Nitroxidergic Neurons in Nuclei of the Medulla Oblongata in Hypertensive and Normotensive Rats

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Distribution of nitroxidergic neurons and neuronal NO-synthase activity in certain medulla oblongata nuclei were studied in normotensive and hypertensive rats with different types of arterial hypertension. In rats with renovascular hypertension, neuronal NO-synthase activity markedly decreased in most nuclei 2 weeks after surgery, while the number of NO-positive cells did not change significantly; after 4 weeks, the percentage of NO-positive neuron markedly decreased and neuronal NO-synthase activity also slightly decreased. No further decrease in neuronal NO-synthase activity was observed 8 week after intervention, but the percentage of NO-neurons decreased compared to that in normotensive rats. In spontaneously hypertensive rats, changes in most nuclei were directed similarly to those observed in 8-week renovascular hypertension, but motor nuclei demonstrated less differentiated reaction to hypertension. In all cases, changes in the parameters observed in sensory nucleus (*n. solitarius*) appeared earlier and were more pronounced than in nuclei of the reticular formation related to bulbar vasomotor center.

Key Words: *nitroxidergic neurons; medulla oblongata; bulbar vasomotor center; hypertension*

NO is proved to participate in blood pressure regulation [1,11,13]. It is generally accepted that the role of NO in this process is mainly associated with endothelium-dependent vasomotor control mechanisms [1-3]. However, the presence of nitroxidergic neurons (NO-neurons) in the medulla oblongata was hypothesized, where they can be involved into central mechanisms of blood pressure control [4,7].

Here we studied NO-neurons and activity of neuronal NO-synthase (nNOS) in these neurons in the medulla oblongata nuclei of normotensive and hypertensive rats.

MATERIALS AND METHODS

Experiments were carried out on mongrel rats with normal blood pressure (control group; n=5), rats with experimental renovascular hypertension (RVH; n=18), and spontaneously hypertensive rats (SHR; n=6) weighting 200-240 g. Rats with RVH reproduced as described elsewhere [5] were examined 2, 4, and 8 weeks after surgery (6 animals per point). Systolic blood pressure was measured by tail cuff method using an ML U/4c501 system (MedLab) for noninvasive blood pressure monitoring for rats. The rats were anesthetized with urethane 125 mg/100 g body weight.

Nuclei related to the vasomotor center were investigated [6,12]: nucleus tractus solitarius, reticular giant-cell nucleus, reticular small-cell nucleus, reticular lateral nucleus.

NO-neurons were labeled for NADPH-diaphorase (NADPH-d) as described previously [10]. Specificity of hystochemical staining was verified by incubation of control sections in solutions containing no HCT or NADPH, and in solution where NADPH was replaced with NADP. Since the amount of diformazan precipitate is proportional to molecular content of nNOS, precipitate density reflects enzyme activity in neurons. The absolute amount of Nissl-stained neurons, the per-

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centage of NO-neurons, and mean enzyme activity for one cell in each nucleus separately were estimated on sections of each nuclei using an Allegro MC automated image analysis system. The data were processed statistically using Student's *t* test.

RESULTS

Blood pressure in normotensive rats was 114.7±5.4 mm Hg and in SHR 175.4±4.2 mm Hg. In rats with RVH, blood pressure gradually increased after intervention and reached 148.0±5.6 mm Hg by the second week, 167.5±8.8 mm Hg by the fourth week, and stabilized at the level of 186.6±5.1 mm Hg by the eighth week.

In all investigated nuclei of normotensive rats we found NO-positive neurons differing by the structure and density of precipitate (their cytoplasm was stained in different blue tones, Fig. 1). In nuclei with high content of these neurons they were located close to each other, while in other nuclei they were situated separately. Neuronal processes and capillaries located in close proximity to neurons were also labeled. The percentage of NO-neurons in the studied nuclei varied from 24.4% (*n. tractus solitarius*) to 49.4% in lateral reticular nucleus (Fig. 2). In some studies [13], higher proportion of NO-neurons was provided for *n. tractus solitarius*, probably because nonspecifically stained neurons were also counted. nNOS activity per neuron in various nuclei also differed and was maximum in giant-cell and lateral reticular nuclei (Fig. 2).

