Changes in Neurons of Medulla Oblongata Nuclei under Conditions of Chronic NO-Synthase Inhibition

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny,* Vol. 151, No. 1, pp. 116-120, January, 2011
Original article submitted October 12, 2009

Nucleus tractus solitarius and giant-cell and lateral reticular nuclei were studied using the reaction to NADPH-diaphorase in 7-, 10-, 14-, 30-, 45-, 60-day-and 3- and 6-month-old rats receiving L-NAME (50 μg/kg, 2 times a day) on days 1-6 of life. In 7-14-day-old rats, the compound reduced NO-synthase activity in the majority of NO-neurons and the total number and to a lesser degree the relative number of these neurons, while cell cross-section areas remained practically unchanged. The differences in the corresponding quantitative parameters between the control (D-NAME administration) and experimental groups decreased with time after the last L-NAME injection and became undetectable starting from the age of 30-45 days. In the nucleus tractus solitarius, the changes in metric parameters after exposure to NO-synthase inhibitor were more pronounced than in the reticular formation nuclei.

Key Words: *nitroxidergic neurons; medulla oblongata nuclei; age-related changes*

The role of NO in the nervous system functioning is commonly associated with two main processes: transneuronal interaction (highly active second messenger) and cerebral blood flow regulation (neurotransmitter) [4,7,8,14]. The studies demonstrated the possibility of NO involvement into the mechanisms of nervous system development. NO hyperactivity was shown to inhibit cell proliferation in nerve cell culture, whereas the decrease in NO activity, alternatively, intensifies this process and stimulates axon and dendrite growth and synapse formation [2]. Chronic NO-synthase inhibition in sheep fetuses determines elevated blood pressure, increased tonus of the sympathetic nervous system in newborn animals [10], and increases the number of NO-positive cells in the nucleus tractus solitarius [3].

The objective of this study was to investigate nitroxidergic neurons (NO-neurons) in certain medulla oblongata nuclei in rats of different age after chronic NO-synthase inhibition in perinatal period.

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MATERIALS AND METHODS

Experiments were carried out on albino Wistar rats receiving subcutaneous injections of NO-synthase inhibitor N^{ω}-nitro-L-arginine methyl ester (L-NAME) 50 μg/kg twice a day during days 1-6 of life (experimental group). The animals were intraperitoneally anesthetized with nembutal (5 mg/100 g) and examined on days 7 (n=8), 10 (n=7), 14 (n=9), 30 (n=8), 45 (n=8), and 60 (n=8) and 3 (n=8) and 6 (n=10) months after birth. In parallel, we examined age-matched control rats (at least 5 animals in each age group) receiving the same dose of N^{ω}-nitro-D-arginine methyl ether (D-NAME) not inhibiting NO-synthase.

In each group, medulla oblongata sections were prepared at three levels for more complete microscopy of the nuclei. Nucleus tractus solitarius (NTS), lateral reticular nucleus (LRN), and giant-cell reticular nucleus (GRN) were studied (Fig. 1). NO-neurons were detected as described previously [9]. To reveal all the neurons within the nucleus projection, the sections were stained with 0.5% methylene blue. The material was quantitatively processed using an Allegro MC automated image processing system [6] by calcu-

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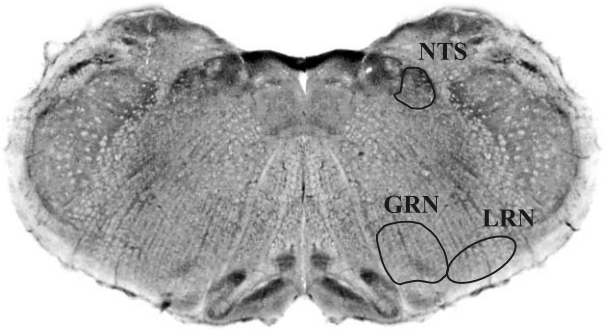

Fig. 1. Localization of NTS, LRN, and GRN on frontal section of rat medulla oblongata at the level of the middle third of the inferior olive.

lating profile area of neurons in each nucleus, mean NO-synthase activity in them, relative (per 1 mm^2) and absolute neuron number in the nucleus projection, and the percent of NO-neurons. The method for material preparation and for histochemical and morphometric investigations was described previously [7]. To simplify comparison of quantitative data between rats in different age groups, the corresponding data were expressed in percents from the value in definitive (6-month-old) animals. Student's *t* test was used to assess statistical significance of obtained data.

