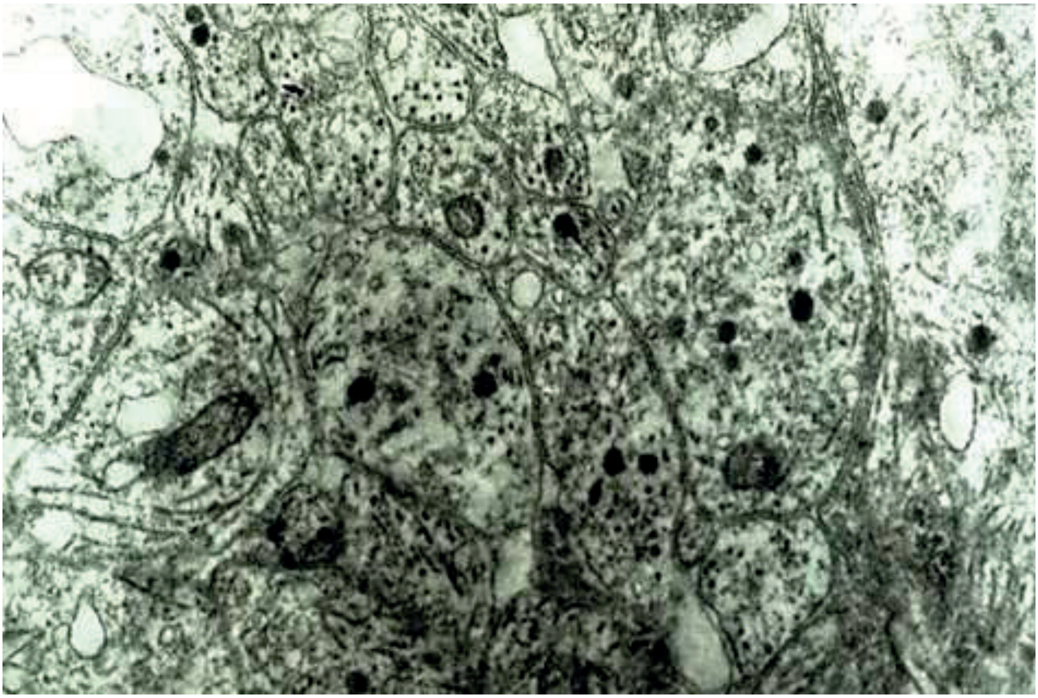

Figure 3. Neuropil of the MHC ganglion of the colon wall of a 9-month-old calf. Zoom 32000

Throughout the fetal and newborn stages of development, the ganglia retain this localization. The ganglia include neuroblasts, single differentiating neurocytes, and neuroglial cells.

Starting from 6-7 months the age of the fetus, in the intestinal wall neuroblasts are found localized in the lamina propria of the mucous membrane of the crypts and the base of the villi.

At the late fetal stage of development, the number of neuroblasts, differentiating neurocytes and neurocytes of types 1 and 2 according to Dogel increases. Some of the nerve fibers are surrounded by the myelin sheath. Under the crypts, in the proper lamina of the mucous membrane, small nerve nodules are found, consisting on transverse sections of 1-2 neurocytes, 3-4 neuroblasts and 9-12 glial cells. These nodules are found in greater numbers in the rectum and cecum than in the colon.

At the late fetal stage of development, the nerve fibers and ganglia of the intermuscular nerve plexus form a large-looped network, and its largest loops are located in the colon, in comparison with the rectum and cecum. Mature neurocytes of types 1 and 2 according to Dogel, in comparison with neuroblasts, differentiating neurocytes and neuroglial cells, have a larger cell area and a high level of development. In the middle and late stages of fetal development, first in the ganglia of the intermuscular nerve plexus, and later in the ganglia of the submucosal nerve plexus, synaptic connections are formed. Polyvalent receptors are found in the mucous and muscular membranes. As a rule, in the ganglia, Dogel type 1 neurocytes are located separately from type 2 neurocytes. Nerve cells located together, intertwined with dendrites, form dendric tangles. Before birth, as before, glial cells and neuroblasts predominate in the ganglia of the intermuscular and submucosal nerve plexuses. In the serous membrane, along the nerve fibers, there are single nerve nodules.

CONCLUSIONS

The morphological changes observed during the development of intestinal ganglia have the following sequence: 1 - aggregation of neural cells of the anlage of ganglia, coinciding with the growth of preganglionic fibers; 2 - the beginning of differentiation of neural crest cells; 3 - the appearance of cells with glial and neuronal phenotypes (the formation of processes in neural cells); 4 - progressive complication of the shape of neuroblasts, due to the growth and branching of processes; 5 - the appearance of synaptic contacts, ensuring the expansion of interneuronal connections; 6 - some isolation of neurons in the ganglion due to the formation of glial membranes and the connective tissue capsule of the node; 7 - the development of ganglia due to quantitative changes in nerve cells (cell growth, complication of the neuropil, the formation of numerous and diverse synaptic contacts).

The specialization of the intermuscular nerve plexus in black-and-white cattle is formed from 3-4 months, the age of the fetus, and the organ specialization of the submucosal nerve plexus is carried out for 5-6 months.

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