

DANIEL ŻUROWSKI<sup>1</sup>, ŁUKASZ NOWAK<sup>1</sup>, JERZY WORDLICZEK<sup>2</sup>, JAN DOBROGOWSKI<sup>3</sup>,  
PIOTR J. THOR<sup>1</sup>

## EFFECTS OF VAGUS NERVE STIMULATION IN VISCERAL PAIN MODEL

**Abstract:** *Effects of vagus nerve stimulation in visceral pain model.*

Visceral pain is an important therapeutic problem. A number of studies have established that abdominal vagal afferents modulate somatic pain behavior. Although it is not clear if vagal afferents transmit nociceptive information, a change in their activity can increase or decrease nociceptive transmission in visceral pain. Aims of the present study were to determine whether the subdiaphragmatic vagus nerves play a role in the endogenous pain inhibitory mechanisms in visceral pain model and whether it involves opioidergic pathways.

Data obtained in our studies show that vagus nerve plays the direct role in conveying the nociceptive information in the peritonitis model of visceral pain. We have shown, that vagal afferents exhibit an increase in excitability and subdiaphragmatic vagotomy decrease nociceptive behavior in visceral pain in rats. We have also tested two different stimulation parameters of chronic subdiaphragmatic vagal nerve stimulation: VNS1 (high-intensity) and VNS2 (low-intensity) in visceral pain model in rats. Both stimulation parameters increased pain threshold but VNS1 was more effective than VNS2. Naloxone inhibited the antinociceptive effects of VNS, reversing partially increase in the pain threshold in rats and increases number of writhes in visceral pain model. Therefore, our data indicate that this analgesic effect of the VNS is mediated, at least in part, by descending opioidergic pathways.

The present study has confirmed the importance of vagal afferents for nociception in general and proven that this role is not limited to somatic pain but also extends to visceral pain.

**Key words:** vagal nerve stimulation, visceral pain, subdiaphragmatic vagotomy

The vagus nerve contains mostly afferent fibers that innervate thoracic and abdominal viscera conveying information important for the feedback modulation of homeostatic and allostatic functions such as circulation, respiration, gastrointestinal, and neuroendocrine systems [1]. The activity in these afferents also modulate the inflammatory process, including inflammatory hyperalgesia

[2–4]. Our previous work has helped delineate details of how vagal afferent activity affects components of the inflammatory response, and recently, we have detected several aspects of this modulation [5–7]. Several previous studies have also documented the role of vagally mediated afferent neural signals in modulation of pain perception [8–11]. Although it is not clear if vagal afferents transmit nociceptive information, a change in their activity can increase or decrease nociceptive transmission in visceral pain. The effects of vagotomy or simulation of the cut nerve were studied acutely on behavioral responses to painful stimuli and overall conclusion was that under certain conditions vagal afferent stimulation had an analgesic potential [12–14].

Vagotomy studies demonstrate that vagal afferent integrity is essential to the efficacy of different analgesic treatments like morphine [13]. Furthermore, subdiaphragmatic vagotomy decreases the threshold for mechanically induced hind paw withdrawal in rats [8, 12], increases sensitivity to various noxious lesions [15] and enhances hyperalgesia induced by bradykinin [8, 16]. Vagotomy resulting in a chronic elevation in plasma concentration of epinephrine mediate the effects of the adrenal medulla on pain signaling [17]. However, vagotomy also prevents the establishment of kainic acid-induced hyperalgesia in mice [18] and reduces nociception in the formalin test in male rats [9].