RESULTS

Administration of D-NAME to rats did not substantially change metric parameters of NO-neurons in comparison with the previously reported values for the same nuclei in intact age-matched animals [7]. In both cases, quantitative changes in neurons are most

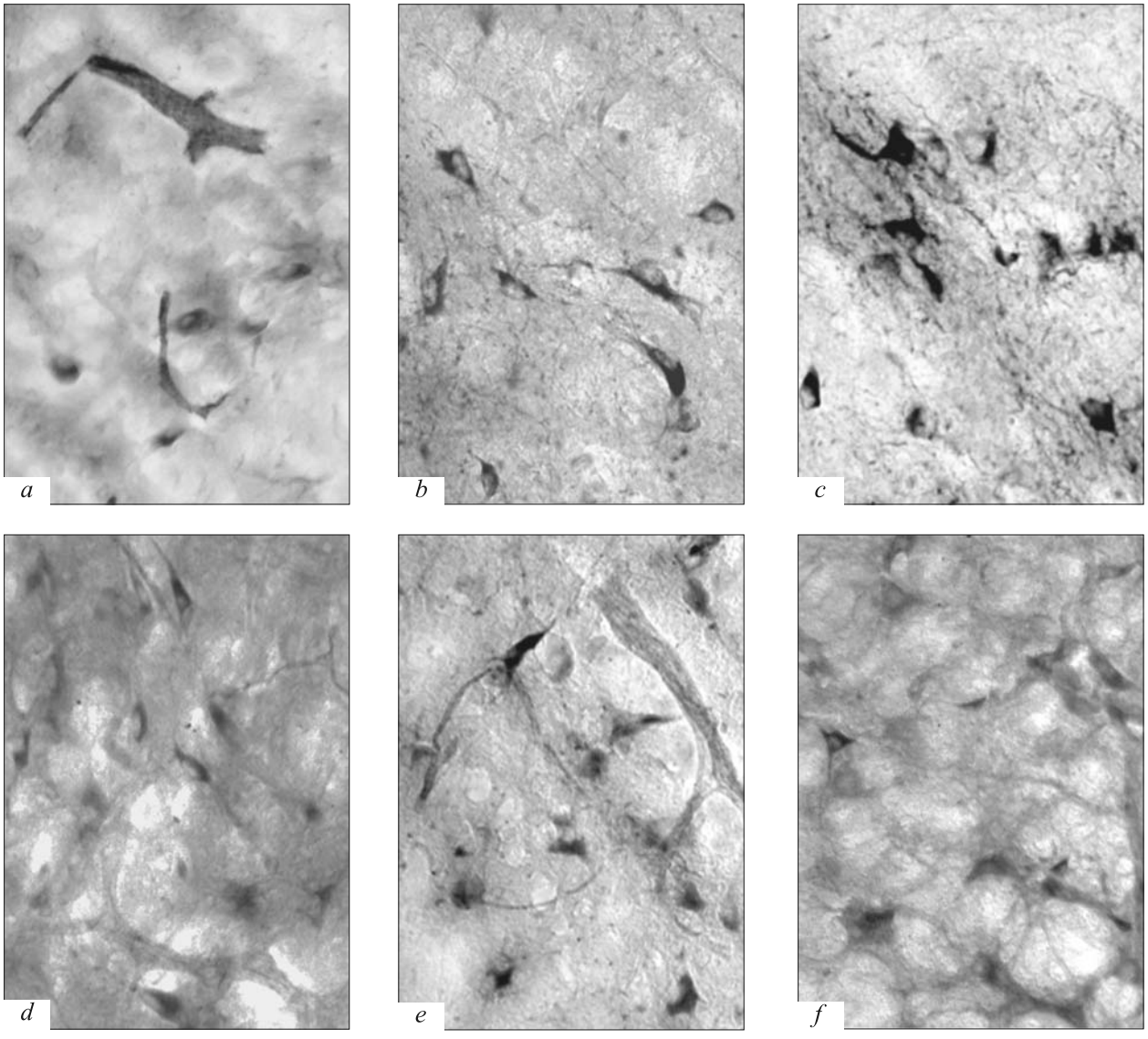

Fig. 2. NTS neurons in control rats on days 7 (*a*), 30 (*b*), and 60 (*c*) of life and in experimental rats on days 7 (*d*), 30 (*e*), and 60 (*f*) of life, ×100.

