NO-ergic Neurons of Rat Nucleus Gracilis during Acute Pain

I. V. Dyuizen

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 135, No. 1, pp. 215-218, January, 2003
Original article submitted July 10, 2002

Histochemical staining for NADPH diaphorase showed that the number of NO-ergic neurons in the nucleus gracilis of rat brain increased within the first few hours of pain stimulation. Initially, the changes were noted on the side of lesion, but then involve both halves of the nucleus. It was assumed that nucleus gracilis participates in processing of acute pain information.

Key Words: *NADPH diaphorase, nitric oxide, pain,* nucleus gracilis

Gracile and cuneate nuclei of the posterior funiculi participate in the transmission of total somatic afferent impulses, form lemniscal projections to the thalamic nuclei, and are responsible for discriminant analysis of tactile perception [7]. According to modern notions, the posterior funiculi play an important role in not only proprio-, but also nociperceptive processes [2]. Painful stimulation activates neurons of the gracile and cuneate nuclei and changes neurochemical profile of their afferents [10]. However, cellular mechanisms underlying these changes remain unstudied. NO acts as a modulator of neuronal chains during painful stimulation [1,13]. In the present study we examined changes in NO-ergic function of nucleus gracilis neurons induced by acute pain.

MATERIALS AND METHODS

Male Wistar rats (mean body weight 280 g) were used in the experiments. The rats were injected with 0.2 ml 4% formaldehyde into the left hindpaw. This test is widely used in studies of the mechanisms of acute inflammatory pain [8]. Experiments were carried out 1, 3, and 6 h postinjection, each group included 5 animals. Five intact rats served as controls.

Laboratory of Pharmacology, Institute of Sea Biology, Far East Department of the Russian Academy of Sciences; Department of histology, Vladivostok State Medical University

NO synthesizing neurons were visualized and their NO-ergic activity [11] was analyzed using histochemical reaction for NADPH diaphorase (NADPH-d). The animals were anesthetized by sodium thiopental and decapitated. The brain was fixed by 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.2) at 4°C for 2 h; 30-μ cryostat sections were incubated in a medium containing 0.1 mg/ml β-NADPH, 0.1 mg/ml nitroblue tetrazolium, 0.3% Triton X-100 on 50 mM Tris-HCl buffer (pH 8.0) [11]. The number of NADPH-d-positive neurons in the ipsi- and contralateral nuclei gracilis was determined on serial sections and their percentage from the total number of Nissl-stained neurons was calculated. The preparations were photographed on an Olympus BH2-RFCA microscope, model BHS. Enzyme activity was evaluated cytospectrophotometrically and expressed in optical density units. Sigma reagents were used in the study.

RESULTS

In intact rats, the nucleus gracilis contained few NADPH-d-positive neurons, which form two populations. The caudal part of the nucleus contains 14.2% NO-positive neurons (Fig. 1, *a*) with round bodies (8-10 μ in diameter) and intense histochemical reaction (143.3±8.2 opt. density units). The rostral part of the nucleus gracilis contains larger (10-12-μ) oval or triangular cells with 2-3 primary dendrites and lower

0007-4888/03/1352-0187$25.00

enzyme activity (69.1±3.4 opt. density units). These neurons constitute 44.4% of the total cell population.

The number of NO-positive cells in the caudal and rostral parts of the nucleus significantly increased within 1 h of painful stimulation (Fig. 1, *c, d*). The changes involved both the ipsi- and contralateral parts, but were more pronounced on the side of lesion: the absolute number of NO-positive neurons in the caudal and rostral parts on the side of lesion increased 7.5- and 1.4-fold, respectively (Table 1). Three hours after formalin injection the number of NADPH diaphorase-positive neurons continued to increase in both the ipsi- and contralateral portions of the nucleus. On the contrary, in the caudal part of the nucleus gracilis the number of NO-positive cells decreased, but still 4.5-fold surpassed the normal. Six hours after formalin injection the number of NO-positive neurons decreased and approached the normal in both portions of the nucleus gracilis.

Thus, peripheral painful stimulation induced short-term but pronounced neurochemical changes in nucleus gracilis neurons enhancing their NO producing activity. Pain is always associated with activation of NO synthesis in relay neurons modifying pain signal [1,7,13]. The increased number of NO-ergic neurons in the nucleus gracilis probably attests to its involvement into processing of nociceptive signal. The gracilis and cuneate nuclei of the posterior funiculi were for a long time regarded as components of the proprioceptive analyzer, because their main sensory input is formed by thick myelin Aβ-type fibers normally not conducting pain impulses [3]. But later, nerve fibers releasing substance P, a marker and a transmitter of nociceptive afferents, upon stimulation were identified in these fibers [4]. It was also shown that long-term stimulation of these fibers induces peculiar metabolic changes (similar to pain-induced changes) in nucleus gracilis neurons [10]. This process was associated with enhanced physiological activity in thalamic structures receiving direct projections from the nucleus gracilis and responsible for discriminant analysis of pain sensitivity [6]. Moreover, electrical stimulation of nucleus gracilis neurons induced activation of endogenous analgesic reactions [6]. These facts suggest that funiculus posterior nuclei can be considered as important components of integrative nociceptive reaction of the organism [2].

