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Expression of Serotonin Transporter Protein in the Dorsal Raphe Nucleus during the Early Postnatal Period in Rats

L. I. Khozhai

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We report here our studies on expression of serotonin transporter protein (5-HTT) in the dorsal, ventral, and lateral subnuclei of the dorsal raphe nucleus (DRN) in Wistar rats (n = 15) during the early postnatal period. Histological methods were used, along with the immunocytochemical reaction for 5-HTT. Most neurons in the subnuclei studied here were found to express 5-HTT intensely on day 5 of the postnatal period. However, by day 10, the level of expression decreased sharply, though there was a subsequent age-related (by day 20) increase in expression, with a rise in the number of cells in the 5-HTT-positive neuron population and an increase in the density of the plexus formed by their processes. These changes in 5-HTT expression provide evidence of different levels of the functional activity of serotonin in the DRN during the early postnatal period.

Keywords: dorsal raphe nucleus, neurons, neuron processes, serotonin transport protein.

The serotonin transporter (5-HTT) is the main factor controlling the intracellular serotonin level. Studies of the characteristics of changes in its synthesis during different periods of ontogeny and the tight interaction with different types of neurons are important for understanding the regulation of serotonin activity both during CNS development and in the execution of a variety of physiological functions.

Existing data provide evidence that there are significant differences in the distributions of 5-HTT in the plasma membranes of the bodies of serotoninergic neurons between embryos and adults [2]. An immunocytochemical reaction for 5-HTT showed that the plasma membranes of neuron bodies and dendrites show insignificant numbers of 5-HTT binding sites in adult animals. In embryos and fetuses, significant quantities of granules containing 5-HTT are present at the peripheries of neuron bodies and on the membranes of large dendrites in the raphe nuclei. 5-HTT on neuron bodies, dendrites, and terminal processes mediates serotonin reuptake, simultaneously facilitating the triggering of active release of serotonin in the developing brain [2, 3]. However, there are virtually no reports on the distribution of 5-HTT binding sites in the raphe nuclei and early postnatal development. Thus, the aim of the present work was to study 5-HTT expression in the dorsal (d), ventral (v), and lateral (lat) subnuclei of the dorsal raphe nucleus (DRN) during the early postnatal period in rats.

Materials and Methods

Studies were performed on Wistar laboratory rats. Animal keeping and all experiments procedures were performed in compliance with the "Regulations for Studies Using Experimental Animals" (USSR Ministry of Health Decree No. 755 of August 12, 1977). Brains from rat pups were studied on days 5, 10, and 20 of postnatal development (five animals in each age group); specimens were fixed in zinc-ethanol-formaldehyde pH 7.4 and embedded in paraffin using a standard method; serial transverse sections of thickness 5 μm were cut at the level of the bregma – 7.92–8.04 mm.

Immunocytochemical reactions for 5-HTT were performed using primary antibodies (anti-serotonin transporter antibody, AbCam, UK) and secondary reagents from an LSAB2 System-HRP (Dako, Denmark). Reaction product was visualized using DAB+ chromogen (Dako, Denmark). After the immunocytochemical reaction, some sections were

Laboratory for Nervous System Ontogeny, Pavlov Institute of Physiology, Russian Academy of Sciences, St. Petersburg, Russia; e-mail: astarta0505@mail.ru.

