Nitric Oxide Contributes to the Spinal Nociceptive Processing

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INTRODUCTION

Nitric oxide (NO) is an agent with a small reactive molecule, which is produced in the nervous system in NO synthase (NOS)-containing neurons. It plays a crucial role, similarly to N-methyl-D-aspartate (NMDA) receptors, in multisynaptic local circuits connected with processing of nociceptive information in the spinal cord. Subpopulations of NOS-containing neurons were found in many regions of the central and peripheral nervous system. These cells (about 2% of the total number) were identified in different laminae of the spinal cord [1]. Nitric oxide is suggested to act as both a classical neurotransmitter and a retrograde messenger [2]. Under conditions of tonic and chronic pain, when high-frequency afferent signals arrive at the spinal cord, NMDA receptors are activated. As a result, a substantial influx of calcium into NOS-containing neurons and production of NO are observed. This leads to potentiation of synaptic transmission in spinal neurons manifested as hyperalgesia to thermal and mechanical stimulation, facilitation of the transsynaptic effects, and central sensitization [3]. Modulation of NOS activity in the spinal cord with the use of novel drugs inhibits the development of these processes. Possible involvement of NOS-containing cells in the central transmission of spinothalamic information and pain modulation recently became a subject of increasing interest [4, 5].

The working hypothesis of our study was as follows. Spinal NOS-containing neurons might not only indirectly influence the nociceptive processing in the spinal cord via NO production, but also directly transmit nociceptive information to the thalamus and analgesic zones of the periaqueductal gray (PAG). Neuronal nicotinamide adenine dinucleotide phosphate diaphorase (NADPH-d) has been shown to be a form of NOS [6]. Thus, in our study we used combined retrograde axonal transport of Fluoro-Gold (FG) and an NADPH-d histochemistry technique to estimate the role of NOS-containing spinal neurons in direct nociceptive transmission and pain modulation in rats.

METHODS

The experiments were carried out on two groups of Sprague-Dawley rats (250-320 g, five animals in each group). Spinal neurons were retrogradely labelled with FG 2% solution (Fluorochrome Inc., USA) by two unilateral thalamic or PAG injections (750 nl each, leftside). Serial 50-μm-thick sections were cut through the injected zone and the cervical enlargement (C6-C7), thoracic cord (Th1-Th2), lumbar enlargement (L5-L6), and sacral spinal cord (S1-S2). Sections were sub-
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sequent processed with NADPH-d histochemistry [7]. FG-labelled, NADPH-d-reactive (FG(+) and NADPH-b(+)s, respectively) neurons and double-labelled cells were examined with the use of a fluorescence microscope and appropriate lock filter systems for ultraviolet light (360-420 nm) and/or normal light (for additional information about the technique, see also the earlier study [8]). The data are represented as the means ± s.e.m.

RESULTS

A halo of dye injection and a diffusion zone in the thalamus covered the central lateral nucleus, ventrobasal nuclear complex, and posterior nuclear group of the thalamus (Fr -3.5). Spreading of the dye in the PAG was observed within its dorsolateral and lateral subdivisions (Fr -6.5) [9]. A consistent pattern of NADPH-d(+) neurons was observed in layers 2-5 and in zone 10 throughout the entire spinal cord. NADPH-d(+) cells were also found in the intermediolateral cell column (IML) of the thoracic spinal cord. Scattered positive neurons in layers 1 and 6-8 were also observed. Most of the FG(+) neurons were located at the side contralateral to the tracer administration (Fig. 1).

In the sacral spinal cord a lot of the FG(+) cells were localized within the layers 7 and 10. Sources of the spinothalamic pathways to the thalamus or PAG (i.e., FG(+) cells) were mixed together with NADPH-d(+) neurons in the dorsal horn (layers 1-6), layer 10, and medial part of the intermediate zone (layer 7). Spinal neurons unequivocally fitting the criteria for double labelling (Fig. 2A, B), i.e., having the NADPH-d-stained cytoplasm (visible in normal light) and containing a bright fluorescent nucleus, nucleolus, or the faint diffuse blue luminescence of the cytoplasm (visible in ultraviolet light) were also noticed. However, the number of double-labelled spinal neurons was very limited. They were identified exclusively in the cervical and lumbar enlargements within layers 3, 4, and 10 (Fig. 1).

The size and morphology of the spinothalamic tract (STT) cells (Fig. 2) or PAG-projecting neurons (Fig. 3) were found to vary, depending on their laminar location and segmental distribution (Figs. 2 and 3). Thus,