The dynamics of changes in parameters in different medulla oblongata nuclei in rats with RVH is presented (Fig. 3, *a*). During the second week after surgery, significant reduction of nNOS activity was observed, while the relative content of NO-neurons in most nuclei remained unchanged. Changes observed in *n. tractus solitarius* were more pronounced than in reticular formation nuclei. It is known that NO under physiological conditions facilitates sensory information transduction within *n. tractus solitarius*, intensifies baroreceptor inhibition of sympathetic vasomotor activity, thus determining blood pressure decrease [3,9,14]; an opposite effect is observed under condition of arterial hypertension [5,12]. During the fourth week after surgery, a marked decrease in the percent of NO-neurons was observed, nNOS activity also decreased (Fig. 3, *a*, *b*). In *n. tractus solitarius*, the studied parameters decreased by almost 2 times in comparison with those in normotensive rats and by more than 16% in animals with 2-week RVH ($p<0.05$). The most pronounced changes in both the percentage of NO-neurons and nNOS activity among reticular nuclei were observed in lateral and giant-cell nuclei. The relative content of neurons in these nuclei decreased by more than 30% and activity decreased by 40% in comparison with control values, and by 18-20% and 12-14%, respectively, in comparison with previous time point (2 weeks). Changes in the small-cell nucleus and central nucleus were not so marked, but significant in comparison with those in normotensive animals ($p<0.05$). Eight weeks after RVH induction, nNos activity in nuclei remained unchanged or slightly increased, while the content of NO-neurons decreased by 2-5% compared to the previous time point. The relative content of NO-neurons and mean activity remained below the control ($p<0.05$). It is known that the reduced activity of the nitroxidergic system serves as a cause of hyperactivation of the sympathetic nervous system and blood pressure increase [4,6], which in turn induces vascular wall remodeling

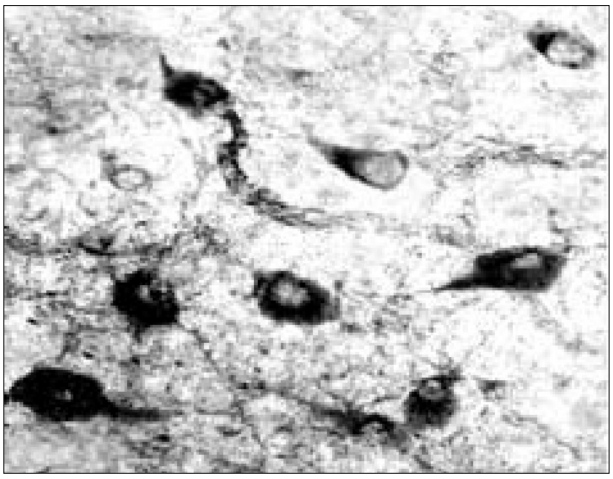

Fig. 1. NO-neurons in *n. tractus solitarius* with low, middle, high and very high nNOS activity in control rats. ×400.

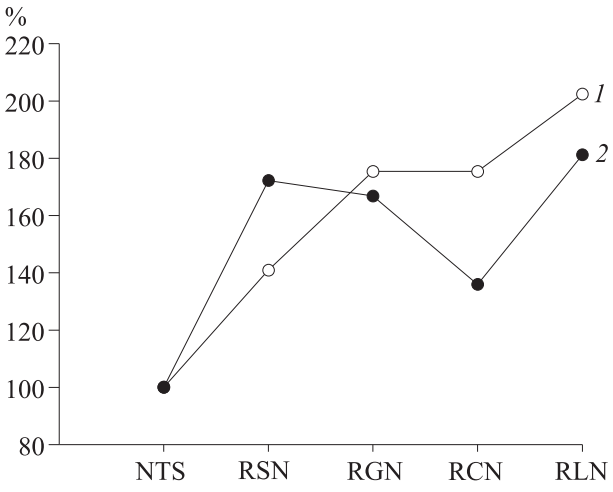

Fig. 2. Percent of NO-neurons in the medulla oblongata nuclei and nNOS activity in normotensive rats. *1*) percent of NO-neurons from the total number of neurons; *2*) mean nNOS activity. Here and on Fig. 3: NTS: *n. tractus solitarius*, RSN: reticular small-cell nucleus, RGN: reticular giant-cell nucleus, RCN: reticular central nucleus, RLN: reticular lateral nucleus.

