A STUDY OF RAT DORSAL ROOT GANGLION GLIAL CELLS USING IMMUNOHISTOCHEMICAL DETECTION OF GLUTAMINE SYNTHETASE

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The main structural elements of the dorsal root ganglion (DRG) are sensory neurons and glial cells. The satellite glial cells (SGCs) are most poorly studied glial element of DRG. Currently, a wide range of immunohistochemical markers are used to detect SGCs: glial fibrillar acidic protein (GFAP), S100 protein, vimentin and the most selective marker of satellite glial cells - glutamine synthetase (GS) [Kolos, Korzhevskii, 2018]. It is known that SGCs play an important role in maintaining glutamate homeostasis by participating in the deactivation of the perineuronal neurotransmitter. There are a few studies on detection of glutamine synthetase-contaning cells of the adult sensory ganglion [Miller et al.,2002; Saitoh, Araki, 2010], while studies on these cells in the prenatal period have not been performed.

The *aim* of this study is to detect glutamine synthetase-containing glial cells of the rat DRG in embryogenesis and after birth using immunohistochemical methods.

Materials and Methods. We used embryos of Wistar rats at embryonic days 12–19 (E12-E19, n = 20), newborn (n = 5) and mature (n = 5) rats. Cervical dorsal root ganglia (segments 3–5) were used for the study. Fragments of the rat spinal cord were fixed in zinc-ethanol-formaldehyde solution, dehydrated, and embedded in paraffin. Anti-glutamine synthetase antibody (clone GS-6, Chemicon, USA) was used.

Results and discussion

Satellite glial cells

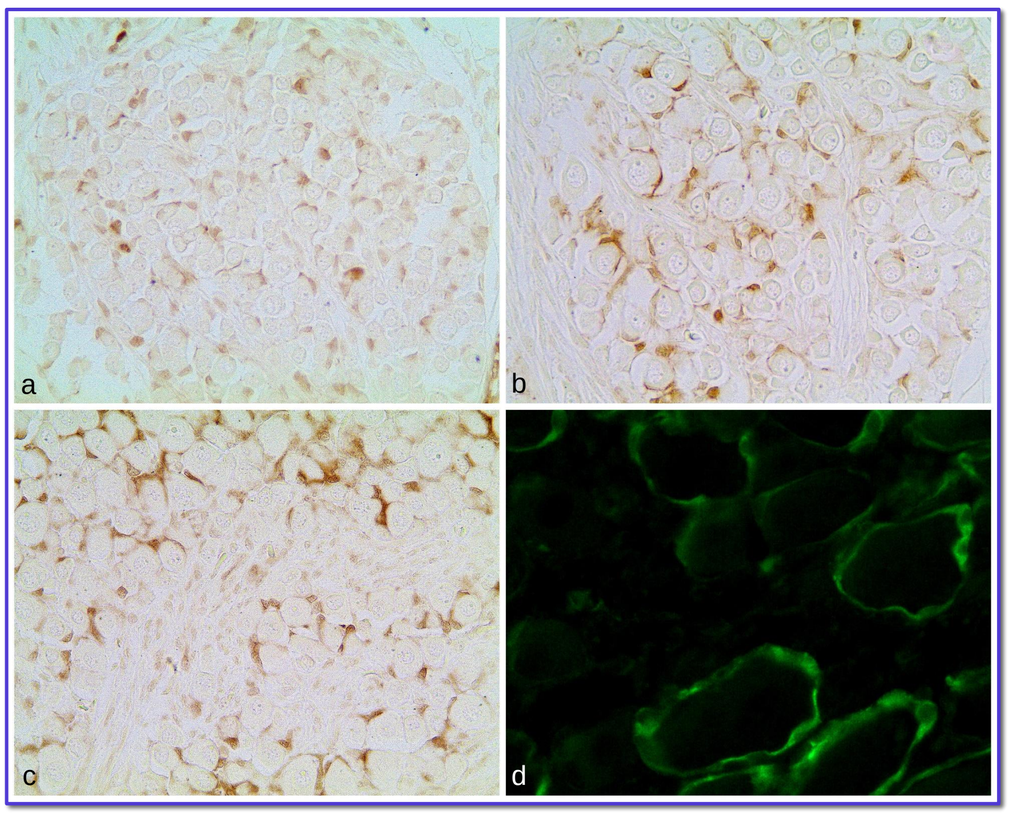

Fig. 1. Glutamine synthetase-immunopositive satellite glial cells of the DRG of 18-day rat embryo (a), 19-day rat embryo (b) newborn rat (c) and mature rat (d). Immunohistochemical staining for glutamine synthetase (a, b, c); immunofluorescence reaction to glutamine synthetase (Cy2 - green staining) (d). Magnification: (a, b, c) x400; (d) x1000.

