DOI 10.1007/s11055-022-01319-y
Neuroscience and Behavioral Physiology, Vol. 52, No. 6, July, 2022

Monoamine Oxidase B in Developing Histaminergic Neurons in the Rat Brain

S. M. Zimatkin and A. V. Zaerko

Translated from Morfologiya, Vol. 159, No. 1, pp. 13–19, January–February, 2021. Original article submitted January 19, 2021. Accepted December 10, 2021. Published March 4, 2022.

Background. Brain histaminergic neurons play an important role in regulating many body functions, systems, and reactions, and also in the pathogenesis of many pathological states and diseases. Histamine in the brain functions as a neurotransmitter and is located mainly in histaminergic neurons. All histaminergic neurons in the hypothalamus, in contrast to other types of neurons, have high monoamine oxidase type B (MAO B) activity, this being a key enzyme in histamine metabolism in the brain. **Objective.** Parallel assessment of MAO B activity and immunoreactivity in histaminergic neurons in the rat hypothalamus during postnatal ontogeny. **Materials and methods.** Experiments were run on hypothalamus specimens from 5-, 10-, 20-, 45-, and 90-day offspring of white rats (45 rat pups) in compliance with the "Guidelines for Studies Using Experimental Animals." Hypothalamus sections were processed histochemically to detect MAO B activity and immunohistochemically using antibodies to MAO B. **Results.** MAO B, the enzyme catalyzing the oxidative deamination of histamine and functioning as a marker enzyme for histaminergic neurons in the hypothalamus, showed no cytoplasmic activity or immunoreactivity on day 5 after birth, after which these measures increased in parallel from day 10 to day 90 of postnatal ontogeny. **Conclusions.** The synchronicity of postnatal development of the MAO B activity and immunoreactivity in histaminergic neurons in the brain is evidence of the parallel accumulation of MAO B protein in these cells and increases in its enzymatic activity, reflecting the establishment of their specific neurotransmitter metabolism.

Keywords: monoamine oxidase B, histaminergic neurons, brain, hypothalamus.

Histaminergic neurons in the brain play an important role in regulating many body functions, systems, and reactions: neuroendocrine and cardiovascular, cerebral blood flow, body temperature, sleep and waking, feeding and drinking behavior, and memory and learning, and they also have roles in the pathogenesis of many pathological states and diseases [1–5]. In the brain, histamine functions as a neurotransmitter and is located mainly in histaminergic neurons; small quantities of histamine are present in mast cells in the meninges and interlayers of loose connective tissue [2, 4]. The bodies of histaminergic neurons in the brain in mammals are located solely in the posterior hypothalamus, where they form five groups, i.e., nuclei (E1–E5) [1, 6]. The axons of histaminergic neurons are distributed to all parts of the brain, where they may coordinate other neural systems [7–9].

Monoamine oxidase type B (MAO B) is the key enzyme in histamine metabolism in the brain, where histamine oxidase (histaminase) is absent, and oxidizes up to 40% of the histamine at the periphery. Metabolism is the only means of removing histamine after completion of neurotransmission by histaminergic neurons in the brain, as there is no histamine reuptake system, which contrasts with other types of aminergic neurons, which have specific transporters for their transmitters to mediate reuptake [1, 5]. Histamine is metabolized by the enzyme histamine-N-methyltransferase to tele-methylhistamine, which is then converted by MAO B (oxidative deamination) to N-tele-methylimidazole acetaldehyde. All histaminergic neurons in the hypothalamus, in contrast to other types of neurons, have high MAO B activity [1]. Other types of hypothalamic neuron do not contain

Grodno State Medical University, Grodno, Belarus;
e-mail: smzimatkin@mail.ru, wersall_91@mail.ru.