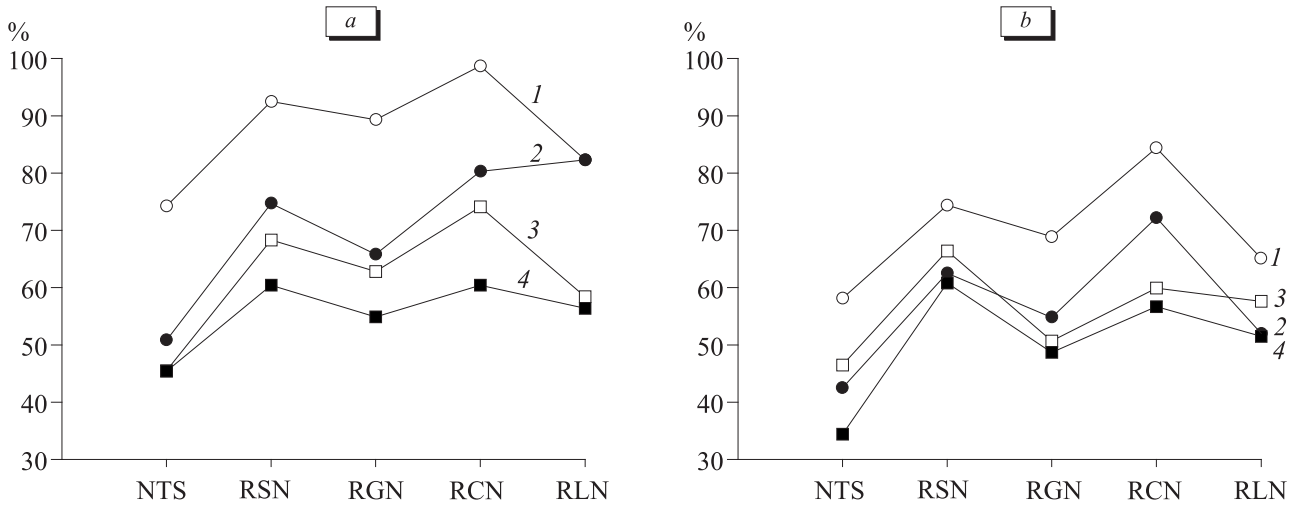

Fig. 3. Percent of NO-neurons from their total number (*a*) and mean nNOS activity (*b*) in medulla oblongata nuclei in rats with RVH 2, 4, and 8 weeks after intervention and in spontaneously hypertensive animals. *1*) rats with RVH 2 weeks after intervention; *2*) rats with RVH 4 weeks after intervention; *3*) rats with RVH 8 weeks after intervention; *4*) SHR.

and development of stable arterial hypertension [2,8]. Similar processes seem to take place in rats with induced RVH during weeks 6-8 after surgery and are accompanied by thickening of the arterial muscle layer and appearance of "paradoxical" vasoconstrictor reaction [2,4].

Changes observed in most of nuclei of SHR were similar to those in rats with 8-week RVH (Fig. 3, *a*, *b*). However, the differences between reticular nuclei in SHR were less pronounced, *i. e.* the response of central nitroxidergic mechanisms to hypertension was less differentiated than in animals with RVH. At the same time, mean enzyme activity in *n. tractus solitarius* of SHR was significantly lower (by 12%, $p<0.05$) and the percent of NO-neurons was virtually the same as in animals with 8-week RVH ($p>0.05$), the differences in mean activity were significant only in comparison with those in normotensive animals.

Thus, certain peculiarities of quantitative indicators characterizing the state of the nitroxidergic system in medulla oblongata nuclei were observed in SHR and RVH rats. It is quite possible that in rats with genetically determined disturbances in NO-synthesis, the absence of differentiated response of efferent nuclei of the vasomotor center to a decrease in afferent stimulation is a potential mechanism leading to the development of arterial hypertension.

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