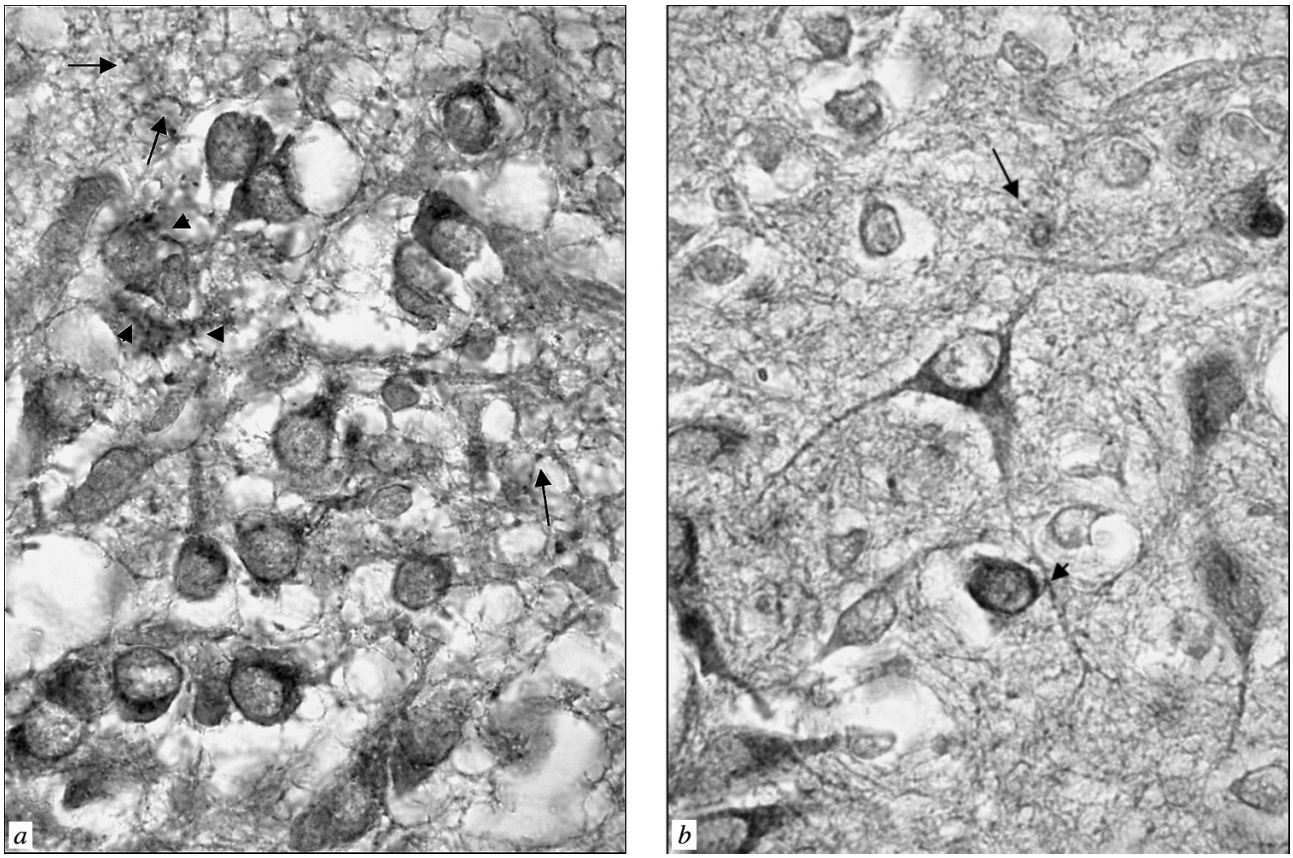

Fig. 1. Rat dorsal raphe nucleus on day 5 (*a*) and day 10 (*b*) of the postnatal period of development. Long arrows show terminal varicose dilations; arrowheads show immunopositive granules. Immunocytochemical reaction for serotonin transporter protein. Objective ×100, ocular ×10.

counterstained with Meyer's hematoxylin (Bio-Optica, Italy) or thionine (Serva, USA, Germany) and embedded in synthetic Permount medium (Termo, USA). All immunocytochemical reaction procedures on histological brain sections from animals at different stages of development were standardized and performed simultaneously. Analysis of reaction results consisted of assessing immunocytochemical staining of the cytoplasm of neurons, the presence of varicose dilations of terminals, and the distribution of immunopositive granules – as presumptive 5-HTT binding sites [3].

Neurons were counted on histological sections passing through the central part of the nucleus. Morphological analysis and quantitative assessments were performed using digital images of serial sections taken using a Leica DME (Leica, Germany) light microscope and a Leica EC3 digital camera (Leica, Germany). Numbers of immunopositive neurons per standard unit area were counted as a percentage of the total number, taken as 100%.

