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Nitric Oxide Synthase in Damaged Sensory Neuron

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NADPH diaphorase colocalized with nitric oxide synthase was studied in damaged protoneurons of the nodose ganglion of rabbit vagus nerve. The number and activity of nitric oxide synthase-expressing neurons increased. Nitric oxide is involved in neuron remodeling.

Key Words: *nitric oxide synthase; nitric oxide; protoneuron remodeling*

Recent studies suggest the involvement of nitric oxide (NO) in neuronal regeneration [7-9]. However, there are no morphological signs confirming this hypothesis.

Here we studied NO synthase (NOS) and determined the interrelation between its activity and parameters of remodeling of damaged protoneurons.

MATERIALS AND METHODS

In adult rabbits, the vagus nerve was ligated 1 mm distally to the nodose ganglion. Experiments were performed on 12 rabbits: 4 rabbits served as the control, while others were examined 5 (n=4) and 10 (n=4) days after ligation. These periods corresponded to most pronounced but ambiguous changes in protoneurons observed after peripheral nerve ligation [2,3]. In each group, nodose ganglia from 3 rabbits were examined for the presence of NADPH diaphorase; and in 1 rabbit, a half of the nodose ganglion was stained with toluidine blue by the Nissl method, and another half was impregnated by Cajal method.

NADPH diaphorase colocalized with NOS was studied as described elsewhere [6]. Functions of these enzymes change similarly and simultaneously, and their activities correspond to NO secretion. Hence, the state of NOS and NOergic functions of cells can be evaluated by NADPH diaphorase content [6].

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The material was fixed in cold (4°C, 2h) formaldehyde in 0.1 M phosphate buffer (pH 7.4). Under these conditions, only NADPH diaphorase, but not other diaphorases retained their activity [6]. The material was then washed in 15% sucrose at the same temperature for 24 h. Slices (10 μ thick) prepared from frozen samples were mounted on slides, incubated in a medium containing 50 mM Tris-buffer (pH 8.0), 1 mM NADPH (Sigma), 0.5 mM NBT (Sigma), and 0.2% Triton X-100 (Serva) at 37°C for 30 min, washed with distilled water, dehydrated, and embedded into a balsam.

The number of NO-positive neurons in 3 slices taken from the middle part of the ganglia from 3 rabbits was estimated. NADPH diaphorase activity was measured using a VICKERS M-85 microdensitometer in 100 neurons. There were 4 types of cells displaying low (type 1), average (type 2), high (type 3), and extremely high (type 4) activity. The relative number of each type of cells was determined. The results were analyzed using Student's t test.

RESULTS

Five days after vagus nerve ligation, the number of NOS-expressing neurons increased (p<0.05) compared with the control (Figs. 1, *a* and *b*). Type 1 neurons with low enzyme activity were prevalent in the control ganglion, but absent in damaged ganglia. Type 4

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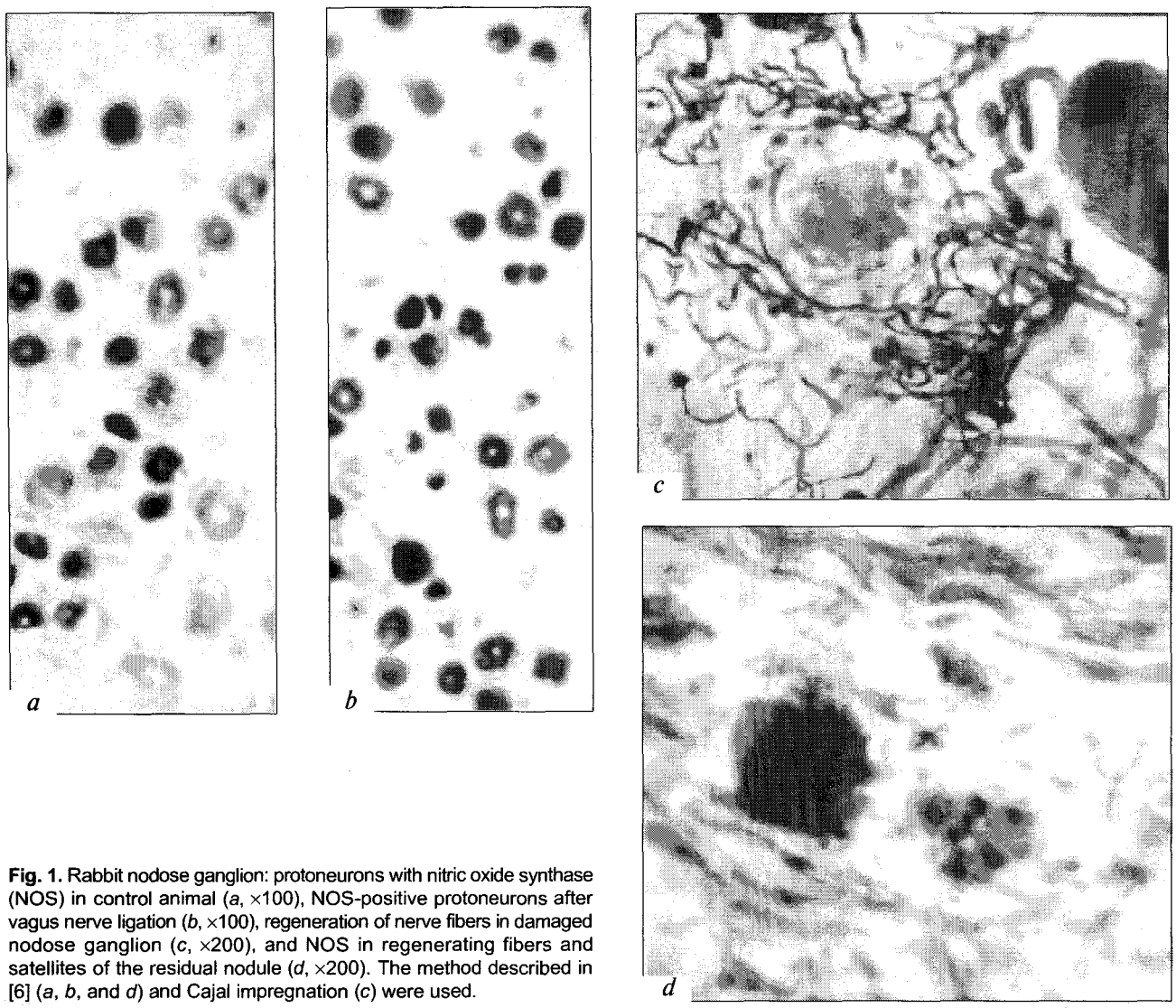