Stimulation of vagal afferents (VNS) in rodents can raise or lower baseline nociceptive thresholds and can either increase or decrease the magnitude of hyperalgesia [13]. The analgesic effect of VNS seems to depend on a critical stimulation intensity that activates C fibers [19]. Vagus nerve stimulation (VNS) therapy for treatment of epilepsy enabled assessment of its effect on pain in human. Like in the animal study, the clinical data showed contradictory results. In one study, VNS induced a significant decrease of thermal pain threshold [20]. On the contrary, in another study, VNS produced an increase in mechanical pain threshold but no change of heat pain thresholds [21]. In addition, pain perception has also been assessed in healthy human volunteers after vagal afferent activation by a rapid filling of the stomach with water [22]. Heat pain threshold was increased after such gastric distension while mechanical pain threshold was not significantly altered. On the other hand, Holtmann *et al.* [23] reported lower thresholds for the perception of pain in patients who had previously undergone vagotomy in course of a Billroth I gastrectomy, compared to those in healthy controls. Moreover, Ajao *et al.* [24] reported that vagotomy definitely reduces the pain associated with terminal upper gastrointestinal neoplasms.

However, there is no report to investigate whether subdiaphragmatic vagotomy changes the pain threshold in inflammatory visceral pain. Furthermore, the influences of chronic electrical stimulation of subdiaphragmatic vagal afferent nerves on visceral pain evoked by inflammatory stimuli have not been investigated.

Persistent abdominal pain or discomfort represent the most frequently mentioned complaints of patients and is important therapeutic problem [25]. Therefore, we decided to use in our study the “Writhing Test” (visceral pain model). Aims of the present study were to determine: whether the subdiaphragmatic vagus nerves play a role in the endogenous pain inhibitory mechanisms following inflammatory visceral pain and whether it involves opioidergic pathways.

## MATERIALS AND METHODS

Male Wistar rats weighting 250–300 g were housed at a room temperature ( $22 \pm 2\text{C}^\circ$ ) and on a 12 hours light/day cycle. Standard laboratory food (Labofeed B, Poland) and tap water were provided ad libitum. Before every experiment rats were food deprived for 12 hours. Studies were approved by the Ethic Committee for Animal Research of Jagiellonian University (92/2010).

### Experimental visceral pain model (WR)

Nociceptive activity was tested in rats using the writhing model [7]. We used acetic acid (10 ml/kg 0.6% solution), stimulus known to produce inflammation as well as writhing response in rats. The rats had the nociceptive stimuli injected into the peritoneal cavity. Controls were injected with saline. The intensity of nociception was quantified by counting the number of writhes occurring during 30 min after acetic acid injection. The writhing response consists of a contraction of the abdominal muscles together with a stretching of hind limbs.

### c-Fos expression in nodose ganglion (NG)

After Vetbutal overdosing inferior vagal ganglions (NG, nodose ganglions) were bilaterally prepared and sampled to histochemical evaluations. The preparations were fixed and then frozen. Frozen preparation was sliced by using cryostat and subsequently submitted to the immunohistochemical staining. The specimens were incubated with rabbit c-Fos antibody (K-25; Sc-253) (Santa Cruz Biotechnology). After 3 times repeated PBS washing the specimens were incubated with second degree biotinylated goat anti-rabbit antibody (Jackson ImmunoResearch, West Grave, PA). Afterwards the specimens were incubated with streptavidin — Cy3 complex (Jackson ImmunoResearch, West Grave, PA). Then the specimens were embedded in fluorescent specimen’s medium (DAKOCytomation, Denmark). Negative controls were conducted without the first antibody. The specimens were analyzed under the Zeiss Axsioscop fluorescent microscope.

## Neuroelectrophysiological studies

The activity of single vagal afferents was recorded as previously described [26]. Rats were anesthetized with pentobarbital sodium (40 mg/kg, i.p.) and placed in a supine position with the head gently clamped in place. Body temperature was monitored by a rectal thermal probe and was maintained at 36–37°C by a warm-water heating pad. Artificial ventilation was carried out throughout the experiments *via* a tracheal cannula. The left trunk of the vagus was gently separated from the carotid artery. To electrically isolate the nerve, a piece of Teflon tape was placed under it and the site was filled with paraffin oil (37°C). A small bundle of nerve fibers was peeled off and cut free from the main trunk, and the cut end was placed on a bipolar platinum wire electrode (A-M Systems, Carlsborg, WA). A reference electrode was placed in the epineurium. Neural activity was sampled at 20 kHz and amplified using standard techniques (PowerLab/8SP ADInstruments, Australia) and fibers with obvious spontaneous activity were recorded for the 30 min periods on PC hard disc. Vagal afferents discharges were analysed with dual time-amplitude windows discriminator software (Spike Histogram v.2 ADInstruments). Then, the activities (spikes/s) of vagal afferent fibers were analyzed with ANOVA followed by Tukey's tests. Results were expressed as means  $\pm$  SEM and  $p < 0.05$  considered significant.