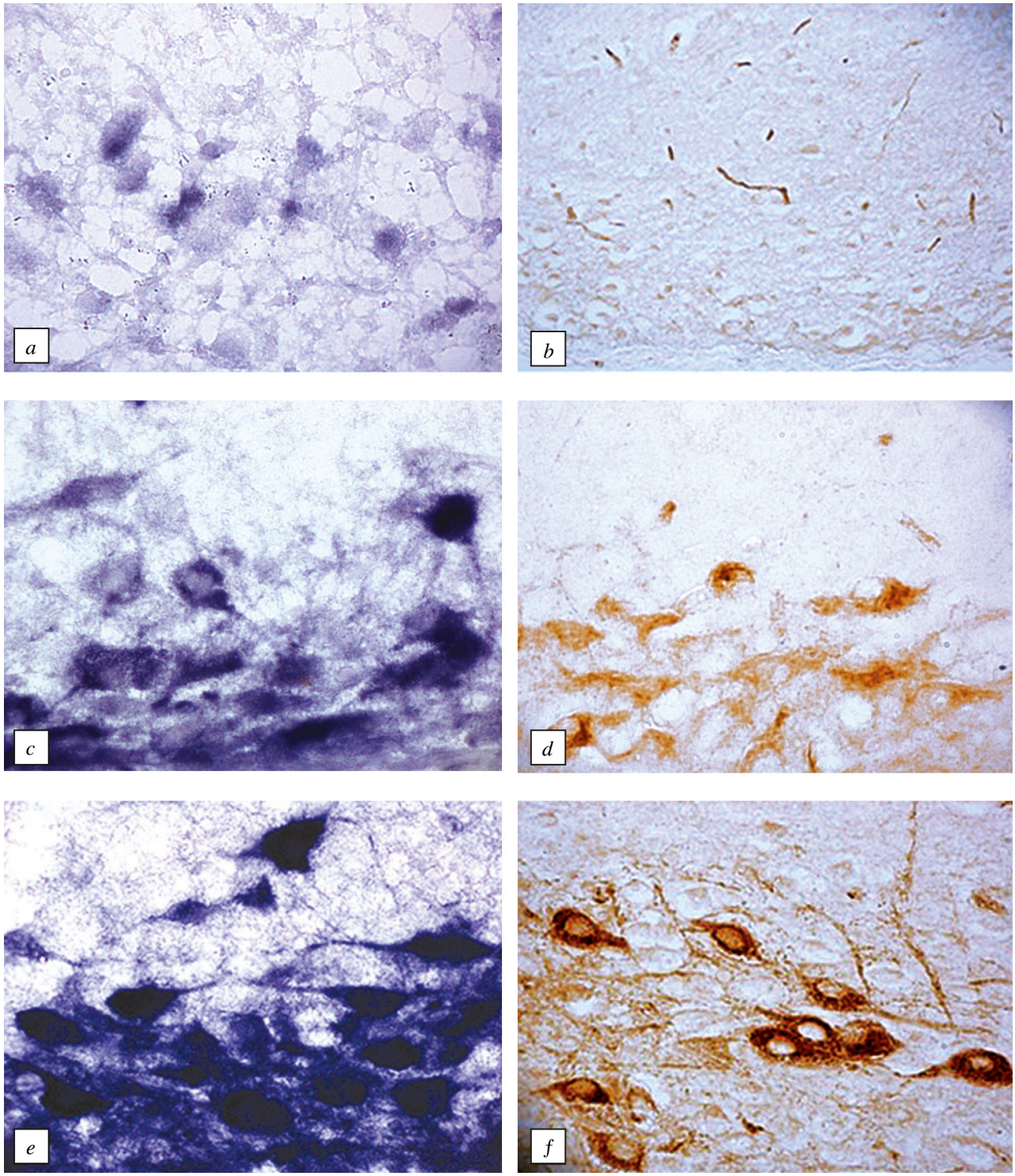

Fig. 1. MAO B in histaminergic neurons in rat hypothalamus nucleus E2 in postnatal ontogeny: *a*, *c*, *e*) histochemical detection of MAO B activity (Zimatkin and Tsydik method); *b*, *d*, *f*) immunohistochemical detection of MAO B protein (Zimatkin and Zaerko method); *a*, *b*) day 10; *c*, *d*) day 20; *e*, *f*) day 90 of postnatal development. Digital microphotography. Magnification ×400.

MAO B, which provided the grounds for us to develop histochemical and immunohistochemical methods of detecting histaminergic neurons in the hypothalamus, in cryostat and paraffin sections, respectively [1, 10]. Combined use of these two methods is of interest for parallel detection of the content of enzyme protein and MAO B activity to assess the establishment of specific transmitter metabolism in developing histaminergic neurons in the rat brain.

Objective. To carry out a parallel assessment of MAO B activity and immunoreactivity in histaminergic neurons in the rat hypothalamus during postnatal ontogeny.

