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VAGAL AFFERENT MODULATION OF SPINAL TRIGEMINAL NEURONAL RESPONSES TO DURAL ELECTRICAL STIMULATION IN RATS

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Abstract—Vagus nerve stimulation (VNS) is an approved antiepileptic and antidepressant treatment, which has recently shown promise as a therapy for drug-resistant primary headaches. Specific neurobiological mechanisms underlying its anticephalgic action are not elucidated, partly because of the deficiency of research-related findings. The spinal trigeminal nucleus (STN) plays a prominent role in pathophysiology of headaches by modulating pain transmission from intracranial structures to higher centers of the brain. To determine whether vagal stimulation may affect trigeminovascular nociception, we investigated the effects of VNS on the STN neuronal activity in the animal model of headache. In anesthetized rats the spike activity of the STN neurons with convergent orofacial and meningeal inputs was monitored, and the changes in neuronal responses to electrical stimulation of the dura mater under preconditioning or under continuous electrical stimulation of the left cervical vagus nerve were studied. Preconditioning vagal afferent stimulation (200– ms train of pulses at 30 Hz applied before each dural stimulus) did not produce substantial changes in the STN spike activity. However, continuous VNS with frequency of 10 Hz in 48% of cases significantly suppressed trigeminal neuronal responses to dural electrical stimulation. In line with the decrease in evoked activity, the VNS-induced depression of ongoing neuronal firing was observed. Although the inhibitory effect was prevailing, 29.5% of STN neurons were facilitated by VNS, whereas 22.5% were unresponsive to the stimulation. These results provide an evidence of VNS-induced modulation of trigeminovascular nociception, and therefore contribute to a deeper understanding of neurophysiological mechanisms underlying effects of vagal stimulation in chronic drug-resistant headaches.

Key words: vagus nerve stimulation, headache, dural electrical stimulation, trigeminal pathway, neuronal activity.

INTRODUCTION

In the last two decades, the vagus nerve stimulation (VNS) has become the most widely used non-pharmacological treatment for refractory epilepsy and depression (Kosel and Schlaepfer, 2002; Albert et al., 2009; Bajbouj et al., 2010; Ruffoli et al., 2011). Furthermore, VNS is currently considered by clinicians as a valid therapy for the prevention of drug-resistant primary headaches (Lenaerts et al., 2008; Broggi et al., 2009, 2010). It has been reported that at least half of patients receiving VNS had reductions in frequency and intensity of their chronic cluster, migraine or daily headaches which were not only associated with epilepsy or depression (Sadler et al., 2002; Hord et al., 2003; Cecchini et al., 2009), but also referred to as primary disorders (Mauskop, 2005). Furthermore, a case has been described in which mechanical (prolonged finger pressure) stimulation of the cervical portion of the vagal nerve trunk was able to abort migraine attacks (Di Stani et al., 2007). Additionally, the use of VNS as an *adjunctive* therapy allowed decreasing the numbers and dosages of prophylactic anticephalgic drugs received by the patients (Hord et al., 2003; Mauskop, 2005).

The current explanations of the VNS-induced relief of primary headaches remain highly speculative and are mostly based on evidences of general antinociceptive action of VNS. The analgesic effect of the vagal afferent stimulation has been reported in numerous behavioral pain tests in laboratory animals. For instance, the VNS-induced inhibition of the nociceptive digastric reflex induced by intense tooth-pulp stimulation (Maixner et al., 1991; Bossut et al., 1992), reduction of the cumulative duration of rubbing and scratching the injection site in the orofacial formalin test (Bohotin et al., 2003b), and latency increase of the tail-flick or the hind paw withdrawal response to noxious heat (Ren et al., 1989; Aicher et al., 1991; Thurston and Randich, 1991; Bohotin et al., 2003a) were reported. In epileptic patients treated with VNS, an increase in mechanical pain threshold was noted (Kirchner et al., 2000, 2006).

The exact mechanisms by which stimulation of vagal afferents may reduce pain are not clear. Several neurophysiological studies reported that VNS had predominantly inhibitory effect on activity of lumbar and thoracic spinal neurons (Thies and Foreman, 1983; Hobbs et al., 1989; Ren et al., 1991; Evans et al., 1994). In addition, the vagal afferent stimulation has been found to suppress the spike frequency

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Abbreviations: GABA, gamma-amino butyric acid; STN, spinal trigeminal nucleus; VNS, vagus nerve stimulation.

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and c-fos expression occurring in trigeminal and trigeminothalamic neurons in response to noxious orofacial stimulation (Bossut and Maixner, 1996; Bohotin et al., 2003b). Furthermore, the VNS-induced inhibition of activity of tooth pulp-responsive units in the trigeminal nuclei oralis and caudalis (Takeda et al., 1998; Tanimoto et al., 2002), as well as in the ventral posteromedial nucleus of the thalamus (Nishikawa et al., 1999) has been reported.

These findings indicate that VNS may inhibit nociceptive neurotransmission both at the spinal and at the supraspinal level. However, the neurobiological mechanisms underlying the anticephalgic action of VNS remain unclear. Currently it is hypothesized that VNS, through widespread ascending pathways of the nucleus of the solitary tract, could alter activity of pain-modulating brain structures responsible for headache (Hord et al., 2003; Mauskop, 2005; Lenaerts et al., 2008; Broggi et al., 2009, 2010). Direct experimental evidence for this concept is lacking, mainly due to the deficiency of animal model studies pursuing understanding of VNS action in headache.