### Vagotomy

Subdiaphragmatic vagotomy were performed on rats ( $n = 8$ ) as previously described [27]. Briefly, after an overnight fast, rats were anesthetized using pentobarbital (40 mg/kg ip). After a midline laparotomy, the stomach and lower esophagus were gently retracted from the abdominal cavity. For the total vagotomy, the two vagal trunks were exposed and cut. All neural and connective tissue surrounding the esophagus immediately below the diaphragm was removed to transect all small vagal branches. Sham animals were also prepared ( $n = 8$ ). For the sham vagotomy, the viscera were similarly handled, but no nerves were cut. The incisions were closed in layers. Postoperatively, the animals were observed and weighed daily to monitor the general health of the animals. Rats that failed to gain body mass were euthanized with pentobarbital. Studies were done after 7 days after surgery.

### Vagus nerve stimulation (VNS)

To asses VNS antinociceptive effect on visceral pain (WR), four experimental groups of eight animals were established:

1. Animals with WR
2. Animals with WR receiving VNS (divided in two subgroups with different parameters: VNS1 and VNS2)

3. Animals with WR treated with naloxone (1 mg/kg sc)

4. Animals with WR receiving VNS treated with naloxone (1 mg/kg sc)

In groups 2 and 4 in visceral pain model left vagus nerve below diaphragm was dissected from esophagus and electrodes were wrapped around the nerve with the positive electrode placed distally. The stimulator device (Institute of Electron Technology, Cracow, Poland) was implanted in a subcutaneous pocket on the back of the rats [28]. At least five days were allowed for recovery before starting experiments. After pilot study, following stimulation parameters were used:

VNS1 — 2 mA, 20 Hz, 0.5 msec pulse with, 20 s on/18 off duty cycle

VNS2 — 40  $\mu$ A, 20 Hz, 0.5 msec pulse with, 30 s on/30 s off duty cycle

## RESULTS

### Effect of subdiaphragmatic vagotomy

To determine whether subdiaphragmatic vagus nerves are involved in the modulation of visceral pain chronic bilateral subdiaphragmatic vagotomy was performed. Data were collected from 8 sham-operated control rats and 8 rats after vagotomy. Chronic subdiaphragmatic vagotomy produced a marked decrease in number of abdominal muscle contractions compared to sham operation ( $p < 0,05$ ). Our results provide evidence that subdiaphragmatic vagotomy decreases nociceptive behavior in visceral pain.

### Effects of visceral pain on vagal afferents activity and c-Fos expression in nodose ganglia

Visceral pain increased both vagal afferents discharges frequency and c-Fos expression in NG ( $p < 0.05$ ). Our data indicate that vagal afferents are directly involved in visceral hyperalgesia. Intraperitoneal acetic acid injection induces chemical peritonitis and this inflammatory process gives the way to hyperalgesia. We have shown, that in the presence of inflammation, vagal afferents exhibit an increase in excitability. Naloxone — a nonselective opioid receptors antagonist did not affect vagal afferents activity.