Materials and Methods. Experiments were carried out on 12 male mongrel white rats with starting weight 230 ± 20 g and their offspring (a total of 45 rat pups). All experiments were performed in compliance with the "Guidelines for Studies Using Experimental Animals." This study was approved by the Biomedical Ethics Committee, Grodno State Medical University (protocol No. 1 of January 30, 2018). Rat pups were decapitated on days 5, 10, 20, 45, and 90 after birth. Brains were rapidly removed and the hypothalamus was dissected out and frozen in liquid nitrogen. A CM 1850 cryostat (Leica Microsystems GmbH, Germany) was used to cut serial cryostat sections of thickness 12 μm, which were processed histochemically to detect MAO B activity [Zimatkin, 2015]. Other hypothalamus specimens were fixed in zinc–ethanol–formaldehyde at +4°C (overnight) and then embedded in paraffin. A Leica RM 2125 RTS microtome (Leica, Germany) was used to prepare serial paraffin sections of thickness 5 μm, which were mounted onto slides. Sections were then processed with primary polyclonal rabbit antibodies to MAO B (Elabscience, Cat. No. EPP15673, China) at a dilution of 1:100 at +4°C for 20 h in a humid chamber. Bound primary antibody was detected using an Elabscience Cat. No. E-IR-R213 (China) detection kit. Histological and histochemical preparations were examined, photographed, and analyzed using an Axioskop 2 Plus microscope (Zeiss, Germany) fitted with a Leica DFC 320 digital video camera (Leica, Germany) with a ×40 objective and computer image analysis program Image Warp (Bit Flow, USA). The data were processed using nonparametric statistics in Statistica 10.0 (StatSoft Inc., USA). Single-parameter group comparisons were with the Mann–Whitney U test for independent sets. Between-group differences were regarded as statistically significant if the probability of erroneous assessments was no greater than 5% ($p < 0.05$, where p is the critical significance level).

Results. Histochemical studies showed that the activity of the marker enzyme for hypothalamic histaminergic neurons MAO B was not detected on day 5 after birth, was very low on day 10, and then progressively increased such that on day 90 it reached a level six times that on day 10. Similar data were obtained in immunohistochemical studies: MAO B immunoreactivity in histaminergic neurons increased four-fold from day 10 to day 90 of postnatal development (Figs. 1 and 2).

Discussion. Our data are consistent with results reported by Leung et al. (1993), who measured differences in regional MAO A and MAO B activities during postnatal development in Wistar rats and found that MAO B activity in the hypothalamus, corpus striatum, midbrain, and cerebral cortex was low on day 5 and then increased significantly with development [11]. Thus, MAO B, the enzyme metabolizing histamine and operating as a marker for histaminergic neurons, was not detected on day 5 of postnatal development, which may be evidence of the low level of oxidative deamination of histamine in the neurons of interest. However, the brain shows a peak histamine concentration during this time [12], which can be explained in terms of the sharp increase in the number of mast cells [5, 13] occurring in the velum interpositum between the developing thalamus and hippocampus [13]. The role of mast cell histamine in the brain is unclear, though in early postnatal ontogeny these cells are responsible for forming a significant proportion of the total pool of this biogenic amine in the brain [5]. The total content of histamine produced by mast cells at age about two weeks of postnatal development gradually decreases to the level typical of adult individuals. Decreases in histamine synthesis in mast cells result from maturation of histaminergic neurons in the tuberomammillary region, which also start to produce it [14]. This is consistent with our data on progressive increases in MAO B in these neurons in the period from day 10 to day 90 of postnatal development in rats. This reflects the establishment of transmitter activity in developing histaminergic neurons in the brain. The synchronicity of the postnatal development of MAO B activity and immunoreactivity in histaminergic neurons in the brain is evidence of parallel accumulation of MAO B protein in neurons and increases in its enzymatic activity. With the development of histochemical and immunohistochemical methods, this approach can also be used for studies of other enzymes.

