NO-Positive Neurons in Some Nuclei of Human Bulbar Vasomotor Center in Arterial Hypertension

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The distribution of nitroxidergic neurons and activities of neuronal NO synthase in them in some nuclei of the bulbar vasomotor center were studied in patients with early forms of arterial hypertension. Activity of neuronal NO synthase is reduced significantly in the majority of nuclei in patients with early forms of arterial hypertension, while the content of NO-positive cells was only slightly changed. More pronounced changes in this parameter were detected in the solitary tract nuclei in comparison with the reticular formation nuclei, which had efferent relationships with the intermediate lateral spinal nucleus.

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

Experimental studies [1,2] showed that molecular regulators synthesized in the nervous tissue, including NO, are involved in neurogenic mechanisms of arterial hypertension (AH) formation. At the central level, NO inhibits sympathetic activity and reduced blood pressure [2,10,12]. Suppression of NO synthesis causes an opposite effect: increase in vascular tone [3,4,12]. The sympathoinhibitory effects are realized in the nuclei of the so-called bulbar vasomotor center [4,9]. Though the involvement of NO in the realization of the function of the vasomotor center is acknowledged by many scientists [7,8,9], we found no morphological data on the location and counts of nitroxidergic neurons (NO neurons) in the medulla oblongata nuclei in healthy individuals and on changes in these parameters in AH.

We studied the distribution of NO neurons and activity of neuronal NO synthase (nNOS) in them in some nuclei of the bulbar vasomotor center in patients suffering from AH.

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MATERIALS AND METHODS

The material for the study was collected at forensic medical autopsy of men aged 18-44, victims of traffic accidents. The arteries of the pia mater were collected in 8 normal subjects (control) and 6 age-matched victims dead from other than cerebral injuries, in whom first-degree AH was diagnosed during life.

Sections of the medulla oblongata were made at two levels in order to study the nuclei more amply. The preparations were examined separately under two microscopes fitted with similar grids with squares of the same size in the oculars. The target nucleus was oriented in both microscopes by the characteristic signs in the saggittal and frontal planes and drawn on the millimeter paper in accordance with the position in the grid coordinates. The dorsomedial part of the solitary tract nucleus (STN), ventral reticular giant cell nucleus (RGN), reticular central nucleus (RCN), reticular small-cell nucleus (RSN), and reticular lateral nucleus (RLN) were studied. NO neurons were labeled as described previously [6] for detection of NADPH diaphorase (NADPH-d). In order to evaluate enzyme (NADPH-d; EC 1.6.99.1) activity, the material was fixed in

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4% paraformaldehyde solution in 0.1 M Na phosphate buffer (pH 7.4) for 2 h at 4°C and washed in 15% sucrose solution for 24 h. The specimens were incubated for 1 h at 37°C in a medium containing 50 mM Tris buffer, 0.2% Triton X-100, 0.8 mg/ml p-NADPH, and 0.4 mg/ml NBT (pH 8.0).

The specificity of histochemical reaction was verified by incubation of several sections in solutions without NBT or NADPH and in solution containing NADP instead of NADPH. Since chemical basis of the reaction consists in the formation of formasan precipitate during reduction of tetrazolium salts in the presence of NADPH-d, no histochemical reaction should take place in the incubation medium in the absence of any of its main components.

The absolute counts of Nissl-positive neurons, the percent of NO neurons, and the mean activity of NADPH-d for cells of each nucleus individually (in optical density units) were evaluated in the projection of section of each nucleus by the Allegro MC automated system for image analysis. The percent of neurons with low (type I), medium (type II), high (type III), and very high (type IV) enzyme activities were evaluated for enzyme-positive cells. The significance of differences between the values was evaluated using Student's *t* test.