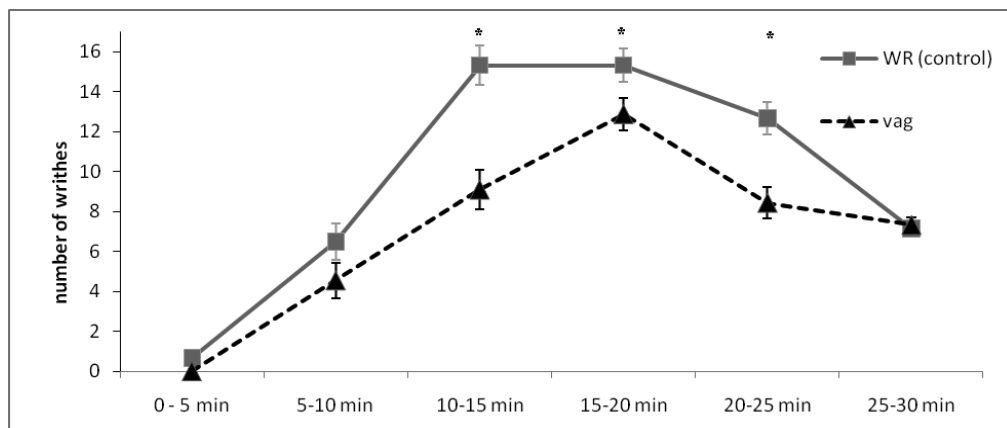


Fig 1. Effect of subdiaphragmatic vagotomy (vag) on the writhing response (WR) induced by acetic acid in conscious rats. The number of writhes was determined for the interval of 0–30 min, after i.p. injection of acetic acid (10 ml/kg 0.6% solution).

Results are expressed as means  $\pm$ SD for groups of eight rats;

\*  $p < 0.05$

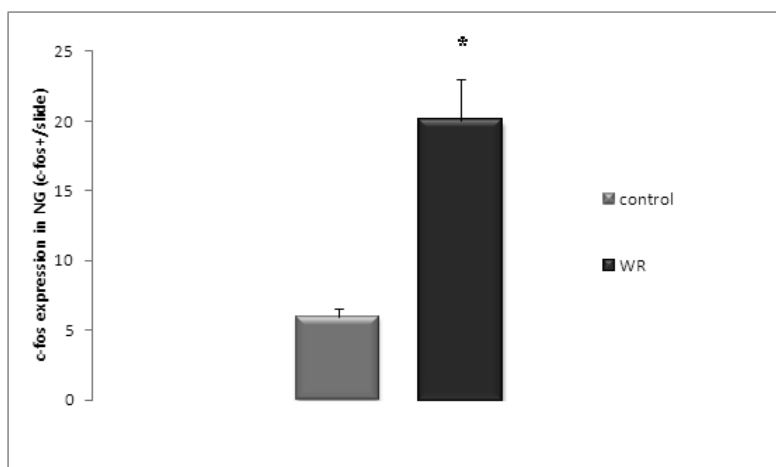


Fig. 2. c-Fos expression in NG (nodose ganglion) in visceral pain (WR — writhing response);

\*  $p < 0.05$

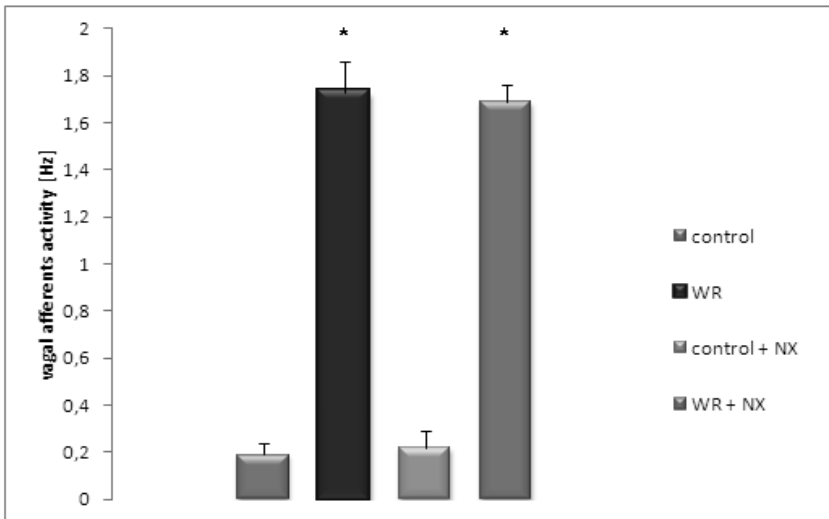


Fig. 3. Frequency of vagal afferents discharge in visceral pain (WR — writhing response; NX — naloxone);  
\*  $p < 0.05$