Several lines of evidence suggest a prominent role of the spinal trigeminal nucleus (STN) in pathophysiology of headaches. The second-order nociceptive neurons in the STN are shown to be intimately involved in pain transmission from intracranial structures to higher *centers of the brain* (Goadsby, 2005; Goadsby et al., 2009; Messlinger, 2009). To date, no studies have addressed a question whether vagal afferent stimulation modifies the activity of the STN neurons associated with meningeal nociception. Such data could provide an important contribution to understanding of neurophysiological mechanisms underlying VNS action in drug-resistant headaches.

Therefore, in the present work we investigated the effects of VNS on the STN neuronal activity in the animal model of headache. In order to determine whether vagal stimulation may affect trigeminovascular nociception, we monitored the spike activity of the STN neurons with convergent orofacial and meningeal inputs and studied the changes in their responses to electrical stimulation of the dura mater under electrical stimulation of the left cervical vagus nerve.

EXPERIMENTAL PROCEDURES

Twenty adult male Wistar rats (body weight 300–380 g) were used for the study. The animals were housed in the vivarium of the Pavlov Institute of Physiology (St. Petersburg, Russia) and maintained 2–5 animals per cage on a 12-h light/dark schedule with free access to food and water. All experiments were performed according to the Ethical Guidelines of the International Association for the Study of Pain and European Community Council Directive (86/609/EEC). The study protocol and experimental design were approved by the Institutional Animal Care and Use Committees of the Pavlov Institute of Physiology and the Saint-Petersburg Pavlov State Medical University. All efforts were made to reduce the number of animals used and to minimize any possible suffering.

Anesthesia and surgical preparation

Rats were anesthetized with urethane (1.5 g/kg, i.p; ICN Biomedicals, Aurora, OH, USA). Experimental procedures used were described in detail previously (Lyubashina and Panteleev, 2009; Sokolov et al., 2010, 2012). Briefly, the rat under the surgical level of anesthesia was placed on a thermostatically controlled heating pad. Catheters were placed into the femoral vein for administration of anesthetics and myorelaxants, and into the femoral artery for continuous monitoring of blood pressure. The trachea was intubated and the head of the animal was fixed in a stereotaxic frame. The cervical portion of the left vagal nerve trunk was isolated and cut; its central end was displaced dorsally, put on silver bipolar stimulating electrodes and kept moist with warm paraffin oil (37 °C).

The neck muscles overlying the cisterna magna were separated along the midline and C1 laminectomy was performed. The dura mater was removed to expose the medulla and C1 spinal cord. A longitudinal parietal craniotomy was performed, and the bipolar stimulating electrodes were placed on the dura mater in close proximity to the superior sagittal sinus or visible blood vessels. The electrodes had resistance of 50 KΩ and consisted of two varnish-insulated silver wires with beads (0.3 mm in diameter) at the end. The animal was paralyzed using the pipecuronium bromide (i.v., 1.2 mg/kg initially, maintenance 0.6 mg/kg as required; Gedeon Richter, Budapest, Hungary) and artificially ventilated with room air (75–100 cycles/min, 2–3 ml per cycle) using a small animal ventilator. Rectal temperature was maintained between 37 and 38 °C. The depth of anesthesia was assessed by monitoring blood pressure responses to noxious stimulation; supplementary anesthetic was administered when necessary to ensure the absence of gross (> 20% from the baseline level) blood pressure fluctuations.

Extracellular recordings

Neuronal activity was recorded by varnish-insulated tungsten microelectrodes (Science Products, Hofheim, Germany) with a tip diameter of 5 μm and a resistance of 12 MΩ. The electrodes were lowered into the left STN at the level of C1 spinal cord in 4-μm steps using a microdrive unit. The signals from the recording electrode were amplified and passed to the analogue input of the computer A/D converter by means of the multifunctional acquisition card (sampling period 25 μs). For online acquisition, processing and displaying the data, the custom-written software was used. To isolate the activity of single units from stimulus artifacts, adjacent cell potentials and noise, three-level amplitude discrimination was used online. Recordings of neuronal activity were analyzed as peristimulus time histograms, such that signals gated through the amplitude discrimination were collected in successive bins of 1 ms. The histograms had a sweep length of 500 ms and were created automatically from 50 recordings (one per 1 s). Ongoing activity (if analyzed) and electrically evoked responses of neurons were estimated within 250 ms before and 50 ms after stimulation of the dura mater, correspondingly. Apart from responses to the dural electrical stimulation, all recorded units were tested for responses to mechanical stimulation of their dural and facial cutaneous receptive fields by von Frey filaments (North Coast Medical, Morgan Hill, CA, USA). Only neurons demonstrating all three kinds of responses were selected for further testing.

Dural electrical stimulation and VNS

The dura mater was stimulated using single rectangular pulses of 300–800 μA (15–40 V) with a duration of 0.8 ms delivered by a computer-controlled stimulator. The stimulus intensity was 1.5 times the response threshold. The intensity of the current used to stimulate the vagus nerve was approximately 0.8 of its threshold to induce 10–15% changes (typically decrease) in arterial blood pressure and was not more than 350 μA. Most frequently, the rectangle current pulses of 100–300 μA (5–15 V) with a duration of 0.5 ms were used. In order to compare the post-effect of a short *high-frequency stimulus train with the action of repetitive stimulation of a lower frequency, two corresponding* settings of