RESULTS

NO-positive neurons were detected in all nuclei. These neurons differed by the structure and compactness of the precipitate, as a result of which their cytoplasm was colored a variety of blue shades, from light blue to violet. The reaction to NADPH-d not only identified nNOS in cells [3,6], but also showed quantitative proportion of NO-positive neurons differing by enzyme activity. The compactness of the resultant diformazan precipitate was proportional to the molecular content of nNOS, due to which it was possible to evaluate enzyme activity in neurons [13]. Type I cell precipitate was finely granular and colored blue. In the cytoplasm, it precipitated by solitary or fusing granules, their density increasing from the cell periphery to the

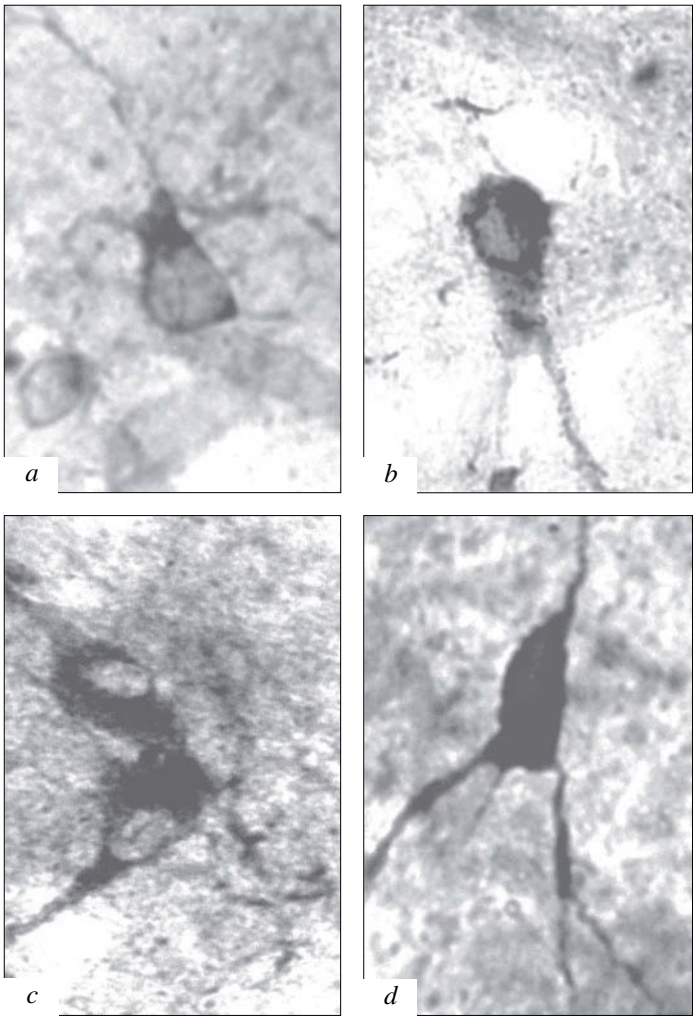

Fig. 1. Neurons with low (*a*), medium (*b*), high (*c*), and very high (*d*) activity of NADPH-d, ×400.

TABLE 1. Distribution of Nitroxidergic Neurons in Some Nuclei of Human Medulla Oblongata ($X \pm S_x$)

Object of study		Percent of NO neurons in total neuron population	NADPH-d MAI, opt. dens. units	Percent of neurons with NADPH-d activity			
				type I	type II	type III	type IV
STN	control	20.6±3.1	24.3±3.3	34.8±3.7	55.6±5.8	9.6±2.0	0
	AH	15.3±2.4*	12.1±2.7*	63.2±2.0*	36.8±3.7*	0	0
RSN	control	34.4±3.4	44.2±4.3	10.2±2.6	28.4±3.2	50.1±3.3	7.8±2.8
	AH	34.7±4.1	32.4±3.5*	28.6±4.4*	52.4±4.0*	14.9±3.1*	4.1±1.2*
RGN	control	38.2±3.3	52.4±3.5	14.7±2.3	22.4±2.2	52.6±5.7	10.3±2.8
	AH	36.5±4.8	37.4±3.7*	30.4±4.2*	49.6±5.0*	15.6±4.2*	4.4±1.4*
RCN	control	39.2±4.4	42.7±3.1	11.2±2.3	30.4±3.6	50.1±3.3	8.3±2.1
	AH	40.4±3.1	38.4±3.5	15.8±3.5	37.8±5.2	42.3±3.7*	4.1±1.7*
RLN	control	44.9±3.2	56.9±4.5	9.0±2.0	31.8±4.1	47.9±6.0	11.3±3.4
	AH	40.7±4.8	39.3±3.4*	16.9±3.1*	49.7±4.0*	27.4±2.1*	6.0±1.0*