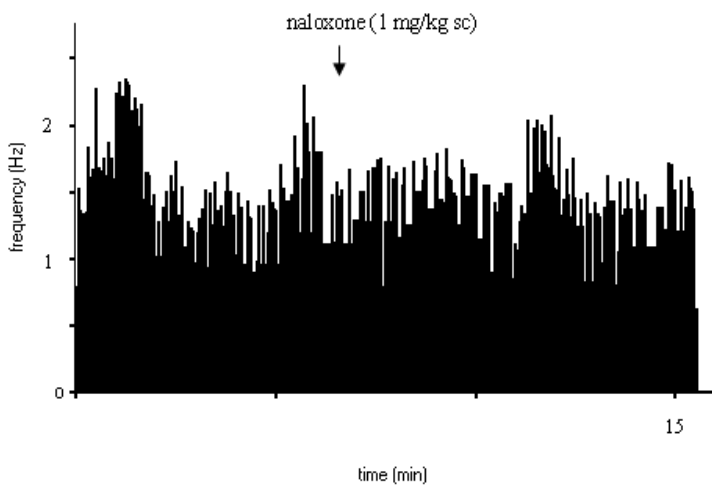


Fig. 4. Rate histogram of vagal afferents discharge in visceral pain model (WR). Number of spikes is plotted against time. Naloxone did not affect spontaneous vagal afferents activity

### Effects of vagal nerve stimulation on visceral pain

We have tested two different stimulation parameters of chronic subdiaphragmatic vagal nerve stimulation: VNS1 (high-intensity) and VNS2 (low-intensity) in visceral pain model in rats. Both stimulation parameters increased pain threshold ( $p < 0.05$ ). VNS1 was more effective than VNS2 in visceral pain. Naloxone — a nonselective opioid receptors antagonist — partially reversed

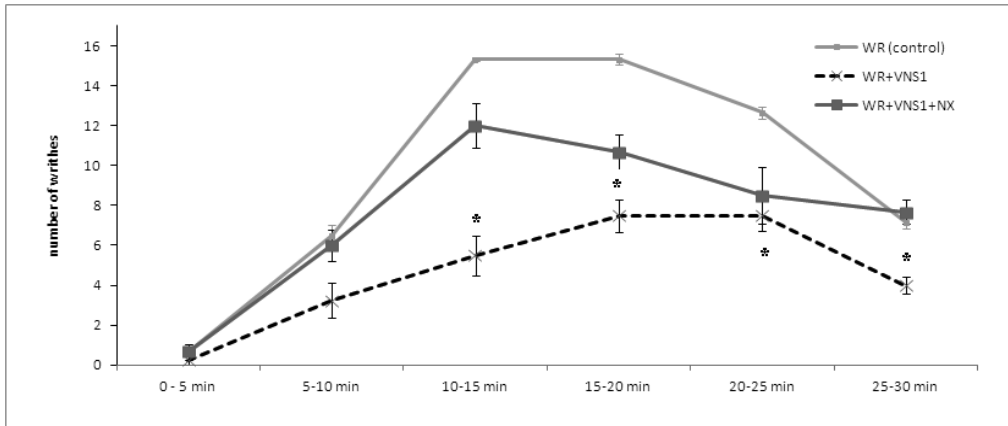


Fig. 5. Influence of VNS1 [2 mA, 20 Hz, 0.5 msec pulse with, 20 s on/18 off duty cycle] on visceral pain (WR — writhing response; VNS — vagal nerve stimulation; NX — naloxone)  
\*  $p < 0.05$