Results

On day 5 of postnatal development, virtually all large neurons in the DRN-d, DRN-v, and DRN-lat were 5-HTT-immunopositive and the cytoplasm of these neurons showed intense staining (Fig. 1). Reactions to 5-HTT showed small numbers of immunopositive granules on the plasma membranes of neuron bodies in the DRN-d, though there were no such granules on the bodies of neurons in the DRN-v or DRN-lat. The neuropil of the DRN-d and DRN-v contained a quite dense network of fine immunopositive terminal processes with large numbers of varicose dilations. The surfaces of membranes of both dendrites and axons (along their lengths) bore immunopositive granules.

On day 10, around 50% of neurons in the DRN-d, DRN-v, and DRN-lat were immunopositive, mainly large neurons. The volume of stained cell cytoplasm was significantly greater than at the preceding time point and staining intensity was identical. Dendrite cytoplasm was stained but axon cytoplasm was not. Immunopositive granules were absent from the membranes of neuron bodies, though they were seen throughout the lengths of processes, in smaller numbers than at the preceding time point.

The density of the network of fine, weakly immunopositive terminals in the neuropil was significantly lower than at five days of development; there were also fewer varicose terminal dilations (see Fig. 1, *b*).

On day 20, most large neurons in the DRN-d, DRN-v, and DRN-lat (almost 80%) were immunopositive, and these

neurons were mainly large in size. There were no granules on the plasma membranes of neuron bodies.

The neuropil contained a quite dense network of immunopositive processes. Terminal varicose dilations were rare in the DRN-d and DRN-lat, though the neuropil contained many small immunopositive granules. In the DRN-v, the neuropil contained a dense network of immunopositive processes and many terminal varicose dilations and granules, though they were fewer in number than on day 5 of development.

Discussion

The results obtained here showed that most neurons in the subnuclei (DRN-d, DRN-v, and DRN-lat) of the DRN intensely expressed 5-HTT on day 5 of the postnatal period. However, the expression level decreased sharply by day 10 but then showed an increase with age (by day 20), this being due to an increase in the number of neurons in the population expressing 5-HTT, an increase in the density of the network of immunopositive processes, and the appearance of many very small (dust-like) immunopositive granules, evidently representing presynaptic 5-HTT binding sites [3, 4].

The presence of a large number of 5-HTT on the bodies, dendrites, and terminal processes of DRN neurons [5] is probably evidence for a process of efficient reuptake of both extracellular and synaptic serotonin.

These results suggest that serotonin reuptake facilitates triggering of its active release, which is a prerequisite for the development and maturation of the CNS and the formation of serotonin-regulated functions [3]. The changes in 5-HTT expression seen here provide evidence that serotonin has different levels of functional activity in the DRN in the early postnatal period.

REFERENCES

1. G. Bruning, O. Liangos, and H. G. Baumgarten, "Prenatal development of the serotonin transporter in mouse brain," *Cell Tiss. Res.*, **289**, No. 2, 211–221 (1997).
2. H. Tao-Cheng and F. C. Zhou, "Differential polarization of serotonin transporters in axons vs. soma-dendrites: am immunogold electron microscopy study," *Neuroscience*, **94**, 821–830 (1999).
3. F. C. Zhou, Y. Sari, and J. K. Zhang, "Expression of serotonin transporter protein in developing rat brain," *Dev. Brain*, **119**, 33–45 (2000).
4. F. C. Zhou, J. H. Tao-Cheng, L. Segu, et al., "Serotonin transporters are located on the axons beyond the synaptic junction: anatomical and functional evidence," *Brain Res.*, **805**, 241–254 (1998).
5. F. C. Zhou, Y. Xu, S. Bledsoe, et al., "Serotonin transporter antibodies: production, characterization, and localization in the brain," *Mol. Brain Res.*, **43**, 267–278 (1996).