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Research Report

Effect of peripheral axotomy on pain-related behavior and dorsal root ganglion neurons excitability in NPY transgenic rats

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Abstract

In order to clarify the physiologic role of NPY in sensory processing, we obtained intracellular recordings of DRG neurons from wild type (WT) and NPY overexpressing transgenic rats (NPY-TG) before and after injury. We investigated medium and large diameter DRG neurons since upregulation of NPY peptide following the nerve injury occurs primarily in those cells. Neurons were classified as Aα/β and Aδ using conduction velocity and action potential duration. Prior to the injury, Aα/β neurons of NPY-TG rats conducted more slowly and had a more brief AHP than similar cells from the WT group. Aδ neurons at baseline conducted faster in TG animals compared to WT. Ligation of the 5th lumbar spinal nerve (SNL) produced certain changes in Aα/β cells that were evident only in the TG group. These include increased refractory period, increased input resistance, AHP prolongation and a depolarizing shift in threshold for AP initiation. The expected injury-induced CV slowing was not seen in NPY-TG Aα/β cells. In the Aδ cell group, injury produced a depolarizing shift in the resting membrane potential, an increase in AP duration and decrease in AHP and refractory period duration only in WT rats, while NPY-TG cells lacked these injury-induced changes. Behavior tests showed diminished sensory response to nerve injury in NPY-TG rats, i.e., shorter duration of enhanced pain-related behavior and attenuation of contralateral effect. In conclusion, our observations suggest that NPY overexpression leads to reduced neuronal activity following nerve injury in a cell-specific manner.

Theme: Sensory system
Topic: Neuropathic pain, pain modulation, neuropeptide

Keywords: Neuropathic pain; Neuropeptide Y; Transgenic rat; Spinal nerve injury; Dorsal root ganglion; Intracellular recording

1. Introduction

NPY is a highly conserved peptide belonging to the pancreatic polypeptide family which has marked and diverse biological effects [5]. Besides its effects on the regulation of neuronal activity, cardiovascular performance and food intake, it is also critically involved in pain processing [37].

However, precise mechanisms of its action and the role in neuropathic pain is insufficiently understood with conflicting results being reported [18]. It is documented that pharmacologically applied NPY induces analgesia in naïve rodents [17,24] and reverses hyperalgesia after inflammation [27,48,60]. Interestingly, antinociceptive effects can only be elicited with small doses of NPY [30]. Also, NPY-deficient mice exhibit exaggerated autotomy, demonstrating an analgesic effect of the NPY [46]. These NPY actions are probably mediated by the Y_1 receptor (Y_1R) since enhanced nociception is observed in some behavioral tests performed in mice lacking Y_1 receptor [39]. The same receptor is responsible for antihyperalgesic effect of NPY observed in inflammatory pain models [48]. Beneficial effect of NPY

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after peripheral nerve injury has been shown only in two studies of flexor reflexes in normal and axotomized rats [64,65].

In contrast to these observations, other studies have found that intrathecal NPY application exacerbates hyperalgesia following a nerve injury via activation of Y_1Rs located on myelinated nerve fibers [61], and that NPY administered subcutaneously into the hind paw of animals with chronic nerve injury exacerbates hyperalgesic responses to nociceptive stimuli [51]. In addition to this, recent data show that NPY injection into the gracile nerve of uninjured rats elicited reversible tactile hypersensitivity in the ipsilateral hindpaw [40].

The conflicting findings in these reports may result from the participation of different subtypes of NPY receptors and application of NPY to different sites. Our approach with NPY transgenic rat model overcomes the application problems and allows reliable comparison with the abundant existing data obtained from experiments in which rat model was used. The analysis of phenotypes of transgenic overexpressing and knockout animal models has revealed significant roles of NPY and its receptors in a variety of different physiological processes. However, both techniques have limitations, and data from those studies should be interpreted with caution [26].

This is the first study describing effects of endogenous NPY in the context of the neuropathic pain. We assume that a cell-specific upregulation of endogenous NPY following the peripheral nerve injury can reduce pain-related behavior by reducing excitability in a cell-specific manner. In order to test this hypothesis, we investigated the influence of NPY overexpression on sensory processing and neuronal excitability after spinal nerve ligation (SNL) in NPY-TG rats. We have also confirmed transgene expression pattern in DRG of transgenic rats. Parts of this study have been previously reported in form of an abstract at Society for Neuroscience's 33rd Annual Meeting.

2. Material and methods

2.1. Animal preparation

Behavioral experiments were performed on NPY transgenic (NPY-TG, $n=18$) Sprague–Dawley rats and non-transgenic littermates (control wild type–WT rats, $n=18$). The main phenotypic traits of these rats have been well described [31,33–35,50,52]. The procedure for development of transgenic NPY rats is described in detail elsewhere [32]. Spinal nerve ligation was performed during halothane anesthesia in a manner similar to the original method of Kim and Chung [19]. Briefly, after exposure of the right paravertebral region, the sixth lumbar transverse process was removed, and the ventral rami of the right L5 and L6 spinal nerves were ligated with 6–0 silk thread and cut distal to the ligature. After surgery, the rats were returned to the animal colony where they were kept under normal housing conditions. All experimental procedures and protocols have been pre-approved by MCW Animal Care Committee.

2.2. Behavioral testing

Sensory testing included behavioral tests covering a range of sensory modalities, including cold sensitivity, heat withdrawal latency, mechanical hyperalgesia, mechanical allodynia and mechanical summation with von Frey probes. Our overall sensory testing strategy is in detail described elsewhere [16]. Briefly, after short familiarization with the testing environment on the day prior to the first sensory evaluation, testing sessions were performed on the day preceding surgery and on the fourth, eleventh and eighteenth days after surgery. All testing was blinded. Cold and heat sensitivity was tested by applying an acetone drop three times to each foot [4] and by method of Hargreaves et al. [14], respectively. Three determinations of withdrawal latency for each paw were separated by 1 min. Mechanical allodynia was tested with von Frey fibers. All regions of the right and left paw were tested, in random sequence, before going on to the next stiffer fiber. The withdrawal response was scored either as none or positive if the paw was removed. The test was applied three times to each paw, separated by intervals of at least 1 min. Threshold was determined by interpolation after logit transformation. Temporal summation to the mechanical stimulation was tested by presenting a von Frey stimulus in the center of the plantar aspect of the paw 6 times at 1 Hz and determining withdrawal threshold as the first fiber strength with 50% or more response. The response was scored either as none or positive if the paw was removed. During each testing session, one such determination was performed for each fiber on each paw. Mechanical hyperalgesia was evaluated using a 22-g spinal anesthesia needle applied to the center of the paw with enough force to indent the skin but not to puncture it. Responses were of two types, either a brisk simple withdrawal with immediate return of the foot to the cage floor or a hyperalgesia-type sustained elevation with licking and grooming. The response type was noted for each of three applications to each paw separated by at least 2 min.

2.3. Tissue preparation

Right L5 ganglia were removed between the 18th to the 21st days after surgery. Rats were anesthetized with halothane (2% in oxygen), and a laminectomy was performed while the surgical field was bathed with oxygenated artificial cerebrospinal fluid (aCSF, in mM: NaCl 128, KCl 3.5, $MgCl_2$ 1.2, $CaCl_2$ 2.3, NaH_2PO_4 1.2, $NaHCO_3$ 24.0, glucose 11.0). The L5 ganglia and attached dorsal roots were removed and the connective tissue capsule dissected away. Dorsal root ganglia (DRGs) were transferred to a recording chamber and bathed with 35 °C aCSF