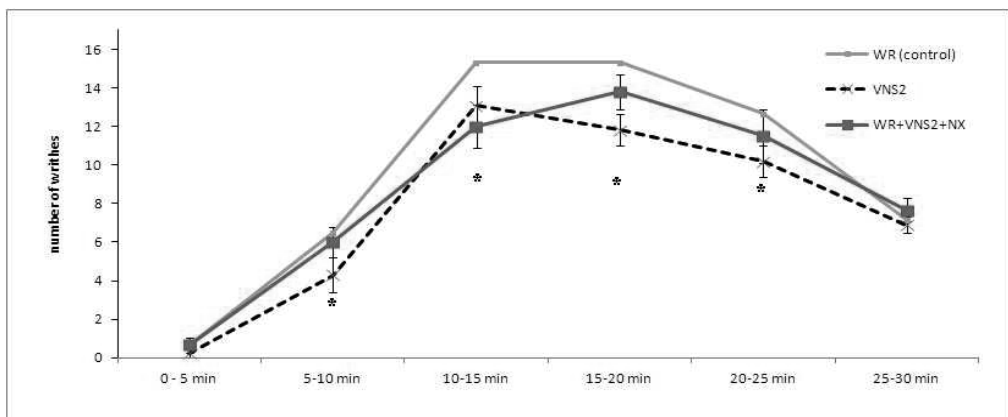


Fig. 6. Influence of VNS2 [40  $\mu$ A, 20 Hz, 0.5 msec pulse with, 30 s on/30 s off duty cycle] on visceral pain (WR — writhing response; VNS — vagal nerve stimulation; NX — naloxone)  
\*  $p < 0.05$



antinociceptive effects of VNS, causing the decrease in the pain threshold and the increase nociceptive behavior ( $p < 0.05$ ).

## DISCUSSION

The key findings of the our study are as follows: visceral pain increased excitability of vagal sensory fibres; subdiaphragmatic vagotomy decrease nociceptive behavior in visceral pain; chronic VNS may be effective to alleviate visceral pain and this analgesic effect is mediated in part, by opioidergic pathways.

Although it is commonly assumed that vagal afferents are not involved in nociception and pain, there is growing evidence that they play a complex role in these processes [1]. The change of the activity in the vagus nerve, especially the subdiaphragmatic vagus nerve, affects nociceptive responses [8, 13, 29]. The present study has confirmed the importance of vagal afferents for nociception in general and proven that this role is not limited to somatic pain but also extends to visceral pain. We have shown that subdiaphragmatic vagotomy decrease nociceptive behavior in visceral pain. We have also shown, that in the presence of inflammation, vagal afferents exhibit an increase in excitability (visceral pain increased both vagal afferents discharges frequency and c-Fos expression in NG).

Vagotomy also prevents the establishment of kainic acid-induced hyperalgesia [18] and reduces nociception in the formalin test in rats [9]. On the other hand several studies demonstrate that subdiaphragmatic vagotomy decreases the threshold for mechanically induced hind paw withdrawal in rats [8, 12, 16].

Limited information is available about contribution of the vagus nerve to visceral pain. Chen *et al.* [27] reported that vagotomy enhanced visceral pain responses in rats. Furuta and coworkers [28] have shown that subdiaphragmatic vagal dysfunction caused chronic muscle hyperalgesia accompanied by visceral pain. The results of these experiments are contradictory to our results.

In our experiments, animals that had undergone subdiaphragmatic vagotomy 7 days prior to the “*writhing test*” showed decreased nociceptive responses to the inflammatory visceral stimulus. Data obtained in our studies show that vagus nerve plays the direct role in conveying the nociceptive information in the peritonitis model of visceral pain. Several studies reported similar conclusions. Noxious gastric distention resulted in c-Fos expression in the nucleus of the solitary tract which is even more pronounced than that in the spinal cord [30]. This c-Fos expression could be largely ascribed to activation of vagal afferents and to a minor extent activation of a spino-solitary pathway. Esophageal distension has been shown to elicit cardiovascular reflex responses mediated by vagal afferents and this has been interpreted as a nociceptive event [1].

Finally, and most relevant to the overall theme of immunosensation, Watkins *et al.* [4] have demonstrated that LPS- and IL-1 $\beta$ -induced hyperalgesia depends on the integrity of the vagus nerve, particularly its common hepatic branch.

We have tested two different stimulation parameters of chronic subdiaphragmatic vagal nerve stimulation (high intensity — VNS1 and low intensity — VNS2) in experimental visceral pain model in rats. Both stimulation parameters increased pain threshold in visceral pain. However VNS1 was more effective than VNS2. Our findings are in accordance with previous studies of the effects of VNS.