Fig. 1. Rabbit nodose ganglion: protoneurons with nitric oxide synthase (NOS) in control animal (*a*, ×100), NOS-positive protoneurons after vagus nerve ligation (*b*, ×100), regeneration of nerve fibers in damaged nodose ganglion (*c*, ×200), and NOS in regenerating fibers and satellites of the residual nodule (*d*, ×200). The method described in [6] (*a*, *b*, and *d*) and Cajal impregnation (*c*) were used.

neurons with extremely high enzyme activity appeared after ligation, while the content of types 2 and 3 cells was higher than in the control (Table 1).

Examination of Nissl-stained ganglia showed that 77.3% neurons were in the state of primary excitation, had swollen bodies and ectopic (peripherally positioned) nuclei, and displayed central chromatolysis. These changes are typical of peripheral nerve injury [2]. We assumed that high activity of the enzyme (especially in type 4 cells) corresponded to retrogradely reacting neurons.

Ten days after surgery, the relative number of NOS-containing neurons increased by 92.9%, but these changes were insignificant (compared with day 5 after ligation). Type 1 neurons were not found, while the contents of types 2, 3, and 4 neurons were the same as on day 5 after ligation (Table 1). However, morphological signs of NOS-containing neurons differed on days 5 and 10 after ligation. Ten days after surgery, 86% reactive neurons were in the state of repair (by contrast to primary neuronal excitation observed on day 5). The nucleus was centrally positioned, perinuclear basophilic substance was colored blue, and coarse lumps radiated from this substance toward the periphery [2].

Cajal impregnation showed thin and intensely impregnated nerve processes of the reactive genesis [3] containing small diformazan granules in the ganglion (Fig. 1, *c*). The enzyme was expressed by satellites of residual nodules appeared at the site of dead nerve cells (Fig. 1, *d*). Ganglionic neuroglial cells of control animals did not react to NOS.

Hence, the highest NOS activity corresponded to repair processes in the nodose ganglion.

Under normal conditions, protoneurons express constitutive NOS, while damage leads to the appearance of the inducible enzyme [8,9], which can be detected by immunohistochemical assay. The increased

TABLE 1. Absolute and Relative Contents of NO-Positive Neurons and NOS Activity in Nodose Ganglion after Vagus Nerve Ligation ($x \pm Sx$)

Time, days	Number of neurons		NOS activity			
			type of neurons			
	Nissl staining	with NOS	1	2	3	4
Control	226±11.5	43.4±6.3 (19.2)	8.8±0.7 (56.3)	14.6±1.2 (29.1)	22.5±0.8 (14.6)	—
5	— (77.3)	175±12.1 (77.4)	—	18.6±0.12 (23.9)	24.8±0.17 (31.6)	31.6±1.6 (44.5)
10	— (86.0)	210±16.6 (92.9)	—	17.8±0.9 (21.6)	26.7±1.4 (33.6)	34.9±1.8 (44.8)

Note. Percentage is shown in parentheses.

activity and accumulation of NO-positive neurons after trauma suggest expression of inducible NOS.

NO can act as an autocrine and paracrine regulator during primary excitation and repair of violated nervous associations, respectively.

Retrograde reactions are accompanied by inhibition of oxidative phosphorylation and cytochrome oxidase activity in neurons [1]. Under these conditions, NOS compensates synthetic functions of cytochrome oxidase, and respiration of neurons partially changes to the ancient nitrate pathway [5]. Our findings confirm this interrelation between NOS and cytochrome oxidase.

The involvement of NO as a universal signal molecule in the regulation of opposite functions depends on spatial information [4]. Therefore, activation of NOS can be related to primary excitation (the early neuronal response to injury) and late nerve cell remodeling (the involvement of NO in repair of cell structure).

These data indicate that NO is involved in both the initial degeneration and the late repair phase of neuronal response to injury.

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