Note. MAI: mean activity index. *$p<0.05$ compared to the control.

perinuclear zone (Fig. 1, *a*). These neurons constitutes at least $^1/_3$ of the total number of nerve cells in STN (Table 1). Type II neurons formed a more coarse cytoplasmic precipitate, sometimes fusing into a uniform perinuclear ring (Fig. 1, *b*). An almost homogenous violet precipitate spot presenting as an arch-shaped accumulation near the nucleus was often seen above the nucleus and a part of the cytoplasm, with rare scattered granules in the remaining part of the cytoplasm. The content of type II neurons was maximum in STN (about 60%). In type III neurons, blue-violet diformazan granules evenly filled the cell cytoplasm, except the nucleus (Fig. 1, *c*). The precipitate clearly labeled long processes protruding in the dorsomedial direction. The content of type III neurons was maximum in reticular nuclei, reaching 50% of all cells (Table 1). In neurons with very high activity, massive homogenous violet precipitate filled the entire body of the cell and processes except the nuclear zone (Fig. 1, *d*). Type IV neurons were permanently detected only in the reticular nuclei and constituted 8-12%. They were extremely rare in STN.

The studied nuclei contain different levels of NO neurons: from 17.4 to 44.9% (Table 1). In areas with maximum number of these neurons they were located close to each other. Irrespective of the density of NO-positive cells, neuronal processes, capillaries, sometimes glial cells were also labeled (Fig. 2).

In AH, nNOS activity in neurons was significantly lower than in the control, though the number of NO-positive cells did not change much (Table 1). The number of neurons expressing nNOS decreased significantly (by almost 26%) only in STN (area of secondary afferent neurons of the pressure-receptive arch [5]). The spaces between NO neurons increased (Fig. 3, *a*). The majority of cells in this nucleus were type I neurons. The percent of type II cells was reduced significantly, while type III neurons virtually disappeared from the visual fields: just 1-2 cells of this kind were seen in few sections. As a result of redistribution of cells in the nucleus in favor of type I neurons, the mean index of enzyme activity in AH patients decreased by more than 50% in comparison with normal subjects ($p<0.05$). Normally, NO facilitating transmission of sensory information into the solitary tract nucleus stimulates the pressure-receptive inhibition of sympathetic vasomotor activity, thus promoting reduction of blood pressure [3,14]. Disorders in this neurotransmitter synthesis lead to an opposite effect. Injection of NO synthesis inhibitors to the nucleus causes an increase of arterial pressure [8].

The sequence of changes in the studied parameters in the reticular nuclei was largely similar to that in STN, but less pronounced. The numerical density of NO-positive neurons in these nuclei slightly decreased (by 5-8%, similarly as in RLN and RGN) or remained at the basal level (RSN and RCN; Table 1). Glucose label showed that they formed direct bonds with cells of the spinal intermediate lateral nucleus, participating in the formation of the neurogenic vascular tone [12]. The greatest changes in the parameters of this group of nuclei were detected in the RLN. The differences between the mean indexes of enzyme activity and percentage of types I-IV cells in this nucleus in normal subjects and AH patients reached 25-30%. Types III and IV NO neurons were common in RLN in patients with