The number of NO-positive neurons increased during the first hours of nociceptive stimulation. During the first hours of painful stimulation these changes were similar in the rostral and caudal segments of the nucleus with the prevalence of NO response on the ipsilateral side. The mechanisms of this activation are probably associated with enhanced glutamatergic transmission in afferent pathways playing a key role in potentiation of NO synthesis [11,13]. Published data

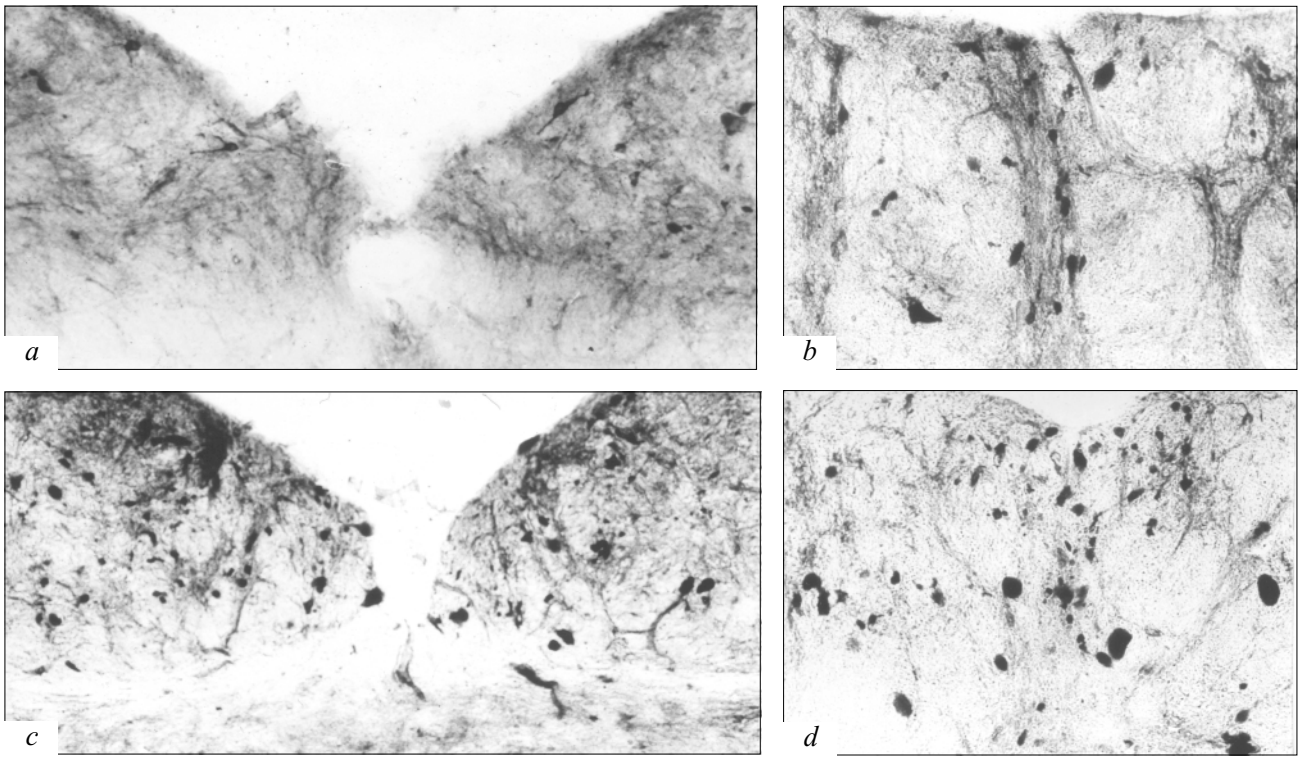

Fig. 1. NADPH diaphorase in neurons of the rostral (*a, c*) and caudal (*b, d*) parts of nucleus gracilis in the control (*a, b*) and after 1-h painful stimulation (*c, d*). Histochemical method of S. Vincent and H. Kimura, ×100.

TABLE 1. Quantitative Changes in NADPH Diaphorase Activity in Neurons of Nucleus Gracilis ($M \pm m$, n=5)

Portion of nucleus gracilis		Control	Painful stimulation, h		
			1	3	6
Rostral	on the side of lesion	27.7	38.9*	43.9*	34.8
	on the contralateral side	27.8	28.3	41.6*	32.2
Caudal	on the side of lesion	3.1	23.9*	14.4*	6.2
	on the contralateral side	3.04	15.7*	13.1*	4.8

Note. *p<0.05 compared to the control.

on the sources of nucleus gracilis innervation can explain subsequent bilateral activation of NADPH diaphorase. In addition to direct somatosensory projections from spinal ganglion neurons, the nucleus gracilis receives postsynaptic fibers from neurons of the deep layers of the spinal cord. The former innervate the ipsilateral half of the nucleus gracilis, while the latter form afferents to both the ipsi- and contralateral parts of this nucleus [3]. Successive transmission of the pain signal in these neuronal chains determines asynchronous reaction of the ipsi- and contralateral parts of the nucleus gracilis. Previous experiments showed that 40 days after sciatotomy enhanced NO production was observed only on the side of lesion and primarily in the rostral segment of the nucleus gracilis [5]. These differences can be explained by peculiar neurochemical modifications accompanying acute nociceptive reactions or chronic pains. This is confirmed in our experiments showing short-term (within 3 h) activation of NADPH-d induced by acute pain in contrast to neuropathic changes associated with more persistent changes in the neurochemical profile of nucleus gracilis cells [5].

Neurotransmitter specificity of NADPH diaphorase-positive cells in the nucleus gracilis is unknown. It can be assumed that similarly to the cuneate nucleus containing GABA and glycine-synthesizing NO-ergic cells, which do not form thalamic projections [12], NADPH-d-positive cells in the nucleus gracilis also belong to inhibitory interneurons. Their NO-ergic hyperactivity caused by painful stimulation can promote changes in the functional properties of neurons forming projections to the ventrobasal thalamus. Under these conditions the structures of the nucleus gracilis can double the spinal-thalamic sensory discriminative pathways responsible for the analysis of acute somatic pain.

The study was supported by the Russian Foundation for Basic Research (grant No. 02-04-49644) and Far East Department of the Russian Academy of Sciences (ISB, No. A-5).

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