Boundary cap cells

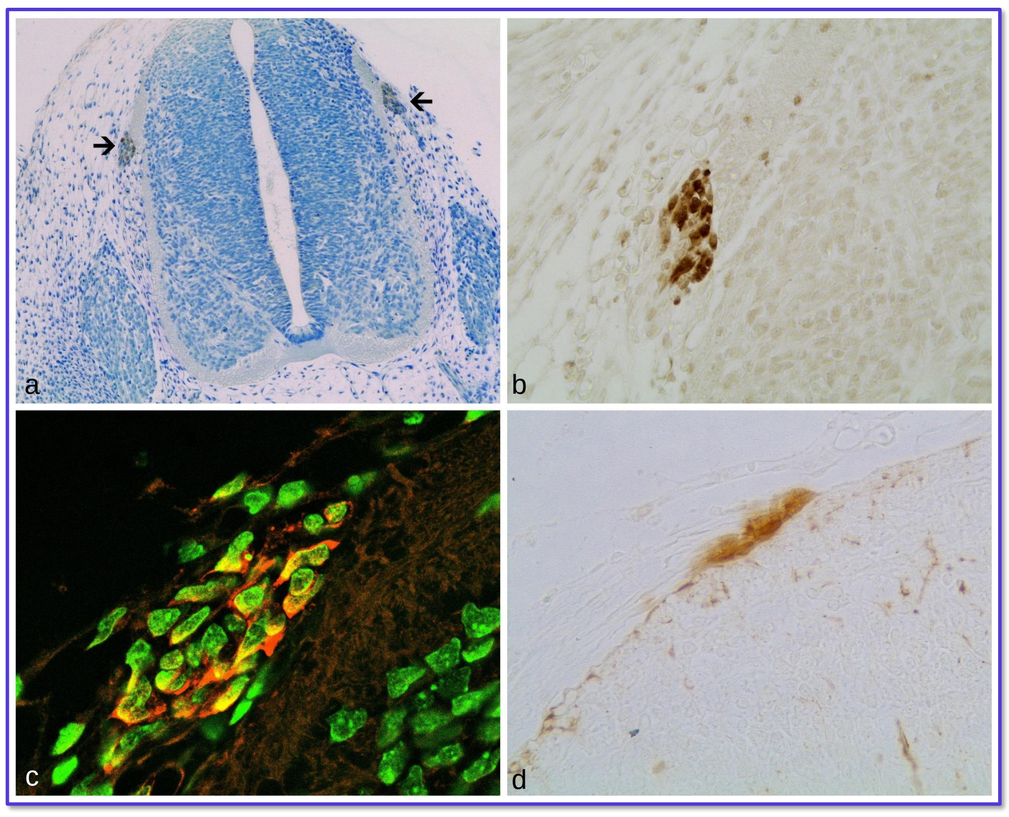

Fig. 2. Glutamine synthetase-immunopositive boundary cap cells of a rat embryos of 14 (a), 15 (b), 16 (c) days of development and a newborn rat (d). Arrows indicate boundary cap cells of the forming posterior root of the spinal cord. Immunohistochemical reaction to glutamine synthetase (a, b, d); doublestained with toluidine blue (a); immunofluorescence reaction to glutamine synthetase (Rhodamine Red-X—red staining) with additional staining for cell nuclei SYTOX Green dye (green staining) (c). Magnification: (a) x100; (b) x400; (c) x1000 and (d) x400.

In the present study, we have demonstrated that glutamine synthetase is present in forming satellite glial cells of the rat DRG, starting from 18 days of embryonic development (Fig. 1). On embryonic day 18, the enzyme could be revealed only in the perinuclear region of the forming small SGCs, located in close vicinity to sensory neurons. On embryonic day 19, GS was present in the cytoplasm of young SGCs, located around the forming neurons of the DRG.

It is known that the initial afferents of sensory neurons reach the spinal cord at days 15–16 of embryogenesis; the branching of axons and the formation of synapses, including glutamatergic synapses, on the spinal neurons starts from day 18 of development. Thus, it is clear that the processes of enzyme synthesis, providing for the glutamate recirculation within the DRG, start in the cytoplasm of SGCs during the formation of the contacts of neurons with their target cells in the spinal cord.

In this study, it was shown for the first time that boundary cap cells (BCC) synthesize glutamine synthetase. BCC are a temporary population of cells, but the duration of their existence in the area of spinal cord roots remains unknown. We have shown that BCC containing GS are present in the dorsal root entry zone (DREZ) starting from the E14 and until birth (Fig. 2). The functional significance of the expression of glutamine synthetase by the BCC that form a barrier in the border zone requires additional research. During E13–E14, the growing axons of the emerging glutamatergic neurons of the DRG are directed to the dorsolateral part of the spinal cord and enter the marginal layer through the DREZ. The release of glutamate from the growing sensory axons probably reduces their response to repulsive guidance molecules. The regulation of the concentration of glutamate released by growing axons in the transition zones of the spinal cord can be provided by BCC together with the glia limitans.

***Conclusion.* Thus, this study demonstrates for the first time that differentiating satellite glial cells of the rat DRG contain glutamine synthetase from day 18 of embryonic development. Immunohistochemical detection of this enzyme allows differentiate them from developing Schwann cells and neuroblasts. It was established for the first time that the boundary cap cells, located at the border with the PNS, synthesize glutamine synthetase. It was noted that such cells are present in the area of dorsal root entry zone during the period from the 14th day of embryonic development to birth.**

Литература: Kolos, E.A. and Korzhevskii, D.E., Glutamine synthetase-containing cells of the dorsal root ganglion at different stages of rat ontogeny, Russ. J. Dev. Biol., 2018, vol. 49, no. 3, pp. 179–183.
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