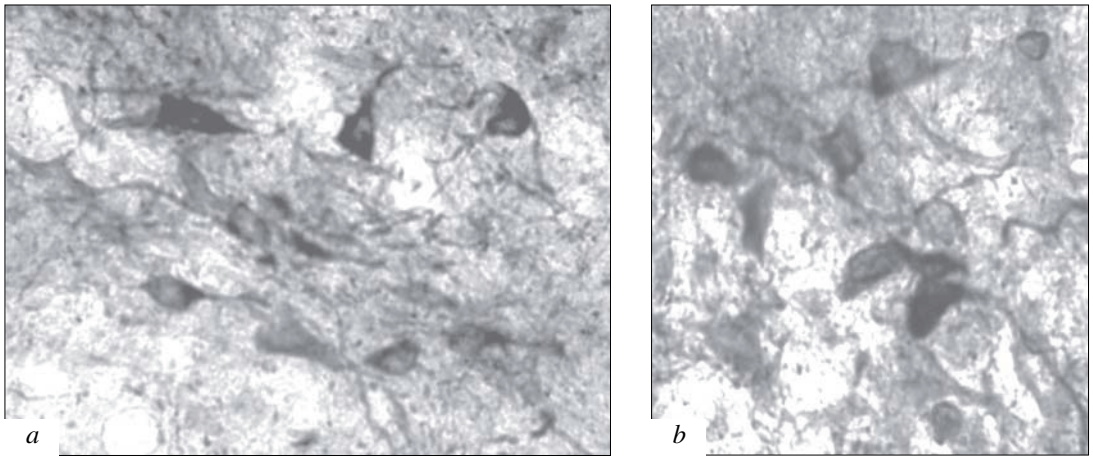

Fig. 2. Neurons of STN (*a*) and RLN (*b*) in healthy individuals, ×100.

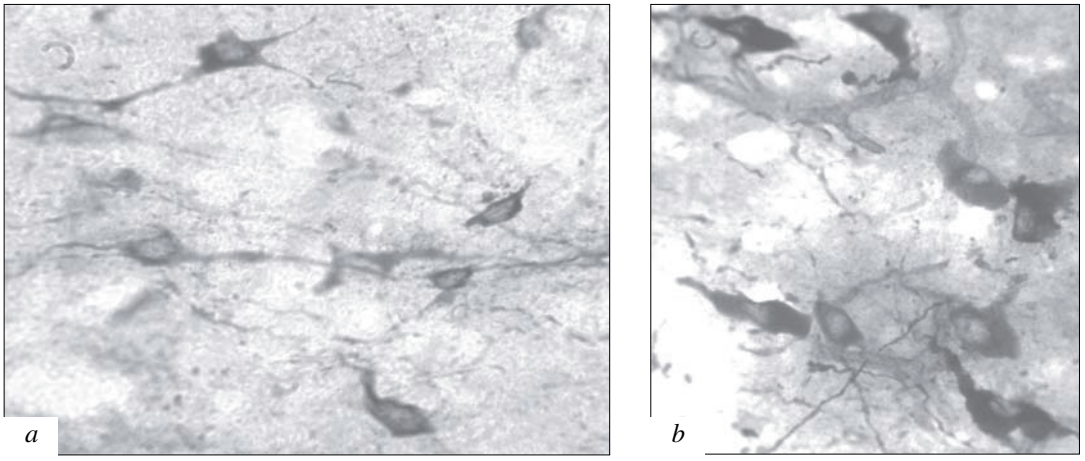

Fig. 3. Neurons of STN (*a*) and RLN (*b*) in AH patients, ×100.

AH, in contrast to STN. Type II cells were most numerous there (Fig. 3, *b*). Significant differences between the studied parameters in patients and controls were detected also for RSN and RGN, but these differences were not so great as for RLN (Table 1). In the RCN, the changes in the studied parameters were minimum and significant only for the mean index of the enzyme activity (Table 1).

Hence, we detected specific features of the reaction of NO neurons in the studied nuclei of the bulbar vasomotor center in AH. First, most quantitative parameters of the nitroxidergic system were reduced in all cases; this was presumably a cause of hyperactivation of the sympathetic nervous system and moderate elevation of blood pressure observed in patients with early forms of the disease [2]. Second, more pronounced shifts in the STN parameters were detected in comparison with other nuclei. This was presumably due to more serious disorders in the nitroxidergic mechanisms (primarily NO synthesis) in the afferent component of the reflex arch in first-degree AH. Third, the degree of these shifts differs in different reticular nuclei, which suggests different contribution of each of these nuclei into activation of the preganglionic sympathetic neurons, which was shown by previous experiments [4]. Fourth, an appreciable decrease in the percent of NO neurons was detected only for STN, but not for other nuclei in our study. This can be the substratum for recovery of the initial vascular tone as a result of timely correction of function of the sympathetic nervous system.

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