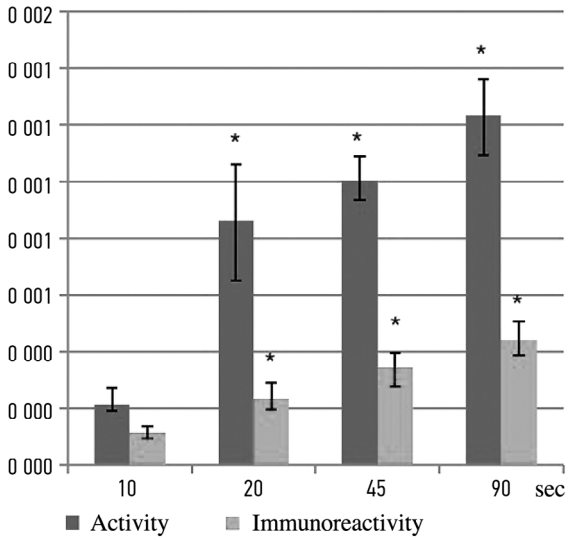

Fig. 2. MAO B activity and immunoreactivity in histaminergic neurons in rat hypothalamus nucleus E2 during postnatal ontogeny. Neuron cytoplasm optical density in the histochemical and immunohistochemical MAO B detection methods. *$p < 0.05$ for each period compared with the preceding period.

Conclusions. From day 5 to day 90 of postnatal ontogeny, the cytoplasm of histaminergic neurons in the rat hypothalamus showed parallel increases in the activity and immunoreactivity of the enzyme carrying out the oxidative deamination of histamine, MAO B, which reflects the establishment of specific transmitter metabolism in these cells.

The authors declare that there was no external funding for this study.

The authors declare that they have no conflicts of interests associated with publication of this article.

Authors' contributions: concept and study design: S. M. Zimatkin; specimen collection and processing, statistical analysis: A. V. Zaerko; data analysis and interpretation, writing of text: S. M. Zimatkin and A. V. Zaerko.

REFERENCES

1. S. M. Zimatkin, *Histaminergic Brain Neurons*, Novoe Znanie, Moscow (2015).
2. R. E. Brown, D. R. Stevens, and H. L. Haas, "The physiology of brain histamine," *Prog. Neurobiol.*, **63**, 637–672 (2001).
3. H. L. Haas, O. A. Sergeeva, and O. Selbach, "Histamine in the nervous system," *Physiol. Rev.*, **88**, 1183–1241 (2008).
4. H. Haas and P. Panula, "The role of histamine and the tuberomamillary nucleus in the nervous system," *Nat. Rev. Neurosci.*, **4**, 121–130 (2003), https://doi.org/10.1038/nrn1034.
5. P. Panula and S. Nuutinen, "The histaminergic network in the brain: basic organization and role in disease," *Nat. Rev. Neurosci.*, **14**, 472–487 (2013), https://doi.org/10.1038/nrn3526.
6. N. Inagaki, K. Toda, I. Taniuchi, et al., "The five subgroups of the tuberomammillary nucleus of the rat: an analysis of the histaminergic efferent projections to the medial preoptic area and inferior colliculus," *Exp. Brain Res.*, **80**, 374–380 (1990).
7. M. S. Airaksinen, G. Flügge, E. Fuchs, and P. Panula, "Histaminergic system in the tree shrew brain," *J. Comp. Neurol.*, **286**, 289–310 (1989).
8. N. Inagaki, A. Yamatodani, K. Shinoda, et al., "Histaminergic nerve fibers in the median eminence and hypophysis of rats demonstrated immunocytochemically with antibodies against histidine decarboxylase and histamine," *Brain Res.*, **439**, 402–405 (1988).
9. P. Panula, H. Y. Yang, and E. Costa, "Histamine-containing neurons in the rat hypothalamus," *Proc. Natl. Acad. Sci. USA*, **81**, 2572–76 (1984).
10. S. M. Zimatkin and A. V. Zaerko, "A method for detecting histaminergic neurons in the hypothalamus," *Morfologiya*, **156**, No. 4, 102–105 (2019).
11. T. K. Leung, L. Lim, and J. C. Lai, "Brain regional distributions of monoamine oxidase activities in postnatal development in normal and chronically manganese-treated rats," *Metab. Brain. Dis.*, **8**, No. 3, 137–149 (1993).
12. K. Karlstedt, A. Senkas, M. Ahman, and P. Panula, "Regional expression of the histamine H-2 receptor in adult and developing rat brain," *Neuroscience*, **102**, 201–208 (2001).
13. S. Auvinen and P. Panula, "Development of histamine-immunoreactive neurons in the rat brain," *J. Comp. Neurol.*, **276**, No. 2, 289–303 (1988).
14. P. Panula, M. Sundvik, and K. Karlstedt, "Developmental roles of brain histamine," *Trends Neurosci.*, **37**, 159–168 (2014), https://doi.org/10.1016/j.tins.2014.01.001.