active during 30 days of postnatal ontogeny. NO-neurons were found in all age groups (Fig. 2), but during the first days after birth their number was low and they were characterized by low NO-synthase activity (Fig. 2, *a*). Then, rapid enlargement and increase in absolute and relative number of NO-neurons were observed in nucleus projection (Fig. 3, *a*, *b*). The percent of NO-neurons from the total number of nerve cells (Fig. 3, *c*) detected by methylene blue also increased, although slower than other parameters: adult values ($p>0.05$) were reached only between 45 days and 3 months. Age-related changes in NO-synthase activity also had some peculiarities (Fig. 3, *d*). In most nuclei, this parameter rapidly increased to the age of 30 days exceeding definitive values by 20-25%, remained at that level until postnatal day 45-60 ($p>0.05$), and then gradually decreased.

Although all three nuclei are functionally connected into an integral system of hemodynamics regulation [5,7], each of them has its own age change scale, as it was shown in our experiments (Fig. 3). In 7-day-old animals, enzyme activity in NTS was substantially higher than in other nuclei (~70% of the definitive value) and did not significantly differ from the definitive value on day 14 ($p>0.05$). In GRN, these values on days 7 and 14 of life were 55 and 68%, respectively, in LRN 47 and 59% ($p<0.01$). Local differences in nucleus development were also noted for other quantitative parameters. Analysis of age-related changes and their values indicated that NTS is characterized by the most rapid development. However, metric values of NO-neurons also reach definitive level ($p<0.05$) in other nuclei by the age of 3 month.

Treatment with NO-synthase inhibitor L-NAME little changed cell shape and size, but reduced the intensity of histochemical reaction in most neurons. At the same time, nonuniform accumulation of the reaction product in neurons located in the nucleus pro-