Bohotin *et al.* [14] tested the analgesic effect of two different stimulation protocols, one with a stringent duty cycle and one with the duty cycle used in epilepsy. VNS reduced significantly formalin-evoked nociceptive behaviour in rats [14, 31]. The VNS protocol with the most stringent duty cycle, produced the most rapid and pronounced antinociceptive effect. However, the less stringent duty cycle was also able to induce antinociception, but only after days of stimulation. Antinociceptive effect starts after 24 h of stimulation and increases over the next 2 days to reach plateau. Several studies have demonstrated that vagus nerve stimulation (VNS) alters pain perception. A recent study showing changes in pain perception as a function of different VNS settings hints at the promise of using VNS for some form of pain modulation. Findings suggest that, in general, low-intensity VNS is associated with pronociceptive effects, but high-intensity VNS is associated with antinociceptive effects [13]. Moreover, acutely, VNS increases pain perception [20], but used chronically VNS results in a decrease in pain perception in humans [21].

Our results suggest, that VNS can influence the pain threshold via the modulation of the opioid system. Naloxone — a nonselective opioid receptors antagonist — inhibited the antinociceptive effects of VNS, reversing partially increase in the pain threshold and increases number of writhes in visceral pain model. Therefore, our data indicate that this analgesic effect of the VNS is mediated, at least in part, by descending opioidergic pathways.

The exact mechanisms by which VNS may reduce pain remain to be determined. The central projections of the vagus nerve suggest that the upper cervical spinal cord could be involved [32]. The observation that vagal stimulation inhibits spinal cord neurons in segments below C3 but excites C1-C3 neurons lead to the hypothesis that propriospinal neurons from high cervical segments could be an important component of vagally mediated antinociception in distant spinal segments [33]. Direct projections of primary vagal afferents to the upper cervical spinal cord have been demonstrated also in tracing studies [1].

Central terminals of primary vagal afferents are largely found in the nucleus of the solitary tract [1] which may be the initial relay for vagal afferent inhibition of pain [13]. Experiments using local anesthetic blockade of nucleus tractus solitarius but also raphe magnus, locus ceruleus and periaqueductal

gray suggests that these structures mediate part of the antinociceptive effects of VNS [34, 35]. Stimulation of vagal afferent fibers has been reported to affect the basal activity of raphe magnus neurons [36]. Different neurotransmitter systems have been implicated in VNS-induced analgesia, in particular serotonin [37], noradrenaline [38] and opioids [38, 39]. GABA was found increased in the cerebrospinal fluid of epileptic patients treated with VNS [40]. Vagal afferent impulses could also modulate pain perception via an indirect activation of the paraventricular nucleus, which in turn modifies adrenaline release from the adrenal medulla [17]. Finally, VNS is able to increase plasma levels of ACTH and corticosterone [41] which could mediate some of its antinociceptive and anti-inflammatory effects.

The exact pathways of antinociceptive action of vagal afferents remain unclear. Therefore further investigations are needed to establish the exact mechanisms of vagal nerve stimulation in a different forms of pain and subsequently to compile the brand new antinociceptive therapeutical strategies.

#### CONFLICT OF INTERESTS STATEMENT

None declared.

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<sup>1</sup> Department of Pathophysiology  
Jagiellonian University Medical College  
ul. Czysta 18, 31-121 Kraków, Poland

<sup>2</sup> Department of Pain Treatment and Palliative Care  
Jagiellonian University Medical College  
ul. Śniadeckich 10, 31-531 Kraków, Poland

<sup>3</sup> Department of Pain Research and Treatment  
Jagiellonian University Medical College  
ul. Śniadeckich 10, 31-531 Kraków, Poland

**Corresponding author:**

Daniel Żurowski, MD, PhD  
Department of Pathophysiology  
Jagiellonian University Medical College  
ul. Czysta 18, 31-121 Kraków, Poland  
E-mail: [danski@mp.pl](mailto:danski@mp.pl)