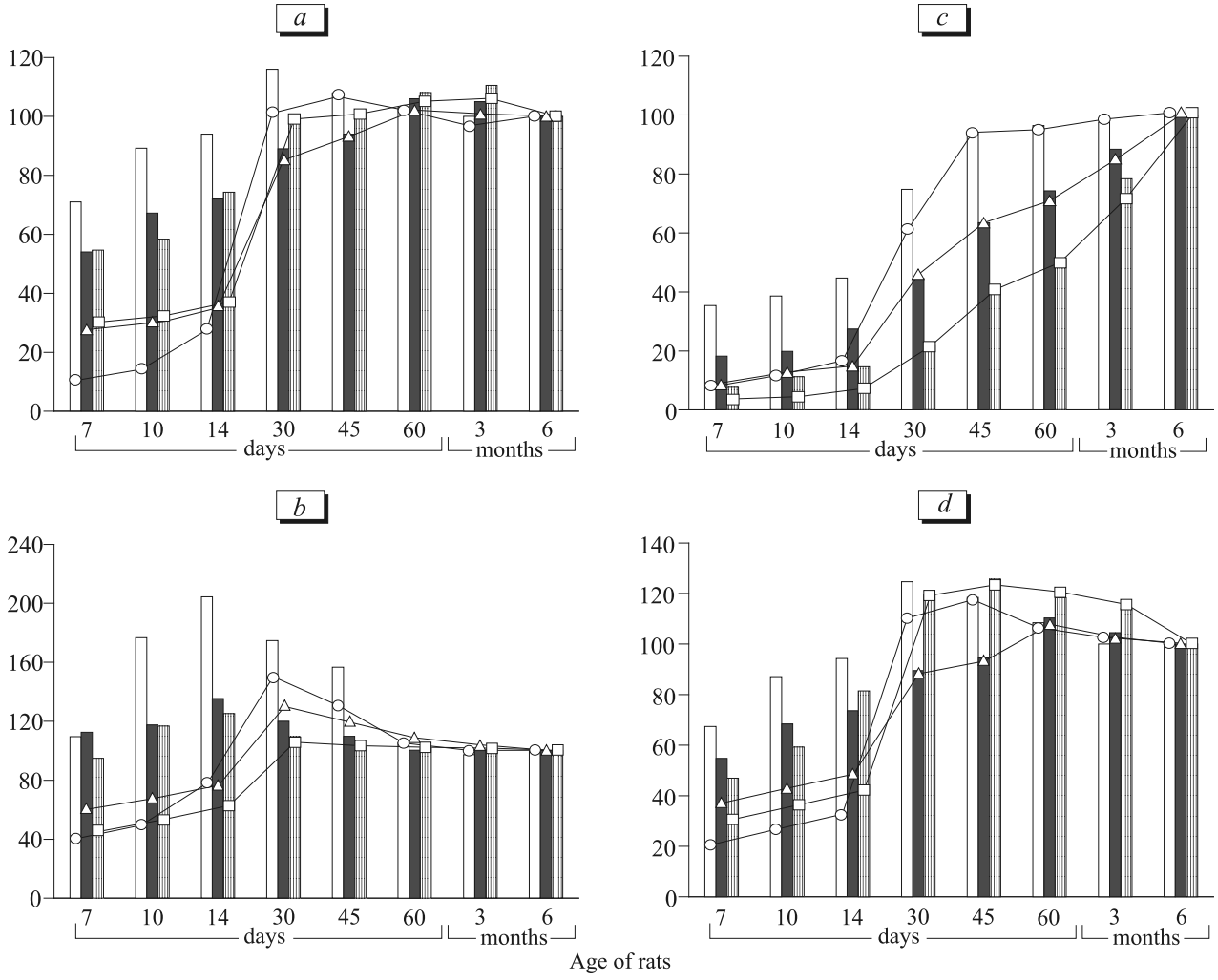

Fig. 3. Changes in NTS, GRN, and LRN neurons in control (histogram) and experimental (graph) rats. *a*) absolute number of neurons; *b*) relative number of neurons; *c*) percent of NO-neurons from the total cell number in the studied nuclei; *d*) mean NO-synthase activity. Values of 6-month-old rats were taken as 100%. Light bars (circles): NTS, dark bars (triangles): GRN, shaded bars (squares): LRN.

jection was observed (Fig.2, *d-f*). The intensity and direction of changes in quantitative parameters largely depended on animal age and nucleus investigated (Fig. 3). In 7-14-day-old rats of the experimental group, NO-synthase activity, the percentage of NO-neurons, and the absolute and, to a lesser degree, relative number of NO-neurons decreased in comparison with the control ($p<0.05$), while cross-section areas of these cells did not differ significantly from the control ($p>0.05$). In NTS, these parameters more markedly differed from the control than in nuclei of the reticular formation. Similar changes in metric values were noted for the distribution of NO-neurons in rats with elevated blood pressure [1]. The changes observed in the medulla oblongata of experimental rats seem to be determined by the hypertensive effect of L-NAME [12]. It is well known that suppression of nitroxidergic mechanisms activates the sympathetic nervous system and increases blood pressure, while activation of nitroxidergic processes produces an opposite effect [10,12,13]. With increasing the time from the last L-NAME injection, blood pressure gradually returned to normal (over 3-4 weeks) and the differences between the control and experimental groups in most studied metric parameters decreased in all nuclei (Fig. 3). In 30-day-old animals of the experimental group, NO-synthase activity, percentage of NO-neurons, and their absolute number in LRN and GRN remained below the control ($p<0.05$), while in NTS most parameters reached the control values (Fig. 3, *d*). In 45-60-day-old as well as in 3- and 6-month old rats from experimental group, the values for all nuclei corresponded to those in the control group ($p>0.05$).

Thus, administration of NO-synthase inhibitor during the perinatal period reduced NO-synthase activity in most NO-neurons for 30 days after withdrawal and reduced their relative and absolute numbers. With increasing the time from the last L-NAME injection, the differences in the corresponding quantitative parameters between the control and experimental groups decreased and became undetectable starting from the age of 30-45 days. Changes in metric parameters after exposure to NO-synthase inhibitor in NTS were more pronounced than in the reticular formation nuclei. In contrast to previous studies [10] we found no morphological signs of stimulation of NO-neuron development in the medulla oblongata of mature animals after suppression of the nitroxidergic system.

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